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Diagnosis, prevention and treatment in diabetic nephropathy, volume III

Edited by
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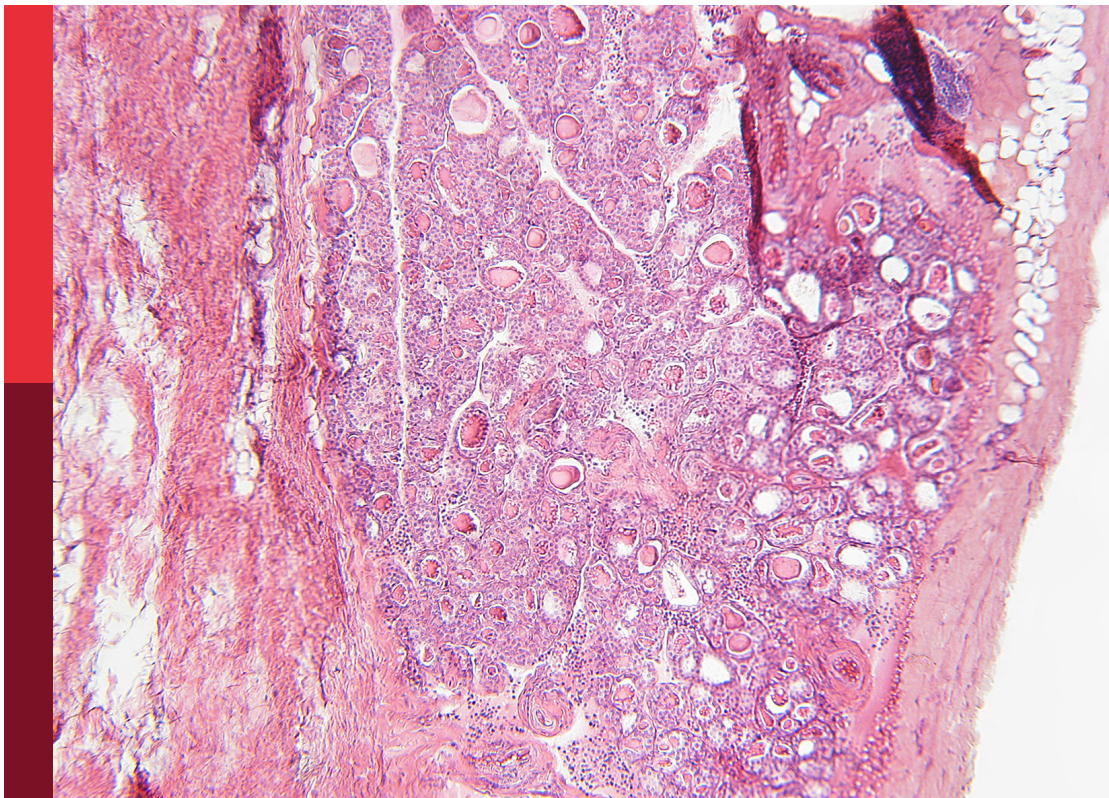

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Diagnosis, prevention and treatment in diabetic nephropathy, volume III

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Editorial: Diagnosis, prevention and treatment in diabetic nephropathy, volume III

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KEYWORDS

diabetes mellitus, kidney, diabetic nephropathy, chronic kidney disease, end-stage kidney disease

Editorial on the Research Topic
Diagnosis, prevention and treatment in diabetic nephropathy, volume III

Diabetic nephropathy (DN) represents the leading cause of end-stage kidney disease (ESKD), affecting approximately 30-40% of diabetic patients. It is characterized by progressive kidney damage, which manifests with albuminuria, declining glomerular filtration rate (GFR), and structural changes such as glomerulosclerosis and tubulointerstitial fibrosis. Contemporary medical research has made great strides in understanding diabetes mellitus (DM) complications including DN. From new biomarkers to treatments based on nanomedicine and the interaction between gut microbiota and kidney disease, the studies included in this Research Topic offer a comprehensive and multidimensional vision to improve diagnosis, prevention and management of diabetic kidney disease.

Zhang et al. provide an overview of the state of the art on DN pathogenesis and treatment, focusing on connection of DN with cardiovascular conditions like hypertension and coronary artery disease. Thus, a correct and holistic management of this condition is crucial to reduce mortality and improve quality of life.

In this regard, even in this third Research Topic on DN, the emphasis remains strong on the need for innovative biomarkers to facilitate early detection and monitoring disease progression.

Of interest, given the interplay between diabetes and its microvascular and macrovascular complications, several works explored the role of innovative inflammatory biomarker in the setting of DN.

In particular, Jiang et al. found that vascular endothelial growth factor (VEGF) was strongly associated with DN progression and glycemic control in a population of patients with type 2 DM, suggesting a predictive role of VEGF for glycemic control and DN in older adults with type 2 DM.

Nie et al., in a study conducted on the National Health and Nutrition Examination Survey (NHANES) database, showed an association between the systemic immune-

inflammation index (SII), an inflammatory biomarker which integrates neutrophils, lymphocytes, and platelets, and higher prevalence of diabetes; specifically, each unit increase in SII/100 increased the likelihood of having diabetes by 4%.

Moreover, in a Chinese population, Yan et al. showed that higher SII is independently associated with an increased risk of DN and its severity, suggesting that SII might be a promising biomarker for DN and its distinct phenotypes in the Chinese population.

Compared to other inflammatory markers, including SII, monocyte-to-lymphocyte ratio (MLR) and systemic inflammatory response index (SIRI), Li et al. showed instead that neutrophil-to-lymphocyte ratio (NLR) seems to be more effective in identifying the risk of DN, albuminuria, and low-eGFR in type 2 DM patients. However, type 2 DM patients with elevated levels of NLR, SII, MLR and SIRI should be closely monitored for their potential risk to renal function.

Another potential prognostic marker for renal endpoint events in individuals with DN, has been explored by Sun et al. which showed that serum secretory leukocyte protein inhibitor (SLPI), a protein with antiprotease, anti-inflammatory and immunomodulatory activity, expressed in distal renal tubular cells was associated with DN progression and clinical parameters of DN.

Among innovative biomarkers, Cao et al. analyzed the role of retinol-binding protein 4 (RBP4) as a marker for the detection of renal impairment, showing its potential as a diagnostic tool with good sensitivity and specificity for patients with type 2 DM and DN.

Even tumoral markers, such as CEA, SCC, and CA211, have been explored in a study by Chen et al. showing a potential in the early detection of microalbuminuria in patients with type 2 DM.

In a study based on 4888 subjects of the National Nutrition and Health Examination Survey (NHANES) database Tang et al. showed instead that higher levels of lactate dehydrogenase (LDH), an easy-to-obtain marker, were an independent risk factor for the risk of DN in patients with type 2 DM.

Interestingly, this Research Topic also presents us with works that explore the correlation between fecal microbiota and DN.

In particular, alterations in the gut bacterial composition and metabolites, including Trimethylamine (TMA) and trimethylamine-N-oxide (TMAO), have been correlated with progression of DN, as showed by the studies of Yu et al. and Huo et al.

Specifically, as described by Yu et al., kidney injury in diabetic patients can enhance dysbiosis of the gut microbiota, which can itself further impair renal function through several metabolites. For this reason, enhancing gut microbiota stability, improving glucose metabolism, and reducing uremic toxin production through dietary adjustments, fecal microbiota transplantation (FMT), and the use of probiotics or prebiotics can help delay the progression of DN.

Moreover, as demonstrated by Lu et al., genetic variants associated with gut microbiota composition can also influence the susceptibility to DN, opening new avenues for personalized approaches.

Another point addressed in this topic includes the relationship between lifestyle and DN.

We already know that obesity and sleep duration are associated with cardiovascular risk factors, including insulin resistance, hyperglycemia, hypertension, dyslipidemia, and inflammatory response. Liu et al., showed that BMI changes and sleep duration were determinants of ND progression. Indeed, excessive sleep and increased BMI were associated with the risk of ND.

Moreover, Liu et al., in a cross-sectional study including 5389 participants, showed that higher levels of oxidative balance score (OBS), a comprehensive indicator that considers various dietary components and lifestyle factors to assess an individual's exposure to pro-oxidants and antioxidants, and dietary OBS were associated with a reduced risk of diabetic kidney disease, low-eGFR, and albuminuria, highlighting the importance of an antioxidant-rich diet and lifestyle among diabetic patients and suggesting that the integration of OBS into clinical practice may represent an innovative strategy to mitigate the burden of DN.

The management of DN is based on a holistic approach aimed at controlling blood glucose and blood pressure, reducing albuminuria, slowing disease progression and managing underlying comorbidities associated with DM.

If DN progresses to ESKD, the only available treatments are dialysis and kidney transplantation. However, neither significantly improves patient survival. As a result, increasing research efforts are focused on identifying new therapeutic targets and diagnostic markers.

At present, among the treatment available for DN, we find medications such as insulin or sodium-glucose transport protein 2 (SGLT2) inhibitors, renin-angiotensin-aldosterone system (RAAS) inhibitors and statins, which are often used to address dyslipidemia.

Interestingly, Guo et al. showed that long-term treatment with statins increased the risk of DN.

Zhou et al. clarify the role the potential targets of RAAS inhibitors for the treatment of DN.

Among cutting-edge treatments, nanotherapies are emerging as one of the most promising therapeutic approaches, as showed by (Paul et al.). In particular, nanotherapeutic platforms enable targeted drug delivery to renal tissues, improving therapeutic efficacy and reducing systemic side effects. This represents a significant advance over conventional therapies.

However, future research should focus on developing new treatments, particularly targeting inflammation, oxidative stress, and vascular dysfunction. Advances in technology will enable personalized and precision approaches of treatment.

In conclusion, this Research Topic underline that advances in biomarkers, innovative therapies and understanding the gut microbiota offer new hope to improve the quality of life of patients with DN. However, it is essential to promote longitudinal and multidimensional studies to validate these findings and translate them into personalized and effective clinical interventions.

Therefore, investing in translational research and implementing targeted prevention programs are essential. A comprehensive approach that integrates advanced therapies, lifestyle modifications, and continuous monitoring is crucial for effectively managing this complex pathology.

Author contributions

MR: Conceptualization, Writing – original draft, Writing – review & editing. FB: Conceptualization, Writing – original draft, Writing – review & editing. AF: Conceptualization, Writing – original draft, Writing – review & editing.

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.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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Effects of sleep duration and changes in body mass index on diabetic kidney disease: a prospective cohort study

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Aims: To examine the associations of sleep duration and changes in BMI with the onset of diabetic kidney disease (DKD).

Materials and methods: 2,959 participants with type 2 diabetes were divided into three groups based on sleep duration: short (<7 h/day), intermediate (7-9 h/day), or long (>9 h/day). Changes in BMI during follow-up were trisected into loss, stable, or gain groups. DKD was defined as either the urinary albumin/creatinine ratio (UACR) ≥ 3.39 mg/mmol or the estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73m^2, or both. Cox regression models were used to assess hazard ratios (HRs) and 95% confidence intervals (CIs).

Results: During a mean follow-up of 2.3 years, DKD occurred in 613 participants (20.7%). A J-shaped curve was observed between sleep duration and DKD. Compared to intermediate sleep duration, long sleep duration was associated with higher risks of DKD (HR 1.47; 95% CI: 1.19-1.81). In the joint analyses, compared to participants with intermediate sleep duration and stable BMI, long sleep duration with BMI gain had the highest risks of DKD (HR 2.04; 95% CI: 1.48-2.83). In contrast, short or intermediate sleep duration accompanied by decrease in BMI was associated with a reduced risk of DKD, with HRs of 0.50 (95% CI: 0.31-0.82) and 0.61 (95% CI:0.47-0.80), respectively.

Conclusions: Long sleep duration is significantly associated with an increased risk of DKD, which is further amplified by obesity or BMI gain. These findings suggest that both proper sleep duration and weight control are essential to preventing DKD.

KEYWORDS

sleep duration, obesity, changes in BMI, type 2 diabetes, diabetic kidney disease

Introduction

Diabetes affects 537 million adults globally in 2021 and is responsible for an estimated 2 million deaths, including those related to diabetic kidney disease (DKD) (1). It can lead to end-stage renal disease (ESRD) as kidney function deteriorates over time, ultimately necessitating interventions such as dialysis or kidney transplantation (2). The progression of DKD is heavily influenced by hyperglycemia, and common comorbidities such as hypertension and hyperlipidemia also play significant roles in the pathogenesis of DKD (3). These factors lead to glomerular endothelial dysfunction and podocyte damage, ultimately resulting in glomerulosclerosis and the formation of renal unit loss (4, 5). While the complex pathophysiological mechanisms involved are still being explored.

Sleep duration has been demonstrated as a critical factor influencing a variety of health outcomes like hyperglycemia, cardiovascular disease (CVD), and cognitive decline (6–8). The guidelines provided by the American Diabetes Association (ADA) indicate a correlation between inadequate or excessive sleep duration and elevated levels of glycated hemoglobin (HbA1c) (9). A previous cross-sectional study found both short and long sleep durations were associated with decreased estimated glomerular filtration rate (eGFR) as well as increased albuminuria (10). Another cross-sectional study suggested that longer daytime sleep among individuals with type 2 diabetes was linked to albuminuria (11). However, these cross-sectional studies cannot establish a causal relationship between sleep duration and DKD. Moreover, there is a notable scarcity of prospective cohort studies that have specifically investigated this association. Additionally, prior investigations have demonstrated a positive correlation between higher BMI and DKD, whereas a reduction in BMI has been linked to a decreased risk of DKD (12). This indicates that alterations in BMI contribute significantly to the onset of DKD. There is a growing recognition of the significance of comprehensive management of lifestyle and metabolic health status. Both of these aspects are incorporated as modifiable factors in the care goals for individuals with diabetes. It is highly meaningful to perform risk stratification for DKD by combining sleep duration and changes in BMI as it enhances the identification of high-risk populations and enables targeted interventions.

Therefore, the present study aimed to extend the existing knowledge by investigating the relationship between sleep duration and DKD, and exploring the potential influence of obesity and changes in BMI on this association.

Materials and methods

Study design and participants

This prospective cohort was part of a pilot and standard system, the National Metabolic Management Center (MMC) at Ruijin Hospital, Shanghai Jiao Tong University School of Medicine. The details of MMC have been previously described (13, 14). A total of 5,655 individuals aged 18 years or older, who had received a confirmed diagnosis of type 2 diabetes, were followed from June 2017 to December 2022. Out of these individuals, we excluded participants with a follow-up period of fewer than 6 months (n = 750), existing DKD at baseline (n = 1,327), or lacking information on sleep duration (n = 318), urinary albumin/creatinine ratio (UACR), or eGFR (n = 301). The final analysis comprised a total of 2,959 participants (the flowchart of participant inclusion appears in Supplementary Figure 1). Written informed consent was obtained from each participant, and the study protocol received ethical approval from the Institutional Review Board of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine.

General characteristics and outcomes

Participants underwent an extensive physical examination, blood sample collection, and completion of baseline questionnaires to obtain lifestyle-related information. The questionnaires included questions on night sleep duration, nap duration, and sleep quality. Participants' total sleep duration of twenty-four hours was obtained by combining both nocturnal and midday sleep periods. Three self-reported options were available to assess sleep quality based on subjective evaluation: 1) good, 2) poor, and 3) requiring medication. The definition of ideal smoking status and alcohol consumption, as well as the standards for physical measurements, were detailed in our previously published studies (15, 16). Blood pressure was measured after a minimum of 5 minutes of rest. Blood samples were collected from each participant following an overnight fasting period to obtain HbA1c, fasting plasma glucose (FPG) and 2-hour post-load plasma glucose (PG), lipid profile, and other laboratory parameters. The UACR was determined by dividing the concentration of urinary albumin by the concentration of urinary creatinine. The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation to assess renal function (17).

Definitions

Based on previous studies, the classification of sleep duration was as follows: intermediate (7-9 h/day), short (<7 h/day), and long (>9 h/day) (18). BMI was divided into three categories according to the criteria recommended by the National Health Commission of the People's Republic of China: normal weight, <24 kg/m^2; overweight, 24-27.9 kg/m^2 and obese, ≥28 kg/m^2. Central obesity was defined as waist circumference ≥90 cm for men and ≥85 cm for women (19). To perform the joint analysis, we categorized the study participants based on their changes in BMI from baseline to the final visit. Participants with the lowest one-third change in BMI were classified as the "loss" group; those with the middle one-third change were classified as the "stable" group; and the upper thirds were classified as the "gain" group (20). Considering the potential impact of baseline BMI status on DKD, we categorized participants into four groups based on changes in BMI status: "Remained normal BMI" for those with normal BMI at both baseline and the

last examination, "Remained overweight or obese" for those who were overweight or obese at both examinations, "Became normal BMI" for those who were overweight or obese at baseline but had a normal BMI at the last examination, and "Became overweight or obese" for those with a normal BMI at baseline but were overweight or obese at the last examination. The details of the characteristics of BMI changes in the different groups, categorized based on changes in BMI or BMI status, have been provided in the Appendix (Table S1). The definition of diabetes was fasting plasma glucose ≥7.0 mmol/L, or 2-hour plasma glucose ≥11.1 mmol/L, or HbA1c ≥6.5%, or previously diagnosed by their healthcare provider (21). Albuminuria was defined as UACR ≥3.39 mg/mmol. The presence of albuminuria or eGFR <60 mL/min/1.73m^2 was defined as the presence of DKD (22).

Statistical analyses

Continuous variables were presented as mean ± standard deviation or median with interquartile range, while categorical variables were presented as counts and proportions. To assess differences among groups, P-values were calculated using the ANOVA method for continuous variables and chi-square tests for categorical variables.

We employed Cox proportional hazards models to investigate the associations between sleep duration and the incidence of DKD. The Schoenfeld residuals method was utilized to evaluate the proportional hazards assumptions of the Cox models. According to previous studies on risk factors for kidney diseases, we selected potential confounding factors and adjusted for them in the multivariable model (23–25). Model 1 adjusted for age and sex, while in Model 2, additional adjustments were made for the duration of diabetes, HbA1c, smoking status, alcohol intake, BMI, eGFR, use of angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blockers (ARB); model 3 was further adjusted for history of hypertension, CVD and cancer, and sleep quality (good or poor/requiring medication) based on model 2. Restricted cubic splines with three knots placed at the 5th, 50th, and 95th percentiles were employed to examine the associations between sleep duration and DKD. We performed stratified analyses by age (<50 years, 50-60 years, or >60 years), sex, duration of diabetes (<5 years, or ≥5 years), glycemic control (HbA1c <7%, or ≥7%), BMI (<24 kg/m^2, 24-27.9 kg/m^2, or ≥28 kg/m^2), changes in BMI (stable, loss, or gain), changes in BMI status (remained normal, remained overweight or obese, became normal, or became overweight or obese), and waist circumference (normal, or central obesity). Adjustments to the model were the same as in model 3 except for the stratification variables. The statistical significance of interactions was assessed using the likelihood ratio test, comparing models with and without cross-product terms between sleep duration categories (<7, 7-9, >9 h/day) and the stratification variables. To examine whether the association between sleep duration and DKD was modified by BMI, changes in BMI, or changes in BMI status, we conducted joint analyses. The model accounted for the covariates included in model 3, except for BMI.

Sensitivity analyses were conducted. Initially, the participants were categorized into three groups according to the percentage variation in BMI to test the robustness of the results: Individuals with a BMI reduction of 5.0% or higher were designated as the "BMI loss" group (n=636). This threshold aligns with the weight loss recommendation of ≥5% for individuals who are overweight or obese and diagnosed with type 2 diabetes, as routinely advocated by the American Diabetes Association (ADA) (26). As BMI decreased by an average of 1.3% for all participants during the follow-up period while the number of participants with a ≥ 5% increase in BMI was less than one in ten (n=286), the "BMI gain" group consisted of individuals who experienced a BMI increase of 3.0% or higher (n = 495). The participants in the "BMI stable" group had a BMI decrease of < 5.0% or an increase of < 3.0% (n=1805). Furthermore, we analyzed the effect of nighttime sleep on DKD with an additional adjustment for the duration of daytime naps.

The statistical analysis was conducted using R version 4.1.1 (R Foundation, Vienna, Austria). Two-tailed P-values were reported, and a significance level of 0.05 was used to determine statistical significance.

Results

Baseline characteristics

The baseline characteristics of the study participants according to sleep duration are presented in Table 1. The mean age of the participants was 55.2 (SD, 11.7) years, and 1,765 were males (59.7%). Of all the 2,959 participants involved, 15%, 70%, and 15% reported sleeping <7 hours, 7-9 hours, and >9 hours per day, respectively. Participants who reported sleeping >9 hours per day were slightly older with a longer duration of diabetes and poorer glycemic control compared to the other two groups ($p < 0.05$).

Independent association of sleep duration with DKD and albuminuria

Throughout a mean (± SD) follow-up period of 2.3 (± 1.4) years, a total of 613 incidents (20.7%) of DKD were detected. Table 2 demonstrates a significant association between sleep duration and the risks of DKD. Participants with long sleep duration had higher risks of DKD (26.4%) compared to those with intermediate (19.5%) and short sleep duration (20.5%) (P = 0.006). Similarly, the incidence of albuminuria was higher in participants with long sleep duration, with 23.5% developing albuminuria compared to 16.6% in the reference group and 19.2% in the short sleep duration group (P = 0.003).

We did not detect any evidence of a violation of the proportional hazard assumption, employing a p-value threshold of 0.05 (Figure S2). After adjusting for age and gender, long sleep duration was associated with an increased risk of developing DKD (HR 1.45; 95% CI:1.18-1.79) compared with intermediate sleep duration (model 1). The association was prominent after additional

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TABLE 1 Baseline characteristics of the study population according to sleep duration.

		Habitual sleep duration, h/day			
	Total	< 7	7 - 9	> 9	P
	(N=2,959)	(N=443)	(N=2,073)	(N=443)	
Age, years	55.19 ± 11.74	54.47 ± 11.17	55.02 ± 11.80	56.73 ± 11.90	0.008
Males	1765 (59.65%)	247 (55.76%)	1248 (60.20%)	270 (60.95%)	0.186
Duration of diabetes, years	7.49 ± 7.41	7.39 ± 7.36	7.28 ± 7.34	8.58 ± 7.69	0.003
Education, high school and above, n (%)	2199 (74.80%)	328 (74.55%)	1545 (75.11%)	326 (73.59%)	0.793
History of hypertension, n (%)	1148 (38.93%)	154 (34.92%)	809 (39.16%)	185 (41.86%)	0.099
History of cardiovascular disease, n (%)	359 (12.19%)	52 (11.82%)	233 (11.29%)	74 (16.74%)	0.006
History of any cancer, n (%)	135 (4.58%)	19 (4.32%)	93 (4.51%)	23 (5.20%)	0.784
Ideal smoking status, n (%)	2278 (77.22%)	346 (78.28%)	1597 (77.26%)	335 (75.96%)	0.712
Drinking, n (%)	279 (9.44%)	46 (10.38%)	195 (9.42%)	38 (8.60%)	0.661
Systolic blood pressure, mmHg	126.38 ± 16.56	126.12 ± 17.24	126.48 ± 16.52	126.15 ± 16.07	0.870
Diastolic blood pressure, mmHg	73.56 ± 10.42	73.94 ± 10.95	73.65 ± 10.35	72.74 ± 10.19	0.177
BMI, kg/m^2	25.60 ± 3.99	25.93 ± 4.63	25.57 ± 3.86	25.38 ± 3.90	0.104
Waist circumference, cm	91.19 ± 10.17	91.63 ± 11.15	91.10 ± 9.93	91.17 ± 10.25	0.618
HbA1c, %	7.71 ± 1.68	7.54 ± 1.59	7.71 ± 1.69	7.89 ± 1.67	0.009
HbA1c < 7%, n (%)	1190 (40.37%)	189 (42.76%)	851 (41.23%)	150 (33.94%)	0.010
Fasting blood glucose, mmol/L	8.83 ± 3.12	8.73 ± 2.82	8.83 ± 3.18	8.94 ± 3.11	0.605
2-h postload glucose, mmol/L	14.81 ± 4.80	14.62 ± 4.68	14.72 ± 4.77	15.40 ± 5.00	0.019
Triglycerides, mmol/L	1.47 [1.03;2.11]	1.51 [1.04;2.23]	1.45 [1.02;2.11]	1.48 [1.07;2.03]	0.304
Total cholesterol, mmol/L	4.95 ± 1.18	4.97 ± 1.10	4.96 ± 1.17	4.90 ± 1.28	0.635
HDL cholesterol, mmol/L	1.26 ± 0.32	1.25 ± 0.31	1.26 ± 0.33	1.24 ± 0.31	0.400
LDL cholesterol, mmol/L	3.05 ± 0.97	3.07 ± 0.94	3.05 ± 0.96	3.03 ± 1.05	0.863
ACEI/ARB, n (%)	791 (27.75%)	103 (24.52%)	553 (27.57%)	135 (31.84%)	0.056

Data are shown as mean ± SD or median (interquartile range) for continuous variables and count (percentage) for categorical variables. P values refer to comparisons among groups using the ANOVA statistical method for comparing continuous variables and the chi-square tests for comparing categorical variables.
BMI, body mass index; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein;
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blockers.

