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Advances in diabetic kidney disease: Pathophysiology, clinical characteristics, and future directions

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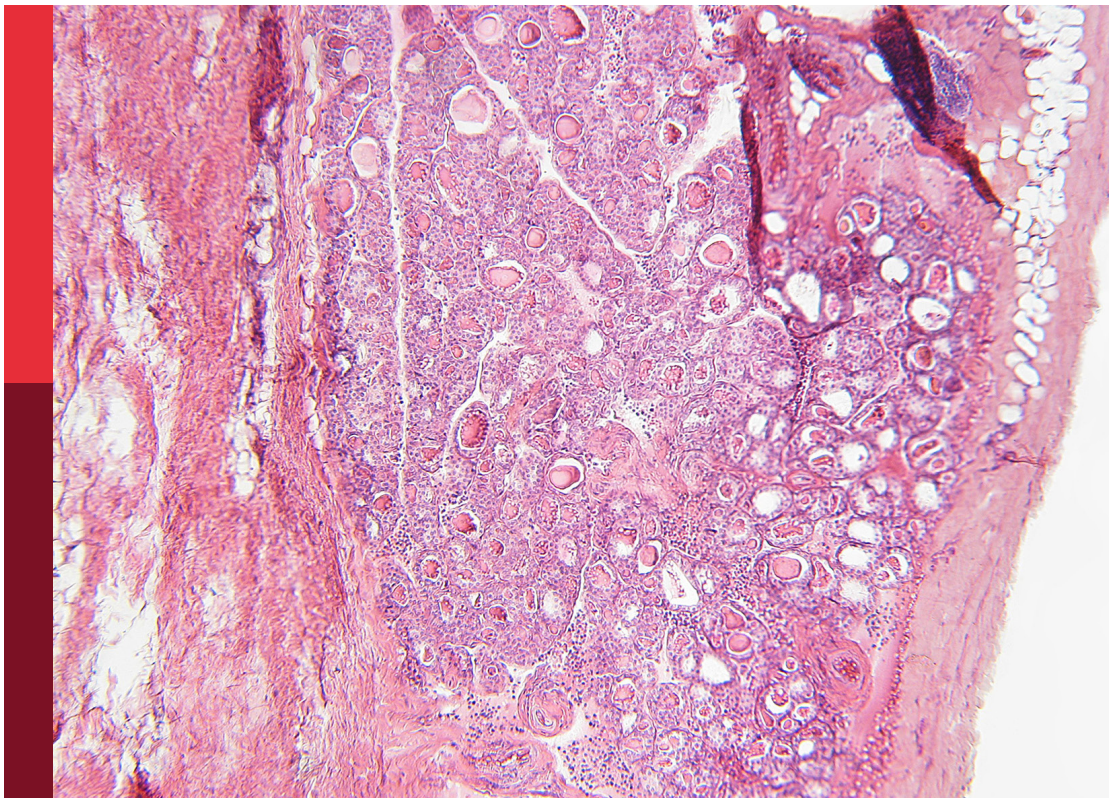

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Advances in diabetic kidney disease: Pathophysiology, clinical characteristics, and future directions

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Naoxintong capsule delay the progression of diabetic kidney disease: A real-world cohort study

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Introduction: Diabetic kidney disease (DKD) is a severe and growing health problem, associated with a worse prognosis and higher overall mortality rates than non-diabetic renal disease. Chinese herbs possess promising clinical benefits in alleviating the progression of DKD due to their multi-target effect. This real-world retrospective cohort trial aimed to investigate the efficacy and safety of Naoxintong (NXT) capsules in the treatment of DKD. Our study is the first real-world study (RWS) of NXT in the treatment of DKD based on a large database, providing a basis for clinical application and promotion.

Methods: The data was collected from Tianjin Healthcare and Medical Big Data Platform. Patients with DKD were enrolled from January 1, 2011, to March 31, 2021. NXT administration was defined as the exposure. The primary outcome was the change in estimated glomerular filtration rate (eGFR). We employed the propensity score matching (PSM) method to deal with confounding factors.

Results: A total of 1,798 patients were enrolled after PSM, including 899 NXT users (exposed group) and 899 non-users (control group). The eGFR changes from baseline to the end of the study were significantly different in the exposed group compared to the control group (-1.46 $\pm$ 21.94 vs -5.82 $\pm$ 19.8 mL/(min·1.73m^2), P< 0.01). Patients in the NXT group had a lower risk of composite renal outcome event (HR, 0.71; 95%CI, 0.55 to 0.92; P = 0.009) and deterioration of renal function (HR, 0.74; 95% CI, 0.56 to 0.99; P = 0.039).

Conclusion: NXT can significantly slow the decline of eGFR and reduce the risk of renal outcomes. However, large cohort studies and RCTs are needed to further confirm our results.

KEYWORDS

Naoxintong capsule, diabetic kidney disease, retrospective cohort study, propensity score matching, real-world study

Introduction

Diabetic kidney disease (DKD) is a severe and growing health problem globally, which is reported to be the leading cause of the end-stage renal disease (ESRD) (1). There is accumulating evidence that DKD is associated with a worse prognosis and higher overall mortality rates than non-diabetic renal disease (2). Efforts to reduce ESRD prevalence and burden, therefore, include the prevention of DKD before its occurrence and the delay of progression of established DKD. Recent guidelines on the treatment of DKD recommended that lifestyle interventions and the blocking of the renin-angiotensin–aldosterone system (RAAS) should be considered for individuals with DKD (3). Despite the certain effects these recommendations achieved, many individuals still suffer glomerular filtration rate (GFR) decline and renal damage due to the lack of satisfactory therapeutic strategies (4).

Traditional Chinese Medicine (TCM) has a long history of clinical practice and is becoming a promising option worldwide as complementary medicine (5). TCM therapies, especially Chinese herbs, possess promising clinical benefits in alleviating the progression of DKD due to their multi-target effect (6). Naoxintong (NXT) capsule is one of the classic prescription drugs for treating cardiovascular disease caused by Qi stagnation and blood stasis (7). The formula has been reported to have a renal protective effect and be effective in ameliorating glucose metabolism and delaying DKD progression (8, 9). However, to our knowledge, NXT capsule has never been investigated in DKD patients in the setting of a clinical trial. The prior studies were limited to the empirical summaries of physicians and had a small sample size. Therefore, we hypothesize that NXT capsule may be a safe and effective treatment for DKD. We designed a retrospective observational cohort study to evaluate the efficacy and safety of NXT in patients with DKD. Our study is the first real-world study (RWS) of NXT in the treatment of DKD based on a large database, providing a basis for clinical application and promotion.

Methods

Data collection

The present retrospective observational cohort study was conducted using data from Tianjin Healthcare and Medical Big Data Platform. This platform contains information from 42 tertiary hospitals and 40 secondary hospitals, supplemented by data from 274 primary and community hospitals, covering 17,420,097 patients' medical records in Tianjin, China. Patient data were retrieved from the electronic medical record, Hospital Information System (HIS), Laboratory Information System (LIS), and the home page of the medical record, including the date of birth, sex, BMI, diagnostic codes, course of the disease, medication, and laboratory indices. The data were extracted by two researchers to avoid bias and were validated by a third researcher to ensure their accuracy.

This study was approved by the ethics committee of Guang'anmen Hospital, China Academy of Chinese Medical Sciences on November 23, 2020 (2020-056-KY). Since the study was designed retrospectively, informed consent was not required. All clinical studies were conducted in accordance with the Helsinki Declaration.

Study design and participants

Patients with confirmed DKD from 82 hospitals were enrolled from January 1, 2011, to March 31, 2021. The diagnostic criteria of DKD were according to the Kidney Disease Outcomes Quality Initiative (KDOQI) clinical practice guidelines (10). Key inclusion criteria included estimated glomerular filtration rate<90 mL/(min·1.73m^2) and sufficient serum creatinine records or endpoint events. Patients with a severe lack of clinical diagnosis and treatment data were excluded. The inclusion and exclusion criteria are as follows:

Inclusion criteria

(1) Patients in the database who met any of the following diagnoses:

a. Diagnostic names included "diabetic nephropathy", "diabetic kidney disease", "diabetes with renal complications" or disease ICD is "E10.2", "E11.2", "E12.2", "E13.2", "E14.22";

b. Patients who met both of the following diagnoses: ①Diagnostic names included "diabetes", "hyperglycemia", or disease ICD is "E14.901", "R73.901"; ②Diagnostic names included "arteriosclerotic nephropathy", "hypertensive nephropathy", "renal disease", "nephropathy", or disease ICD is "I12.903", "N04.901", "N28.901", "I12.901";

(2) Baseline glomerular filtration rate was less than 90 mL/(min·1.73m^2);

(3) The visit time was from January 1, 2011 to March 31, 2021;

(4) Two serum creatinine tests were recorded or at least one serum creatinine test with one endpoint event.

Exclusion criteria

1. Patients with serious lack of clinical diagnosis and treatment data;
2. Patients with NXT medication interval of more than 1 year.

The patients were divided into two groups according to the NXT prescription. The exposed group included patients who had taken NXT for more than 60 days, and it was further divided into high, medium, and low exposed groups based on the duration of administration. The control group included patients who were diagnosed with DKD but did not take NXT (including capsule, decoction, granules, and traditional Chinese medicine that with the same efficacy as NXT). Since the data in this study are real-world data, propensity score matching (PSM) was conducted to reduce the interference of confounding factors. The final study cohort comprised 1,798 DKD patients, with 899 patients in each group (Figure 1). Details on the study design and screening process can be found in Supplement 1.

Outcomes

The primary outcome was the change in estimated glomerular filtration rate. The eGFR was calculated by CKD-EPI equation using gender, age, and serum creatinine. The formula is as follows:

$$\text{Female} \le 62(\le 0.7)\mu\text{mol/L(mg/dL)} \quad \text{eGFR}\ [\text{mL}/(\text{min}\cdot 1.73\text{m}^2)] = 144 \times (\text{Scr}/0.7)^{-0.329} \times (0.993)^{\text{age}} > 62(>0.7)\mu\text{mol/L(mg/dL)} \quad \text{eGFR}\ [\text{mL}/(\text{min}\cdot 1.73\text{m}^2)] = 144 \times (\text{Scr}/0.7)^{-1.209} \times (0.993)^{\text{age}}$$

$$\text{Male} \le 80(\le 0.9)\mu\text{mol/L(mg/dL)} \quad \text{eGFR}\ [\text{mL}/(\text{min}\cdot 1.73\text{m}^2)] = 141 \times (\text{Scr}/0.9)^{-0.411} \times (0.993)^{\text{age}} > 80(>0.9)\mu\text{mol/}L\text{(mg/dL)} \quad \text{eGFR}\ [\text{mL}/(\text{min}\cdot 1.73\text{m}^2)] = 141 \times (\text{Scr}/0.9)^{-1.209} \times (0.993)^{\text{age}}$$

The secondary outcomes included kidney composite endpoint (end-stage renal disease (11), renal replacement therapy, doubling of serum creatinine (12), death due to kidney disease), 50% decrease in eGFR, major adverse cardiovascular events (MACE) (cardiovascular death, non-fatal myocardial infarction or non-fatal stroke), hospitalization for heart failure, and the change of CKD stages.

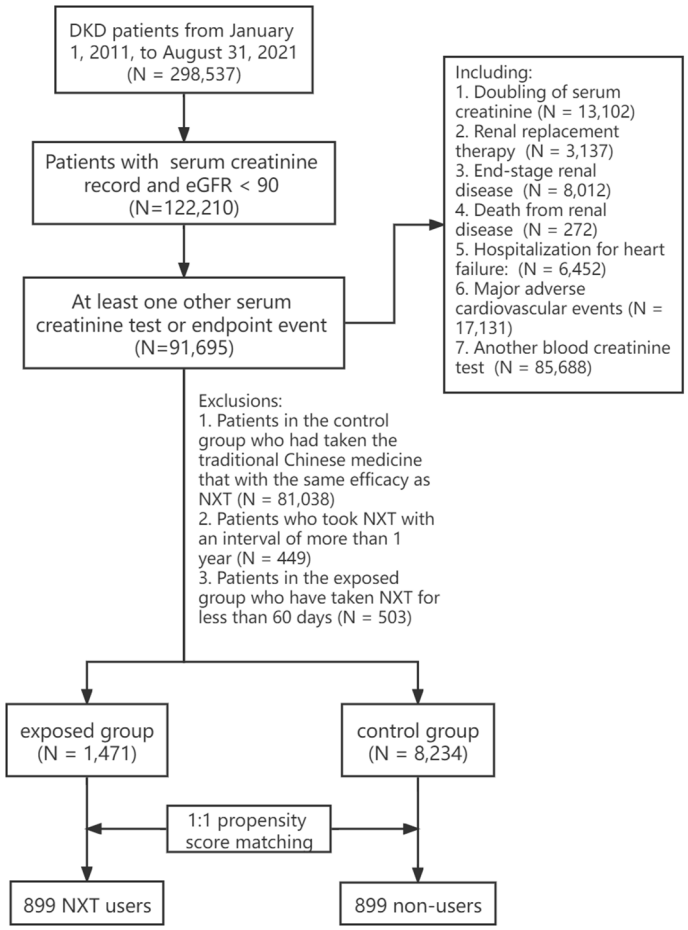

FIGURE 1
Workflow of the retrospective cohort study.

The stage of CKD refers to the 2021 definition and staging criteria of CKD proposed by Kidney Disease: Improving Global Outcomes (KDIGO). Safety indicators included blood routine, serum electrolyte, and liver function, which were assessed by calculating the difference between the endpoint and baseline.

Statistical analysis

For quantitative data, descriptive statistical analysis was carried out by the number of cases (N), Mean, standard deviation (Std), minimum (Min), Maximum (Max), median (Med), upper quartile (Q1), and lower quartile (Q3). For qualitative data, descriptive statistical analysis was performed using frequency tables, percentages, or constituent ratios. Differences between continuous normally-distributed variables were tested with the Student t-test; non-normally distributed variables were compared by the rank-sum test (13). Categorical variables were compared with the chi-squared test (14). For survival data or time-to-event data, the Kaplan-Meier survival analysis model was used to calculate the average survival time. Survival curves (Kaplan-Meier curves) were drawn to describe the survival status of patients in each group, with HRs and 95% CIs estimated using a Cox proportional hazards model.

To eliminate the influence of baseline covariates, we employed the PSM method to deal with potential confounding factors (15, 16). Literature search and stepwise logistic regression were used to select the confounders with relatively large impact on the results. Finally, the following confounding factors were included in the PSM model:

Age, gender, smoking history, alcohol consumption history, BMI, blood pressure classification, observation days, baseline eGFR, baseline medication (Insulin and its analogs, glinides, sulfonylurea, biguanides, thiazide diuretics, β-blockers, ACEI/ARB), underlying diseases (hypertension, cardiovascular and cerebrovascular diseases, chronic respiratory diseases, metabolic diseases, urinary system diseases, anemia).

The propensity score matching method was carried out by the MatchIt package of R software (17). According to whether NXT was taken or not, the above factors were used as covariables to construct the propensity score (PS) by logistic regression analysis. We used the 1:1 nearest neighbor matching method to match the propensity scores, and the maximum caliper width was set to 0.25 of the PS standard deviation (18). After the completion of PSM, the standardized mean differences were used to assess the balance of covariates before and after matching, with an absolute value less than 0.1 indicating acceptable balance and reasonable control of confounding factors (19).

For all tests, significance was defined as $P \leq 0.05$. SPSS 22.0 and R 3.6.2 software were employed for statistical analysis (20, 21).

Results

Demographic and clinical characteristics

A total of 9,705 patients were enrolled in the final cohort, with 1,471 (15.2%) patients in exposed group and 8,234 (84.8%) patients in control group (Figure 1). Before PSM, NXT users had lower eGFR (72.97 ± 17.53 vs 69.61 ± 21.10 mL/(min·1.73m^2)), longer observation days (1636.37 days vs 926.40 days), and a higher proportion with comorbidities and concomitant medication. We found a significantly higher percentage of patients in the exposed group had a history of hypertension (99.3% vs 69.8%, P< 0.001), cardio-cerebrovascular diseases (99.8% vs 73.9%, P< 0.001), and metabolic diseases (77.8% vs 43.6%, P< 0.001) compared with the control group. And the proportion of patients using Insulin and its analogs (77% vs 46.5%, P< 0.001), glinides (43.8% vs 16.7%, P< 0.001), sulfonylurea (30.6% vs 15.7%, P< 0.001), biguanides (57.0% vs 30.7%, P< 0.001), thiazide diuretics (9.9% vs 1.4%, P< 0.001), β-blockers (31.3% vs 13.9%, P< 0.001), ACEI (69.8% vs 13.6%, P< 0.001), and ARB (18.6% vs 4.1%, P< 0.001) was significantly higher in the exposed group than in the control group.

Considering the interference of confounding factors, the PSM method was employed to balance the baseline covariates (22). Of 8,234 patients eligible for analysis in the control group, 899 were matched to a patient from NXT exposed group. After 1:1 propensity score–matching, all variables were balanced between the exposed group and control group (Table 1). The standardized mean differences were calculated to assess the balance in the PS model and were<0.1. In the exposed group, the median time of NXT taken was 224 days. Estimated glomerular filtration rate in exposed group and analysis-eligible control group were 72.18 ± 18.49 mL/(min·1.73m^2) and 71.99 ± 18.98mL/(min·1.73m^2), respectively. In subgroup analysis, the high, middle, and low exposed groups contained 306, 233, and 360 patients, respectively. All variables were balanced between the three subgroups and their respective matched control groups. There were no significant differences in baseline eGFR levels among the 3 subgroups. Details on baseline characteristics of each subgroup before and after PSM could be found in Supplement 2 (Table I-IV).

Primary outcome

Table 2 presented the changes in eGFR at the endpoint compared to the baseline. The mean baseline eGFR is 72.18 ± 18.49 mL/(min·1.73m^2) in exposed group and 71.99 ± 18.98mL/(min·1.73m^2) in control group, with no statistical difference between the two groups (P = 0.96). After NXT administration, the exposed group had higher eGFR (70.73 ± 27.68 vs 66.17 ± 26.43 mL/(min·1.73m^2)) compared to non-exposed group, with

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TABLE 1 The baseline characteristic before and after propensity score matching.

Group	Before PSM				After PSM			
	Exposed group (1471)	Control group (8234)	P Value	Std. Mean Diff.	Exposed group (899)	Control group (899)	P Value	Std. Mean Diff.
Age (mean (SD))	66.71 (9.18)	63.37 (11.00)	<0.001	0.3643	66.17 (9.17)	66.13 (10.38)	0.923	0.0049
Male (%)	936 (63.6)	4629 (56.2)	<0.001	0.1541	555 (61.7)	531 (59.1)	0.267	0.0549
Smoking history (%)	364 (24.7)	879 (10.7)	<0.001	0.326	163 (18.1)	177 (19.7)	0.668	-0.0404
Drinking history (%)	272 (18.5)	721 (8.8)	<0.001	0.2507	135 (15.0)	137 (15.2)	0.971	-0.0062
BMI (%)			0.005				0.99	
obese (BMI≥25)	194 (13.2)	1168 (14.2)		-0.0295	128 (14.2)	128 (14.2)		0
overweight (23≥BMI<25)	366 (24.9)	2003 (24.3)		0.0128	211 (23.5)	211 (23.5)		0
normal (18.5≥BMI<23)	311 (21.1)	1424 (17.3)		0.0942	182 (20.2)	177 (19.7)		0.0138
underweight (BMI<18.5)	5 (0.3)	26 (0.3)		0.0041	2 (0.2)	3 (0.3)		-0.0236
Blood pressure classification (%)			<0.001				0.92	
Grade 1 hypertension	208 (14.1)	835 (10.1)		0.1148	113 (12.6)	116 (12.9)		-0.0101
Grade 2 hypertension	80 (5.4)	338 (4.1)		0.0588	47 (5.2)	53 (5.9)		-0.03
Grade 3 hypertension	23 (1.6)	123 (1.5)		0.0056	14 (1.6)	10 (1.1)		0.0359
High normal	668 (45.4)	3426 (41.6)		0.0764	395 (43.9)	383 (42.6)		0.0269
Normal	57 (3.9)	357 (4.3)		-0.0239	37 (4.1)	35 (3.9)		0.0112
Baseline eGFR (mean (SD))	72.97 (17.53)	69.61 (21.10)	<0.001	0.1917	72.18 (18.49)	71.99 (18.98)	0.825	0.0106
Observation days (mean (SD))	1636.37 (1037.36)	926.40 (1004.00)	<0.001	0.6844	1373.86 (997.67)	1392.85 (1029.45)	0.691	-0.019
Baseline medication								
Insulin and its analogs (%)	1132 (77.0)	3826 (46.5)	<0.001	0.724	608 (67.6)	614 (68.3)	0.8	-0.0143
Glinides (%)	645 (43.8)	1374 (16.7)	<0.001	0.5474	293 (32.6)	301 (33.5)	0.726	-0.019
Sulfonylurea (%)	450 (30.6)	1294 (15.7)	<0.001	0.3228	212 (23.6)	213 (23.7)	1	-0.0026
Biguanides (%)	839 (57.0)	2526 (30.7)	<0.001	0.5325	426 (47.4)	421 (46.8)	0.85	0.0111
Thiazide diuretics (%)	145 (9.9)	117 (1.4)	<0.001	0.283	47 (5.2)	50 (5.6)	0.835	-0.015
β-blockers (%)	460 (31.3)	1143 (13.9)	<0.001	0.3751	256 (28.5)	263 (29.3)	0.755	-0.0173
ACEI (%)	1027 (69.8)	1122 (13.6)	<0.001	1.224	484 (53.8)	483 (53.7)	1	0.0022
ARB (%)	273 (18.6)	337 (4.1)	<0.001	0.3721	98 (10.9)	97 (10.8)	1	0.0036
Underlying diseases								
Hypertension (%)	1461 (99.3)	5748 (69.8)	<0.001	3.5916	889 (98.9)	888 (98.8)	1	0.0106
Cardio-cerebrovascular diseases (%)	1468 (99.8)	6085 (73.9)	<0.001	5.7399	896 (99.7)	893 (99.3)	0.504	0.0579
Chronic respiratory diseases (%)	385 (26.2)	880 (10.7)	<0.001	0.3523	189 (21.0)	194 (21.6)	0.818	-0.0136
Metabolic diseases (%)	1145 (77.8)	3593 (43.6)	<0.001	0.8235	607 (67.5)	610 (67.9)	0.92	-0.0071
Urinary system diseases (%)	379 (25.8)	1284 (15.6)	<0.001	0.2326	207 (23.0)	198 (22.0)	0.652	0.0238
Anemia (%)	316 (21.5)	1469 (17.8)	<0.001	0.0887	184 (20.5)	197 (21.9)	0.489	-0.0358

SD, Standard Deviation; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

TABLE 2 The change of eGFR before and after study.

	Exposed group	Control group	W statistic	P-Value
Baseline eGFR	72.18±18.49	71.99±18.98	403532	0.958846
Endpoint eGFR	70.73±27.68	66.17±26.43	351593	0.000002
eGFR change	-1.46±21.94	-5.82±19.8	350706	0.000001

p-value< 0.01. The eGFR changes from baseline to the end of the study were significantly different in the exposed group compared to the control group (-1.46 ± 21.94 vs -5.82 ± 19.8 mL/(min·1.73m^2), P< 0.01). In the exposed group, the overall rate of decline in eGFR was 0.07 ± 13.42mL/min/1.73 m^2 per year, compared with 1.91 ± 19.34 mL/min/1.73 m^2 per year in the control group (P< 0.01).

The trend of eGFR in the study period was presented in Table 3 and Figure 2. Starting from the baseline, we defined a period of 180 days as a follow-up time point. The mean eGFR was calculated for each period. From the overall trend, the eGFR in the NXT group improved to a certain extent, with P values of 0.0003, 0.0005, and 0.023 in the third, fourth and sixth study periods, respectively.

Secondary outcome

Significant benefit in the time to the first occurrence of composite renal outcome was observed in the NXT group (hazard ratio [HR], 0.71; 95% confidence interval [CI], 0.55 to 0.92; P = 0.009) (Figure 3A). The median occurrence time was 929.5 (95% CI, 838 to 1118) days in the exposed group versus 575.5 (95% CI, 463 to 662) days in the control group. There were 100 patients presented the outcome of deterioration of renal function in the exposed group and 98 in the control group (HR, 0.74; 95% CI, 0.56 to 0.99; P = 0.039) (Figure 3B). The median occurrence time was significantly longer in the exposed group (1020 days; 95% CI, 919 to 1200 versus 660 days; 95% CI, 568 to 712). Longer survival time of MACE was observed in NXT group (HR, 0.61; 95% CI, 0.45 to 0.82; P = 0.001) (Figure 3C). However, the incidence of MACE was higher in the exposed group (8.1%) than in the control group (11.9%). There was no significant difference in the time to hospitalization for heart failure (P = 0.63) (Figure 3D).

In terms of the change of CKD stages, more patients in the exposed group were in CKD stage 1 at the endpoint (269 vs 159) compared with the control group, exhibiting a noticeable improvement in eGFR. (Table 4). The overall improvement proportion of CKD stage was 34.04% in the exposed group and 22.36% in the control group, while the overall progression proportion of CKD stage was 21.25% in the exposed group and 24.92% in the control group.

The difference in safety indices between baseline and the end of the study is shown in Table 5. NXT capsule did not cause any abnormality of safety indicators including blood routine, serum electrolyte, and liver function (P>0.05).

Discussion

DKD has emerged as a worldwide medical catastrophe, bringing tremendous pressure on medical resources and the economy. According to data from the International Diabetes Federation (IDF), by the end of 2021, the number of diagnosed diabetes mellitus (DM) reached 537 million globally (23), which has a great impact on the prevalence rate of DKD. Much effort has been made to slow the reduction of GFR and maintain renal function, including glycemic control, blood pressure regulation, and lipid lowering (24). However, the long-term impact of these strategies is still not optimistic (25).

Based on the current treatment status, TCM treatment of diabetic kidney disease needs to be paid more attention. Our study is the first real-world study (RWS) of TCM treatment of DKD based on a large electronic information system. The reason we conducted RWS was that it is designed to evaluate the outcomes of all interventions in a real clinical setting to achieve results that are more closely related to clinical reality. Moreover, although Naoxintong capsule has been commonly used clinically for the treatment of DKD, it has only been approved for the treatment of cardiovascular and cerebrovascular diseases, which makes it impossible to conduct RCTs related to DKD. RWS could provide basis for NXT off-label drug use, further expand drug indications, and lay a

TABLE 3 The trend of eGFR in study period.

Study period	Control group	NXT group	number of patients in control group	number of patients in NXT	W statistic	P-Value
baseline	71.99 ± 18.98	72.18 ± 18.49	899	899	403532	0.958846
1	71.45 ± 24.8	74.15 ± 24.29	447	357	74341.5	0.095922
2	72.54 ± 24.56	73.83 ± 24.76	413	373	72696	0.173313
3	69.24 ± 24.85	74.43 ± 25.49	340	332	47436	0.000346
4	65.92 ± 26.4	72.67 ± 25.01	299	323	40433	0.000452
5	67.5 ± 27.04	70.53 ± 27.11	216	309	30360.5	0.078346
6	64.98 ± 27.18	70.13 ± 27.3	170	281	20825.5	0.02258
7	63.71 ± 26.42	67.13 ± 29.13	148	228	14422.5	0.061622
8	63.35 ± 28.37	67.38 ± 28.37	123	194	10868	0.181503

1 study period = 180 days.

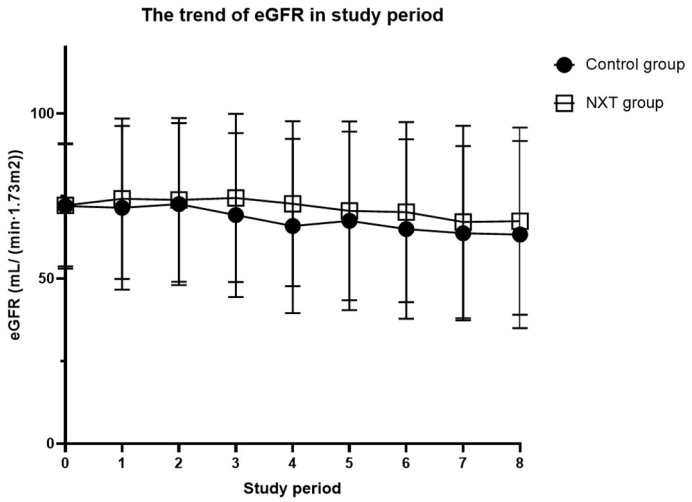

FIGURE 2
The trend of eGFR in study period. 1 study period = 180 days.

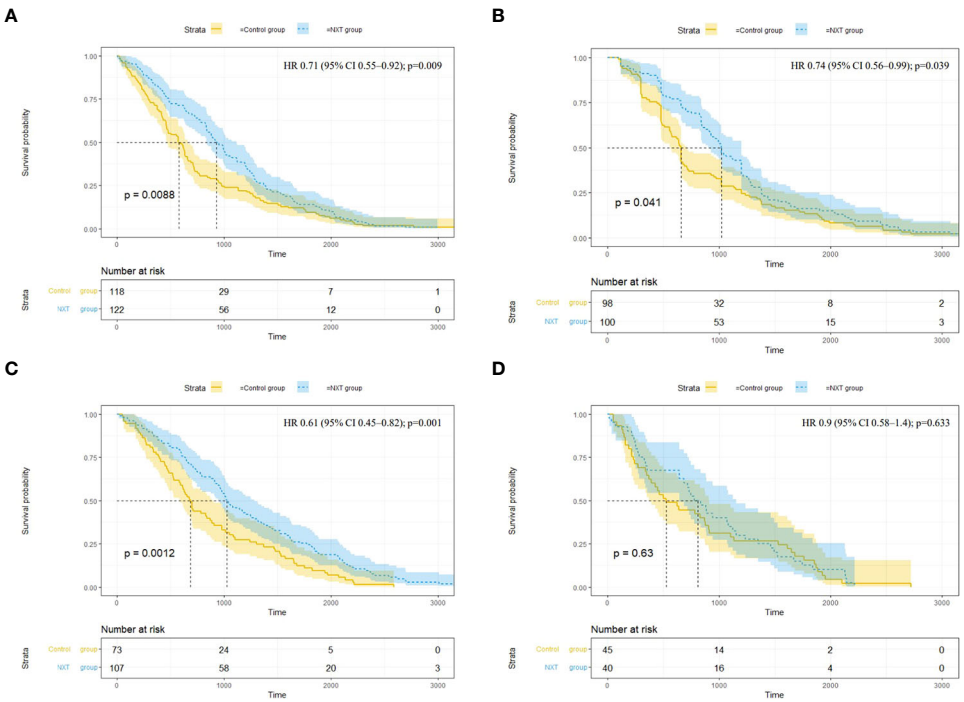

FIGURE 3
Survival analysis of endpoint events. **(A)** Composite renal outcome (end-stage renal disease11, renal replacement therapy, doubling of serum creatinine, death due to kidney disease); **(B)** 50% decrease in eGFR; **(C)** Major adverse cardiovascular events (MACE) (cardiovascular death, non-fatal myocardial infarction or non-fatal stroke); **(D)** Hospitalization for heart failure. HR, hazard ratio; CI, confidence interval; Time (days).

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TABLE 4 The change of CKD stages.

a. The change of CKD stages in exposed group

Endpoint Baseline	CKD1	CKD2	CKD3a	CKD3b	CKD4	CKD5
CKD2	249	346	52	34	30	11
CKD3a	13	23	25	16	10	5
CKD3b	5	4	4	8	8	12
CKD4	2	1	1	2	5	13
CKD5	0	0	0	0	2	18
Total	269	374	82	60	55	59

b. The change of CKD stages in control group

Endpoint Baseline	CKD1	CKD2	CKD3a	CKD3b	CKD4	CKD5
CKD2	156	399	93	32	25	17
CKD3a	3	34	28	19	13	5
CKD3b	0	1	4	12	5	6
CKD4	0	1	0	2	5	9
CKD5	0	0	0	0	0	30
Total	159	435	125	65	48	67

c. The proportion of changes in CKD stages

		Improvement	maintenance	progression
Exposed group	Total	306 (34.04%)	402 (44.72%)	191 (21.25%)
	CKD2-CKD3a	285 (35.01%)	371 (45.58%)	158 (19.41%)
	CKD3b-CKD5	21 (24.7%)	31 (36.47%)	33 (38.82%)
Control group	Total	201 (22.36%)	474 (52.73%)	224 (24.92%)
	CKD2-CKD3a	193 (23.42%)	427 (51.82%)	204 (24.76%)
	CKD3b-CKD5	8 (10.67%)	47 (62.67%)	20 (26.67%)

foundation for follow-up randomized controlled studies. Our study demonstrated that TCM was efficacious in slowing eGFR decline and reducing the progression of early-stage DKD to ESRD. Thus, TCM can be an alternative treatment in addition to glucose-lowering, antihypertensive, and lipid-lowering therapies for the treatment of DKD patients.

In this retrospective cohort study, patients with DKD who used NXT capsules had less eGFR decline (1.46 ± 21.94 vs 5.82 ± 19.8 mL/(min·1.73m^2)) than those who did not take NXT. Although the incidence rates were similar, patients in the NXT group had a lower risk of composite renal outcome events (end-stage renal disease, renal replacement therapy, doubling of

TABLE 5 The difference of safety indices before and after treatment.

	Control group	NXT group	Number of patients in control group	Number of patients in NXT group	W statistic	P-Value
RBCs	-0.17 ± 0.56	-0.15 ± 0.68	605	664	192327.5	0.190696
WBCs	0.36 ± 2.91	0.46 ± 3.57	605	665	200460.5	0.914422
PLT	-4.67 ± 53.16	1.79 ± 64.67	604	664	191494	0.165402
HbA1c	-5.01 ± 17.24	-5.26 ± 20.98	559	541	148217	0.570007
K+	-0.02 ± 0.6	0.01 ± 0.59	376	441	80694.5	0.510382
ALT	4.76 ± 73.76	4.16 ± 75.86	502	520	127785	0.562135
AST	10.2 ± 64.83	16.96 ± 174.09	336	346	53247	0.057786
ALP	1.92 ± 54.07	2.3 ± 53.07	484	534	124114.5	0.275087
TBIL	0.68 ± 8.98	0.07 ± 8.15	512	552	141388	0.987972
TP	5.59 ± 66.58	5.27 ± 52.83	451	485	103663	0.167512

RBCs, red blood cells; WBCs, white blood cells; PLT, platelets; HbA1c, Hemoglobin A1c; K+, potassium; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, Alkaline phosphatase; TBIL, total bilirubin; TP, transpeptidase.

serum creatinine, death due to kidney disease), and deterioration of renal function (50% decrease in eGFR). These results suggest that NXT capsules may be an effective therapy for kidney protection in DKD patients. A longer survival time of MACE was also observed in patients who used NXT (HR, 0.61; 95% CI, 0.45 to 0.82; P = 0.001). However, we found a higher incidence of MACE in the NXT group compared with the control group (11.9% vs 8.1%). This may be due to the fact that clinicians prescribe NXT capsules mainly for patients with cardiovascular diseases (CVD). Therefore, patients who were administrated NXT were more likely to have worse cardiovascular situations. Our study focuses more on the therapeutic effect of NXT on DKD, confounding factors related to cardiovascular disease may not be well balanced.

Moreover, NXT administration was significantly associated with the improvement of CKD stages both in the subgroup of patients with CKD stage 2-3a and CKD stage 3b-5. The number of patients with improved CKD stage in the NXT group and the control group was 306 and 201 (34.04% vs 22.36%), respectively. In terms of CKD progression, NXT showed clinical benefits in patients with CKD stage 2-3a (19.41% vs 24.76%). While in patients with CKD stage 3b-5, NXT capsules did not achieve ideal results (38.82% vs 26.67%). This suggests that NXT may have greater benefit in improving renal function in early-stage DKD patients. For patients with CKD stage 3b-5, the proportion of progression and improvement was higher in the NXT group than in the control group. At the same time, due to the relatively small sample size of late-stage DKD patients, there may be some bias in the results.

In addition, the patients in the exposed group were divided into three subgroups according to exposure time, and the average duration of medication in the high, medium, and low exposed groups was 700.28 days, 253.20 days, and 106.82 days, respectively (Supplement 2 Table IV). we observed that NXT administration ameliorated the eGFR decline and improved the CKD stages, irrespective of the level of exposure to NXT (Supplement 2 Table V-VI).

NXT capsule was developed from the classical formula "Bu-Yang-Huan-Wu-Tang (BYHWT)", which has been approved by the China Food and Drug Administration (CFDA) for the treatment of cardiovascular disease (7). Previous studies have shown that NXT takes a variety of protective effects on CVD including coronary artery disease, myocardial infarction, atherosclerosis, acute coronary syndrome, ischemic stroke, and so on (26–29). In addition, researchers found that NXT may also have renoprotective effects by improving insulin sensitivity and regulating glucose and lipid metabolism. Yang et al. found that NXT protects db/db diabetic mice from DN by reducing renal inflammation and podocyte injury (8). Moreover, NXT decreased the deposition of extracellular matrix proteins by increasing the expression of MMP2/9 through inhibition of the TGF-β/Smad pathway and CTGF expression (9). Yan et al. demonstrated that NXT exerted therapeutic efficacy against diabetes and its complications by improving insulin sensitivity, glucose metabolism, and energy expenditure (30). However, the above-mentioned studies mainly focused on animal experiments, without the support of clinical studies. Due to the off-label use of NXT in the treatment of DKD, randomized controlled trials (RCTs) cannot be conducted. Therefore, we conducted a retrospective study to explore the therapeutic effect of NXT, providing scientific evidence and a research basis for expanding the indications of NXT capsules. The research database we selected covers all hospitals in Tianjin area, with wide data coverage and sufficient number of patients. The patient data captured in this database are representative, providing reliable resources for us to carry out the study. Our study showed that NXT is effective in improving eGFR in DKD patients, especially in patients with early-stage DKD. NXT also had a beneficial effect on the risk of renal events, as demonstrated by the increase in survival time of the renal composite outcome.

NXT contains sixteen Chinese materia medica: Radix Astragali (Huangqi), Radix Paeoniae Rubra (Chishao), Salviae miltiorrhizae Radix et Rhizoma (Danshen), Radix Angelicae Sinensis (Danggui wei), Chuanxiong Rhizoma (Chuanxiong), Persicae Semen (Taoren), Carthami Flos (Honghua), Olibanum (Ruxiang), Myrrha (Moyao), Spatholobi Stem (Jixueteng), Cinnamomi Ranulus (Guizhi), Achyranthis bidentatae Radix (Niuxi), Scorpio (Quanxie), Pheretima (Dilong), Mori Ramulus (Sangzhi), and Hirudo (Shuizhi) (7). In TCM theory, these prescriptions can tonify qi, activate blood circulation, remove stasis, and dredge collaterals. Thus, the indications of NXT coincide with the main pathogenesis of DKD, which is characterized by the presence of qi deficiency, blood stasis, and turbidity. Studies have shown that many components of NXT possess anti-inflammation properties and could improve renal function (8). The main identified components in the NXT capsule include ferulic acid, paeoniflorin, hydroxysafflor yellow A, amygdalin, salvianolic acid B, and astragaloside IV (31, 32), all of which have the pharmacological effects of anti-inflammation, anti-oxidation, and renoprotection against diabetes. Astragaloside IV in Radix Astragali (Huangqi) exhibits anti-inflammatory, anti-oxidative stress, and anti-fibrotic properties, effectively alleviating the renal injury induced by high glucose (33, 34). Ferulic acid is known as an anti-oxidative agent, which relieves inflammation, decreases oxidative stress markers, and attenuates excessive autophagy to protect podocytes against injury (35, 36). Astragaloside IV and ferulic acid have also been found to have hypoglycemic effects. Paeoniflorin and hydroxysafflor yellow A alleviate renal injury mainly through inhibiting inflammatory responses (37–39). Amygdalin, hydroxysafflor yellow A, and Salvianolic acid B could ameliorate renal fibrosis by inhibiting the expression of transforming growth factor (TGF)–β1 and the proliferation of renal interstitial fibroblasts (40, 41). The pharmacological effects of these components suggest that the renal protective efficacy of NXT may be a direct effect on the kidney or an indirect anti-diabetic effect.

Collectively, our results provided clinical evidence for the treatment of DKD with NXT capsules. This study was a good attempt for the real-world research of traditional Chinese medicine, which not only proved its feasibility, but also showed the scientific nature. It will serve as a demonstration for the development, marketing and indication expansion of Chinese patent medicines in the future.

However, there are still some limitations in our current study. Firstly, as a retrospective study, although many important confounders were accounted for, residual unknown or unmeasured confounding factors cannot be eliminated. Secondly, the medication data of participants was collected only from hospital records, which may be incomplete because some patients bought drugs at pharmacies. Therefore, the current findings will need to be confirmed in large cohort studies and RCTs.

Conclusion

NXT can significantly slow the decline of eGFR and reduce the risk of renal outcomes. However, large cohort studies and RCTs are needed to further confirm our results.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

YQZ: Conceptualization, Methodology, Writing - Original Draft; YHZ: Data curation, Investigation; CQY: Software, Validation; YYD: Writing-Reviewing and Editing. LLJ: Writing- Reviewing and Editing; DJ: Data curation, Validation; FML: Methodology, Supervision, Project administration; XLT: Supervision, Project administration. All data were generatedin-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1037564/full#supplementary-material

References

1. Molitch ME, Adler AI, Flyvbjerg A, Nelson RG, So W-Y, Wanner C, et al. Diabetic kidney disease: a clinical update from kidney disease: Improving global outcomes. *Kidney Int* (2015) 87:20–30. doi: 10.1038/ki.2014.128

2. Schroijen MA, Luijtgaarden van MWM, Noordzij M, Ravani P, Jarraya F, Collart F, et al. Survival in dialysis patients is different between patients with diabetes as primary renal disease and patients with diabetes as a co-morbid condition. *Diabetologia* (2013) 56:1949–57. doi: 10.1007/s00125-013-2966-1

3. American Diabetes Association. 11. microvascular complications and foot care: Standards of medical care in diabetes-2020. *Diabetes Care* (2020) 43:S135–51. doi: 10.2337/dc20-S011

4. Hajhosseiny R, Khavandi K, Jivraj N, Mashayekhi S, Goldsmith DJ, Malik RA, et al. Have we reached the limits for the treatment of diabetic nephropathy? *Expert Opin Investig Drugs* (2014) 23:511–22. doi: 10.1517/13543784.2014.892580

5. Eisenberg DM, Davis RB, Ettner SL, Appel S, Wilkey S, Rompay Van M, et al. Trends in alternative medicine use in the united states, 1990-1997: results of a

follow-up national survey. *JAMA* (1998) 280:1569–75. doi: 10.1001/jama.280.18.1569

6. Tang G, Li S, Zhang C, Chen H, Wang N, Feng Y, et al. Clinical efficacies, underlying mechanisms and molecular targets of Chinese medicines for diabetic nephropathy treatment and management. *Acta Pharm Sin B* (2021) 11:2749–67. doi: 10.1016/j.apsb.2020.12.020

7. Han J, Tan H, Duan Y, Chen Y, Zhu Y, Zhao B, et al. The cardioprotective properties and the involved mechanisms of NaoXinTong capsule. *Pharmacol Res* (2019) 141:409–17. doi: 10.1016/j.phrs.2019.01.024

8. Yang S, Chen Y, Duan Y, Ma C, Liu L, Li Q, et al. Therapeutic potential of NaoXinTong capsule on the developed diabetic nephropathy in db/db mice. *BioMed Pharmacother* (2019) 118:109389. doi: 10.1016/j.biopha.2019.109389

9. Yang S, Liu M, Chen Y, Ma C, Liu L, Zhao B, et al. NaoXinTong capsules inhibit the development of diabetic nephropathy in db/db mice. *Sci Rep* (2018) 8:9158. doi: 10.1038/s41598-018-26746-1

10. KDOQI. KDOQI clinical practice guidelines and clinical practice recommendations for diabetes and chronic kidney disease. *Am J Kidney Dis* (2007) 49:S12–154. doi: 10.1053/j.ajkd.2006.12.005

11. Krolewski AS, Skupien J, Rossing P, Warram JH. Fast renal decline to end-stage renal disease: an unrecognized feature of nephropathy in diabetes. *Kidney Int* (2017) 91:1300–11. doi: 10.1016/j.kint.2016.10.046

12. Lambers Heerspink HJ, Perkovic V, de Zeeuw D. Is doubling of serum creatinine a valid clinical 'hard' endpoint in clinical nephrology trials? *Nephron Clin Pract* (2011) 119:c195–199; discussion c199. doi: 10.1159/000327614

13. Mishra P, Singh U, Pandey CM, Mishra P, Pandey G. Application of student's t-test, analysis of variance, and covariance. *Ann Card Anaesth* (2019) 22:407–11. doi: 10.4103/aca.ACA_94_19

14. Jung S-H. Stratified fisher's exact test and its sample size calculation. *Biom J* (2014) 56:129–40. doi: 10.1002/bimj.201300048

15. Deb S, Austin PC, Tu JV, Ko DT, Mazer CD, Kiss A, et al. A review of propensity-score methods and their use in cardiovascular research. *Can J Cardiol* (2016) 32:259–65. doi: 10.1016/j.cjca.2015.05.015

16. Austin PC. An introduction to propensity score methods for reducing the effects of confounding in observational studies. *Multivariate Behav Res* (2011) 46:399–424. doi: 10.1080/00273171.2011.568786

17. Zhang Z. Propensity score method: a non-parametric technique to reduce model dependence. *Ann Transl Med* (2017) 5:7. doi: 10.21037/atm.2016.08.57

18. Rassen JA, Shelat AA, Myers J, Glynn RJ, Rothman KJ, Schneeweiss S, et al. One-to-many propensity score matching in cohort studies. *Pharmacoepidemiol Drug Saf* (2012) 21 Suppl 2:69–80. doi: 10.1002/pds.3263

19. Pustejovsky JE, Rodgers MA. Testing for funnel plot asymmetry of standardized mean differences. *Res Synth Methods* (2019) 10:57–71. doi: 10.1002/jrsm.1332

20. Dudley WN, Benuzillo JG, Carrico MS. SPSS And SAS programming for the testing of mediation models. *Nurs Res* (2004) 53:59–62. doi: 10.1097/00006199-200401000-00009

21. Chan BKC. Data analysis using r programming. *Adv Exp Med Biol* (2018) 1082:47–122. doi: 10.1007/978-3-319-93791-5_2

22. Baumfeld Andre E, Reynolds R, Caubel P, Azoulay L, Dreyer NA. Trial designs using real-world data: The changing landscape of the regulatory approval process. *Pharmacoepidemiol Drug Saf* (2020) 29:1201–12. doi: 10.1002/pds.4932

23. Home, et al. *IDF diabetes atlas | tenth edition*. Available at: https://diabetesatlas.org/.

24. Kaplan NM. Management of hypertension in patients with type 2 diabetes mellitus: guidelines based on current evidence. *Ann Intern Med* (2001) 135:1079–83. doi: 10.7326/0003-4819-135-12-200112180-00012

25. Sasso FC, Nicola De L, Carbonara O, Nasti R, Minutolo R, Salvatore T, et al. Cardiovascular risk factors and disease management in type 2 diabetic patients with diabetic nephropathy. *Diabetes Care* (2006) 29:498–503. doi: 10.2337/diacare.29.03.06.dc05-1776

26. Xu J, Wang T, Guo F, Ji E, Zhang Y, Wu H, et al. Systematical identification of the protective effect of danhong injection and BuChang NaoXinTong capsules on transcription factors in cerebral ischemia mice brain. *Oxid Med Cell Longev* (2020) 2020:5879852. doi: 10.1155/2020/5879852

27. Liu X, Wang Q, Cui Y, Hussain M, Yang H, Li X, et al. Multiple protein and mRNA expression correlations in the rat cerebral cortex after ischemic injury and repair due to buchang naoxintong jiaonang (BNJ) intervention. *BioMed Pharmacother* (2020) 125:109917. doi: 10.1016/j.biopha.2020.109917

28. Zhang W-J, Su W-W, Li P-B, Rao H-Y, Lin Q-W, Zeng X, et al. Naoxintong capsule inhibits the development of cardiovascular pathological changes in bama minipig through improving gut microbiota. *Front Pharmacol* (2019) 10:1128. doi: 10.3389/fphar.2019.01128

29. Li J, Zhao X, Zhang Y, Wan H, He Y, Li X, et al. Comparison of traditional Chinese medicine in the long-term secondary prevention for patients with ischemic stroke: A systematical analysis. *Front Pharmacol* (2021) 12:722975. doi: 10.3389/fphar.2021.722975

30. Yan Z, Wu H, Zhou H, Chen S, He Y, Zhang W, et al. Integrated metabolomics and gut microbiome to the effects and mechanisms of naoxintong capsule on type 2 diabetes in rats. *Sci Rep* (2020) 10:10829. doi: 10.1038/s41598-020-67362-2

31. Wang H, Jiang Y, Ding M, Li J, Hao J, He J, et al. Simultaneous determination and qualitative analysis of six types of components in naoxintong capsule by miniaturized matrix solid-phase dispersion extraction coupled with ultra high-performance liquid chromatography with photodiode array detection and quadrupole time-of-flight mass spectrometry. *J Sep Sci* (2018) 41:2064–84. doi: 10.1002/jssc.201701411

32. Ma X, Lv B, Li P, Jiang X, Zhou Q, Wang X, et al. Identification of 'Multiple components-multiple targets-multiple pathways' associated with naoxintong capsule in the treatment of heart diseases using UPLC/Q-TOF-MS and network pharmacology. *Evid Based Complement Alternat Med* (2016) 2016:9468087. doi: 10.1155/2016/9468087

33. Wang E, Wang L, Ding R, Zhai M, Ge R, Zhou P, et al. Astragaloside IV acts through multi-scale mechanisms to effectively reduce diabetic nephropathy. *Pharmacol Res* (2020) 157:104831. doi: 10.1016/j.phrs.2020.104831

34. Zhang Y, Tao C, Xuan C, Jiang J, Cao W. Transcriptomic analysis reveals the protection of astragaloside IV against diabetic nephropathy by modulating inflammation. *Oxid Med Cell Longev* (2020) 2020:9542165. doi: 10.1155/2020/9542165

35. Choi R, Kim BH, Naowaboot J, Lee Young M, Hyun MR, Cho EJ, et al. Effects of ferulic acid on diabetic nephropathy in a rat model of type 2 diabetes. *Exp Mol Med* (2011) 43:676–83. doi: 10.3858/emm.2011.43.12.078

36. Chowdhury S, Ghosh S, Das AK, Sil PC. Ferulic acid protects hyperglycemia-induced kidney damage by regulating oxidative insult, inflammation and autophagy. *Front Pharmacol* (2019) 10:27. doi: 10.3389/fphar.2019.00027

37. Liu Q, Lin X, Li H, Yuan J, Peng Y, Dong L, et al. Paeoniflorin ameliorates renal function in cyclophosphamide-induced mice *via* AMPK suppressed inflammation and apoptosis. *Biomed Pharmacother* (2016) 84:1899–905. doi: 10.1016/j.biopha.2016.10.097

38. Wang P, Wang W, Shi Q, Zhao L, Mei F, Li C, et al. Paeoniflorin ameliorates acute necrotizing pancreatitis and pancreatitis–induced acute renal injury. *Mol Med Rep* (2016) 14:1123–31. doi: 10.3892/mmr.2016.5351

39. Bai J, Zhao J, Cui D, Wang F, Song Y, Cheng L, et al. Protective effect of hydroxysafflor yellow a against acute kidney injury *via* the TLR4/NF-κB signaling pathway. *Sci Rep* (2018) 8:9173. doi: 10.1038/s41598-018-27217-3

40. Guo J, Wu W, Sheng M, Yang S, Tan J. Amygdalin inhibits renal fibrosis in chronic kidney disease. *Mol Med Rep* (2013) 7:1453–7. doi: 10.3892/mmr.2013.1391

41. Hu N, Duan J, Li H, Wang Y, Wang F, Chu J, et al. Hydroxysafflor yellow a ameliorates renal fibrosis by suppressing TGF-β1-Induced epithelial-to-Mesenchymal transition. *PloS One* (2016) 11:e0153409. doi: 10.1371/journal.pone.0153409

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Association of metabolic dysfunction-associated fatty liver disease, type 2 diabetes mellitus, and metabolic goal achievement with risk of chronic kidney disease

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Background: Although type 2 diabetes mellitus (T2DM) plays a significant role in the association between metabolic dysfunction-associated fatty liver disease (MAFLD) and chronic kidney disease (CKD), how T2DM development and glycemic deterioration affect CKD and its renal function indicators, estimated glomerular filtration rate (eGFR) and urine albumin-to-creatinine ratio (UACR), remains unknown. We aimed to assess the association between MAFLD, along with T2DM, and risk of CKD, and then evaluate the effect of metabolic goal achievement in MAFLD on the risk of CKD.