TABLE 2 Associations of sleep duration with DKD among people with type 2 diabetes.

	Habitual sleep duration, h/day			
	< 7	7 - 9	> 9	P
DKD incidence, n (%)	91 (20.54%)	405 (19.54%)	117 (26.41%)	0.006
Model 1	1.01 (0.80-1.26)	1.00 (Ref)	1.45 (1.18-1.79)	
Model 2	0.96 (0.75-1.23)	1.00 (Ref)	1.47 (1.19-1.82)	
Model 3	0.97 (0.75-1.24)	1.00 (Ref)	1.47 (1.19-1.81)	
Albuminuria incidence, n (%)	85 (19.19%)	345 (16.64%)	104 (23.48%)	0.003
Model 1	1.10 (0.87-1.40)	1.00 (Ref)	1.54 (1.24-1.92)	
Model 2	1.04 (0.81-1.34)	1.00 (Ref)	1.59 (1.27-1.99)	
Model 3	1.06 (0.81-1.37)	1.00 (Ref)	1.55 (1.24-1.95)	

Hazard ratios and 95% confidence intervals of the associations of sleep duration with DKD and albuminuria. Model 1 was adjusted for age and sex; model 2 was further adjusted for duration of diabetes, HbA1c, smoking status, alcohol intake, BMI, eGFR, use of angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blockers (ARB) based on model 1; model 3 was further adjusted for history of hypertension, CVD and cancer, and sleep quality (good or poor/requiring medication) based on model 2.

adjustment for diabetes duration, smoking status, alcohol intake, BMI, eGFR, and use of ACEI/ARB (HR 1.47; 95% CI:1.19-1.82) (model 2). Furthermore, the observed association between longer sleep and risk of DKD remained significant after controlling for sleep quality and history of hypertension, CVD, and cancer (HR 1.47; 95% CI: 1.19-1.81) (model 3). Short sleep duration did not show an association with increased risks of DKD or albuminuria. The restricted cubic spline regression analysis confirmed a J-shaped curve, with >9 h/day of sleep being associated with a higher risk of DKD and albuminuria (Figure 1). The same covariates as in Model 3 were included in the analysis. In addition, consistent results were observed in subgroup analyses (all interaction P-values > 0.05) (see Figure S3).

Joint association of sleep duration and BMI with DKD and albuminuria

Figures 2A, B; Table S2 show the joint association of sleep duration and BMI with DKD and albuminuria. Individuals with long sleep duration and overweight had higher risks of DKD (HR 2.12; 95% CI: 1.52-2.94) and albuminuria (HR 2.45; 95% CI: 1.72-3.48) compared to the reference group, which consists of individuals with intermediate sleep duration and BMI < 24kg/m^2. Similarly, individuals with long sleep duration and obesity were at higher risk of DKD (HR 1.83; 95% CI: 1.17-2.86) and albuminuria (HR 2.06; 95% CI: 1.27-3.35) compared to the reference group. Additionally, participants with short or intermediate sleep duration and obesity were also associated with an elevated risk of DKD and albuminuria.

Joint association of sleep duration and changes in BMI with DKD and albuminuria

Figures 3A, B; Table S3 illustrate the joint association of sleep duration and changes in BMI with DKD and albuminuria. Participants who had long sleep duration and experienced BMI gain faced the highest risks of DKD (HR 2.04; 95% CI: 1.48-2.83) and albuminuria (HR 2.09; 95% CI: 1.48-2.93) compared to the reference group, comprising individuals with intermediate sleep duration and stable BMI. In contrast, participants with short sleep patterns and experiencing BMI loss were found to have lower risks of DKD (HR 0.50; 95% CI: 0.31-0.82) and albuminuria (HR 0.55; 95% CI: 0.34-0.91) compared to the reference group. Similarly, participants with intermediate sleep patterns and BMI loss also had a protective effect on the development of DKD (HR 0.61; 95% CI: 0.47-0.80) and albuminuria (HR 0.57; 95% CI: 0.43-0.77).

Joint association of sleep duration and changes in BMI status with DKD and albuminuria

Figures 4A, B; Table S4 depict the joint association of sleep duration and changes in BMI status with DKD and albuminuria. Participants who had long sleep duration and became overweight or obese faced the highest risks of DKD (HR 2.49; 95% CI:1.14-5.40), in contrast to the reference group consisting of individuals with intermediate sleep durations and remained BMI normal. Similarly, participants who had long sleep durations and remained overweight or obese were also at an increased risk of DKD (HR 2.46; 95% CI: 1.79-3.39). Conversely, participants with intermediate sleep patterns who transitioned to a normal BMI exhibited reduced risks of DKD compared to the reference group (HR 0.58; 95%CI: 0.34-0.97). Furthermore, the group with short sleep patterns that transitioned to a normal BMI had no participants who developed DKD.

Sensitivity analyses

The results remained largely consistent in all sensitivity analyses (Figures S4-5; Table S5).

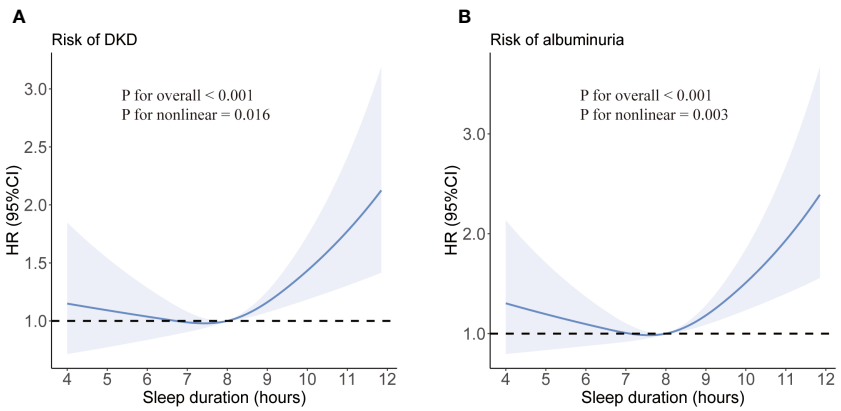

FIGURE 1
Multivariate-adjusted spline curves for associations of sleep duration with DKD **(A)** and albuminuria **(B)**. Sleep duration was fitted as a smooth term using a restricted cubic spline with 3 knots. Shading indicates 95% confidence intervals. The model was adjusted for age, sex, duration of diabetes, HbA1c, smoking status, alcohol intake, BMI, eGFR, use of ACEI/ARB, history of hypertension, CVD and cancer, and sleep quality.

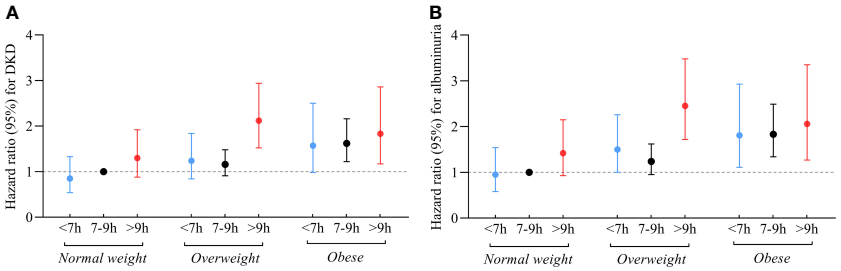

FIGURE 2
Relationship between sleep duration and risk of DKD **(A)** and albuminuria **(B)** among participants with varying BMI. Those who slept for 7-9 hours/day and had a BMI < 24 kg/m^2 were referenced. All models were adjusted for age, sex, duration of diabetes, HbA1c, smoking status, alcohol intake, eGFR, use of ACEI/ARB, history of hypertension, CVD and cancer, and sleep quality.

Discussion

This prospective cohort study examined the relationship between sleep duration and the occurrence of DKD in individuals diagnosed with T2DM. Our findings revealed a significant link between long sleep duration (>9 h/day) and an elevated risk of DKD, which remained after adjusting for potential confounding variables. We identified a nonlinear relationship between sleep duration and the onset of DKD, characterized by a J-shaped curve, which was also present in the occurrence of albuminuria. Furthermore, we found that participants with both long sleep duration and BMI gain faced the greatest risks of DKD compared to individuals with intermediate sleep duration and stable BMI. To our knowledge, this is the first prospective cohort to report that long sleep duration was associated with a higher risk of DKD when compared to those who slept 7-9 hours per day. Moreover, our results emphasize the influence of BMI changes on this association, suggesting that the combination of longer sleep and BMI gain may synergistically contribute to the development of DKD.

Although the association between long sleep duration and the risk of DKD has been proposed, relevant studies published thus far are sparse (10, 11). Several studies have examined the connection between sleep duration and CKD in the general population; however, the findings have yielded inconsistent results. A previous investigation identified a U-shaped relationship between both insufficient sleep duration (≤4 hours) and excessive sleep duration (>10 hours) and CKD in middle-aged and older people (27), while the Nurses' Health Study (NHS) did not observe a correlation between longer sleep (≥9 hours) and rapid decline in eGFR (decrease of more than 30%) (28). Furthermore, research conducted in Japan discovered that longer sleep duration (>8 hours) was an important predictor of end-stage kidney disease (ESKD) (29). The disparities between our findings and the conclusions of previous research may be attributed, in part, to variations in race, geographical locations, underlying disease, and baseline renal function among these study populations.

The mechanisms involved in the negative effects of long sleep duration on the onset of DKD have not been sufficiently appreciated. To begin with, prolonged periods of sleep may trigger an upsurge in high-sensitivity C-reactive protein (hs-CRP) levels and stimulate an inflammatory response, which further leads to cellular injury, glomerular endothelial dysfunction, proliferation of mesangial cells, and increased vascular permeability (30, 31). These factors can ultimately contribute to the development of albuminuria and DKD (32). Second, longer sleep duration is associated with several metabolic abnormalities, including hypertension, dyslipidemia, and insulin resistance (33, 34), all of which are closely associated with the onset of DKD (35). Furthermore, numerous studies have reported that individuals with long sleep durations often have other unhealthy daily behaviors, such as sedentary behavior and lack of physical activity, which are well-established risk factors for diabetes complications, including DKD (36–38).

Consistent with previous studies suggesting that obesity is an independent risk factor for DKD (39), our study extends this

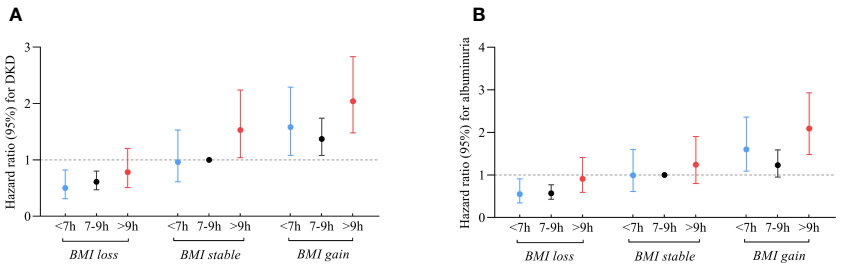

FIGURE 3
Relationship between sleep duration and risk of DKD **(A)** and albuminuria **(B)** among participants with varying changes in BMI. Those who slept 7-9 hours/day and had stable BMI during follow-up were referenced. All models were adjusted for age, sex, duration of diabetes, HbA1c, smoking status, alcohol intake, eGFR, use of ACEI/ARB, history of hypertension, CVD and cancer, and sleep quality.

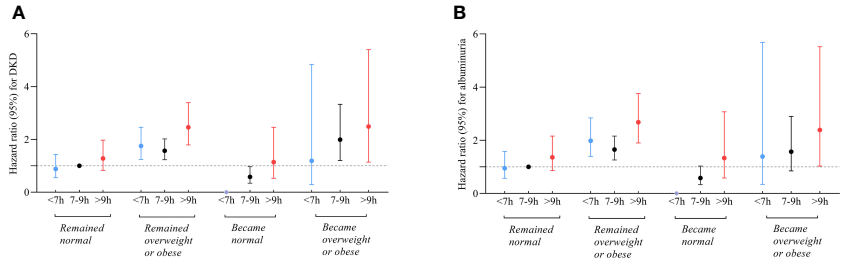

FIGURE 4
Relationship between sleep duration and risk of DKD **(A)** and albuminuria **(B)** among participants with varying changes in BMI status. Those who slept 7-9 hours/day and remained normal BMI during follow-up were referenced. All models were adjusted for age, sex, duration of diabetes, HbA1c, smoking status, alcohol intake, eGFR, use of ACEI/ARB, history of hypertension, CVD and cancer, and sleep quality. * The group with short sleep patterns that transitioned to a normal BMI had no participants who developed DKD.

understanding through joint analyses of sleep duration and variability in BMI and BMI status. In our study, the effect of long sleep duration on the incidence of DKD was more pronounced among those who experienced BMI gain, transitioned to overweight or obese, or remained overweight or obese. The biological mechanisms underlying the joint effects are still unclear. On one hand, previous studies have indicated that prolonged sleep has been associated with reduced physical activity, decreased energy expenditure, and subsequent weight gain (40). On the other hand, evidence suggests that obese individuals tend to have a higher risk of obstructive sleep apnea (OSA), a condition that adversely affects the quality of sleep and contributes to excessive daytime sleepiness (41, 42). This bidirectional association between sleep and obesity forms a detrimental cycle that exacerbates the risk of DKD. In addition, both long sleep duration and obesity are related to risk factors of DKD, like insulin resistance, hypertension, dyslipidemia, and inflammatory response (43, 44). On the contrary, participants with short or intermediate sleep duration and a decrease in BMI during follow-up were observed to be related to a reduction in the risk of DKD. The finding suggests that maintaining appropriate sleep duration and achieving weight loss may have a protective effect against DKD. A previous randomized controlled trial has demonstrated that weight loss ameliorates insulin resistance and results in lower HbA1c and systolic blood pressure in individuals with obesity, thereby delaying the onset of the microvascular complications of diabetes (45). This finding coincides with the outcomes of our analysis. Further exploration is needed to understand the underlying mechanisms of this relationship. Additionally, further research is warranted to establish specific and effective strategies targeting sleep duration and weight management to prevent or delay the onset and progression of DKD.

The strengths of our study include its large sample size and the implementation of a longitudinal study design, which provides a more robust approach compared to cross-sectional studies. Our finding extends previous research by exploring the combined effects of sleep duration and changes in BMI on the occurrence of DKD. Additionally, we fully adjusted for potential confounding factors to ensure the reliability of the outcomes. In particular, we adjusted for sleep quality to assess the independent risk of sleep duration for DKD, which has rarely been considered in previous studies.

Despite the novel insights provided by this study, several potential limitations should also be acknowledged. Firstly, although the association between sleep duration and BMI changes and DKD incidence reached statistical significance, our conclusions still need to be validated in populations of other genetic backgrounds. Secondly, our assessment of sleep duration is self-reported while those measured using polysomnography are more objective. However, self-reported questionnaires have been widely utilized as a more feasible form of epidemiological investigation in large-scale population studies.

Conclusion

Long sleep duration was significantly associated with increased risks of DKD. Notably, compared to participants with intermediate sleep duration and stable BMI, long sleep duration with BMI gain had higher risks of DKD. Conversely, short or intermediate sleep duration with loss of BMI was associated with decreased risks of DKD, indicating that both appropriate sleep duration and weight control are required to prevent the development of DKD. Future investigations are warranted to elucidate the underlying mechanisms of this association and develop better intervention strategies for sleep habits and weight management.

STROBE statement

This study was reported in accordance with STROBE guidelines for cohort studies.

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 humans were approved by the Institutional Review Board of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

CL: Conceptualization, Data curation, Visualization, Writing – original draft. JZ: Methodology, Validation, Writing – original draft. XW: Data curation, Methodology, Visualization, Writing – original draft. JS: Formal Analysis, Methodology, Writing – review & editing. QF: Conceptualization, Software, Writing – original draft. WZ: Data curation, Validation, Writing – review & editing. LS: Data curation, Investigation, Software. ZH: Data curation, Investigation, Validation. JH: Writing – review & editing. WG: Formal Analysis, Validation, Writing – review & editing. WW: Funding acquisition, Resources. YP: Supervision, Writing – review & editing. YZ: Funding acquisition, Supervision, Project administration.

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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.2023.1278665/full#supplementary-material

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Association between systemic immune-inflammation index and diabetes: a population-based study from the NHANES

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Background: Systemic Immune-Inflammation Index (SII) has been reported to be associated with diabetes. We aimed to assess possible links between SII and diabetes.

Methods: Data were obtained from the 2017-2020 National Health and Nutrition Examination Survey (NHANES) database. After removing missing data for SII and diabetes, we examined patients older than 20 years. Simultaneously, the relationship between SII and diabetes was examined using weighted multivariate regression analysis, subgroup analysis, and smooth curve fitting.

Results: There were 7877 subjects in this study, the average SII was 524.91 ± 358.90, and the prevalence of diabetes was 16.07%. Weighted multivariate regression analysis found that SII was positively associated with diabetes, and in model 3, this positive association remained stable (OR = 1.04; 95% CI: 1.02–1.06; p = 0.0006), indicating that each additional unit of SII, the possibility of having diabetes increased by 4%. Gender, age, BMI, regular exercise, high blood pressure, and smoking did not significantly affect this positive link, according to the interaction test (p for trend>0.05).

Discussion: Additional prospective studies are required to examine the precise connection between higher SII levels and diabetes, which may be associated with higher SII levels.

KEYWORDS

systemic immune-inflammation index, diabetes, NHANES, cross-sectional study, population-based study

1 Introduction

The chronic metabolic disorder known as diabetes is characterized by persistently high blood sugar levels (1). Worldwide, the prevalence of diabetes is rising each year and has emerged as a severe public health issue (2). More than 460 million people worldwide have diabetes, and type 2 diabetes affects more than 90% of these individuals. Diabetes harms patients' health but also has a significant financial impact on families and society (3). Diabetic microangiopathy, one of the most prevalent early consequences of diabetes, is also a risk factor for heart and renal disease (4). Additionally, the study finds levels of systemic inflammation may explain the increased prevalence of diabetes (5).

Systemic Immunity-Inflammation Index (SII) is the platelet count multiplied by the neutrophil count divided by the lymphocyte count, which is considered to be a new and reliable indicator (6) for comprehensively measuring the systemic immunity and inflammation level of the subject (7). Inflammatory factor indicators have been linked to diabetes, according to an increasing number of research (8). Jie Wang et al. investigated the relationship between NLR and depression in patients with diabetes and found that NLR increased and the prevalence of depression increased (9). Bartosz Hudzik et al. find a link between PLR and diabetes (10). In diabetic patients, Dan Yu et al. discovered that LMR can be used as an index to predict the recurrence of rectal cancer (11). These indicators, which only represent two different types of immune cells, could not be very predictive. According to Jie Wang et al., SII is a risk factor for diabetes depression (12). It can be assumed that there may be a relationship between SII and diabetes since SII has been suggested as an index to detect depression in diabetes. However, no one has independently looked into the connection between SII and diabetes.

Liu Yongming et al. found that middle-aged and elderly diabetes caused by arteriosclerosis may be mediated by white blood cell count (13). Xiang Fang et al. Diabetes is related to the expression of inflammatory genes and white blood cell count, and blood glucose is positively correlated with inflammatory genes and white blood cell count (14). Francesco Zaccardi et al. found that compared with patients without diabetes, there was no difference in platelet count in patients with diabetes. However, this study still took into account the image of platelet count in the experiment and included it in the covariate to design the experiment (15).

The link between SII and diabetes will be examined in this article. We examine the association between SII and diabetes using data from the NHANES database for the years 2017 to 2020. We think that there may be a connection between the rise in SII and the rise in diabetes prevalence. Researchers and medical professionals offer references.

2 Materials and methods

2.1 Population research

The National Health and Nutrition Examination Survey (NHANES) is a research program designed to assess adults' and children's health and nutritional status in the United States (16). The NHANES database contains population data, questionnaire data, laboratory data, and dietary data. This study is used to determine the prevalence and pathogenic factors of diseases, etc., and provides national standard references to help design sound public health policies (17). In total, 15,560 participants from the 2017–2018 and 2019–2020 research years were included in this study, whereas 6,328 participants under the age of 20 were omitted. Following the exclusion of 1111 people with missing SII data and 244 participants with missing diabetes data, 7877 participants were ultimately included in the study. Subjects were given the go-ahead to participate in the NHANES by the NCHS Ethics Review Board, and each participant gave their written informed consent (18) (Figure 1).

2.2 Exposure variables and outcome variables

SII is a composite index made up of platelet count, neutrophil count, and lymphocyte count that is used as an exposure variable and is assessed using an automated hematology analysis instrument (CoulterDxH 800 analyzer). calculated by dividing the lymphocyte count by the platelet count, and then multiplying by the neutrophil count (19).

Did your doctor inform you that you had diabetes? Are these other questions asked by a professional interviewer utilizing a computer-assisted personal interview (CAPI) system at home? The subject was deemed to have diabetes if his response was yes. Having diabetes diagnosed by a doctor was intended to be an outcome variable (20).