Methods: In this cross-sectional study, 5,594 participants were included. Multivariate logistic regression and linear regression were used to examine the association between MAFLD with its T2DM status and metabolic goal achievement and risk of CKD, as well as eGFR and UACR.

Results: The MAFLD group had a higher prevalence of CKD (16.2 vs. 7.6%, $P < 0.001$) than the non-MAFLD group. MAFLD was independently associated with an increased risk of CKD (odds ratio [OR]: 1.35, 95% CI: 1.09–1.67) and increased eGFR and UACR. Among the three MAFLD subtypes, only the T2DM subtype exhibited significant associations with increased risk of CKD (OR: 2.85, 95% CI: 2.24–3.63), as well as increased eGFR and UACR. Glycemic deterioration in MAFLD was dose-dependently associated with an increased risk of CKD (P-trend < 0.001). Achieved metabolic goals in MAFLD decreased the risk of CKD, eGFR, and UACR; MAFLD with 2 or 3 achieved metabolic goals was not significantly associated with the risk of CKD (OR: 0.81, 95% CI: 0.59–1.12) and albuminuria.

Conclusion: MAFLD was independently associated with an increased risk of CKD, as well as increased eGFR and UACR. This association is strongly driven by T2DM status. Glycemic deterioration in MAFLD was dose-dependently

associated with an increased risk of CKD. Achieved metabolic goals in MAFLD decreased the risk of CKD by reducing the risk of albuminuria.

KEYWORDS

albuminuria, chronic kidney disease, estimated glomerular filtration rate, metabolic dysfunction-associated fatty liver disease, metabolic goal achievement, type 2 diabetes mellitus, urine albumin-to-creatinine ratio

Introduction

Nonalcoholic fatty liver disease (NAFLD) represents the most common chronic liver disorder worldwide, with a prevalence of 29.2% in China (1, 2). NAFLD is closely associated with metabolic disorders, such as type 2 diabetes mellitus (T2DM) (3), hypertension (4), and obesity (5), and other extra-hepatic diseases, such as chronic kidney disease (CKD) (6) and cardiovascular disease (CVD) (7). The newly propagated name, metabolic dysfunction-associated fatty liver disease (MAFLD), might be a more appropriate term to describe the liver disease associated with underlying metabolic dysfunction (8). The association between MAFLD and other diseases is still not fully understood.

Chronic kidney disease, another leading public health problem affecting nearly 10% of the Chinese population (9), is defined by estimated glomerular filtration rate (eGFR) and urine albumin-to-creatinine ratio (UACR) (10). Diabetes is the leading cause of CKD, and CKD is considered to share common metabolic risk factors with NAFLD, such as T2DM (6). The association between NAFLD and CKD is established in several epidemiological studies (11–15), two of which are based on patients with diabetes (11, 12). The risk of CKD progression increased with the severity of NAFLD in patients with T2DM (11). Putative mechanisms linking NAFLD with CKD include T2DM and metabolic syndrome, dysbiosis and perturbed intestinal function, and aging (6). Recent studies have shown that MAFLD is independently associated with CKD (16–18). Although T2DM plays a significant role in the association between NAFLD and CKD, how T2DM development and glycemic deterioration affect CKD and its renal function indicators, eGFR and UACR, remains unknown.

Metabolic dysfunction-associated fatty liver disease criteria identify an additional portion of people with more metabolic comorbidities and a higher risk of CKD compared to NAFLD (17, 19), so metabolic management is more important to reduce the risk of CKD than before. "ABCs" metabolic goal, including glycated hemoglobin A1c (HbA1c), blood pressure (BP), and low-density lipoprotein cholesterol (LDL-C) (20, 21), was first proposed to alleviate the complications of diabetes. A recent study revealed that metabolic goal achievement among NAFLD reduced the risk of CKD, and there was a significant interaction between poor glycemic control and NAFLD on the risk of CKD (12). However, whether overall and specific metabolic goal achievement among MAFLD has effects on CKD, as well as eGFR and UACR, still needs to be explored.

Therefore, we conducted a cross-sectional study based on the general population in southern China to evaluate the associations of MAFLD and its T2DM status with CKD, eGFR, and UACR, and then to check whether metabolic goal achievement among MAFLD reduces the risk of CKD.

Materials and methods

Study population

The study was based on baseline survey data of Fuqing Cohort from the general population of Fuqing City in southeast China. From July 2020 to June 2021, 7,009 eligible participants aged 35–75 years who had lived in Fuqing City for at least 5 years were recruited. After exclusion, a total of 5,594 participants were included for analysis (Figure 1). This study was conducted in compliance with the regulations of the Declaration of Helsinki and was approved by the Ethical Committee of Fujian Medical University (approval number [2020-58]). All participants provided written informed consent.

Data collection

Face-to-face interviews for study participants were performed by a team of trained interviewers using an electronic structured questionnaire. The following information was collected: demographics, smoking, alcohol and tea drinking, physical activities, medical history, and medication use.

Height, weight, waist circumference, hip circumference, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured by trained staff. Body mass index (BMI) was calculated as body weight in kilograms divided by height squared in meters (kg/m^2). BP was measured using an electronic sphygmomanometer (OMRON U30; Omron Company, Kyoto, Japan) according to a standard protocol. The average of the three readings was calculated for analysis. Daily physical metabolic

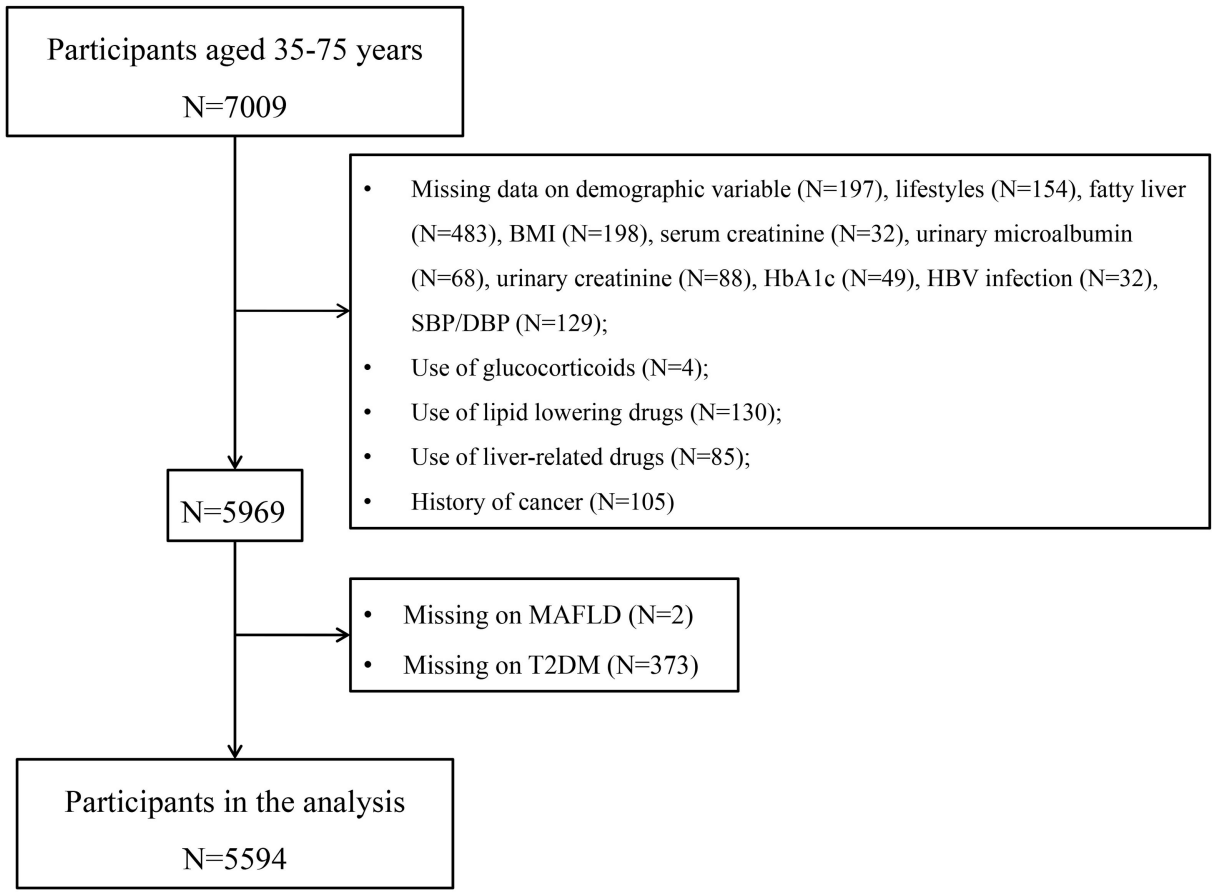

FIGURE 1
Flowchart of selection of study participants.

equivalent (MET/day) was calculated for the quantification of physical activity intensity.

Type 2 diabetes mellitus was defined as a fasting plasma glucose level ≥ 7.0 mmol/L or 2-h post-load glucose level ≥ 11.1 mmol/L or HbA1c ≥ 6.5% or use of antidiabetic drugs or self-reported history of diabetes. Prediabetes was defined as nondiabetic individuals having a fasting plasma glucose level of 5.6–6.9 mmol/L or 2-h post-load glucose level of 7.8–11.1 mmol/L or HbA1c of 5.7%−6.4%. Hypertension was defined as mean SBP ≥ 140 mmHg or mean DBP ≥ 90 mmHg or use of hypotension.

Overnight fasting venous blood samples and random urine samples were collected. Serum creatinine, glucose level, lipid profile, urinary creatinine, and microalbumin were measured using standard laboratory procedures (Toshiba automatic biochemical analyzer, TBA-120FR, Japan). HbA1c was measured using a high-performance liquid chromatography method (fully automated glycohemoglobin (HbA1c) analyzer, HA8180, Japan). Urinary creatinine and microalbumin were used to calculate UACR.

Abdominal ultrasonography was performed using an ultrasonic instrument (Prosoundα7, Hitachi Medical Company, Tokyo, Japan) by experienced ultrasonographists.

Definition of metabolic dysfunction-associated fatty liver disease

Metabolic dysfunction-associated fatty liver disease was defined as evidence of fatty liver based on ultrasound with at least one of the following three criteria (8): (1) overweight or obesity (BMI ≥ 23.0 kg/m^2 in Asians); (2) T2DM; and (3) metabolic dysregulation among non-overweight individuals (BMI < 23.0 kg/m^2 in Asians). Metabolic dysregulation was defined as the presence of at least two of the following metabolic risk abnormalities: (1) waist circumference ≥ 90 cm for Asian men and 80 cm for Asian women; (2) BP ≥ 130/85 mmHg or specific drug treatment; (3) triglycerides (TG) ≥ 1.70 mmol/L or specific drug treatment; (4) high-density lipoprotein cholesterol (HDL-C) < 1.0 mmol/L for men and < 1.3 mmol/L for women;

(5) prediabetes; (6) insulin resistance (HOMA-IR) score $\geq$ 2.5; and (7) plasma high-sensitivity C-reactive protein (hs-CRP) level > 2 mg/L.

Definition of CKD

The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to calculate eGFR(22). The formula is given as follows: eGFR = 141 × min (Scr/κ, 1)$^{\alpha}$ × max (Scr/κ, 1)$^{-1.209}$ × 0.993^{Age} × 1.018 [if female], where Scr is serum creatinine concentration in milligrams per deciliter, κ is 0.9 for males and 0.7 for females, α is −0.411 for males and −0.329 for females, min represents the minimum of 1 or Scr/κ, and max represents the maximum of 1 or Scr/κ. CKD was defined as eGFR < 60 ml/min/1.73 m^2 or the presence of albuminuria. Albuminuria was defined as UACR $\geq$ 30 mg/g.

Definition of ABCs' metabolic goal

"ABCs" metabolic goal was defined as HbA1c < 6.5% (A), SBP/DBP < 140/90 mmHg (B), and LDL-C < 100 mg/dl (C) (12, 20, 23).

Statistical analysis

Continuous variables were expressed as median (25th and 75th percentiles) due to skewed distribution, and categorical variables were presented as frequency (percentage). Differences between non-MAFLD group and MAFLD group were assessed using the Wilcoxon rank sum test for continuous variables and Pearson's chi-squared test for categorical variables.

Multivariate logistic regression was used to estimate odds ratios (ORs) and corresponding 95% confidence intervals (95% CIs) for associations between MAFLD and CKD. Multivariate linear regression was used to estimate regression coefficients (βs) and corresponding 95% CIs for associations of MAFLD with eGFR and UACR. UACR values were log-transformed for analysis. The following models were constructed. Model 1 was unadjusted. Model 2 was adjusted for age, sex, and BMI. Model 3 was further adjusted for education level (0/1–6/7–9/$\geq$10 years), occupation (farmer or unemployment/worker/sales or service/office job/others), alcohol drinking (never/former/current), tea drinking (never/former/current), HBV infection (yes/no), T2DM (yes/no, removed when conflicting with independent variable), and physical activity (MET/day).

Subgroup analysis was performed to assess potential effect modifiers by stratifying sex, age (<65/$\geq$65 years), BMI (<28/$\geq$28 kg/m^2), alcohol drinking (never/ever), tea drinking (never/ever), smoking (never/ever), HBV infection, T2DM, hypertension, and dyslipidemia.

Statistical significance was defined as a 2-tailed $P < 0.05$. All statistical analyses were conducted using R version 4.0.5 (Foundation, Vienna, Austria).

Results

General characteristics of participants

The general characteristics of 5,594 participants are presented in Table 1. The median age of the participants was 57 (50, 65) years and 35.1% were men. Of them, 1,878 (33.6%) and 589 (10.5%) were diagnosed with MAFLD and CKD.

Compared to the non-MAFLD group, participants with MAFLD were more likely to be older, men, to have significantly higher levels of BMI, waist circumference, hip circumference, SBP, DBP, UACR, fasting and postprandial plasma glucose, HbA1c, TC, TG, and LDL-C. In addition, the prevalence of T2DM, hypertension, hyperlipidemia, albuminuria, and CKD in the MAFLD group is significantly higher than that in the non-MAFLD group (Table 1).

Association of MAFLD with CKD, EGFR, and UACR

As shown in Table 3, MAFLD was independently associated with an increased risk of CKD (OR: 1.35, 95% CI: 1.09–1.67) after adjusting for age, sex, BMI, education level, occupation, alcohol drinking, tea drinking, HBV infection, T2DM, and MET/day (Table 2). Considering the heterogeneity of MAFLD, MAFLD was categorized into three subtypes based on the definition (24, 25). A significant association was observed only in the T2DM subtype (OR: 2.85, 95% CI: 2.24–3.63). The same analysis was performed with eGFR and UACR, two indexes in the definition of CKD, as outcomes. Linear regression analysis showed that MAFLD was independently associated with increased eGFR (β: 1.37, 95% CI: 0.73–2.01) and UACR (β: 0.15, 95% CI: 0.09–0.21) (Supplementary Table S1). MAFLD subtype analysis showed that a significant association for eGFR was observed in the overweight/obesity subtype (β: 0.94, 95% CI: 0.19–1.69) and T2DM subtype (β: 3.10, 95% CI: 2.25–3.95), whereas for UACR, a significant association was observed only in the T2DM subtype (β: 0.53, 95% CI: 0.45–0.61). Collectively, these data suggest that T2DM is a crucial factor in the association between MAFLD and CKD.

Subgroup analysis found no significant interaction in the association between MAFLD and CKD (Supplementary Table S2). Significant interactions were observed for eGFR and UACR as outcomes (Supplementary Table S3). For eGFR, the association was

TABLE 1 General characteristics of subjects with and without MAFLD.

	Total $N = 5,594$	Non-MAFLD $N = 3,716$	MAFLD $N = 1,878$	P value
Age, median (25, 75th percentiles), year,	57 (50,65)	57 (49,65)	58 (52,66)	**< 0.001**
Male, n (%)	1,961 (35.1)	1,275 (34.3)	686 (36.5)	0.101
Education level, years				0.114
0	1,921 (34.3)	1,245 (33.5)	676 (36.0)	
1-6	1,900 (34.0)	1,273 (34.3)	627 (33.4)	
7-9	1,303 (23.3)	894 (24.1)	409 (21.8)	
≥10	470 (8.4)	304 (8.2)	166 (8.8)	
Occupation				**0.028**
Farmer or unemployment	4,074 (72.8)	2,684 (72.2)	1,390 (74.0)	
Worker	590 (10.5)	425 (11.4)	165 (8.8)	
Sales or service	358 (6.4)	242 (6.5)	116 (6.2)	
Office job	511 (9.1)	326 (8.8)	185 (9.9)	
Other	61 (1.1)	39 (1.0)	22 (1.2)	
Alcohol drinking status				0.266
Never	4,976 (89.0)	3,320 (89.3)	1,656 (88.2)	
Former	186 (3.3)	114 (3.1)	72 (3.8)	
Current	432 (7.7)	282 (7.6)	150 (8.0)	
Smoking status				0.372
Never	4,129 (73.8)	2,762 (74.3)	1,367 (72.8)	
Former	491 (8.8)	314 (8.4)	177 (9.4)	
Current	974 (17.4)	640 (17.2)	334 (17.8)	
Tea drinking status				**< 0.001**
Never	4,270 (76.3)	2,926 (78.7)	1,344 (71.6)	
Former	61 (1.1)	34 (0.9)	27 (1.4)	
Current	1,263 (22.6)	756 (20.3)	507 (27.0)	
T2DM, n (%)	1,248 (22.3)	515 (13.9)	733 (39.0)	**< 0.001**
Hypertension, n (%)	2,609 (46.6)	1,447 (38.9)	1,162 (61.9)	**< 0.001**
Hyperlipidemia, n (%)	1,986 (35.5)	1,118 (30.1)	868 (46.2)	**< 0.001**
HBV infection, n (%)	787 (14.1)	590 (15.9)	197 (10.5)	**< 0.001**
BMI, kg/m^2	23.9 (21.8,26.0)	22.7 (21.0,24.6)	26.2 (24.6,28.2)	**< 0.001**
Waist circumference, cm	82.8 (76.2,89.2)	79.4 (73.8,85.0)	89.6 (84.1,95.0)	**< 0.001**
Hip circumference, cm	93.7 (89.8,98.0)	92 (88.4,95.8)	97.2 (93.6,101.2)	**< 0.001**
SBP, mmHg	131.8 (119.0,146.5)	128.5 (116.5,143.0)	138.5 (125.5,152.9)	**< 0.001**
DBP, mmHg	84.0 (77.0,92.0)	82.5 (75.5,89.5)	88.0 (81.0,95.5)	**< 0.001**
Uric acid, umol/L	341.5 (291.5,404.5)	329.7 (282.9,389.4)	368.6 (314.9,433.6)	**< 0.001**
Creatinine, umol/L	61.7 (54.5,72.4)	62.0 (55.2,71.8)	60.7 (53.1,73.7)	**0.015**
eGFR, mL/min/1.73 m^2	97.17 (90.08,104.07)	97.18 (89.97,104.19)	97.15 (90.27,103.75)	0.4328
eGFR category				0.476
eGFR <60	49 (0.9)	36 (1.0)	13 (0.7)	
60≤ eGFR<90	1,333 (23.8)	894 (24.1)	439 (23.4)	
eGFR ≥90	4,212 (75.3)	2,786 (75.0)	1,426 (75.9)	
UACR, mg/g	7.22 (4.46,13.46)	6.57 (4.24,11.35)	9.09 (5.26,18.14)	**< 0.001**
Albuminuria, n (%)	561 (10.0)	261 (7.0)	300 (16.0)	**< 0.001**
CKD, n (%)	589 (10.5)	284 (7.6)	305 (16.2)	**< 0.001**
FPG, mmol/L	4.95 (4.58,5.46)	4.84 (4.52,5.25)	5.24 (4.77,6.13)	**< 0.001**
Postprandial GLU, mmol/L	7.25 (6.00,9.01)	6.92 (5.80,8.41)	8.23 (6.67,10.48)	**< 0.001**

(Continued)

TABLE 1 (Continued)

	Total $N = 5,594$	Non-MAFLD $N = 3,716$	MAFLD $N = 1,878$	P value
HbA1c, %	5.9 (5.7,6.2)	5.8 (5.6,6.0)	6.1 (5.8,6.6)	**< 0.001**
TC, mmol/L	5.65 (4.96,6.36)	5.58 (4.90,6.27)	5.78 (5.11,6.51)	**< 0.001**
TG, mmol/L	1.11 (0.82,1.56)	0.98 (0.74,1.29)	1.47 (1.09,2.09)	**< 0.001**
HDL-C, mmol/L	1.57 (1.36,1.83)	1.64 (1.43,1.90)	1.45 (1.25,1.64)	**< 0.001**
LDL-C, mmol/L	3.17 (2.70,3.69)	3.12 (2.66,3.60)	3.30 (2.82,3.79)	**< 0.001**
MET/day	9.95 (6.65,16.05)	10 (6.65,16.60)	9.79 (6.65,15.09)	0.4014

All continuous variables were expressed as median (25, 75th percentiles), categorical variables were presented as frequency (percentage).
BMI, body mass index; CKD, chronic kidney disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; GLU, plasma glucose; HbA1c, glycated hemoglobin A1c; HBV, hepatitis B virus; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MAFLD, metabolic associated fatty liver disease; MET/day, daily physical metabolic equivalent; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triglyceride; UACR, urinary albumin to creatinine ratio. Boldface type indicates statistical significance (P value < 0.05).

TABLE 2 Association between MAFLD subgroups and CKD.

	No.	CKD	Model 1† (OR and 95% CI)	Model 2‡ (OR and 95% CI)	Model 3§ (OR and 95% CI)
Non-MAFLD	3,716	284	1.00 (ref)	1.00 (ref)	1.00 (ref)
MAFLD	1,878	305	**2.34 (1.97,2.78)**	**1.74 (1.42,2.13)**	**1.35 (1.09,1.67)**
T2DM (-)¶ & overweight/obesity (BMI>23)	1,046	106	**1.36 (1.08,1.72)**	1.08 (0.83,1.40)	1.07 (0.82,1.40)
T2DM (-)¶ & BMI<23 & ≥2 metabolic disorders	99	9	1.21 (0.60,2.42)	1.26 (0.63,2.54)	1.22 (0.60,2.45)
T2DM (+)††	733	190	**4.23 (3.45,5.19)**	**2.89 (2.27,3.67)**	**2.85 (2.24,3.63)**

BMI, body mass index; CI, confidence interval; CKD, chronic kidney disease; MAFLD, metabolic associated fatty liver disease; OR, odds ratio; T2DM, type 2 diabetes mellitus. †Unadjusted model. ‡Age, sex and BMI adjusted. §Adjusted for age, sex, BMI, education level, occupation, alcohol drinking status, tea drinking status, HBV infection, T2DM (removed in MAFLD subgroup analysis) and MET/day. ¶Absence of T2DM. ††Presence of T2DM. Boldface type indicates statistical significance (P value < 0.05).

stronger for individuals who were female, nonsmokers, and had T2DM. For UACR, the association was stronger for individuals who had T2DM and hypertension.

Association of MAFLD and T2DM with CKD, EGFR, and UACR

To further study the role of T2DM in the association between MAFLD and CKD, MAFLD and T2DM were combined for analysis (Table 3). Using non-MAFLD without T2DM group as the reference, MAFLD without T2DM (OR: 1.40, 95% CI: 1.07–1.83), non-MAFLD with T2DM (OR: 2.87, 95% CI: 2.18–3.78), and MAFLD with T2DM (OR: 3.72, 95% CI: 2.88, 4.79) groups were all associated with an increased risk of CKD. To better understand the association between glycemic status in MAFLD and CKD, MAFLD was categorized by T2DM status. The unadjusted ORs of normal glucose group, prediabetes group, and T2DM group among MAFLD for CKD were 0.86 (95% CI: 0.43–1.72), 1.42 (95% CI: 1.12–1.79), and 4.23 (95% CI: 3.45–5.19), respectively. Only the T2DM group remained statistically significant after full adjustment (OR: 2.83, 95% CI: 2.23–3.58). Similarly, for eGFR and UACR, a significant association was observed in the T2DM group (Supplementary Table S4). The T2DM group was further divided into newly diagnosed T2DM and pre-existing T2DM to explore the effect of T2DM duration on CKD. The pre-existing T2DM group had a higher OR (OR: 3.75, 95% CI: 2.80–5.02) than newly diagnosed T2DM (OR: 2.21, 95% CI: 1.65–2.96), indicating that the risk of CKD increases with T2DM duration (Table 3).

Association of MAFLD and metabolic goal achievement with CKD, EGFR, and UACR

"ABCs" metabolic goal was introduced to study whether the metabolic goal achievement reduces the risk of CKD among MAFLD (Figure 2). MAFLD with poorly controlled HbA1c, BP, and LDL was associated with an increased risk of CKD. MAFLD with HbA1c (OR: 1.20, 95% CI: 0.95–1.52) and BP goal achievement (OR: 0.69, 95% CI: 0.50–0.95) decreased the risk

TABLE 3 Association of MAFLD and T2DM with CKD.

	No.	CKD	Model 1[†] (OR and 95% CI)	Model 2[‡] (OR and 95% CI)	Model 3[§] (OR and 95% CI)
Combination of MAFLD and T2DM					
MAFLD (-)[¶] & T2DM (-)[††]	3,201	191	1.00 (ref)	1.00 (ref)	1.00 (ref)
MAFLD (+)[‡‡] & T2DM (-)[††]	1,145	115	**1.76 (1.38,2.24)**	**1.41 (1.08,1.84)**	**1.40 (1.07,1.83)**
MAFLD (-)[¶] & T2DM (+)[§§]	515	93	**3.47 (2.66,4.54)**	**2.87 (2.19,3.78)**	**2.87 (2.18,3.78)**
MAFLD (+)[‡‡] & T2DM (+)[§§]	733	190	**5.51 (4.42,6.88)**	**3.76 (2.93,4.84)**	**3.72 (2.88,4.79)**
P-trend			**< 0.001**	**< 0.001**	**< 0.001**
MAFLD subgroups by different T2DM status					
Non-MAFLD	3,716	284	1.00 (ref)	1.00 (ref)	1.00 (ref)
MAFLD without T2DM	1,145	115	**1.35 (1.07,1.69)**	1.10 (0.85,1.41)	1.09 (0.84,1.40)
MAFLD with normal glucose	135	9	0.86 (0.43,1.72)	0.86 (0.42,1.72)	0.84 (0.42,1.70)
MAFLD with prediabetes	1,010	106	**1.42 (1.12,1.79)**	1.12 (0.86,1.45)	1.11 (0.85,1.44)
MAFLD with T2DM	733	190	**4.23 (3.45,5.19)**	**2.87 (2.26,3.63)**	**2.83 (2.23,3.58)**
MAFLD with newly diagnosed T2DM[¶¶]	420	89	**3.25 (2.50,4.23)**	**2.23 (1.67,2.98)**	**2.21 (1.65,2.96)**
MAFLD with pre-existing T2DM[†††]	313	101	**5.76 (4.41,7.51)**	**3.82 (2.86,5.11)**	**3.75 (2.80,5.02)**
P-trend			**< 0.001**	**< 0.001**	**< 0.001**

CI, confidence interval; CKD, chronic kidney disease; MAFLD, metabolic associated fatty liver disease; OR, odds ratio; T2DM, type 2 diabetes mellitus. [†]Unadjusted model. [‡]Age, sex, and BMI adjusted. [§]Adjusted for age, sex, BMI, education level, occupation, alcohol drinking status, tea drinking status, HBV infection, and MET/day. [¶]Absence of MAFLD. [††]Absence of T2DM. [‡‡]Presence of MAFLD. [§§]Presence of T2DM. [¶¶]Newly diagnosed T2DM is defined by laboratory indicators without pre-existing T2DM, meaning relative short duration of T2DM. [†††]Pre-existing T2DM is defined as self-reported T2DM and use of antidiabetic drug, meaning relative long duration of T2DM. Boldface type indicates statistical significance (*P* value < 0.05).

of CKD, while LDL goal achievement has no effect on CKD (OR: 1.50, 95% CI: 1.04–2.15). Notably, BP goal achievement reversed the increased risk to a decreased one (OR: 0.69, 95% CI: 0.50–0.95), suggesting the importance of BP control to reduce CKD risk in subjects with MAFLD. A combination of HbA1c and BP control in MAFLD exhibited gradually decreased risks of CKD. The group achieving both HbA1c and BP control had the lowest risk (OR: 0.60, 95% CI: 0.41–0.89). With more achieved metabolic goals in MAFLD, the risk of CKD decreased more obviously. MAFLD with 2 or 3 achieved metabolic goals exhibited no statistically significant association with CKD (OR: 0.81, 95% CI: 0.59–1.12).

Using eGFR and UACR as outcomes, metabolic goal achievement in MAFLD significantly decreased eGFR and UACR (Supplementary Table S5). We also assessed the association with albuminuria, and the ORs were similar to those for CKD (Supplementary Table S6).

Discussion

In this cross-sectional study based on the general population in southern China, we demonstrated that the presence of MAFLD significantly increased the risk of CKD by increasing both eGFR and UACR. This significant association was observed only in the T2DM subtype among MAFLD. Categorized by glycemic status, MAFLD with T2DM group rather than prediabetes and normal glucose groups was associated with an increased risk of CKD. Furthermore, metabolic goal achievement in MAFLD decreased the risk of CKD by reducing the risk of albuminuria.

In our population, the prevalence of MAFLD was 33.6%, higher than 29.2% and 26.1% in two studies based on the populations of southern China (26, 27). This may be attributed to demographic differences and increasing prevalence over time. The prevalence of eGFR <60 ml/min/1.73 m^2, albuminuria, and CKD was 0.9, 10.0, and 10.5%, respectively, compared to those of 1.7, 9.4, and 10.8% from a nationally representative sample of the Chinese adults (9).

Numerous studies have reported a significant association between NAFLD and CKD (6, 11–14). Recently, a significant association between MAFLD and CKD was established in cross-sectional and cohort studies (16–18). Our study further examined this association. To the best of our knowledge, MAFLD has great heterogeneity due to its definition and can be classified into different subtypes (24, 25). There is currently no study that explores the association between MAFLD subtypes and CKD. In our study, a significant association was observed only in the T2DM subtype among the three MAFLD subtypes. Furthermore, results indicated that the T2DM subtype significantly increased eGFR and UACR, two renal function indexes defining CKD. We speculated from the definition of CKD that increased albuminuria by the T2DM subtype in MAFLD exceeds increased eGFR, leading to CKD.

Diabetes is the leading cause of CKD, referred to as diabetic kidney disease (DKD), developing in around 40%

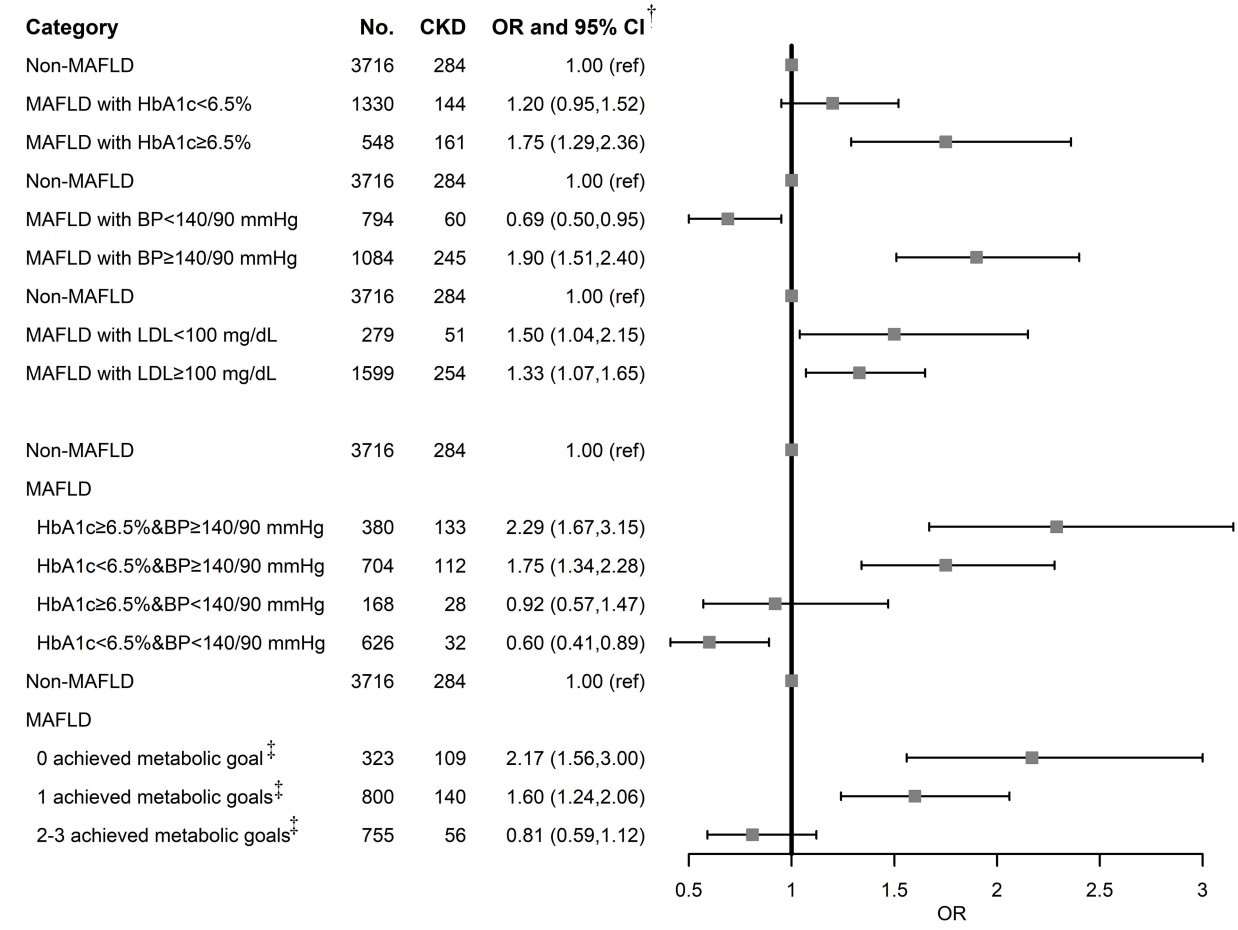

FIGURE 2
Association of MAFLD and metabolic goal achievement with CKD. BP, blood pressure; CI, confidence interval; CKD, chronic kidney disease; MAFLD, metabolic associated fatty liver disease; OR, odds ratio; T2DM, type 2 diabetes mellitus. †Adjusted for age, sex, BMI, education level, occupation, alcohol drinking status, tea drinking status, HBV infection, T2DM, and MET/day. ‡Achieved metabolic goal is defined as HbA1c<6.5%, SBP/DBP<140/90 mmHg, and LDL-C<100 mg/dl.

of patients with T2DM (28). Recent evidence showed that MAFLD complicated with T2DM had a higher prevalence of albuminuria and CKD than MAFLD without T2DM (17). We observed a synergy effect of MAFLD and T2DM on CKD, as well as eGFR and UACR. Further categorized into normal glucose, prediabetes, and T2DM, only MAFLD with T2DM group was significantly associated with CKD. In addition, the pre-existing T2DM group had a higher risk of CKD than the newly diagnosed T2DM, suggesting that T2DM progression among MAFLD elevated the risk of CKD. MAFLD with glycemic deterioration was associated with increased eGFR and UACR. It is noteworthy that MAFLD with prediabetes was still associated with increased eGFR, rather than UACR. The natural pathway of DKD includes glomerular hyperfiltration, progressive albuminuria, declining GFR, and finally, end-stage renal disease (ESRD) (29). Glomerular hyperfiltration, characterized by increased GFR, was observed in prediabetes, early stage of DKD (29, 30), and NAFLD(31, 32), consistent with our findings that MAFLD with prediabetes and T2DM significantly increased eGFR. Research showed that baseline high eGFR or glomerular hyperfiltration was associated with faster decline over time and worse renal outcomes (30, 33, 34). Whether increased eGFR in MAFLD and T2DM will lead to faster decline and CKD development and progression needs to be explored in our follow-up studies. Albuminuria is present in around 30% of patients with T2DM and does not occur in the absence of hyperglycemia (29). MAFLD itself is also independently associated with an increased risk of albuminuria (17). Consistent with these studies, our data showed that only MAFLD with T2DM group significantly increased UACR, and UACR continued to increase with the duration of diabetes. Taken together, it is reasonable that we found that

both eGFR and UACR were increased in the MAFLD with T2DM group.

As the new definition of MAFLD incorporates more metabolic risk factors and confers a high risk of CKD than NAFLD (17, 19), metabolic management is essential for reducing the risk of CKD. Our results demonstrated that the achievement of any 2 "ABCs" metabolic goals comprising HbA1c, BP, and LDL decreased the adverse effect of MAFLD on albuminuria and CKD risk to null. As we know, it is very difficult to achieve comprehensive metabolic control in practice, so it makes more sense to identify a specific metabolic goal that reduces enormous risks for diseases. Our findings revealed that MAFLD with glycemic and BP control, rather than LDL control, exhibited no statistically significant associations with CKD. According to the Global Burden of Disease study data from 1990 to 2016, diabetes and hypertension are the first two important causes of CKD(35). A meta-analysis of four randomized controlled trials (RCTs) showed that more intensive glucose control in adults with T2DM over 5 years reduced kidney events (36). Several long-term follow-up studies found that glycemic control in patients with diabetes reduced the risk of DKD and albuminuria (37, 38), in line with our findings. Research suggested that intensive BP control decreased the risk of incident CKD in patients with hypertension or diabetes (39). Interestingly, our study showed that an increased risk of CKD was reversed to a decreased one after BP control, suggesting BP serves as an important bridge between MAFLD and CKD. MAFLD with both well-controlled HbA1c and BP conferred the lowest risk compared to other groups. Similarly, there is evidence that a combination of poorly controlled blood glucose and BP was associated with a higher risk of incident CKD than having either alone (40, 41). Metabolic goal achievement in MAFLD significantly decreased eGFR and risk of albuminuria, suggesting that this decreased risk of CKD by metabolic goal achievement was basically attributed to decreased albuminuria. The real effect of metabolic goal achievement on eGFR may need to be assessed in our follow-up studies. Moreover, we found that MAFLD with HbA1c $< 6.5\%$ significantly reduced eGFR, consistent with studies showing that improved HbA1c mitigates glomerular hyperfiltration in the early-stage DKD (30).

The strength of our study is that we explored MAFLD with different glycemic statuses on the risk of CKD and identified the T2DM subtype which conferred the highest risk of CKD among MAFLD subtypes. Furthermore, the introduction of "ABCs" metabolic goal highlighted the importance of metabolic management in reducing the risk of CKD in MAFLD. We also used eGFR and UACR as outcomes to evaluate their impacts on CKD. However, there are limitations in this study. First, this cross-sectional study prevents us from making inferences about a causal relationship. Second, ultrasound, rather than a gold-standard liver biopsy, was performed to diagnose fatty liver in our study. Although insufficient accuracy is a problem, ultrasound is still the first choice in large-scale epidemiological studies for its accessibility and feasibility. Third, GFR and albuminuria have a dynamic, fluctuating progressive process (29), so only one sampling may not represent the real condition. Future follow-up studies are needed to explore the long-term changes in these renal function indexes and CKD development and progression among the three MAFLD subtypes. Whether increased eGFR in the T2DM subtype will lead to a faster decline and CKD progression is an interesting topic.

In conclusion, we demonstrated that MAFLD significantly increased the risk of CKD by increasing both eGFR and UACR. The T2DM subtype is the only MAFLD subtype driving this relationship. MAFLD with T2DM group rather than prediabetes and normal glucose groups was associated with an increased risk of CKD, as well as increased eGFR and UACR. Moreover, metabolic goal achievement in MAFLD significantly decreased the risk of CKD by reducing the risk of albuminuria.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary materials, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Fujian Medical University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

WS: conceptualization, data curation, formal analysis, methodology, and writing—original draft. LXi: conceptualization, investigation, validation, and writing—original draft. MC: formal analysis, visualization, and writing—review and editing. SD: data curation and project administration. LXu: investigation and validation. RF: data curation and writing—review and editing. WY: conceptualization, funding acquisition, project administration, and writing—review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1047794/full#supplementary-material

References

1. Zhou F, Zhou J, Wang W, Zhang XJ Ji YX, Zhang P, et al. Unexpected rapid increase in the burden of Nafld in China from 2008 to 2018: a systematic review and meta-analysis. *Hepatology.* (2019) 70:1119–33. doi: 10.1002/hep.30702

2. Wang XJ, Malhi H. Nonalcoholic fatty liver disease. *Ann Intern Med.* (2018) 169:ITC65-ITC80. doi: 10.7326/AITC201811060

3. Samuel VT, Shulman GI. Nonalcoholic fatty liver disease, insulin resistance, and ceramides. *N Engl J Med.* (2019) 381:1866–9. doi: 10.1056/NEJMcibr1910023

4. Cai J, Zhang XJ Ji YX, Zhang P, She ZG Li H. Nonalcoholic fatty liver disease pandemic fuels the upsurge in cardiovascular diseases. *Circ Res.* (2020) 126:679–704. doi: 10.1161/CIRCRESAHA.119.316337

5. Polyzos SA, Kountouras J, Mantzoros CS. Obesity and nonalcoholic fatty liver disease: from pathophysiology to therapeutics. *Metabolism.* (2019) 92:82–97. doi: 10.1016/j.metabol.2018.11.014

6. Byrne CD, Targher G. Nafld as a driver of chronic kidney disease. *J Hepatol.* (2020) 72:785–801. doi: 10.1016/j.jhep.2020.01.013

7. Guerreiro GTS, Longo L, Fonseca MA, de Souza VEG, Alvares-da-Silva MR. Does the risk of cardiovascular events differ between biopsy-proven Nafld and Mafld? *Hepatol Int.* (2021) 15:380–91. doi: 10.1007/s12072-021-10157-y

8. Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. *J Hepatol.* (2020) 73:202–9. doi: 10.1016/j.jhep.2020.03.039

9. Zhang L, Wang F, Wang L, Wang W, Liu B, Liu J, et al. Prevalence of chronic kidney disease in china: a cross-sectional survey. *Lancet.* (2012) 379:815–22. doi: 10.1016/S0140-6736(12)60033-6

10. Levey AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, et al. Definition and classification of chronic kidney disease: a position statement from kidney disease: improving global outcomes (Kdigo). *Kidney Int.* (2005) 67:2089–100. doi: 10.1111/j.1523-1755.2005.00365.x

11. Aubert L, Sandino J, Gutierrez-Solis E, Garcia-Martin F, Segura J, Porrini E, et al. Role of non-alcoholic fatty liver disease in the evolution of renal function in patients with diabetes mellitus. *Nephrol Dial Transplant.* (2021). doi: 10.1093/ndt/gfab176

12. Li M, Zhao Z, Qin G, Chen L, Lu J, Huo Y, et al. Non-alcoholic fatty liver disease, metabolic goal achievement with incident cardiovascular disease and egfr-based chronic kidney disease in patients with prediabetes and diabetes. *Metabolism.* (2021) 124:154874. doi: 10.1016/j.metabol.2021.154874

13. Nabi O, Boursier J, Lacombe K, Mathurin P, de Ledinghen V, Goldberg M, et al. Comorbidities are associated with fibrosis in nafld subjects: a nationwide study (Nash-Co Study). *Dig Dis Sci.* (2021). doi: 10.1007/s10620-021-07032-z

14. Cai X, Sun L, Liu X, Zhu H, Zhang Y, Zheng S, et al. Non-alcoholic fatty liver disease is associated with increased risk of chronic kidney disease. *Ther Adv Chronic Dis.* (2021) 12:20406223211024361. doi: 10.1177/20406223211024361

15. Chen PC, Kao WY, Cheng YL, Wang YJ, Hou MC, Wu JC, et al. The correlation between fatty liver disease and chronic kidney disease. *J Formos Med Assoc.* (2020) 119:42–50. doi: 10.1016/j.jfma.2019.02.010

16. Liang Y, Chen H, Liu Y, Hou X, Wei L, Bao Y, et al. Association of Mafld with diabetes, chronic kidney disease, and cardiovascular disease: a 46-year cohort study in China. *J Clin Endocrinol Metab.* (2022) 107:88–97. doi: 10.1210/clinem/dgab641

17. Sun DQ, Jin Y, Wang TY, Zheng KI, Rios RS, Zhang HY, et al. Mafld and risk of Ckd. *Metabolism.* (2021) 115:154433. doi: 10.1016/j.metabol.2020.154433

18. Hashimoto Y, Hamaguchi M, Okamura T, Nakanishi N, Obora A, Kojima T, et al. Metabolic associated fatty liver disease is a risk factor for chronic kidney disease. *J Diabetes Investig.* (2021) 13:308–16. doi: 10.1111/jdi.13678

19. Nguyen VH, Le MH, Cheung RC, Nguyen MH. Differential clinical characteristics and mortality outcomes in persons with Nafld and/or Mafld. *Clin Gastroenterol Hepatol.* (2021) 19:2172–81. doi: 10.1016/j.cgh.2021.05.029

20. Stark Casagrande S, Fradkin JE, Saydah SH, Rust KF, Cowie CC. The prevalence of meeting A1c, blood pressure, and Ldl goals among people with diabetes, 1988-2010. *Diabetes Care.* (2013) 36:2271–9. doi: 10.2337/dc12-2258

21. American Diabetes A. Standards of medical care in diabetes—2011. *Diabetes Care.* (2011) 34:S11–61. doi: 10.2337/dc11-S011

22. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* (2009) 150:604–12. doi: 10.7326/0003-4819-150-9-200905050-00006

23. Cai X, Hu D, Pan C, Li G, Lu J, Ji Q, et al. The risk factors of glycemic control, blood pressure control, lipid control in chinese patients with newly diagnosed type 2 diabetes _ a nationwide prospective cohort study. *Sci Rep.* (2019) 9:7709. doi: 10.1038/s41598-019-44169-4

24. Chen X, Chen S, Pang J, Tang Y, Ling W. Are the different mafld subtypes based on the inclusion criteria correlated with all-cause mortality? *J Hepatol.* (2021) 75:987–9. doi: 10.1016/j.jhep.2021.06.013

25. Sohn W, Kwon HJ, Chang Y, Ryu S, Cho YK. Liver fibrosis in asians with metabolic dysfunction-associated fatty liver disease. *Clin Gastroenterol Hepatol.* (2021). doi: 10.1016/j.cgh.2021.06.042

26. Fan J, Luo S, Ye Y, Ju J, Zhang Z, Liu L, et al. Prevalence and risk factors of metabolic associated fatty liver disease in the contemporary South China population. *Nutr Metab (Lond).* (2021) 18:82. doi: 10.1186/s12986-021-00611-x

27. Chen YL Li H, Li S, Xu Z, Tian S, Wu J, et al. Prevalence of and risk factors for metabolic associated fatty liver disease in an urban population in china: a cross-sectional comparative study. *BMC Gastroenterol.* (2021) 21:212. doi: 10.1186/s12876-021-01782-w

28. Anders HJ, Huber TB, Isermann B, Schiffer M. Ckd in diabetes: diabetic kidney disease versus nondiabetic kidney disease. *Nat Rev Nephrol.* (2018) 14:361–77. doi: 10.1038/s41581-018-0001-y

29. Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: challenges, progress, and possibilities. *Clin J Am Soc Nephrol.* (2017) 12:2032–45. doi: 10.2215/CJN.11491116

30. Tonneijck L, Muskiet MH, Smits MM, van Bommel EJ, Heerspink HJ, van Raalte DH, et al. Glomerular hyperfiltration in diabetes: mechanisms, clinical significance, and treatment. *J Am Soc Nephrol.* (2017) 28:1023–39. doi: 10.1681/ASN.2016060666

31. Abbate M, Mascaro CM, Montemayor S, Casares M, Gomez C, Ugarriza L, et al. Non-alcoholic fatty liver disease is associated with kidney glomerular hyperfiltration in adults with metabolic syndrome. *J Clin Med.* (2021) 10:1717. doi: 10.3390/jcm10081717

32. Yodoshi T, Arce-Clachar AC, Sun Q, Fei L, Bramlage K, Xanthakos SA, et al. Glomerular hyperfiltration is associated with liver disease severity in children with nonalcoholic fatty liver disease. *J Pediatr.* (2020) 222:127–33. doi: 10.1016/j.jpeds.2020.03.038

33. Melsom T, Nair V, Schei J, Mariani L, Stefansson VTN, Harder JL, et al. Correlation between baseline Gfr and subsequent change in Gfr in Norwegian adults without diabetes and in Pima Indians. *Am J Kidney Dis.* (2019) 73:777–85. doi: 10.1053/j.ajkd.2018.11.011

34. Ruggenenti P, Porrini EL, Gaspari F, Motterlini N, Cannata A, Carrara F, et al. Glomerular hyperfiltration and renal disease progression in type 2 diabetes. *Diabetes Care.* (2012) 35:2061–8. doi: 10.2337/dc11-2189

35. Xie Y, Bowe B, Mokdad AH, Xian H, Yan Y, Li T, et al. Analysis of the global burden of disease study highlights the global, regional, and national trends of chronic kidney disease epidemiology from 1990 to 2016. *Kidney Int.* (2018) 94:567–81. doi: 10.1016/j.kint.2018.04.011

36. Zoungas S, Arima H, Gerstein HC, Holman RR, Woodward M, Reaven P, et al. Effects of intensive glucose control on microvascular outcomes in patients with Type 2 diabetes: a meta-analysis of individual participant data from randomised controlled trials. *Lancet Diabetes Endocrinol.* (2017) 5:431–7. doi: 10.1016/S2213-8587(17)30104-3

37. Gubitosi-Klug RA, Group DER. The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: summary and future directions. *Diabetes Care.* (2014) 37:44–9. doi: 10.2337/dc13-2148

38. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med.* (2008) 359:1577–89. doi: 10.1056/NEJMoa0806470

39. Beddhu S, Rocco MV, Toto R, Craven TE, Greene T, Bhatt U, et al. Effects of intensive systolic blood pressure control on kidney and cardiovascular outcomes in persons without kidney disease: a secondary analysis of a randomized trial. *Ann Intern Med.* (2017) 167:375–83. doi: 10.7326/M16-2966

40. He J, Li Z, Wang R, Nie H, Wang F, Yuan J, et al. Association of blood pressure and long-term change with chronic kidney disease risk among chinese adults with different glucose metabolism according to the 2017 acc/aha guidelines. *J Clin Hypertens (Greenwich).* (2021) 23:2080–8. doi: 10.1111/jch.14371

41. Derakhshan A, Bagherzadeh-Khiabani F, Arshi B, Ramezankhani A, Azizi F, Hadaegh F. Different combinations of glucose tolerance and blood pressure status and incident diabetes, hypertension, and chronic kidney disease. *J Am Heart Assoc.* (2016) 5:e003917. doi: 10.1161/JAHA.116.003917

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Mendelian randomization study supports the causal association between serum cystatin C and risk of diabetic nephropathy

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Aims: Cystatin C, an inhibitor of cysteine protease, has been used as a biomarker for estimating glomerular filtration rate. However, the causal relation between cystatin C and diabetic nephropathy remains uncertain.