2.3 Covariates

We summarized potential covariates that might confound the association between SII and diabetes. The covariates selected for this study were: age(year), Albumin refrigerated serum, White blood cell count, Platelet count, blood urea nitrogen content, chloride content, dietary protein intake, carbohydrate intake, total sugar intake, total fat intake, cholesterol intake, gender, race, education level (divided into no high school education, high school education, high school education or above), marital status (married with a partner, divorced and separated, widowed, never married), poverty-income ratio (0-1.5, 1.5-3.5, >3.5), BMI (0-25, 25-30, >30), whether you have high blood pressure, whether you smoke (21), whether you exercise regularly (22). Among them, the BMI classification corresponds to three groups of normal weight, overweight, and obese. Refrigerated serum albumin is measured in g/L, and albumin concentration is measured using the dye bromocreol violet (BCP). The PH value binds to albumin in the PH range of 5.2-6.8, and the color change is measured at 600nm, with secondary measurements at 700nm. Albumin value measurement is used in the diagnosis and treatment of certain liver and heart diseases as well as diabetes (23). Both the White blood cell count (24) and the Platelet count (25) are measured in 1000 cells/uL. A complete blood count on a blood specimen is measured at a mobile testing center using a Beckman Coulter DxH 800 instrument. The VCS (volume, conductivity, and dispersion)

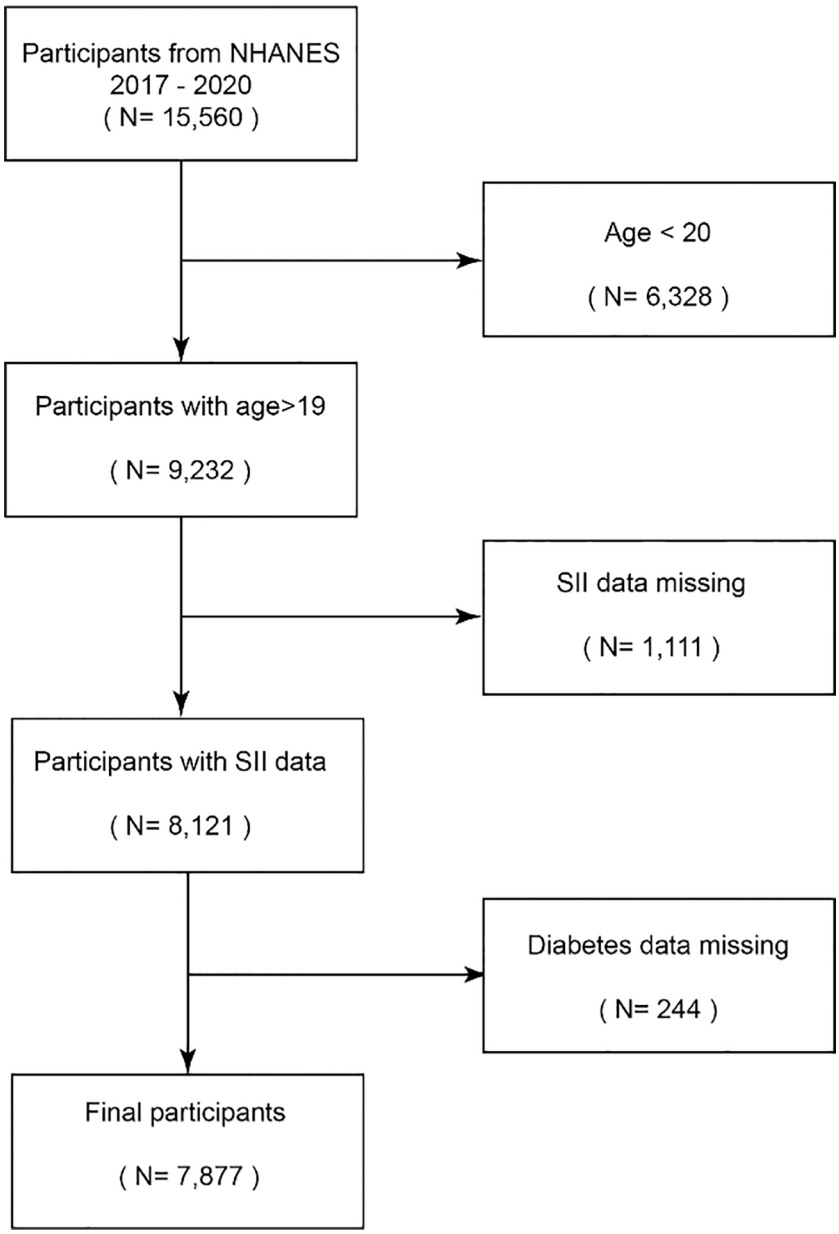

FIGURE 1
Flowchart of the sample selection from NHANES 2017–2020.

method was used to determine the leucocytes. Blood urea nitrogen was measured at 340nm using a coupled enzyme reaction in the unit of mmol/L (26). The chloride content was measured in mmol/L, and the serum electrolyte concentration was determined by the indirect ion selective electrode (ISE) method. Sample dilution 1:31 (27). Dietary protein intake, carbohydrate intake, total sugar intake, total fat intake, and cholesterol intake, these data are from the dietary section of the database. Dietary recall interviews were collected at a mobile test center (MEC), and participants' total nutrient intake on day one was used in this study (28). High blood pressure was determined by the source of the questionnaire part, whether or not the doctor considered having high blood pressure (29). Whether you smoke or not, according to the part of the questionnaire, whether you have smoked 100 cigarettes so far (30).

2.4 Statistical methods

Continuous variables were expressed as means with standard deviations in the description of the study population, while categorical variables were expressed as percentages. The association between participants in the SII/100 quartile group and the presence of diabetes was assessed using the t-test for continuous variables and the chi-square test for categorical variables. To investigate the relationship between SII and the prevalence of diabetes, multivariate logistic regression was performed. Model 1 had no covariates, model 2 added covariates age, sex, and race, and model 3 adjusted variables: age, sex, race, urine albumin content (31), refrigerated serum content, blood urea nitrogen content (32), chloride content, dietary protein intake, carbohydrate intake, total

sugar intake, total fat intake, cholesterol intake, education Degree (divided into no high school education, high school education, high school education or above), marital status (married with a partner, divorced and separated, widowed, never married), poverty-income ratio (0-1.5, 1.5-3.5, >3.5), BMI (0-25, 25-30, >30), whether you have high blood pressure (33), whether you smoke, whether you exercise regularly. SII and diabetes were assessed in the model using odds ratios (ORs) and 95% confidence intervals (CIs). Create a multivariate test by controlling variables and fitting smooth curves to three models. The relationship and inflection point between SII and diabetes were examined using a threshold effect analysis model. R Studio (version 4.2.2) and empowered stats (version 2.0) were used to do the statistical study. 0.05 was the threshold for significance. We employ a weighting approach to lower the dataset's volatility.

3 Results

3.1 Baseline characteristics of the study population

A total of 7877 subjects were included in this study, of which 48.14% were male and 51.86% were female, with an average age of 50.87 ± 17.56. The mean value of SII was 524.91 ± 358.90, and the prevalence of diabetes was 16.07%.

Table 1 is a table of diabetes-based weighted demographic baseline characteristics. The average age of subjects with diabetes was 61.09 ± 13.16, and the average age of subjects without diabetes was 46.55 ± 17.15. The average age of subjects with diabetes was higher than that of subjects without diabetes. Diabetes is used as a stratified variable. The presence or absence of diabetes is related to age, gender, education level, marital status, poverty-income ratio, BMI, hypertension, smoking or not, regular exercise, SII, albumin content, blood urea nitrogen content, Chloride content, dietary carbohydrate intake, dietary total sugar intake, and dietary cholesterol intake were significantly correlated ($p<0.05$). Compared with people without diabetes, people with diabetes tended to be older, high school educated, married or in a partner, had higher SII, higher albumin, higher blood urea nitrogen, lower chloride, carbohydrate non-Hispanic white males with lower intake, lower sugar intake, higher cholesterol intake, poverty-income ratio >3.5, BMI >30 kg/m2, hypertension, smoking, and no regular exercise. People with diabetes have higher white blood cell counts and lower platelet counts than those without diabetes.

Table 2 is a table of SII-based weighted demographic baseline characteristics. SII quartile subjects have significant differences in age, albumin content, chloride content, protein content, gender, race, education level, income-poverty ratio, BMI, hypertension, diabetes, etc. ($p<0.05$). Subjects in the fourth quartile of the SII quartile tend to be older, have lower protein intake, lower carbohydrate intake, high school education or higher, poverty-income ratio>3.5, BMI>30 kg/m2, have Hypertensive, non-Hispanic white females without diabetes. SII was also significantly different in the quartile group from white blood cell count and platelet count, with the fourth quartile having the highest count.

TABLE 1 Baseline characteristics of study population according to Diabetes, weighted.

Characteristics	Diabetes	Non-Diabetes	*p*-Value
	N=1266	N=6611	
Age(years)	61.09 ± 13.16	46.55 ± 17.15	<0.0001
SII	597.58 ± 419.76	532.32 ± 334.90	<0.0001
White blood cell count (1000 cells/uL)	7.61 ± 2.24	7.17 ± 5.38	0.004
Platelet count (1000 cells/uL)	239.47 ± 71.08	247.11 ± 64.54	<0.001
Albumin, refrigerated serum (g/L)	39.71 ± 3.52	41.13 ± 3.33	<0.0001
Blood Urea Nitrogen (mmol/L)	6.40 ± 2.78	5.18 ± 1.80	<0.0001
Chloride (mmol/L)	100.43 ± 3.44	101.36 ± 2.64	<0.0001
Protein (gm)	79.53 ± 38.53	82.07 ± 42.82	0.0989
Carbohydrate (gm)	225.10 ± 107.48	249.89 ± 128.06	<0.0001
Total sugars (gm)	94.82 ± 70.74	108.12 ± 78.70	<0.0001
Total fat (gm)	87.98 ± 46.28	89.88 ± 49.46	0.2875
Cholesterol (mg)	341.46 ± 269.35	317.66 ± 254.35	0.0108
Gender (%)			<0.0001
Male	54.99	46.95	
Female	45.01	53.05	
Race/Ethnicity (%)			0.1288
Mexican American	9.29	8.36	
Non-Hispanic White	61.00	63.53	
Non-Hispanic Black	12.02	10.80	
Other Hispanic	6.50	7.80	
Other Race	11.20	9.50	
Education (%)			<0.0001
Less than high school	15.85	10.30	
High school	32.29	26.13	
More than high school	51.86	63.57	
Marital status (%)			<0.0001
Married/Living with partner	66.34	61.43	
Widowed/Divorced/Separated	25.90	17.66	
Never married	7.76	20.91	
Income to poverty ratio (%)			<0.0001

(Continued)

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TABLE 1 Continued

Characteristics	Diabetes	Non-Diabetes	*p*-Value
	N=1266	N=6611	
0–1.5	24.69	22.14	
1.5–3.5	27.08	32.94	
>3.5	62.77	38.99	
BMI (kg/m^2) (%)			<0.0001
0–25	10.14	28.08	
25–30	27.08	32.94	
>30	62.77	38.99	
High blood pressure (%)			<0.0001
Yes	68.85	27.14	
No	31.15	72.86	
Smoke (%)			<0.0001
Yes	51.14	41.60	
No	48.86	58.40	
Vigorous work activity (%)			<0.0001
Yes	20.53	28.05	
No	79.47	71.95	

Mean ± SD for continuous variables: the p-value was calculated by the weighted linear regression model. % for categorical variables: the p-value was calculated by a weighted chi-square test. BMI, body mass index; SII, SII, Systemic Immune-Inflammation Index.

3.2 Relationship between SII and diabetes

Since the effect size was not obvious, we magnified the value of SII by 100 times to compare the relationship between SII/100 and diabetes. Table 3 shows the multivariate regression analysis between SII/100 and diabetes. For diabetes, a positive association between SII/100 and diabetes was observed. In model 3, this positive association remained stable (OR = 1.04; 95% CI: 1.02–1.06; p = 0.0006), indicating that each unit increase in SII/100 increased the likelihood of having diabetes by 4%. In a sensitivity analysis, a fully adjusted model for the SII/100 quartile (OR = 1.31; 95% CI: 1.05-1.63; p = 0.0187) indicated a stable relationship between elevated SII and increased odds of developing diabetes. Compared with the first quartile, participants in quartile 4 had a 31% increased risk of developing diabetes. At the same time, the p for trend of the three models were all <0.05, which was statistically significant.

A subgroup analysis of the association between SII and diabetes is shown in Figure 2. SII was significantly correlated with male sex, age less than 60, 0<BMI<25, and irregular exercise (p<0.05). The interaction test showed that there was no statistical difference in the relationship between SII and diabetes in each category, and gender, age, BMI, regular exercise, hypertension, and smoking had no significant impact on this positive relationship (p for trend>0.05).

The nonlinear association between SII and diabetes was then described using smooth curve fitting (Figures 3, 4). Adjusted variables: age, sex, education, marital status, poverty-income ratio, BMI, hypertension, smoking, regular exercise, albumin level, blood urea nitrogen level, chloride level, dietary carbohydrate intake, dietary total sugar intake, and dietary cholesterol intake, and high blood pressure. Interaction test showed that there was no statistically significant difference in the association between SII and diabetes among different stratification groups, and all interactions were p>0.05, indicating that age, sex, BMI, sampling, regular exercise, and hypertension had no significant dependence on this positive association. After stratified analysis by sex, it was found that the female stratification presents an inverted U-shaped curve.

4 Discussion

Our research revealed an association between increased SII and a higher prevalence of diabetes. After stratified analysis by gender, it was discovered that female stratification likewise exhibits an inverted U-shaped curve in the connection between SII and diabetes.

To our knowledge, few studies have individually assessed the association between SII and diabetes. Ahmet Elbeyli et al. found that SII can be used as a diagnostic marker for diabetic macular edema and improve diabetic retinopathy (34). Jie Wang et al. found that SII can be used as a diagnostic marker for diabetic depression (12). Wencong Guo et al. found that higher SII levels were significantly associated with diabetic nephropathy (35). Kübra Özata Gündoğdu et al. studied the association of diabetic macular edema with serous macular detachment and SII, and elevated SII levels may increase the incidence of serous macular detachment (36). Safak Ozer Balin et al. reported that SII can be used as a predictive marker for diabetic foot osteomyelitis (37). Yohanes Andy Rias et al. surveyed 294 Indonesian diabetic patients and found that low levels of SII can regulate psychological problems in diabetic patients (38). In contrast, Yohanes Andy Rias et al.'s research results showed a benign effect of low-level SII, which is different from the general high-level SII. Therefore, it is found that the level of SII may have different effects on the judgment of diabetes, and it is meaningful for us to study the relationship between SII and diabetes. Research by Jingxin Zhou et al. suggested that SII may be a potential marker for the treatment of diabetic macular edema (39). We hypothesize that there may be a relationship between SII and diabetes, which may be a good prospective marker, in light of the studies' confirmation of SII's predictive power. We discovered through our research that there is currently no research on the relationship between SII and diabetes alone, but there is research on the relationship between SII and diabetes-related diseases like the aforementioned diabetic macular edema, diabetic nephropathy, and diabetic depression. The studies mentioned above utilized several survey techniques and study populations at the same time. Our investigation discovered a potential link between greater SII levels and a higher chance of developing diabetes, which is consistent with the majority of studies.

Despite being a novel inflammatory marker, diabetes has not been examined with SII alone. However, diabetes has been linked in

TABLE 2 Baseline characteristics of study population according to Systemic Immune-Inflammation Index quartiles, weighted.

Characteristics	SII Quartiles				*p*-Value
	Q1	Q2	Q3	Q4	
	N=1969	N=1969	N=1969	N=1970	
Age (years)	47.44 ± 17.03	46.97 ± 17.08	48.68 ± 17.60	49.91 ± 17.57	<0.0001
White blood cell count (1000 cells/uL)	6.27 ± 9.25	6.72 ± 1.71	7.41 ± 1.82	8.58 ± 2.37	<0.001
Platelet count (1000 cells/uL)	206.44 ± 51.65	234.37 ± 50.68	254.93 ± 56.85	287.78 ± 72.62	<0.001
Albumin, refrigerated serum (g/L)	41.46 ± 3.30	41.38 ± 3.07	40.87 ± 3.18	40.21 ± 3.78	<0.0001
Blood Urea Nitrogen (mmol/L)	5.34 ± 1.92	5.25 ± 1.68	5.39 ± 2.02	5.34 ± 2.25	0.1865
Chloride (mmol/L)	101.44 ± 2.64	101.26 ± 2.63	101.38 ± 2.69	100.94 ± 3.03	<0.0001
Protein (gm)	83.02 ± 41.49	84.82 ± 45.16	82.11 ± 41.00	77.36 ± 41.08	<0.0001
Carbohydrate (gm)	250.17 ± 123.91	246.80 ± 120.98	247.20 ± 131.08	244.14 ± 127.46	0.5832
Total sugars (gm)	106.06 ± 76.30	103.07 ± 70.45	107.94 ± 83.80	108.95 ± 79.97	0.1046
Total fat (gm)	90.00 ± 48.35	91.36 ± 50.80	89.34 ± 47.47	87.99 ± 49.52	0.2070
Cholesterol (mg)	325.85 ± 260.72	324.46 ± 257.25	320.42 ± 256.25	312.20 ± 251.48	0.3786
Gender (%)					<0.0001
Male	56.33	51.01	45.06	40.99	
Female	43.67	48.99	54.94	59.01	
Race/Ethnicity (%)					<0.0001
Mexican American	8.12	9.84	8.50	7.41	
Non-Hispanic White	52.65	62.78	66.09	69.31	
Non-Hispanic Black	19.97	10.02	8.76	6.78	
Other Hispanic	7.16	8.33	7.11	7.90	
Other Race	12.10	9.02	9.54	8.60	
Education (%)					0.0438
Less than high school	10.27	11.34	11.40	10.73	
High school	26.69	25.60	27.41	27.71	
More than high school	63.04	63.06	61.19	61.56	
Marital status (%)					0.0974
Married/Living with partner	60.88	63.15	63.31	60.56	
Widowed/Divorced/Separated	18.42	16.56	18.90	20.61	
Never married	20.69	20.29	17.79	18.83	
Income to poverty ratio (%)					0.0002
0–1.5	23.87	21.76	20.49	23.94	
1.5–3.5	29.09	29.59	31.91	34.06	
>3.5	47.04	48.65	47.60	41.99	
BMI (kg/m^2) (%)					<0.0001
0–25	32.67	26.26	22.68	23.47	
25–30	34.15	34.41	30.86	29.98	
>30	33.18	39.33	46.45	46.55	
High blood pressure (%)					<0.0001

(Continued)

TABLE 2 Continued

Characteristics	SII Quartiles				*p*-Value
	Q1	Q2	Q3	Q4	
	N=1969	N=1969	N=1969	N=1970	
Yes	30.16	28.73	31.25	37.97	
No	69.84	71.27	68.75	62.03	
Smoke					0.0894
Yes	41.81	40.40	43.60	44.92	
No	58.19	59.60	56.40	55.08	
Vigorous work activity (%)					0.4710
Yes	28.00	27.35	28.30	25.13	
No	72.00	72.65	71.70	74.86	
Diabetes (%)					<0.0001
Yes	10.13	11.69	11.06	14.84	
No	89.87	88.31	88.94	85.16	

Mean ± SD for continuous variables: the p-value was calculated by a weighted linear regression model. % for categorical variables: the p-value was calculated by a weighted chi-square test. Q, quartile; BMI, body mass index; SII, Systemic Immune-Inflammation Index.

clinical studies with several traditional inflammatory indicators. Zhao-tong Jia et al. measured serum IMA and hs-CRP concentrations in patients with diabetic retinopathy by the rate-nephelometric method. There may be a positive correlation between hs-CRP concentration and the prevalence of diabetic retinopathy (r = 0.617, P < 0.01) (40). Hai-hang Liu et al. believed that serum hs-CRP concentration can predict the incidence of diabetes, and inflammatory factors play an important role in diabetes research (41). Klisic et al. studied the association of type 2 diabetes with some inflammatory factors, platelet-to-neutrophil ratio (PNR), monocyte/granulocyte-to-lymphocyte ratio (M/GLR), derived neutrophils to lymphocytes Cell ratio (dNLR), all three indicators are independently associated with type 2 diabetes (42). Klisic's study affirmed the role of traditional inflammatory factors in the prediction of diabetes but did not specifically explore the correlation between the two, so in-depth research in this study is necessary. Si-Yang Wang et al. demonstrated that the ratio of neutrophils to lymphocytes can be used as a marker for predicting diabetes (43). Si-Yang Wang's research also affirmed the outstanding work of inflammatory factors in the diagnosis of diabetes but did not conduct specific correlation studies. Mohamad Akbari's group (44) and Dan Qu's group (45) both considered elevated levels of interleukin 6 (IL-6) to be an independent predictor of diabetes. Compared with traditional inflammatory factors, SII binds three types of immune cells, reflects the inflammatory state well comprehensively, and has shown better prognostic value in several studies (46). For example, Afiat Berbudi et al. studied the effect of SII, neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and monocyte/lymphocyte ratio (MLR) in predicting the impact of type 2 diabetes on the immune system. Their ROC curve analysis confirmed that among these markers, SII was more effective and accurate in predicting the impact of T2DM on the immune system (47). Huaping Huang et al. found that SII can predict the postoperative survival rate of patients with cervical cancer, and it is more accurate and effective than other inflammatory factors (7). Hongmei Zhang et al. studied the relationship between leukocytes, centriocytes, lymphocytes, and diabetes, and the P values were all <0.001, and the final

TABLE 3 Association between systemic immune-Inflammation index and diabetes.

	Crude Model (Model 1)	Partially Adjusted Model (Model 2)	Fully Adjusted Model (Model 3)
	OR (95% CI) *p*-Value	OR (95% CI) *p*-Value	OR (95% CI) *p*-Value
SII/100	1.04 (1.02, 1.05) ***	1.04 (1.02, 1.05) ***	1.04 (1.02, 1.06) ***
SII/100 quartiles			
Quartiles 1	Reference	Reference	Reference
Quartiles 2	1.06 (0.89, 1.27)	1.17 (0.97, 1.41)	1.10 (0.88, 1.37)
Quartiles 3	1.09 (0.92, 1.30)	1.21 (1.00, 1.46) *	1.10 (0.88, 1.37)
Quartiles 4	1.40 (1.19, 1.66) ***	1.59 (1.33, 1.91) ***	1.31 (1.05, 1.63) *
p for trend	<0.0001	<0.0001	0.0187

Model 1, no covariates were adjusted. Model 2, age, sex, and race were adjusted. Model 3, age, sex, race, marital status, income to poverty ratio, education level, drink, smoke, BMI, high blood pressure, blood urea nitrogen, White blood cell count, Platelet count, chloride, protein, carbohydrate, total sugars, total fat, cholesterol, and vigorous work activity were adjusted. 95% CI, 95% confidence interval; OR, odds ratio; SII, Systemic Immune-Inflammation Index. * p < 0.05, ** p < 0.01, *** p < 0.001; a p < 0.05 was considered statistically significant.

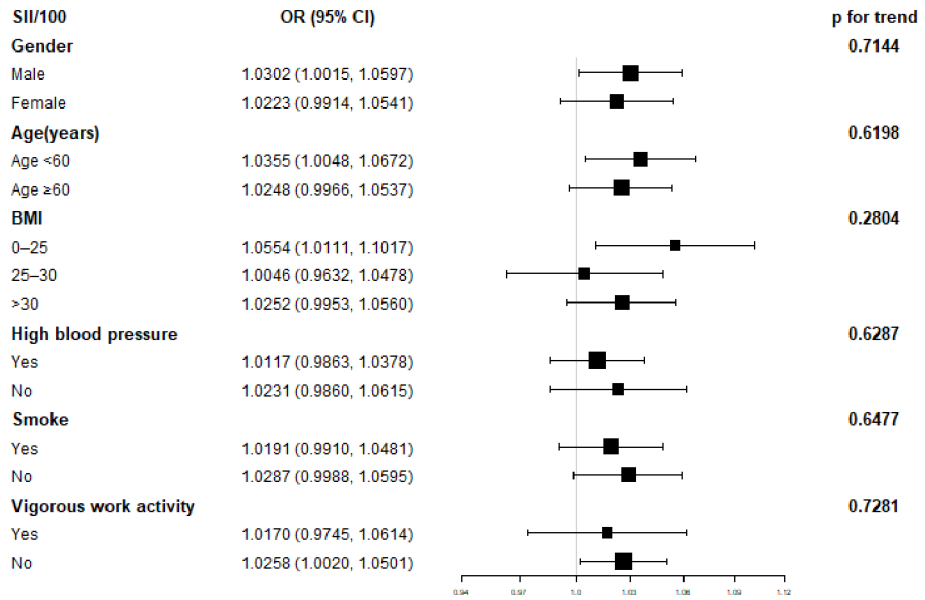

FIGURE 2
Subgroup analysis for the association between Systemic Immune-Inflammation Index and Diabetes.

experiment found that the increase in leukocyte level was related to hyperglycemia. The research conclusion of Hongmei Zhang et al. is the same as ours (48). Saori Kashima et al., using the data from Yuport Medical Examination Center, also found that increased white blood cell count levels may increase the probability of diabetes, which is also consistent with our conclusion (49). Jin-Young Hwang et al. found that the prevalence of diabetes may increase with the increase of platelet count (50). This is consistent

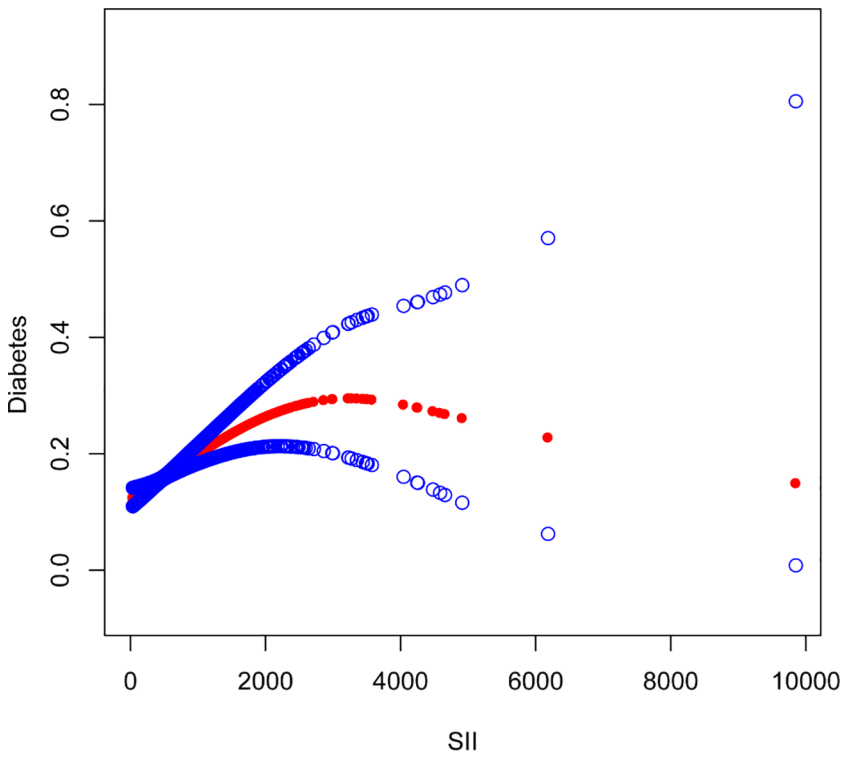

FIGURE 3
The association between Systemic Immune-Inflammation Index and Diabetes. The solid red line represents the smooth curve fit between variables. Blue bands represent the 95% confidence interval from the fit.

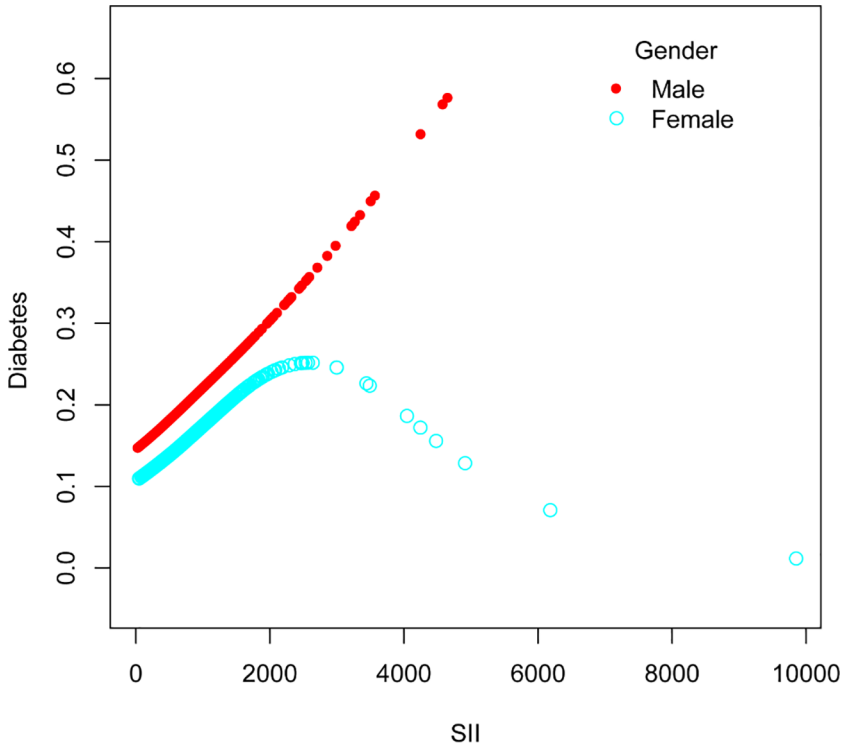

FIGURE 4
The association between Systemic Immune-Inflammation Index and Diabetes stratified by sex.

with our results. Yuqin Qian et al. found that platelet count may be related to diabetic peripheral neuropathy and is a potential risk marker (51). The SII selected in this study included platelet count, and the experimental results could truly reflect the burden of inflammation and accurately reflect the relationship between SII and diabetes.

The mechanisms underlying the positive association between inflammation and diabetes are unclear. Hitomi Usui Kataoka et al. suggested that endoplasmic reticulum stress may affect the pathogenesis of diabetes (52). Haichen Zhang et al. found epigenetic abnormalities in diabetic patients, which may provide information for target drug prediction (53). Mina Wang et al. summarized two causes of diabetes mellitus: impaired insulin action and impaired insulin secretion or a combination of both factors (54).

Our research has some advantages. The sample size is sufficient to be representative, and the years chosen are the most recent two data sets. We also adjusted for confounding factors to produce robust results. For example, previous studies have mentioned that dietary intake (55), physical activity (56) and protein intake (57) increase the prevalence of diabetes. As a result, we added protein intake and physical activity as factors to the fully adjusted model, which strengthened our findings. Our study does, however, have certain drawbacks. There is no causal association because it is a cross-sectional study, hence several prospective studies are required to explain the causative relationship. Confounding effects cannot be ruled out, even though we accounted for covariates to lessen their impact on the results.

5 Conclusion

More research is required to confirm our findings, which showed that SII levels are strongly related to diabetes.

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

In 2003, the NHANES Institutional Review Board (IRB) changed its name to the NCHS Research Ethics Review Board (ERB). In 2018, the name was changed from NCHS Research Ethics Review Board to NCHS Ethics Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

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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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Nanomedicines for the management of diabetic nephropathy: present progress and prospects

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Diabetic nephropathy (DN) is a serious microvascular consequence of diabetes mellitus (DM), posing an encumbrance to public health worldwide. Control over the onset and progress of DN depend heavily on early detection and effective treatment. DN is a major contributor to end-stage renal disease, and a complete cure is yet to be achieved with currently available options. Though some therapeutic molecules have exhibited promise in treating DN complications, their poor solubility profile, low bioavailability, poor permeation, high therapeutic dose and associated toxicity, and low patient compliance apprehend their clinical usefulness. Recent research has indicated nano-systems as potential theranostic platforms displaying futuristic promise in the diagnosis and treatment of DN. Early and accurate diagnosis, site-specific delivery and retention by virtue of ligand conjugation, and improved pharmacokinetic profile are amongst the major advantages of nano-platforms, defining their superiority. Thus, the emergence of nanoparticles has offered fresh approaches to the possible diagnostic and therapeutic strategies regarding DN. The present review corroborates an updated overview of different types of nanocarriers regarding potential approaches for the diagnosis and therapy of DN.

KEYWORDS

diabetic nephropathy, glomerular filtration barrier, nanocarriers, nanotheranostics, renal retention, targeted delivery

1 Introduction

Diabetes mellitus (DM) is linked to long-term damage and failure of various organs to function correctly and is a major contributor to the onset of many associated complications (1, 2). Diabetic nephropathy (DN) is one of the most frequent and severe forms of diabetic complications linked to diabetes-related microvascular damage, renal failure, and overt albuminuria. In most cases, renal complications are accompanied by cardiovascular irregularities contributing to a shortened lifespan of the patients (3). DN affects nearly one-third of DM patients, and its prevalence is increasing alarmingly with time (4). As the number of people with DM increases, so does the number of people with DN. DN is also the major cause of end-stage renal diseases, and diabetes-related mortality worldwide (5, 6). Nephromegaly and a modified Doppler are early morphological markers of renal injury, although proteinuria and glomerular filtration rate are the greatest indicators of severity (7). DN is often accompanied by other diabetic microvascular complications like diabetic retinopathy and diabetic neuropathy, which further worsen the situation (8). In addition to cumbersome pathophysiological aspects, DN simultaneously causes financial draining,

Nanoparticles are small nano-sized particles with unique physical, chemical, and biological properties that make them useful as diagnostic and therapeutic tools (9). Nanoparticles have exhibited promising potential regarding the management of DN due to their targeting ability along with improved pharmacokinetic attributes (10). Several nanoformulations have come up with exciting promises in the diagnosis and treatment of DN. Nanoparticles offer several advantages like improvement of pharmacokinetic profiles, stability, bioavailability, trackability, biocompatibility, and therapeutic efficacy of potent chemotherapeutic agents (11). The current article aims to articulate the role of different nanotheranostic tools with utilization promises for the treatment of DN. In this review, present-day advances in the synthesis of DN-selective nanotherapeutics as well as their areas of application have been discussed. The impact of various formulation factors has also been discussed, along with a summary of recent nanotheranostic developments including the passive and active targeting strategies pertinent to DN. The article also discusses present day challenges and/or limitations regarding renal targeting for DN, and attempts to dig out probable remedies for the same. Consequently, prospects of nanomedicine regarding DN management with respect to probable clinical translation has also been discussed in a comprehensive manner.

2 DN at a glance

Among diabetic patients, DN is possibly the most life-threatning complication, and leading cause behind renal failure (12). DN is a chronic complication arising from DM over a span of few years (13). The global prevalence of diabetes has risen substantially from 108 million in 1980 to 537 million in 2021, and predicted to rise to 643 million by 2030 and 783 million by 2045 (14). DM-associated hyperglycemia slowly causes hypertension and kidney dysfunction. The developed hypertension further worsens kidney's functionality, and ultimately results in renal failure. Albuminuria, glomerular lesions, tubulointerstitial fibrosis, and decreased renal filtration rate are the hallmarks of the multifunctional degenerative condition known as DN. Patients with type I DM seldom develop diabetic kidney damage prior to 10 years of the disease, whereas nearly 3% of people with type II DM already have overt nephropathy at the time of first diagnosis (15).

Class I glomerular lesions involve thickening of glomerular basement membrane mainly due to accumulation of extracellular matrix components e.g. collagen IV, laminin, and fibronectin membrane as an early indication of kidney damage. Class II glomerular lesions comprise of mesangial expansions of mild (IIa) to severe (IIb) grades distorting glomerular capillaries. Class III glomerular lesions include mesangiolysis, and detachment of endothelial cells from glomerular basement membrane. Class IV glomerular lesions involve advanced diabetic glomerulosclerosis caused by excessive accumulation of extracellular matrix components. Tubulointerstitial lesions comprise of interstitial fibrosis and tubular atrophy, and interstitial inflammation. Vascular lesions distinguish DN from hypertensive nephropathy. In addition, insudative lesions can be located in Bowman's capsule, glomerular capillary, and glomerulotubular junction (16). Measures currently used to determine the existence and progression of diabetic nephropathy include blood urea nitrogen, serum creatinine, formulae to estimate glomerular filtration rate, proteinuria, and albuminuria. These measurements, however, are not exact, do not directly assess renal tissue damage, and are comparatively insensitive to minute variations in renal functions. Consequently, the availability of novel biomarkers that are sensitive, specific, and accurate as well as capable of detecting kidney damage and forecasting clinically relevant outcomes would be greatly beneficial in DN.

The three layer filtration system within the Malpighian corpuscle consists of vascular endothelium, glomerular basement membrane, and podocytes of the visceral epithelium. Mesangial cells produce a collagen network that structurally supports the capillaries. Filtration occurs within glomerulus across mesangium. The primary contributors to the development of DN have been identified as hyperglycemia, overproduction of advanced glycation end products (AGEs), activation of protein kinase C (PKC), increased oxidative stress, inflammation, and activation of poly (ADP-ribose) polymerase (PARP) (12, 17, 18). Figure 1 provides a holistic overview of DN-associated signaling cascades. An increased pressure state within the nephron is the first development towards DN. DM-associated hypertension results in increased pressure throughout arteriolar-vascular system, including the afferent arteriole of glomerulus. This, in turn increases the glomerular filtration rate. Hyperglycemia-mediated direct intrarenal activation of the renin-angiotensin-aldosterone system (RAAS), and subsequent vasoconstriction of the efferent arteriole further adds to the increased pressure state. Angiotensin II, binding with AT1R receptors causes the smooth muscles of the arteries to contract, raising blood pressure. Additionally the adrenal cortex produces more aldosterone, and sodium absorption is enhanced. During DM associated hypertension, vasoconstriction of efferent artery of glomerulus is greater than that of afferent artery (19). Rise

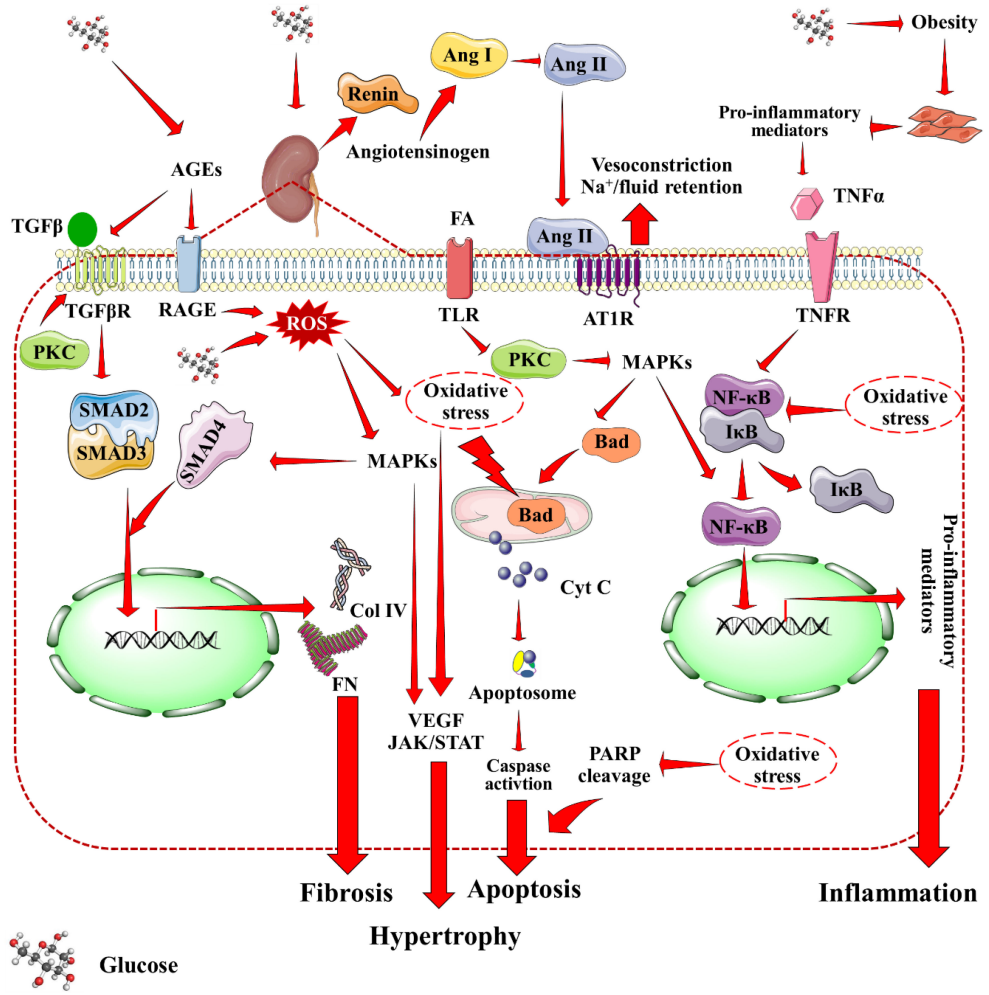

FIGURE 1
Mechanistic insights into DN-associated signaling cascades. Red arrow indicates downstream cellular events. AGEs, Advanced glycation end products; Ang I, Angiotensin I; Ang II, Angiotensin II; AT1R, Angiotensin II receptor type I; Col IV, Collagen type IV; Cyt C, Cytochrome C; MAPKs, Mitogen activated protein kinases; PARP, Poly (ADP-ribose) polymerase, PKC, Protein kinase C; RAGE, Receptor for AGEs; ROS, Reactive oxygen species; TNFR, Tumor necrosis factor receptor; VEGF, Vascular endothelial growth factor.

of pressure within the glomerulus results in mesangial expansion. The increased pressure leads to trauma and damage to the mesangium. In response to this damage, mesangial cells start secreting proinflammatory cytokines, and oxygen free radicals that lead to inflammation, oxidative stress, and endothelial dysfunction further damaging the nephron vasculature (20). CD80 upregulation enhances inflammatory cytokine production and mortality. Concurrently, vascular permeability is worsened by AGEs and PKC, which harms the basal membrane. Oxidative stress acts as the trigger for many signaling pathways associated with DN that causes activation of inflammatory, apoptotic and fibrotic pathways especially through the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), mitogen-activated protein kinase (MAPK), mammalian target of rapamycin (mTOR), janus kinase/signal transducers and activators of transcription (JAK/STAT), and (transforming growth factor beta/suppressor of mothers against decapentaplegic) TGF-β/Smad signaling (21, 22). All of these somewhat combine into hypertrophy and matrix accumulation within mesangium, which is known as mesangial expansion (23). TGF-β1, in particular, is linked to glomerular hypertrophy and extracellular matrix accumulation in the mesangium, which reduces glomerular filtration rate and contributes to renal impairment. TGF-β2 overexpression in diabetic kidneys has been linked to renal fibrosis and extracellular matrix accumulation during early stages of DN. Interestingly, hyperglycemia enhances the interaction of dipeptidyl peptidase-4 (DPP-4) with cation-independent mannose 6-phosphate receptors, which results in TGF-β activation in turn leading to renal fibrosis, glomerulosclerosis, and proteinuria. Concurrently with mesangial expansion, the fenestrations between podocyte foot processes also expand, decreasing the available surface area for filtration. Furthermore, monocyte chemoattractant protein-1 (MCP1) attracts activated macrophages/monocytes into the renal tubulointerstitium, leading to glomerular endothelial membrane leakage and renal injury. Polyol pathway, and phosphoinositide-3-kinase/protein kinase B (PI3K/Akt) signaling are implicated in the

process of glomerular hypertrophy and podocyte injury. Under high glucose conditions, mesangial cells cumulate polyols, and produce an excess of matrix proteins. The accumulation of extracellular matrix proteins, specially collagen and fibronectin, has been attributed for thickening of glomerular membrane, and mesangial matrix expansion in DN. Upregulation of collagen expression in renal tissues promotes accumulation of mesangial matrix proteins, thereby inducing glomerulonecrosis. Moreover, dilation of the fenestrations tend to make the filtration system leaky. Thus, larger molecules e.g. proteins filter out of the blood leading to one of the prime markers of DN, i.e. albuminuria (24). CD80, an immune-related molecule, is directly involved in focal segmental glomerulo-sclerosis and proteinuria via integrin downregulation.

As a combined result of all the factors, ischemia, cell death, and atrophy of the vasculature supporting glomerulus and tubules come into effect over time. This, inevitably results in decline of kidney's ability to filter blood, gradually leading to renal failure. Interestingly, expression of extracellular matrix proteins in kidney is negatively regulated by store-operated Ca^{2+} channels in mesangial cells, which may act as an endogenous renoprotective strategy in diabetics (25). Importantly, all the contributing factors to DN are directly associated with underlying hyperglycemia. Therefore, progress of DN can potentially be slowed down and/or prevented by controlling DM.

3 Rationale to use nanotheranostics in DN

The use of nanotechnology for the diagnosis and treatment of human diseases has increased dramatically in the recent decades owing chiefly to their capacity to function as theranostic tools that can carry a therapeutic load, and/or improve image contrast in diagnostics (26). Nanoparticles are utilized to deliver both small-molecules and big macromolecular (proteins, and nucleic acids) medicaments, as well as to diagnosis and detect disease progression. Nanoparticles have gained importance as novel designed nanomedicine due to their outstanding biocompatibility, tiny size to cross the cell barrier, wide surface area to carry effective drugs with high loading, and possibility of selective targeting of the drug to the affected area (27). Emerging evidence revealed that nanoformulations are able to reduce the necessary dose, increase permeability, and alter solubility to achieve maximal bioavailability and therapeutic efficacy (28, 29). Nanoparticles, which are preferable candidates for loading and delivering poorly soluble drugs, have been found to improve bioavailability and maintain long-term blood circulation to facilitate drug(s)' sustained release. Because of the kidneys' natural ability to choose nanoparticles within a stipulated size range, limiting the size of nanoparticles within a specific range is a design criterion for creating nanoparticle-based therapeutics targeting renal diseases. Particularly, nanoparticles with sizes smaller than 10 nm pass past the glomerular filtration barrier, whereas those larger than 100 nm seldom disseminate into the kidney because they are primarily retained by the liver and spleen. On the contrary, during DN, the kidney's mesangium can sequester nanoparticles between 30 and 80 nm in size, reducing liver retention and hepatotoxicity (30).

Many potential therapeutic candidates exhibit poor therapeutic index and pose difficulties for formulation scientists. In an effort to address these issues, nanosized formulations are seen as superior and safer alternatives to conventional formulations. Nanotechnology-based techniques not only increase the surface area of pharmaceuticals, but also somewhat change the physiochemical characteristics of the active components (31). The overall benefit is shown in improved efficacy, improved dose management, and lower dose. Nanoparticles are well suited to improve absorption for drugs that undergo extensive first-pass metabolism on oral administration. The physiochemical features of the nanoparticles, their distribution and binding characteristics, plasma concentration, urine pH, biological variables, diseased state, and blood flow to the kidneys all influence clearance. Overall pharmacokinetic properties depend on particle size, surface charge, shape, surface morphology, surface engineering etc. Pharmacokinetic studies that take into account the materials of nanoparticles as well as the loaded drugs are the need of the hour to better predict the future of nanomedicine.