Methods: We assessed the causal effect of cystatin C together with other five serum biomarkers including KIM-1, GDF-15, TBIL, uric acid, and Scr on diabetic nephropathy by Mendelian randomization (MR) analysis. 234 genetic variants were selected as instrumental variables to evaluate the causal effect of cystatin C (N_{GWAS}=361194) on diabetic nephropathy (Ncase/Ncontrol up to 3283/210463). Multivariable MR (MVMR) was performed to assess the stability of cystatin C's causal relationship. Two-step MR was used to assess the mediation effect of BMI and SBP.

Results: Among the six serum biomarkers, only cystatin C causally associated with diabetic nephropathy (IVW OR: 1.36, 95%CI [1.15, 1.61]). After adjusting for the potential confounders BMI and SBP, cystatin C maintained its causal effect on the DN (OR: 1.17, 95%CI [1.02, 1.33]), which means that the risk of DN increased by 17% with an approximate 1 standard deviation (SD) increment of serum cystatin C level. Two-step MR results indicated that BMI might mediate the causal effect of cystatin C on diabetic nephropathy.

Interpretation: Our findings discovered that cystatin C was a risk factor for diabetic nephropathy independent of BMI and SBP in diabetes mellitus patients. Future research is required to illustrate the underlying mechanism and prove targeting circulating cystatin C could be a potential therapy method.

KEYWORDS

Mendelian randomization, cystatin C, diabetic nephropathy, biomarker, glomerular filtration rate

Introduction

Diabetic nephropathy (DN), a most common complication of diabetes mellitus (DM), is the main causes of end-stage renal disease, and it occurs in 25% to 40% of DM patients worldwide (1, 2). DN is often clinically diagnosed based on persistently increased albuminuria with a ratio of microalbumin and urine creatinine more than 300 mg/g or estimated glomerular filtration rate (eGFR) less than 60 ml/min/1.73m^2 (3). The therapeutic options for DN were very limited. Therefore, constant search for potential novel therapeutic targets is in big need.

Many risk factors have been recognized to be related with the development and progression of DN in the recent decade (1). Several studies identified plasma kidney injury molecule 1(KIM-1) as a positive predictor of ESRD in T1DM patients and it could predict the early decline of eGFR as well as progression to chronic kidney disease stage 3 without macroalbuminuria (4, 5). Growth differentiation factor-15 (GDF-15) was reported to be a predictor of the rapid deterioration of renal function (6, 7). A recent meta-analysis indicated that total bilirubin level was negatively correlated with the risk of DN (8). Another meta-analysis of 25741 T2DM patients revealed that each increase of 1mg/dl of serum uric acid could increase the risk of DN by 24% (9). Serum creatinine (Scr) and cystatin C are routinely utilized to estimate eGFR. However, a recent study suggested that high level of baseline cystatin C and high velocity of increase of cystatin C in T2DM patients were more likely to develop DN in later life (10). Recent study also reported that high serum creatinine (Scr) variability could independently predict the onset of albuminuria in T2DM patients (11). However, these observational studies couldn't conclude the causal association between these risk factors and DN.

Mendelian randomization (MR) can explore whether risk factors are causally linked to the outcome by analyzing genetic variants as instrumental variables, which represents with single nucleotide polymorphisms (SNPs). Since the gene randomly distributed at conception, MR can mimic randomized trials and minimize the effect of confounders biasing observational studies (12). Benefit by the recent comprehensive meta-analysis with the GWAS of DN, we performed MR analysis to access the possible causal effect of these risk factors on DN. In this study, we used MR to evaluate whether the following six serum biomarkers of renal function or renal injury (cystatin C, KIM-1, GDF-15, TBIL, uric acid, and Scr) casually associate with DN. Interestingly, it turned out only serum cystatin C was casually linked to DN. Subsequently, we analyzed the SNPs relative to cystatin C and found that the majority of them were related to body mass index (BMI) and systolic blood pressure (SBP). Thus, we further validated the causal relationship between serum cystatin C and DN by multivariable MR using SBP and BMI as confounders, which indicated the slightly alleviated detrimental casual effect. Besides, two-step MR indicated that BMI might play as a mediator between cystatin C and DN. Thus, we concluded that cystatin C was a risk factor in the development of DN independent of BMI and SBP in diabetes mellitus patients.

Materials and methods

Overall study design

MR analysis was used to evaluate the causal association between serum biomarkers and diabetic nephropathy, which is based on three assumptions: Assumption 1, the selected genetic variants should be robustly correlated with serum biomarkers; Assumption 2, the genetic variants should not associate with the confounders between the relationship of biomarkers and DN; Assumption 3, the genetic variants should only associate with DN *via* serum biomarkers. Two-sample univariable Mendelian randomization was implemented to evaluate the causal association between multiple serum biomarkers (cystatin C, Scr, urate, total bilirubin, KIM-1, GDF-15) and DN. Biomarkers with significant causal effects on the outcome will be further searched for potential confounders from published articles and Phenoscanner V2 (http://www.phenoscanner.medschl.cam.ac.uk/). Multivariable Mendelian randomization that included biomarkers with significant causal effect and their confounders will be implemented to validate their causal association. Once a causal relationship was established, two-step MR was used to investigate whether confounder plays as a mediator between cystatin C and DN (Figure 1).

Ethics

The summary-level data of GWAS used in this study are publicly accessible, and the original study have acquired ethical approval and informed consent.

Data source and instrumental variable selection

Instrumental variables (IV) were extracted from GWAS data and SNPs with high linkage disequilibrium were removed. Independent SNP is defined by $r^2 < 0.001$ and clumping distance >1Mb using 1,000 genomes reference panel for Europeans (https://www.internationalgenome.org/). Genetic variants that were highly associated with cystatin C, creatinine, urate, and total bilirubin were selected from a GWAS cohort conducted by the Neale Lab Consortium including 361194 individuals of European ancestry (http://www.nealelab.is/uk-biobank). IVs for KIM-1 and GDF-15 were obtained from a meta-analysis including up to 21,758 individuals of European

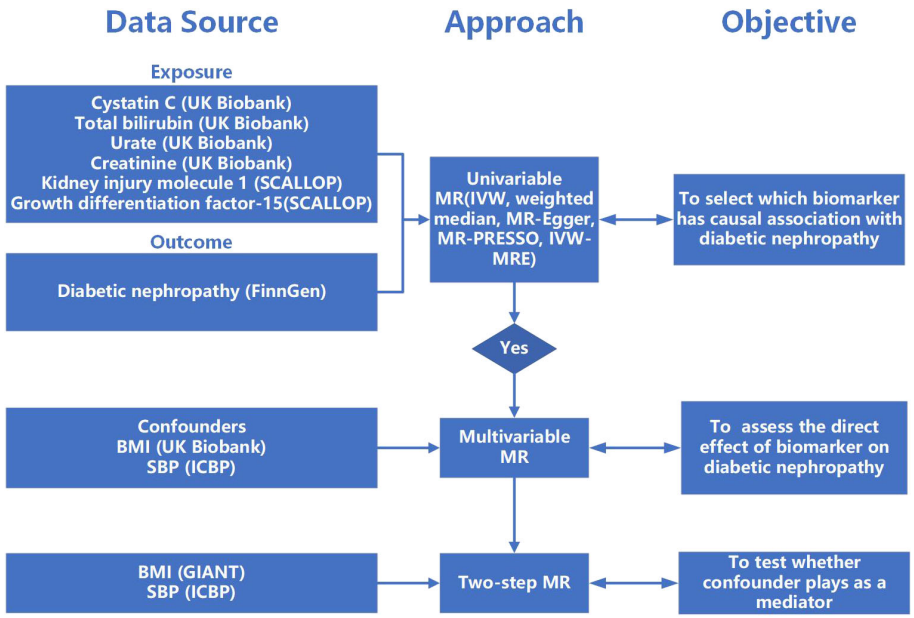

FIGURE 1
The flow of study. MR, Mendelian randomization; IVW, inverse-variance weighted; MR-PRESSO, Mendelian randomization pleiotropy residual sum and outlier; IVW-MRE, multiplicative random effects inverse variance weighted; BMI, body mass index; SBP, systolic blood pressure.

ancestry (13). Genetic variants of confounder SBP were obtained from a GWAS of a meta-analysis that contains over one million European samples from International Consortium for Blood Pressure (ICBP) and UK Biobank (UKB) (14). Genetic variants of confounder BMI were retrieved from GWAS performed by the Neale Lab consortium including 336,107 European individuals (http://www.nealelab.is/uk-biobank). The BMI GWAS used for two-step MR was obtained from the Genetic Investigation of Anthropometric Traits (GIANT) Consortium including 681,275 samples of European ancestry (15).

Diabetic nephropathy as outcome was defined when there was glomerular disorders in the patients with diabetes mellitus with the criterion of ICD-10 (code: N08.3*), summary statistics of which was from FinnGen biobank including 213,746 European individuals (3283 cases and 210463 controls) (16). Except for GDF-15, IVs from other exposures were extracted with a genome-wide significant threshold ($p<5E^{-8}$). Genetic variants of GDF-15 were obtained with a lower threshold ($p<1E^{-5}$) since few IVs were identified with the original threshold. Palindromic SNPs were further excluded from the IV list. For those instruments that are missing in the outcome, proxy SNP with LD score>0.8 was used. In order to satisfy MR assumption three, SNPs with significant association with the outcome were excluded. The F statistic was calculated using the formula: $F = beta^2/se^2$, where beta represents the effect of SNP on the exposure and se is the standard error of the beta, to assess whether there is a possibility of weak instrument bias (17). R^2 calculated by the following formula: $2 \times EAF \times (1\text{-}EAF) \times beta^2$, where EAF represents the effect allele frequency of the SNP, represents the proportion of variance of the exposure explained by SNPs (18).

Statistical analysis

In this study, inverse variance weighted (IVW) analysis was utilized as the major statistic method. Meanwhile, MR-Egger regression, weighted median, and MR-PRESSO were also performed as complementary methods to validate the IVW result. The IVW method could combine each genetic variant's Wald estimate in a meta-analysis model and produce unbiased result if horizontal pleiotropy is balanced (19). MR-Egger regression can detect pleiotropy through the intercept it produces while its causal estimate can be largely affected by outliers (20). The weighted median method can provide consistent results even if as many as 50% of instrumental variables are invalid. Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) (21) could detect outliers with horizontal pleiotropy ($p<0.05$), and return a corrected causal estimate after removing them. To validate the robustness of the MR result, Cochran's Q statistic was performed to detect heterogeneity among instrumental variables. If heterogeneity exists, the multiplicative random effects inverse variance weighted method was further performed (22) to

validate the previous MR estimates. Leave-one-out analysis was used to determine whether the SNPs strongly affect the stability of causal estimates.

Multivariable MR (MVMR) was performed to assess the stability of the significant causal relationships, which could estimate the causal relationship between each exposure and a single outcome, producing a causal estimate of direct effect and adjusting pleiotropy caused by other exposures that were included in the MVMR analysis (23). Two-step MR was used to assess the confounder's mediation effect (24). Firstly, IVs of cystatin C were utilized to perform UVMR analysis against confounder. Secondly, MVMR analysis was used to estimate the causal effect of confounder on DN adjusted for cystatin C. Potential confounders were obtained based on published papers and the online search with Phenoscanner V2. We further calculated the proportion of the mediation effect of confounders by utilizing the product of coefficients method. We first estimated the causal effect of cystatin C on individual confounder, then multiplying the confounder's effect on DN adjusted for cystatin C, which produced the indirect effect. Finally, we assessed the proportion of mediation effect through dividing the indirect effect by the total effect which in this case is the causal effect of cystatin C on DN. The standard errors were generated by using the delta method. Results were displayed in the form of odds ratio (OR) per an approximate 1 standard deviation (SD) increment. In this study, the statistical power of Mendelian randomization is calculated using mRnd (https://shiny.cnsgenomics.com/mRnd/) with a type 1 error rate of 0.05 (25). Two-sided $p<0.05$ level of significance is used in all estimates. Statistical analysis was carried out with ''TwosampleMR'' and ''MR-PRESSO'' packages in R version 4.1.3.

Results

Selection of genetic instrumental variables of exposures

Summary information of selected IVs of 6 exposures was presented in Table 1. The mean concentration of cystatin C, creatinine, urate, and total bilirubin obtained from UK biobank's website which contain the same cohort from GWAS database used in this study but with more participants were 0.908 ± 0.176 mg/L, 72.407 ± 18.524 μmol/L, 309.398 ± 80.394 μmol/L and 9.119 ± 4.425 μmol/L, respectively. The number of IVs varies from 11 to 243, explaining the 4.15% ~ 28.21% variance of corresponding exposure. General F statistics of all exposures and each selected SNP was greater than 10, suggesting that instrumental variables were valid and robust to be included in further MR analysis. The detailed information of all selected SNPs of six exposures was presented in Tables S1-S6.

The significant causal effect of serum cystatin C on DN with univariable MR

Among 6 serum biomarkers, only cystatin C has a significant causal effect on diabetic nephropathy as a risk factor (IVW OR: 1.19, 95%CI [1.04, 1.35], p=0.009) (Figure 2). The same causal direction was observed in MR-Egger, weighted median, and MR-PRESSO analysis (Figure S1). Hence, the risk of diabetic nephropathy would increase by 19% with per SD increase of cystatin C. Cochran's Q test of cystatin C indicates that there is evidence of heterogeneity (IVW $p<0.05$), while no indication of pleiotropy in MR-Egger (p for intercept>0.05) (Table S7). Multiplicative random effects IVW method returned a result resembled to IVW (OR: 1.19, 95%CI [1.04, 1.35], p=0.009) (Figure S1). Leave-one-out analysis indicated a similar result to Cochran's Q-test, suggesting some SNPs might influence the causal estimate. We further identified rs734801, a cystatin C gene (CST3), as a significant IV that could strongly affect the MR result from the leave-one-out analysis (Figure S2).

Although horizontal pleiotropy may exist with a $p<0.05$ as suggested with the global test of MR-PRESSO, the distortion test showed that there is no difference whether the pleiotropic outliers were removed or not (p=0.91). Therefore, we further removed all SNPs identified by MR-PRESSO which may cause horizontal pleiotropy (rs10200647, rs36207014, rs734801, rs77924615, rs80138475). The resulting data showed that cystatin C has a greater causal association with diabetic nephropathy (IVW OR: 1.36, 95%CI [1.15, 1.61], p=0.0004),

TABLE 1 A summary of GWAS summary statistics for six different serum biomarkers.

Exposures	Dataset source	Sample size	NSNP	R2(%)	F
Cystatin C	UK Biobank	361194	234	9.59	163.6084
Total bilirubin	UK Biobank	361194	99	28.21	1433.199
Urate	UK Biobank	361194	188	7.35	152.2644
Creatinine	UK Biobank	361194	243	4.15	64.37972
Kidney injury molecule 1 levels	SCALLOP	21,758	11	13.68	313.1804
Growth differentiation factor-15 levels	SCALLOP	21,758	18	5.67	72.59881

NSNP, the number of SNP included in the MR analysis; R2(%), the proportion of variance explained by included SNPs of each exposure; F, the general F statistic for each biomarker.

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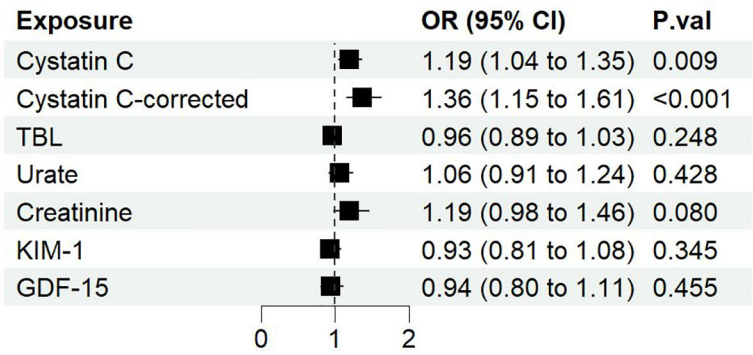

FIGURE 2
The forest plot of different serum biomarkers' effect on diabetic nephropathy. OR, odds ratio; CI, confidence interval; P.val, the p-value of IVW MR analysis.

and Cochran's Q-test no longer showed evidence of heterogeneity (IVW p>0.05) (Table S7). On the basis of the sample size of 213746 individuals (3283 cases and 210463 controls) and setting the explained variance of 4.76%, our study has 99% power to detect effect of serum cystatin C on DN with an OR of 1.36.

Association of rs734801 in the CST3 gene with cystatin C and DN

As mentioned previously, rs734801 of CST3 gene, which encoded the most plentiful extracellular inhibitor of cysteine proteases (26), strongly affect the MR result based on the leave-one-out analysis. We further examined rs734801, finding that it contributed most to the genetic control of serum cystatin C, explaining 4.7% of the variance. This SNP strongly associated with cystatin C (beta=- 0.37, $p<1E^{-200}$), but not with DN (OR =0.99, p=0.767) (Table S1).

The significant causal effect of serum cystatin C on DN with MVMR

Next, we performed multivariable MR to further analyze the direct effect of cystatin C on diabetic nephropathy. Through online searching IVs selected for serum cystatin C with Phenoscanner V2, we found several potential confounding phenotypes (e.g. BMI, SBP, DBP, log eGFR, hypertension, cholesterol). Finally, we chose BMI and SBP as adjusted confounders in the MR analysis for the following reasons: First, BMI and SBP were the most frequent phenotypes in the process of searching among all the potential confounding phenotypes. Second, BMI and SBP were reported to be risk factors in published MR analysis (27, 28). Subsequently, we implemented three rounds of MVMR: cystatin C against diabetic nephropathy adjusted for (1) BMI alone (2) SBP alone (3) BMI and SBP combined. It showed that cystatin C maintained its causal effect on the outcome no matter adjusted for BMI alone (OR: 1.17, 95%CI [1.03, 1.33], p=0.019), SBP alone (OR: 1.20, 95%CI [1.05, 1.37], p=0.009) or both (OR: 1.17, 95%CI [1.02, 1.33], p=0.02) (Figure 3).

BMI could be a mediator between cystatin C and DN

Next, we performed two-step MR to further investigate whether BMI, SBP functioned as mediator between cystatin C and DN. It showed that there was a casual association between cystatin C and BMI (β=0.05, 95%CI [0.01, 0.09], p=0.024). After adjusted for cystatin C, BMI showed detrimental effect on DN (OR: 1.70, 95%CI [1.39, 2.07], p<0.001). The proportion mediated by BMI was 15.2% (95%CI [4.93%, 22.6%] (Figure 4).

Although SBP was previously shown to be a risk factor of DN, we failed to make a conclusion that it played as a mediator between cystatin C and DN, since a causal relationship between cystatin C and SBP was not significant (p=0.81) (Figure 4).

Discussion

In the present study, we took advantage of MR analysis to thoroughly examine the causal association between six serum biomarkers (Cystatin C, KIM-1, GDF-15, TBIL, Urate, and Scr) and DN. Among the six biomarkers, only cystatin C positively associated with the risk of DN. There was no statistical significance for the genetic relationship among the other five biomarkers and DN.

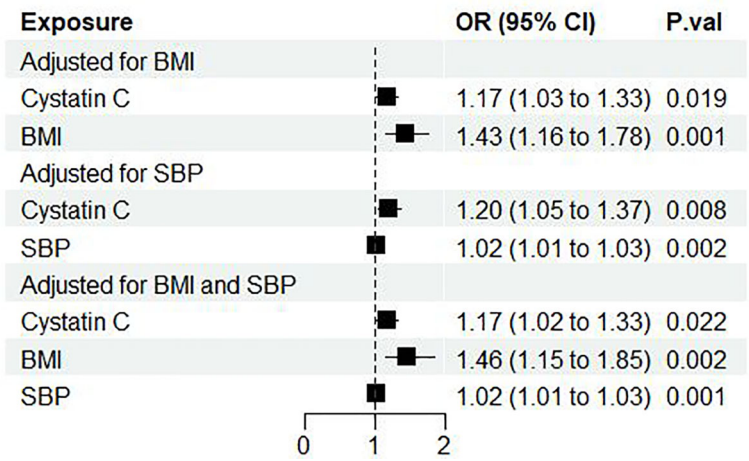

FIGURE 3
Forest plot of cystatin C's effect on diabetic nephropathy adjusted for BMI and/or SBP. OR, odds ratio; CI, confidence interval; P.val, the p-value of IVW MR analysis. BMI, body mass index; SBP, systolic blood pressure.

Several studies have shown that high levels of serum urate might cause CKD progression through a mechanism such as excessive production of nitric oxide, activating the renin-angiotensin system, stimulating the proliferation of vascular smooth muscle cell, and obstruction from urate crystals. However, a previous MR study showed that there is no causal relation between serum urate levels and CKD (29) and randomized, controlled trials (RCTs) consistently show that lowering serum urate with allopurinol treatment has no benefits on kidney outcomes among patients with early-to-moderate DN (30). Consistent with this, our results suggest that serum uric acid level didn't casually link to DN.

Although KIM-1, GDF-15, and TBIL were previously reported to contribute to tubular injury (31, 32) and predict the progression of CKD (4–8). Our data did not show the causally link of these molecules with DN. The reasons of these discrepancies might be as follows: First, the sample sizes reported in the previous studies are too small. Second, most of these studies are observational, which may not reveal a causal relationship but may arise reverse causality because of confounding factors. Third, the experimental results from animal studies may not fully translate into patients with CKD.

Cystatin C, a cysteine protease inhibitor, regulating the activity of cathepsins S and K, have multiple functions in human vascular pathophysiology (33), which is usually used as a measure for GFR. Elevated serum cystatin C routinely serves as an early and sensitive biomarker of impaired renal function (34). Interestingly, our data indicated that serum cystatin C is causally correlated with DN. Additionally, we found that a single SNP (rs734801) in the CST3 gene had a strong association with cystatin C. Besides, we performed another MR analysis excluding this SNP along with another four IVs (rs10200647, rs36207014, rs77924615, rs80138475) which were identified by MR-PRESSO, and obtained a more significant causal effect on

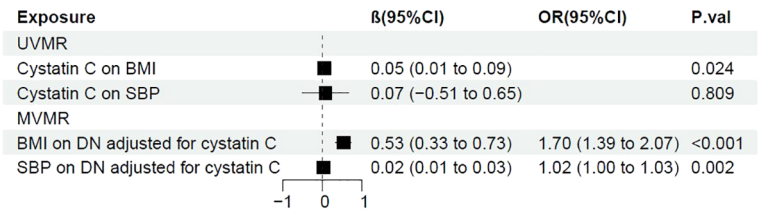

FIGURE 4
Forest plot of two-step MR with BMI and SBP. OR, odds ratio; CI, confidence interval; P.val, the p-value of IVW MR analysis. BMI, body mass index; SBP, systolic blood pressure.

DN. Consistent with us, several observational studies demonstrated that cystatin C levels are correlated with the prevalence of T2DM (35) and obesity in adolescents with age of 14-17 years independently of other confounding risk factors (36).

The mechanisms underlying the casual relationship between cystatin C and DN are unclear. There are several potential explanations. Firstly, cystatin C function as a cysteine proteinase inhibitor, which regulated the protease-antiprotease activities of the vascular wall. Thus, the imbalance between cystatin C and cysteine cathepsins might lead to the remodeling of the vascular wall (37). Second, cystatin C was previously reported to be involved in the amplification of cytokines and neuroinflammation in microglia and vascular endothelial injury (38). As inflammation is the hallmark of DN and endothelium injury plays a pivotal role in the occurrence and progression of DN, cystatin C may participate in in the pathological process of DN. Third, cystatin C promotes the proliferation of T cells and differentiation of T cells towards Th1/Th17 cell, which promotes the immune response (39). There is evidence that cystatin C is implicated in several inflammatory autoimmune diseases such as rheumatoid arthritis (40). Since both innate and adaptive immune systems and renal inflammation contribute to the development and progression of DN (41), cystatin C may promote inflammation in DN. However, whether elevated serum cystatin C results in DN progression through endothelium injury, remodeling of vascular wall, and the immune response requires further investigations.

Our study has some limitations: First, since the GWAS derives from European ancestry, generalizability to other ethnicities is limited. Second, due to lacking of individual-level data of GWAS, we cannot explicitly present the baseline data of participants or further stratify serum cystatin C to calculate more detailed causal effect. Third, despite we adjust potential pleiotropy, there is still chance for other confounders to influence the causal estimate. Hence, future research is warranted for further validating our findings.

Conclusions

Our results suggest that there was a causal relationship between serum cystatin C and DN in diabetic patients, which warns us that cystatin C is not only a biomarker bur also a risk factor for DN progression.

Data availability statement

Summary statistics are available from each consortium (details in Materials and methods) or via the MR-Base platform (https://gwas.mrcieu.ac.uk/).

Author contributions

BF and AC designed the study and drafted the manuscript. BF, YL, LY, LJY, YZ, and AC acquired the data, performed statistical analysis and manuscript revision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1043174/full#supplementary-material

References

1. Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: Challenges, progress, and possibilities. *Clin J Am Soc Nephrol* (2017) 12(12):2032–45. doi: 10.2215/CJN.11491116

2. Burrows NR, Zhang Y, Hora I, Pavkov ME, Sheff K, Imperatore G, et al. Sustained lower incidence of diabetes-related end-stage kidney disease among American indians and Alaska natives, blacks, and hispanics in the U.S., 2000-2016. *Diabetes Care* (2020) 43(9):2090–7. doi: 10.2337/dc20-0495

3. Lerma EV, Batuman V. *Diabetes and kidney disease. 2nd.* Cham: Springer International Publishing AG (2021).

4. Nowak N, Skupien J, Niewczas MA, Yamanouchi M, Major M, Croall S, et al. Increased plasma kidney injury molecule-1 suggests early progressive renal decline in non-proteinuric patients with type 1 diabetes. *Kidney Int* (2016) 89(2):459–67. doi: 10.1038/ki.2015.314

5. Sabbisetti VS, Waikar SS, Antoine DJ, Smiles A, Wang C, Ravisankar A, et al. Blood kidney injury molecule-1 is a biomarker of acute and chronic kidney injury and predicts progression to ESRD in type I diabetes. *J Am Soc Nephrol* (2014) 25 (10):2177–86. doi: 10.1681/ASN.2013070758

6. Hellemons ME, Mazagova M, Gansevoort RT, Henning RH, de Zeeuw D, Bakker SJ, et al. Growth-differentiation factor 15 predicts worsening of albuminuria in patients with type 2 diabetes. *Diabetes Care* (2012) 35(11):2340–6. doi: 10.2337/dc12-0180

7. Lajer M, Jorsal A, Tarnow L, Parving HH, Rossing P. Plasma growth differentiation factor-15 independently predicts all-cause and cardiovascular mortality as well as deterioration of kidney function in type 1 diabetic patients with nephropathy. *Diabetes Care* (2010) 33(7):1567–72. doi: 10.2337/dc09-2174

8. Zhang D, Zhu B, Zhang W, Wang W, Guo D, Yang L, et al. Total bilirubin level may be a biomarker of nephropathy in type 2 diabetes mellitus: A meta-analysis of observational studies based on MOOSE compliant. *Med (Baltimore)* (2017) 96(1):e5765. doi: 10.1097/MD.0000000000005765

9. Ji P, Zhu J, Feng J, Li H, Yu Q, Qin H, et al. Serum uric acid levels and diabetic kidney disease in patients with type 2 diabetes mellitus: A dose-response meta-analysis. *Prim Care Diabetes* (2022) 16(3):457–65. doi: 10.1016/j.pcd.2022.03.003

10. Wang N, Lu Z, Zhang W, Bai Y, Pei D, Li L. Serum cystatin c trajectory is a marker associated with diabetic kidney disease. *Front Endocrinol (Lausanne)* (2022) 13:824279. doi: 10.3389/fendo.2022.824279

11. Low S, Zhang X, Ang K, Yeo SJD, Lim GJ, Yeoh LY, et al. Discovery and validation of serum creatinine variability as novel biomarker for predicting onset of albuminuria in type 2 diabetes mellitus. *Diabetes Res Clin Pract* (2018) 138:8–15. doi: 10.1016/j.diabres.2017.11.003

12. Emdin CA, Khera AV, Kathiresan S. Mendelian randomization. *JAMA* (2017) 318(19):1925–6. doi: 10.1001/jama.2017.17219

13. Folkersen L, Gustafsson S, Wang Q, Hansen DH, Hedman AK, Schork A, et al. Genomic and drug target evaluation of 90 cardiovascular proteins in 30,931 individuals. *Nat Metab* (2020) 2(10):1135–48. doi: 10.1038/s42255-020-00287-2

14. Evangelou E, Warren HR, Mosen-Ansorena D, Mifsud B, Pazoki R, Gao H, et al. Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. *Nat Genet* (2018) 50(10):1412–25. doi: 10.1038/s41588-018-0205-x

15. Yengo L, Sidorenko J, Kemper KE, Zheng Z, Wood AR, Weedon MN, et al. Meta-analysis of genome-wide association studies for height and body mass index in approximately 700000 individuals of European ancestry. *Hum Mol Genet* (2018) 27(20):3641–9. doi: 10.1093/hmg/ddy271

16. Kurki MI, Karjalainen J, Palta P, Sipilä TP, Kristiansson K, Donner K, et al. FinnGen: Unique genetic insights from combining isolated population and national health register data. *medRxiv* (2022) 2022:2022.03.03.22271360. doi: 10.1101/2022.03.03.22271360

17. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med* (2008) 27(8):1133–63. doi: 10.1002/sim.3034

18. Pierce BL, Ahsan H, Vanderweele TJ. Power and instrument strength requirements for mendelian randomization studies using multiple genetic variants. *Int J Epidemiol* (2011) 40(3):740–52. doi: 10.1093/ije/dyq151

19. Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan N, Thompson J. A framework for the investigation of pleiotropy in two-sample summary data mendelian randomization. *Stat Med* (2017) 36(11):1783–802. doi: 10.1002/sim.7221

20. Burgess S, Thompson SG. Interpreting findings from mendelian randomization using the MR-egger method. *Eur J Epidemiol* (2017) 32(5):377–89. doi: 10.1007/s10654-017-0255-x

21. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from mendelian randomization between complex traits and diseases. *Nat Genet* (2018) 50(5):693–8. doi: 10.1038/s41588-018-0099-7

22. Burgess S, Davey Smith G, Davies NM, Dudbridge F, Gill D, Glymour MM, et al. Guidelines for performing mendelian randomization investigations. *Wellcome Open Res* (2019) 4:186. doi: 10.12688/wellcomeopenres.15555.1

23. Sanderson E. Multivariable mendelian randomization and mediation. *Cold Spring Harb Perspect Med* (2021) 11(2). doi: 10.1101/cshperspect.a038984

24. Relton CL, Davey Smith G. Two-step epigenetic mendelian randomization: A strategy for establishing the causal role of epigenetic processes in pathways to disease. *Int J Epidemiol* (2012) 41(1):161–76. doi: 10.1093/ije/dyr233

25. Brion MJ, Shakhbazov K, Visscher PM. Calculating statistical power in mendelian randomization studies. *Int J Epidemiol* (2013) 42(5):1497–501. doi: 10.1093/ije/dyt179

26. O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, et al. Reference sequence (RefSeq) database at NCBI: Current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res* (2016) 44(D1):D733–45. doi: 10.1093/nar/gkv1189

27. Hou T, Li M, Lin H, Zhao Z, Lu J, Wang T, et al. The causal effect of systolic blood pressure lowering on vascular outcomes in diabetes: A mendelian randomization study. *J Clin Endocrinol Metab* (2022) 107(9):2616–25. doi: 10.1210/clinem/dgac354

28. Todd JN, Dahlstrom EH, Salem RM, Sandholm N, Forsblom C, FinnDiane Study G, et al. Genetic evidence for a causal role of obesity in diabetic kidney disease. *Diabetes* (2015) 64(12):4238–46. doi: 10.2337/db15-0254

29. Jordan DM, Choi HK, Verbanck M, Topless R, Won HH, Nadkarni G, et al. No causal effects of serum urate levels on the risk of chronic kidney disease: A mendelian randomization study. *PloS Med* (2019) 16(1):e1002725. doi: 10.1371/journal.pmed.1002725

30. Doria A, Galecki AT, Spino C, Pop-Busui R, Cherney DZ, Lingvay I, et al. Serum urate lowering with allopurinol and kidney function in type 1 diabetes. *N Engl J Med* (2020) 382(26):2493–503. doi: 10.1056/NEJMoa1916624

31. Eardley KS, Zehnder D, Quinkler M, Lepenies J, Bates RL, Savage CO, et al. The relationship between albuminuria, MCP-1/CCL2, and interstitial macrophages in chronic kidney disease. *Kidney Int* (2006) 69(7):1189–97. doi: 10.1038/sj.ki.5000212

32. Mori Y, Ajay AK, Chang JH, Mou S, Zhao H, Kishi S, et al. KIM-1 mediates fatty acid uptake by renal tubular cells to promote progressive diabetic kidney disease. *Cell Metab* (2021) 33(5):1042-1061.e7. doi: 10.1016/j.cmet.2021.04.004

33. Amin F, Khan MS, Bano B. Mammalian cystatin and protagonists in brain diseases. *J Biomol Struct Dyn* (2020) 38(7):2171–96. doi: 10.1080/07391102.2019.1620636

34. Shlipak MG, Matsushita K, Arnlov J, Inker LA, Katz R, Polkinghorne KR, et al. Cystatin c versus creatinine in determining risk based on kidney function. *N Engl J Med* (2013) 369(10):932–43. doi: 10.1056/NEJMoa1214234

35. Sahakyan K, Lee KE, Shankar A, Klein R. Serum cystatin c and the incidence of type 2 diabetes mellitus. *Diabetologia* (2011) 54(6):1335–40. doi: 10.1007/s00125-011-2096-6

36. Huo YX, Wei W, Liu Y, Ma YN, Tao JM, Wang NN, et al. Serum cystatin c levels are associated with obesity in adolescents aged 14-17 years. *Front Endocrinol (Lausanne)* (2022) 13:816201. doi: 10.3389/fendo.2022.816201

37. Yao D, Li S, Jing J, Cai X, Jin A, Yang Y, et al. Association of serum cystatin c with cerebral small vessel disease in community-based population. *Stroke* (2022) 53 (10):3123–32. doi: 10.1161/STROKEAHA.122.039277

38. Liu Q, Zhang Y, Liu S, Liu Y, Yang X, Liu G, et al. Cathepsin c promotes microglia M1 polarization and aggravates neuroinflammation via activation of Ca (2+)-dependent PKC/p38MAPK/NF-kappaB pathway. *J Neuroinflamm* (2019) 16 (1):10. doi: 10.1186/s12974-019-1398-3

39. Zhang W, Zi M, Sun L, Wang F, Chen S, Zhao Y, et al. Cystatin c regulates major histocompatibility complex-II-peptide presentation and extracellular signal-regulated kinase-dependent polarizing cytokine production by bone marrow-derived dendritic cells. *Immunol Cell Biol* (2019) 97(10):916–30. doi: 10.1111/imcb.12290

40. Zi M, Xu Y. Involvement of cystatin c in immunity and apoptosis. *Immunol Lett* (2018) 196:80–90. doi: 10.1016/j.imlet.2018.01.006

41. Tang SCW, Yiu WH. Innate immunity in diabetic kidney disease. *Nat Rev Nephrol* (2020) 16(4):206–22. doi: 10.1038/s41581-019-0234-4

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Risk factors of chronic kidney disease among type 2 diabetic patients with longer duration of diabetes

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Background: Chronic kidney disease (CKD) in patients with type 2 diabetes mellitus (T2DM) is the major cause of end stage renal disease, characterized by proteinuria with a subsequent decline in glomerular filtration rate. Although hyperglycemia is the major risk factor for the development and progression of kidney disease among diabetic patients, many other risk factors also contribute to structural and functional changes in the kidneys. As recommended by Kidney Disease Improving Global Outcomes (KDIGO), CKD classification based on cause and severity, links to risk of adverse outcomes including mortality and kidney outcomes.

Objective: The aim of this study is to investigate the involvement of risk factors associated with the severity of CKD among participants with longer duration of diabetes. This study also aims to find whether number of risk factors vary among risk of CKD progression categories based on KDIGO classification.

Material and methods: This cross-sectional study retrospectively selected 424 participants from type 2 diabetic cohort and categorized them based on the classifications for the diagnosis of kidney diseases in patients with diabetes, according to the KDIGO guidelines. Odds ratios and 95% CI of each risk factors according to severity of renal disease were determined.

Results: Based on KDIGO classification, participants with type 2 diabetes (T2D) were categorized in to low risk (n=174); moderately increased risk (n=98); and high/very high risk (n=152). Type 2 diabetic participants with risk factors such as, hyperlipidemia, hypertension, DM duration ≥15 years and diabetic retinopathy showed a high/very high risk of CKD progression when compared with low-risk category. While T2D participants with risk factors such as, lack of exercise, hypertension, and diabetic retinopathy showed a moderately increased risk of CKD progression. In addition, participants with highest number of risk factors were significantly distributed among high/very high risk of CKD progression category.

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Conclusion: This study findings conclude that patients with T2DM and duration of ≥15 years, hyperlipidemia, hypertension and diabetic retinopathy have an increased prevalence of advanced CKD. In addition to this, increased number of risk factors could be an indicator of the severity of CKD in T2D.

KEYWORDS

diabetic kidney disease (DKD), diabetic nephropathy, longer duration of diabetes, risk factors, high prevalence of diabetes

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood glucose level and associated with number of complications including acute metabolic and long-term vascular complications. Type 2 diabetes mellitus (T2DM) is the most common type of diabetes worldwide (1). Chronic kidney disease (CKD) in patients with T2DM is the major cause of end stage renal disease, characterized by proteinuria with a subsequent decline in glomerular filtration rate (2). The great increase in the prevalence of diabetes, significant morbidity and mortality associated with kidney disease among patients with diabetes and burden of healthcare cost has led to research imperative in near future (1).

The development and progression of diabetic kidney disease (DKD) involves the complex interplay of many factors contributing to the structural and functional changes in the kidneys. Chronic hyperglycemia is a crucial factor responsible for the development of kidney disease in patients with DM and lead to progressive glomerular and tubular damage, negatively impacting on health outcomes. CKD in patients with DM is defined as elevated urinary albumin excretion or impaired renal function or both and approximately half of the patients with T2D develop renal diseases (3). KDIGO guidelines recommend that the combined assessment of eGFR and albuminuria status provide more accurate evaluation as being at low, moderately increased, high or very high risk of worsening kidney function (4).

Besides hyperglycemic condition, the combination of other factors including hypertension, dyslipidemia, genetic predisposition, obesity, lifestyle factors are also contributing to the development and progression of kidney disease among patients with diabetes. The prevention and management of CKD in patients with DM might depend on lifestyle modifications and pharmacological strategies such as combined targeted therapies for hyperglycemia, hypertension, albuminuria, hyperlipidemia, and regular use of reno protective agents, which might provide synergistic effect in controlling and minimizing the effect of these factors (5). Recently a joint group of American Diabetes Association (ADA) and KDIGO provide evidence-based recommendations and guidelines to improve clinical outcomes of people with diabetes and CKD which includes CKD screening and diagnosis, glycemia monitoring, lifestyle therapies, treatment goals and pharmacologic management (6). The impact of non-modifiable factors such as age, gender and duration of diabetes are also critical (3, 7).

We hypothesized that in high-risk population, the identification and efficient management of the modifiable risk factors may prevent or delay the development of CKD in patients with diabetes. In this population, there is a general lack of consensus regarding the non-modifiable and modifiable risk factors and comorbidities that are associated with risk of CKD in diabetes. This study aims to investigate the involvement of modifiable and non-modifiable risk factors and other comorbid conditions linked with severity of CKD in T2D cohort with longer duration of diabetes. This study also aims to examine whether number of risk factors vary among risk categories of CKD.

Materials and methods

Study population

The data of the study population were collected retrospectively from a previous cohort study conducted at University Diabetes Center, King Saud University Medical City (KSUMC), Riyadh, Saudi Arabia during the year 2014-15 (8). The study was approved by Institutional Review Board (IRB) at College of Medicine, King Saud University (IRB/E-19/3969) and was carried out in accordance with the declaration of Helsinki (8).

The inclusion criteria of this study were as follows: (1) Saudi nationals with T2DM (2) age between 35 and 70 years (3) greater than 10 years of diabetes duration. Exclusion criteria were as follows: (1) pregnant women (2) other causes of renal impairment which includes glomerulonephritis, interstitial nephropathy, vasculitis, malignant hypertension, pelvicalyceal infection, bilateral cortical necrosis, amyloidosis; (3) patients with abnormal liver function (4) patients who take the

medications that might affect kidney functions; and (5) end stage renal disease (ESRD) patients (6) patients with cancer.

Retrospectively selected 424 participants and categorized based on the classifications for the diagnosis of kidney diseases in patients with diabetes, according to KDIGO guidelines. The selected participants were subdivided into three groups according to the severity of risk, namely; low, moderately increased and high/very high risk. Six eGFR categories were included namely; G1, G2, G3a, G3b, G4 and G5 (G1 represents ≥90, G2 represents 60–89, G3a represents 45–59, G3b represents 30–44, G4 represents 15–29 and G5 represents <15 ml/min/1.73 m^2 of eGFR). Similarly, based on the values of ACR, three categories were included namely; A1, A2 and A3 (A1 represents <30 mg/g, A2 represents 30-300 mg/g and A3 represents >300 mg/g of ACR). Low-risk category comprises of G1A1 and G2A1; moderately increased risk category includes G1A2, G2A2 and G3aA1; and high/very high risk category comprises of G1A3, G2A3, G3aA2, G3bA1, G3aA3, G3bA2, G3bA3, G4A3, and G5A3 (9, 10) (Figure 1).

Risk factors of DKD were determined according to National Kidney Foundation guidelines and Rubeaan et al. (11, 12). Data of non-modifiable risk factors (age, gender, diabetes mellitus duration (DM duration)) and modifiable risk factors (glycemic control (fasting blood sugar (FBS), HbA1c), blood pressure (systolic blood pressure (SBP) and diastolic blood pressure (DBP)), lipids (total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol) obesity (body mass index (BMI), smoking, lack of physical activity) were collected from previous study records (8).

Diagnosis of T2D was based on the ADA criteria or reported to be taking treatment for diabetes (13). The diabetic neuropathy was evaluated by assessing upper and lower extremities of nerve conduction velocity. The presence of at least one definite microaneurysm in any field photographed was considered as the criterion for the diagnosis of diabetic retinopathy (14). Hypertension is diagnosed when blood pressure is consistently ≥130 and/or ≥80mm Hg or being managed with antihypertensive medications such as angiotensin II receptor antagonist, thiazide diuretic, angiotensin-converting enzyme inhibitor, beta blocker, and calcium channel blockers (15). CKD-EPI creatinine equation was used to calculate eGFR (16).

Statistical analysis was performed using statistical software, SPSS version 21.0 (IBM Corp., Armonk, NY, USA). The categorical data were summarized as percentage and the continuous data were presented as mean and standard deviation and the difference were analyzed using one-way ANOVA (analysis of variance). The categorical variables were analyzed by using Chi-square test. Odds ratio (OR) were used for association of risk factors with severity of kidney disease. OR was calculated using online statistical calculator (https://www.medcalc.org/calc/odds_ratio.php) and expressed in 95% confidence intervals and graphically represented in the form of forest plot. Low risk group was used as reference group to calculate the OR. The p-value was taken as significant at $p<0.05$.

Results

The selected 424 T2DM participants were subdivided in to three groups according to the severity of kidney diseases (low risk, n=174; moderately increased risk, n=98; high/very high risk, n= 152). The three groups were similar in BMI, DBP, HDL and LDL cholesterol whereas age, DM duration, SBP, glycemic parameters (FBS, HbA1c), and lipid parameters (total cholesterol and triglycerides) were significantly differed among three groups (Table 1).

Figure 2 shows the percentage of distribution of participants according to the number of risk factors and other complications among different risk categories based on KDIGO classification.

eGFR categories (ml/min/1.73m²) Description and range				Persistent albuminuria categories Description and range		
				A1 (Normal to mildly increased)	A2 (Moderately increased)	A3 (Severely increased)
				<30 mg/g	30-300 mg/g	>300 mg/g
	G1	Normal or high	≥ 90	65(15.3%)	0(0%)	0(0%)
	G2	Mildly decreased	60-89	109(25.7%)	76(17.9%)	19(4.5%)
	G3a	Mildly to moderately decreased	45-59	22(5.2%)	54(12.7%)	22(5.2%)
	G3b	Moderately to severely decreased	30-44	4(0.94%)	19(4.5%)	20(4.7%)
	G4	Severely decreased	15-29	0(0%)	0(0%)	9(2.1)
	G5	Kidney failure	<15	0(0%)	0(0%)	5(1.2)

Green: low risk; Yellow: moderately increased risk; Orange: high risk; Red: very high risk.

FIGURE 1
Distribution of participants according to the Kidney Disease Improving Global Outcomes (KDIGO) classification.

TABLE 1 Demographic and clinical characteristics of patients with type 2 diabetes according to KDIGO classification.

Parameters	Low risk (n=174)	Moderately increased risk (n=98)	High/very high risk (n=152)	p value
Age (years)	54 ± 6.0	55.9 ± 6.0	55.9 ± 6.4	0.01
Gender (male, n %)	74 (42.5)	47 (48)	68 (44.7)	0.68
DM duration (years)	16.8 ± 4.5	18.9 ± 6.0	19.5 ± 5.9	<0.001
Hypertension, n (%)	95 (54.6)	75 (76.5)	128 (84.2)	<0.000
Hyperlipidemia, n (%)	139 (79.9)	84 (86.6)	141 (92.8)	0.004
BMI (kg/m^2)	32.7 ± 5.7	31.7 ± 5.1	32.3 ± 5.9	0.38
SBP (mm Hg)	130.4 ± 16.9	138.6 ± 18.0	143.6 ± 20.9	<0.001
DBP (mm Hg)	72.6 ± 10.0	75.2 ± 10.6	74.3 ± 11.7	0.12
FBS (mg/dl)	187.8 ± 74.2	228.5 ± 94.8	243.1 ± 103	<0.001
HbA1c (%)	10.0 ± 1.3	10.5 ± 1.5	10.9 ± 2.1	<0.001
Total cholesterol (mg/dl)	172.5 ± 37	182.7 ± 44.0	198.6 ± 54.2	<0.001
Triglyceride (mg/dl)	151.2 ± 61.0	186.5 ± 89.7	208.7 ± 94	<0.001
HDL cholesterol (mg/dl)	44.8 ± 10.5	46.7 ± 12.1	47.4 ± 13.5	0.13
LDL cholesterol (mg/dl)	130.8 ± 40.6	134 ± 40.5	138.2 ± 50.9	0.32

Data represents in mean ± standard deviation and percentage. DM duration (diabetes mellitus duration), BMI (body mass index), SBP(systolic blood pressure), DBP(diastolic blood pressure), FBS (fasting blood sugar), HDL cholesterol (high density lipoprotein cholesterol), LDL cholesterol (low density lipoprotein cholesterol), P value <0.05 is statistically significant.

Among all risk categories, participants with highest number of risk factors (n= >6) were significantly distributed among high/very high risk category. The percentage of distribution of number of risk factors and other complications (n= >6) in low risk category was 50% while moderately increased risk and high/very high risk categories showed 66.3% and 76.4% respectively (p=<0.001).

Risk factors of DKD associated with the severity of kidney disease

Table 2 and Figure 3 show the odds ratios of risk factors associated with the severity of kidney disease among T2D. In non-modifiable risk factors, DM duration ≥15 years was found to increase the odds of 1.75 in high/very high risk category (p=0.026). Furthermore, in high/very high risk category, presence of hyperlipidemia and hypertension showed an increase in odds of three times (OR=3.22, CI 95% 1.57-6.61); p=0.001) and four times (OR=4.43, CI 95% 2.61-7.52); p=<0.0001) respectively. In addition, presence of diabetic retinopathy remained significant with a higher OR of 4.5 (CI 95% 2.82-7.18); p=<0.0001) in high/very high risk category. In moderately increased risk category, lack of exercise (2.13, CI 95% 1.06-4.30); p=0.033), comorbidities; hypertension (OR=2.71, CI 95% 1.55-4.72); p=0.004), microvascular complication; presence of diabetic retinopathy (OR=2.51, CI 95% 1.50-4.19; p=0.004)

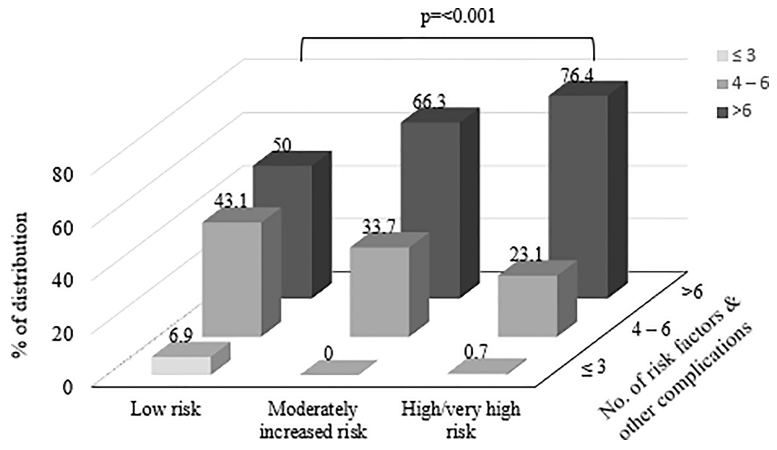

FIGURE 2
Shows the percentage of distribution of participants according to the number of risk factors and other complications among different risk categories based on Kidney Disease Improving Global Outcomes (KDIGO) classification.

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TABLE 2 Odds ratios of risk factors associated with the severity of kidney disease among patients with type 2 diabetes.

Parameters	Low risk (n=174)	Moderately increased risk (n=98)	OR (95% CI)	p value	Low risk (n=174)	High/very high risk (n=152)	OR (95% CI)	p value
	n (%)	n (%)			n (%)	n (%)		
Non-modifiable risk factors								
Age, >45 (years)	156 (89.7)	91 (92.9)	1.50 (0.60-3.72)	0.382	156 (89.7)	142 (93.4)	1.63 (0.73-3.66)	0.229
≤45 (years)	18 (10.3)	7 (7.1)	1.00		18 (10.3)	10 (6.6)	1.00	
Gender, Male	74 (42.5)	47 (48)	1.24 (0.75-2.04)	0.387	74 (42.5)	68 (44.7)	1.09 (0.70-1.69)	0.688
Female	100 (57.5)	51 (52)	1.00		100 (57.5)	84 (55.3)	1.00	
DM duration ≥15 (years)	117 (67.2)	73 (74.5)	1.42 (0.81-2.47)	0.212	117 (67.2)	119 (78.3)	1.75 (1.06-2.89)	0.026
<15 (years)	57 (32.8)	25 (25.5)	1.00		57 (32.8)	33 (21.7)	1.00	
Modifiable risk factors								
Obese, Yes	108 (62.4)	56 (57.7)	0.82 (0.49-1.36)	0.448	108 (62.4)	89 (61.0)	0.93 (0.59-1.47)	0.788
No	65 (37.6)	41 (42.3)	1.00		65 (37.6)	57 (39.0)	1.00	
HbA1c, >8 (%)	158 (91.3)	91 (94.8)	1.72 (0.60-4.91)	0.304	158 (91.3)	144 (94.7)	1.70 (0.70-4.15)	0.236
<8 (%)	15 (8.7)	5 (5.2)	1.00		15 (8.7)	8 (5.3)	1.00	
Exercise, No	134 (77.0)	86 (87.8)	2.13 (1.06-4.30)	0.033	134 (77.0)	123 (80.9)	1.26 (0.74-2.16)	0.389
Yes	40 (23.0)	12 (12.2)	1.00		40 (23.0)	29 (19.1)	1.00	
Smoking, Yes	4 (2.3)	3 (3.1)	1.34 (0.29-6.12)	0.704	4 (2.3)	6 (3.9)	1.74 (0.48-6.30)	0.394
No	170 (97.7)	95 (96.9)	1.00		170 (97.7)	146 (96.1)	1.00	
Comorbidities								
Hyperlipidemia, Yes	139 (79.9)	84 (86.6)	1.62 (0.81-3.24)	0.167	139 (79.9)	141 (92.8)	3.22 (1.57-6.61)	0.001
No	35 (20.1)	13 (13.4)	1.00		35 (20.1)	11 (7.2)	1.00	
Hypertension, Yes	95 (54.6)	75 (76.5)	2.71 (1.55-4.72)	0.004	95 (54.6)	128 (84.2)	4.43 (2.61-7.52)	<0.0001
No	79 (45.4)	23 (23.5)	1.00		79 (45.4)	24 (15.8)	1.00	
Microvascular complications								
Diabetic neuropathy, Yes	84 (48.3)	48 (49)	1.02 (0.62-1.68)	0.911	84 (48.3)	84 (55.3)	1.32 (0.85-2.04)	0.208
No	90 (51.7)	50 (51.0)	1.00		90 (51.7)	68 (44.7)	1.00	
Diabetic retinopathy, Yes	51 (29.3)	50 (51.0)	2.51 (1.50-4.19)	0.004	51 (29.3)	99 (65.1)	4.50 (2.82-7.18)	<0.0001
No	123 (70.7)	48 (49.0)	1.00		123 (70.7)	53 (34.9)	1.00	

Low risk vs moderately increased risk and low risk vs high/very high risk.

had 2 times higher odds than low risk category. Diabetic retinopathy showed an increase in odds of 1.79 (p=0.02) in high/very high risk category than moderately increased risk group (Supplementary Table 1).

Discussion

In this study, according to KDIGO classification, T2D participants with risk factors such as, hyperlipidemia, hypertension, DM duration ≥15 years and diabetic retinopathy showed a high/very high risk of CKD progression when compared with low risk category. While T2D participants with risk factors including, lack of exercise, hypertension, and diabetic retinopathy showed a moderately increased risk of CKD progression. In addition, participants with highest number of risk factors were significantly distributed among high/very high risk of CKD progression category.

T2D is characterized by persistent glycemia associated with insulin deficiency and insulin resistance. The pathogenesis of hyperglycemia is recognized in several organs such as liver, adipose tissue, intestine, kidney, central nervous system etc. (17, 18). The metabolic abnormalities in DKD includes glomerular hyper filtration, progressive albuminuria, declining glomerular filtration rate and eventually end stage renal disease. The metabolic changes associated with diabetes may alter kidney hemodynamics and promote glomerular hyperfiltration and albuminuria (19).

Progression of kidney disease is accelerated by a variety of modifiable and non-modifiable risk factors and associated

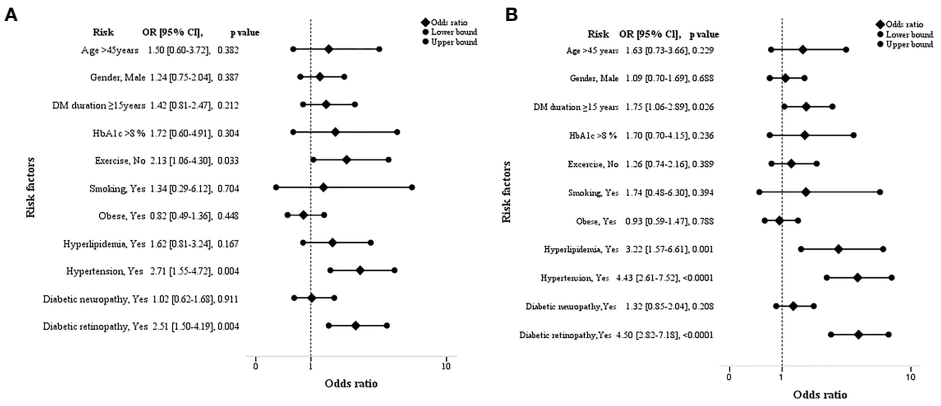

FIGURE 3
Forest plots showing the odds ratios of risk factors associated with the severity of kidney disease among type 2 diabetes. [low vs moderately increased risk **(A)** and low vs high/very high risk group **(B)**].

comorbidities and unhealthy life styles such as, physical inactivity, uncontrolled blood pressure and blood glucose (20–22). The pathogenesis of the long-term persistent hyperglycemia has been associated with functional and structural changes in renal cells (23). A notable finding in this study is that among non-modifiable risk factors, only DM duration ≥15 years revealed a 1.75-fold increase in high/very high risk of CKD progression than low risk category. In a Saudi registry study, the prevalence of diabetic nephropathy was found to be increasing with duration of diabetes and the highest was reported in the duration >15 years (12). In a recent study, an adjusted hazard ratio showed that the patients with duration of diabetes <10 years had reduced the risk of diabetic nephropathy than those having longer duration of >10 years (24).

Hyperlipidemia has been implicated in the pathogenesis of CKD in diabetes. In this cohort high/very high risk category showed a three-fold increase in risk for hyperlipidemia than low risk category. A prospective cohort study of patients with T2D for a period of 5.8 years showed that elevated lipid levels were associated with increased development of albumin creatinine ratio (25). Furthermore, hypertension is widely known as important independent risk factor for CKD in diabetes (26, 27). This study reported a significant increased risk for hypertension among moderately increased category and high/very high risk category. In a previous study, hypertensive patients were found to be at a higher risk of developing DKD as compared to non-hypertensive subjects with an increase in odds of 1.67 (28).

It is well known that the prevalence of CKD and diabetic retinopathy increase proportionally to the duration of disease among T2D (29). This study reported a significant two-fold increased risk for diabetic retinopathy among moderately increased risk category whereas in high/very high risk category it was four-fold. This finding is in line with various population studies where it was reported that diabetic retinopathy is a known risk factors for diabetic nephropathy (12, 30, 31).

In general, physical activity would be effective in patients with diabetes which improves insulin sensitivity, endothelial dysfunction, cellular senescence and interstitial fibrosis which may cause end stage renal damage and renal dysfunction in diabetes (32–35). In this cohort, a significant two-fold increase in risk for lack of exercise was observed among moderately increased risk category than low risk. In an earlier study it was reported that CKD patients with T2D improved kidney function by 6-12% after twelve weeks exercise program (36).

In this study, the increase in number of risk factors (modifiable/non-modifiable) and other co-morbidities were linked with severity of CKD in T2D. In previous observations, risk factors such as family history of DKD, cigarette smoking, uncontrolled blood pressure, presence of low-grade inflammation, advanced glycation end products, lack of physical activity and hyperlipidemia had an influence on the progression to kidney disease in diabetes and combination of these risk factors have been identified as the ones that offer the greatest risk of development and progression of DKD (11, 37).

These study findings highlight the importance of using KDIGO classification to define CKD among patients with diabetes. Furthermore, in clinical practice, regular risk factor assessment could reduce the risk of kidney disease progression and cardiovascular disease. By implementing life style modifications, glycemic monitoring and pharmacological management may help to improve clinical outcomes of people with diabetes and CKD.

The limitations to this study include a cross-sectional-not longitudinal- analysis which impedes any contributing association between CKD and its risk factors. In addition, relatively small sample size and lack of up-to-date data on drugs made it difficult to determine its effect on clinical outcome.

Conclusion

This study findings concludes that duration of diabetes ≥ 15 years, hyperlipidemia, hypertension and diabetic retinopathy have an increased prevalence of advanced CKD in T2DM. In addition to this, increased number risk factors could be an indicator for progression of CKD in T2D. Therefore, in high risk population, implementing screening of known risk factors at outset of disease may help to initiate treatment strategies at early phase and prevent or delay the progression of disease. Further studies in larger population are needed to determine the effect of these risk factors and complications in the progression of renal disease in T2D.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Board (IRB) at College of Medicine, King Saud University (IRB/E-19/3969). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1079725/full#supplementary-material

References

1. International Diabetes Federation. *IDF diabetes atlas* (2021). Brussels, Belgium. Available at: https://www.diabetesatlas.org (Accessed April 21, 2022).

2. Gheith O, Farouk N, Nampoory N, Halim MA, Al-Otaibi T. Diabetic kidney disease: world wide difference of prevalence and risk factors. *J Nephropharmacol* (2015) 5(1):49–56. Available at: http://jnephropharmacology.com/Article/NPJ_20160131181910.

3. Thomas MC, Brownlee M, Susztak K, Sharma K, Jandeleit-Dahm KA, Zoungas S, et al. Diabetic kidney disease. *Nat Rev Dis Primers* (2015) 1:15018. doi: 10.1038/nrdp.2015.18

4. Levin A, Stevens PE, Bilous RW, Coresh J, De Francisco AL, De Jong PE, et al. Kidney disease: Improving global outcomes (KDIGO) CKD work group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Suppl* (2013) 3(1):1–150.

5. Woodhams L, Sim TF, Chalmers L, Yeap B, Green D, Schlaich M, et al. Diabetic kidney disease in type 2 diabetes: A review of pathogenic mechanisms, patient-related factors and therapeutic options. *PeerJ* (2021) 9:e11070. doi: 10.7717/peerj.11070

6. de Boer IH, Khunti K, Sadusky T, Tuttle KR, Neumiller JJ, Rhee CM, et al. Diabetes management in chronic kidney disease: A consensus report by the American diabetes association (ADA) and kidney disease: Improving global outcomes (KDIGO). *Diabetes Care* (2022) 3:dci220027. doi: 10.2337/dci22-0027

7. Roy S, Schweiker-Kahn O, Jafry B, Masel-Miller R, Raju RS, O'Neill LMO, et al. Risk factors and comorbidities associated with diabetic kidney disease. *J Prim*

Care Community Health (2021) 12:21501327211048556. doi: 10.1177/21501327211048556

8. Al-Rubeaan K, Siddiqui K, Alghonaim M, Youssef AM, AlNaqeb D. The Saudi diabetic kidney disease study (Saudi-DKD): Clinical characteristics and biochemical parameters. *Ann Saudi Med* (2018) 38(1):46–56. doi: 10.5144/0256-4947.2018.03.01.1010

9. International Society of Nephrology. Chapter 1: Definition and classification of CKD. *Kidney Int Suppl* (2013) 3(1):19–62. doi: 10.1038/kisup.2012.64

10. Ketteler M, Block GA, Evenepoel P, Fukagawa M, Herzog CA, McCann L, et al. Diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder: Synopsis of the kidney disease: Improving global outcomes 2017 clinical practice guideline update. *Ann Intern Med* (2018) 168 (6):422–30. doi: 10.7326/M17-2640

11. Harjutsalo V, Groop PH. Epidemiology and risk factors for diabetic kidney disease. *Adv Chronic Kidney Dis* (2014) 21(3):260–6. doi: 10.1053/j.ackd.2014.03.009

12. Al-Rubeaan K, Youssef AM, Subhani SN, Ahmad NA, Al-Sharqawi AH, Al-Mutlaq HM, et al. Diabetic nephropathy and its risk factors in a society with a type 2 diabetes epidemic: A Saudi national diabetes registry-based study. *PloS One* (2014) 9(2):e88956. doi: 10.1371/journal.pone.0088956

13. American Diabetes Association. Classification and diagnosis of diabetes. *Diabetes Care* (2015) 38:S8–S16. doi: 10.2337/dc15-S005

14. Early Treatment of Diabetic Retinopathy study research group. Grading diabetic retinopathy from stereoscopic colour fundus photographs-an extension of the modified airlie house classification. ETDRS report number 10. *Ophthalmology* (1991) 98:786–806. doi: 10.1016/S0161-6420(13)38012-9

15. Flack JM, Adekola B. Blood pressure and the new ACC/AHA hypertension guidelines. *Treands Cardiovasc Med* (2020) 30(3):160–4. doi: 10.1016/j.tcm.2019.05.003

16. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med* (2009) 150(9):604–12. doi: 10.7326/0003-4819-150-9-200905050-00006

17. De Fronzo R. From the triumvirate to the ominous octet: A new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes* (2009) 58:773–95. doi: 10.2337/db09-9028

18. Pecoits-Filho R, Abensur H, Betônico CC, Machado AD, Parente EB, Queiroz M, et al. Interactions between kidney disease and diabetes: Dangerous liaisons. *Diabetol Metab Syndr* (2016) 8:50. doi: 10.1186/s13098-016-0159-z

19. Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: Challenges, progress, and possibilities. *Clin J Am Soc Nephrol* (2017) 12(12):2032–45. doi: 10.2215/CJN.11491116

20. Kelly JT, Su G, Carrero JJ. Lifestyle interventions for preventing and ameliorating CKD in primary and secondary care. *Curr Opin Nephrol Hypertens* (2021) 30(6):538–46. doi: 10.1097/MNH.0000000000000745

21. Mahmoodi BK, Matsushita K, Woodward M, Blankestijn PJ, Cirillo M, Ohkubo T, et al. Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without hypertension: A meta-analysis. *Lancet* (2012) 380:1649–6. doi: 10.1016/S0140-6736(12)61272-0

22. Wang TJ, Evans JC, Meigs JB, Rifai N, Fox CS, D'Agostino RB, et al. Low-grade albuminuria and the risks of hypertension and blood pressure progression. *Circulation.* (2005) 111:1370–6. doi: 10.1161/01.CIR.0000158434.69180.2D

23. Amorim RG, Guedes GDS, Vasconcelos SML, Santos JCF. Kidney disease in diabetes mellitus: Cross-linking between hyperglycemia, redox imbalance and inflammation. *Arq Bras Cardiol* (2019) 112(5):577–87. doi: 10.5935/abc.20190077

24. Kebede SA, Tusa BS, Weldesenbet AB, Tessema ZT, Ayele TA. Incidence of diabetic nephropathy and its predictors among type 2 diabetes mellitus patients at university of gondar comprehensive specialized hospital, Northwest Ethiopia. *J Nutr Metab*, 2021:6757916. doi: 10.1155/2021/6757916

25. Gall MA, Hougaard P, Borch-Johnsen K, Parving HH. Risk factors for development of incipient and overt diabetic nephropathy in patients with non-insulin dependent diabetes mellitus: Prospective, observational study. *BMJ.* (1997) 314(7083):783–8. doi: 10.1136/bmj.314.7083.783

26. Tapp RJ, Shaw JE, Zimmet PZ, Balkau B, Chadban SJ, Tonkin AM, et al. Albuminuria is evident in the early stages of diabetes onset: Results from the Australian diabetes, obesity, and lifestyle study (AusDiab). *Am J Kidney Dis* (2004) 44(5):792–8. doi: 10.1016/S0272-6386(04)01079-0

27. Hovind P, Tarnow L, Rossing P, Jensen BR, Graae M, Torp I, et al. Predictors for the development of microalbuminuria and macroalbuminuria in patients with type 1 diabetes: Inception cohort study. *BMJ* (2004) 328(7448):1105. doi: 10.1136/bmj.38070.450891.FE

28. Wagnew F, Eshetie S, Kibret GD, Zegeye A, Dessie G, Mulugeta H, et al. Diabetic nephropathy and hypertension in diabetes patients of sub-Saharan countries: A systematic review and meta-analysis. *BMC Res Notes* (2018) 11 (1):565. doi: 10.1186/s13104-018-3670-5

29. Adler AI, Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR, et al. Development and progression of nephropathy in type 2 diabetes: The united kingdom prospective diabetes study (UKPDS 64). *Kidney Int* (2003) 63(1):225–32. doi: 10.1046/j.1523-1755.2003.00712.x

30. Shen FC, Chen CY, Su SC, Liu RT. The prevalence and risk factors of diabetic nephropathy in Taiwanese type 2 diabetes - a hospital based study. *ActaNephrologica* (2009) 23(2):90–5.

31. Farah RI, Al-Sabbagh MQ, Momani MS, Albtoosh A, Arabiat M, Abdulraheem AM, et al. Diabetic kidney disease in patients with type 2 diabetes mellitus: A cross-sectional study. *BMC Nephrol* (2021) 22(1):1–8. doi: 10.1186/s12882-021-02429-4

32. Agarwal R, Light RP. Physical activity and hemodynamic reactivity in chronic kidney disease. *Clin J Am Soc Nephrol* (2008) 3(6):1660–8. doi: 10.2215/CJN.02920608

33. Bowlby W, Zelnick LR, Henry C, Himmelfarb J, Kahn SE, Kestenbaum B, et al. Physical activity and metabolic health in chronic kidney disease: A cross-sectional study. *BMC Nephrol* (2016) 17(1):187. doi: 10.1186/s12882-016-0400-x

34. Bai Y, Sigala W, Adams GR, Vaziri ND. Effect of exercise on cardiac tissue oxidative and inflammatory mediators in chronic kidney disease. *Am J Nephrol* (2009) 29(3):213–21. doi: 10.1159/000156715

35. Werner C, Fürster T, Widmann T, Pöss J, Roggia C, Hanhoun M, et al. Physical exercise prevents cellular senescence in circulating leukocytes and in the vessel wall. *Circulation.* (2009) 120(24):2438–47. doi: 10.1161/CIRCULATIONAHA.109.861005

36. Nylen ES, Gandhi SM, Kheirbek R, Kokkinos P. Enhanced fitness and renal function in type 2 diabetes. *Diabetes Med* (2015) 32(10):1342–5. doi: 10.1111/dme.12789

37. Macisaac RJ, Ekinci EI, Jerums G. Markers and risk factors for the development and progression of diabetic kidney disease. *Am J Kidney Dis* (2014) 63(2):S39–62. doi: 10.1053/j.ajkd.2013.10.048

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Predictive nomogram model for major adverse kidney events within 30 days in sepsis patients with type 2 diabetes mellitus

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Background: In sepsis patients, Type 2 Diabetes Mellitus (T2DM) was associated with an increased risk of kidney injury. Furthermore, kidney damage is among the dangerous complications, with a high mortality rate in sepsis patients. However, the underlying predictive model on the prediction of major adverse kidney events within 30 days (MAKE30) in sepsis patients with T2DM has not been reported by any study.

Methods: A total of 406 sepsis patients with T2DM were retrospectively enrolled and divided into a non-MAKE30 group (261 cases) and a MAKE30 group (145 cases). In sepsis patients with T2DM, univariate and multivariate logistic regression analyses were conducted to identify independent predictors of MAKE30. Based on the findings of multivariate logistic regression analysis, the corresponding nomogram was constructed. The nomogram was evaluated using the calibration curve, Receiver Operating Characteristic (ROC) curve, and decision curve analysis. A composite of death, new Renal Replacement Therapy (RRT), or Persistent Renal Dysfunction (PRD) comprised MAKE30. Finally, subgroup analyses of the nomogram for 30-day mortality, new RRT, and PRD were performed.

Results: In sepsis patients with T2DM, Mean Arterial Pressure (MAP), Platelet (PLT), cystatin C, High-Density Lipoprotein (HDL), and apolipoprotein E (apoE) were independent predictors for MAKE30. According to the ROC curve, calibration curve, and decision curve analysis, the nomogram model based on those predictors had satisfactory discrimination (AUC = 0.916), good calibration, and clinical application. Additionally, in sepsis patients with T2DM, the nomogram model exhibited a high ability to predict the occurrence of 30-day mortality (AUC = 0.822), new RRT (AUC = 0.874), and PRD (AUC = 0.801).

Conclusion: The nomogram model, which is available within 24 hours after admission, had a robust and accurate assessment for the MAKE30 occurrence, and it provided information to better manage sepsis patients with T2DM.

KEYWORDS

sepsis, type 2 diabetes mellitus, major adverse kidney events within 30 days, nomogram model, early warning

Introduction

Sepsis is a life-threatening organ dysfunction due to a dysregulated host response to infection induced by bacterial, viral, or fungal infection (1). Sepsis is currently the leading cause of mortality for patients, accounting for over 10% of the mortality within hospitals (1, 2). Furthermore, sepsis is associated with a mortality rate of 25–30% (3). It was reported that between 17–20% of the patients with sepsis had diabetes mellitus (4, 5). With the wide adoption of Western food and lifestyles, it is projected that the prevalence of T2DM will exceed 700 million worldwide and may soon reach pandemic levels (6). Due to immune system dysfunction, diabetic patients have an increased propensity to develop infections and are at a higher risk for sepsis (2 - 6 times) (7, 8).

Additionally, sepsis is particularly harmful to the kidney, which makes sepsis-induced acute kidney injury (S-AKI) a risk factor for increased mortality rate (9). Moreover, a growing body of research has revealed that T2DM was associated with an increased risk of S-AKI, but did not increase the overall mortality of sepsis patients (5, 8, 10). The renal damage may be due to increased activation of NF-kappa B, TGF-β and oxidant levels as a result of consistent hyperglycaemia milieu, or it may result from end-organ damage by atherosclerosis (8). Nevertheless, S-AKI cannot be used to assess clinically major renal adverse events such as death, and dialysis dependency, among others. The National Institute of Diabetes and Digestive and Kidney Diseases workgroup on clinical trials in Acute Kidney Injury (AKI) recommended the use of MAKE30 as an endpoint in 2012 (11). Effects of the short-term or longer-term evolution of AKI were captured by MAKE30, a composite of death, new RRT, or PRD (11). Furthermore, MAKE30 was a composite and objective clinical outcome measure for sepsis patients that reflected comprehensive renal outcomes better than just a single complication, AKI. Therefore, early MAKE30 prediction and prompt personalized management may enhance the clinical prognosis.

It is crucial to select appropriate endpoints in clinical trials. Although there is consensus that serum creatinine and urine volume are employed as AKI predictors, the serum creatinine and urine volume have a significant variation until 50% of renal function is lost, indicating that none of them are particularly meaningful to patients. In addition, researchers are beginning to understand that patient-centered outcome, such as mortality, dialysis, and chronic kidney disease development, are more important for patients. With the help of MAKE30, it is possible to detect short-term effects on AKI, improve target therapy, and facilitate the conduct of clinical trials. The MAKE30 has been used as an adequate test endpoint in several clinical trials including the SALT, SMART, and pediatric sepsis trials (12–14). There are no trustworthy or reliable prediction models, however, to identify MAKE30 in sepsis patients with T2DM. We hypothesized that a nomogram model, based on routine biomarkers available within 24 hours of admission, may be of significant clinical value to predict MAKE30 because conventional biochemical indications are intrinsically unstable when used as a single index. To identify high-risk individuals likely to develop MAKE30 in sepsis patients with T2DM, the goal of our study was to identify the risk variables for MAKE30 and build an early prediction model.

Materials and methods

Study design

Between January 2015 and December 2021, 406 sepsis patients with T2DM participated in a retrospective cohort study (project number: 81773128), and anonymized clinical data are obtained from the Biobank of First Affiliated Hospital of Xi'an Jiaotong University (Xi'an, China).

Patients

All sepsis patients with T2DM (18 years old and above) were evaluated for study enrollment. The sepsis 3.0 criteria were used for sepsis diagnosis (1). Furthermore, T2DM patients were identified if one of the following conditions was met: (1) self-reported diagnosis of T2D, (2) fasting plasma glucose (FPG) ≥7.0 mmol/L or (3) having received T2DM medications according to the 2022 American Diabetes Association (ADA) criteria (15). The following were the exclusion criteria: (1) below 18 years old; (2)

hospitalization less than 24 hours; (3) A history of chronic kidney disease (stage 4-5) or renal transplantation, or current hemodialysis; (4) Hematological disorders. Participants were then divided into MAKE30 group and non-MAKE30 group based on their diagnostic criteria (16). Any one of the following criteria can be used to diagnose MAKE30 composite endpoints: (1) death; (2) receiving RRT for the first time; (3) a PRD (defined as a final inpatient serum creatinine value greater than or equal to 200% of baseline). These three components of MAKE30 were removed 30 days after inclusion or at hospital release, whichever came first. Furthermore, baseline serum creatinine was determined as follows: (1) If available, the lowest value was measured between 12 months and 24 hours prior to hospitalization; (2) When measured values were unavailable, an estimate was made using the formula previously described [creatinine(μmol/l)= 88.4 × (0.74 − 0.2 + 0.003 × age) in females, and creatinine (μmol/l) = 88.4 × (0.74 + 0.003 × age) in males] (16, 17).

Data collection

The general information immediately available within 24 hours after admission included age, gender, temperature, Heart Rate (HR), Respiratory Rate (RR), MAP, source of infection, and Sequential Organ Failure Assessment (SOFA). The level of White Blood Cells (WBC), Neutrophil Percentage (NEUT%), lymphocyte, monocyte, PLT, Procalcitonin (PCT), Prothrombin Time Activity (PTA), Thrombin Time (TT), International Normalized Ratio (INR), Fibrinogen Degradation Products (FDP), D-Dimer (D-D), Fibrinogen (FIB), Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT), Globin (GLB); Albumin (ALB); Total Bilirubin (TBiL); Lipoprotein(a) (Lp(a)); apoE, apolipoprotein B (apoB), apolipoprotein A (apoA), Low-Density Lipoprotein (LDL), HDL, Triglycerides (TGs), Total Cholesterol (TC), urinary glucose, Uric Acid (UA), cystatin C, Blood Urea Nitrogen (BUN), Creatinine (Cr), and Total Cholesterol (TC) were also recorded within 24 hours after admission.

Statistical analysis

Mean ± standard deviation was used to express the continuous variables that conformed to the normal distribution, median (interquartile range) was used to express non-normally distributed continuous variables, and categorical variables were expressed by the percentages. To further identify the independent predictors of MAKE30, univariate and multivariate logistic regression models were used. The corresponding nomogram was constructed using the output from multivariate logistic regression analysis, and then we constructed an online dynamic nomogram using the "DynNom" package. The ROC curves were used to evaluate the accuracy of independent predictors of MAKE30. The calibration curves were drawn to assess the consistency of the observed results and predicted probability. Decision Curve Analysis (DCA) was performed to assess the clinical net benefit of the predictive model. Finally, an analysis of the secondary outcomes using ROC curves was performed to assess the discrimination of the nomogram for 30-day mortality.

SPSS 26.0 software and R version 4.1.2 were used for the statistical analysis, at $P < 0.05$.

Results

Basic characteristics

In the final analyses, 406 sepsis patients with T2DM in total were selected (Figure 1). Of the 406, 145 (35.7%) patients

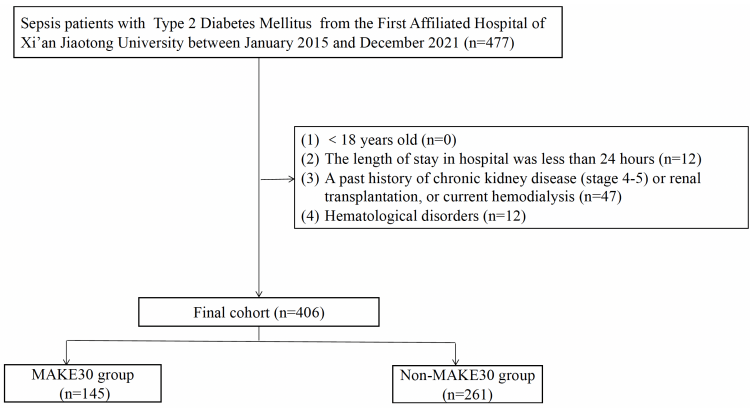

FIGURE 1
The flowchart of patient selection.

reached MAKE30 during hospitalization. For the individual components, the mortality incidence was 92 (22.7%), new RRT was 45 (11.1%), and PRD was 37 (9.11%). Venn diagram shows the relationship among MAKE30 components more intuitively (Figure 2A). The median age of participants was 62 years (range, 57 to 71), with 39.2% of patients being female. Patients with MAKE30 were significantly older than those with non-MAKE30 (Table 1). Moreover, patients with MAKE30 had significantly higher SOFA scores than those with non-MAKE30 (10, 8-13 vs. 5, 3-7; $P < 0.001$). There were significant differences in the MAP (67, 63-90 vs. 93, 80-112; $P < 0.001$), HR, and infection sources between the MAKE30 group and the non-MAKE30 group. Nevertheless, gender, temperature, and RR were not significantly different at $P > 0.05$.

Univariate analyses of clinical biochemical indicators

All the clinical biochemical indicators were available within 24 hours of admission as shown in Table 2. Univariate analyses revealed that the level of NEUT, PCT, TT, INR, FDP, D-D, APTT, PT, TBiL, UA, cystatin C (2.0, 1.1-2.9 vs. 1.1, 0.9-1.5; $P < 0.001$), BUN, and Cr significantly increased in the MAKE30 group compared with the non-MAKE30 group. On the contrary, the level of PLT (97, 58-151 vs. 162, 95-251; $P < 0.001$), PTA, apoE (35.5, 30.3-55.0 vs. 49.5, 34.8-75.2; $P < 0.001$), apoB, apoA, LDL, HDL (0.39, 0.29-0.51 vs. 0.70, 0.58-0.90; $P < 0.001$), and TGs were significantly lower in the S-AKI group, compared with the non-MAKE30 group.

Independent predictors of MAKE30 and nomogram development

The significant different variables, including age, HR, MAP, NEUT, PLT, PCT, PTA, TT, INR, FDP, D-D, APTT, PT, TBiL, apoE, apoB, apoA, LDL, HDL, TGs, UA, BUN, cystatin C and Cr were used in multivariate logistic regression analyses. Then, it revealed that in sepsis patients with T2DM, MAP (0.928,0.906-0.950), PLT (0.995,0.992-0.999), HDL (0.009,0.002-0.036), apoE (0.988,0.979-0.997), and cystatin C (1.960,0.360-2.826) were independent predictors for MAKE30 (Table 3). Furthermore, a nomogram based on these traits was created to predict MAKE30 in sepsis patients with T2DM (Figure 2B). Moreover, we provided an online version of this nomogram using the "DynNom" package for widespread use by physicians and researchers (https://diabetes-s-aki.shinyapps.io/DynNomapp/).

Verification of the prediction model

In sepsis patients with T2DM, the diagnostic value of the nomogram model for MAKE30 was assessed using the ROC curve. The model had a good ability to predict MAKE30 (AUC = 0.916) as demonstrated in Figure 2C. Moreover, the predictive probabilities based on the calibration curve were consistent with the observation results, indicating a successful calibration (Figure 3A). In DCA, it was found that the nomogram has a superior overall net benefit within a wide and practical range of threshold probabilities, implying a high potential for clinical application (Figure 3B). Hence, in sepsis patients with T2DM, the nomogram model may be a robust MAKE30 predictor.

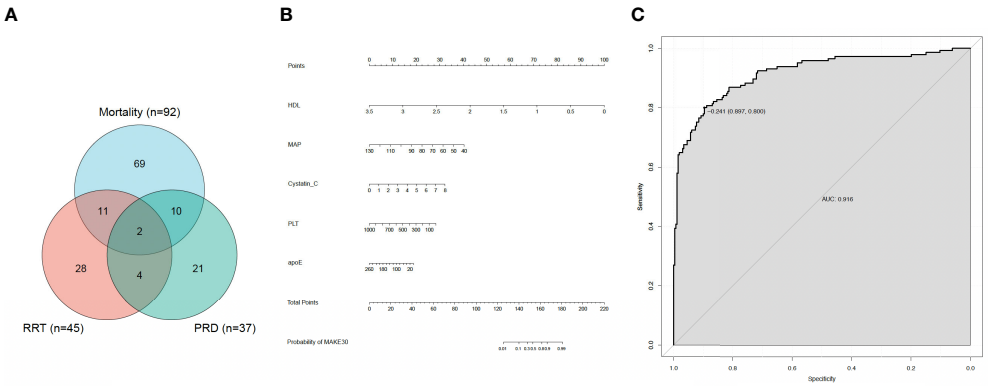

FIGURE 2
MAKE30 in sepsis patients with type 2 diabetes mellitus. **(A)** Venn diagram of major adverse kidney events within 30 days (MAKE30) components; **(B)** Nomogram to estimate the risk of MAKE30 in sepsis patients with type 2 diabetes mellitus; **(C)** The ROC curve of the nomogram for predicting MAKE30 in sepsis patients with type 2 diabetes mellitus.

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TABLE 1 The demographic and clinical data of sepsis patients with diabetes between the non-MAKE30 and MAKE30 groups.

Variables	Total (n=406)	Non-MAKE30 (n=261)	MAKE30 (n=145)	OR (95%CI)	*P* value
Age (years)	62 (51-71)	61 (49-71)	64 (54-71)	1.019 (1.005-1.034)	0.010
Female	159 (39.2%)	101 (38.7%)	58 (40.0%)	0.947 (0.625-1.434)	0.797
Temperature(°C)	36.7 (36.3-37.3)	36.7 (36.3-37.3)	36.6 (36.2-37.5)	1.136 (0.910-1.418)	0.259
HR (bpm)	93 (80-112)	89 (80-105)	97 (82-120)	1.014 (1.004-1.023)	0.004
RR (bpm)	20 (18-22)	20 (18-22)	21 (19-23)	1.018 (0.980-1.057)	0.357
MAP (mmHg)	88 (76-99)	91 (82-100)	67 (63-90)	0.915 (0.897-0.932)	<0.001
Infection source					0.005
Pulmonary infection	94 (23.2%)	49 (18.8%)	45 (31.0%)		
Intra-abdominal infection	177 (43.6%)	131 (50.2%)	46 (31.7%)		
Urinary infection	59 (14.5%)	39 (14.9%)	20 (13.8%)		
Central nervous system infection	24 (5.9%)	14 (5.4%)	10 (6.9%)		
Skin and soft tissue infections	21 (5.2%)	13 (5.0%)	8 (5.5%)		
Cardiovascular system infections	31 (7.6%)	15 (5.7%)	16 (11.0%)		
SOFA	7(4-9)	5(3-7)	10(8-13)	2.000 (1.749-2.287)	<0.001

The subgroup analyses using the prediction model

Figure 4 illustrates how ROC curve analyses revealed that the nomogram model also had a high ability to predict the occurrence of 30-day mortality (AUC = 0.822), new RRT (AUC = 0.874), and PRD (AUC = 0.801) in sepsis patients with T2DM.

Discussion

We constructed a simple nomogram model to predict MAKE30 in sepsis patients with T2DM based on those independent predictors (MAP, PLT, Cystatin C, HDL, and apoE) within 24 hours of admission. The most important thing was that the model worked well and was simple to apply. In addition, doctors must use a quick and precise nomogram model to anticipate MAKE30 to make tailored management decisions, which will improve the outcome and reduce mortality in sepsis patients with T2DM. Since sepsis is one of the most common causes of kidney injury, it is a prominent clinical problem in critically ill patients (18). Furthermore, more sepsis patients with T2DM 145 (35.7%) reached a MAKE30 composite outcome, than patients with acute pancreatitis (16%) and sepsis patients (28.3%) (16, 17).

The classical theories suggest that the primary pathogenic mechanisms causing kidney damage include decreased renal blood flow, secondary tubular epithelial cell death, or acute tubular necrosis. In the current study, sepsis with T2DM and a low level of MAP is an independent risk factor for MAKE30, which indicates renal tissue ischemia. However, more studies have revealed that various mechanisms, such as inflammation, microcirculatory dysfunction, and metabolic reprogramming, contribute to the pathogenesis of kidney injury, and that hemodynamic instability was not a necessary pathogenetic factor for kidney injury (19, 20). It is well known that during the sepsis period, inflammatory mediators are released in the intravascular compartment, causing damage to the vascular endothelium. Then, the coagulation system subsequently hyperactivated as a result of damaged vascular endothelium activating platelets first, which significantly caused the subsequent formation of micro-thrombi contributing to kidney injury (20, 21). Intimate and complex relationships existed between the inflammatory response and coagulation failure, both of which have a significant impact on the etiology of kidney damage in sepsis patients with type 2 diabetes. We also found that PLT was an independent predictor of MAKE30. Previous studies have demonstrated shown that PLT is important for controlling hemostasis as well as for interacting with other immune cells to modulate the immune and inflammatory response (22). Lv et al. also revealed that platelets contributed to kidney injury by inducing renal cell apoptosis (23).

In addition, we demonstrated that in sepsis patients with T2DM, cystatin C was an independent predictor of MAKE30. Protease inhibitors belonging to the cystatin superfamily, serum cystatin C, can pass freely through the glomerular filtration and then be completely reabsorbed and degraded by the renal tubules (24). It is not significantly influenced by biological factors such as age, gender, muscle mass, diet, infection, and tumors, in contrast

TABLE 2 Univariate analyses of clinical biochemical indicators between non-MAKE30 and MAKE30 groups in sepsis patients with T2DM.

Variables	Total (n=406)	Non-MAKE30 (n=261)	MAKE30 (n=145)	OR (95%CI)	*P* value
WBC (x 10^9/L)	10.4 (6.1-14.9)	10.2 (5.9-16.1)	10.9 (6.7-14.1)	0.985 (0.960-1.012)	0.279
NEUT (%)	86.3 (76.5-91.9)	85.5 (73.1-90.9)	89.2 (80.0-93.0)	1.021 (1.004-1.039)	0.017
Lymphocyte (x 10^9/L)	0.75 (0.43-1.20)	0.77 (0.46-1.27)	0.70 (0.37-1.06)	0.838 (0.630-1.115)	0.225
Monocyte (x 10^9/L)	0.39 (0.26-0.64)	0.39 (0.26-0.61)	0.45(0.24-0.69)	1.409 (0.797-2.491)	0.239
PLT (x 10^9/L)	134 (85-209)	162 (95-251)	97 (58-151)	0.992 (0.990-0.995)	<0.001
PCT (ng/ml)	6.5 (1.1-23.0)	3.3 (0.59-17.7)	16.6 (3.9-35.72)	1.013 (1.007-1.020)	<0.001
PTA (%)	72.0 (54.0-87.5)	76.0 (60.0-89.1)	61.1 (44.5-83.4)	0.978 (0.968-0.987)	<0.001
TT (S)	14.7 (1.0-17.2)	14.6 (1.0-16.7)	15.5 (1.03-18.1)	1.011 (1.003-1.019)	0.009
INR	1.2 (1.1-1.4)	1.2 (1.1-1.4)	1.3 (1.1-1.6)	2.156 (1.353-3.436)	0.001
FDP (mg/L)	10.0 (4.5-22.4)	9.0 (4.3-19.1)	12.2 (5.8-30.3)	1.004 (0.999-1.008)	0.106
D-D (mg/L)	3.4 (1.7-7.7)	3.1(1.5-6.5)	4.3 (1.9-9.7)	1.024 (1.003-1.045)	0.025
FIB (g/L)	4.8 (3.3-6.0)	4.9 (3.4-6.1)	4.4 (3.1-6.0)	0.924 (0.840-1.018)	0.109
APTT (S)	39.7 (35.0-46.4)	39.0 (35.0-44.6)	42.2 (35.3-53.7)	1.024 (1.011-1.036)	<0.001
PT (S)	14.8 (13.3-16.8)	14.6 (13.4-16.2)	15.6 (13.1-18.3)	1.034 (1.003-1.066)	0.030
GLB	24.6 (20.3-18.5)	25.0 (20.6,28.4)	24.1 (19.7,29.1)	1.006 (0.979-1.032)	0.682
ALB	30.7 (25.1-39.9)	30.5 (25.4,39.0)	31.2 (24.8,42.4)	1.004 (0.987-1.021)	0.645
TBiL	17.8 (10.7-34.6)	17.0 (10.6-29.0)	18.4 (10.8,43.8)	1.003 (1.000-1.006)	0.024
Lp(a) (mg/L)	103 (35-258)	97 (28-258)	135 (41-223)	1.000 (0.999-1.001)	0.991
apoE (mg/L)	46.8 (33.4-72.3)	49.5 (34.8-75.2)	35.5 (30.3-55.0)	0.984 (0.977-0.991)	<0.001
apoB (g/L)	0.70 (0.52-0.89)	0.74 (0.57-0.94)	0.62 (0.40-0.78)	0.319 (0.156-0.654)	0.002
apoA (g/L)	0.73 (0.50-0.87)	0.77 (0.64-0.87)	0.52 (0.36-0.81)	0.072 (0.031-0.167)	<0.001
LDL (mmol/L)	1.36 (0.90-2.14)	1.58 (1.02-2.21)	1.02 (0.66-1.75)	0.489 (0.371-0.644)	<0.001
HDL (mmol/L)	0.62 (0.44-0.77)	0.70 (0.58-0.90)	0.39 (0.29-0.51)	0.002 (0.01-0.009)	<0.001
TGs (mmol/L)	1.89 (1.11-3.48)	1.79 (1.16-3.97)	1.89 (1.03-2.86)	0.938 (0.900-0.977)	0.002
TC (mmol/L)	2.81 (2.03-3.84)	2.82 (2.08-3.95)	2.69 (1.92-3.72)	0.898 (0.801-1.008)	0.068
Urinary glucose (mmol/L)	11.2 (7.9-15.3)	12.0 (8.1-15.4)	10.4 (7.2-15.2)	0.990 (0.962-1.018)	0.466
UA (umol/L)	317 (213-436)	281 (203-380)	386 (281-531)	1.003 (1.002-1.005)	<0.001
Cystatin C (mg/L)	1.2 (0.9-1.9)	1.1 (0.9-1.5)	2.0 (1.1-2.9)	3.018 (2.262-4.028)	<0.001
BUN (mmol/L)	8.7 (5.4-15.2)	7.8 (5.1-11.5)	12.1 (6.7-23.0)	1.116 (1.083-1.150)	<0.001
Cr (umol/L)	108 (59-239)	89 (55-235)	132 (72-320)	1.005 (1.003-1.007)	<0.001

to Creatinine (Cr). Serum Cr may not be able to detect very early changes in renal function, particularly in sepsis patients with early tubular necrosis (18). Nevertheless, serum cystatin C was able to predict the occurrence of AKI, one to two days earlier than serum Cr (18, 25). Cystatin C has been suggested as a potential predictor for the early diagnosis of S-AKI or was an alternative to the gold standard "creatinine" in growing number of studies in recent years (18, 26). Regardless of the underlying precipitating factors, previous studies have demonstrated that cystatin C played a significant role in the diagnosis and prediction of kidney injury in sepsis or other critical clinical conditions (25, 27). Moreover, in critically ill neonates, cystatin C could be used as a powerful predictor of kidney injury (28). When it was immediately estimated within 24 hours after admission for early detection of kidney injury, hence, serum or urine, cystatin C was very valuable (26).In addition, it was found that serum cystatin C was associated with the recovery, death, or renal replacement therapy of kidney injury (27, 29).

TABLE 3 Multivariate logistic regression analyses of independent predictors for MAKE30 in sepsis patients with T2DM.

Variables	β	SE	Wald	P-value	OR (95% CI)
MAP	-0.075	0.012	39.339	<0.001	0.928 (0.906-0.950)
PLT	-0.005	0.002	7.205	0.007	0.995 (0.992-0.999)
HDL	-4.766	0.737	41.770	<0.001	0.009(0.002-0.036)
apoE	-0.012	0.005	6.485	0.011	0.988(0.979-0.997)
Cystatin C	0.673	0.187	13.005	<0.001	1.960(1.360-2.826)
Constant	8.710	1.245	48.945	<0.001	—

MAP, Mean Arterial Pressure; PLT, platelet; HDL, High-Density Lipoprotein; apoE, apolipoprotein E.

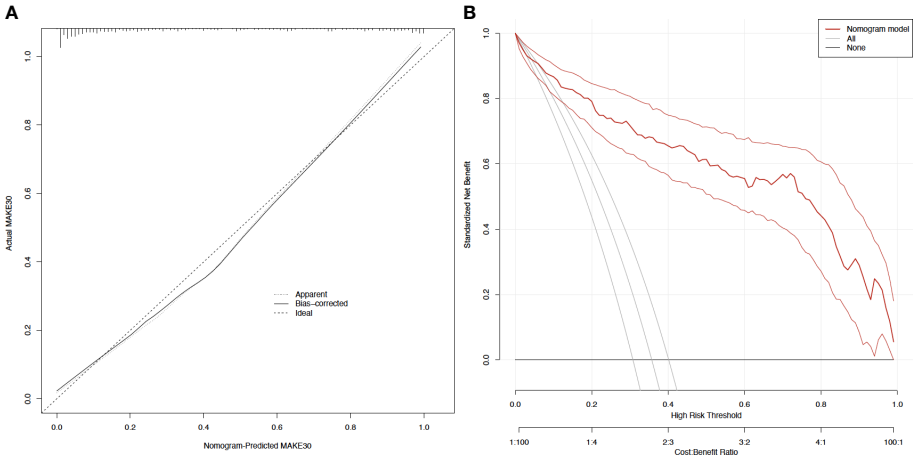

FIGURE 3
Calibration curves and decision curve analysis of the nomogram for predicting MAKE30 **(A)** Calibration curves of the nomogram; **(B)** Decision curve analysis of the nomogram.

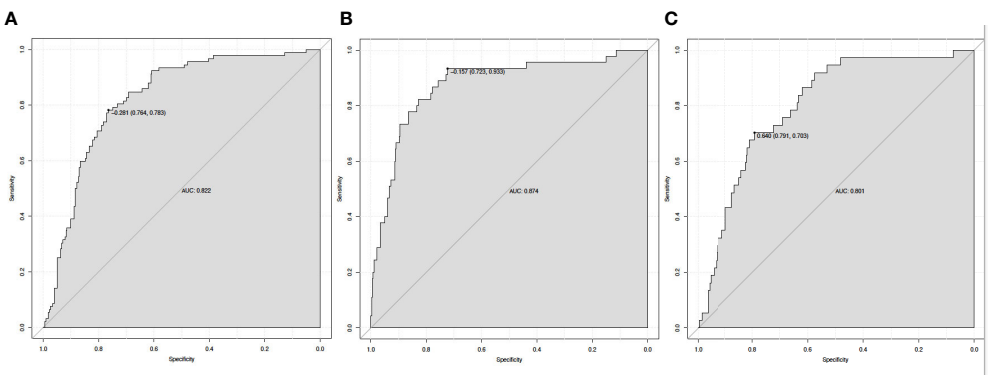

FIGURE 4
The ROC curve of the nomogram for predicting 30-day mortality **(A)**, new RRT **(B)**, and PRD **(C)**. RRT, renal replacement therapy; PRD, persistent renal dysfunction.

Future interventions may be directed at altering the apolipoproteins and cholesterol of sepsis patients, which was revealed by omic methods (30, 31). Proinflammatory cytokines can also alter the hepatic synthesis of apolipoprotein and acute phase reactants in the liver, such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 (IL-1) (32). This study revealed that apoE was an independent predictor for MAKE30. Increasing evidence has demonstrated that apoE is important in the pathophysiological course of anti-infection and anti-inflammation like sepsis (31). Previous studies have demonstrated that apoE 3 could protect porcine proximal tubular cells from gentamicin-induced injury (33), and apoE ϵ4 was protective against the development of kidney injury (34).

Decreased HDL concentrations are commonplace during an acute sepsis episode and are proportional to the degree of inflammation (32). Studies have shown that HDL levels decreased by 40–70% throughout the inflammatory process, which resulted in patients with sepsis having a bad prognosis (35). Furthermore, in patients with sepsis, reduced HDL levels were independently associated with an increased risk of kidney injury onset and decreased Glomerular Filtration Rate (GFR) (36). Chien et al. indicated that to avoid disease progression, multi-organ dysfunction, and renal injury in sepsis, HDL levels were a prognostic factor for making a personalized management plan (37). Reduced cholesterol may be the outcome of elevated erythropoietin brought on by hypoxia signaling activation in patients with ischemia-induced kidney impairment (38). Since kidneys are significant in the recycling of senescent HDL particles, clinical studies have demonstrated that low levels of HDL were associated with increased risk of kidney injury (35). Moreover, polymorphisms in HDL metabolism genes such as rs1800777 (allele A) in the CETP gene, were strongly associated with an increased risk of kidney injury during sepsis (39). Increased HDL levels may activate the endothelial nitric oxide synthase (eNOS) pathway, reducing the production of adhesion molecules, leucocytes activation, and neutrophil infiltration, reducing vascular impairment and renal parenchymal damage (35). The loss of HDL and its components is influenced by renal tubular injury influences tubular reabsorption function and catabolism (35). Extra renal synthesis and metabolism of HDL components can also be influenced by kidney injury (40). Therefore, in sepsis patients with T2DM, lower HDL is significantly associated with MAKE30 prediction

In this study, we developed a simple nomogram model (based on MAP, PLT, Cystatin C, HDL, and apoE) to predict MAKE30 in sepsis patients with T2DM. After verification, it demonstrated good performance in discrimination, calibration, and clinical application. This nomogram can also be used to determine the appropriate treatment options for high-risk patients. The application of the nomogram model is demonstrated by the following example: assuming a sepsis patient with T2DM with a MAP of 70 mmHg, a Cystatin C of 3 mg/L, an HDL of 1 mmol/L, a PLT of 200 x 10^9/L, and an apoE of 40 mg/L. The score assigned to each parameter on the "Points" axis is obtained as shown in Figure 2B. The sum of points for each parameter is used to calculate the final score [27 (MAP) + 12 (Cystatin C) + 72 (HDL) + 23 (PLT) + 15 (apoE) = 149]. This score corresponds to about 33% risk of developing MAKE30. Another option is to use the online version (https://diabetes-s-aki.shinyapps.io/DynNomapp/) to obtain the same result easily. Finally, we also discovered that in sepsis patients with T2DM, the nomogram model had perfect predictive power for predicting 30-day mortality (AUC = 0.822), new RRT (AUC = 0.874), and PRD (AUC = 0.801).

Limitations

Nevertheless, this study had some limitations. Firstly, since it was a single-center study, there was a chance of selection bias influencing the results. Secondly, it was a seven-year retrospective study, so there was significant advancement in the management decision-making process, affecting sepsis development. Thirdly, the model was constructed from a training group with a significantly smaller testing sample. Therefore, additional multi-center prospective studies with an adequate cohort size would be needed to assess its potential and validate the results.

Conclusions

In conclusion, our study demonstrated that in sepsis patients with T2DM, the levels of MAP, PLT, Cystatin C, HDL, and apoE available within 24 hours after admission played a critical role in MAKE30 prediction. Moreover, the predictive nomogram model based on those predictors performed well in the discrimination, calibration, and clinical application for MAKE30, which is crucial for clinicians to make timely personalized management decisions.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

This study was conducted following the Declaration of Helsinki and was approved by the Ethical Committee of the First Affiliated Hospital of Xi'an Jiaotong University. All patient data were analyzed in anonymity. Patient consent was waived by the ethics committee, as no individual data were published, nor was any intervention performed on patients.