Many therapeutic moieties exhibit promise in treating DN-related complications. However, poor solubility profile, poor bioavailability, low permeation, and high therapeutic dose apprehend potential clinical utilization (16, 29). Different nano-scale formulations, present with efficient targeting, and reduce dose-related toxic effects (29, 30). Nano-scale delivery systems offer numerous benefits to overcome the shortcomings of potential therapeutic agents (32). Formulations can be designed for reaching the target site at desirable concentrations. Passive targeting can be achieved by designing formulations to reach the target region mainly by utilizing the enhanced permeability and retention (EPR) effect (Figure 2). Formulations must remain in the circulation for a long time permitting their transmission to the target receptor(s). Certain aspects like pH, temperature, molecular size, microenvironment etc. can aid to achieve the same. External stimuli viz. ultrasound, hyperthermia, light, electric or magnetic fields may also contribute to regulate and/or trigger activity of nanosystems. Regarding renal delivery, due to increased vascular permeability and localized inflammation in the nephritic state, nanoparticles are more likely to house in the glomerulus (33). Active targeting is based on the affinity of ligand to specific receptor. In this approach, delivery of a certain quantity of either a therapeutic agent or a diagnostic agent or both, to target cells is based mainly on ligand-receptor interactions (34). A number of targeting ligands such as antibodies and non-antibodies (transferrin, RGD, folic acid, etc.) have been utilized to endorse selective delivery of the nanoformulations to particular regions (Figure 2). The presence of several targeting windows within the sick nephron, such as size cutoff, presence of charge-bearing components, and availability of particular receptors, can be used to develop functional nanoparticles for targeted therapeutic and diagnostic reasons. Current DN treatment options focus more on blood pressure, glycemia, and cholesterol management compared to molecular progression mechanisms of DN. Given the recognized difficulties, recent research suggests that nanoparticle-based

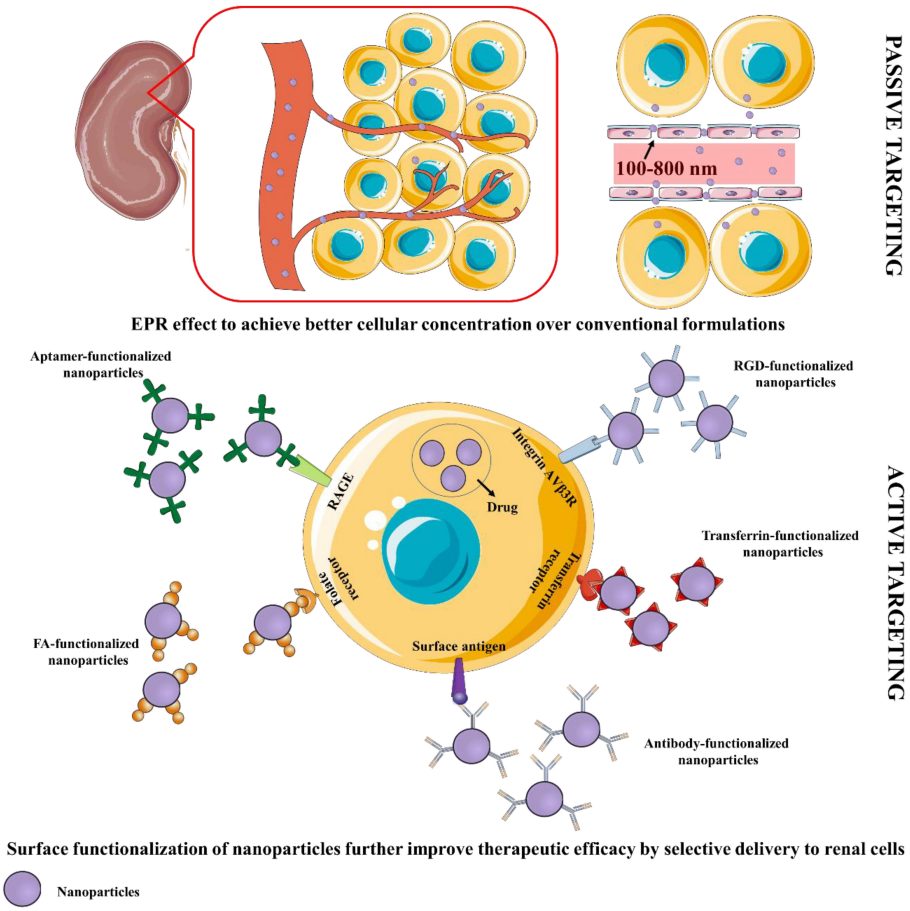

FIGURE 2
Schematic representation of passive and active targeting of renal cells by nanoformulations. EPR, Enhanced permeability and retention; FA, Folic acid; RGD, Arginylglycylaspartic acid.

platforms may provide potential futuristic ways to combat debilitating disorders such as DN. Several nano-systems have been developed that can be tagged/conjugated with certain moieties for improved targetability and/or traceability.

4 Formulation aspects for renal delivery

The glomerular basement membrane, podocytes, mesangial cells, and proximal tubules are the key sections of the kidney implicated in nephropathy. Many parameters, including size, charge, protein conjugation, and receptors, protect the formulations' desirable site of action (Figure 3). Make, size, and interaction of the nanoparticles impact the biodistribution significantly.

4.1 Size

Due to the kidney's natural ability to act as a blood filter, smaller diameter nanoparticles (<10 nm) are quickly eliminated from the kidney. The glomerular endothelium, with pores of about 70 nm, is the initial part of the three layer filtration system in glomerular area of nephron. The glomerular basement membrane comprises of an interwoven meshwork that can filter tiny molecules based on their charge and size. Along with this membrane, podocytes with filtration slits of approximately 30 nm also contribute. Water and minute plasma molecules can pass through the glomerulus, which filters blood depending on size and charge. The blood still contains higher molecular weight compounds and anionic charged components e.g., albumin (Albuminuria is brought on by DN-related disruption of this barrier). Therefore, structural characteristics of nanoparticles are crucial for their ability to reach renal cells. The nanoparticles with the highest plasma half-life have a particle size of about 100 nm. Smaller particles are more likely to pass through the kidneys. Larger nanoparticles (> 100 nm) are unable to reach the renal mesangium because of the size restriction imposed by the fenestrations, whereas nanoparticles with a diameter of about 75 ± 25 nm were specifically aimed towards the renal mesangium. On the contrary, proximal tubules can selectively be targeted with nanoparticles as large as 400 nm (35).

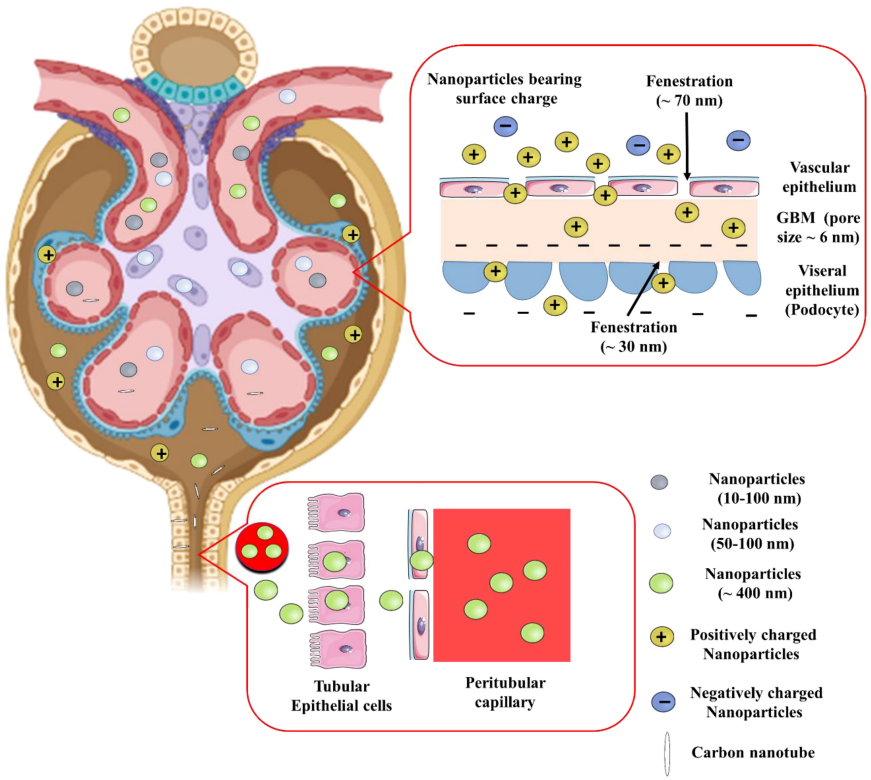

FIGURE 3
Effect of size, charge, and shape of nanoparticles on glomerular filtration barrier for renal retention. GBM, Glomerular basement membrane.

4.2 Charge

Because of their greater ability to attach to proteins and interact with the mononuclear phagocytic system, positively charged nanoparticles are rapidly eliminated from the kidney. Macrophages preferentially absorb negatively charged nanoparticles compared to neutrally charged nanoparticles. Nanoparticles with <15 mV charges are less likely to be taken up by macrophages, and display better circulation in blood (36). Cationic nanoparticles are easily taken in by the cellular membrane, and are capable of endosomal escape. As a result, negatively charged glomerular basement membrane easily absorbs positively charged nanoparticles to neutrally charged nanoparticles due to its anionic composition. On the contrary, ultrasmall (<5nm) nanoparticles with negative surface charge had been discovered to enter the glomerular capillaries, where they were able to bypass the glomerular endothelium and slowly accumulate in mesangial cells for 30 days (37). This, further supports the the primary role of charge in determining renal uptake of nanoparticles smaller than 5.5 nm.

4.3 Shape

Besides the key attributes like particle size and surface charge, the shape of nanoparticles influences drug targeting, even if the particle size is not perfect given the points discussed earlier. This is especially true for carbon nanotubes due to their higher aspect ratio (38). When the spatial orientation of the carbon nanotubes is perpendicular to the basement membrane, their lower diameter and higher aspect ratio enable them to pass through the glomerulus (39). Similar-sized nanoparticles usually have varied densities, which affects the speed and distribution through blood circulation. While low density nanoparticles circulate more quickly in the bloodstream, leading to quicker kidney clearance, shorter blood retention, and lower targeting; most of the dense nanoparticles do tend to have higher buoyancy forces and do not stay in the centre of the bloodstream where the speed is more, thus approaching the blood vessel walls more quickly.

4.4 Materials of construction

Nanoparticles composed of phospholipids, biologically derived lipids, natural polymers and strategically designed biodegradable polymers, and dendrimers are regarded as biocompatible and devoid of unwanted effects. However, polymeric nanoparticles are usually more prone to hepatic metabolism. Polymeric nanoparticles may be effective in overcoming the renal filtration threshold due to their low molecular weight, which allows them to be filtered through the kidneys and retained in the kidneys via post-glomerular processes. The tendency of biological nanoparticles to be more kidney-specific leads to an improvement in efficacy; examples include those that mimic the influenza A virus's sequence recognition technique (40). Poly(vinylpyrrolidone-co-

dimethyl maleic acid) has been demonstrated to have a high accumulation potential in the kidney and can be conjugated with superoxide dismutase for the treatment of renal diseases (41). Poly-l-glutamic acid is a renal polymeric drug carrier that is selective for renal protective drugs and has advantages such as high drug loading, non-immunogenicity, biodegradability, and biocompatibility (42). Many cases, nanoparticles are coated with polymers like polyethylene glycol (PEG), polyethylene oxide, dextran, polysorbates, and starch and small molecules like citrate to elevate biodistribution. Due to their decreased propensity for protein adsorption, PEGs in the mass range of 10 kDa are thought to make for superior coating materials than other PEGs (31, 43). Apolipoprotein E binds to polysorbate 80 to facilitate the transport of polysorbate-coated nanocarriers. Polysorbates also act as P-gp inhibitors to improve permeability of nanoparticles (44). Biodegradable nanoparticles e.g. poly(lactic-co-glycolic acid) (PLGA) nanoparticles are relatively easily metabolized, and the degradation products can be used in biological cycles like the Krebs cycle. Surface modification with poloxamers and/or citrates can enhance the circulation time of nanoparticles.

4.5 Targeting

Various techniques have been utilized for targeting renal cells based on their location in the kidney. Site-specific delivery of customized nanoparticles improves therapeutic impact, and decreased harmful side effects. In many cases, *in vivo* administration of nanoparticles in tumors has been the subject of several investigations, which led to few accidental findings of kidney-selective targeting and accumulation (38, 45). In addition, numerous biological ligands can be utilized to target precise areas, and improve specificity and efficiency, thus avoiding systemic accumulation and potential toxicities. The ligand, on the other hand, may be accountable for an unexpected deviation from the normal physiological pathway required for renal uptake, affecting the clearance and targeting. Indeed, the selection of the ligand is influenced by factors such as size, shape, hydrophilicity, immunogenicity, and stability, in addition to specificity. Nanoparticles can be modified to increase its effectiveness as well as target-specific distribution by polyethylene glycol (PEG)ylation, peptide conjugation, alterations in surface chemistry, and ligand binding. Angiotensin II receptors, megalin receptors, collagen IV, and v3 integrin receptors have demonstrated promise as possible targets for target-specific drug delivery to treat DN (46). Futuristic theranostics using nanoparticles have shown promise for treating a wide range of illnesses and ailments.

5 Nanoformulations in management of DN

Advancements in nanotechnology have enhanced the scope for early diagnosis of a disease including DN. Many therapeutic molecules exhibit promise in treating DN complications. However, clinical relevance is constrained by their poor solubility profile, poor bioavailability, poor permeability, high therapeutic dose, and related toxicity. Numerous studies have demonstrated that nanoparticles, with their enhanced pharmacokinetic properties and targeting capabilities, offer a high potential for treating DN (47, 48). Different potential roles of nanoparticles in management of DN are schematically represented in Figure 4.

5.1 Diagnosis

Since many diabetic complications do not display clinical symptoms immediately, systemic screening based diagnosis at early stages is the need of the hour. The monitoring of kidney status using a number of non-invasive imaging modalities, including as computed tomography (CT), positron emission tomography (PET), and magnetic resonance imaging (MRI), is now being used, however, applications of nanotechnology-hyphenated non-invasive imaging modalities have been found to be proficient enough for accurate diagnosis of DN.

A variety of magnetic nanoparticle-based probes have been created as contrast agents for imaging. Due to their exceptional magnetic and biodegradable qualities, iron oxide nanoparticles are amongst the most researched nanoparticles for enhancing clinical imaging. However, because they are biocompatible, superparamagnetic iron oxide nanoparticles (SPIONs) are more compelling. These nanoparticles can be targeted with magnetism, tracked using MRI, and even be used as magnetic triggers for drug release thanks to their superparamagnetic capabilities (49, 50). Emphasizing the fact that early diagnosis can lead to higher chances of therapeutic success, utilizing the role of low-density lipoprotein receptor 1 (LOX-1) as a potential biomarker, anti-LOX-1 superparamagnetic iron oxide PEG-coated nanoparticles have been designed to identify inflammatory renal lesions during early stages of DN (51). It has been shown that superparamagnetic iron oxide PEG-coated nanoparticles demonstrates good cellular internalization and durable MRI tracking abilities. The study by Luo and colleagues (45) supplies important informations with regard to detection, Characterization, and monitoring of early DN, utilizing the interaction between LOX-1-enriched inflammatory renal lesions and anti-LOX-1 targeted nanoparticles. However, more research is necessary on the toxicity and safety of LOX-1 targeted superparamagnetic iron oxide PEG-coated nanoparticles. Gold nanoparticles (AuNPs) are a promising choice for improving CT imaging because of their capacity to increase X-ray attenuation. AuNPs were found to greatly increase the contrast to noise ratio of CT in an imaging phantom at both low (40–60 kVp) and high (100–140 kVp) tube potentials compared to the clinically utilized contrast agent iodine (52, 53).

Quantam dots (QDs) also display futuristic prospects in diagnosis purpose. QDs can be endorsed as multimodality contrast agents used in CT, PET, MRI, infrared fluorescence, and photoacoustic imaging applications (54). In a study, occurrence of aldose reductase (AR) and toll-like receptor 4 (TLR4) in cells and renal tissues of diabetic rats was identified by a QD-based immunofluorescence technique and compared with the conventional immunohistochemistry method (55). For this

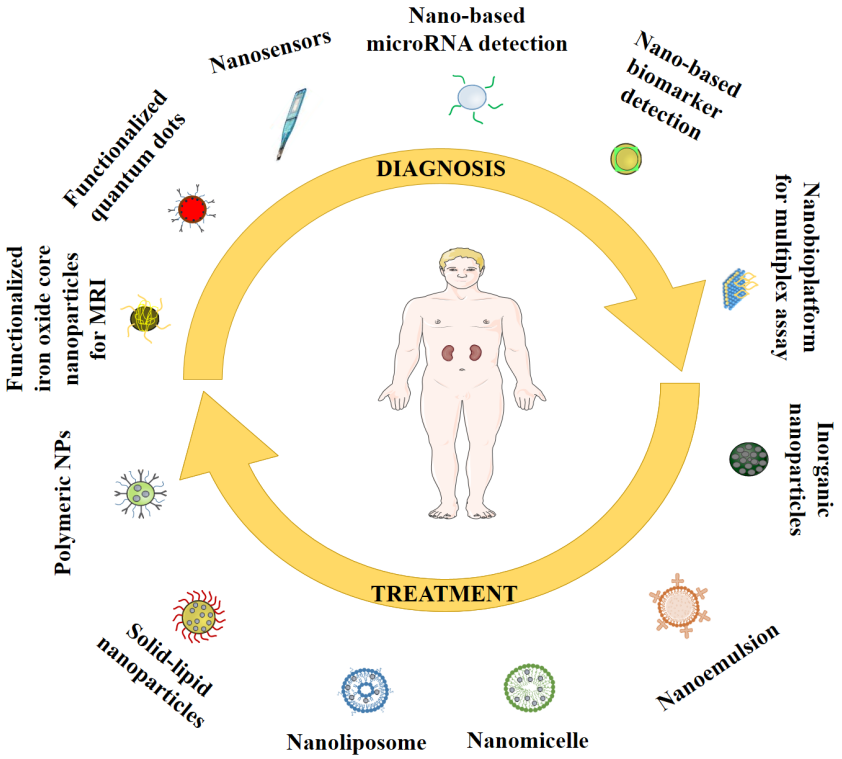

FIGURE 4
Different roles of nanoparticles in the management of DN.

purpose, CdSe/CdS/ZnS QDs were prepared and conjugated with rabbit polyclonal TLR4 antibody and mouse monoclonal AR antibody. Study results explained that both the AR and the TLR4 proteins were upregulated in the renal tissues of diabetic rats. The relationship between AR and TLR4 in the pathogenesis of DN was revealed by the developed dual-colour QD-based immunofluorescence labelled technique to study the expressions of AR and TLR4 in the renal tissues simultaneously. Compared to the conventional immunohistochemistry that require 7-9 h to provide 100% detection rate, the QD-conjugated with primary antibody and conventional immunohistochemistry shows maximum detection rate in minimal time of 3.5 h making it more convenient regarding accuracy (55). Summarily, this QD-based multiplexed imaging method offers new insights into the mechanistic investigation of biological component correlations, as well as possible applications in disease diagnosis and treatment. In another experiment, mono-dispersed and size controllable ^{89}Sr-doped CdSe QDs were prepared and coated with polyamidoamine to improve the accuracy and stability of quantitative analysis of renal injury of DN (56). The biocompatible QDs, besides facilitating urinalysis-based detection of renal injury markers through both fluorescent and radio-analytical methods, did not elicit any side effect. Clearly, the QD-based imaging methods offer a new vision into the mechanistic study of the correlation among biological factors with DN.

Due to its stability and ease of detection in human urine, microRNA (miR) can also be utilized to identify DN (57). Urinary exosomal miR is identified in early-stage DN. MiR-192, miR-21, miR-770, and miR-126 are also employed as biomarkers (58). Exosomal urine miRs shielded from RNase activity have steadily developed into a reliable novel biomarker for the detection of chronic kidney disease in its early stages, a field in which metal nanoparticle-based diagnosis is becoming more and more pronounced. A simple colorimetric assay based upon AuNPs has already been designed by Nossier and colleagues (59). The method relies on quantification of certain urinary miRs viz. MiR-210 and miR-34a to detect DN. In another study, a simple, cost-effective, portable method has been developed to determine DN via silver nanoparticles (AgNPs)-based colorimetric determination of creatinine (60). In a recent development, AuNP-based 3D sensors have proved reliable to detect thiamine in urine which can be correlated with onset and progress of DN (61). These methods present with advantages like simplicity, high reliability, and cost-effectiveness. Even in few cases, portability emerges as an added advantage along with non-invasiveness.

5.2 Treatment

Nanoformulations have drawn growing interest for therapeutic purposes due to their potential to cross biological barriers and boost the bioavailability of the drugs. With advancements in biochemistry and nanotechnology, antidiabetic medications can be disintegrated, implanted, encapsulated, or attached to nanoparticles using a nanoplatform for drug delivery. Additionally, active targeting with nanoparticles might also help with effective and site-specific

delivery of therapeutic agents. Table 1 enlists potentially useful nanocarriers for treatment of DN.

5.2.1 Lipid-based nanoparticles

Lipid-based nanoparticles are regarded as highly promising drug carriers due to their benefits of biodegradability, biocompatibility, higher kidney retention, and minimal immunogenicity. Liposomes, in particular present themselves with certain advantages like improving membrane permeability, sustained release properties, and site-specific delivery via surface functionalization. Among lipid-based formulations, liposomes offer the highest biocompatibility and biodegradability (28). Liposome systems, however, face significant challenges in terms of long-term stability. PEG chains can be covalently linked to increase liposome stability and *in vivo* circulation time (106). Solid lipid nanoparticles (SLNs) have been created as an alternative to liposomes in order to address their stability issues. Surfactants are essential parts of SLNs at the binding interface and lower the interfacial energy between the lipid and aqueous phases. The somewhat imperfect crystalline form of Nanostructured lipid carriers (NLCs), on the other hand allows for more drug loading, prevents drug outflow owing to lesser water content.

Improved renal hemodynamics during early stages of DN has been observed with co-enzyme Q10 containing liposomes in combination with ultrasound targeted microbubbles destruction (64). Upregulation of Bcl-2, and downregulation of Bax and caspase

TABLE 1 Different nanoformulations in DN therapeutics.

S. No.	Nanocarriers	Key components/features of nanoformulations	Therapeutic agents	Outcomes	References
1	Liposomes	SAINT C18	Rapamycin	Improved podocyte targeting with rapamycin	(62, 63)
2	Liposomes	Ultrasound-targeted microbubble destruction	Co-enzyme Q10	Preservation of podocytes, inhibition of apoptosis	(64)
3	Liposomes	soybean phosphatidylcholine, sodium deoxycholate	Eprosartan mesylate	Renal protection via blocking angiotensin II receptors	(65)
4	Liposomes	Ultrasound targeted microbubble destruction	Basic fibroblast growth factor	Intrarenal delivery, inhibition of inflammation	(66)
5	Liposomes	polymeric core and lipidic shell modified with kidney targeted peptide	Rhein	Improved kidney targeted distribution, and bioavailability	(67)
6	Liposomes	Distearoyl phosphatidylcholine	Hirudin	Enhanced accumulation of hirudin in renal tissue to relieve renal injury	(30)
7	Liposomes	Glucose-ligand conjugation	Astaxanthin	Preferential renal distribution via overexpressed GLUT1 receptors on mesangial cells	(68)
8	Liposomes	PEGylation	Quercetin	Renal protection	(69)
9	Nanoliposomes	–	Silymarin	Attenuation of DN-associated renal injury	(70)
10	Nanoliposomes	soybean phosphatidylcholine	Calycosin	Regulation of mitochondrial respiratory activities	(45)
11	SLNs	Compritol	Myricitrin	Improved bioavailability of myricitrin	(71, 72)
12	NLCs	Glyceryl monostearate and decanoyl/octanoyl-glycerides	Ergosterol	Improved oral bioavailability against DN complications	(73)
13	Niosomes	Cholesterol	Gymnemic acid	Inhibition of AGEs and oxidative stress	(74)
14	Niosomes	Cholesterol	Rubiadin	Oral admi istration, improved biomarker status	(75)
15	Polymeric nanoparticles	Chitosan	siRNA	Kidney-specific delivery utilizing megalin receptors	(76)
16	Polymeric nanoparticles	PLA-P85-PLA	Insulin	Protection of insulin from enzymatic breakdown, improved permeability	(77)
17	Polymeric nanoparticles	Cyclodextrin	siRNA	Greater uptake of mannose-targeted nanoparticles in mesangium	(78)
18	Polymeric nanoparticles	PEG-*b*-(PELG-*g*-PZLL)	Quercetin	Downregulation of ICAM1 on renal epithelium	(79)
19	Lysozyme-conjugate PEI nanoplexes	PEI	Gentamycin	Megalin-mediated uptake in proximal cells	(80)

(Continued)