Author contributions

QX conceived of the study and drafted the manuscript. TX, RC, and XZ participated in the statistical analysis. HW participated the design of the study. SW, CL, and JZ participated in its design and coordination and helped to draft the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1024500/full#supplementary-material

References

1. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* (2016) 315(8):801–10. doi: 10.1001/jama.2016.0287

2. Frydrych LM, Fattahi F, He K, Ward PA, Delano MJ. Diabetes and sepsis: Risk, recurrence, and ruination. *Front Endocrinol (Lausanne)* (2017) 8:271. doi: 10.3389/fendo.2017.00271

3. Yue S, Li S, Huang X, Liu J, Hou X, Wang Y, et al. Construction and validation of a risk prediction model for acute kidney injury in patients suffering from septic shock. *Dis Markers* (2022) 2022:9367873. doi: 10.1155/2022/9367873

4. Trevelin SC, Carlos D, Beretta M, da Silva JS, Cunha FQ. Diabetes mellitus and sepsis: A challenging association. *Shock* (2017) 47(3):276–87. doi: 10.1097/SHK.0000000000000778

5. Esper AM, Moss M, Martin GS. The effect of diabetes mellitus on organ dysfunction with sepsis: an epidemiological study. *Crit Care* (2009) 13(1):R18. doi: 10.1186/cc7717

6. Bhutani J, Bhutani S. Worldwide burden of diabetes. *Indian J Endocrinol Metab* (2014) 18(6):868–70. doi: 10.4103/2230-8210.141388

7. Costantini E, Carlin M, Porta M, Brizzi MF. Type 2 diabetes mellitus and sepsis: state of the art, certainties and missing evidence. *Acta Diabetol* (2021) 58 (9):1139–51. doi: 10.1007/s00592-021-01728-4

8. Jiang L, Cheng M. Impact of diabetes mellitus on outcomes of patients with sepsis: an updated systematic review and meta-analysis. *Diabetol Metab Syndr* (2022) 14(1):39. doi: 10.1186/s13098-022-00803-2

9. Poston JT, Koyner JL. Sepsis associated acute kidney injury. *BMJ* (2019) 364: k4891. doi: 10.1136/bmj.k4891

10. Sathananthan M, Sathananthan A, Jeganathan N. Characteristics and outcomes of patients with and without type 2 diabetes mellitus and pulmonary sepsis. *J Intensive Care Med* (2020) 35(9):836–43. doi: 10.1177/0885066619833910

11. Palevsky PM, Molitoris BA, Okusa MD, Levin A, Waikar SS, Wald R, et al. et al: Design of clinical trials in acute kidney injury: report from an NIDDK workshop on trial methodology. *Clin J Am Soc Nephrol* (2012) 7(5):844–50. doi: 10.2215/CJN.12791211

12. Self WH, Semler MW, Wanderer JP, Ehrenfeld JM, Byrne DW, Wang L, et al. et al: Saline versus balanced crystalloids for intravenous fluid therapy in the emergency department: study protocol for a cluster-randomized, multiple-crossover trial. *Trials* (2017) 18(1):178. doi: 10.1186/s13063-017-1923-6

13. Semler MW, Self WH, Wanderer JP, Ehrenfeld JM, Wang L, Byrne DW, et al. et al: Balanced crystalloids versus saline in critically ill adults. *N Engl J Med* (2018) 378(9):829–39. doi: 10.1056/NEJMoa1711584

14. Weiss SL, Balamuth F, Thurm CW, Downes KJ, Fitzgerald JC, Laskin BL. Major adverse kidney events in pediatric sepsis. *Clin J Am Soc Nephrol* (2019) 14 (5):664–72. doi: 10.2215/CJN.12201018

15. American Diabetes Association Professional Practice C and American Diabetes Association Professional Practice C, Draznin B, Aroda VR, Bakris G, Benson G, et al. 2. classification and diagnosis of diabetes: Standards of medical care in diabetes-2022. *Diabetes Care* (2022) 45(Suppl 1):S17–38. doi: 10.2337/dc22-S002

16. Zhang X, Ye B, Mao W, Liu L, Li G, Zhou J, et al. Major adverse kidney events within 30 days in patients with acute pancreatitis: a tertiary-center cohort study. *HPB (Oxford)* (2022) 24(2):169–75. doi: 10.1016/j.hpb.2021.05.012

17. Mele A, Cerminara E, Habel H, Rodriguez-Galvez B, Oldner A, Nelson D, et al. Fluid accumulation and major adverse kidney events in sepsis: a multicenter observational study. *Ann Intensive Care* (2022) 12(1):62. doi: 10.1186/s13613-022-01040-6

18. Al-Amodi HS, Abdelsattar S, Kasemy ZA, Bedair HM, Elbarbary HS, Kamel HFM. Potential value of TNF-alpha (-376 G/A) polymorphism and cystatin c (CysC) in the diagnosis of sepsis associated acute kidney injury (S-AK I) and prediction of mortality in critically ill patients. *Front Mol Biosci* (2021) 8:751299. doi: 10.3389/fmolb.2021.751299

19. Peerapornratana S, Manrique-Caballero CL, Gomez H, Kellum JA. Acute kidney injury from sepsis: current concepts, epidemiology, pathophysiology, prevention and treatment. *Kidney Int* (2019) 96(5):1083–99. doi: 10.1016/j.kint.2019.05.026

20. Fani F, Regolisti G, Delsante M, Cantaluppi V, Castellano G, Gesualdo L, et al. Recent advances in the pathogenetic mechanisms of sepsis-associated acute kidney injury. *J Nephrol* (2018) 31(3):351–9. doi: 10.1007/s40620-017-0452-4

21. Jansen MPB, Florquin S, Roelofs J. The role of platelets in acute kidney injury. *Nat Rev Nephrol* (2018) 14(7):457–71. doi: 10.1038/s41581-018-0015-5

22. Joffre J, Hellman J, Ince C, Ait-Oufella H. Endothelial responses in sepsis. *Am J Respir Crit Care Med* (2020) 202(3):361–70. doi: 10.1164/rccm.201910-1911TR

23. Lv D, Zhang Y, Wang C, Gu Y, Zhang Y, Li X. Platelets derived transthyretin participate in the development of sepsis associated acute kidney injury by inducing oxidative stress and apoptosis of renal tubular epithelial cells. *Shock* (2022) 57 (5):722–31. doi: 10.1097/SHK.0000000000001908

24. Bellos I, Fitrou G, Daskalakis G, Papantoniou N, Pergialiotis V. Serum cystatin-c as predictive factor of preeclampsia: A meta-analysis of 27 observational studies. *Pregnancy Hypertens* (2019) 16:97–104. doi: 10.1016/j.preghy.2019.03.006

25. Herget-Rosenthal S, Marggraf G, Hüsing J, Göring F, Pietruck F, Janssen O, et al. Early detection of acute renal failure by serum cystatin c. *Kidney Int* (2004) 66 (3):1115–22. doi: 10.1111/j.1523-1755.2004.00861.x

26. Bian Z, Zhu R, Chen S. The predict value of serum/urocystatin c on acute kidney injury in elderly patients with sepsis. *Exp Gerontol* (2021) 155:111576. doi: 10.1016/j.exger.2021.111576

27. Leem AY, Park MS, Park BH, Jung WJ, Chung KS, Kim SY, et al. Value of serum cystatin c measurement in the diagnosis of sepsis-induced kidney injury and prediction of renal function recovery. *Yonsei Med J* (2017) 58(3):604–12. doi: 10.3349/ymj.2017.58.3.604

28. Hidayati EL, Utami MD, Rohsiswatmo R, Tridjaja B. Cystatin c compared to serum creatinine as a marker of acute kidney injury in critically ill neonates. *Pediatr Nephrol* (2021) 36(1):181–6. doi: 10.1007/s00467-020-04668-3

29. Nejat M, Pickering JW, Walker RJ, Endre ZH. Rapid detection of acute kidney injury by plasma cystatin c in the intensive care unit. *Nephrol Dial Transplant* (2010) 25(10):3283–9. doi: 10.1093/ndt/gfq176

30. Sharma NK, Tashima AK, Brunialti MKC, Ferreira ER, Torquato RJS, Mortara RA, et al. Proteomic study revealed cellular assembly and lipid metabolism dysregulation in sepsis secondary to community-acquired pneumonia. *Sci Rep* (2017) 7(1):15606. doi: 10.1038/s41598-017-15755-1

31. Shao Y, Zhao T, Zhang W, He J, Lu F, Cai Y, et al. Presence of the apolipoprotein e-ϵ4 allele is associated with an increased risk of sepsis progression. *Sci Rep* (2020) 10(1):15735. doi: 10.1038/s41598-020-72616-0

32. Golucci A, Marson FAL, Ribeiro AF, Nogueira RJN. Lipid profile associated with the systemic inflammatory response syndrome and sepsis in critically ill patients. *Nutrition* (2018) 55-56:7–14. doi: 10.1016/j.nut.2018.04.007

33. Takamoto K, Kawada M, Ikeda D, Yoshida M. Apolipoprotein E3 (apoE3) safeguards pig proximal tubular LLC-PK1 cells against reduction in SGLT1 activity induced by gentamicin c. *Biochim Biophys Acta* (2005) 1722(3):247–53. doi: 10.1016/j.bbagen.2004.12.006

34. MacKensen GB, Swaminathan M, Ti LK, Grocott HP, Phillips-Bute BG, Mathew JP, et al. Preliminary report on the interaction of apolipoprotein e polymorphism with aortic atherosclerosis and acute nephropathy after CABG. *Ann Thorac Surg* (2004) 78(2):520–6. doi: 10.1016/j.athoracsur.2004.02.106

35. Stasi A, Franzin R, Fiorentino M, Squiccimarro E, Castellano G, Gesualdo L. Multifaced roles of HDL in sepsis and SARS-CoV-2 infection: Renal implications. *Int J Mol Sci* (2021) 22(11):5980. doi: 10.3390/ijms22115980

36. Roveran Genga K, Lo C, Cirstea M, Zhou G, Walley KR, Russell JA, et al. Two-year follow-up of patients with septic shock presenting with low HDL: the effect upon acute kidney injury, death and estimated glomerular filtration rate. *J Intern Med* (2017) 281(5):518–29. doi: 10.1111/joim.12601

37. Chien JY, Jerng JS, Yu CJ, Yang PC. Low serum level of high-density lipoprotein cholesterol is a poor prognostic factor for severe sepsis. *Crit Care Med* (2005) 33(8):1688–93. doi: 10.1097/01.CCM.0000171183.79525.6B

38. Li J, Yang M, Yu Z, Tian J, Du S, Ding H. Kidney-secreted erythropoietin lowers lipidemia *via* activating JAK2-STAT5 signaling in adipose tissue. *EBioMedicine* (2019) 50:317–28. doi: 10.1016/j.ebiom.2019.11.007

39. Genga KR, Trinder M, Kong HJ, Li X, Leung AKK, Shimada T, et al. CETP genetic variant rs1800777 (allele a) is associated with abnormally low HDL-c levels and increased risk of AKI during sepsis. *Sci Rep* (2018) 8(1):16764. doi: 10.1038/s41598-018-35261-2

40. Zhong J, Yang H, Kon V. Kidney as modulator and target of "good/bad" HDL. *Pediatr Nephrol* (2019) 34(10):1683–95. doi: 10.1007/s00467-018-4104-2

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Association between hemoglobin glycation index and diabetic kidney disease in type 2 diabetes mellitus in China: A cross-sectional inpatient study

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Objective: To investigate the association between Hemoglobin Glycation Index (HGI) and Diabetic Kidney Disease (DKD) in Chinese type 2 diabetic individuals and to construct a risk score based on HGI to predict a person's risk of DKD.

Methods: We retrospectively analyzed 1622 patients with type 2 diabetes mellitus (T2DM). HGI was obtained by calculating the fasting plasma glucose (FPG) level into the formula, and they were grouped into low HGI group (L-HGI), medium HGI group (H-HGI) and high HGI group (H-HGI) according to tri-sectional quantile of HGI. The occurrence of DKD was analyzed in patients with different levels of HGI. Multivariate logistics regression analysis was used to analyze the risk factors of DKD in patients with T2DM.

Results: A total of 1622 patients with T2DM were enrolled in the study. Among them, 390 cases were DKD. The prevalence of DKD among the three groups was 16.6%, 24.2% and 31.3%. The difference was statistically significant (P = 0.000). There were significant differences in age (P=0.033), T2DM duration (P=0.005), systolic blood pressure (SBP) (P=0.003), glycosylated hemoglobin (HbA1c) (P=0.000), FPG (P=0.032), 2-hour postprandial plasma glucose (2h-PPG) (P=0.000), fasting C-peptide FCP (P=0.000), 2-hour postprandial C-peptide (2h-CP) (P=0.000), total cholesterol (TC) (P=0.003), low density lipoprotein cholesterol (LDL-C) (P=0.000), serum creatinine (sCr) (P=0.001), estimated glomerular filtration rate (eGFR) (P=0.000) among the three groups. Mantel-Haenszel chi-square test showed that there was a linear relationship between HGI and DKD (x2=177.469, p < 0.001). Pearson correlation analysis showed that with the increase of HGI level the prevalence of DKD was increasing (R= 0.445, P=0.000). It was indicated by univariate logistic regression analysis that individuals in H-HGI was more likely to develop DKD (OR: 2.283, 95% CI: 1.708~ 3.052) when compared with L-HGI. Adjusted to multiple factors, this trend still remained significant (OR: 2.660, 95% CI: 1.935~ 3.657). The combined

DKD risk score based on HGI resulted in an area under the receiver operator characteristic curve (AUROC) of 0.702.

Conclusions: High HGI is associated with an increased risk of DKD. DKD risk score may be used as one of the risk predictors of DKD in type 2 diabetic population.

KEYWORDS

diabetes mellitus, type 2, hemoglobin glycation index, diabetic kidney disease, complication

1 Introduction

Diabetic kidney disease (DKD) is an important microvascular complication of diabetes and has become the main cause of chronic kidney disease (CKD) and end stage renal disease (ESRD) (1–3). Early detection of DKD and conducting the most effective targeted intervention are the key steps to offset the development of adverse clinical outcomes of diabetes mellitus (DM). Glycated hemoglobin (HbA1c) level is recommended as the gold standard method to evaluate glycemic control in DM patients. Despite the generally acknowledged role of HbA1c in the management of patients with diabetes, considerable differences in HbA1c exist even in patients with similar mean blood glucose (MBG) profiles (4, 5). Studies have shown that some individuals have persistently higher or lower HbA1c levels than expected. However, recent researches had shown that considerable biological variation of HbA1c was not only affected by blood glucose levels but also influenced by interindividual biological differences and environmental factor (6, 7). That means, even at the same blood glucose level, the level of HbA1c could be different. Therefore, solely relying on HbA1c level to evaluate the risk of DM is not suitable for all populations, which will produce a significant deviation. Hempe et al. (8) described this discrepancy by hemoglobin glycation index (HGI), which was calculated as the difference between an individual's observed HbA1c and the estimated HbA1c.

HGI can identify people with HbA1c levels that are higher or lower than average compared to other people with the same blood glucose concentration (8, 9). It has been found that HGI could promote the development of some microvascular and macrovascular complications in DM patients (10). A recent meta-analysis showed that increased HbA1c variability was associated with increased risk of all-cause mortality, cardiovascular disease (CVD), renal disease, peripheral neuropathy in patients with type 2 diabetes mellitus (T2DM) (11). In the Diabetes Control and Complications Trial (DCCT), a high HGI at the baseline was a predictor of CKD and retinopathy in patients with T1DM after 7 years of follow-up (12). The individual variation in HbA1c observed in the DCCT was attributable to biological variation and not measurement error. In the Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial, a high HGI at the baseline predicted major microvascular events, which comprised new or worsened nephropathy or retinopathy in T2DM (13). Chih-Hung Lin et al. (14) found that HGI independently predicted renal function deterioration in patients with T2DM and a low CKD risk. Although cumulative evidence suggests a role for HGI in diabetes complications, Lachin et al. (15) had contradictory findings on reassessment of HGI for prediction of microvascular complications in the DCCT. They concluded that HGI was not a useful predictor for microvascular complications because it is not statistically independent of HbA1c.

Although the above-mentioned studies have shed light on the potential application of HGI in the management of diabetic complications, the evidence for HGI as a predictor of DKD remains unclear. Therefore, this study intends to analyze the relationship between HGI level and the risk of DKD in Chinese patients with T2DM and to construct a risk score to conveniently predict a person's risk of DKD aiming to provide a new reference for clinical evaluation of diabetic complications.

2 Materials and methods

2.1 Participants

In this retrospective study, 1622 T2DM who were hospitalized in Peking University International Hospital endocrinology department from March 2015 to April 2021 were analyzed. Among them, 1016 (62.64%) were males and 606 (37.36%) were females, with an average age of (55.8 ± 13.47) years. The average duration of T2DM was 9.31 ± 7.73 years. All subjects met the T2DM diagnostic criteria of the World Health Organization (WHO) in 1999 (16). According to the diagnostic criteria of DKD (17), the subjects were divided into 1232 cases of non-DKD and 390 cases of DKD. The exclusion criteria included: (1) Other type of diabetes mellitus; (2) Acute complications of diabetes; (3) With primary renal parenchyma; (4) Recent urinary tract infection, taking drugs that affect renal function, etc.; (5) With severe anemia or blood loss; (6) Pregnant and lactating women; (7) Patients who were hospitalized for twice or more times.

2.2 Methods

2.2.1 General conditions collected

All participants' age, date of birth and diabetic duration (unit by year) were collected and recorded. All participants were asked to take off their shoes and socks and wear light and thin clothes, following which height (cm) and weight (kg) were measured with measuring instrument, and body mass index (BMI) was obtained according to the formula weight/height^2 (kg/m^2). Blood pressure including systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in all participants.

2.2.2 Laboratory measurement

All subjects were asked to fast for at least 8 hours, and venous blood samples were collected in the morning. Chemiluminescence method was then used to test blood glucose and blood lipid profile. Other biochemical indices were then determined. High-pressure liquid chromatography was used to test HbA1c level. The tests were carried out in the biochemical laboratory of Peking University International Hospital. Laboratory measurements included fasting plasma glucose (FPG), 2 hour postprandial plasma glucose (2h-PPG), fasting C-Peptide (FCP), 2 hour C-Peptide (2h-CP), HbA1c, low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), uric acid (UA), serum creatinine (sCr), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and urinary microalbumin/creatinine ratio (UACR). The estimated glomerular filtration rate (eGFR) was estimated according to the sCr level.

2.2.3 HGI calculation

Taking the actual measured value of HbA1c as the dependent variable and FPG as the independent variable, a linear regression equation was established as follows: predict HbA1c= 5.249 + 0.383* FPG (r= 0.636 and p<0.001). Predicted HbA1c was then subtracted from the individual's observed HbA1c to generate HGI (HGI = observed HbA1c – predicted HbA1c). HGI values were divided into three groups by using the tri quantile method: L-HGI, M-HGI and H-HGI (Figure 1).

2.3 Statistical methods

All data were processed by SPSS 22.0. Normal distribution data were shown as mean standard deviation (± s), and nonnormal distribution data were shown as mean median and quartile spacing. When quantitative data were normally distributed and variance was homogeneous, variance analysis was used for comparison among groups. When data were not normally distributed, variance analysis such as Kruskal Wallis test was used for comparison among multiple groups; qualitative data was expressed in percentage (%). Chi-square test was used to compare the qualitative data among the three groups. Pearson correlation analysis was used to analyze the correlation between HbA1c and FPG, and the regression equation was established accordingly. Logistic regression method was used for analysis of the main influencing factors of DKD with T2DM, and p< 0.05 was used for statistical significance. The AUROC was used to evaluate the sensitivity of the proposed risk score for the prediction of DKD.

3 Results

3.1 General characteristics among the 3 groups

There were significant differences in age (P=0.033), T2DM duration (P=0.005), SBP (P=0.003), HbA1c (P=0.000), FPG (P=0.032), 2h-PPG (P=0.000), FCP (P=0.000), 2h-CP (P=0.000), TC (P=0.003), LDL-C (P=0.000), sCr (P=0.001), eGFR (P=0.000) among the three groups. There were no significant differences in sex (P=0.299), DBP (P=0.058), BMI (P=0.274), TG (P=0.932), HDL-C (P=0.327), UA (P=0.089), UACR (P=0.111). The prevalence of DKD among the three groups was 16.60%, 24.20% and 31.30%. The difference was statistically significant (P=0.000). Mantel-Haenszel chi-square test showed that there was a linear relationship between HGI and DKD (x^2 = 31.817, P=0.000). Pearson correlation analysis showed that with the increase of HGI level the prevalence of DKD was increasing (r= 0.140, P=0.000). (Table 1, Figure 2)

3.2 Binary logistic regression analysis of the relationship between DKD and related factors and HGI risk analysis in T2DM

To investigate the potential interactions affecting the prevalence of DKD in T2DM, binary logistic regression analysis was performed as shown in Table 2. It was shown that age, T2DM duration, SBP,

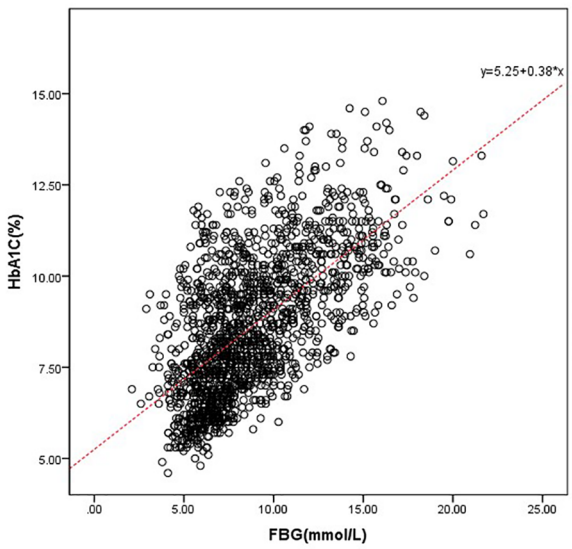

FIGURE 1
Correlation between HbA1c and FPG. We drove the predicted HbA1c, which was defined as follows: predict HbA1c= 5.249 + 0.383* FPG (r= 0.636 and p<0.001).

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TABLE 1 Comparison of general data and biochemical index results in each group.

Variable	L-HGI (*n*=541)	M-HGI (*n*=541)	H-HGI (*n*=540)	$F(X^2)$	*p*
Sex [n(M/F)]	*353/188*	*330/211*	*333/207*	2.416	0.299
Age (yeas)	55.70 ± 12.94	56.86 ± 13.40	54.73 ± 14.00	3.415	0.033
T2DM Duration (yeas)	9.00 ± 7.73	10.17 ± 7.69	8.74 ± 7.69	5.307	0.005
SBP (mmHg)	130.90 ± 16.23	134.53 ± 17.55	132.56 ± 18.06	5.961	0.003
DBP (mmHg)	78.10 ± 11.13	79.31 ± 10.36	79.60 ± 11.30	2.858	0.058
BMI (Kg/m^2)	25.78 ± 3.73	26.14 ± 3.65	26.00 ± 3.65	1.296	0.274
FBG (mmol/L)	8.53 ± 3.27	8.91 ± 3.29	9.01 ± 3.00	3.435	0.032
PBG (mmol/L)	11.66 ± 4.19	12.76 ± 4.38	13.47 ± 4.68	23.119	0.000
HbA1c (%)	6.99 ± 1.34	8.46 ± 1.28	10.43 ± 1.40	937.672	0.000
FCP (ng/ml)	2.66 ± 1.31	2.43 ± 1.31	2.14 ± 1.38	19.637	0.000
2h-CP (ng/ml)	6.81 ± 4.07	5.66 ± 3.36	4.65 ± 3.60	43.579	0.000
TC (mmol/L)	4.23 ± 1.04	4.35 ± 1.15	4.47 ± 1.16	5.976	0.003
TG (mmol/L)	2.08 ± 2.00	2.05 ± 1.60	2.09 ± 1.62	0.080	0.923
HDL-C (mmol/L)	1.02 ± 0.27	1.00 ± 0.27	1.00 ± 0.26	1.119	0.327
LDL-C (mmol/L)	2.43 ± 0.83	2.56 ± 0.90	2.64 ± 0.92	8.059	0.000
UA (umol/L)	345.30 ± 91.16	349.17 ± 96.78	336.85 ± 94.44	2.421	0.089
Crea (umol/L)	68.77 ± 19.49	71.87 ± 35.99	65.66 ± 17.94	7.835	0.000
eGFR ($ml/min/1.73m^2$)	97.42 ± 18.58	95.44 ± 21.78	100.28 ± 18.69	8.196	0.000
UACR (mg/g)	60.79 ± 280.66	103.63 ± 422.70	96.65 ± 363.17	2.202	0.111
DKD (%)	90 (16.6)	131 (24.2)	169 (31.3)	31.817	0.000

FCP, TC, eGFR, HGI were risk factors for DKD. Furthermore, in our study, HGI was found having a strong link with the incidence of DKD. Univariate logistic regression analysis showed that compared with L-HGI group, the risk of DKD in H-HGI group was significantly increased (OR: 2.283, 95% CI: 1.708~ 3.052). After adjusting for age, T2DM duration, SBP, TC, FCP, eGFR, the risk of DKD in H-HGI group was 2.66 times than that in L-HGI group. (Table 3)

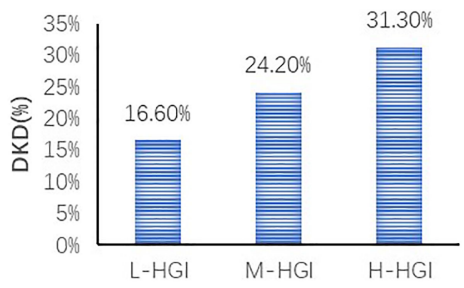

FIGURE 2
Comparison of prevalence of DKD in different HGI groups. We found an increase in the prevalence of DKD in three groups. The difference was statistically significant (P=0.000). Correlation analysis revealed that with the increase of HGI level the prevalence of DKD was increasing (r= 0.140, P=0.000).

3.3 Construction of a risk score for DKD

According to the results, the variables such as HGI, age, T2DM duration, SBP, TC, FCP, eGFR were the key risk factors ($P < 0.05$). We put them into the model, which determined the risk of DKD [DKD risk score =0.212* HGI + 0.042* T2DM duration (year) + 0.023* SBP (mmHg) + 0.164* FCP (ng/ml)– 0.017* age (year)- 0.024* eGFR ($ml/min/1.73m^2$)- 2.301]. The area below the receiver operating characteristics (ROC) curve of this model was 0.702 (95% *CI*: 0.671 - 0.734), which showed good discrimination ability. The sensitivity and specificity corresponding to the maximum Youden index were 0.640% and 0.649%, respectively (Figure 3).

4 Discussion

In this Cross- Sectional study of Chinese adults with T2DM, we found an association between HGI and incident DKD. A dramatic increase in DKD incidence was observed among subjects with higher values of HGI. Our findings suggested that HGI may be a useful predictor of incident DKD among patients with T2DM.

HbA1c value is generally considered the gold standard method for evaluating glycemic control. However, in three large randomized controlled clinical trials (18–20), namely, the Action

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TABLE 2 Binary logistic regression analysis of risk factors for DKD in T2DM.

Variables	DKD in T2DM		
	*β*st	OR (95% CI)	*P*
Age (yeas)	-0.017	0.984 (0.971-0.997)	0.015
T2DM Duration (yeas)	0.042	1.043 (1.024-1.063)	0.000
SBP (mmHg)	0.023	1.023 (1.016-1.030)	0.000
FCP (ng/ml)	0.164	1.179 (1.070-1.299)	0.001
TC (mmol/L)	0.117	1.124 (1.007-1.255)	0.037
eGFR (ml/min/1.73m^2)	-0.024	0.976 (0.968-0.984)	0.000
HGI	0.212	1.236 (1.135-1.346)	0.000

TABLE 3 Logistic regression analysis of the relationship between HGI and DKD in T2DM.

HGI level	Unadjusted model	P^1	Multivariate model[a]	P^2
	OR^1 (95%CI)		OR^2 (95%CI)	
L-HGI	1.000 (ref)	0.000	1.000 (ref)	0.000
M-HGI	1.6019 (1.186-2.161)	0.002	1.414 (1.023-1.995)	0.036
H-HGI	2.283 (1.708-3.052)	0.000	2.660 (1.935-3.657)	0.000

[a]Adjusted for age, T2DM duration, SBP, TC, FCP, eGFR.

to Control Cardiovascular Risk in Patients with Diabetes (ACCORD), ADVANCE and the Veterans Affairs Diabetes Trial (VADT), intensive glycemic control in patients with T2DM did not benefit large blood vessels. Especially in the ACCORD trial, as compared with standard therapy, intensive therapy to target normal HbA1c levels for 3.5 years increased mortality and did not significantly reduce major cardiovascular events (21). Accordingly, Sheng et al. (22) proposed a hypothesis that HbA1c variability may be related to all-cause mortality of intensive therapy, and conducted a *post hoc* analysis of the ACCORD trial. This study showed that long-term follow-up HbA1c variability was a strong predictor of all-cause mortality. Thus, HbA1c is not a one-size-fits-all indicator of blood glucose control. This phenomenon might be attributed to various biological factors, including genetic predisposition, erythrocyte turnover rates, intracellular glucose concentrations, intracellular or extracellular pH, lipid peroxides, inorganic phosphates, hemoglobin oxygenation status, cellular redox status, and the activity of non-enzymatic protein glycation (9, 23).

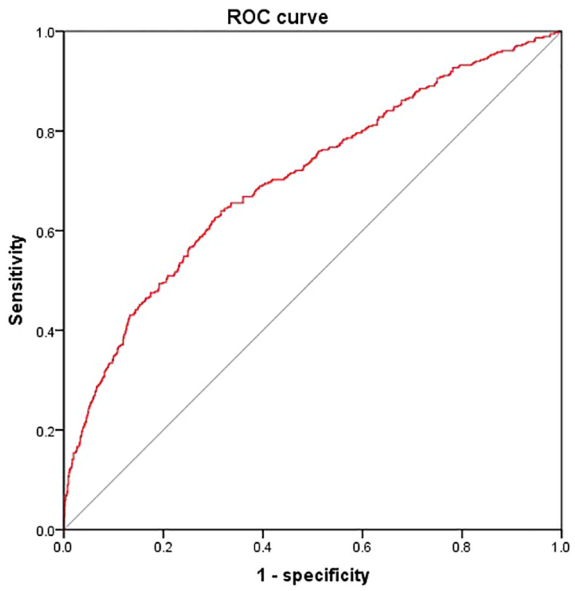

FIGURE 3
Receiver operating characteristics curves of DKD risk score. We put HGI, age, T2DM duration, SBP, TC, FCP and eGFR into the model The area below the ROC curve of this model was 0.702 (95%*CI*: 0.671 - 0.734). The sensitivity and specificity corresponding to the maximum Youden index were 0.640% and 0.649% respectively.

Increasing evidence is supporting the role of glucose variability (GV) in the development of diabetic complications (24). Studies in recent years (25) have shown that HbA1c levels vary greatly among individuals, and some patients may have high or low HbA1c levels inconsistent with blood glucose control levels, which brings some difficulties to clinical prognosis assessment based on this indicator. HGI can identify people with HbA1c levels that are higher or lower than average compared to other people with the same blood glucose concentration (8, 9). Biological sources of HGI variation include genetic and environmental factors that affect person-to-person variation in HbA1c or blood glucose. In our study, we also found that only 40.5% of HbA1c variation can be explained by FPG. In addition, Sabanayagam C. et al. (26) designed a study to determine whether the relationship of HbA1c to diabetic microvascular

complications showed any natural thresholds that could be useful in diagnosing diabetes. There data supported use of an HbA1c cut-off point of between 6.6 and 7.0% in diagnosing diabetes. Any retinopathy, CKD, albuminuria and peripheral neuropathy were less well detected at these cut-off points. Our study also suggested that the incidence of DKD in L-HGI group [HbA1c (6.99 ± 1.34) %] is lowest, similar to previous studies.

Before 2015, several studies shown a positive association between GV and diabetic complications, both macrovascular and microvascular (27). Since 2015, new evidence has also emerged in support of GV as an independent risk factor for total mortality and death due to cardiovascular disease in both type 1 and type 2 diabetes (11, 28–32). R. J. McCarter et al. (12) concluded that individual biological variation in HbA1c, which is distinct from that attributable to mean blood glucose (MBG), was evident among type 1 diabetic patients in DCCT and was a strong predictor of risk for diabetic complications. At 7 years' follow-up, patients in H-HGI had three times greater risk of retinopathy (30 *vs*. 9%, $P < 0.001$) and six times greater risk of nephropathy (6 *vs*.1%, $P < 0.001$) compared with the L-HGI. The individual variation in HbA1c observed in DCCT was attributable to biological variation and not measurement error. Evidence of a link between biological variation in HbA1c and microvascular complications in DCCT suggested that factors responsible for biological variation in nonenzymatic HbA1c may also influence individual susceptibility to diabetic complications. Chih-Hung Lin et al. (14) found that a high HGI predicted rapid renal function decline without or with a resultant eGFR < 60 ml/min/1.73m^2, but not onset of macroalbuminuria followed for a median of 7.3 years. Thus, HGI independently predicted renal function deterioration in patients with T2DM and a low CKD risk. In patients with T2DM, HbA1c variability affects CKD more than average HbA1c (33). Even in nondiabetic individuals, studies have reported the effect on HGI and kidney dysfunction and CKD among the non-diabetic individuals and the adults with hypoglycemic drug naive prediabetes and diabetes. Teresa Vanessa Fiorentino et al. concluded that HGI may be a useful tool to identify nondiabetic individuals with an increased risk of having kidney dysfunction (34). Wonjin Kim et al. found an association between HGI and incident CKD. High HGI was associated with an increased risk of incident CKD. Regardless of HbA1c value, subjects with higher values of HGI were at a higher risk of incident CKD during the 10-year follow-up period (35).

In the ACCORD population, baseline age, BMI, SBP, DBP, fasting serum glucose, TC and SCr had significant difference compared with low HbA1c variability, while sex and LDL-C had not (22). Most of these results have been confirmed in our study but of BMI and LDL-C, suggesting that the influencing factor of HGI in T2DM is complex and that the causes are multifactorial. In addition, further logistic regression analysis showed that the age, T2DM duration, SBP, TC, FCP, 2h-CP, eGFR and HGI were the key risk factors of DKD in this study. The risk of DKD in T2DM patients with high HGI levels is 2.283 times higher than those with low HGI levels. Adjusted to multiple factors, this trend still remained significant (OR: 2.660, 95%CI: 1.935~ 3.657). Furthermore, we constructed a risk score based on HGI to predict a person's risk of DKD, which could be useful for the clinician.

At present, the mechanism of HGI on DKD in T2DM is still unclear. The possible mechanisms involve the following aspects: (1) Fluctuation of blood glucose leads to increased oxidative stress, production of inflammatory cytokines and endothelial dysfunction (36, 37). Compared with persistent hyperglycemia, islet β-cell dysfunction and apoptosis increased significantly in the state of blood glucose fluctuation which leading to decrease of insulin secretion (38). (2) Fluctuated blood glucose deteriorated the progression of DKD by increasing the blood urea nitrogen and sCr, decreasing creatinine clearance, and accelerating renal ultrastructural injury. This adverse result was probably due to its promoting oxidative stress activity and the p-AKT signaling pathway inhibition, which activated its downstream proteins, resulting in severe renal injury (39). (3) One possible explanation is that even periods of sustained hyperglycemia are "remembered," thus conferring an increased risk of microvascular complications (40, 41), hence, the detrimental effect of HbA1c variability may be mediated through the same mechanism underlying the "metabolic memory" phenomenon, including oxidative stress. (4) Because the risk of microvascular complications increases exponentially as HbA1c rises (42), subjects with higher HbA1c variability would "accumulate" a surplus of risk in the periods spent at the upper end of their HbA1c range. This hypothesis might be indirectly supported by GIUSEPPE PENNO's observation that the effect of HbA1c variability is a statistically significant effect in the higher quartile of HbA1c-SD (33).

5 Conclusion

To sum up, HGI may be a reference index for blood glucose control in T2DM patients and could predict the risk of DKD. This study brings important enlightenment for daily diabetes management, that is, diabetes patients should take into account the variability of HbA1c while controlling blood glucose or HbA1c levels. "Beyond HbA1c" is an important concept of diabetes diagnosis and treatment at present (43). Blood glucose, glycosylated albumin and HbA1c variability are important factors for blood glucose control and long-term prognosis. In order to reduce the risk of all-cause death in diabetic patients, measures should be taken as soon as possible to incorporate HbA1c variability into the management objectives of diabetic patients.

This study also has some limitations. As the analysis was conducted on the basis of cross section, the existing database cannot collect relevant data at the time of diagnosis of diabetes. Unfortunately, previous studies have not reported it yet. Next, we will focus on collecting data of new-onset T2DM, and make further exploration on the impact of HGI on DKD. In addition, the subjects have not been followed up to determine the relationship between HGI and DKD in the existing cross-sectional study. However, according to the current research results, HGI has stable disease prediction value. Furthermore, high-quality and large sample prospective cohort studies and randomized controlled clinical trials and even cytological studies will be carried out to clarify the mechanism of HGI and the predictive value of HGI on DKD, and to develop a personalized HbA1c variability control target, so as to

provide new reference indicators for clinical diabetic blood glucose control and reduce the occurrence of complications.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Biomedical Ethics Committee of Peking University International Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

XZhan, SX, XZhao and JD made substantial contributions to the conception and design of the study. SX and JD collected the data. SX and XZhao analyzed the data. SX and XZhan drafted the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Cheng HT, Xu X, Lim PS, Hung KY. Worldwide epidemiology of diabetes-related end-stage renal disease, 2000-2015. *Diabetes Care* (2021) 44:89–97. doi: 10.2337/dc20-1913

2. American Diabetes Association. Microvascular complications and foot care: Standards of medical care in diabetes-2021. *Diabetes Care* (2021) 44:S151–s167. doi: 10.2337/dc21-S011

3. Zhang L, Long J, Jiang W, Shi Y, He X, Zhou Z, et al. Trends in chronic kidney disease in China. *New Engl J Med* (2016) 375:905–6. doi: 10.1056/NEJMc1602469

4. Braga F, Dolci A, Mosca A, Panteghini M. Biological variability of glycated hemoglobin. *Clinica chimica acta;* (2010) 411:1606–10. doi: 10.1016/j.cca.2010.07.030

5. Wilson DM, Xing D, Cheng J, Beck RW, Hirsch I, Kollman C, et al. Persistence of individual variations in glycated hemoglobin: analysis of data from the juvenile diabetes research foundation continuous glucose monitoring randomized trial. *Diabetes Care* (2011) 34:1315–7. doi: 10.2337/dc10-1661

6. Kim MK, Yun KJ, Kwon HS, Baek KH, Song KH. Discordance in the levels of hemoglobin A1C and glycated albumin: Calculation of the glycation gap based on glycated albumin level. *J Diabetes its complications* (2016) 30:477–81. doi: 10.1016/j.jdiacomp.2015.12.022

7. Li K, Song WJ, Wu X, Gu DY, Zang P, Gu P, et al. Associations of serum glucagon levels with glycemic variability in type 1 diabetes with different disease durations. *Endocrine* (2018) 61:473–81. doi: 10.1007/s12020-018-1641-1

8. Hempe JM, Gomez R, McCarter RJJr., Chalew SA. High and low hemoglobin glycation phenotypes in type 1 diabetes: a challenge for interpretation of glycemic control. *J Diabetes its complications* (2002) 16:313–20. doi: 10.1016/S1056-8727(01)00227-6

9. Soros AA, Chalew SA, McCarter RJ, Shepard R, Hempe JM. Hemoglobin glycation index: a robust measure of hemoglobin A1c bias in pediatric type 1 diabetes patients. *Pediatr Diabetes* (2010) 11:455–61. doi: 10.1111/j.1399-5448.2009.00630.x

10. Hempe JM, Liu S, Myers L, McCarter RJ, Buse JB, Fonseca V. The hemoglobin glycation index identifies subpopulations with harms or benefits from intensive treatment in the ACCORD trial. *Diabetes Care* (2015) 38:1067–74. doi: 10.2337/dc14-1844

11. Chen J, Yi Q, Wang Y, Wang J, Yu H, Zhang J, et al. Long-term glycemic variability and risk of adverse health outcomes in patients with diabetes: A systematic review and meta-analysis of cohort studies. *Diabetes Res Clin Pract* (2022) 192:110085. doi: 10.1016/j.diabres.2022.110085

12. McCarter RJ, Hempe JM, Gomez R, Chalew SA. Biological variation in HbA1c predicts risk of retinopathy and nephropathy in type 1 diabetes. *Diabetes Care* (2004) 27:1259–64. doi: 10.2337/diacare.27.6.1259

13. van Steen SC, Woodward M, Chalmers J, Li Q, Marre M, Cooper ME, et al. Haemoglobin glycation index and risk for diabetes-related complications in the action in diabetes and vascular disease: Preterax and diamicron modified release controlled evaluation (ADVANCE) trial. *Diabetologia* (2018) 61:780–9. doi: 10.1007/s00125-017-4539-1

14. Lin CH, Lai YC, Chang TJ, Jiang YD, Chang YC, Chuang LM. Hemoglobin glycation index predicts renal function deterioration in patients with type 2 diabetes and a low risk of chronic kidney disease. *Diabetes Res Clin Pract* (2022) 186:109834. doi: 10.1016/j.diabres.2022.109834

15. Lachin JM, Bebu I, Bergenstal RM, Pop-Busui R, Service FJ, Zinman B, et al. Association of glycemic variability in type 1 diabetes with progression of microvascular outcomes in the diabetes control and complications trial. *Diabetes Care* (2017) 40:777–83. doi: 10.2337/dc16-2426

16. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabetic Med* (1998) 15:539–53. doi: 10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S

17. National Kidney Foundation. KDOQI clinical practice guideline for diabetes and CKD: 2012 update. *Am J Kidney Dis* (2012) 60:850–86. doi: 10.1053/j.ajkd.2012.07.005

18. Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *New Engl J Med* (2008) 358:2560–72. doi: 10.1056/NEJMoa0802987

19. Duckworth W, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, et al. Glucose control and vascular complications in veterans with type 2 diabetes. *New Engl J Med* (2009) 360:129–39. doi: 10.1056/NEJMoa0808431

20. Hemmingsen B, Lund SS, Gluud C, Vaag A, Almdal T, Hemmingsen C, et al. Intensive glycaemic control for patients with type 2 diabetes: systematic review with meta-analysis and trial sequential analysis of randomised clinical trials. *BMJ* (2011) 343:d6898. doi: 10.1136/bmj.d6898

21. Gerstein HC, Miller ME, Byington RP, Goff DCJr., Bigger JT, Buse JB, et al. Effects of intensive glucose lowering in type 2 diabetes. *New Engl J Med* (2008) 358:2545–59. doi: 10.1056/NEJMoa0802743

22. Sheng CS, Tian J, Miao Y, Cheng Y, Yang Y, Reaven PD, et al. Prognostic significance of long-term HbA(1c) variability for all-cause mortality in the ACCORD trial. *Diabetes Care* (2020) 43:1185–90. doi: 10.2337/dc19-2589

23. Fiorentino TV, Marini MA, Succurro E, Andreozzi F, Sciacqua A, Hribal ML, et al. Association between hemoglobin glycation index and hepatic steatosis in non-diabetic individuals. *Diabetes Res Clin Pract* (2017) 134:53–61. doi: 10.1016/j.diabres.2017.09.017

24. Ceriello A, Monnier L, Owens D. Glycaemic variability in diabetes: clinical and therapeutic implications. *Lancet Diabetes Endocrinol* (2019) 7:221–30. doi: 10.1016/S2213-8587(18)30136-0

25. Rodríguez-Segade S, Rodríguez J, Cabezas-Agricola JM, Casanueva FF, Camiña F. Progression of nephropathy in type 2 diabetes: the glycation gap is a significant predictor after adjustment for glycohemoglobin (Hb A1c). *Clin Chem* (2011) 57:264–71. doi: 10.1373/clinchem.2010.144949

26. Sabanayagam C, Liew G, Tai ES, Shankar A, Lim SC, Subramaniam T, et al. Relationship between glycated haemoglobin and microvascular complications: is there a natural cut-off point for the diagnosis of diabetes? *Diabetologia* (2009) 52:1279–89. doi: 10.1007/s00125-009-1360-5

27. Frontoni S, Di Bartolo P, Avogaro A, Bosi E, Paolisso G, Ceriello A. Glucose variability: An emerging target for the treatment of diabetes mellitus. *Diabetes Res Clin Pract* (2013) 102:86–95. doi: 10.1016/j.diabres.2013.09.007

28. Bonke FC, Donnachie E, Schneider A, Mehring M. Association of the average rate of change in HbA1c with severe adverse events: a longitudinal evaluation of audit data from the Bavarian disease management program for patients with type 2 diabetes mellitus. *Diabetologia* (2016) 59:286–93. doi: 10.1007/s00125-015-3797-z

29. Skriver MV, Sandbæk A, Kristensen JK, Støvring H. Relationship of HbA1c variability, absolute changes in HbA1c, and all-cause mortality in type 2 diabetes: a Danish population-based prospective observational study. *BMJ Open Diabetes Res Care* (2015) 3:e000060. doi: 10.1136/bmjdrc-2014-000060

30. Wan EY, Fung CS, Fong DY, Lam CL. Association of variability in hemoglobin A1c with cardiovascular diseases and mortality in Chinese patients with type 2 diabetes mellitus - a retrospective population-based cohort study. *J Diabetes its complications* (2016) 30:1240–7. doi: 10.1016/j.jdiacomp.2016.05.024

31. Lee CL, Sheu WH, Lee IT, Lin SY, Liang WM, Wang JS, et al. Trajectories of fasting plasma glucose variability and mortality in type 2 diabetes. *Diabetes Metab* (2018) 44:121–8. doi: 10.1016/j.diabet.2017.09.001

32. Wightman SS, Sainsbury CAR, Jones GC. Visit-to-visit HbA1c variability and systolic blood pressure (SBP) variability are significantly and additively associated with mortality in individuals with type 1 diabetes: An observational study. *Diabetes Obes Metab* (2018) 20:1014–7. doi: 10.1111/dom.13193

33. Penno G, Solini A, Bonora E, Fondelli C, Orsi E, Zerbini G, et al. HbA1c variability as an independent correlate of nephropathy, but not retinopathy, in patients with type 2 diabetes: the renal insufficiency and cardiovascular events (RIACE) Italian multicenter study. *Diabetes Care* (2013) 36:2301–10. doi: 10.2337/dc12-2264

34. Fiorentino TV, Marini MA, Succurro E, Sciacqua A, Andreozzi F, Perticone F, et al. Elevated hemoglobin glycation index identify non-diabetic individuals at increased risk of kidney dysfunction. *Oncotarget* (2017) 8:79576–86. doi: 10.18632/oncotarget.18572

35. Kim W, Go T, Kang DR, Lee EJ, Huh JH. Hemoglobin glycation index is associated with incident chronic kidney disease in subjects with impaired glucose metabolism: A 10-year longitudinal cohort study. *J Diabetes its complications* (2021) 35:107760. doi: 10.1016/j.jdiacomp.2020.107760

36. Rizzo MR, Barbieri M, Marfella R, Paolisso G. Reduction of oxidative stress and inflammation by blunting daily acute glucose fluctuations in patients with type 2 diabetes: role of dipeptidyl peptidase-IV inhibition. *Diabetes Care* (2012) 35:2076–82. doi: 10.2337/dc12-0199

37. Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *Jama* (2006) 295:1681–7. doi: 10.1001/jama.295.14.1681

38. Kim MK, Jung HS, Yoon CS, Ko JH, Jun HJ, Kim TK, et al. The effect of glucose fluctuation on apoptosis and function of INS-1 pancreatic beta cells. *Korean Diabetes J* (2010) 34:47–54. doi: 10.4093/kdj.2010.34.1.47

39. Ying C, Zhou X, Chang Z, Ling H, Cheng X, Li W. Blood glucose fluctuation accelerates renal injury involved to inhibit the AKT signaling pathway in diabetic rats. *Endocrine* (2016) 53:81–96. doi: 10.1007/s12020-016-0867-z

40. The Writing Team for the Diabetes Control, Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Effect of intensive therapy on the microvascular complications of type 1 diabetes mellitus. *Jama* (2002) 287:2563–9. doi: 10.1001/jama.287.19.2563

41. Ihnat MA, Thorpe JE, Ceriello A. Hypothesis: the 'metabolic memory', the new challenge of diabetes. *Diabetic Med* (2007) 24:582–6. doi: 10.1111/j.1464-5491.2007.02138.x

42. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* (2000) 321:405–12. doi: 10.1136/bmj.321.7258.405

43. Bloomgarden Z. Beyond HbA1c, second take. *J Diabetes* (2019) 11:416–7. doi: 10.1111/1753-0407.12910

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Corrigendum: Association between hemoglobin glycation index and diabetic kidney disease in type 2 diabetes mellitus in China: a cross-sectional inpatient study

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In the published article, there were errors in the list of author affiliations. Instead of "[1]Department of Endocrinology, International Hospital, Peking University, Beijing, China, [2]Department of Nephrology, International Hospital, Peking University, Beijing, China]", it should be "[1]Department of Endocrinology, Peking University International Hospital, Beijing, China, [2]Department of Nephrology, Peking University International Hospital, Beijing, China".

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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Dyslipidemia and serum cystatin C levels as biomarker of diabetic nephropathy in patients with type 2 diabetes mellitus

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Background: Diabetic nephropathy is a leading cause of end-stage renal disease. The diagnostic markers of nephropathy, including the presence of albuminuria and/or a reduced estimated glomerular filtration rate, are not clinically ideal, and most of them are raised after a significant reduction in renal function. Therefore, it is crucial to seek more sensitive and non-invasive biomarkers for the diagnosis of diabetic nephropathy.

Objective of the study: This study aimed to investigate the serum cystatin C levels and dyslipidemia for the detection of diabetic nephropathy in patients with type 2 diabetes mellitus.

Methodology: A hospital-based comparative cross-sectional study was conducted from December 2021 to August 2022 in Tikur, Anbessa specialized teaching hospital with a sample size of 140 patients with type2 diabetes mellitus. Socio-demographic data was collected using a structured questionnaire, and 5

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mL of blood was collected from each participant following overnight fasting for biochemical analyses.

Results: In type 2 diabetes patients with nephropathy, we found significant lipoprotein abnormalities and an increase in serum cystatin C ($P < 0.001$) compared to those without nephropathy. Serum cystatin C, systolic blood pressure, fasting blood glucose, total cholesterol, triglyceride, low density lipoprotein, very low-density lipoprotein, high density lipoprotein, and duration of diabetes were identified as being significantly associated with diabetic nephropathy ($P < 0.05$) in multivariable logistic regression analysis. The mean values of total cholesterol levels, triglyceride levels, and high-density lipoprotein cholesterol levels were also found to be significantly higher ($P < 0.05$) in females as compared to male type-2 diabetic patients. The fasting blood glucose levels and lipid profiles of the participants were found to be significantly associated with serum cystatin C levels.

Conclusion: The present study found significant serum cystatin C and lipoprotein abnormalities in T2DM patients with diabetic nephropathy when compared with those without diabetic nephropathy, and these lipoprotein abnormalities were significantly associated with serum cystatin C levels.

KEYWORDS

diabetes, diabetic nephropathy, serum cystatin C, serum creatinine, dyslipidemia

Background

Diabetes mellitus (DM) is a common metabolic disorder resulting from insufficient insulin secretion or insulin action that leads to elevated blood glucose levels (1). The metabolic disorders associated with DM cause secondary pathophysiological changes in multiple organ systems caused by acute and chronic complications (2). Diabetic nephropathy is one of the major chronic microvascular complications of diabetic and a leading cause of end-stage renal disease (ESRD) (3), and it is predominantly due to type 2 diabetes mellitus (T2DM) (4). Hyperglycemia is the main driving force behind the development of chronic complications of diabetic, including DKD (5). There are hypotheses that have been widely supported by scholars about how hyperglycemia causes diabetic complications (including DN), which are the polyol pathway, the hexosamine pathway (HBP), the production of AGEs, and PKC. It stimulates the resident and non-resident renal cells to produce humoral mediators and cytokines that can lead to functional and phenotypic changes in renal cells and tissues, interference with cell growth, interacting proteins, advanced glycation end products (AGEs), etc., ultimately resulting in glomerular and tubular damage and the onset of kidney disease. Therefore, poor blood glucose control is a particularly important risk factor for the development of DN.

T2DM Patients with diabetic nephropathy are often associated with abnormal lipid metabolism and lipid deposition (6). This altered lipid profile is termed "diabetic dyslipidemia," which includes high plasma levels of total cholesterol, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and triglycerides (TG) and a lower concentration of high-density lipoprotein (HDL). Several studies suggest that diabetic dyslipidemia plays a major role in the pathogenetic development of diabetic nephropathy in T2DM patients (7, 8). However, this has not been well studied in most of the sub-Saharan African countries, including Ethiopia.

The diagnostic markers of nephropathy, including the presence of albuminuria and/or a reduced estimated glomerular filtration rate (eGFR), are not clinically ideal, and most of them are raised after a significant reduction in renal function (9, 10). Kidney biopsy is the gold standard for differentiating diabetic nephropathy from non-diabetic nephropathy (11), but it is invasive and not suitable for routine clinical practice. Therefore, it is crucial to investigate the pathogenesis and seek more sensitive and non-invasive biomarkers for the diagnosis of diabetic nephropathy.