TABLE 1 Continued

S. No.	Nanocarriers	Key components/features of nanoformulations	Therapeutic agents	Outcomes	References
20	Polymeric nanoparticles	Polyethyleneglycol-co-polycaprolactone-co-polyethylenimine	Rhein	Improved solubility, and renal distribution of rhein	(81)
21	Polymeric nanoparticles	PLGA	Phe-Tyr dipeptide	Improved bioavailability of peptide	(82)
22	Polymeric nanoparticles	Chitosan	Insulin	Oral delivery reduced fasting blood glucose level	(83)
23	Polymeric nanoparticles	PLGA	Crocetin	Anti-inflammatory, antifibrotic activities	(84)
24	Polymeric nanoparticles	Chitosan	Polydatin	Stronger hypoglycemic response and amelioration of DN compared to native polydatin	(85)
25	Polymeric nanoparticles	PLA	*Tinospora cordifolia* stem extract	Reduced expression of proinflammatory cytokines	(86)
26	Polymeric nanoparticles	Chitosan	Berberine	Improved permeability of berberine	(87)
27	Polymeric nanoparticles	p(AAPBA-b-HPA)	Insulin	Glucose and pH-sensitive delivery of insulin	(88)
28	Polymeric nanoplex	Dendrimer-templated polymer, albumin	Si RNA	Enhanced serum stability, avoidance of degradation by RNase, cytosolic delivery of siRNA following endosomal escape	(48)
29	Lipid-polymer hybrid nanoparticles	Chitosan, tripolyphosphate	All-trans retinoic acid	Improved solubility profile of retinoic acid	(1)
30	Metallic nanoparticles	AuNPs	Pomegranate peel extract	Downregulation of ROS production	(89)
31	Metallic nanoparticles	AgNPs	*Momordica charantia* leaf extract	Normalization of KIM1 levels	(90)
32	Metallic nanoparticles	AgNPs	*Momordica charantia* leaf extract	Reversal of DN-mediated alterations of gene expressions	(91)
33	Nanoparticles	*In situ* AuNPs onto chitosan functionalized PLGA nanoparticles	Insulin	Long-term insulin release upon oral delivery	(92)
34	Mesoporous silica nanoparticles	Silica, cerium oxide	Metformin	Long-term cyclic scavenging of free radicals	(93)
35	Biological nanoparticles	Virus	Cinaciguat	Active accumulation of cinaciguat at mesangial sites	(40)
36	Cationic nanoparticles	Cyclodextrin, adamantine-PEG	siRNA	Electrostatic interaction with negatively charged glomerular 4444rrrr4basement membrane	(36)
37	Immunoliposomes	Hydrogenated soybean phosphatidylcholine	Mycophenolate mofetil	Size-dependent retention in mesangium resulting in decreased cell expansion	(94)
38	Nab-paclitaxel	Albumin	Paclitaxel	Renoprotective effects	(95)
39	Nanoparticles	PF-A299-585	Triplotide	Renal targeting	(96)
40	Nanoparticles	Albumin	Methylprednisolone	Neonatal Fc receptor-targeted delivery improved cellular uptake in podocytes	(97)
41	Nanoparticles	Melanin@Glc-NCM	Melanin	Improved bioavailability of melanin, photo-thermal and glucose-sensitive delivery to mesangial cells	(98)
42	QDs	Semiconductor	RGDfC motif	Enhanced binding to αvβ3 receptors on podocytes	(99)

(Continued)

TABLE 1 Continued

S. No.	Nanocarriers	Key components/features of nanoformulations	Therapeutic agents	Outcomes	References
43	Metallic nanoparticles	ZnO nanoparticles	–	Activation of autophagy, elevated antoxidant and anti-inflammatory effects	(100)
44	Metallic nanoparticles	AuNPs	AuNPs combined with dapagliflozin	Renoprotective effect by targeting miR-21 and miR-192	(101)
45	Polymeric nanoparticles	PGA-coated polymeric nanoparticles	Rhein	Improved renal drug distribution and cellular uptake	(102)
46	Metallic nanoparticles	SeNPs	SeNPs combined with bee venom	Improvements in biochemical, histological, and molecular parameters	(103)
47	Nanoparticles	p-Coumaric acid nanoparticles	Chitosan	Improved nephroprotection	(104)
48	Metallic nanoparticles	AuNPs	–	Attenuation of high glucose-induced cytotoxicity on renal tubular epithelial cells	(105)

3 indicated low to no cytotoxicity, and preservation of podocytes. Another group of researchers claimed improved delivery of co-enzyme Q10 to kidneys by formulating into liposomes, also utilizing ultrasound targeted microbubbles destruction for site-specific delivery (107). With an entrapment effectiveness of 86.15%, co-enzyme Q10 was efficiently encapsulated within the liposomes exhibiting hydrodynamic diameter of about 180 nm and a negative surface charge. Coupling of liposomes with ultrasound-mediated microbubble destruction clearly improved kidney-specific distribution. These studies establish co-enzyme Q10-liposomes in combination with ultrasound microbubbles as a potential strategy to prevent and/or reverse the progress of early DN. Similar improvement was also reported with intra-renal delivery of basic fibroblast growth factor-loaded liposomes utilizing ultrasound targeted microbubbles destruction (66). Inflammation-mediated cellular apoptosis of renal tubular cells was significantly minimized too, on treatment with the formulated liposomes. Clearly, developed formulation played crucial roles to overcome the earlier known shortcomings of basic fibroblast growth factor i.e. short half-life, low stability and poor penetration.

Oral delivery of eprosartan mesylate-incorporated bilosomes exhibited better therapeutic efficacy to protect murine diabetic kidneys evidenced by the improved reduction of serum creatinine, total albumin, urea, lactate dehydrogenase, and malondialdehyde levels over eprosartan mesylate suspension, which has been achieved by the superior blockade of angiotensin II receptor by this novel nanoformulation as compared to conventional suspension (65). Bearing negative surface charge, the bilosomes were favourably taken up by Payer's patches. In the future, the produced eprosartan mesylate loaded nano-bilosomes could serve as a feasible oral formulation for DN and may give prospective benefits in situations of hypertension and renal illness. Liposomal hirudin has been found to be more effective than native hirudin in DN, acting by inhibiting the expression of vascular endothelial growth factor (VEGF) and TGF-β1 in the diabetic kidneys (30). In another interesting experiment, targeted delivery of astaxanthin has been achieved by using glucose ligand-conjugated astaxanthin-loaded liposomes that are transported by overexpressed glucose transporter 1 (GLUT1) transporter on the cell membrane of glomerular mesangial cells (68). Glucose-PEG600-DSPE ligand modified liposomes could specifically transport on the membrane of glomerular mesangial cells by overexpressed GLUT1, and achieved excellent kidney-targeted drug delivery. PEGylated liposome containing quercetin exhibited renal protective effects in DN, by attenuating oxidative stress, reducing AGE expression, and delaying the progression of DN (69). Lipid nanovesicles have been prepared with l-α-phosphatidylcholine, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[biotinyl(polyethylene glycol)-2000] and cholesterol that selectively target Tm cells. However, the kidney biopsies from the patients with lupus nephritis, patients with DN, and healthy controls demonstrated that the kidneys of patients with lupus nephritis are highly infiltrated with active CD8+ Tm cells with increased Kv1.3 expression (108). Higher expression of GLUT occurs in kidneys of diabetic population, which has been targeted by developing liposomes modified by mannose-PEG-distylacylphosphatidyl ethanolamine. Mannose aids in improving the therapeutic efficacy by augmenting the aqueous solubility of a drug (109). Kidney-targeted delivery of astaxanthin improved the bioavailability of astaxanthin and led to amelioration of renal pathological conditions by potentiating antioxidant capacity of the drug. Incorporation of glutathione into liposomal formulation improved therapeutic efficacy of glutathione by preventing oxidation of glutathione, thus enhanced antioxidant activity to suppress oxidative stress to nephrons involving polyol pathway (110). Silymarin nanoliposomes exhibited *in vivo* therapeutic potential in attenuating DN evidenced by decreasing renal fibrosis, inflammation and oxidative stress in DN rats mediated through TGF-β/Smad and JAK2/STAT3/suppressor of cytokine signaling (SOCS)1 co-suppression, leading to improved survival rate (70). This study demonstrated that the gap between *in vitro* and *in vivo* models arising due to poor pharmacokinetic attributes of a drug could be overturned through employing nanocarrier-mediated mediated advanced drug delivery tool. Incorporation of calycosin within liposomes improves the solubility and bioavailability (~2.3

times) than that of the free calycosin and thus enhanced therapeutic efficacy against DN (45).

SAINT-o-some, a modified liposome have already demonstrated improved serum stability and drug release characteristics. In addition, functionalization of SAINT-o-somes with particular antibodies like anti- vascular cell adhesion molecule 1 (VCAM-1) could improve therapeutic efficacy of SAINT-o-somes via active targeting to the podocytes (62). SAINT-O-somes generated tiny particles (106 nm) with 71% siRNA encapsulation effectiveness. SAINT-O-somes were serum stable at 37°C, protected siRNA from destruction by serum RNases, and had pharmacokinetics equivalent to standard long circulating liposomes following intravenous administration. These anti-VCAM-1 and anti-E-selectin SAINT-O-Somes are thus a unique drug delivery system capable of delivering siRNA into inflammed primary endothelium cells and possessing physicochemical properties to meet the needs of potential clinical application. VCAM-1 has been regarded to be expressed in association with tumor necrosis factor α (TNF-α) activation. Thus, cellular absorption of targeted SAINT-o-somes would to be greater than that of non-targeted SAINT-o-somes during TNF-α overexpression. In this aspect, rapamycin could be used for active targeting TNF-α-overexpressed podocytes (63). Clearly, anti-VCAM-1-rapamycin-SAINT-O-Somes reduced AB8/13 cell migration more effectively than free rapamycin and non-targeted rapamycin.-SAINT-O-Somes demonstrating the viability of VCAM-1 focused drug delivery to podocytes. Myricitrin is well-known to decrease oxidative stress-mediated cytotoxicity; however, it has poor bioavailability issues to meet with desired therapeutic effect *in vivo*. In a study, mycitrin-loaded SLNs improved DN altercations more prominently by lowering oxidative stress and raising antioxidant enzyme levels, compared to native myricitrin, which clearly demonstrated that the mycitrin-loaded SLNs could abrogate the bioavailability issue with mycitrin (71). The DN model used in this study is not well established, which sparks ambiguity over the practical impact of the outcomes (72). However, that does not seem to impact the improved efficacy of myricitrin on being encapsulated within SLNs. Formulation into NLCs also enhanced the therapeutic efficacy of ergosterol in DN by improving solubility profile as well as oral bioavailability of the drug (73). The spherical, negative surface charge-bearing formulation encapsulated ergosterol to a very high extent, and exhibited promise to improve therapeutic efficacy of the same.

5.2.2 Niosomes

Niosomes are liposome-like vesicles composed of single chain surfactant molecules along with cholesterol. Niosomes are physically and chemically more stable with additional cost-effectiveness, and ease of scale up (111, 112). Gymnemic acid niosomes have been proven to be a successful drug delivery tool to attenuate DN in rats by inhibiting oxidative stress, inflammation, and AGE formation (74). In another preclinical study, oral administration of rubiadin-loaded niosomes exhibited therapeutic promise against DN in rats (75).

5.2.3 Polymeric nanoparticles

Polymeric nanoparticles have piqued the interest of many researchers in the realm of medical biology owing to their high efficiency, ease of synthesis, long circulation time, biocompatibility, low toxicity, ability to absorb/encapsulate, and carry other molecules, and possibility of active targeting via surface modification (113, 114). Polymeric nanoparticles can deliver a wide range of drug(s) that offer high surface to volume ratio and have enormous opportunity to ameliorate DN by virtue of their versatile physicochemical properties. A wide array of polymers are promising choices because of their adaptability and flexibility in order to satisfy the needs of nanotechnology-based drug delivery. Additionally, they can be designed with a variety of desirable properties, which enhances their appeal, as does their capacity to tolerate physiological stress and their high biological stability. Polymeric nanoparticles can also be altered by ligand attachment and/or functionalization with surface modification for site-specific drug delivery.

Insulin-loaded PLA-P85-PLA vesicles have been proven to be effective in maintaining blood glucose homeostasis for a longer period of time (77). Insulin is protected from enzymatic breakdown in the gastrointestinal tract by PLA-P85-PLA vesicles. Moreover, P85 displays significant cell membrane permeability in the intestines due to its amphiphilic characteristics. A study showed quercetin-loaded polymeric nanoparticles to successfully ameliorate DN by downregulating intercellular adhesion molecular-1 (ICAM1) on endothelium of kidney, and reducing the CD11b+ myeloid cells accumulation (79). *Tinospora cordifolia* stem extract-loaded poly-lactic acid (PLA) nanoparticles have been reported to ameliorate DN by downregulating expressions of proinflammatory cytokines (86). PLGA nanoparticles loaded with Phe-Tyr dipeptide improved the bioavailability of the peptide drug to open up the scope to treat DN along with hypertension and cardiovascular diseases (82). Emerging evidence showed that PLGA-based nanoparticles engineered with kidney injury molecule 1 (KIM1) could improve renal targeting (46). In an interesting development, PLGA nanoparticles with particle sizes that are on the larger side could be retained in the kidneys (115). The nanoparticles were PEGylated to improve circulation time, and resulted in enhanced accumulation in the kidney. Rhein-loaded polyethyleneglycol-co-polycaprolactone-co-polyethylenimine nanoparticles were found to be effective in DN management, and enhance the therapeutic efficacy via kidney-targeted distribution of rhein (81). By resolving the limitations of low aqueous solubility and low kidney distribution of rhein, the formulated nanoparticles not only reduced the fasting blood glucose, serum creatinine, blood urea nitrogen, and urinary protein levels, but also decreased the intensity of oxidative stress by inhibiting TGF-β1 and endorsing the dephosphorylation of Smad2/3 signaling pathways. Rhein-loaded polycaprolactone-polyethyleneimine nanoparticles improved renal medication distribution and cellular uptake via poly-γ-glutamic acid (PGA)-mediated receptor-ligand interaction with –glutamyltranspeptidase (102). The PGA coating not only assured the stability, continuous drug release, and biocompatibility of the

formed nanoparticles, but it also increased renal cellular absorption by interacting with-glutamyltranspeptidase on the renal cells. The PGA coating prevented the formulation from being recognized by the reticuloendothelial system, which resulted in a longer circulation period. As a result, the PGA coating method opens up a new path for the use of nanomedicine in the treatment of renal disorders including DN. Similarly, crocetin-loaded PLGA nanoparticles exhibited better therapeutic efficacy in the management of DN compared to free crocetin evidenced by superior anti-inflammatory, and antifibrotic effects compared to native drug (84). Recently, novel glucose-responsive nanoparticles for insulin delivery has been developed with a copolymer made of acrylic acid-P-hydroxyphenethyl anisate and a newly synthesized macromolecular initiator p(AAPBA) by block copolymerization (88). The nanoparticles made of the copolymer p(AAPBA-b-HPA) exhibited high sensitivity for both pH and glucose, high biocompatibility, good insulin loading and sufficient stability under physiological conditions. Furthermore, these nanoparticles showed therapeutic promise in controlling hyperglycemia and restoring renal function by mitigating oxidative and inflammation-induced renal injury in DN mice (88).

Chitosan, a non-toxic mucoadhesive polymer with biocompatibility and easy modifiability, has been found to be a reliable, valid intestinal permeability enhancer promoting protein drug absorption. Especially, low molecular weight chitosan has displayed selective accumulation in kidneys aiding in renal-targeted drug delivery (116). Chitosan nanoparticles have opened up a new possibility of oral insulin delivery to overcome drawbacks like needle phobia, and peripheral hyperinsulinemia. Oral delivery of insulin-loaded trimethyl chitosan nanoparticles depicted better solubility profile compared to chitosan nanoparticles. Study results indicated comparable reduction in fasting blood glucose levels with oral delivery of insulin-loaded trimethyl chitosan nanoparticles and injection of insulin. Additionally, nanoparticles also exhibited decrease in serum TGF-β1 levels (83). These findings indicated trimethyl chitosan nanoparticles loaded with insulin to be somewhat more effective regarding therapeutic intervention than insulin injection. This can be linked to the fact that, chitosan enhances mucosal adhesion and increases absorption through an ionic contact between its cationic amine groups and the anionic groups on the surface of epithelial cells,. As a result, intestinal epithelial tight junctions intermittently open to allow for inter-epithelial insulin transfer. When treating DN, polydatin-loaded chitosan nanoparticles have been demonstrated to improve therapeutic efficacy evidenced by stronger hypoglycemic, antioxidant, and anti-inflammatory effects over free polydatin (85). To achieve good clearance from the system followed by effective drug delivery, biodegradable chitosan nanoparticles came up with attractive outcomes. The improved therapeutic efficacy has been achieved through improving absorption and prolonged-release properties of polymeric nanoparticles are all part of the mechanism of the renal protective effects against DN. Encapsulation into chitosan nanoparticles has been demonstrated to successfully respond to biopharmaceutical limitations of berberine as an antidiabetic agent (87). Chitosan, by facilitating paracellular transport via transient opening of intercellular tight junctions, could do away with the problem of high P-gp efflux associated with berberine in native form.

5.2.4 Lipid-polymer hybrids

These hybrid nanostructures usually consist of a polymeric core covered by lipid layer. They are constructed from at least two distinct materials to overcome the limitations of single component, and/or to achieve multiple functionalities for single nano-architectures. Hybrid nanoparticles offer distinct advantages like prolonged circulation time, high stability, biocompatibility etc. whereby lipidic and polymeric materials complement each-other. In a contextual study, rhein-loaded liponanoparticles (consisting of polymeric core and lipidic shell modified with kidney targeted peptide) improved cellular internalization by renal tubular cells, endothelial cells, mesangial cells, and podocytes via non-lysosomal pathways (61) (30). Novel two-step nanoparticular cascade of size control and enhancement of renal cellular uptake could successfully overcome the delivery obstacles to renal regions. In a later study, oral delivery of all-trans retinoic acid encapsulated chitosan/tripolyphosphate lipid hybrid nanoparticles improved therapeutic efficacy of retinoic acid against DN, as well as improved its solubility profile by nanoencapsulation (1). In comparison with free drug and drug-loaded lipidic nanoformulation, the hybrid nanoparticles under discussion exhibited superiority in ameliorating DN evidenced by the significant reduction of urea, creatinine, TNF-α, granulocyte-macrophage colony-stimulating factor, VEGF and ICAM-1 levels with increase in LKB1 and AMPK levels.

5.2.5 Metallic nanoparticles

During the past few decades, metal-based nanoparticles have gained increasing attention in the biomedicinal field owing mainly to ease of synthesis, and long-term stability answering the low mechanical properties of polymeric nanoparticles. To avoid complexation of metal ions in the body, modification with sodium citrate can be very useful (117). In diabetic kidneys, selenium nanoparticles (SeNPs) treatment increased levels of heat shock protein (HSP-70), longevity protein Sirtuin 1 (SIRT-1), and regulated the apoptotic proteins Bax and Bcl-2 (118). SeNPs successfully inhibited apoptosis in kidney cells and prevented the course of DN not only by reducing oxidative stress and increasing the levels of cytoprotective protein HSP-70 and longevity protein SIRT-1. Chitosan-stabilized SeNPs have evolved as a potential therapeutic tool to ameliorate DN by downregulating expression levels of TGF-β1 and aldose reductase (119). Though vascular and glomerular congestion was observed in renal tissues of treated groups, the risk-benefit ratio seemed favorable. Mucosal adherence and increased absorption, results due to interaction of the cationic amine groups of chitosan with the anionic groups at the epithelial cell membrane (120). In a more recent study, citric acid and ascorbic acid-protected SeNPs ameliorated DN during pregnancy, and improve histopathological features of the kidney (121). Given that, the formulated SeNPs accelerated the start of gestation and improved rate of successful pregnancy among diabetics, the formulation seems to be a potential futuristic asset

on establishing safety profile. SeNPS, in a very interesting study complemented bee venom to protect from DN by improving biochemical, histological, and molecular parameters (103).

Pomegranate peel extract-stabilized AuNPs downregulated production of reactive oxygen species (ROS) via blockade of protein glycation, and dephosphorylation of MAPK/NF-κB/STAT3-mediated proinflammatory response, thus creating an economic option to treat DN (89). AuNPs have been observed to limit expansion of mesangial matrix, and ameliorate podocyte damage to maintain permeability and integrity of glomerular filtration barrier (117). In another study, citrate-stabilized AuNPs exerted therapeutic effects against DN *via* ameliorating oxidative stress (105). Similarly, chitosan/sodium lignosulfonate AuNPs exhibited protective effects against DN (122). Clearly, fabrication of AuNPs enhanced antioxidant response while at the same time maintaining glycemic homeostasis. AuNPs (2.5 mg/kg/day, intravenous) can substantially ameliorate DN, in combination with dapagliflozin (2 mg/kg/day) (101). The treatment regimen successfully alleviated renal fibrosis, minimized apoptosis, and activated autophagy. They seemed to mitigate DN by targeting miR-192 and miR-21 and their downstream pathways i.e. fibrosis, apoptosis, autophagy, and oxidative stress. In line with the findings, AuNPs of about 30 nm diameter attenuated high glucose-induced cytotoxicity on renal tubular epithelial cells by downregulating free radicals, AGEs, and apoptotic marker proteins (105). However, the cytoprotective effects minimized in presence of SIRT-3 inhibitor.

In a study, *Momordica charantia* leaf extract-loaded AgNPs significantly elevated the level of HMG-CoA reductase, PPARα and PPARγ in DN subjects (90). Nanoparticles at a dose of 50 mg/kg for 12 days significantly upregulated PPARs compared to diabetic controls. The AgNPs also normalized level of KIM1. In another study, oral administration of *Momordica charantia* leaf extract-loaded AgNPs reversed DN-mediated upregulation of PI3K, Akt, TGF-β, JAK2, STAT3 and glucokinase genes, and consequent downregulation of phosphatase and tensin homolog (PTEN), suppressor of cytokine signaling proteins (SOCS)3 and SOCS4 genes (91). Clearly, formulation into AgNPs played crucial roles to counteract biopharmaceutical limitations of traditional medicinal herb.

In a study, ZnO nanoparticles attenuated oxidative stress, and inflammation to exert protective effects against DN (123). Another study was performed to explore the mechanistic renoprotective benefits of ZnO nanoparticles (124). Results revealed that ZnO nanoparticles to be effective against DN by increasing Nrf2-DNA-binding activity and downregulating thioredoxin-interacting protein (TXNIP) gene expression, which leads to oxidative stress suppression, and impairing inflammatory response by reducing NLRP3 inflammasome activation. ZnO nanoparticles reduce glucose absorption by inhibiting the intestinal α-glucosidase enzyme, improve hepatic glycogenesis by acting on insulin signaling pathways, and enhance glycolysis, thereby improving glucose disposal. They have a potential antiglycation effect by inhibiting the formation of AGEs and preventing changes in protein structure that may occur as a result of covering amino groups in proteins. In a recent study, ZnO-nanoparticles at an oral dose of 10/mg/kg/day for 4 weeks exhibited promise against DN (100). They retained constancy of the glomerular filtration barrier, and restored almost normal renal structure. These ZnO nanoparticles exerted anti-apoptotic, anti-inflammatory, and antioxidant activities to hint at a new therapeutic modality of DN.

Citrate-stabilized Mn_3O_4 nanoparticles protect mitochondria, the master regulator of cellular red-ox homeostasis, by scavenging intracellular ROS, inhibiting apoptotic triggers, and preventing antioxidant enzyme loss to alleviate diabetes-associated chronic kidney disease (125). Due to its free radical scavenging activity and high biocompatibility, Mn_3O_4 nanoparticles have emerged as one of the most affordable and effective materials for oxidative stress. By avoiding ATP depletion and the opening of the mitochondrial permeability transition pore, Mn_3O_4 nanoparticles, exerts protective benefits, by regulating red-ox homeostasis and autocatalysis, Mn_3O_4 nanoparticles can lessen oxidative stress, and functionalizing citric acid considerably improves its biocompatibility. The Mn^{2+} ions simultaneously form harmless free radicals that can be effectively managed by biological systems. Adding absence of toxic side effects to it, Mn_3O_4 nanoparticles might become superior to oral hypoglycemic drugs currently in use (125).

Metallic nanoparticle posses advantages of stability, easy storability, and sustained availability, thus surpassing the low mechanical properties associated with polymeric nanoparticles. However, metal-based nanoparticles tend to build up in the kidneys since heavy metals are difficult to eliminate via biodegradation. Therefore, searching ways to avoid the unwanted effects by heavy metals has become a focus of current nanoparticle research. There is a possibility that ions produced by metallic nanoparticles may cause injury to the organs involved during excretion. Hence, rigorous research into the toxicity of these nanoparticles to organs during metabolism is required, while quick clearance considerably minimizes the harmful effect of nanomaterials on the system. Nanoparticles larger than 6 nm in size or containing heavy metals could be readily absorbed by the reticuloendothelial system. Because their diameters fall below the threshold necessary for kidney filtration, nanoparticles with small sizes (5.5 nm) are promptly excreted in the urine (126). Some large-sized nanoparticles may be removed by the kidneys following breakdown during prolonged circulation in the body whereby the modified sodium citrate approach might be useful for certain metallic nanoparticles to avoid complexation and enhance circulation time. Again, renal accumulation of metal-based nanoparticles might actually turn up to be a positive indication in terms of therapeutic accumulation at renal sites. The probable toxicity of metallic nanoparticles to the CNS can be minimized by virtue of selective targeting to renal regions (127). Again, to solve solubility issues of some metallic nanoparticles, water-soluble ligands immobilized on nanoparticle surfaces can be utilized to dissolve noble metal nanoparticles in water (128).

5.2.6 Biological nanoparticles

Biomimetic nanomedicine combines the benefits of biomaterials, particularly human cells and pathogens, with the physicochemical features of diverse functional materials. Good biocompatibility, and high accumulation capacity make biological

nanoparticles superior candidates among futuristic drug delivery cargos. Viral nanoparticles can integrate the virus' nucleic acid into the host cell's nucleic acid with high sensitivity and targeting specificity (129). Influenza A virus nanoparticles have already been used to deliver cinaciguat (degraded by endolysosomes) into renal mesangial cells (40). Cinaciguat from viral nanoparticles can specifically activate the soluble form of guanylate cyclase, which in turn converts guanosine triphosphate to guanosine monophosphate, thereby downregulating glomerular fibrosis. In the experiment under discussion, equivalent efficacy could be observed with merely 10% dose administration of cinaciguat loaded within nanoparticles, compared to free cinaciguat. The dose reduction further aided in reducing biological toxicity, potentially improving patient compliance (40). Like other carriers, Long circulation times tend to improve efficacy and tissue-specific accumulation in case of biological nanoparticles too. Viral nanoparticles bearing negative charge exhibit short half-lives while those with positive surface charge stay in systemic circulation for longer period (130–132). PEGylation has evolved as an effective strategy to minimize biospecific interactions and immunogenicity, and improve circulation time (133). There is a paucity of information on the effectiveness of specifically designed viral nanoparticles *in vivo*. Many studies have demonstrated proof of concept, to underline the strong potential of viral nanoparticles as novel candidates in biomedicinal fields. The next challenge is to better comprehend the outcome and any potential long-term negative effects.

5.2.7 Others

Zuckerman and colleagues (36) have fabricated cyclodextrin-based cationic nanoparticles for delivering siRNA to glomerular basement membrane. These nanoparticles were subsequently coated with an adamantane-PEG conjugate to increase their hydrophilicity, and induce a positive charge. The net positive surface charge has been ascribed to effective drug transport to glomerular basement membrane, and enabled accumulation in the glomeruli. The study has further affirmed that siRNA nanoparticles did not disintegrate in blood; rather, siRNA nanoparticles accumulated and disassembled in the glomerular basement membrane while free siRNA failed to do so. RGDfC (Arg-Gly-Asp-DPhe-Cys)-conjugated QDs have been evaluated for kidney targeting (99). RGDfC-conjugated QDs have been taken up selectively by podocytes that express αvβ3 integrin receptors. A novel mechanism for renal targeting involved altering the size of nanoparticles to meso-scale formulations, as well as altering their opsonization potential (134). Meso-scale nanoparticles for renal proximal tubule targeting have offered significantly enhanced localization in the kidney, as well as remained within kidney for a longer period of time in comparison with other organs. This targeting strategy can be utilized to cater to proximal renal tubule as pressure drop in the nephron, and the large absorptive pressure of the capillaries aid in endocytosis of meso-scale nanoparticles with low opsonization potential into endothelial cells of peritubular capillaries. Human serum albumin peptide fragments can be created as possible renal targeting carriers. Triplotide has been conjugated with PF-A299-585, a peptide fragment of human serum albumin (96). Membranous nephropathy model study has confirmed the usefulness of PF-A299-585 as a carrier for renal targeting, compared to free triplotide. An albumin-bound nanoformulation, nab-paclitaxel has partially ameliorated the microscopic structural changes encountered in the renal cortex during DN, and decreased the immune expression of the fibrogenic mediator TGF-β1 to exert renoprotective effects (95). However, the researchers did not compare the efficacy of native paclitaxel with that of nab-paclitaxel, thus disallowing us to arrive at a definitive conclusion on potential benefits of nanoformulation over native drug. In another study, by interacting with neonatal Fc receptors, albumin nanoparticles specifically entered into renal podocytes, demonstrating the methyl prednisolone-loaded nanoparticles to be specifically absorbed by the podocytes (97). Neonatal Fc receptors -expressing human podocytes displayed an improvement of nearly 36 folds, compared to the uptake in the non-expressing control cells. Co-localization further confirmed that uptake of the uniform, preferable-sized (10 nm) nanoconjugates involved receptor-mediated endocytosis followed by lysosome associated transportation. Another research group reported that nanoparticles of particle size 95 nm were optimum for mesangial accumulation (135). Moreover, compared to free celastrol, celastrol-albumin nanoparticles have exhibited decreased drug accumulation in non-target organs and tissues, thus reducing the systemic toxicity of celastrol. The primary mechanism for the uptake of nanoparticles by mesangial cells was thought to be clathrin-mediated endocytosis. The systemic pressure of the blood flowing into the kidneys and the fenestration of the glomerular capillaries' endothelium, which allowed the nanoparticles to reach the mesangial cells, were additional factors for the site-specific accumulation of nanoparticles. The cut-off for getting through the filtration barrier is 10 nm, though; as a result, the targeted delivery was successful because nanoparticles were able to localize in the mesangial cells while being unable to cross the barrier (135). To address the obstacles associated with siRNA delivery, cationic dendrimer-templated polymeric nanoplex was conjugated with anionic albumin for delivery of siRNA (48). The approach enhanced serum stability, avoided *in vivo* degradation of free siRNA by RNase and achieved cytosolic delivery of siRNA following endosomal escape. The nanoplex-mediated delivery of siRNA has successfully knocked down targeted gene in both *in vitro* and *in vivo* models of DN. Inhibition of histone deacetylase 4 (HDAC4) by the cargo, in DN subjects makes promise to alleviate podocyte injury, and downregulate HDAC4-STAT1 mediated inflammatory processes. Multifunctional nanoparticles containing metformin have been developed whereby hollow mesoporous silica nanocomposite particles have been doped with trace cerium oxide (for inherent renoprotecvtive activity) were formulated (93). The nanoparticles could mitigate oxidative stress, suppress cellular apoptosis, protect from DN-associated renal injury significantly chiefly by improving renal accumulation over free metformin. Trace cerium oxide has been doped with a multifunctional nanocomposite, to accomplish long-term cyclic scavenging of free radicals in the body. Cerium oxide has a high oxygen storage capacity and improves red-ox characteristics. The hollow silica structure has been etched to retain metformin. Novel

photothermal nanoparticles have been prepared that offer outstanding kidney-targeted therapy in presence of near-infrared irradiation for the management of DN (98). In this work, glucose ligand conjugated neutrophil-like cell membrane-coated melanin nanoparticles (Melanin@Glc-NCM) have been synthesized to improve the bioavailability and antidiabetic property of native melanin. Consequently, the formulation could precisely be transported by overexpressed GLUT1 on glomerular mesangial cells. Furthermore the photothermal nanoparticles have restrained hyperproliferation of glomerular mesangial cells after near-infrared irradiation to overcome and/or protect from kidney damage especially during gestational period (98). In this context, a novel attempt to evaluate chitosan-loaded p-coumaric acid nanoparticles hinted at a potential nano-based delivery approach to provide greater nephroprotective effect against DN (104).

6 Current challenges and future prospects

DN has emerged as a global impediment associated with DM. For management of DN, renal targeted drug delivery has generated interest among researchers. The use of nanoformulations is an emerging strategy to reach the targeted site efficiently. Polymers, lipids metal-based carriers etc. have been explored for targeted drug delivery to the kidney. The molecular targets of DN are important for site-specific delivery. Nanoformulations are able to overcome constraints like short duration of action, low bioavailability, and dose-related toxic adverse effects, thus hinting at effective strategies for DN management (29). Since it is a progressive nephropathy, early detection and treatment of DN is highly desirable. Therefore, new methods aided with nanoparticulates may offer novel horizon regarding diagnosis of DN.

In the case of polymeric carriers, the renal filtration threshold plays an important role. The renal retention of polymers with molecular weight lower than the renal filtration threshold progresses with post-glomerular processes (10). On the other hand, renal retention of polymers with higher molecular weight generally involves extravasation from the renal vasculature leading to accumulation in the parenchymal tissues (16). From a synergistic therapy perspective, nano-based approaches present unique opportunities to treat complex heterogeneous diseases (136). However, nanocarriers for therapeutic purposes must be safe *in vivo*. Further evaluation in DN is required regarding pharmacokinetics and pharmacodynamics of characteristic proteinuria and altered glomerular filtration rate during pathological conditions (137). Biological nanoparticles e.g. viral carriers seem to be more kidney specific, thus enhancing their bioavailability for renal disorders. However, extensive trials, and *in vitro* and *in vivo* studies are required to evaluate biosafety of such nanocarriers. Compared to other modalities, the aim of nanoplatform-based drug delivery is to deliver drugs precisely to renal sites to interact with multiple pathogenic pathways. The application of multifunctional targeted nanoparticles in DN diagnosis is a development trend, whereby successful clinical translation would require long-term efficacy and safety assessments. Certain carriers have been designed with ligands for efficient, site-specific delivery of diagnostic or therapeutic agents into the renal region.

The propensity of the kidney to expel drugs out of the system makes developing a platform for drug administration within the kidneys for the treatment of DN highly challenging for formulation scientists. Though treatment modalities involving mRNA seem to be a promising approach, mRNAs face obstacles such as fast nuclease destruction, macrophage phagocytosis removal, and renal filtration clearance. Nanoencapsulation can be an effective approach to solve major issues. However, safety and adjuvancity of mRNA-nanocarriers is still a matter of concern (138). Since the ground-breaking mRNA COVID-19 vaccines have already shown the great potential of mRNA nanomedicines, we anticipate that ongoing innovation will result in novel and extremely effective mRNA-based therapeutics for other diseases (including DN) too. The vast number of nanoformulations at preclinical and clinical stages of evaluation make it clear that the field of nanomedicines is destined to gain a sizable market share in the near future. In terms of DN, specialized biophysical properties of nanomedicine-based techniques enable improved contact with renal cells to promote improved retention as well as improved cellular uptake inside the basal membrane epithelial cells, podocytes, mesangial cells, and proximal tubule cells. New insights into the pathophysiology of DN and knowledge of the structure and function of the kidney serve as effective tools for optimizing medication delivery systems for DN research. It's time for nephrology specialists and nanotechnological research teams to work together to create energizing nanomedicine strategies for the clinical translation of specific targeting and therapy of DN.

7 Discussions

DN has become a significant issue in both type 1 and type 2 DM globally. The course of DN may be potentially slowed down and/or halted by treating underlying DM because all of the processes are linked to underlying hyperglycemia. Oral hypoglycemics, presently in use often aggravate kidney damage (139). By eliminating undesirable traits of potential therapeutic agents, including short duration of action, low bioavailability, and toxic adverse effects, nanoparticles can offer effective therapeutic methods for the treatment of DN. Moreover, early detection and treatment of DN are particularly desirable due to the fact that it is a progressive disorder. Novel methods for the diagnosis of DN might also be provided by nanoparticle-based techniques. Figure 5 explains roles of different nanoformulations regarding diagnosis and treatment of DN.

Polymeric nanoparticles may be useful in overcoming the renal filtration threshold due to their low molecular weight, which permits them to be filtered through the kidneys and be kept in the kidneys via post-glomerular processes. Because of their extended retention time, ultrafine size, and capacity to release medications for prolonged periods of time, chitosan nanoparticles have become highly desirable as oral delivery vehicles. As was previously mentioned, research on cutting-edge nano-drug delivery systems is already on the way, but more study is required to evaluate the typical proteinuria in

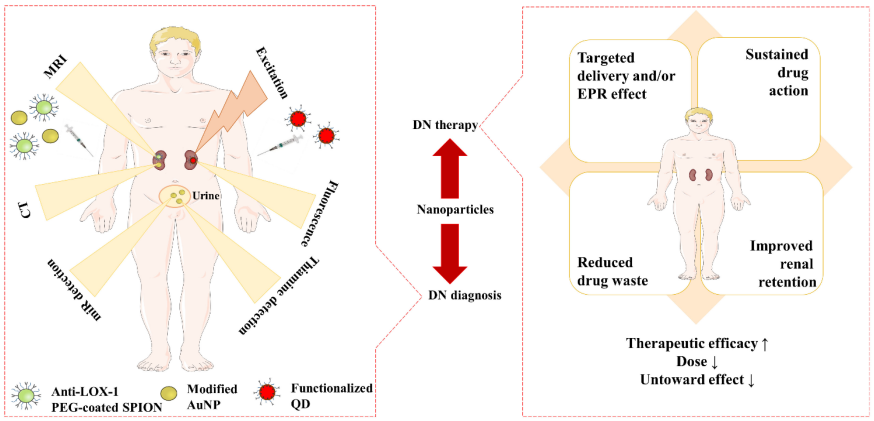

FIGURE 5
Advantageous roles of nanoformulations in DN diagnosis, and DN therapy. '↑' indicates increase, '↓' indicates decrease. AuNP, Gold nanoparticle; DN, Diabetic nephropathy; EPR, Enhanced permeability and retention; PEG, Polyethylene glycol; QD, Quantum dot; SPION, Super paramagnetic iron oxide nanoparticle.

pathological conditions, as well as changes in glomerular filtration rate in pathological conditions, and to design an optimal drug delivery system for DN. The tendency of biological nanoparticles to be more kidney-specific leads to an increase in their efficacy, examples include those that imitate the sequence recognition approach of the influenza A virus (40). However, extensive trials, as well as *in vitro* and *in vivo* research, are still needed to assess the toxicity and biosafety of viral nanoparticles. Though there exist chamces of AgNPs-mediated inflammation and cell damage in the kidneys, AgNPs still represent a promising nanoformulation for diagnostic applications. The main site for accumulatrion is liver (not kidney), which can again possibly be minimized via citrate modification (140). Moreover, silver can be cleared from most of the organs within 8 weeks post-dosing (141). Even, evidence suggests that acutely ingested AgNPs, irrespective of size or coating are well-tolerated (142). On this note, in a study, no harmful effects in liver and kidney were observed efter 90 days exposure to AgNPs (143). Another study reported a dose upto 10 mg/kg of AgNPs to rats to be safe while higher doses exerted toxic effects (144). Apart from dose, particle surface area, surface chemistry, and meticulous, accurate characterization of particle size and morphologic properties continue to be key issues regarding efficacy and toxicity, particularly in the physiological environment (145).

The presence of several targeting windows within the sick nephron, such as size cutoff, presence of charge-bearing components, and availability of particular receptors, can be used to construct functional nanoparticles for targeted therapeutic and/or diagnostic reasons (Table 2). Glucose or other sugar base ligands may be employed to target GLUT1 on glomerular mesangial cells for effective and targeted drug delivery in DN, resulting in improved bioavailability of several drugs (68, 98, 109, 139). A major trend that will require long-term efficacy and safety assessments, is the use of multifunctional targeted nanoparticles in DN diagnosis. Although the practicality of utilizing nanoparticles in kidney-targeted distribution has been established using excellent size-control and mesangial filtration, the nanoparticles would be excreted in urine quickly if they are not internalized swiftly by renal cells. Furthermore, most renal cells in DN patients, particularly renal tubular cells, exhibit cellular uptake dysfunctions of glucose, protein, and mineral salt, as well as nanoparticles, impeding improvements in the therapeutic efficacy of nanoparticles (146). Thus, efforts to overcome the challenges of inadequate cellular absorption by renal cells and fast urinary excretion of nanoparticles in DN treatment remain necessary.

Clinically, microbubbles are utilized as ultrasound contrast agents, and investigated as an ultrasound therapy mediator. To enhance the theranostic effect, microbubbles can be attached to the nanoparticles with the intention of targeting, permeation, imaging, and enhancing acoustic response under ultrasound waves. The particle size of microbubbles, like that of nanoparticles, and surface display of targeting ligands, are significant aspect for drug delivery to the kidney (64, 66). Researchers are still working on the microbubble therapy technology for drug delivery to the kidney as an emerging delivery mechanism.

Although biodegradable polymers like PLA are widely thought to be benign, their immunotoxicological potential should be carefully assessed as smaller sized particles tend to cause more damage to normal cells (147). Potential toxicological hazards of metallic nanoparticles also need to be carefully addressed before rampant clinical utilization. One of the most difficult problems in the clinical use of nano-biomedicine is how to deal with the biotoxicity of bio-nano drug-loaded particles and their breakdown products, which requires more investigation.

Poly(vinylpyrrolidone-co-dimethyl maleic acid) has been shown to have a high accumulation potential in the kidney and can be conjugated with superoxide dismutase for the treatment of renal illnesses. The molecular weight and charge of the copolymer effect its distribution. Copolymer with a molecular weight of 6-8 kDa accumulates in the kidneys; the stronger the negative charge, the longer it takes for it to be eliminated from the kidney (41). The copolymer poly(vinylpyrrolidone-co-dimethyl maleic anhydride) has been reported to be quickly removed from circulation, while accumulating in proximal tubular cells (148). For the treatment of renal disease the copolymer was conjugated with superoxide

TABLE 2 Targeting strategies of nanoformulations to renal cells.

S No.	Targeting types	Targeting mechanisms/strategies	Outcomes	References
1	Active	VCAM-1 and E-selectin specific antibodies	Selective delivery of siRNA to inflamed cells with amphiphile-modified liposomes	(62)
2	Active	Anti-VCAM-1 antibody	Targeted delivery to TNF-α activated podocytes	(63)
3	Passive	Ultrasound-mediated targeting	Directed delivery to target region	(64, 66)
4	Active	Kidney-targeted peptide	Kidney-specific distribution	(67)
5	Active	Interaction of glucose-ligand with GLUT1 receptor	Preferential renal distribution	(68)
6	Active	Megalin-mediated uptake of polymeric nanoparticles	Kidney-specific delivery of loaded cargo	(76, 80)
7	Active	Mannose or transferrin targeted delivery	Mesangium-specific delivery of siRNA	(78)
8	Passive	Brij-functionalized polymeric nanocarriers	Improved permeability	(87)
9	Passive	pH and glucose responsive delivery	Reduction of blood glucose to ameliorate DN	(88)
10	Passive	Size-dependent retention in mesangium	Amelioration of mesangial proliferative glomerulonephritis	(94)
11	Active	Peptide fragments of human serum albumin	Improved renal delivery of triplotide	(96)
12	Active	Neonatal Fc receptors targeted delivery	Specific absorption by podocytes	(98)
13	Passive	Photo-thermal nanoparticles	Improved distribution to mesangia0l cells	(99)
14	Passive and active	Anti-LOX-1, PEG-coated SPIONs	Detection, Characterization, and monitoring of early DN	(51)
15	Active	Anti-TLR4 and anti-AR antibody-tagged QDs	Fast, accurate detection	(55)

dismutase to suppress ROS production and the extracellular signal-regulated kinase (ERK) signaling pathway in DN (148). Poly-l-glutamic acid is a renal polymeric drug carrier that is selective for renal protective medicines with advantages like high drug-loading, non-immunogenicity, biodegradability, and biocompatibility. The selective absorption of fluorescence-tagged poly-l-glutamic acid of molecular weight 41 kDa by kidney epithelial cells was reported in a study (42). While the renal retention of higher molecular-weight polymers typically involves extravasation from the renal vasculature and deposition in the parenchymal tissues, the renal retention of polymers with a molecular weight lower than the renal filtration threshold involves active reuptake of polymers by the proximal tubules. Hence, there exists strong possibility of renal-targeted delivery of polymeric nanoparticles utilizing such copolymers. In addition, antibody and peptide ligands showing promise in renal targeting can further improve the site-specificity (16).

A randomized, placebo-controlled double-blind clinical trial evaluated efficacy of curcumin-loaded nanoformulations prepared using polysorbate 80 (149). Encapsulation of curcumin into nanoformulation enhanced its bioavailability, resulting in increased antioxidant activity of curcumin along with better therapeutic response against DM. The safety and tolerability of the nanoformulation was also ascertained (80 mg nanocurcumin for 8 weeks) hinting at possibility of commercialization. In another relevant trial, significant improvements were observed regarding DM parameters in patients, using curcumin nanomicelles at a dose of 80 mg/day for 3 months (150). The therapeutic effects found in this study are thought to be attributable to the improved bioavailability of curcumin when delivered as nano-micelles. Future studies should concentrate on the production processes and thorough clinical studies of nanoformulations leading to their quick availability for utilization. A multidisciplinary strategy combined with clinical and ethical considerations is needed to integrate nanotechnology into ordinary clinical practice. Additionally, the synergistic effect of combining flavonoid nanoformulations with conventional medication therapy for diabetes could be examined. However, successful clinical translation still lies in the womb of the future.

8 Conclusion

Because of the kidney's propensity to expel medications from the body, targeting the kidneys in DN is a huge challenge. Since DN therapy necessitates high renal drug concentrations, the development of concentration-dependent targeted drug delivery devices is required. The development of nanotheranostic platforms improves treatment efficacy and safety while also providing new precision therapy measures for patients with DN. This field of nanomedicine is quickly expanding, as indicated by the various nanoparticles that have already been produced. Nanomedicine-based techniques with specific biophysical properties could result in highly regulated nanocarriers for kidney targeting. Nanoparticles would be able to connect with renal cells for an extended period of time, improving retention and cellular uptake in numerous cell types. However, additional significant

efforts have to be initiated by the researchers to develop clinically effective targeted nanoformulations containing diagnostic and/or therapeutic moieties. Future research is anticipated to progressively concentrate on drug delivery systems based on nanoparticles, with the molecular targets of DN being essential for site-specific targeted delivery.

Author contributions

SD, RK, SJ and NJ contributed to the conceptualization of the manuscript. PP, LC, TD, VP, and UP designed the first draft of the manuscript. SJ, NJ, RK, and PC edited and corrected the manuscript. The final correction and editing were done by SD, PC, and RK. All figures were formulated by SD All authors contributed to the article and approved the submitted version.

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Conflict of interest

Author LC is employed by the company BioAnalytical Lab, Meso Scale Discovery.

The remaining 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

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Enhanced trimethylamine metabolism and gut dysbiosis in type 2 diabetes mellitus with microalbumin

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Background: Abnormal gut microbiota and blood trimethylamine-N-oxide (TMAO) metabolome have been reported in patients with type 2 diabetes mellitus (T2DM) and advanced diabetic nephropathy. This study aimed to investigate the gut microbiota profiles and a group of targeted urine metabolic characteristics in T2DM patients with or without microalbuminuria, to determine the correlation between the gut microbiota composition, trimethylamine (TMA) metabolism, and the clinical features during progression of diabetic kidney disease (DKD)

Methods: This study included 26 T2DM patients with microalbuminuria (Micro), 26 T2DM patients with normoalbuminuria (Normo), and 15 healthy controls (HC). Urine and Fecal samples were detected using ultra performance liquid chromatography tandem mass spectrometry and 16S ribosomal DNA gene sequencing, respectively.

Results: The TMAO/TMA ratio decreased gradually during the HC-Normo-Micro transition. The levels of TMA, choline and betaine were significantly different between the HC group and the T2DM patients belonging to both Normo and Micro groups. At the operational taxonomic unit (OTU) level, the gut microflora diversity was significantly reduced in the Micro groups compared to the HC groups and the Normo groups. Taxonomic analyses revealed significant consumption in the relative abundances of eight bacterial genera and significant enrichment of two bacterial genera during the HC-Normo-Micro transition. Furthermore, the relative abundances of six bacterial genera, namely, Ruminococcus_1, [Eubacterium]_ruminantium_group, Roseburia, Faecalibacterium, Fusicatenibacter and Coprococcus_3 exhibited significant differences, and were associated with elevated urinary albumin creatinine ratio (UACR), TMAO/TMA, TMA and its precursors in the Micro group compared with the other groups.