Abbreviations: DM, Diabetes mellitus, T2DM, type 2 diabetes mellitus; ESRD, end-stage renal disease, very VLDL, low-density lipoprotein; LDL, low-density lipoprotein, TG, triglycerides; HDL, high-density lipoprotein, TC, total cholesterol, eGFR, estimated glomerular filtration rate; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; rmp, revolutions per minute; FBG, fasting blood glucose; BUN, blood urea nitrogen; EPHI, Ethiopian Public Health Institute; SPSS, Statistical Package for Social Sciences, CI, confidence interval; DRERC, Department of Ethics and Research Committee; Cys-C, Cystatin C; Scr; Serum creatinine, AOR, adjusted odds ratio; SD, standard deviation.

Human cystatin C is a member of the cystatin superfamily (inhibitors of cysteine proteinases) and a nonglycosylated basic protein with a low molecular mass (13 kD) that is freely filtered by the glomerulus and produced by all nucleated cells (12, 13). Studies have reported that unlike serum creatinine, serum cystatin C is not influenced by age, sex, muscle mass, or dietary intake (14, 15). It is currently under investigation as a promising marker and a replacement for serum creatinine and other renal markers for the early detection of renal impairment (16). Recent studies showed that cystatin C is a more sensitive serum marker for renal function assessment compared to serum creatinine (15, 17). Increased cystatin C and disorders of lipid metabolism are associated with diabetic nephropathy in T2DM patients (18). Therefore, this study aimed to explore the clinical significance of serum cystatin C and lipid profile abnormalities for the differential diagnosis of diabetic nephropathy and non-diabetic nephropathy in patients with T2DM.

Methods and materials

Study design, settings, and participants

A hospital-based comparative cross-sectional study was conducted from December 2021 to August 2022 in Tikur, Anbessa specialized teaching hospital, Addis Ababa, Ethiopia, with the aim of evaluating the serum cystatin C and lipid profiles as markers of diabetic nephropathy in known type 2 diabetic patients who were on regular follow-up at the diabetic clinic of Tikur, Anbessa specialized hospital. A total of 140 eligible T2DM patients who had follow-ups at the diabetic center of Tikur Anbesa specialized hospital during the study period were recruited for this study. We classified the T2DM participants into those with and without nephropathy. The first group included a total of 83 T2DM patients without nephropathy, and the second group included 57 T2DM patients with nephropathy diagnosed by physicians. We excluded patients who had any of the other complications of diabetic, medical or surgical problems, were pregnant women, or had taken lipid-lowering drugs from the study.

A structured questionnaire was used to collect socio-demographic data, including age, sex, address, history, and duration of diabetes. Anthropometric measurements such as weight, height, body mass index (BMI), and blood pressure (BP) were determined using appropriate standardized devices. The height and weight of the participants were measured following standard protocols. Then, BMI was calculated *via* weight in kilograms divided by height in meters squared (BMI = Kg/m^2). Furthermore, blood pressure was measured using a digital automatic BP monitor apparatus for three consecutive times (five minutes apart), and the average values for both diastolic and systolic BP were considered for this study.

Maintaining all aseptic precautions, 5 mL of blood was collected following overnight fasting from the ante-capital vein of each volunteer participant by two trained laboratory technicians for biochemical analyses. The blood sample was collected using an appropriate test tube and transferred to a clean, dry serum separator tube. Then it was centrifuged at 3000 revolutions per minute (rpm) at room temperature (20 to 25°C). A pure serum sample was separated and placed in the nunc tube, where it was stored at -20 until biochemical analysis was done.

Fasting blood glucose (FBG) level, serum TC, HDL cholesterol, and TG levels were determined using Dimension EXL 200 System chemistry analyzer through enzymatic method from the serum sample using commercially available kit of auto analyzer. Serum creatinine and blood urea nitrogen (BUN) were also estimated by the enzymatic method. LDL cholesterol and VLDL cholesterol concentrations were calculated using the Friedewald formula (19). The cystatin C levels of serum were measured by a turbidimetric immunoassay test. All biochemical investigations were done on a fully automated analyzer at the National Reference Laboratory for Clinical Chemistry, Ethiopian Public Health Institute (EPHI).

Statistical analysis

Data was entered and analyzed using SPSS version 25. Categorical variables were presented in frequency and percentages. Continuous variables including demographics, clinical and biomedical data were expressed as mean ± standard deviation. An independent sample t-test or Mann–Whitney U test was used for the comparison of variables as appropriate. An adjusted logistic regression and multiple linear regression models were employed to examine the association of predictor variables with diabetic nephropathy. All statistical tests were two-tailed, and p-values <0.05 at a 95% confidence interval (CI), were taken as statistically significant.

Result

The socio-demographic characteristics of the study participants

A total of 140 T2DM patients were recruited for this study (83 without diabetic nephropathy and 53 with diabetic nephropathy). The mean age of all patients was 45.2 ± 13.2 years. From all the subjects involved in the study, 74 (52.9%) were males and the rest 66 (47.1%) were females. Majority of T2DM patients 116(82.9%) were living in urban areas. In all patients, the mean ± SD of BMI was 26.3 ± 3.7 kg/m^2. The mean ± SD of the duration of patients with T2DM was 13.3 ± 8.6 years with the maximum and minimum duration of 36 years and 1year respectively. In T2DM patients with diabetic nephropathy, we found that the TG, TC, LDL, and VLDL cholesterol levels were significantly increased, whereas HDL cholesterol levels were significantly lower ($P < 0.001$) in those patients. The detailed basic characteristics of the study participants are depicted in Table 1.

Clinical parameters and independent predictors of diabetic nephropathy in T2DM patients

Out of 140 T2DM patients, 85 (60.7%) used oral medication, 25 (17.86%) used insulin injection, and 30 (21.4%) used both oral medication and insulin injection. After adjusting for all possible

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TABLE 1 Socio-demographic and clinical profiles of T2DM patients with and without nephropathy.

Variables		All T2DM patients (n=140)	T2DM patients with nephropathy (n=57)	T2DM patients without nephropathy (n=83)	p-value
Age (year), mean ± SD		45.0 ± 13.2	44.0 ± 15.12	46.2 ± 13.18	0.875
Sex	Male n (%)	74 (52.9)	32 (56.1)	42 (50.6)	0.127
	Female n (%)	66 (47.1)	25 (43.9)	41 (49.4)	0.136
Residence	Urban n (%)	116 (82.86)	48 (84.2)	68 (81.9)	0.471
	Rural n (%)	24 (17.14)	9 (15.8)	15 (18.1)	0.812
BMI, mean ± SD		26.3 ± 3.7	26.4 ± 5.98	27.5 ± 3.42	0.052
Duration of T2DM		13.3 ± 8.6	15.3 ± 7.6	12.3 ± 7.6	0.077
FBG		135 ± 14.7	175.2 ± 16.4	87.3 ± 7.1	0.071
SBP (mmHg), mean ± SD		129.2 ± 21.29	139.7 ± 19.29	128.7 ± 21.7	0.025
DBP (mmHg), mean ± SD		80.9 ± 10.24	82.9 ± 9.40	80.8 ± 11.61	0.459
TC (mg/dL) mean ± SD		197.6 ± 42.5	229.2 ± 64.8	164.7 ± 21.4	<0.001
TG (mg/dL) mean ± SD		104.8 ± 47.4.	127.8 ± 39.5	77.3 ± 56.6	<0.001
HDL (mg/dL) mean ± SD		34.7 ± 10.7	27.6 ± 8.91	41.3 ± 9.5	<0.001
LDL (mg/dL) mean ± SD		147 ± 27.1	159.3 ± 38.9	121.7 ± 24.6	<0.001
VLDL (mg/dL) mean ± SD		39 ± 11.5	51.5 ± 29.7	23.9 ± 8.5	<0.001
BUN (mg/dL) mean ± SD		29 ± 7.8	30.6 ± 8.9	26.3 ± 5.7	0.031
Scr (mg/dL) mean ± SD		0.97 ± 0.31	1.1 ± 0.4	0.8 ± 0.3	0.75
Cys-C (mg/dL mean ± SD		1.15 ± 0.6	1.3 ± 0.7	0.73 ± 0.3	<0.001

Comparisons were made using independent sample t-test, Mann-Whitney test, or Chi-square test as appropriate. Categorical variables are presented in frequency and percentage while continuous variables are presented as mean ± SD, P-value<0.05 was considered statistically significant. SD, Standard Deviation; %, percentage; BMI, Body Mass Index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; FBG, Fasting Blood Glucose; TC, Total Cholesterol; TG, Triglyceride; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; VLDL, Very Low Density Cholesterol; BUN, Blood Urea Nitrogen; Cys-C, Cystatin C; Scr, Serum creatinine.

confounders using multivariable logistic regression analysis serum cystatin C (P <0.001), SBP (P = 0.02), FBG (P =0.001), TC (P <0.01), TG (P <0.001), LDL (P <0.001), VLDL (P <0.001), HDL (P<0.061) and duration of T2DM (P =0.04) were identified to be significantly associated with diabetic nephropathy. Increasing serum cystatin C levels by one unit was associated with 1.41 times (AOR: 1.41, 95% CI: 1.09, 2.77) higher odds of having diabetic nephropathy. For a unit increase in the duration of T2DM, there was a 1.32 times (AOR: 1.32, 95% CI: 1.11, 2.87) greater likelihood of having diabetic nephropathy. The odds of T2DM patients with nephropathy significantly increased per a unit rise in SBP, FBG, TC, TG, LDL, and VLDL levels (Table 2).

Comparison of clinical and biomedical parameters between males and females

An independent sample t-test showed that the mean values of TC (P = 0.021), TG (P = 0.011), and HDL cholesterol levels (P = 0.01) were significantly higher in females as compared to male type-2 diabetic patients, but significantly lower in serum creatinine levels (P = 0.03) as compared to males (Table 3).

Multiple linear regression analysis of serum cystatin C with clinical and biomedical parameters in type 2 diabetic patients

In the multiple linear regression analyses, the SBP, FPG, TC, TG, HDL, LDL, and VLDL of the T2DM participants were significantly associated with serum cystatin C levels (Table 4).

Discussion

This study aimed to evaluate serum cystatin C and lipid profiles for assessing the potential predictor markers of diabetic nephropathy in patients with T2DM. High serum creatinine and low cytostatin C levels were found in males, whereas low creatinine and high cystatin C levels were found in females. It is well known that the creatinine level is highly affected by muscle mass, which means that males are more muscular than females. But the cystatin C difference needs further study as it is a promising marker currently under investigation.

The study found significantly higher serum cystatin C levels and SBP and lipid profile abnormalities in type 2 diabetics with

TABLE 2 Independent factors for predicting diabetic nephropathy in T2DM patients.

Variables	AOR (95% CI)	P - value
Age (in years)	0.97 (0.87-1.07)	0.381
BMI (kg/m2)	1.32 (0.44-1.89)	0.125
SBP (mmHg)	1.731 (1.01-1.072)	0.02
DBP (mmHg)	0.92 (0.95-1.022)	0.17
FBG (mg/dL)	1.30 (1.11-2.87)	0.01
TC (mg/dL)	1.5 (1.01-2.91)	<0.001
TG (mg/dL)	1.41 (1.21-2.66)	<0.001
HDL (mg/dL)	0.67 (1.23-2.57)	0.061
LDL (mg/dL)	1.21 (1.07-2.47)	<0.001
VLDL (mg/dL)	1.3 (1.11-2.87)	<0.001
BUN (mg/dL)	0.96 (0.71-1.95)	0.131
Scr (mg/dL)	0.86 (0.91-2.95)	0.07
Cys-C C (mg/dL)	1.41 (1.09-2.77)	<0.001
Diabetes duration (in years)	1.32 (1.11-2.87)	0.04

Mean + SD is significant at the p< 0.05; AOR, adjusted odds ratio; CI, confidence interval; BMI, Body Mass Index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; FB, Fasting Blood Glucose; TC, Total Cholesterol; TG, Triglyceride; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; VLDL, Very Low Density Cholesterol; BUN, Blood Urea Nitrogen; Cys-C, Cystatin C; Scr, Serum creatinine.

nephropathy compared to those without nephropathy. These findings are similar to other previous studies (18, 20). In contrast to these findings, the study conducted by Jing Wei et al. revealed that the serum parameters, including HDL and LDL, were not significantly different between T2DM patients with nephropathy and those without diabetic nephropathy (21). However, in the case of serum cystatin C levels, TC, and TG, this study agreed with the findings of the present study, and unlike our finding, it found a significant difference in serum creatinine in type 2 diabetics with nephropathy compared to those without nephropathy.

There is growing evidence that abnormalities in lipid metabolism contribute to renal disease progression (6). In T2DM patients with diabetic nephropathy, we found that HDL cholesterol levels were significantly low in T2DM patient with nephropathy and other parameters of lipoproteins were significantly higher. Numerous experiments studies explained the mechanism as the hyperlipidemia can cause glomerulosclerosis and tubulointerstitial sclerosis by stimulating resident renal cells to produce fibrotic cytokines and chemokines, leading to infiltration of monocytes or macrophages into glomerular tissue to promote extracellular matrix deposition and have shown that lipid accumulation, lipid toxicity and lipid metabolism disorders are related to diabetic kidney damage (6, 22).

Several studies found that the levels of serum cystatin C rose earlier than creatinine (22, 23). In this study we found that the cystatin C level of the type 2 DM is increased significantly and associated with lipoproteins in T2DM patients diagnosed nephropathy. This showed that the serum cystatin C level is related to subclinical tubular impairment and can be an earlier marker of renal disease in diabetic patients before the onset of other renal markers. This is in agreement with some other earlier studies (23, 24).

In the multivariate regression analysis, we found that the serum cystatin C level is significantly associated with FBG, TC, TG, HDL, LDL, and VLDL ($P < 0.001$). However, it is insignificantly associated with serum creatinine, duration of DM, BMI, BUN,

TABLE 3 Comparison of clinical and biomedical parameters between males and females. .

Variables	Sex of participants		P - value
	Male (n=74)	Female (n=66)	
Age (in years)	53.7 ± 8.1	48.9 ± 5.8	0.27
BMI (kg/m^2)	25.78 ± 5.44	23.6 ± 7.3	0.15
DBP (mmHg)	85.7 ± 10.74	82.9 ± 9.94	0.21
SBP (mmHg)	128.3 ± 20.32	125.8 ± 18.72	0.49
FBG (mg/dL)	89.9 ± 6.1	129.2 ± 16.2	0.07
TC (mg/dL)	182.7 ± 20.4	227.2 ± 63.8	0.021
TG (mg/dL)	121.8 ± 38.5	168.3 ± 76.6	0.011
HDL (mg/dL)	32.3 ± 6.5	42.6 ± 8.9	0.01
LDL (mg/dL)	138.7 ± 24.6	158.3 ± 48.9	0.501
VLDL (mg/dL)	24.9 ± 8.5	50.5 ± 28.7	0.101
BUN (mg/dL)	26.3 ± 6.7	28.6 ± 8.7	0.24
Scr (mg/dL)	1.2 ± 0.3	0.8 ± 0.4	0.03
Cys-C (mg/dL)	0.73 ± 0.2	1.3 ± 0.4	0.081

Comparisons were made using independent sample t-test, P-value<0.05 was considered statistically significant. BMI, Body Mass index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; FB, Fasting Blood Glucose; TC, Total Cholesterol; TG, Triglyceride; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; VLDL, Very Low Density Cholesterol; BUN, Blood Urea Nitrogen; Cys-C, Cystatin C; Scr, Serum creatinine.

TABLE 4 Multiple linear regression analysis of serum cystatin C with clinical and biomedical parameters in type 2 diabetic patients.

Variables	Serum cystatin C	
	β (95% CI)	P-value
Sex of participant	-0.061	0.481
Age of participant	0.009	0.215
Duration of DM	0.066	0.303
BMI (kg/m^2)	0.011	0.613
SBP (mmHg)	0.12	0.021
DBP (mmHg)	0.07	0.571
FPG (mg/dL)	0.54	<0.001
TC (mg/dL)	0.75	<0.001
TG (mg/dL)	0.87	<0.001
HDL (mg/dL)	-0.25	0.023
LDL (mg/dL)	0.46	0.016
VLDL (mg/dL)	0.43	0.003
BUN (mg/dL)	0.06	0.324
Scr (mg/dL)	0.032	0.451

Analysis was made using multiple linear regression analysis, P-value<0.05 was considered statistically significant. BMI, Body Mass index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; FBG, Fasting Blood Glucose; TC, Total Cholesterol; TG, Triglyceride; HDL-C, High-Density Lipoprotein Cholesterol; LDL-C, Low-Density Lipoprotein Cholesterol; VLDL, Very Low-Density Cholesterol; BUN, Blood Urea Nitrogen; Cys-C, Cystatin C; Scr, Serum creatinine.

and the age of the participant. This was in line with study conducted by Kachhawa et al. (18).

This study also revealed the mean values of TC, and TG were significantly higher ($p < 0.05$) in females as compared to male type-2 diabetic patients, but low in HDL and serum creatinine levels as compared to males. Another study conducted by Radzeviciene et al., support these results on type one diabetic patients (19, 25).

Conclusion

We found that serum cystatin C is not significantly affected by sex, unlike serum creatinine, and a significant difference was found between T2DM patients with nephropathy and those without nephropathy. The present study also found significant lipoprotein abnormalities in T2DM patients with diabetic nephropathy when compared with those without diabetic nephropathy, and these lipoprotein abnormalities were significantly associated with serum cystatin C levels.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Addis Ababa University Department of Ethics and Research Committee (DRERC). The patients/participants provided their written informed consent to participate in this study.

Author contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, data acquisition, analysis, and interpretation, or all these areas; took part in drafting, revising, or critically reviewing for important intellectual content. All authors have agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Asmamaw T, Genet S, Menon M, Tarekegn G, Chekol E, Geto Z, et al. Early detection of renal impairment among patients with type 2 diabetes mellitus through evaluation of serum cystatin c in comparison with serum creatinine levels: A cross-sectional study. *Diabetes Metab Syndrome Obesity: Targets Ther* (2020) 13:4727–35. doi: 10.2147/DMSO.S279949

2. Zhou B, Zou H, Xu G. Clinical utility of serum cystatin c in predicting diabetic nephropathy among patients with diabetes mellitus: a meta-analysis. *Kidney Blood Pressure Res* (2016) 41(6):919–28. doi: 10.1159/000452593

3. Zhang XX, Kong J, Yun K. Prevalence of diabetic nephropathy among patients with type 2 diabetes mellitus in China: A meta-analysis of observational studies. *J Diabetes Res* (2020) 2020:2315607. doi: 10.1155/2020/2315607

4. Shahwan MJ, Gacem SA, Zaidi SK. Prevalence of diabetic nephropathy and associated risk factors among type 2 diabetes mellitus patients in ramallah, Palestine. *Diabetes Metab Syndrome* (2019) 13(2):1491–6. doi: 10.1016/j.dsx.2019.02.017

5. Amorim RG, Guedes GDS, Vasconcelos SML, Santos JCF. Kidney disease in diabetes mellitus: Cross-linking between hyperglycemia, redox imbalance and inflammation. *Arq Bras Cardiol* (2019) 112(5):577–87. doi: 10.5935/abc.20190077

6. Chen SC, Tseng CH. Dyslipidemia, kidney disease, and cardiovascular disease in diabetic patients. *Rev Diabetic Studies: RDS* (2013) 10(2-3):88–100. doi: 10.1900/RDS.2013.10.88

7. Hager MR, Narla AD, Tannock LR. Dyslipidemia in patients with chronic kidney disease. *Rev Endocrine Metab Disord* (2017) 18(1):29–40. doi: 10.1007/s11154-016-9402-z

8. Opazo-Ríos L, Mas S, Marín-Royo G, Mezzano S, Gómez-Guerrero C, Moreno JA, et al. Lipotoxicity and diabetic nephropathy: Novel mechanistic insights and therapeutic opportunities. *Int J Mol Sci* (2020) 21(7):2632. doi: 10.3390/ijms21072632

9. Thipsawat S. Early detection of diabetic nephropathy in patient with type 2 diabetes mellitus: A review of the literature. *Diabetes Vasc Dis Res* (2021) 18 (6):14791641211058856. doi: 10.1177/14791641211058856

10. Pugliese G, Penno G, Natali A, Barutta F, Di Paolo S, Reboldi G, et al. Diabetic kidney disease: new clinical and therapeutic issues. joint position statement of the Italian diabetes society and the Italian society of nephrology on "The natural history of diabetic kidney disease and treatment of hyperglycemia in patients with type 2 diabetes and impaired renal function". *J Nephrol* (2020) 33(1):9–35. doi: 10.1007/s40620-019-00650-x

11. Zhang W, Liu X, Dong Z, Wang Q, Pei Z, Chen Y, et al. New diagnostic model for the differentiation of diabetic nephropathy from non-diabetic nephropathy in Chinese patients. *Front Endocrinol* (2022) 13:913021. doi: 10.3389/fendo.2022.913021

12. Sapkota S, Khatiwada S, Shrestha S, Baral N, Maskey R, Majhi S, et al. Diagnostic accuracy of serum cystatin c for early recognition of nephropathy in type 2 diabetes mellitus. *Int J Nephrol* (2021) 2021. doi: 10.1155/2021/8884126

13. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al. Estimating glomerular filtration rate from serum creatinine and cystatin c. *New Engl J Med* (2012) 367(1):20–9. doi: 10.1056/NEJMoa1114248

14. Tangri N, Stevens LA, Schmid CH, Zhang YL, Beck GJ, Greene T, et al. Changes in dietary protein intake has no effect on serum cystatin c levels independent of the glomerular filtration rate. *Kidney Int* (2011) 79(4):471–7. doi: 10.1038/ki.2010.431

15. Baxmann AC, Ahmed MS, Marques NC, Menon VB, Pereira AB, Kirsztajn GM, et al. Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin c. *Clin J Am Soc Nephrol* (2008) 3(2):348–54. doi: 10.2215/CJN.02870707

16. Arceo ES, Dizon GA, Tiongco REG. Serum cystatin c as an early marker of nephropathy among type 2 diabetics: A meta-analysis. *Diabetes Metab Syndrome* (2019) 13(6):3093–7. doi: 10.1016/j.dsx.2019.11.007

17. Amer AH, Haridas N. Early diagnostic markers in diabetic nephropathy patients. *J Clin Diagn Res* (2018) 12(11). doi: 10.7860/JCDR/2018/36574.12203

18. Knopfholz J, Disserol CC, Pierin AJ, Schirr FL, Streisky L, Takito LL, et al. Validation of the friedewald formula in patients with metabolic syndrome. *Cholesterol* (2014) 2014:261878. doi: 10.1155/2014/261878

19. Stankute I, Radzeviciene L, Monstaviciene A, Dobrovolskiene R, Danyte E, Verkauskiene R. Serum cystatin C as a biomarker for early diabetic kidney disease and dyslipidemia in young type 1 diabetes patients. *Medicine (Kaunas, Lithuania)* (2022) 58 (2):218 doi: 10.3390/medicina58020218

20. Wei J, Wang B, Shen FJ, Zhang TT, Duan Z, Zhou DM. Diagnostic value of triglyceride and cystatin c ratio in diabetic kidney disease: a retrospective and prospective cohort study based on renal biopsy. *BMC Nephrol* (2022) 23(1):270. doi: 10.1186/s12882-022-02888-3

21. Su W, Cao R, He YC, Guan YF, Ruan XZ. Crosstalk of hyperglycemia and dyslipidemia in diabetic kidney disease. *Kidney Dis* (2017) 3(4):171–80. doi: 10.1159/000479874

22. Murty MS, Sharma UK, Pandey VB, Kankare SB. Serum cystatin c as a marker of renal function in detection of early acute kidney injury. *Indian J Nephrol* (2013) 23 (3):180–3. doi: 10.4103/0971-4065.111840

23. Woo KS, Choi JL, Kim BR, Kim JE, Han JY. Clinical usefulness of serum cystatin c as a marker of renal function. *Diabetes Metab J* (2014) 38(4):278–84. doi: 10.4093/dmj.2014.38.4.278

24. Papadopoulou-Marketou N, Chrousos GP, Kanaka-Gantenbein C. Diabetic nephropathy in type 1 diabetes: a review of early natural history, pathogenesis, and diagnosis. *Diabetes/metabol Res Rev* (2017) 33(2). doi: 10.1002/dmrr.2841

25. Nayak BS, Butcher DM, Bujhawan S, Chang D, Chang S, Cabral-Samaroo D, et al. Association of low serum creatinine, abnormal lipid profile, gender, age and ethnicity with type 2 diabetes mellitus in Trinidad and Tobago. *Diabetes Res Clin Pract* (2011) 91(3):342–7. doi: 10.1016/j.diabres.2010.12.017

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Role of sex hormones in diabetic nephropathy

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Diabetic nephropathy (DN) is the most common microvascular complication in diabetes and one of the leading causes of end-stage renal disease. The standard treatments for patients with classic DN focus on blood glucose and blood pressure control, but these treatments can only slow the progression of DN instead of stopping or reversing the disease. In recent years, new drugs targeting the pathological mechanisms of DN (e.g., blocking oxidative stress or inflammation) have emerged, and new therapeutic strategies targeting pathological mechanisms are gaining increasing attention. A growing number of epidemiological and clinical studies suggest that sex hormones play an important role in the onset and progression of DN. Testosterone is the main sex hormone in males and is thought to accelerate the occurrence and progression of DN. Estrogen is the main sex hormone in females and is thought to have renoprotective effects. However, the underlying molecular mechanism by which sex hormones regulate DN has not been fully elucidated and summarized. This review aims to summarize the correlation between sex hormones and DN and evaluate the value of hormonotherapy in DN.

KEYWORDS

sex hormones, estrogen, testosterone, diabetic nephropathy, metabolism

Introduction

Diabetic nephropathy (DN) is one of the most common and serious complications of diabetes mellitus and a major cause of chronic kidney disease and end-stage renal disease (ESRD) (1–4). The occurrence and progression of DN are closely related to patient blood glucose levels, blood pressure, genetic background and age (5, 6). Unlike other renal diseases, once macroalbuminuria occurs, DN will remain throughout life, which makes DN a major cause of death in patients with diabetes. DN patients at the end stage of renal failure rely on dialysis and kidney transplantation. Therefore, preventing and treating DN has become a pressing problem worldwide. Many studies have shown that the occurrence and development of DN are closely correlated with sex (7). In addition to social roles, psychological cognition and behavioral habits, the most important difference between the sexes is sex hormones. Especially in women, sex hormones vary greatly throughout life, from infancy to adolescence, sexual maturity, pregnancy, perimenopause and

postmenopause. However, the underlying molecular mechanism by which sex hormones regulate DN has not been fully elucidated. Moreover, based on the impact of sex hormone imbalances on the development of DN, hormone therapy in patients with diabetes may alleviate diabetic kidney injury to a certain extent and is a potentially valuable therapeutic strategy for DN patients.

In this article, we summarized the effects of sex hormone changes on DN development by searching and reviewing published articles. We hope our work will provide information on the correlation between sex hormones and DN and provide new clues for the treatment of DN.

Sex hormones

Sex hormones are steroidal hormones synthesized mainly by the gonads, the placenta, and the reticular cortex of the adrenal gland in animals. In female animals, the ovaries mainly secrete two types of sex hormones: estrogen and progesterone. In male animals, the testes secrete androgens, mainly testosterone.

The synthesis of sex hormones is based on cholesterol, which is converted to pregnenolone by cytochrome P-11A (CYP11A). Pregnenolone can be converted to progesterone by 3βHSDI and transported from the outer mitochondrial membrane to the inner mitochondrial membrane by transporters (8). There are two ways to synthesize androstenedione. First, pregnenolone is converted to dehydroepiandrosterone by CYP17 and then to androstenedione; second, progesterone is converted to 17α-hydroxyprogesterone and then to androstenedione (8). Androstenedione is converted to testosterone by the enzyme 17HSD3, which is converted to estradiol *via* aromatase (CYP19) (8, 9). Figure 1 shows the synthesis of sex hormones.

Most sex hormones are metabolically inactivated in a similar manner: by forming more water-soluble conjugates, such as glucuronides or sulfate esters, in metabolic organs, such as the liver and kidneys. These conjugates are then excreted in urine or secreted into the intestine with bile and excreted in feces (10, 11).

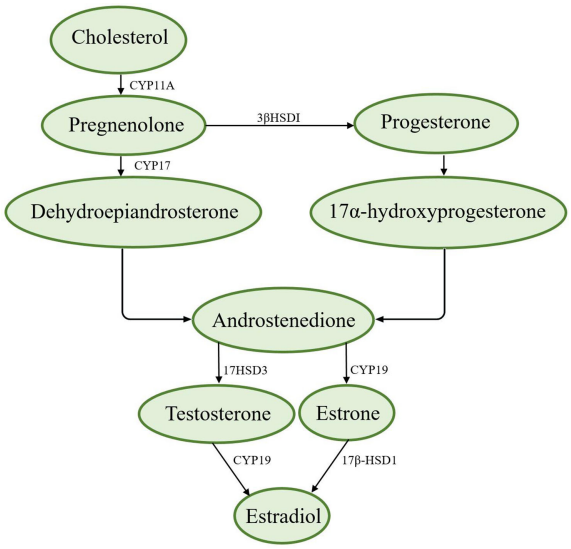

FIGURE 1
Synthesis of sex hormones.

Testosterone

Testosterone is a steroid hormone. It is the main sex hormone and anabolic hormone in the male body and is mainly synthesized by the interstitial cells of the testicles. Other organs, such as the adrenal glands and ovaries, also produce small amounts of testosterone.

Androgen receptor (AR) is encoded by the AR gene on the X chromosome and is widely distributed in various tissues and organs, including the endothelium and kidney (12). AR plays an important role in the development and maintenance of the reproductive, musculoskeletal, cardiovascular, immune, neurological and hematopoietic systems (12, 13). When not bound to testosterone, AR is bound in the cytoplasm by heat shock protein (HSP) and chaperone proteins. When interacting with testosterone or dihydrotestosterone, AR is released from HSP and chaperone proteins and translocates to the nucleus to produce the corresponding biological effects (14). Sankar et al. reported that AR was a key determinant of the response to testosterone, and circulating levels of testosterone can influence spatial cognition in adult males (15).

Estrogen

Estrogens are produced by the placenta and ovaries of female animals and promote the development of secondary sexual characteristics and the maturation of sexual organs in females. There are three main types of estrogens in females, estrone (E1), estradiol (E2) and estriol (E3). These estrogens play important roles in regulating many physiological functions, such as cell proliferation and differentiation, development, body homeostasis and metabolism (16–20). Under physiological and pathophysiological conditions, the effects of estrogen are mediated by estrogen receptors α/β and G protein-coupled estrogen receptors (GPER). These receptors are involved in the development of many diseases, including DN, cancer, neurodegenerative diseases, and cardiovascular, metabolic and autoimmune diseases (21–24).

Alterations in sex hormones in diabetes

Alterations in sex hormones between the sexes

Under physiological conditions, sex hormone levels and their functions in men and women alter with increasing age. Both testosterone and estrogen have been found to decline with age in men and women (25–28). Gambineri et al. summarized the reasons for the difference in circulating sex hormone levels between the

sexes, and they believed that it is due to the difference in the synthesis site of sex hormones, the conversion rate of sex hormones to each other, and the binding degree of sex hormones to sex hormone binding globulin (SHBG) in the two sexes (29).

Alterations in sex hormones in diabetes

Diabetes can cause an imbalance in sex hormones in patients (30). Studies have shown that compared with men without diabetes, men with diabetes have decreased levels of testosterone and increased levels of E2. However, testosterone levels are higher and E2 levels are lower in women with diabetes than in those without diabetes, suggesting that diabetes is associated with an imbalance in sex hormones (30–37). In females with diabetes (compared with females without diabetes), the decreased level of E2 may reduce creatinine clearance and increase urine albumin excretion and tubular fibrosis in the kidney, which may increase the risk of developing renal complications (38).

Insulin levels have a significant impact on the functional regulation of the hypothalamic-pituitary-gonadal axis (HPGA) (39). Normally, insulin is secreted by pancreatic β cells. Then, it binds to insulin receptors and activates intracellular protein tyrosine kinase (PTK). Activated PTK can phosphorylate and activate insulin receptor substrates (IRS) to activate phosphoinositide 3-kinase (PI3K). The activated PI3K signaling cascade enhances gonadotropin-releasing hormone (GnRH) secretion in the hypothalamus, which stimulates the pituitary secretion of luteotropic hormone (LH) and follicle-stimulating hormone (FSH) and eventually induces the release of sex hormones by the gonads (40). Approximately 5% of sex steroids are present in the blood and enter cells through specific receptors on the plasmalemma (41). During diabetes, altered levels of SHBG, increased levels of oxidative stress and increased levels of CYP19 activity are present in adipose tissue. This results in the conversion of testosterone and androstenedione to estradiol and estrone, respectively, which contribute to reducing serum testosterone concentrations in men with diabetes (42–47). In addition, the disruption of glucolipid metabolism, the reduced bioavailability of insulin during diabetes and the reduced activity of CYP19 in the ovaries of diabetic rats, as determined by Bozkurt et al., might be responsible for the reduced levels of estradiol in females with diabetes (48–50).

The level of insulin can be affected by leptin. Leptin is a type of adipokine that is secreted by adipose tissue. It can regulate energy metabolism and may regulate reproductive function by regulating the release of GnRH in the hypothalamus (39, 51, 52). The level of insulin can also affect the generation of leptin (53, 54). Under normal circumstances, leptin phosphorylates IRS-2 on hypothalamic leptin receptors, activating PI3K and stimulating the release of GnRH (55, 56). Under diabetic circumstances, the feedback between insulin and leptin is disordered, thus impairing the release of GnRH and ultimately reducing sex hormone secretion.

In contrast, altered levels of sex hormones may be a predisposing factor for diabetes. CYP19 is the limiting enzyme for estradiol synthesis. Jones et al. found that in aromatase-knockout (ArKO) female mice, glucose oxidation was decreased and obesity and insulin levels were increased (57). A study showed that decreased CYP19 activity combined with low concentrations of dihydrotestosterone (DHT) downregulates the expression of transforming growth factor-β (TGF-β) and type IV collagen and inhibits the level of glomerulosclerosis and tubular interstitial fibrosis, thus attenuating the progression of renal complications in male diabetic rats (35). Takeda et al. showed that a short-term E2 treatment could reverse the development of glucose intolerance and insulin resistance by enhancing lipid metabolism in male ArKO mice (58).

Role of sex hormones in the development of DN

Sex differences in the development of DN

The occurrence and development of DN are affected by sex to a great extent (59–61). Observations in humans and animals showed that the level of sex steroids in males and females are altered by DN. Plasma testosterone levels in men were decreased to levels similar to those in women, while plasma estradiol levels in women were decreased to levels similar to those in men. In many DN models, male animals tend to progress more quickly than female animals. In type 1 and type 2 diabetes, the prevalence of microproteinuria and macroproteinuria is higher in males than in females, and the risk of microproteinuria and progression to macroproteinuria is also higher (7, 62–65). This phenomenon is also seen in nondiabetic renal diseases. Neugarten et al. found that men with chronic renal disease show a more rapid decline in renal function than women with chronic renal disease (66).

However, other studies showed an opposite result, as they reported that women with diabetes have a higher risk of progressing to ESRD than men with diabetes (67). When the women with diabetes included in the statistics were older (postmenopausal), they had a higher rate of progression to ESRD (68). In the Irbesartan DN trial and the angiotensin II (AngII) receptor antagonist Losartan study, postmenopausal women with diabetes developed end-stage renal disease at a faster rate than men with diabetes (69, 70). In addition, age at diagnosis of type 1 diabetes also has an impact on the timing of the onset of ESRD in both sexes. Men diagnosed with type 1 diabetes before puberty had a delayed onset of ESRD, while women diagnosed at puberty face a higher risk of ESRD (71, 72).

Role of testosterone in DN

The risk of renal complications in men with diabetes is higher than that in premenopausal women with diabetes. Testosterone is considered to be more conducive to the genesis of DN in males. Kang et al. reported that men have a higher risk of renal complications (73). Sharon et al. reported that the decrease in testosterone may partly attenuate kidney injury in males (74). Jan et al. reported that men with type 1 diabetes have a higher risk of ESRD and mortality (75). In contrast, the effect of testosterone on

the progression of DN in females with diabetes is rarely mentioned, and females are considered to be less influenced by testosterone (73).

Role of estrogen in DN

Changes in estrogen levels affect the occurrence of DN, and estrogen may have different effects in males and females with diabetes (76, 77). As mentioned above, the level of circulating testosterone in men with diabetes is decreased, while the level of E2 is increased (33, 36, 37). The increased level of E2 may increase the risk of renal complications in men (38, 60, 78). In male STZ-induced diabetic rats, inhibition of testosterone transformation to estradiol attenuates inflammation and the expression of type IV collagen and TGF-β; hence, the progression of DN is reduced (78).

As the most important sex hormone in women, estrogen has been shown to prevent podocyte apoptosis. Estrogen can also inhibit type I/IV collagen synthesis in mesangial cells and promote the degradation of the extracellular matrix, which are critical factors that induce tubular fibrosis (79–81). The effect of estrogen on the female kidney may vary at the postmenopause stage. William et al. reported that women at the postmenopause stage have a higher risk of renal complications (70). Lewis et al. found that kidney function was reduced in women with diabetes with an average age of 58 (69). Studies have shown that women who undergo ovariectomy (OVX) have a higher risk of diabetes and other complications (82–84). Mankhey et al. reported that in STZ-induced diabetic female rats, OVX could enhance DN, whereas 17-β-estradiol replacement therapy could attenuate DN (38). Therefore, estrogen is considered to have a renal protective function in women with diabetes.

Sex hormones affect the genesis of DN and its underlying mechanisms

Patients with diabetes who progress to nephropathy have significantly higher initial mean blood pressure, cholesterol, HbA1c, low-density lipoprotein (LDL) cholesterol and triglyceride levels (85). The development of DN includes renal hemodynamic changes, sugar/lipid metabolic disorders, and the effects of oxidative stress and inflammation. These changes cause glomerular basement membrane thickening, mesangial matrix accumulation, glomerular sclerosis and tubular epithelial cell injury, which eventually lead to renal tubular fibrosis, proteinuria and the leakage of large molecules (86–88).

●Oxidative stress and inflammation

In the diabetic state, NADPH oxidases (Nox proteins) are activated to produce excess reactive oxygen species (ROS) through the electron transport chain (89). When too many ROS accumulate, they attack organs, including the kidney, and this is accompanied by the depletion of antioxidants. Additionally, the oxidative/antioxidant system balance is disrupted, resulting in oxidative stress (89, 90). The kidney contains a high density of mitochondria. Excess ROS lead to oxidative damage to mitochondrial proteins and mitochondrial DNA (mtDNA). Then, the kidney fails to filter and reabsorb Na+, glucose and other metabolites from the urine, and vascular permeability is increased (91, 92). Testosterone may reduce the activation of STAT3 to increase the production of ROS (93). Mustafa and Mehmet found that estradiol had positive effects on the antioxidant defense system and tissue lipid peroxidation in OVX diabetic rats, possibly by enhancing the antioxidant activities in the kidney, thus protecting against diabetes (94). Hong et al. found that estrogen can inactivate Nox, inhibit the production of superoxide anions, and reduce oxidative stress in the kidney, thus reducing kidney injury (95, 96).

The high glucose environment of diabetes also leads to increased production of advanced glycation end products (AGEs), which interact with their receptor RAGE to activate NF-κB. Then, inflammatory responses occur, producing multiple proinflammatory and profibrotic molecules (97–100). T and B lymphocytes are subsequently activated (101). Activated T lymphocytes can produce proinflammatory cytokines (e.g., IL-17, IL-6, TNF-α and IFN-γ) or recruit and activate macrophages (102–108). Activated B lymphocytes can induce the formation of inflammatory immune complexes and produce proinflammatory cytokines (e.g., IL-6, IL-10 and TNF) (106, 109–111). After proinflammatory cytokines are released, the cascade amplifies the NF-κB signal, produces more proinflammatory cytokines and recruits adjacent macrophages to the inflammatory site in tubules, which leads to kidney infiltration, increases the expression of proinflammatory and profibrotic molecules (e.g. type I/IV collagen and TGF-β), and exacerbates renal tubular fibrosis (101, 111).

In the diabetic state, testosterone can phosphorylate and activate C-jun (a molecule that functions in renal inflammation) (112–114). Activated C-jun may upregulate monocyte chemoattractant protein-1 (MCP-1) expression. This promotes tubular epithelial cells to attract macrophages to the injury site of tubules, causing local inflammation and tubular cell apoptosis. The activation of C-jun can also upregulate the expression kidney injury molecule-1 and directly induce tubular fibrosis (114, 115). In SD male rats, once inflammation occurs in the kidney, testosterone can upregulate the expression of the proinflammatory cytokine TNF-α to exacerbate the inflammatory response and increase the expression of profibrotic substances to promote tubule epithelial-mesenchymal transition (EMT) and promote renal fibrosis (116).

Tubular fibrosis is the outcome of the inflammatory response in the kidney and is led by TGF-β (a key molecule that can stimulate the production of several extracellular matrix proteins that accumulate in the diabetic kidney, including type IV collagen, fibronectin and laminin). EMT of the renal tubular epithelium leads to tubular fibrosis (117, 118). In the state of diabetes, DHT upregulates the expression of TGF-β in diabetic male rats and accelerates the production of the early fibrosis marker connective tissue growth factor (CTGF). Additionally, epithelial cells acquire a fibroblast phenotype, leading to the genesis of tubular fibrosis (60).

Estrogen can interfere with the expression of TGF-β and its downstream signaling pathway *via* members of the small mother against decapentaplegic (Smad) protein family (Smad2/Smad3/

Smad6/Smad7) (80, 119). Studies have shown that in STZ-induced diabetic female rats, E2 regulates the activity of TGF-β by downregulating profibrotic signaling molecules (Smad2, Smad3) and upregulating antifibrotic signaling molecules (Smad6, Smad7) (80). Thus, E2 can reduce proteinuria and ECM protein expression associated with diabetic glomerulosclerosis and renal tubular fibrosis and play a renoprotective role in females with diabetes (80). Regulation of casein kinase II (CK2) is another mechanism by which E2 may regulate TGF-β activity. CK2 is a serine/threonine protein kinase that, when activated, phosphorylates early growth reactivity 1 (EGR-1). EGR-1 typically binds to specific protein 1 (Sp1), preventing Sp1 from binding to target sequences. Ck2 induces EGR-1 phosphorylation in response to TGF-β to prevent the formation of the EGR-1/Sp1 complex, and the level of free Sp1 increases. Sp1, in turn, binds to target sequences in the promoters of type IV collagen and increases its synthesis. In murine mesangial cells, E2 treatment prevented the TGF-β-induced increase in CK2 expression and activity, thereby inhibiting TGF-β signaling and type IV collagen upregulation (120).

In addition to regulating TGF-β expression and activity in renal cells, E2 can also indirectly regulate TGF-β in the kidney by regulating macrophage infiltration. Macrophages are a key source of TGF-β in diabetic kidneys. In a spontaneously hypertensive rat model of kidney disease, the level of macrophage infiltration in the kidney was higher in males than in females, and OVX in females increased the number of macrophages. Similarly, OVX in diabetic female rats increased macrophage infiltration, and this effect could be normalized by E2 treatment (80, 121). These data suggest that E2 inhibits macrophage infiltration, thereby preventing the production of TGF-β by a major source and potentially protecting the kidney from injury.

●Hemodynamic changes

Increases in ROS are generated by persistent hyperglycemia and can lead to dilatation of the afferent glomerular arteriole, hyperfiltration, hypertransfusion and high internal pressure in the kidney in the early stages of diabetes (122). A prolonged high filtration load due to high glucose increases sodium-glucose cotransporter protein 2 levels in the proximal tubules, and the resorption of glucose and sodium chloride increases. This leads to dysfunctional tubuloglomerular feedback and results in the disruption of the afferent/efferent arteriole balance and increased glomerular unit plasma flow (123). This abnormal status ultimately increases the renal glomerular filtration rate (GFR) and causes glomerulosclerosis.

Before adolescence, sex does not play a significant role in the incidence of DN (124). With aging and the occurrence of chronic complications associated with diabetes mellitus, DN tends to begin earlier in men than in women because testosterone can activate the renin-angiotensin-aldosterone system (RAAS) (73). The RAAS is one of the primary control systems that regulates the balance of blood pressure and fluids, and the kidney is the organ that activates the RAAS. The major bioactive hormone in the RAAS is AngII, which is cleaved from angiotensinogen and can promote vasoconstriction, fibrosis, inflammation and apoptosis (125–128). AngII receptors can be divided into two types according to their length: ATR1 (40 kDa) and ATR2 (41 kDa). ATR1 is considered to be associated with increased blood pressure and vasoconstriction, while AT2R is considered to be associated with reduced blood pressure and inflammation inhibition (127, 128). DHT upregulates ATR1 expression in sexually mature SD male rats (73). The activity of AngII might be modulated by angiotensin-converting enzyme 2 (ACE2) or 3β-HSD4 in males. ACE2 is a zinc metalloproteinase that may degrade AngII to Ang-(1-7) (128–130). Oudit et al. found that the loss of ACE2 exacerbated the degree of glomerulosclerosis in male mice (131). 3β-HSD4 is a ketone reductase whose activity is regulated by angiotensin; it can reduce testosterone and progesterone to inactive metabolites. Under normal conditions, 3β-HSD4 protects the kidney from the potential negative effects of testosterone; in patients with diabetes with increased AngII levels, the loss of 3β-HSD4 activity may increase the susceptibility of the kidney to testosterone-induced damage (132).

Estrogen has a regulatory effect on the RAAS. It can attenuate AngII-induced hypertension and reduce renal insufficiency (73, 130, 133–135). Nitric oxide (NO) can dilate blood vessels, and endothelial cells produce NO through endothelial nitric oxide synthase (eNOS) to regulate vascular tone (136). NO can counteract the vasoconstrictive effects of AngII (137). Acute hyperglycemia induces a state of oxidative stress in the endothelium, which reduces NO production and leads to endothelial dysfunction (137). Estrogen can upregulate eNOS expression to accelerate NO release or increase NO bioavailability to relax blood vessels and lower blood pressure, thereby reducing glomerular sclerosis (138–141). Estrogen can also stimulate NO release and attenuate glomerular sclerosis and renal fibrosis by upregulating ATR2 expression in the renal medulla (142).

●Metabolic disorders

There are two aspects of abnormal glucose metabolism in patients with diabetes. AGEs bind to their receptors to activate the NF-κB pathway and stimulate the production of vascular endothelial growth factor (VEGF), TGF-β and MCP, leading to glomerular podocyte loss, expansion of the glomerular extracellular matrix and progressive glomerulosclerosis (143). Second, protein kinase C is activated by high glucose levels. This results in decreased production of eNOS and increased production of VEGF, which destabilize the endothelial microenvironment and activate the NF-κB pathway. The NF-κB-mediated inflammatory response leads to tubular fibrosis (99).

Persistent hyperglycemia in patients with diabetes can promote fatty acid synthesis and triglyceride accumulation. Excessive lipid accumulation in the glomerulus and renal tubules leads to podocyte dysfunction and damage to proximal tubular epithelial cells and tubular interstitial tissue (144). In addition, proteinuria in patients with diabetes may also serve as a carrier of fatty acids in urine. This leads to the accumulation of fatty acids in the kidney, thus exacerbating renal tubular injury in patients with diabetes (145). In OVX diabetic female rats, due to the lack of estrogen, lipid metabolism disorders occur, and fasting blood glucose levels and the insulin resistance value (HOMA-IR) were significantly increased compared with those in the control group (146).

Generally, glucose/lipid metabolic disorders may induce DN through oxidative stress, inflammation and hemodynamic changes.

Therefore, the role of sex hormones in the modulation of these processes is the same as stated above.

The effects of sex hormones that may function in the occurrence of DN are illustrated in Figure 2, and the molecules affected by sex hormones in the progression of DN are listed in Table 1.

Effects of sex hormone replacement therapies for DN

Effects of sex hormone replacement therapies in females with DN

Using E2 supplementation therapy for DN obtains good results in reducing kidney injury in women; for example, Szekacs et al. reported that in postmenopausal women with DN, estradiol supplementation reduces albuminuria (147). Raloxifene is a type of selective estrogen receptor modulator. It may attenuate glomerulosclerosis and albuminuria in women with DN and slow the progression of nephropathy (148–151). In addition, Bahaa et al. also found that progesterone treatment can attenuate DN in females (152). However, the risk or side effects of sex hormone therapies are nonnegligible. Eliassen et al. reported that E2 supplementation in premenopausal women increases their risk of breast cancer, but Dixon et al. found that raloxifene does not have side effects similar to those of E2 (149, 153). Moreover, the side effects of progesterone in the treatment of DN have been less frequently reported (152).

Effects of sex hormone replacement therapies in males with DN

Using sex hormone therapy for males with DN has been less commonly reported. Qin Xu et al. found that DHT has a dose-dependent effect in DN male rats. DHT at low concentrations (0.75 mg) can partly ease the progression of nephropathy, while DHT at high concentrations (2.0 mg) has the opposite effects in the kidney (154).

Icariin is a recently discovered GPER agonist. Qi et al. reported that icariin has antioxidative stress and antifibrotic effects in DN male rats, but whether it has side effects is unclear and not reported (155).

Table 2 summarizes the existing preclinical/clinical/animal experiments using sex hormone replacement therapies and their roles in the treatment of DN models.

Conclusions

In summary, many studies have shown that the occurrence and progression of DN are closely related to sex hormones. Testosterone can exacerbate DN by activating the RAAS or phosphorylating C-jun to induce tubular fibrosis, so DN usually progresses faster in male patients than in female patients. Estradiol can upregulate the expression of eNOS and increase the level of NO to alleviate the vasoconstriction effect of AngII to reduce tubular fibrosis. In addition, estradiol can alter the level of Smad family members and reduce macrophage infiltration and CK2 activation to alleviate

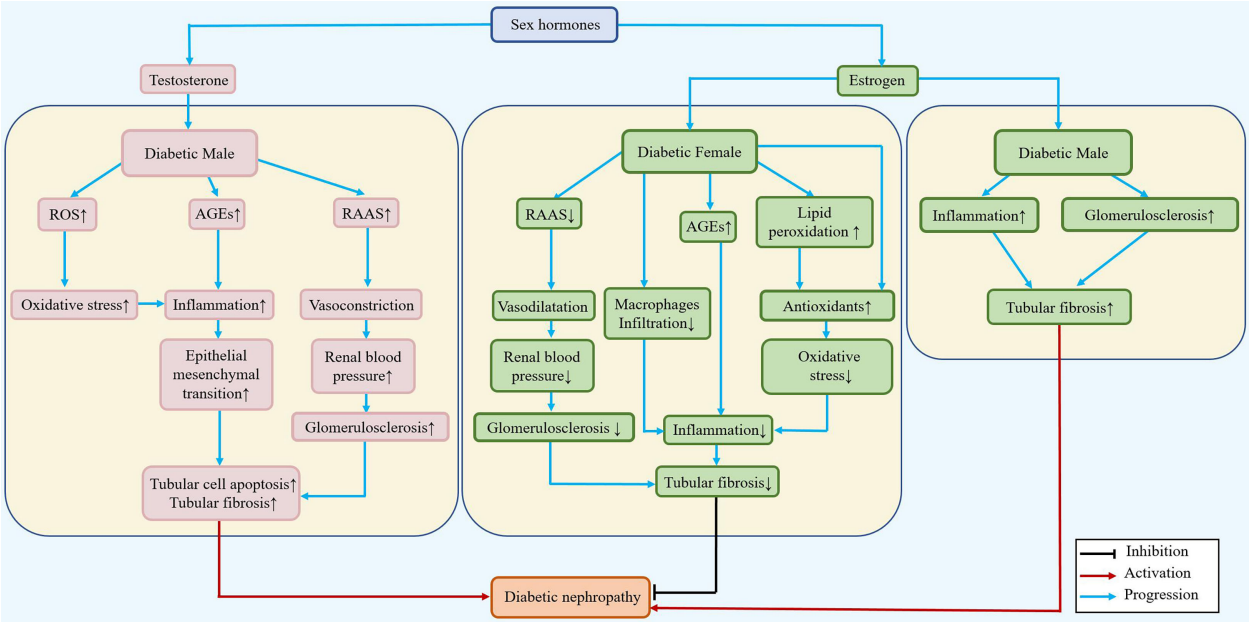

FIGURE 2
Differential roles of estrogen/testosterone in the pathogenesis of DN. During diabetes, testosterone can increase the renal blood pressure and inflammatory response, accelerate the epithelial mesenchymal transition, and lead to renal tubular fibrosis in males. In females, estradiol can reduce renal tubular fibrosis by reducing blood pressure, downregulating the inflammatory response and epithelial mesenchymal transition, and playing a renoprotective role.

TABLE 1 Sex hormones affect the pathogenesis of DN and related molecules.

Sex hormones	Changes in the molecules involved in DN pathogenesis			Outcomes	Reference
	Oxidative stress	Inflammation	Renal haemodynamics		
Testosterone	STAT3↓ ROS↑	C-jun↑ MCP-1↑ TNF-α↑ CTGF↑ Type IV collagen↑ TGF-β↑	ATR1↑ 3β-HSD4↓ AngII↑	Fibrosis↑ Kidney injury↑	(60, 73, 93, 112–116, 131, 132)
Estrogen	Antioxidants (e. g. GSH-Px, GSH and SOD) ↑ Nox↓ ROS↓	Smad 2/3↓ Smad 6/7↑ CK2↓ Type IV collagen↓ TGF-β↓	eNOS↑ NO↑ ATR2↑ AngII↓	Fibrosis↓ Kidney injury↓	(73, 80, 94–96, 119–121, 130, 133–135, 138–142)

Annotation: STAT3, signal transducer and activator of transcription-3; ROS, reactive oxygen species; Nox, NADPH oxidases; GSH-Px, glutathione peroxidase; GSH, glutathione; SOD, superoxide dismutase; MCP-1, monocyte chemoattractant proteins-1; TNF-α, tumor necrosis factor-α; CTGF, connective tissue growth factor; TGF-β, transforming growth factor-β; CK2, casein kinase II; ATR1, angiotensin II receptors-1; AngII, angiotensin II; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; ATR2, angiotensin II receptors-2.
The symbol "↑" means upregulation.
The symbol "↓" means downregulation.

TABLE 2 Sex hormone replacement therapies in DN.

Drug	Research category	Object	Method	Outcome	Reference
Estradiol	Clinical research	Postmenopausal women with DN	Oral estradiol (2mg/day) combined with norgestrel (0.5mg/day)	Albuminuria↓ CrCl↑	(147)
	Preclinical research/animal experiment	Female rats with DN	Estradiol pellets implanting after OVX (10μg/day)	Albuminuria↓ GSI↓ TIFI↓ Blood glucose level↓	(38)
	Preclinical research/animal experiment	db/db female mouse	Subcutaneous implantation of estradiol pellets after OVX(8.3μg/day)	UAE↓ Mesangial expansion↓ Fibronectin↓ Blood glucose level↓	(156)
Raloxifene	Clinical research	Postmenopausal women with DN	Oral (60mg/day)	Albuminuria↓ Risk of vertebral fracture↓ No effect on fasting blood glucose with short-term raloxifene treatment	(148, 150, 151)
	Preclinical research/animal experiment	Female rats with DN	Administering in the phytoestrogen-free chow (10mg/kg/day)	UAE↓ GSI↓ TITF↓ Type I/IV collagen↓ TGF-β↓ IL-6↓	(149)
	Preclinical research/animal experiment	db/db female mouse	Subcutaneous treatment (10mg/kg/day)	Mesangial area↓ TGF-β↓ Fibronectin↓	(156)
Progesterone	Preclinical research/animal experiment	Female rats with DN	Progesterone treatment after OVX (10mg/kg)	UACR↓ GSI↓ Fibronectin↓ ATR1↓ TGF-β↓	(152)
Dihydrotestosterone	Preclinical research/animal experiment	Male rats with DN	Dihydrotestosterone in low concentrations subcutaneous implantation (0.75mg/day)	UAE↓ Glomerular sclerosis↓ TITF↓ Type IV collagen↓ TGF-β↓ IL-6↓	(154)

(Continued)

TABLE 2 Continued

Drug	Research category	Object	Method	Outcome	Reference
	Preclinical research/animal experiment	Male rats with DN	Dihydrotestosterone in high concentrations subcutaneous implantation (2.0mg/day)	Opposite results compared with dihydrotestosterone in 0.75mg/day concentrations (low concentration)	(154)
Icariin	Preclinical research/animal experiment	Male rats with DN	Oral (80mg/kg)	MDA↓ Type IV collagen↓ TGF-β↓	(155)
	Preclinical research/animal experiment	Male rats with DN	Gavage (20, 40, and 80 mg/kg/day)	Blood urea nitrogen↓ Urine protein↓ Urinary creatinine↓ CrCl↑ TITF↓	(157)

Annotation: CrCl, creatinine clearance rate; GSI, glomerulosclerotic index; TIFI, the index of tubulointerstitial fibrosis; UAE, urinary albumin excretion; TITF, tubulointerstitial fibrosis; TGF-β, transforming growth factor-β; IL-6: interleukin-6; UACR, urinary albumin to creatinine ratio; ATR1, angiotensin II receptor 1; MDA, malondialdehyde.
The symbol "↑" means upregulation.
The symbol "↓" means downregulation.

tubular fibrosis. Thus, estradiol is thought to play a protective role in DN. Along with that for new targets for treatment, understanding the effect of sex hormones will provide a new combined therapeutic strategy for DN. Particular challenges are presented and placed within the context of future treatments against DN.

Author contributions

WW and DY conceived the manuscript. JL and DY drafted the manuscript. JL drew the figures. JL, WS and WW proofread the manuscript and made revisions. LL and XA collected the references. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Erfanpoor S, Etemad K, Kazempour S, Hadaegh F, Hasani J, Azizi F, et al. Diabetes, hypertension, and incidence of chronic kidney disease, is there any multiplicative or additive interaction? *Int J Endocrinol Metab* (2021) 19:e101061. doi: 10.5812/ijem.101061

2. Ahmed MA, Ferede YM, Takele WW. Incidence and predictors of chronic kidney disease in type-II diabetes mellitus patients attending at the amhara region referral hospitals, Ethiopia, a follow-up study. *PLoS One* (2022) 17:e0263138. doi: 10.1371/journal.pone.0263138

3. Caramori ML, Rossing P. Diabetic kidney disease. In: Feingold KR, Anawalt B, Blackman MR, Boyce A, Chrousos G, Corpas E KR, Anawalt B, Blackman MR, Boyce A, Chrousos G, Corpas E, et al. edtors. *Endotext* [Internet] South Dartmouth MA: MDText.com, Inc. (2022) 2000.

4. Mishriky BM, Cummings DM, Powell JR. Diabetes-related microvascular complications - a practical approach. *Prim Care* (2022) 49:239–54. doi: 10.1016/j.pop.2021.11.008

5. Weldegiorgis M, Woodward M. The impact of hypertension on chronic kidney disease and end-stage renal disease is greater in men than women, a systematic review and meta-analysis. *BMC Nephrol* (2020) 21:506. doi: 10.1186/s12882-020-02151-7

6. Banerjee D, Winocour P, Chowdhury TA, De P, Wahba M, Montero R, et al. Management of hypertension and renin-angiotensin-aldosterone system blockade in adults with diabetic kidney disease, association of British clinical diabetologists and the renal association UK guideline update 2021. *BMC Nephrol* (2022) 23:9. doi: 10.1186/s12882-021-02587-5

7. Sridhar VS, Yau K, Benham JL, Campbell DJT, Cherney DZI. Sex and gender related differences in diabetic kidney disease. *Semin Nephrol* (2022) 42:170–84. doi: 10.1016/j.semnephrol.2022.04.007

8. Payne AH, Hales DB. Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. *Endocr Rev* (2004) 25:947–70. doi: 10.1210/er.2003-0030

9. Papadopoulos V, Baraldi M, Guilarte TR, Knudsen TB, Lacapere JJ, Lindemann P, et al. Translocator protein (18kDa), new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. *Trends Pharmacol Sci* (2006) 27:402–9. doi: 10.1016/j.tips.2006.06.005

10. Tsuchiya Y, Nakajima M, Yokoi T. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. *Cancer Lett* (2005) 227:115–24. doi: 10.1016/j.canlet.2004.10.007

11. Schmidtova E. [Testosterone–effects, metabolism and genetic determination]. *Cesk Fysiol* (2008) 57:61–75.

12. Rana K, Davey RA, Zajac JD. Human androgen deficiency, insights gained from androgen receptor knockout mouse models. *Asian J Androl* (2014) 16:169–77. doi: 10.4103/1008-682X.122590

13. Kelly DM, Jones TH. Testosterone, a metabolic hormone in health and disease. *J Endocrinol* (2013) 217:R25–45. doi: 10.1530/JOE-12-0455

14. Davey RA, Grossmann M. Androgen receptor structure, function and biology, from bench to bedside. *Clin Biochem Rev* (2016) 37:3–15.

15. Sankar JS, Hampson E. Androgen receptor polymorphism, mental rotation, and spatial visualization in men. *Psychoneuroendocrinology* (2021) 129:105239. doi: 10.1016/j.psyneuen.2021.105239

16. Kitajima Y, Ono Y. Estrogens maintain skeletal muscle and satellite cell functions. *J Endocrinol* (2016) 229:267–75. doi: 10.1530/JOE-15-0476

17. Arnal JF, Lenfant F, Metivier R, Flouriot G, Henrion D, Adlanmerini M, et al. Membrane and nuclear estrogen receptor alpha actions, from tissue specificity to medical implications. *Physiol Rev* (2017) 97:1045–87. doi: 10.1152/physrev.00024.2016

18. Wilkinson HN, Hardman MJ. The role of estrogen in cutaneous ageing and repair. *Maturitas* (2017) 103:60–4. doi: 10.1016/j.maturitas.2017.06.026

19. Trenti A, Tedesco S, Boscaro C, Trevisi L, Bolego C, Cignarella A. Estrogen, angiogenesis, immunity and cell metabolism, solving the puzzle. *Int J Mol Sci* (2018) 15:19. doi: 10.3390/ijms19030859

20. El-Gendy AA, Elsaed WM, Abdallah HI. Potential role of estradiol in ovariectomy-induced derangement of renal endocrine functions. *Ren Fail* (2019) 41:507–20. doi: 10.1080/0886022X.2019.1625787

21. Vrtacnik P, Ostanek B, Mencej-Bedrac S, Marc J. The many faces of estrogen signaling. *Biochem Med (Zagreb)* (2014) 24:329–42. doi: 10.11613/BM.2014.035

22. Hara Y, Waters EM, McEwen BS, Morrison JH. Estrogen effects on cognitive and synaptic health over the lifecourse. *Physiol Rev* (2015) 95:785–807. doi: 10.1152/physrev.00036.2014

23. Kovats S. Estrogen receptors regulate innate immune cells and signaling pathways. *Cell Immunol* (2015) 294:63–9. doi: 10.1016/j.cellimm.2015.01.018

24. Arao Y, Korach KS. The physiological role of estrogen receptor functional domains. *Essays Biochem* (2021) 65:867–75. doi: 10.1042/EBC20200167

25. Landgren BM, Collins A, Csemiczky G, Burger HG, Baksheev L, Robertson DM. Menopause transition, annual changes in serum hormonal patterns over the menstrual cycle in women during a nine-year period prior to menopause. *J Clin Endocrinol Metab* (2004) 89:2763–9. doi: 10.1210/jc.2003-030824

26. Horstman AM, Dillon EL, Urban RJ, Sheffield-Moore M. The role of androgens and estrogens on healthy aging and longevity. *J Gerontol A Biol Sci Med Sci* (2012) 67:1140–52. doi: 10.1093/gerona/gls068

27. Yasui T, Matsui S, Tani A, Kunimi K, Yamamoto S, Irahara M. Androgen in postmenopausal women. *J Med Invest* (2012) 59:12–27. doi: 10.2152/jmi.59.12

28. Ketchem JM, Bowman EJ, Isales CM. Male Sex hormones, aging, and inflammation. *Biogerontology* (2023) 24:1–25. doi: 10.1007/s10522-022-10002-1

29. Gambineri A, Pelusi C. Sex hormones, obesity and type 2 diabetes, is there a link? *Endocr Connect* (2019) 8:R1–9. doi: 10.1530/EC-18-0450

30. Maric C. Sex, diabetes and the kidney. *Am J Physiol Renal Physiol* (2009) 296: F680–688. doi: 10.1152/ajprenal.90505.2008

31. Nilsson S, Makela S, Treuter E, Tujague M, Thomsen J, Andersson G, et al. Mechanisms of estrogen action. *Physiol Rev* (2001) 81:1535–65. doi: 10.1152/physrev.2001.81.4.1535

32. Salonia A, Lanzi R, Scavini M, Pontillo M, Gatti E, Petrella G, et al. Sexual function and endocrine profile in fertile women with type 1 diabetes. *Diabetes Care* (2006) 29:312–6. doi: 10.2337/diacare.29.02.06.dc05-1067

33. Grossmann M, Thomas MC, Panagiotopoulos S, Sharpe K, Macisaac RJ, Clarke S, et al. Low testosterone levels are common and associated with insulin resistance in men with diabetes. *J Clin Endocrinol Metab* (2008) 93:1834–40. doi: 10.1210/jc.2007-2177

34. Vikan T, Schirmer H, Njolstad I, Svartberg J. Low testosterone and sex hormone-binding globulin levels and high estradiol levels are independent predictors of type 2 diabetes in men. *Eur J Endocrinol* (2010) 162:747–54. doi: 10.1530/EJE-09-0943

35. Manigrasso MB, Sawyer RT, Hutchens ZMJr., Flynn ER, Maric-Bilkan C. Combined inhibition of aromatase activity and dihydrotestosterone supplementation attenuates renal injury in male streptozotocin (STZ)-induced diabetic rats. *Am J Physiol Renal Physiol* (2012) 302:F1203–1209. doi: 10.1152/ajprenal.00569.2011

36. Dhindsa S, Reddy A, Karam JS, Bilkis S, Chaurasia A, Mehta A, et al. Prevalence of subnormal testosterone concentrations in men with type 2 diabetes and chronic kidney disease. *Eur J Endocrinol* (2015) 173:359–66. doi: 10.1530/EJE-15-0359

37. Hackett G, Heald AH, Sinclair A, Jones PW, Strange RC, Ramachandran S. Serum testosterone, testosterone replacement therapy and all-cause mortality in men with type 2 diabetes, retrospective consideration of the impact of PDE5 inhibitors and statins. *Int J Clin Pract* (2016) 70:244–53. doi: 10.1111/ijcp.12779

38. Mankhey RW, Bhatti F, Maric C. 17β-estradiol replacement improves renal function and pathology associated with diabetic nephropathy. *Am J Physiology-Renal Physiol* (2005) 288:F399–405. doi: 10.1152/ajprenal.00195.2004

39. Schoeller EL, Schon S, Moley KH. The effects of type 1 diabetes on the hypothalamic, pituitary and testes axis. *Cell Tissue Res* (2012) 349:839–47. doi: 10.1007/s00441-012-1387-7

40. Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, et al. Role of brain insulin receptor in control of body weight and reproduction. *Science* (2000) 289:2122–5. doi: 10.1126/science.289.5487.2122

41. Saito K, Cui H. Emerging roles of estrogen-related receptors in the brain, potential interactions with estrogen signaling. *Int J Mol Sci* (2018) 19:1091. doi: 10.3390/ijms19041091

42. Amory JK, Bremner WJ. Regulation of testicular function in men, implications for male hormonal contraceptive development. *J Steroid Biochem Mol Biol* (2003) 85:357–61. doi: 10.1016/S0960-0760(03)00205-X

43. Maneesh M, Jayalakshmi H, Singh TA, Chakrabarti A. Impaired hypothalamic-pituitary-gonadal axis function in men with diabetes mellitus. *Indian J Clin Biochem* (2006) 21:165–8. doi: 10.1007/BF02913088

44. Rochira V, Zirilli L, Genazzani AD, Balestrieri A, Aranda C, Fabre B, et al. Hypothalamic-pituitary-gonadal axis in two men with aromatase deficiency, evidence that circulating estrogens are required at the hypothalamic level for the integrity of gonadotropin negative feedback. *Eur J Endocrinol* (2006) 155:513–22. doi: 10.1530/eje.1.02254

45. Costanzo PR, Suarez SM, Scaglia HE, Zylbersztein C, Litwak LE, Knoblovits P. Evaluation of the hypothalamic-pituitary-gonadal axis in eugonadal men with type 2 diabetes mellitus. *Andrology* (2014) 2:117–24. doi: 10.1111/j.2047-2927.2013.00163.x

46. Cheung KK, Luk AO, So WY, Ma RC, Kong AP, Chow FC, et al. Testosterone level in men with type 2 diabetes mellitus and related metabolic effects, a review of current evidence. *J Diabetes Investig* (2015) 6:112–23. doi: 10.1111/jdi.12288

47. Schianca GP, Fra GP, Brustia F, Bellan M, Pirovano A, Gualerzi A, et al. Testosterone plasma concentration is associated with insulin resistance in Male hypertensive patients. *Exp Clin Endocrinol Diabetes* (2017) 125:171–5. doi: 10.1055/s-0042-121492

48. Burul-Bozkurt N, Pekiner C, Kelicen P. Diabetes alters aromatase enzyme levels in gonadal tissues of rats. *Naunyn Schmiedebergs Arch Pharmacol* (2010) 382:33–41. doi: 10.1007/s00210-010-0518-5

49. Burul-Bozkurt N, Pekiner C, Kelicen P. Diabetes alters aromatase enzyme levels in sciatic nerve and hippocampus tissues of rats. *Cell Mol Neurobiol* (2010) 30:445–51. doi: 10.1007/s10571-009-9469-0

50. Heppner KM, Baquero AF, Bennett CM, Lindsley SR, Kirigiti MA, Bennett B, et al. GLP-1R signaling directly activates arcuate nucleus kisspeptin action in brain slices but does not rescue luteinizing hormone inhibition in ovariectomized mice during negative energy balance. *eNeuro* (2017) 4(1):ENEURO.0198-16.2016. doi: 10.1523/ENEURO.0198-16.2016

51. Quennell JH, Mulligan AC, Tups A, Liu X, Phipps SJ, Kemp CJ, et al. Leptin indirectly regulates gonadotropin-releasing hormone neuronal function. *Endocrinology* (2009) 150:2805–12. doi: 10.1210/en.2008-1693

52. German JP, Wisse BE, Thaler JP, Oh IS, Sarruf DA, Ogimoto K, et al. Leptin deficiency causes insulin resistance induced by uncontrolled diabetes. *Diabetes* (2010) 59:1626–34. doi: 10.2337/db09-1918

53. Wabitsch M, Jensen PB, Blum WF, Christoffersen CT, Englaro P, Heinze E, et al. Insulin and cortisol promote leptin production in cultured human fat cells. *Diabetes* (1996) 45:1435–8. doi: 10.2337/diab.45.10.1435

54. Barr VA, Malide D, Zarnowski MJ, Taylor SI, Cushman SW. Insulin stimulates both leptin secretion and production by rat white adipose tissue. *Endocrinology* (1997) 138:4463–72. doi: 10.1210/endo.138.10.5451

55. Carvalheira JB, Torsoni MA, Ueno M, Amaral ME, Araujo EP, Velloso LA, et al. Cross-talk between the insulin and leptin signaling systems in rat hypothalamus. *Obes Res* (2005) 13:48–57. doi: 10.1038/oby.2005.7

56. Childs GV, Odle AK, MacNicol MC, MacNicol AM. The importance of leptin to reproduction. *Endocrinology* (2021) 162:bqaa204. doi: 10.1210/endocr/bqaa204

57. Jones ME, Thorburn AW, Britt KL, Hewitt KN, Wreford NG, Proietto J, et al. Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. *Proc Natl Acad Sci U.S.A.* (2000) 97:12735–40. doi: 10.1073/pnas.97.23.12735

58. Takeda K, Toda K, Saibara T, Nakagawa M, Saika K, Onishi T, et al. Progressive development of insulin resistance phenotype in male mice with complete aromatase (CYP19) deficiency. *J Endocrinol* (2003) 176:237–46. doi: 10.1677/joe.0.1760237

59. Zhang XX, Kong J, Yun K. Prevalence of diabetic nephropathy among patients with type 2 diabetes mellitus in China, a meta-analysis of observational studies. *J Diabetes Res* (2020) 2020:2315607. doi: 10.1155/2020/2315607

60. Ziller N, Kotolloshi R, Esmaeili M, Liebisch M, Mrowka R, Baniahmad A, et al. Sex differences in diabetes- and TGF-beta1-Induced renal damage. *Cells* (2020) 9:2236. doi: 10.3390/cells9102236

61. Chesnaye NC, Dekker FW, Evans M, Caskey FJ, Torino C, Postorino M, et al. Renal function decline in older men and women with advanced chronic kidney disease-results from the EQUAL study. *Nephrol Dial Transplant* (2021) 36:1656–63. doi: 10.1093/ndt/gfaa095

62. Orchard TJ, Dorman JS, Maser RE, Becker DJ, Drash AL, Ellis D, et al. Prevalence of complications in IDDM by sex and duration. Pittsburgh epidemiology of diabetes complications study II. *Diabetes* (1990) 39:1116–24. doi: 10.2337/diab.39.9.1116

63. Raile K, Galler A, Hofer S, Herbst A, Dunstheimer D, Busch P, et al. Diabetic nephropathy in 27,805 children, adolescents, and adults with type 1 diabetes, effect of diabetes duration, A1C, hypertension, dyslipidemia, diabetes onset, and sex. *Diabetes Care* (2007) 30:2523–8. doi: 10.2337/dc07-0282

64. Maric C, Sullivan S. Estrogens and the diabetic kidney. *Gend Med* (2008) 5 Suppl A:S103–113. doi: 10.1016/j.genm.2008.03.010

65. Dong W, Zhao Y, Liu D, Liu Y, Li F, Li M. Sex-specific association between type 1 diabetes and the risk of end-stage renal disease, a systematic review and meta-analysis. *Endocrine* (2020) 69:30–8. doi: 10.1007/s12020-020-02255-y

66. Neugarten J, Acharya A, Silbiger SR. Effect of gender on the progression of nondiabetic renal disease, a meta-analysis. *J Am Soc Nephrol* (2000) 11:319–29. doi: 10.1681/ASN.V112319

67. Shen Y, Cai R, Sun J, Dong X, Huang R, Tian S, et al. Diabetes mellitus as a risk factor for incident chronic kidney disease and end-stage renal disease in women compared with men, a systematic review and meta-analysis. *Endocrine* (2017) 55:66–76. doi: 10.1007/s12020-016-1014-6

68. Yu MK, Lyles CR, Bent-Shaw LA, Young BA, Pathways A. Risk factor, age and sex differences in chronic kidney disease prevalence in a diabetic cohort, the pathways study. *Am J Nephrol* (2012) 36:245–51. doi: 10.1159/000342210

69. Lewis EJ, Hunsicker LG, Rodby RA, Collaborative Study G. A clinical trial in type 2 diabetic nephropathy. *Am J Kidney Dis* (2001) 38:S191–194. doi: 10.1053/ajkd.2001.27442

70. Keane WF, Brenner BM, de Zeeuw D, Grunfeld JP, McGill J, Mitch WE, et al. The risk of developing end-stage renal disease in patients with type 2 diabetes and nephropathy, the RENAAL study. *Kidney Int* (2003) 63:1499–507. doi: 10.1046/j.1523-1755.2003.00885.x

71. Mollsten A, Svensson M, Waernbaum I, Berhan Y, Schon S, Nystrom L, et al. Cumulative risk, age at onset, and sex-specific differences for developing end-stage renal disease in young patients with type 1 diabetes, a nationwide population-based cohort study. *Diabetes* (2010) 59:1803–8. doi: 10.2337/db09-1744

72. Harjutsalo V, Maric C, Forsblom C, Thorn L, Waden J, Groop PH, et al. Sex-related differences in the long-term risk of microvascular complications by age at onset of type 1 diabetes. *Diabetologia* (2011) 54:1992–9. doi: 10.1007/s00125-011-2144-2

73. Kang AK, Miller JA. Effects of gender on the renin-angiotensin system, blood pressure, and renal function. *Curr Hypertens Rep* (2002) 4:143–51. doi: 10.1007/s11906-002-0039-9

74. Anderson S, Chapman JG, Oyama TT, Komers R. Effect of orchiectomy on renal function in control and diabetic rats with chronic inhibition of nitric oxide. *Clin Exp Pharmacol Physiol* (2010) 37:19–23. doi: 10.1111/j.1440-1681.2009.05206.x

75. Skupien J, Smiles AM, Valo E, Ahluwalia TS, Gyorgy B, Sandholm N, et al. Variations in risk of end-stage renal disease and risk of mortality in an international study of patients with type 1 diabetes and advanced nephropathy. *Diabetes Care* (2019) 42:93–101. doi: 10.2337/dc18-1369

76. Wells CC, Riazi S, Mankhey RW, Bhatti F, Ecelbarger C, Maric C. Diabetic nephropathy is associated with decreased circulating estradiol levels and imbalance in the expression of renal estrogen receptors. *Gend Med* (2005) 2:227–37. doi: 10.1016/S1550-8579(05)80052-X

77. Ma HY, Chen S, Du Y. Estrogen and estrogen receptors in kidney diseases. *Ren Fail* (2021) 43:619–42. doi: 10.1080/0886022X.2021.1901739

78. Manigrasso MB, Sawyer RT, Marbury DC, Flynn ER, Maric C. Inhibition of estradiol synthesis attenuates renal injury in male streptozotocin-induced diabetic rats. *Am J Physiol Renal Physiol* (2011) 301:F634–640. doi: 10.1152/ajprenal.00718.2010

79. Kwan G, Neugarten J, Sherman M, Ding Q, Fotadar U, Lei J, et al. Effects of sex hormones on mesangial cell proliferation and collagen synthesis. *Kidney Int* (1996) 50:1173–9. doi: 10.1038/ki.1996.425

80. Dixon A, Maric C. 17beta-estradiol attenuates diabetic kidney disease by regulating extracellular matrix and transforming growth factor-beta protein expression and signaling. *Am J Physiol Renal Physiol* (2007) 293:F1678–1690. doi: 10.1152/ajprenal.00079.2007

81. Kummer S, Jeruschke S, Wegerich LV, Peters A, Lehmann P, Seibt A, et al. Estrogen receptor alpha expression in podocytes mediates protection against apoptosis *in-vitro* and *in-vivo*. *PLoS One* (2011) 6:e27457. doi: 10.1371/journal.pone.0027457

82. Appiah D, Winters SJ, Hornung CA. Bilateral oophorectomy and the risk of incident diabetes in postmenopausal women. *Diabetes Care* (2014) 37:725–33. doi: 10.2337/dc13-1986

83. Appiah D, Winters SJ, Allison MA, Baumgartner RN, Groves FD, Myers JA, et al. Cardiovascular disease among women with and without diabetes mellitus and bilateral oophorectomy. *Diabetes Res Clin Pract* (2015) 108:473–81. doi: 10.1016/j.diabres.2015.02.017

84. Honigberg MC, Zekavat SM, Aragam K, Finneran P, Klarin D, Bhatt DL, et al. Association of premature natural and surgical menopause with incident cardiovascular disease. *JAMA* (2019) 322:2411–21. doi: 10.1001/jama.2019.19191

85. Ravid M, Brosh D, Ravid-Safran D, Levy Z, Rachmani R. Main risk factors for nephropathy in type 2 diabetes mellitus are plasma cholesterol levels, mean blood pressure, and hyperglycemia. *Arch Intern Med* (1998) 158:998–1004. doi: 10.1001/archinte.158.9.998

86. Caramori ML, Kim Y, Huang C, Fish AJ, Rich SS, Miller ME, et al. Cellular basis of diabetic nephropathy, 1. study design and renal structural-functional relationships in patients with long-standing type 1 diabetes. *Diabetes* (2002) 51:506–13. doi: 10.2337/diabetes.51.2.506

87. Chen YM, Miner JH. Glomerular basement membrane and related glomerular disease. *Transl Res* (2012) 160:291–7. doi: 10.1016/j.trsl.2012.03.004

88. Herman-Edelstein M, Doi SQ. Pathophysiology of diabetic nephropathy. In: *Proteinuria, basic mechanisms, pathophysiology and clinical relevance* (Switzerland: Springer International Publishing Switzerland) (2016). p. 41–65.

89. Jha JC, Banal C, Chow BS, Cooper ME, Jandeleit-Dahm K. Diabetes and kidney disease, role of oxidative stress. *Antioxid Redox Signal* (2016) 25:657–84. doi: 10.1089/ars.2016.6664

90. Singh DK, Winocour P, Farrington K. Oxidative stress in early diabetic nephropathy, fueling the fire. *Nat Rev Endocrinol* (2011) 7:176–84. doi: 10.1038/nrendo.2010.212

91. Forbes JM, Thorburn DR. Mitochondrial dysfunction in diabetic kidney disease. *Nat Rev Nephrol* (2018) 14:291–312. doi: 10.1038/nrneph.2018.9

92. Wei PZ, Szeto CC. Mitochondrial dysfunction in diabetic kidney disease. *Clin Chim Acta* (2019) 496:108–16. doi: 10.1016/j.cca.2019.07.005

93. Reed DK, Arany I. Sex hormones differentially modulate STAT3-dependent antioxidant responses during oxidative stress in renal proximal tubule cells. *In Vivo* (2014) 28:1097–100.

94. Ulas M, Cay M. 17beta-estradiol and vitamin e modulates oxidative stress-induced kidney toxicity in diabetic ovariectomized rat. *Biol Trace Elem Res* (2011) 144:821–31. doi: 10.1007/s12011-011-9025-x

95. Ji H, Zheng W, Menini S, Pesce C, Kim J, Wu X, et al. Female protection in progressive renal disease is associated with estradiol attenuation of superoxide production. *Gend Med* (2007) 4:56–71. doi: 10.1016/S1550-8579(07)80009-X

96. Ronis MJ, Blackburn ML, Shankar K, Ferguson M, Cleves MA, Badger TM. Estradiol and NADPH oxidase crosstalk regulates responses to high fat feeding in female mice. *Exp Biol Med (Maywood)* (2019) 244:834–45. doi: 10.1177/1535370219853563

97. Li JH, Wang W, Huang XR, Oldfield M, Schmidt AM, Cooper ME, et al. Advanced glycation end products induce tubular epithelial-myofibroblast transition through the RAGE-ERK1/2 MAP kinase signaling pathway. *Am J Pathol* (2004) 164:1389–97. doi: 10.1016/S0002-9440(10)63225-7

98. Tan AL, Forbes JM, Cooper ME. AGE, RAGE, and ROS in diabetic nephropathy. *Semin Nephrol* (2007) 27:130–43. doi: 10.1016/j.semnephrol.2007.01.006

99. Navarro-Gonzalez JF, Mora-Fernandez C, Muros de Fuentes M, Garcia-Perez J. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. *Nat Rev Nephrol* (2011) 7:327–40. doi: 10.1038/nrneph.2011.51

100. Xu Y, Nie L, Yin YG, Tang JL, Zhou JY, Li DD, et al. Resveratrol protects against hyperglycemia-induced oxidative damage to mitochondria by activating SIRT1 in rat mesangial cells. *Toxicol Appl Pharmacol* (2012) 259:395–401. doi: 10.1016/j.taap.2011.09.028

101. Ruiz-Ortega M, Rayego-Mateos S, Lamas S, Ortiz A, Rodrigues-Diez RR. Targeting the progression of chronic kidney disease. *Nat Rev Nephrol* (2020) 16:269–88. doi: 10.1038/s41581-019-0248-y

102. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* (2006) 126:1121–33. doi: 10.1016/j.cell.2006.07.035

103. Eller K, Kirsch A, Wolf AM, Sopper S, Tagwerker A, Stanzl U, et al. Potential role of regulatory T cells in reversing obesity-linked insulin resistance and diabetic nephropathy. *Diabetes* (2011) 60:2954–62. doi: 10.2337/db11-0358

104. Liu G, Ma H, Qiu L, Li L, Cao Y, Ma J, et al. Phenotypic and functional switch of macrophages induced by regulatory CD4+CD25+ T cells in mice. *Immunol Cell Biol* (2011) 89:130–42. doi: 10.1038/icb.2010.70

105. Moon JY, Jeong KH, Lee TW, Ihm CG, Lim SJ, Lee SH. Aberrant recruitment and activation of T cells in diabetic nephropathy. *Am J Nephrol* (2012) 35:164–74. doi: 10.1159/000334928

106. Duran-Salgado MB, Rubio-Guerra AF. Diabetic nephropathy and inflammation. *World J Diabetes* (2014) 5:393–8. doi: 10.4239/wjd.v5.i3.393

107. Lei L, Mao Y, Meng D, Zhang X, Cui L, Huo Y, et al. Percentage of circulating CD8+ T lymphocytes is associated with albuminuria in type 2 diabetes mellitus. *Exp Clin Endocrinol Diabetes* (2014) 122:27–30. doi: 10.1055/s-0033-1358666

108. Zhang C, Xiao C, Wang P, Xu W, Zhang A, Li Q, et al. The alteration of Th1/Th2/Th17/Treg paradigm in patients with type 2 diabetes mellitus, relationship with diabetic nephropathy. *Hum Immunol* (2014) 75:289–96. doi: 10.1016/j.humimm.2014.02.007

109. Navarro-Gonzalez JF, Mora-Fernandez C. The role of inflammatory cytokines in diabetic nephropathy. *J Am Soc Nephrol* (2008) 19:433–42. doi: 10.1681/ASN.2007091048

110. Smith MJ, Simmons KM, Cambier JC. B cells in type 1 diabetes mellitus and diabetic kidney disease. *Nat Rev Nephrol* (2017) 13:712–20. doi: 10.1038/nrneph.2017.138

111. Albvr VR, Tan SH, Candasamy M, Bhattamisra SK. Diabetic nephropathy, an update on pathogenesis and drug development. *Diabetes Metab Syndr* (2019) 13:754–62. doi: 10.1016/j.dsx.2018.11.054

112. Derijard B, Hibi M, Wu IH, Barrett T, Su B, Deng T, et al. JNK1, a protein kinase stimulated by UV light and ha-ras that binds and phosphorylates the c-jun activation domain. *Cell* (1994) 76:1025–37. doi: 10.1016/0092-8674(94)90380-8

113. Sabapathy K, Wagner EF. JNK2, a negative regulator of cellular proliferation. *Cell Cycle* (2004) 3:1520–3. doi: 10.4161/cc.3.12.1315

114. De Borst MH, Prakash J, Melenhorst WB, van den Heuvel MC, Kok RJ, Navis G, et al. Glomerular and tubular induction of the transcription factor c-jun in human renal disease. *J Pathol* (2007) 213:219–28. doi: 10.1002/path.2228

115. Verzola D, Villaggio B, Procopio V, Gandolfo MT, Gianiorio F, Fama A, et al. Androgen-mediated apoptosis of kidney tubule cells, role of c-jun amino terminal kinase. *Biochem Biophys Res Commun* (2009) 387:531–6. doi: 10.1016/j.bbrc.2009.07.056

116. Metcalfe PD, Leslie JA, Campbell MT, Meldrum DR, Hile KL, Meldrum KK. Testosterone exacerbates obstructive renal injury by stimulating TNF-alpha production and increasing proapoptotic and profibrotic signaling. *Am J Physiol Endocrinol Metab* (2008) 294:E435–443. doi: 10.1152/ajpendo.00704.2006

117. Carew RM, Wang B, Kantharidis P. The role of EMT in renal fibrosis. *Cell Tissue Res* (2012) 347:103–16. doi: 10.1007/s00441-011-1227-1

118. Sun YB, Qu X, Caruana G, Li J. The origin of renal fibroblasts/myofibroblasts and the signals that trigger fibrosis. *Differentiation* (2016) 92:102–7. doi: 10.1016/j.diff.2016.05.008

119. Doublier S, Lupia E, Catanuto P, Periera-Simon S, Xia X, Korach K, et al. Testosterone and 17beta-estradiol have opposite effects on podocyte apoptosis that precedes glomerulosclerosis in female estrogen receptor knockout mice. *Kidney Int* (2011) 79:404–13. doi: 10.1038/ki.2010.398

120. Zdunek M, Silbiger S, Lei J, Neugarten J. Protein kinase CK2 mediates TGF-β1-stimulated type IV collagen gene transcription and its reversal by estradiol1. *Kidney Int* (2001) 60:2097–108. doi: 10.1046/j.1523-1755.2001.00041.x

121. Sullivan JC, Semprun-Prieto L, Boesen EI, Pollock DM, Pollock JS. Sex and sex hormones influence the development of albuminuria and renal macrophage infiltration in spontaneously hypertensive rats. *Am J Physiology-Regulatory Integr Comp Physiol* (2007) 293:R1573–9. doi: 10.1152/ajpregu.00429.2007

122. Sugahara M, Pak WLW, Tanaka T, Tang SCW, Nangaku M. Update on diagnosis, pathophysiology, and management of diabetic kidney disease. *Nephrol (Carlton)* (2021) 26:491–500. doi: 10.1111/nep.13860

123. Tuttle KR. Back to the future, glomerular hyperfiltration and the diabetic kidney. *Diabetes* (2016) 66:14–6. doi: 10.2337/dbi16-0056

124. Harvey JN. The influence of sex and puberty on the progression of diabetic nephropathy and retinopathy. *Diabetologia* (2011) 54:1943–5. doi: 10.1007/s00125-011-2185-6

125. Miller JA, Anacta LA, Cattran DC. Impact of gender on the renal response to angiotensin II. *Kidney Int* (1999) 55:278–85. doi: 10.1046/j.1523-1755.1999.00260.x

126. Labandeira-Garcia JL, Rodriguez-Pallares J, Villar-Cheda B, Rodriguez-Perez AI, Garrido-Gil P, Guerra MJ. Aging, angiotensin system and dopaminergic degeneration in the substantia nigra. *Aging Dis* (2011) 2:257–74.

127. Sparks MA, Crowley SD, Gurley SB, Mirotsou M, Coffman TM. Classical renin-angiotensin system in kidney physiology. *Compr Physiol* (2014) 4:1201–28. doi: 10.1002/cphy.c130040

128. Rianto F, Hoang T, Revoori R, Sparks MA. Angiotensin receptors in the kidney and vasculature in hypertension and kidney disease. *Mol Cell Endocrinol* (2021) 529:111259. doi: 10.1016/j.mce.2021.111259

129. Clotet S, Soler MJ, Rebull M, Gimeno J, Gurley SB, Pascual J, et al. Gonadectomy prevents the increase in blood pressure and glomerular injury in angiotensin-converting enzyme 2 knockout diabetic male mice. *Effects renin-angiotensin system J Hypertens* (2016) 34:1752–65. doi: 10.1097/HJH.0000000000001015

130. Clotet-Freixas S, Soler MJ, Palau V, Anguiano L, Gimeno J, Konvalinka A, et al. Sex dimorphism in ANGII-mediated crosstalk between ACE2 and ACE in diabetic nephropathy. *Lab Invest* (2018) 98:1237–49. doi: 10.1038/s41374-018-0084-x

131. Oudit GY, Herzenberg AM, Kassiri Z, Wong D, Reich H, Khokha R, et al. Loss of angiotensin-converting enzyme-2 leads to the late development of angiotensin II-dependent glomerulosclerosis. *Am J Pathol* (2006) 168:1808–20. doi: 10.2353/ajpath.2006.051091

132. Cai Q, Keck M, McReynolds MR, Klein JD, Greer K, Sharma K, et al. Effects of water restriction on gene expression in mouse renal medulla, identification of 3βHSD4 as a collecting duct protein. *Am J Physiology-Renal Physiol* (2006) 291:F218–24. doi: 10.1152/ajprenal.00413.2005

133. Xue B, Pamidimukkala J, Lubahn DB, Hay M. Estrogen receptor-alpha mediates estrogen protection from angiotensin II-induced hypertension in conscious female mice. *Am J Physiol Heart Circ Physiol* (2007) 292:H1770–1776. doi: 10.1152/ajpheart.01011.2005

134. Pingili AK, Davidge KN, Thirunavukkarasu S, Khan NS, Katsurada A, Majid DSA, et al. 2-methoxyestradiol reduces angiotensin II-induced hypertension and renal dysfunction in ovariectomized female and intact Male mice. *Hypertension* (2017) 69:1104–12. doi: 10.1161/HYPERTENSIONAHA.117.09175

135. Ogola BO, Zimmerman MA, Sure VN, Gentry KM, Duong JL, Clark GL, et al. G Protein-coupled estrogen receptor protects from angiotensin II-induced increases in pulse pressure and oxidative stress. *Front Endocrinol (Lausanne)* (2019) 10:586. doi: 10.3389/fendo.2019.00586

136. Albrecht EW, Stegeman CA, Heeringa P, Henning RH, van Goor H. Protective role of endothelial nitric oxide synthase. *J Pathol* (2003) 199:8–17. doi: 10.1002/path.1250

137. Takahashi T, Harris RC. Role of endothelial nitric oxide synthase in diabetic nephropathy, lessons from diabetic eNOS knockout mice. *J Diabetes Res* (2014) 2014:590541. doi: 10.1155/2014/590541

138. Dantas AP, Tostes RC, Fortes ZB, Costa SG, Nigro D, Carvalho MH. *In vivo* evidence for antioxidant potential of estrogen in microvessels of female spontaneously hypertensive rats. *Hypertension* (2002) 39:405–11. doi: 10.1161/hy0202.102993

139. Tostes RC, Nigro D, Fortes ZB, Carvalho MH. Effects of estrogen on the vascular system. *Braz J Med Biol Res* (2003) 36:1143–58. doi: 10.1590/S0100-879X2003000900002

140. Chang Y, Han Z, Zhang Y, Zhou Y, Feng Z, Chen L, et al. G Protein-coupled estrogen receptor activation improves contractile and diastolic functions in rat renal interlobular artery to protect against renal ischemia reperfusion injury. *BioMed Pharmacother* (2019) 112:108666. doi: 10.1016/j.biopha.2019.108666

141. Giandalia A, Giuffrida AE, Gembillo G, Cucinotta D, Squadrito G, Santoro D, et al. Gender differences in diabetic kidney disease, focus on hormonal, genetic and clinical factors. *Int J Mol Sci* (2021) 22:5808. doi: 10.3390/ijms22115808

142. Neugarten J, Golestaneh L. Gender and the prevalence and progression of renal disease. *Adv Chronic Kidney Dis* (2013) 20:390–5. doi: 10.1053/j.ackd.2013.05.004

143. Rabbani N, Thornalley PJ. Advanced glycation end products in the pathogenesis of chronic kidney disease. *Kidney Int* (2018) 93:803–13. doi: 10.1016/j.kint.2017.11.034

144. Herman-Edelstein M, Scherzer P, Tobar A, Levi M, Gafter U. Altered renal lipid metabolism and renal lipid accumulation in human diabetic nephropathy. *J Lipid Res* (2014) 55:561–72. doi: 10.1194/jlr.P040501

145. Opazo-Rios L, Mas S, Marin-Royo G, Mezzano S, Gomez-Guerrero C, Moreno JA, et al. Lipotoxicity and diabetic nephropathy, novel mechanistic insights and therapeutic opportunities. *Int J Mol Sci* (2020) 21:2632. doi: 10.3390/ijms21072632

146. Tawfik SH, Mahmoud BF, Saad MI, Shehata M, Kamel MA, Helmy MH. Similar and additive effects of ovariectomy and diabetes on insulin resistance and lipid metabolism. *Biochem Res Int* (2015) 2015:567945. doi: 10.1155/2015/567945

147. Szekacs B, Vajo Z, Varbiro S, Kakucs R, Vaslaki L, Acs N, et al. Postmenopausal hormone replacement improves proteinuria and impaired creatinine clearance in type 2 diabetes mellitus and hypertension. *BJOG* (2000) 107:1017–21. doi: 10.1111/j.1471-0528.2000.tb10406.x

148. Andersson B, Johannsson G, Holm G, Bengtsson BA, Sashegyi A, Pavo I, et al. Raloxifene does not affect insulin sensitivity or glycemic control in postmenopausal women with type 2 diabetes mellitus, a randomized clinical trial. *J Clin Endocrinol Metab* (2002) 87:122–8. doi: 10.1210/jcem.87.1.8168

149. Dixon A, Wells CC, Singh S, Babayan R, Maric C. Renoprotective effects of a selective estrogen receptor modulator, raloxifene, in an animal model of diabetic nephropathy. *Am J Nephrol* (2007) 27:120–8. doi: 10.1159/000099837

150. Hadjadj S, Gourdy P, Zaoui P, Guerci B, Roudaut N, Gautier JF, et al. Effect of raloxifene – a selective oestrogen receptor modulator – on kidney function in post-menopausal women with type 2 diabetes, results from a randomized, placebo-controlled pilot trial. *Diabetes Med* (2007) 24:906–10. doi: 10.1111/j.1464-5491.2007.02165.x

151. Ishani A, Blackwell T, Jamal SA, Cummings SR, Ensrud KE, Investigators M. The effect of raloxifene treatment in postmenopausal women with CKD. *J Am Soc Nephrol* (2008) 19:1430–8. doi: 10.1681/ASN.2007050555

152. Al-Trad B, Ashankyty IM, Alaraj M. Progesterone ameliorates diabetic nephropathy in streptozotocin-induced diabetic rats. *Diabetol Metab Syndr* (2015) 7:97. doi: 10.1186/s13098-015-0097-1

153. Eliassen AH, Missmer SA, Tworoger SS, Spiegelman D, Barbieri RL, Dowsett M, et al. Endogenous steroid hormone concentrations and risk of breast cancer among premenopausal women. *J Natl Cancer Inst* (2006) 98:1406–15. doi: 10.1093/jnci/djj376

154. Xu Q, Prabhu A, Xu S, Manigrasso MB, Maric C. Dose-dependent effects of dihydrotestosterone in the streptozotocin-induced diabetic rat kidney. *Am J Physiol Renal Physiol* (2009) 297:F307–315. doi: 10.1152/ajprenal.00135.2009

155. Qi MY, Kai C, Liu HR, Su YH, Yu SQ. Protective effect of icariin on the early stage of experimental diabetic nephropathy induced by streptozotocin *via* modulating transforming growth factor beta1 and type IV collagen expression in rats. *J Ethnopharmacol* (2011) 138:731–6. doi: 10.1016/j.jep.2011.10.015

156. Chin M, Isono M, Isshiki K, Araki S, Sugimoto T, Guo B, et al. Estrogen and raloxifene, a selective estrogen receptor modulator, ameliorate renal damage in db/db mice. *Am J Pathol* (2005) 166:1629–36. doi: 10.1016/S0002-9440(10)62473-X

157. Jia Z, Wang K, Zhang Y, Duan Y, Xiao K, Liu S, et al. Icariin ameliorates diabetic renal tubulointerstitial fibrosis by restoring autophagy *via* regulation of the miR-192-5p/GLP-1R pathway. *Front Pharmacol* (2021) 12:720387. doi: 10.3389/fphar.2021.720387

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Metabolic phenotypes and risk of end-stage kidney disease in patients with type 2 diabetes

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Background: Obesity often initiates or coexists with metabolic abnormalities. This study aimed to investigate the pathological characteristics and the independent or mutual relations of obesity and metabolic abnormalities with end-stage kidney disease (ESKD) in patients with type 2 diabetes (T2D) and associated diabetic kidney disease (DKD).

Methods: A total of 495 Chinese patients with T2D and biopsy-confirmed DKD between 2003 and 2020 were enrolled in this retrospective study. The metabolic phenotypes were based on the body weight index (BMI)-based categories (obesity, BMI ≥ 25.0 kg/m^2) and metabolic status (metabolically unhealthy status, ≥ 1 criterion National Cholesterol Education Program Adult Treatment Panel III (NCEP/ATP III) excluding waist circumference and hyperglycemia) and were categorized into four types: metabolically healthy non-obesity (MHNO), metabolically healthy obesity (MHO), metabolically unhealthy non-obesity (MUNO), and metabolically unhealthy obesity (MUO). The pathological findings were defined by the Renal Pathology Society classification. Cox proportional hazards models were used to estimate hazard ratios (HRs) for ESKD.

Results: There are 56 (11.3%) MHNO patients, 28 (5.7%) MHO patients, 176 (35.6%) MUNO patients, and 235 (47.5%) MUO patients. The high prevalence of the Kimmelstiel–Wilson nodule and severe mesangial expansion were associated with obesity, whereas severe IFTA was related to metabolically unhealthy status. In the multivariate analysis, the adjusted HR (aHR) was 2.09 [95% confidence interval (CI) 0.99–4.88] in the MHO group, 2.16 (95% CI 1.20–3.88) in the MUNO group, and 2.31 (95% CI 1.27–4.20) in the MUO group compared with the MHNO group. Furthermore, the presence of obesity was insignificantly associated with ESKD compared with non-obese patients (aHR 1.22, 95% CI 0.88–1.68), while the metabolically unhealthy status was significantly associated with ESKD compared to the metabolically healthy status in the multivariate analysis (aHR 1.69, 95% CI 1.10–2.60).

Conclusion: Obesity itself was insignificantly associated with ESKD; however, adding a metabolically unhealthy status to obesity increased the risk for progression to ESKD in T2D and biopsy-proven DKD.

KEYWORDS

diabetic kidney disease, type 2 diabetes, metabolic phenotype, end-stage kidney disease, prognostic factor

1 Introduction

Diabetes has emerged as one of the most severe non-communicable diseases, affecting approximately 536.6 million adults worldwide according to the 10th edition of the International Diabetes Federation (IDF) Diabetes Atlas (1). China ranks first in diabetes prevalence, with estimates of 140 million affected individuals in 2021 (1). Decreasing mortality among those with diabetes is accomplished by improving medical care; however, the prevalence of developing diabetic kidney disease (DKD) has been estimated at approximately 25%–40% (2). DKD has become the leading cause of end-stage kidney disease (ESKD) in China and worldwide. Delaying the development and progression of DKD remains one of the most important fields in China.

The risk of developing and progressing to DKD is associated with genetic, environmental, and lifestyle factors. These include age, sex, race, as well as modifiable risk factors such as obesity. Several studies have shown that abdominal obesity increases the risk of the progression of chronic kidney disease (CKD) (3, 4). Metabolic syndrome, characterized by a cluster of metabolic abnormalities, has also been identified as an independent risk factor for ESKD (5). Obesity often occurs with metabolic disorders, including hyperglycemia, dyslipidemia, and elevated blood pressure. The role of obesity in renal insufficiency is controversial (6–8), and partly depends on the clustering of metabolic abnormality factors. Obesity and metabolic status are categorized into four types of metabolic phenotypes. Metabolically unhealthy was based on the presence of metabolic syndrome and encompasses three of the five components outlined by the criteria from the National Cholesterol Education Program (NCEP) Adult Treatment Panel-III (ATP-III) (9). Previous studies have shown that the prevalence of CKD slowly increased in metabolically healthy non-obesity (MHNO), metabolically healthy obesity (MHO), metabolically unhealthy non-obesity (MUNO), and metabolically unhealthy obesity (MUO) subgroups within a Taiwanese population (10). Several studies have also reported that, compared to the MHNO group, the MHO, MUNO, and MUO groups progressively increased the CKD risk (11, 12). However, the effect of combined effects of the obesity based on body mass index (BMI) and metabolic status on the risk of future ESKD is lacking. Moreover, stratification by obesity and metabolic status management would have a strong impact on both the individual (stigmatization, self-esteem) and society (attention by healthcare professionals or politicians) (13). Thus, it is important to determine whether obesity itself, metabolic abnormalities, or both conditions contribute to the increased risk of ESKD.

Obesity-related glomerulopathy is characterized by glomerulomegaly as well as disorders of podocytes in the presence of focal and segmental glomerulosclerosis lesions with the underlying mechanisms of insulin resistance and blood pressure salt sensitivity (14). While the metabolic syndrome-induced renal injury mechanism is complex, a high prevalence of microvascular disease was observed in patients with metabolic syndrome. The renal pathological findings in patients with T2D and different metabolic phenotypes have not been well described. Based on the above, this study was used to determine the effect of metabolic phenotype on the risk of ESKD and illustrated the renal pathological characteristics of different metabolic phenotypes in patients with T2D and biopsy-proven DKD.

2 Materials and methods

2.1 Study design and patient selection

A total of 732 patients with diabetes who underwent percutaneous renal biopsy between 2003 and 2020 at the West China Hospital of Sichuan University were reviewed. Indications for renal biopsy include diabetes and renal damage with persistent albuminuria or renal dysfunction, sudden onset overt proteinuria, or hematuria (15). We excluded patients with the following conditions at enrollment: type 1 diabetes (n = 11), missing information on BMI or metabolic abnormalities (n = 23), those with a BMI <18.5 kg/m^2 (n = 6), progression to ESKD at the time of renal biopsy (n = 12), and co-existing non-diabetic renal disease, like membranous nephropathy or immunoglobulin A nephropathy (IgAN) (n = 185) (Figure 1). Finally, 495 adult patients with T2D and biopsy-confirmed DKD were included in the present study. All patients provided informed consent. The study was approved by the Institutional Review Board at the West China Hospital of Sichuan University. This study also complied with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

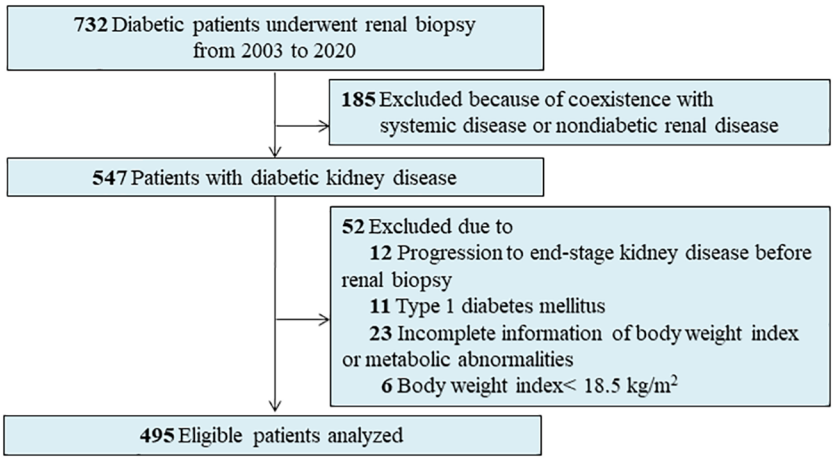

FIGURE 1
Flowchart of patients in this study.

2.2 Metabolic phenotype

According to the previous studies (16, 17), we defined the metabolic status using the ATP-III metabolic syndrome definition, excluding the waist circumference ≥80 cm component due to its significant collinearity with BMI. As all the patients in our study had T2D, patients with one or more of the three following components were classified as metabolically unhealthy (18): triglyceride level ≥1.7 mmol/L; high-density lipoprotein (HDL) cholesterol level <1.0 mmol/L in men or <1.3 mmol/L in women; or lipid-lowering medication use; systolic blood pressure ≥130 mmHg or diastolic pressure ≥85 mmHg; or antihypertensive medication use. Patients taking lipid-lowering agents with fibrates and/or statins were recorded as fulfilling the lipid criterion, while patients using antihypertensive drugs were recorded as satisfying the blood pressure criterion. Based on the Asian criteria, the general obesity status was assessed by BMI. Obesity was defined as a BMI of ≥ 25.0 kg/m^2 (19, 20). Patients with a BMI of 18.5–<23.0 were considered normal weight; for those with a BMI of 23.0–<25.0 were overweight. The patients with normal weight and those who were overweight were collapsed into the non-obesity group. Hence, we categorized four metabolic phenotypes based on the BMI-based categories (non-obesity, obesity) and metabolic status (metabolically healthy status or metabolically unhealthy status): MHNO, MHO, MUNO, and MUO (12).

2.3 Clinical and pathological covariates

Baseline demographics and clinical information at the time of the renal biopsy were extracted from the hospital's electronic medical records system. The estimated glomerular filtration rate (eGFR) was evaluated using the Chronic Kidney Disease Epidemiology Collaboration formula (21). The use of renin–angiotensin–aldosterone system inhibitors, new classes of glucose-lowering agents such as glucagon-like peptide-1 receptor agonists (GLP-1RA), dipeptidyl peptidase-4 (DDP-4) inhibitors, and sodium–glucose cotransporter 2 (SGLT2) inhibitors by the patient for more than half of the follow-up period was defined as treatment. Patients attend follow-up appointments two to four times annually, depending on their clinical condition (15, 22, 23).

Renal biopsy tissues were routinely prepared for light microscopy, immunofluorescence, and electron microscopy using standard procedures. The original immunofluorescence, microscopic, and electron microscopy images were used to confirm a diagnosis of DKD according to the basis of the Renal Pathology Society (RPS) classification (24). The glomerular classifications were categorized into five classes, which were class I, class IIa, class IIb, class III, and class IV. Interstitial fibrosis and tubular atrophy (IFTA) and interstitial inflammation were scored from 0 to 2. Arteriolar hyalinosis and arteriosclerosis were assessed and scored according to the RPS-DKD classification (24). In addition to the RPS-DKD classification, other pathological lesions, including global glomerulosclerosis, segmental sclerosis, extracapillary hypercellularity (EXHC), the Kimmelstiel–Wilson (KW) lesion, mesangial proliferation, and capillary microaneurysms, were evaluated (25). The detailed definition of pathological parameters is listed in Supplementary Table 1.

2.4 Renal outcomes

Renal outcome was defined by the progression to ESKD, which was defined as eGFR <15 ml/min/1.73 m^2 or the need for chronic renal replacement therapy (21). All patients were followed up until January 2022.

2.5 Statistical analysis

Continuous datasets were expressed as the mean and standard deviation (SD) if normally distributed, or as the median and interquartile range (IQR) if not normally distributed. Categorical datasets were expressed as counts and percentages. In patients with

different types of metabolic phenotypes, differences in the continuous datasets were analyzed using one-way analysis of variance (ANOVA). Categorical datasets were analyzed by the chi-squared test or Fisher's exact test. Differences in the continuous datasets between patients with or without obesity or metabolically healthy were analyzed by Student's *t*-test or the Wilcoxon test, while the categorical datasets were analyzed using the chi-squared test or Fisher's exact test (26).

Survival curves for different metabolic phenotypes were generated using the Kaplan–Meier method and log-rank tests. The Cox proportional hazard model was to explore hazard ratios (HRs) for ESKD. Baseline hemoglobin A1c (HbA1c) levels were missing for eight individuals. Multiple imputation methods were used to derive multivariable models. Three multivariable proportional hazard models, both of which included clinical parameters (age, sex, baseline eGFR, and proteinuria), were used in the present study. Age and sex were chosen based on biological plausibility. The second multivariable model incorporated the above parameters plus smoking status, HbA1c, hemoglobin, serum albumin concentration, usage of renin–angiotensin–aldosterone system inhibitors, and new classes of glucose-lowering agents. Clinical covariates such as HbA1c and serum albumin were selected as potential confounders because of their significance in the univariate analysis or association with ESKD in previous studies (27). The third model was the covariates in model 2 plus pathological parameters. Parameters with p <0.05 in the third adjusted model were significant predictors of prognosis.

We performed a series of sensitivity analyses to test the robustness of the results. First, we repeated the main analysis after excluding the first year of follow-up to minimize the potential for reverse causality (28). Second, we reexamined the metabolic phenotypes using the NCEP ATPIII definition of metabolic syndrome (having three of the five criteria) for the determination of metabolically unhealthy status. If the waist circumference was not obtained, the BMI was considered a risk factor for adiposity (29).

All statistical analyses were performed using Stata version 14.0 (Stata Corp. LLC, College Station, TX, USA). Statistical significance was accepted at p <0.05.

3 Results

3.1 Baseline clinical characteristics

Table 1 shows the baseline clinical characteristics of patients. The mean age of the patients was 51 years, and 346 (69.9%) were men. The median baseline eGFR was 59.7 ml/min/1.73 m^2, and the median 24-hour proteinuria was 4.20 g/d. The mean BMI was 25 kg/m^2, and the mean Hb1Ac was 7.6%. A total of 53.1% (263) patients were obese, and 46.9% (232) patients were non-obese. Approximately 17.0% (84) were in a metabolically healthy status, and 83.0% (411) were in a metabolically unhealthy status. Obese patients have a higher prevalence among men, and higher systolic/diastolic blood pressure than non-obese patients. Compared with non-obese patients, obese patients have a higher percentage of Tibetans and higher hemoglobin levels. The eGFR and proteinuria levels showed no significant difference between the non-obese group and the obese group (Supplementary Table 2). When patients were stratified by metabolic status, the sex and race distributions were similar between the patients with a metabolically healthy status and those with a metabolically unhealthy status (Supplementary Table 3). However, the eGFR level was significantly lower in patients with a metabolically unhealthy status than in those with a metabolically healthy status. Proteinuria showed no difference between the two groups (Supplementary Table 3).

When stratified by metabolic phenotypes, there are 56 (11.3%) MHNO patients, 28 (5.7%) MHO patients, 176 (35.6%) MUNO patients, and 235 (47.5%) MUO patients. Table 1 displays the baseline clinical characteristics among the four groups, with age being similar among all groups. However, patients with MHO and MUO have a higher percentage of males than the MHNO and MUNO groups. The systolic and diastolic blood pressure levels slowly increased in patients with MHNO compared to those with MUO. The median eGFR was 67.5 ml/min/1.73 m^2 in MHNO, 67.3 ml/min/1.73 m^2 in MHO, 58.0 ml/min/1.73 m^2 in MUNO, and 58.0 ml/min/1.73 m^2 in MUO. The median eGFR decreased slowly from the MHNO group to the MUO group. Of note, the triglyceride concentration slowly increased while the HDL concentration slowly decreased from the MHNO group to the MHO, MUNO, and MUO groups. However, proteinuria, duration of diabetes, HbA1c, and fasting plasma glucose showed no significant difference among the four groups.

3.2 Renal pathological changes

Supplementary Table 4 shows the renal pathological changes in obese patients and non-obese patients. The KW nodule is a typical renal structural change in DKD. The results showed that obese patients have severe mesangial expansion and a higher prevalence of KW nodules than non-obese patients. However, RPS glomerular classification, percentages of global glomerulosclerosis or segmental sclerosis, presence of EXHC, microaneurysm, and IFTA score showed no differences between the two groups.

Supplementary Table 5 shows renal pathological changes in patients with a metabolically unhealthy and a metabolically healthy status. Patients with a metabolically unhealthy status have higher IFTA, arteriosclerosis, and arteriolar hyalinosis scores compared to those with a metabolically healthy status. However, none of the glomerular lesions, such as mesangial expansion, KW nodule, and EXHC, showed any significant difference between patients with metabolically unhealthy status and those with metabolically healthy status.

Table 2 shows renal pathological findings among the MHNO, MHO, MUNO, and MUO groups. The results demonstrated that patients with MUO have the highest prevalence of KW nodules among the four groups. Compared with the MHNO group, mesangial expansion was much more severe in patients in the MHO, MUNO, and MUO groups. Furthermore, the prevalence of severe IFTA (scores 2 and 3) was significantly higher in the MUNO

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TABLE 1 Clinical characteristics of patients with different metabolic phenotypes.

Characteristics	MHNO (n = 56)	MHO (n = 28)	MUNO (n = 176)	MUO (n = 235)	*P*-value
Age, mean (SD), y	51 (10)	51 (8)	51 (9)	51 (10)	0.98
Sex, Male, n (%)	38 (67.9)	24 (85.7)	111 (63.1)	173 (73.6)	0.03
Race, Tibetan, n (%)	6 (10.7)	7 (25.0)	15 (8.5)	34 (14.5)	0.06
Smoking, Never/Ex/Current, (n)	34/5/17	16/3/9	103/25/48	116/42/77	0.405
Body mass index, mean (SD), kg/m^2	22 (2)	24 (3)	22 (2)	28 (3)	<0.001
Systolic blood pressure, mean (SD), mmHg	121 (9)	122 (9)	146 (24)	148 (23)	<0.001
Diastolic blood pressure, mean (SD), mmHg	78 (6)	79 (6)	85 (14)	88 (13)	<0.001
History of diabetic retinopathy, n (%)	31 (55.4)	11 (39.3)	100 (56.8)	120 (51.1)	0.31
Duration of diabetes, median (IQR), months	96 (36–120)	84 (36–132)	96 (36–144)	96 (40–132)	0.73
Hemoglobin A1c, median (IQR), %	7 (5.9–7.9)	6.9 (5.7–8.4)	7.3 (6.3–8.8)	7.4 (6.3–8.6)	0.29
Hemoglobin A1c, median (IQR), mmol/mol	53 (41–63)	52 (39–68)	56 (45–73)	57 (45–70)	0.29
Hemoglobin, mean (SD), g/L	114 (23)	124 (27)	115 (25)	124 (28)	<0.01
Serum albumin, mean (SD), g/L	33.8 (7.1)	34 (7)	33 (7.5)	34.5 (8)	0.25
Fasting plasma glucose, median (IQR), mg/dL	127 (94.5–190.8)	110.4 (90.6–141.6)	132.6 (101.5–187)	135.7 (104.8–176.4)	0.08
Estimated glomerular filtration rate, median (IQR), ml/min/1.73 m^2	67.5 (49.1–98.7)	67.3 (52.3–103.7)	58 (42.8–86)	58 (38.5–90.2)	0.04
24-h proteinuria, median (IQR), g/d	3.76 (1.33–5.56)	3.81 (1.75–7.5)	4.59 (2.28–7.04)	4.26 (1.84–7.92)	0.42
Hematuria, n (%)	26 (46.4)	15 (53.6)	78 (44.3)	90 (38.3)	0.30
Uric acid, mean (SD), mg/dl	0.6 (0.1)	0.6 (0.1)	0.6 (0.1)	0.7 (0.2)	0.03
Triglyceride, median (IQR), mg/dl	114.7 (82.4–134.6)	115.2 (84.1–140.4)	160.3 (117.8–224.1)	172.7 (124–263.1)	<0.001
Cholesterol, median (IQR), mg/dl	181.7 (163–210.4)	188.1 (163.8–228.3)	198 (165.7–245.6)	194.5 (160.5–241.7)	0.11
High-density lipoprotein cholesterol, median (IQR), mg/dl	58.4 (50.3–79.5)	51.3 (42.2–63)	50.7 (39.1–69.2)	45.2 (37.1–59.9)	<0.001
Low-density lipoprotein cholesterol, median (IQR), mg/dl	101.5 (72.1–123.5)	113.7 (96.7–143.9)	114.8 (79.7–148.1)	105.2 (76.6–141.9)	0.07
Renin–angiotensin–aldosterone system inhibitors, n (%)	43 (76.8)	22 (78.6)	135 (76.7)	185 (78.7)	0.96
New oral hypoglycemic agents, n (%)	14 (25.0)	10 (35.7)	61 (34.7)	78 (33.2)	0.59
Insulin therapy, n (%)	37 (66.1)	19 (67.9)	121 (68.8)	157 (66.8)	0.95

Data are presented as the mean (standard) for continuous variables with symmetric distribution, median (25th-75th percentiles) for continuous variables with asymmetric distribution, or percentages for categorical variables.
SD, standard deviation; IQR, interquartile range.

and MUO groups compared to the MHNO and MHO groups. However, interstitial inflammation, arteriosclerosis score, or arteriolar hyalinosis scores showed no significant difference among the four groups (Table 2).

3.3 Metabolic phenotype and risk of ESKD

During the median follow-up of 29 months, a total of 231 (46.7%) patients reached ESKD, and Figure 2A shows that the survival rate for renal outcome decreased from the MHNO group to the MUO group. Figure 2B shows that the renal survival rate was similar between non-obese and obese patients. When stratified by metabolic status, patients who were metabolically unhealthy demonstrated a lower renal survival rate than those who were metabolically healthy (Figure 2C). An increase in the number of cardiometabolic risk components was associated with an increased incidence of progression to ESKD (log-rank test, p = 0.01) (Figure 2D).

Table 3 shows the crude and multivariate-adjusted HRs for progression to ESKD based on the baseline cohort, using the MHNO group as the reference category. Univariate analysis showed that, compared with MHNO, the HR of progression to ESKD in diabetic patients with MHO, MUNO, and MUO was 1.60,

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TABLE 2 Renal pathological characteristics of patients with and without metabolic phenotype.

Characteristics	MHNO (n = 56)	MHO (n = 28)	MUNO (n = 176)	MUO (n = 235)	*P*-value
Renal Pathology Society classification†, n (%)					0.26
I + IIa	15 (26.8)	8 (28.6)	43 (24.4)	54 (23.0)	
IIb	16 (28.6)	7 (25.0)	36 (20.5)	35 (14.9)	
III	20 (35.7)	11 (39.3)	73 (41.5)	103 (43.8)	
IV	5 (8.9)	2 (7.1)	24 (13.6)	43 (18.3)	
Global glomerulosclerosis, (%)	25.9 (10–38.5)	14.3 (9.2–39.2)	25 (11.8–47.1)	29.4 (14.3–50)	0.91
Segmental sclerosis, (%)	0 (0–6.9)	0 (0–9.6)	0 (0–11.1)	0 (0–10.8)	0.93
Presence of Kimmelstiel–Wilson nodule, n (%)	20 (35.7)	13 (46.4)	74 (42.0)	134 (57.0)	<0.01
Presence of extracapillary hypercellularity, n (%)	6 (10.7)	1 (3.6)	15 (8.5)	15 (6.4)	0.54
Presence of microaneurysm, n (%)	27 (48.2)	6 (21.4)	81 (46.0)	101 (43.0)	0.09
Mesangial expansion					<0.001
score 0	3 (5.4)	0 (.0)	4 (2.3)	6 (2.6)	
score 1	36 (64.3)	4 (14.3)	80 (45.5)	87 (37.0)	
score 2	17 (30.4)	24 (85.7)	92 (52.3)	142 (60.4)	
Interstitial fibrosis and tubular atrophy†, n (%)					0.01
score 0	1 (1.8)	4 (14.3)	6 (3.4)	10 (4.3)	
score 1	34 (60.7)	17 (60.7)	93 (52.8)	100 (42.6)	
score 2	17 (30.4)	6 (21.4)	52 (29.5)	93 (39.6)	
score 3	4 (7.1)	1 (3.6)	25 (14.2)	32 (13.6)	
Interstitial inflammation†, n (%)					0.16
score 0	1 (1.8)	3 (10.7)	5 (2.8)	8 (3.4)	
score 1	44 (78.6)	16 (57.1)	119 (67.6)	172 (73.2)	
score 2	11 (19.6)	9 (32.1)	52 (29.5)	55 (23.4)	
Arteriosclerosis†, n (%)					0.16
score 0	9 (16.1)	5 (17.9)	18 (10.2)	30 (12.8)	
score 1	32 (57.1)	15 (53.6)	77 (43.8)	104 (44.3)	
score 2	15 (26.8)	8 (28.6)	81 (46.0)	101 (43.0)	
Arteriolar hyalinosis†, n (%)					0.09
score 0	9 (16.1)	7 (25.0)	18 (10.2)	27 (11.5)	
score 1	21 (37.5)	9 (32.1)	46 (26.1)	63 (26.8)	
score 2	26 (46.4)	12 (42.9)	112 (63.6)	145 (61.7)	

Data are presented as percent for categorical variables. † Defined by Renal Pathology Society Diabetic Kidney Disease Classification.

95% confidence interval [CI] 0.99–3.25, 1.92, 95% CI 1.12–3.29, and 1.95, 95% CI 1.16–3.28, respectively. The crude HR of HbA1c and diabetes duration for the progression to ESKD was 0.93 (95% CI 0.86–1.01) and 1.01 (95% CI 0.99–1.01) in patients with T2D, respectively. In the multivariate analysis, the adjusted HRs were 2.09 (95% CI 0.99–4.88) in the MHO, 2.16 (95% CI 1.20–3.88) in the MUNO, and 2.31 (95% CI 1.27–4.20) in the MUO groups compared with the MHNO group used as a reference. Additionally, in the multivariate analysis, HbA1c was not associated with future ESKD (HR 1.05, 95% CI 0.97–1.12).

To illustrate whether obesity or metabolic status is associated with renal outcome, we conducted Cox proportional HRs stratified by obesity or metabolic status (Table 3). The results showed that in the multivariate analysis, the presence of obesity itself was insignificantly associated with ESKD in patients with T2D compared with non-obese patients (aHR 1.22, 95% CI 0.88–1.68). When stratified by metabolic status, the metabolically unhealthy status significantly increased the risk for ESKD compared with the metabolically healthy status (aHR 1.69, 95% CI 1.10–2.60). Moreover, no significant interactions were

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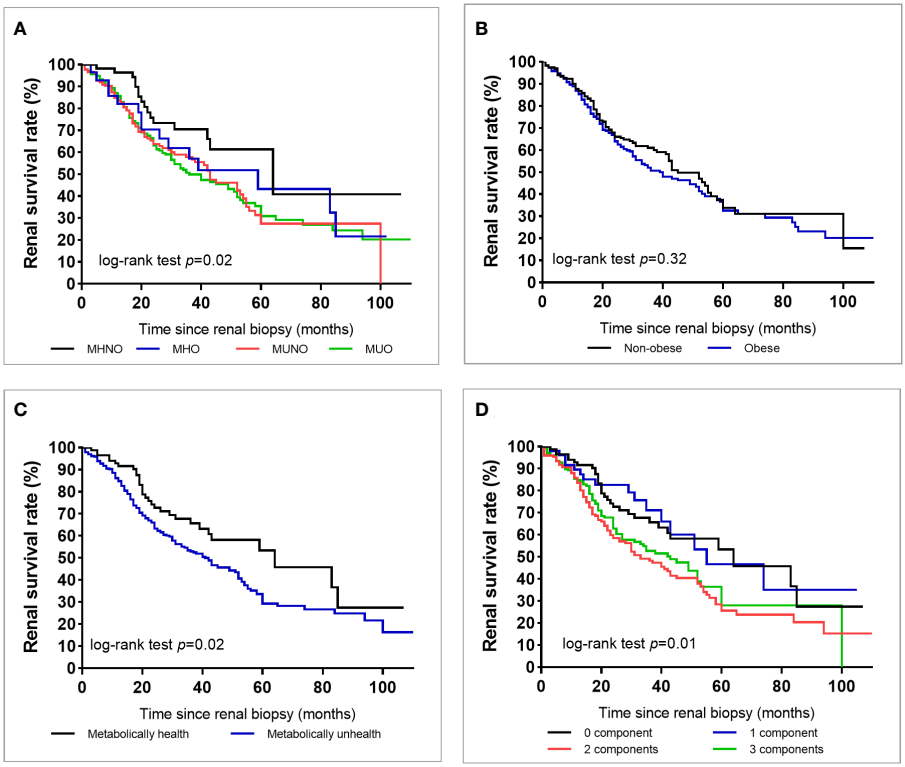

FIGURE 2
Survival rate of end-stage kidney disease in all patients with type 2 diabetes according to the metabolic phenotype **(A)**, obesity or not **(B)**, metabolically healthy status **(C)**, or according to the number of metabolic abnormalities except for diabetes **(D)**.

TABLE 3 Univariate and multivariate analyses for ESKD in patients with type 2 diabetes and diabetic kidney disease.

Variables	Univariate Models hazard ratio (95% confidence interval)	*p*-value	Multivariable model 1[a] adjusted hazard ratio (95% confidence interval)	*p*-value	Multivariable model 2[b] adjusted hazard ratio (95% confidence interval)	*p*-value	Multivariable model 3[c] adjusted hazard ratio (95% confidence interval)	*p* value
Metabolic phenotype								
MHNO	1 (reference)		1 (reference)		1 (reference)		1 (reference)	
MHO	1.60 (0.99–3.25)	0.05	1.86 (0.86–4.03)	0.12	3.17 (1.44–6.97)	<0.01	2.09 (0.99–4.88)	0.06
MUNO	1.92 (1.12–3.29)	0.02	1.76 (1.00–3.08)	0.05	2.15 (1.22–3.79)	0.01	2.16 (1.2–3.88)	0.01
MUO	1.95 (1.16–3.28)	0.01	1.86 (1.08–3.2)	0.03	2.86 (1.62–5.06)	<0.001	2.31 (1.27–4.2)	0.01
Obesity	1.14 (0.88–1.48)	0.33	1.19 (0.9–1.56)	0.22	1.57 (0.97–2.10)	0.06	1.22 (0.88–1.68)	0.22
Metabolically unhealthy status[†]	1.59 (1.09–2.32)	0.02	1.45 (0.96–2.19)	0.08	1.71 (1.12–2.6)	0.01	1.69 (1.10–2.60)	0.02

[a]Adjusted for age, sex, baseline estimated glomerular filtration rate, 24-h proteinuria, uric acid, hemoglobin A1c, and serum albumin concentration.
[b]Adjusted for the parameters in multivariable model a plus hemoglobin A1c, hemoglobin, and serum albumin concentration, usage of renin-angiotensin-aldosterone system inhibitors, new oral hypoglycemic agent, and smoking.
[c]Adjusted for the parameters in multivariable model b plus pathological parameters (Renal Pathology Society glomerular classifications, interstitial fibrosis and tubular atrophy, interstitial inflammation, arteriosclerosis, arteriolar hyalinosis, Kimmelstiel–Wilson nodule, and mesangial expansion).
[†]Comparison to metabolically healthy status.

found between obesity and metabolic status for predicting future ESKD (Supplementary Table 6).

3.4 Subgroup analysis and sensitivity analysis

In the subgroup analysis, the associations between metabolic phenotypes and renal outcome were slightly stronger among those aged >51 years, male, patients with a baseline HbA1c >7%, or with a baseline eGFR <60 ml/min/1.73 m^2. The adjusted HR was progressively higher in MHO, MUNO, and MUO as compared to the MHNO phenotype (Supplementary Figure 1).

In the sensitivity analyses, the relationships between metabolic phenotypes and renal outcome persisted after excluding the first one-year follow-up, which showed consistent positive associations with MHO, MUNO, MUO, and ESKD when compared to the MHNO group (Supplementary Table 7). Furthermore, we defined a metabolically unhealthy status as the presence of metabolic syndrome. We performed sensitivity analyses when using the metabolic syndrome NCEP ATPIII definition and obtained similar results (Supplementary Table 8).

4 Discussion

In this retrospective cohort of patients with T2D and biopsy-proven DKD, approximately half of the patients were obese and had a metabolically unhealthy status. The adjusted HR was progressively higher in MHO, MUNO, and MUO as compared to the MHNO phenotype. Of note, the presence of obesity was not significantly associated with ESKD compared with non-obesity patients, while a metabolically unhealthy status was significantly associated with ESKD compared with a metabolically healthy status in multivariate analysis. Regarding renal pathological characteristics, the high prevalence of KW nodules and severe mesangial expansion was associated with obesity, whereas severe IFTA was associated with a metabolically unhealthy status.

To our knowledge, this is the first study to investigate the association between metabolic phenotype and renal outcome in biopsy-proven DKD patients. Patients with biopsy-confirmed DKD were recruited to uniformly assess renal pathological changes, while the underlying effect of non-diabetic renal diseases on renal outcome was excluded. The link between progressive kidney disease in obese patients and metabolic abnormalities is of enormous public health importance. The role of obesity in renal insufficiency in diabetes is controversial according to the literature. Chung et al. (7) conducted a longitudinal study on 1,187 diabetic patients in Taiwan, showing that diabetic patients who are obese were more likely to have CKD. However, a prospective 5-year study conducted in the eastern region of Morocco showed that the decline of eGFR in DKD of T2D is not directly influenced by BMI (6). Another large epidemiological study conducted in Manchester, UK, demonstrated that there was no statistically significant difference in the rate of progression of CKD between obese and non-obese T2D patients (30). The results of our study showed that obesity itself was not an independent risk factor for ESKD in T2D. This indicated that obesity increased intraglomerular pressure and played an important role in the incidence of DKD, while it was not an independent risk factor for the progression of DKD in T2D. The major risk factors contributing to eGFR decline remain traditional factors such as a low baseline eGFR. Moreover, our data showed that metabolic abnormalities were significantly associated with the risk of ESKD in T2D after adjusting for traditional factors. Similarly, a cross-sectional study enrolled 14,983 general population participants in Taiwan and reported that a metabolically unhealthy status, but not obesity, was associated with a higher risk of CKD (10). Data from a cohort of 3.5 million individuals recorded by The Health Improvement Network in the United Kingdom showed that although cardiometabolic risk increased from normal weight to overweight and obesity, it was more pronounced with an increasing number of metabolic abnormalities (31). It was suggested that metabolic abnormalities could be the intermediate factors linking obesity to future ESKD. Our results showed that obesity itself was insignificantly associated with ESKD; however, their association was enhanced and became significant when adding metabolic status, supporting this suggestion. Furthermore, a significant relationship was found between metabolically unhealthy status and the risk of ESKD, demonstrating their simultaneous contributions to the disease.

MHO, due to its low prevalence (only 5.7% in our cohort), was often ignored by physicians. MHO is characterized as obesity with a low burden of metabolic abnormality, which has been considered a "benign condition" in the past few years. In different European countries, the prevalence of MHO varied from 2% to 28% (13). Meta-analyses of prospective studies demonstrated that MHO is associated with a significantly lower incidence of T2D (32) and cardiovascular disease (32, 33). However, our results revealed that MHO did not associate with renal outcome, which was consistent with a previous study (34). Despite these, we reveal that in subgroups of ages greater than 51 years old, male, HbA1c >7%, and eGFR <60 ml/min/1.73 m^2, MHO significantly increased the risk for progression to ESKD compared with MHNO. This result indicates that MOH still needs rapid intervention in this patient group.

MHO, MUNO, and MUO are reported to be transient phenotypes (13). Previous studies reported that the cardiovascular disease risk increased in women who converted from MHO to MUO compared to those with stable MHO, which was primarily driven by the incidence of T2D and hypertension (33). In this study, we found that systolic/diastolic blood pressure was significantly higher in patients with MUO than in those with MHO, which highlights the importance of intensive blood pressure and blood glucose therapy in a pragmatic approach. Compared with MHO, MUO was considered an alteration in the distribution of ectopic fat. The ectopic fat accumulation triggered an inflammatory cascade, and lipotoxicity was considered an inducer of metabolic dysfunction (35). The transitions between MHO and MUO are not unidirectional and may change over time. Patients who underwent obesity surgery or strict weight-loss interventions may

lead the MUO phenotype transition to MHO or even the MHNO phenotype (36). A nationwide population-based study reported that patients who converted to MHNO had a 2% decrease in risk, whereas patients who evolved to MUNO or MUO had a 60%–68% increased risk of CKD incidence compared to the stable MHNO group (28). Thus, we performed a sensitivity analysis by excluding the first one-year follow-up to test the robustness of the results, which showed consistent positive associations with MHO, MUNO, MUO, and ESKD. Based on the above results, combination therapy for both diabetes and obesity appear to be a promising tool for the future pharmacotherapy of DKD. Indeed, the first glucagon-like peptide 1 (GLP-1)/glucagon coagonist (cotadutide) improved glycemic control, weight loss, and even improved metabolic profiles in patients who were overweight or obese and had T2D in a Phase 2b clinical trial (37). Another novel dual glucose-dependent insulinotropic polypeptide (GIP) and GLP-1 receptor agonist, tirzepatide, not only reduces body weight but also improves the metabolite profile in patients with T2D (38, 39). The *post-hoc* analysis of the SURPASS-4 study showed that tirzepatide showed a delayed effect on eGFR decline in patients with T2D (40).

The obesity-related glomerulopathy shares several pathophysiologic factors relevant to renal damage in DKD. The present study showed that the presence of KW nodules and a mesangial expansion degree were severe in obese patients compared to non-obese patients. The mechanism may be related to the renal hemodynamic changes due to obesity. Animal models of obesity-related glomerulopathy showed that the glomerular tuft volume increased exponentially in relation to body weight gain (41). The numerical density of podocytes decreases as the glomerular diameter increases under persistent renal hyperfusion, thereby causing the decreased extension of podocytic processes to cover the expanded area. This induced a loss in protein selectivity and triggered matrix deposition (42). However, the pathological findings were much different when stratified by metabolic status. Patients with a metabolically unhealthy status have more severe interstitial and microvascular changes (arteriosclerosis and arteriolar hyalinosis) than those with a metabolically healthy status, which is consistent with previous studies (29, 43). The underlying mechanism of metabolic abnormalities induced renal tubular injury may be associated with oxidative stress and cytochrome-C-induced apoptosis (44). The microvascular changes may be linked to greater blood pressure observed in patients with a metabolically unhealth status (43). Coexisting metabolic abnormalities and renal vascular changes synergistically aggravate tubular mitochondrial damage and dysfunction, ultimately leading to renal structural injury and renal insufficiency (45).

Albuminuria is one of the early warning signs of CKD. A previous study showed a relationship between metabolic syndrome and albuminuria, especially in non-East Asian populations (46). But in patients with renal transplant, albuminuria was more associated with elevated systolic blood pressure and hyperglycemic status than with metabolic syndrome (47). Thus, the absence of an association between proteinuria and unhealthy metabolic status may partly be related to the different ethnicities and advanced DKD in this study.

Univariate and multivariate analysis showed that diabetes duration and baseline HbA1c were not associated with progression to ESKD in patients with T2D (data not shown), which was consistent with previous studies (21, 25, 48). In a prospective analysis of patients with T2D, the coefficient of HbA1c variation, not the mean of serially measured fasting plasma glucose, was predictive of the development of renal complications and diabetes-related outcomes (49). A wide variance of HbA1c reflects a more complicated clinical course, suboptimal use of medications, and/or self-management (49). The absence of an association between diabetes duration and renal outcome may be explained by the inability to establish the time of onset of T2DM with certainty. Many individuals have undiagnosed diabetes and impaired glucose tolerance for extended periods, leading to inaccurate assessments of diabetes duration (50).

There are several limitations to this study. First, due to the retrospective observational design, causal relationships between metabolic phenotype and DKD progression cannot be inferred from the results. A prospective study conducted among patients with T2D will be useful to validate our conclusions. Second, our study consisted of a single-center cohort, thus lacking external validity. However, biochemical measurements using the same standard method improved the reliability of our data. Third, no phenotypic changes were recorded during the follow-up time. A limited patient sample size of individuals with MHO was another limitation of this study. The negative association between MHO and renal outcome in patients with T2D cannot be excluded, with the limited number of patients and low incidence of renal events resulting in insufficient power. However, the absence of an interaction between obesity and a metabolically unhealthy status led to the potentially impactful conclusion that obesity itself was not significantly associated with renal outcome in T2D. A large cohort sample size would be useful to validate our conclusion.

In conclusion, this retrospective cohort study demonstrated that the combination of obesity and metabolic health status was associated with an increased risk of ESKD in T2D and biopsy-proven DKD. The highest risk was observed in participants with both obesity and a metabolically unhealthy status. Specifically, MUNO and MUO were both associated with increased risk of ESKD, suggesting that metabolic profiles need to be regularly monitored to reduce future ESKD risk.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by The institutional review board at the West China

Hospital of Sichuan University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

LZ analyzed the data, interpreted the results, and drafted the manuscript. FL analyzed and interpreted data, edited/revised, and approved the final version of the manuscript. YTZ, YCW, HR, YTW, YCZ, XX, JY, QY, and LC carried out the data collecting and recording. NT contributed to the discussion. FL is the guarantor of this work and had full access to all the data in this study and takes responsibility for the integrity of the data. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1103251/full#supplementary-material

References

1. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF diabetes atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract* (2022) 183:109119. doi: 10.1016/j.diabres.2021.109119

2. Jung CY, Yoo TH. Pathophysiologic mechanisms and potential biomarkers in diabetic kidney disease. *Diabetes Metab J* (2022) 46(2):181–97. doi: 10.4093/dmj.2021.0329

3. Bae EH, Lim SY, Jung JH, Oh TR, Choi HS, Kim CS, et al. Obesity, abdominal obesity and chronic kidney disease in young adults: A nationwide population-based cohort study. *J Clin Med* (2021) 10(5):1065–78. doi: 10.3390/jcm10051065

4. Evangelista LS, Cho WK, Kim Y. Obesity and chronic kidney disease: a population-based study among south koreans. *PloS One* (2018) 13(2):e0193559. doi: 10.1371/journal.pone.0193559

5. Lee YB, Han K, Kim B, Jun JE, Lee SE, Ahn J, et al. Risk of end-stage renal disease from chronic kidney disease defined by decreased glomerular filtration rate in type 1 diabetes: a comparison with type 2 diabetes and the effect of metabolic syndrome. *Diabetes Metab Res Rev* (2019) 35(8):e3197. doi: 10.1002/dmrr.3197

6. Bentata Y, Latrech H, Abouqal R. Does body mass index influence the decline of glomerular filtration rate in diabetic type 2 patients with diabetic nephropathy in a developing country? *Ren Fail* (2014) 36(6):838–46. doi: 10.3109/0886022x.2014.899472

7. Chung HF, Al Mamun A, Huang MC, Long KZ, Huang YF, Shin SJ, et al. Obesity, weight change, and chronic kidney disease in patients with type 2 diabetes mellitus: a longitudinal study in Taiwan. *J Diabetes* (2017) 9(11):983–93. doi: 10.1111/1753-0407.12514

8. Shibata I, Shibata M, Sato KK, Takeuchi Y, Okamura K, Koh H, et al. Body mass index and the risk of persistent proteinuria in middle-aged men: the Kansai healthcare study. *Am J Nephrol* (2022) 53(2-3):191–8. doi: 10.1159/000521885

9. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult treatment panel III). *Jama* (2001) 285(19):2486–97. doi: 10.1001/jama.285.19.2486

10. Chen HY, Lu FH, Chang CJ, Wang RS, Yang YC, Chang YF, et al. Metabolic abnormalities, but not obesity per se, associated with chronic kidney disease in a Taiwanese population. *Nutr Metab Cardiovasc Dis* (2020) 30(3):418–25. doi: 10.1016/j.numecd.2019.09.029

11. Alizadeh S, Esmaeili H, Alizadeh M, Daneshzad E, Sharifi L, Radfar H, et al. Metabolic phenotypes of obese, overweight, and normal weight individuals and risk of chronic kidney disease: a systematic review and meta-analysis. *Arch Endocrinol Metab* (2019) 63(4):427–37. doi: 10.20945/2359-3997000000149

12. Wang Y, Sun B, Sheng LT, Pan XF, Zhou Y, Zhu J, et al. Association between weight status, metabolic syndrome, and chronic kidney disease among middle-aged and elderly Chinese. *Nutr Metab Cardiovasc Dis* (2020) 30(11):2017–26. doi: 10.1016/j.numecd.2020.06.025

13. Blüher M. Metabolically healthy obesity. *Endocr Rev* (2020) 41(3):1–16. doi: 10.1210/endrev/bnaa004

14. Ritz E, Koleganova N, Piecha G. Is there an obesity-metabolic syndrome related glomerulopathy? *Curr Opin Nephrol Hypertens* (2011) 20(1):44–9. doi: 10.1097/MNH.0b013e3283414ca1

15. Zhao L, Zou Y, Zhang J, Zhang R, Ren H, Li L, et al. Serum transferrin predicts end-stage renal disease in type 2 diabetes mellitus patients. *Int J Med Sci* (2020) 17 (14):2113–24. doi: 10.7150/ijms.46259

16. Liang X, Margolis KL, Hendryx M, Rohan TE, Groessl EJ, Thomson CA, et al. Metabolic phenotype and risk of colorectal cancer in normal-weight postmenopausal women. *Cancer Epidemiol Biomarkers Prev* (2017) 26(2):155–61. doi: 10.1158/1055-9965.Epi-16-0761

17. Schweitzer ML, Stengel B, Legrand K, Briançon S, Jacquelinet C, Combe C, et al. Obesity phenotype and patient-reported outcomes in moderate and severe chronic kidney disease: a cross-sectional study from the CKD-REIN cohort study. *Qual Life Res* (2019) 28(7):1873–83. doi: 10.1007/s11136-019-02110-2

18. Cho YK, Lee J, Kim HS, Park JY, Lee WJ, Kim YJ, et al. Metabolic health is a determining factor for incident colorectal cancer in the obese population: a nationwide population-based cohort study. *Cancer Med* (2021) 10(1):220–9. doi: 10.1002/cam4.3607

19. Chen J, Kong X, Jia X, Li W, Wang Z, Cui M, et al. Association between metabolic syndrome and chronic kidney disease in a Chinese urban population. *Clin Chim Acta* (2017) 470:103–8. doi: 10.1016/j.cca.2017.05.012

20. Zheng X, Peng R, Xu H, Lin T, Qiu S, Wei Q, et al. The association between metabolic status and risk of cancer among patients with obesity: metabolically healthy obesity vs. metabolically unhealthy obesity. *Front Nutr* (2022) 9:783660. doi: 10.3389/fnut.2022.783660

21. Zhao L, Ren H, Zhang J, Cao Y, Wang Y, Meng D, et al. Diabetic retinopathy, classified using the lesion-aware deep learning system, predicts diabetic end-stage renal disease in Chinese patients. *Endocr Pract* (2020) 26(4):429–43. doi: 10.4158/EP-2019-0512

22. Zhao L, Wang X, Wang T, Fan W, Ren H, Zhang R, et al. Associations between high-altitude residence and end-stage kidney disease in Chinese patients with type 2 diabetes. *High altitude Med Biol* (2020) 21(4):396–405. doi: 10.1089/ham.2020.0076

23. Zhao L, Zhang J, Lei S, Ren H, Zou Y, Bai L, et al. Combining glomerular basement membrane and tubular basement membrane assessment improves the prediction of diabetic end-stage renal disease. *J Diabetes* (2021) 13(7):572–84. doi: 10.1111/1753-0407.13150

24. Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB, et al. Pathologic classification of diabetic nephropathy. *J Am Soc Nephrol* (2010) 21(4):556–63. doi: 10.1681/ASN.2010010010

25. Zhao L, Liu F, Li L, Zhang J, Wang T, Zhang R, et al. Solidified glomerulosclerosis, identified using single glomerular proteomics, predicts end-stage renal disease in Chinese patients with type 2 diabetes. *Sci Rep* (2021) 11(1):4658. doi: 10.1038/s41598-021-83856-z

26. Zhao L, Li L, Ren H, Zou Y, Zhang R, Wang S, et al. Association between serum alkaline phosphatase and renal outcome in patients with type 2 diabetes mellitus. *Renal Failure* (2020) 42(1):818–28. doi: 10.1080/0886022x.2020.1804402

27. Zhang J, Zhang R, Wang Y, Li H, Han Q, Wu Y, et al. The level of serum albumin is associated with renal prognosis in patients with diabetic nephropathy. *J Diabetes Res* (2019) 2019:7825804. doi: 10.1155/2019/7825804

28. Cho YK, Lee J, Kim HS, Park JY, Lee WJ, Kim YJ, et al. Impact of transition in metabolic health and obesity on the incident chronic kidney disease: A nationwide cohort study. *J Clin Endocrinol Metab* (2020) 105(3):e148–e157. doi: 10.1210/clinem/dgaa033

29. Zhao L, Zou Y, Bai L, Zhou L, Ren H, Wu Y, et al. Prognostic value of metabolic syndrome in renal structural changes in type 2 diabetes. *Int Urol Nephrol* (2022) 54(8):2005–14. doi: 10.1007/s11255-021-03051-x

30. Mohsen A, Brown R, Hoefield R, Kalra PA, O'Donoghue D, Middleton R, et al. Body mass index has no effect on rate of progression of chronic kidney disease in subjects with type 2 diabetes mellitus. *J Nephrol* (2012) 25(3):384–93. doi: 10.5301/jn.5000062

31. Caleyachetty R, Thomas GN, Toulis KA, Mohammed N, Gokhale KM, Balachandran K, et al. Metabolically healthy obese and incident cardiovascular disease events among 3.5 million men and women. *J Am Coll Cardiol* (2017) 70(12):1429–37. doi: 10.1016/j.jacc.2017.07.763

32. Bell JA, Kivimaki M, Hamer M. Metabolically healthy obesity and risk of incident type 2 diabetes: a meta-analysis of prospective cohort studies. *Obes Rev* (2014) 15(6):504–15. doi: 10.1111/obr.12157

33. Eckel N, Li Y, Kuxhaus O, Stefan N, Hu FB, Schulze MB. Transition from metabolic healthy to unhealthy phenotypes and association with cardiovascular disease risk across BMI categories in 90 257 women (the nurses' health study): 30 year follow-up from a prospective cohort study. *Lancet Diabetes Endocrinol* (2018) 6(9):714–24. doi: 10.1016/s2213-8587(18)30137-2

34. Uehara S, Sato KK, Koh H, Shibata M, Kinuhata S, Yamada A, et al. The association between metabolically healthy obesity and the risk of proteinuria: the Kansai healthcare study. *J Epidemiol* (2018) 28(8):361–6. doi: 10.2188/jea.JE20170082

35. Martin-Piedra L, Alcala-Diaz JF, Gutierrez-Mariscal FM, Arenas de Larriva AP, Romero-Cabrera JL, Torres-Peña JD, et al. Evolution of metabolic phenotypes of obesity in coronary patients after 5 years of dietary intervention: from the CORDIOPREV study. *Nutrients* (2021) 13(11):4046–59. doi: 10.3390/nu13114046

36. van Vliet-Ostaptchouk JV, Nuotio ML, Slagter SN, Doiron D, Fischer K, Foco L, et al. The prevalence of metabolic syndrome and metabolically healthy obesity in Europe: a collaborative analysis of ten large cohort studies. *BMC Endocr Disord* (2014) 14:9. doi: 10.1186/1472-6823-14-9

37. Nahra R, Wang T, Gadde KM, Oscarsson J, Stumvoll M, Jermutus L, et al. Effects of cotadutide on metabolic and hepatic parameters in adults with overweight or obesity and type 2 diabetes: a 54-week randomized phase 2b study. *Diabetes Care* (2021) 44(6):1433–42. doi: 10.2337/dc20-2151

38. Pirro V, Roth KD, Lin Y, Willency JA, Milligan PL, Wilson JM, et al. Effects of tirzepatide, a dual GIP and GLP-1 RA, on lipid and metabolite profiles in subjects with type 2 diabetes. *J Clin Endocrinol Metab* (2022) 107(2):363–78. doi: 10.1210/clinem/dgab722

39. Thomas MK, Nikooienejad A, Bray R, Cui X, Wilson J, Duffin K, et al. Dual GIP and GLP-1 receptor agonist tirzepatide improves beta-cell function and insulin sensitivity in type 2 diabetes. *J Clin Endocrinol Metab* (2021) 106(2):388–96. doi: 10.1210/clinem/dgaa863

40. Heerspink HJL, Sattar N, Pavo I, Haupt A, Duffin KL, Yang Z, et al. Effects of tirzepatide versus insulin glargine on kidney outcomes in type 2 diabetes in the SURPASS-4 trial: Post-hoc analysis of an open-label, randomised, phase 3 trial. *Lancet Diabetes Endocrinol* (2022) 10(11):774–85. doi: 10.1016/s2213-8587(22)00243-1

41. Guo YP, Jiang HK, Jiang H, Tian HY, Li L. Lipoxin A4 may attenuate the progression of obesity-related glomerulopathy by inhibiting NF-κB and ERK/p38 MAPK-dependent inflammation. *Life Sci* (2018) 198:112–8. doi: 10.1016/j.lfs.2018.02.039

42. Wei L, Li Y, Yu Y, Xu M, Chen H, Li L, et al. Obesity-related glomerulopathy: from mechanism to therapeutic target. *Diabetes Metab Syndr Obes* (2021) 14:4371–80. doi: 10.2147/dmso.S334199

43. Alexander MP, Patel TV, Farag YMK, Florez A, Rennke HG, Singh AK. Kidney pathological changes in metabolic syndrome: a cross-sectional study. *Am J Kidney Dis Off J Natl Kidney Foundation* (2009) 53(5):751–9. doi: 10.1053/j.ajkd.2009.01.255

44. Eirin A, Woollard JR, Ferguson CM, Jordan KL, Tang H, Textor SC, et al. The metabolic syndrome induces early changes in the swine renal medullary mitochondria. *Transl Res* (2017) 184:45–56.e9. doi: 10.1016/j.trsl.2017.03.002

45. Nargesi AA, Zhang L, Tang H, Jordan KL, Saadiq IM, Textor SC, et al. Coexisting renal artery stenosis and metabolic syndrome magnifies mitochondrial damage, aggravating poststenotic kidney injury in pigs. *J hypertension* (2019) 37(10):2061–73. doi: 10.1097/HJH.0000000000002129

46. Rashidbeygi E, Safabakhsh M, Delshad Aghdam S, Mohammed SH, Alizadeh S. Metabolic syndrome and its components are related to a higher risk for albuminuria and proteinuria: evidence from a meta-analysis on 10,603,067 subjects from 57 studies. *Diabetes Metab Syndr* (2019) 13(1):830–43. doi: 10.1016/j.dsx.2018.12.006

47. Sipahioglu MH, Unal A, Yazgac H, Tunca O, Arikan T, Kocyigit I, et al. Relationships between metabolic syndrome, microalbuminuria, and c-reactive protein in Turkish kidney transplant recipients. *Transplant Proc* (2015) 47(5):1408–12. doi: 10.1016/j.transproceed.2015.04.037

48. Zhuo L, Zhang N, Zou G, Chen D, Li W. Clinical characteristics and outcomes of biopsy-proven diabetic nephropathy. *Front Med* (2017) 11(3):386–92. doi: 10.1007/s11684-017-0574-z

49. Luk AO, Ma RC, Lau ES, Yang X, Lau WW, Yu LW, et al. Risk association of HbA1c variability with chronic kidney disease and cardiovascular disease in type 2 diabetes: prospective analysis of the Hong Kong diabetes registry. *Diabetes Metab Res Rev* (2013) 29(5):384–90. doi: 10.1002/dmrr.2404

50. Zimmet P. The burden of type 2 diabetes: are we doing enough? *Diabetes Metab* (2003) 29(4 Pt 2):6s9–18. doi: 10.1016/s1262-3636(03)72783-9

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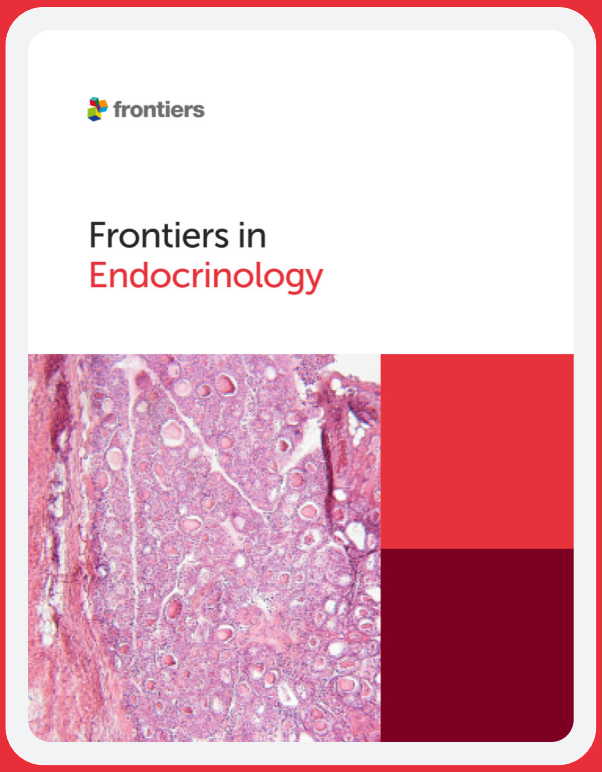

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