Conclusion: The imbalance of gut microbiota has occurred in patients with early-stage DKD, and the consumption of short-chain fatty acid-producing bacteria were associated with the accumulation of TMA and UACR.

KEYWORDS

type 2 diabetes mellitus with microalbuminuria, gut microbiota, trimethylamine, trimethylamine-n-oxide, urinary albumin creatinine ratio

1 Introduction

Diabetic kidney disease (DKD) is the most concerning microvascular complication of diabetes mellitus (DM) and a leading cause of end-stage renal disease (ESRD) and cardiovascular disease (CVD) (1, 2). Up to 40% of type 2 diabetes mellitus(T2DM) patients develop to DKD, and the all-cause mortality is significantly higher in DKD patients compared to DM patients without kidney disease (3). DKD patients demonstrate a higher risk of developing CVD compared to the risk of the disease progressing to ESKD (4). The early detection of DKD is limited, and most of the DKD patients are diagnosed at advanced stages III or IV, which is largely irreversible (5). Current approaches, including lifestyle changes, management of glycemic and blood pressure control, provide limited effects in reducing the incidence and progression of DKD. Therefore, identifying early causes of DKD and developing intervention strategies are of great significance.

The gut microbiota has gained increasing attention as a significant environmental factor in various chronic diseases, including obesity, DM, CVD, nonalcoholic fatty liver disease and chronic kidney disease (CKD) (6, 7). Recent research has revealed differences in the gut microbiota composition between DKD patients and healthy individuals (8, 9). However, most of these studies have focused on the mid to late stages of DKD, with limited investigations in the early stages.

Recent studies have reported that the gut microbiota-derived metabolites play a significant role in disease development by interacting with the host via multiple pathways. Trimethylamine (TMA) is a crucial gut microbiota-derived metabolite that is primarily converted to trimethylamine-N-oxide (TMAO) by the liver enzyme flavin-containing monooxygenase 3. Approximately 95% of TMAO is eliminated by the kidneys. Furthermore, accumulation of TMAO is associated with renal dysfunction. In CKD, elevated levels of TMAO are associated with renal insufficiency and increased risk of mortality (10, 11). Higher TMAO levels are associated with decreased glomerular filtration rate and impaired renal function. Gut dysbiosis aggravated renal dysfunction by increasing the levels of toxic metabolites in the blood (12). TMAO levels are elevated in cases of gut dysbiosis. Haluzik et al. (13) reported that alterations in the intestinal flora of patients with CKD resulted in an increased ability to metabolize choline and elevated transcription of choline monooxygenase and other genes, which increase the levels of TMAO; furthermore, elevated TMAO levels were associated with poorer prognosis of patients with DKD. Higher TMAO levels are associated with increased inflammation (14), abnormal renal function, and renal fibrosis. TMAO promoted renal interstitial fibrosis through the TGF-β/Smad3 signaling pathway. Moreover, TMAO levels and the TMA/TMAO ratio are independent risk factors of DKD (15). T2DM patients with advanced CKD showed increased abundance of TMA-producing bacteria and elevated serum TMAO levels (16). However, very few studies have investigated the roles of TMA and TMAO in early-stage DKD.

In this study, we measured the levels of urine TMAO and its precursors in the T2DM patients with microalbuminuria (Micro). Furthermore, we analyzed the differences in the gut microbiota composition between healthy individual and T2DM patients with Micro to determine the relationship between gut microbiota composition, TMAO levels, and clinical features associated with early-stage DKD.

2 Methods

2.1 Research participants and sample collection

A total of 67 participants, including 15 healthy controls (HC) and 52 patients with type 2 diabetes mellitus (T2DM), were recruited from the First Affiliated Hospital of Huzhou University. The diagnosis of T2DM was based on specific criteria, including fasting plasma glucose (FBG) ≥ 7.0 mmol/L, two-hour plasma glucose level of oral glucose tolerance test (OGTT) ≥ 11.1 mmol/L, glycosylated hemoglobin (HbA1c%) ≥ 6.5%, and estimated glomerular filtration rate (eGFR) ≥ 60 mL/min/1.73 m^2. T2DM patients were then further divided into two groups based on the random urinary albumin creatinine ratio (UACR) levels: patients with normoalbuminuria (n=26; Normo; UACR ≤ 30 mg/g) and patients with Micro (n=26; 30 mg/g < UACR ≤ 300 mg/g). The patients group were recruited consecutively from the Endocrinology Department, while age- and gender-matched healthy individuals were recruited from the Health Examination Center. All participants belonged to the Huzhou Han nationality. They were from similar geographic areas and had similar eating habits.

Exclusion criteria included pregnant diabetes patients, patients with type 1 diabetes or diabetes ketoacidosis, patients with other specific types of diabetes, non-diabetic kidney disease, intestinal diseases, usage of antibiotics within the past month, acute/chronic

infections, patients with malignant tumors, and any mental state that would restrict the subject from consenting to the study.

The following data was extracted from the hospital's public clinical database for the included patients: (1) clinical data, including age, gender, and history of diabetes; (2) laboratory indices, including FBG, insulin (INS), C-peptide (C-p), HbA1c%, total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), hemoglobin (Hb), serum creatinine (Scr), blood urea nitrogen (BUN), eGFR (CKD-EPI, ml/min/1.73m2), albumin (ALB), Urine RBC count, UACR. The HC group also underwent various tests, including blood analysis, urine analysis, hepatitis B surface antigen (HBsAg), anti-hepatitis C antibody (HCV), liver and kidney function, and ultrasound Doppler examination. The results of these tests were found to be within the normal range.

Fasting urine and fecal samples from all participants were collected in the morning, stored in ice bags, transferred within 2 hours to the laboratory and frozen at -80°C until further analysis. It is important to note that all subjects followed an omnivorous diet, and none of them reported having any special eating habits.

2.2 Quantification of the urine TMAO, TMA, and the precursors concentrations

The concentrations of TMAO, TMA, betaine, choline, L-carnitine and creatinine (Cr) in the urine samples were quantified using stable isotope dilution Ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS, SCIEX 6500 QTRAP+ triple quadrupole mass spectrometer). Briefly, 40 μL of 0.1% formic acid aqueous solution was added to 10 μL sample. Subsequently, 200 μL of extraction solution (0.1% formic acid acetonitrile containing isotopically-labeled internal standard mixture, including TMAO-d9, TMA-d9, betaine-d3, choline-d9, L-carnitine-d9, and Cr-d3 at 10 mmol/L) that had been precooled at -20°C was added to the above mentioned urine-formic acid mixture. The mixture was vortexed, sonicated in an ice-water bath for 15 minutes, and then incubated at -40°C for 1 hour. After centrifugation, an 80 μL supernatant was transferred to an auto-sampler vial, and 1 µL supernatant was injected onto a Waters ACQUITY BEH Amide column (100 × 2.1 mm, 1.7 µm, Waters) at a flow rate of 0.5 mL/min using 77% solvent A (10 mmol/L ammonium formate and 1% formic acid in water) and 23% solvent B (1% formic acid in acetonitrile). Mass spectrometry analysis was conducted in positive multiple reaction monitoring (MRM) mode. The acquired MRM data were processed using SCIEX Analyst Work Station Software (version 1.6.3) and Sciex MultiQuant™ Software (version 3.0.3).

2.3 Fecal DNA extraction and 16S ribosomal DNA gene sequencing

Genomic DNA was extracted from fecal samples using the PowerFecal® DNA Isolation Kit (MoBio) according to the manufacturer's protocol. The quantity of genomic DNA was determined using a TBS-380 fluorometer (Turner BioSystems Inc., Sunnyvale, CA), and high-quality DNA (OD260/280 = 1.8~2.0, >1 µg) was used for further analysis.

The V3-V4 variable regions of the bacterial 16S ribosomal DNA gene (16S rDNA) were amplified by polymerase chain reaction (PCR) using the barcoded primers 338F (ACTCCTACGGGAGGCAGCA) and 806R (GGACTACHVGGGTWTCTAAT). The PCR products were extracted, purified, and sequenced with a high throughput sequencer using the MiSeq platform (Illumina Miseq PE300, USA) according to the manufacturer's protocol. The above procedures were completed by Shanghai Majorbio Technology Co., Ltd., Shanghai, China.

2.4 Statistical analyses

2.4.1 Descriptive analyses and metabolome analyses

Statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). The data were presented as either mean ± standard deviation (SD) or median (quartile) for continuous variables, and as absolute values and percentages (%) for categorical variables. Independent t-tests or analysis of variance (ANOVA) were used to compare continuous variables among groups, depending on the normal or non-normal distribution characteristics, respectively. Mann-Whitney U test was employed for analyzing the non-normally distributed data. Categorical variables were evaluated using the chi-squared test. Two-tailed P <0.05 was considered statistically significant.

2.4.2 Microbiome analyses

All statistical analyses were conducted using the R software (version 3.6.3). Alpha-diversity (community richness indices: sobs, chao, ace, and community indices: shannon, simpson, bergerparker) and beta-diversity (Bray-Curtis dissimilarity) indices were generated using the vegdist and diversity functions in the vegan R package. One-way ANOVA was used to evaluate the differences in alpha-diversity between groups. Permutational analysis of variance (PERMANOVA) for the Bray-Curtis dissimilarity, implemented in the adonis R function (17), was performed to evaluate the multidimensional centroid differences in beta-diversity among groups. The Kruskal-Wallis H test was employed to evaluate the differential abundance of the genera. Differences in the relative abundances of microbial features were determined using linear discriminant analysis (LDA) effect size (LEfSe). LEfSe and the non-parametric Kruskal-Wallis (KW) sum-rank test were used to analyze the differences in the relative abundance between groups from the phylum to the genus level, and to identify significantly different species. Subsequently, LDA was used to obtain species with a score greater than 2.5. The correlation between biochemical indicators, metabolites, and various microorganisms was calculated using Spearman's rank correlation coefficient. The results were visualized using the "pheatmap" software package through a heatmap in R.

3 Results

3.1 Clinical characteristics of all participants

After quality control, a total of 67 participants were included in this study. The key clinical characteristics at the time of sample collection are presented in Table 1 and Figure 1. Compared to the HC, patients with T2DM (both Normo and Micro) showed significantly increased levels of FBG, Scr, and BUN, as well as significantly reduced levels of eGFR(CKD-EPI, ml/min/1.73m^2). The UACR of T2DM patients with Micro were significantly different from the T2DM patients with Normo, but the other clinical and laboratory indicators between the 2 groups did not show significant differences. There were no significant differences in age and gender among the three participant groups.

3.2 Concentrations of TMAO, TMA, and the precursor metabolites in all participants

The concentrations of six urine metabolites, namely TAM, TMAO, choline, betaine, L-carnitine and Cr, were quantified using Stable Nuclide Dilution UPLC-MS/MS. After adjusting for baseline Cr levels, the relative levels of the methylamine metabolites in urine samples from the three groups were estimated and shown in Table 2 and Figure 2. Compared to the HC, Normo and Micro patients exhibited significantly higher levels of TAM, choline and betaine in urine, along with significantly lower TMAO/TMA ratios. Additionally, TMAO levels were slightly increased in the Normo group and slightly decreased in the Micro group compared with the HC group, but the differences between the 3 groups were not statistically significant. The data showed significant differences in TMAO/TMA, TMA and its precursor metabolites among the three groups ($p < 0.001$).

3.3 Gut microbiota diversity among healthy controls, T2DM with normoalbuminuria, and T2DM with microalbuminuria

Gut microbiome analysis was conducted on 67 samples from the HC, Normo, and Micro groups. A total of 3,738,982 high-quality 16S rDNA reads were obtained, with a median reading count of 54,394 (ranging from 40,805 to 73,942). Subsequently, clustering analysis of sequences with a similarity greater than 97%, 1 domain, 1 kingdom, 11 phyla, 17 classes, 24 orders, 49 families, 177 genera, 350 species, and 523 operational taxonomic units (OTUs) were obtained.

To evaluate the α diversity of the gut microflora between the 3 groups, various indices including Sobs, Chao, ACE (community

TABLE 1 Key clinical characteristics and laboratory data of the participants.

	HC (n=15)	Normo (n=26)	Micro (n=26)	*P*
Age (yr)	48.73 ± 7.04	53.65 ± 9.22	52.92 ± 9.19	0.1245
Male (%)	8(53.33%)	15(57.69%)	16(61.54%)	0.2679
Diabetes duration(yr)	0.00 ± 0.00	6.89 ± 5.70	8.28 ± 6.3	<0.001
FBG (mmol/L)	4.88 ± 0.42	9.86 ± 3.15[a]	11.28 ± 4.19[b]	<0.001
INS (uU/ml)	NA	6.55(4.85,12.53)	8.9(6.55,15.58)	0.2003
C-p (ng/ml)	NA	1.48 ± 0.69	1.78 ± 0.73	0.1420
HbA1c(%)	NA	9.20 ± 2.58	9.59 ± 2.23	0.5637
TC (mmol/L)	4.77 ± 0.68	4.52 ± 0.75	5.02 ± 0.91	0.0875
TG (mmol/L)	1.21 ± 0.41	1.39 ± 0.46	1.63 ± 0.52[b]	0.0210
LDL-C (mmol/L)	2.57 ± 0.5	2.39 ± 0.63	2.53 ± 0.70	0.6404
HDL-C (mmol/L)	1.39 ± 0.32	1.23 ± 0.28	1.15 ± 0.25[b]	0.0343
Hb (g/L)	142.9 ± 16.07	142.7 ± 15.31	146.4 ± 18.32	0.6876
Scr (μmol/L)	69.2 ± 12.68	82.42 ± 11.61[a]	86.12 ± 14.35[b]	<0.001
BUN(mmol/L)	4.49(3.78,5.41)	5.76(5.07,6.49)[a]	5.72(5.11,6.52)[b]	0.0019
eGFR	103(92,114.4)	81(73.25,97.5)[a]	76(69,94.25)[b]	<0.001
ALB (g/L)	44.2(43.4,46.3)	44.1(41.08,49.4)	47.15(45.93,50.28)	0.0711
Urine RBC count (n/ul)	0(0,0)	0(0,0)	0(0,0.35)	0.7345
UACR (mg/g)	NA	15.84(7.04,21.03)	40.92(34.51,104.1)[c]	<0.001

Categorical variables are expressed as n (%); continuous variables are expressed as median (interquartile range). HC, Healthy Controls; Normo, T2DM patients with normoalbuminuria; Micro, T2DM patients with microalbuminuria. [a]P <0.05: HC versus Normo; [b]P <0.05: HC versus Microa; [c]P <0.05: Normo versus Micro.

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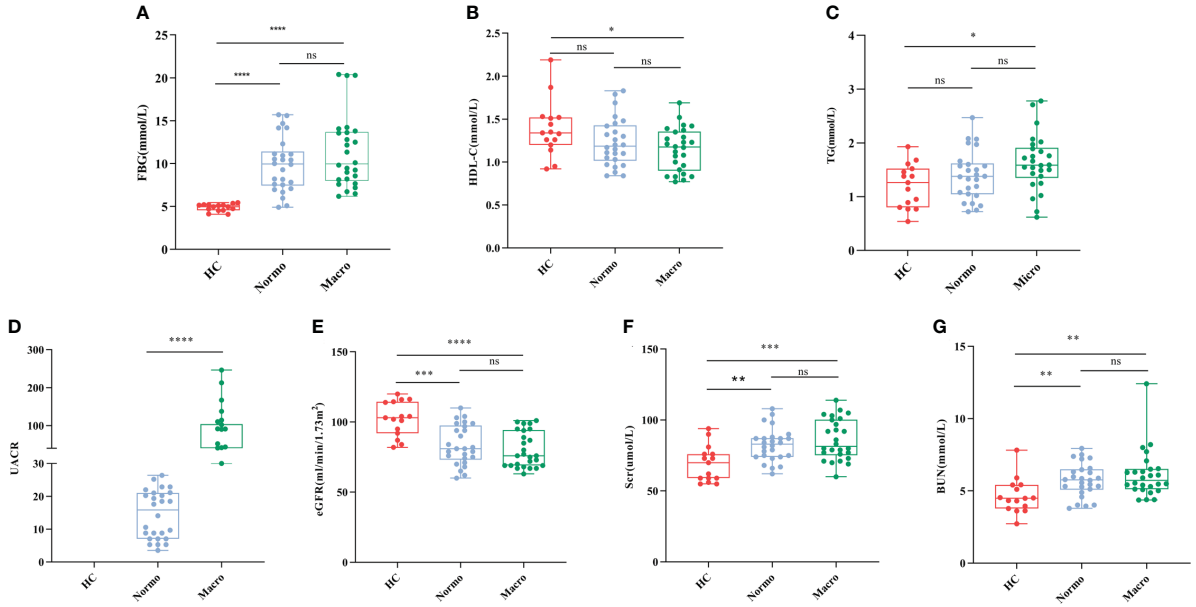

FIGURE 1
Key biochemical indicators between the three groups. **(A)** FBG, **(B)** HDL-C, **(C)** TG, **(D)** UACR, **(E)** eGFR, **(F)** Scr, and **(G)** BUN. *P <0.05; **P <0.01; ***P <0.001; ****P <0.0001. HC, Healthy Controls; Normo, T2DM patients with normoalbuminuria; Micro, T2DM patients with microalbuminuria.

richness), Shannon, Simpson, and Berger-Parker (community diversity) were calculated based on the OTU profiles. The α diversity results (Figure 3) demonstrated that Sobs, Chao, and ACE indices were significant reduced in the Micro group compared with the HC group and Normo group. However, there were no singnificant diffrerences in Simpson, Shannon, and Berger-Parker indices in the 3 groups. These findings suggested that the gut microflora richness was decreased significantly in the Micro group compared to the HC and Normo groups, while the diversity remained unchanged. No statistical difference in α diversity was observed between the HC and Normo groups.

Principal Coordinate Analysis (PCoA) based on Jaccard dissimilarity and Bray-Curtis dissimilarity was performed to assess the β diversity and identify significant differences in microbial community structure. Figure 3G showed the separation of microbiota composition at the OTU level among patients with HC, Normo, and Micro (PERMANOVA, p = 0.004). Furthermore, partial least squares discriminant analysis (PLS-DA, Figure 3H) demonstrated clear distinction and clustering of HC, Normo and Micro samples, indicating a significant differences in intestinal flora composition.

3.4 Gut microbiota composition among healthy controls, T2DM with normoalbuminuria, and T2DM with microalbuminuria

Bacteria with relative abundances greater than 1% at the phylum and genus levels were compared between the HC, Normo, and Micro groups. At the phylum level (Figure 4A), Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria and Fusobacteria accounted for 99% of the bacteria. Firmicutes was the dominant phylum, representing over 50% in each group. At the genus level (Figure 4B), among the bacteria with relative abundances greater than 3%, Bacteroides, Faecalibacterium,

TABLE 2 Concentrations of TAM, TMAO, choline, betaine, and L-carnitine in the participants.

mmol/mol Cr in Urine	HC (n=15)	Normo (n=26)	Micro (n=26)	*P*
TMA	1.68(1.33,1.83)	6.75(4.27,8.08)[a]	7.23(4.31,15.52)[b]	<0.001
TMAO	53.64(23.69,78.35)	63.53(39.97,106)	46.48(34.59,66.73)	0.1665
TMAO/TMA(mol/mol)	24.61(16.76,37.39)	12.12(7.24,18.48)[a]	7.39(2.57,12.28)[b,c]	<0.001
Choline	3.19(2.79,4.6)	8.72(5.96,14.33)[a]	13.18(7.84,21.91)[b]	<0.001
Betaine	9.13(7.29,13.25)	35.61(17.23,74.5)[a]	50.11(22.62,82.64)[b]	<0.001
L-Carnitine	4.95(2.9,12)	3.95(1.51,14.41)	4.46(1.26,14.24)	0.9204

HC, Healthy Controls; Normo, T2DM patients with normoalbuminuria; Micro, T2DM patients with microalbuminuria. [a]P <0.05: HC versus Normo; [b]P <0.05: HC versus Microa; [c]P <0.05: Normo versus Micro.

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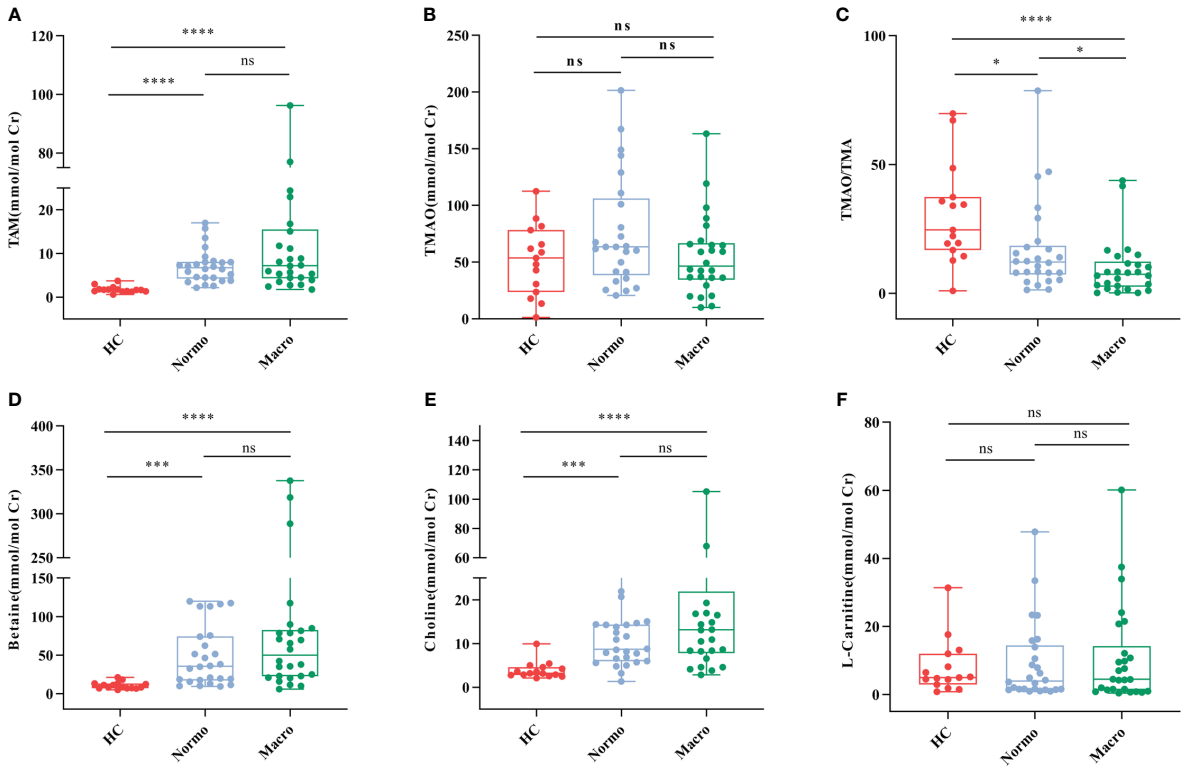

FIGURE 2
Urine metabolic indicators in the three groups. **(A)** TMA, **(B)** TMAO, **(C)** TMAO/TMA, **(D)** Betaine, **(E)** Choline, and **(F)** L-Carnitine. *P <0.05; ***P <0.001; ****P <0.0001. HC, Healthy Controls; Normo, T2DM patients with normoalbuminuria; Micro, T2DM patients with microalbuminuria.

Prevotella_9, Megamonas and Blautia were common to allthe three groups. The HC group additionally included [Eubacterium]_rectale_group, Lactobacillus, Klebsiella and Roseburia; the Normo group included Escherichia-Shigella and Dialister; the Micro group featured Escherichia-Shigella and Bifidobacterium.

LDA-LEfSe analysis was performed to determine significant differences in the bacterial abundances between the 3 groups and identify the bacterial genera that are associated with the Micro group. Based on the LDA score threshold of above 2.5, a total of 24 distinguishing taxa with differential abundances were labeled between the three groups (Figure 4C), ranging from phylum to genus level. At the genus level, Faecalibacterium, Roseburia, [Eubacterium]_ruminantium_group, Lachnoclostridium, [Eubacterium]_eligens_group, Ruminococcus_1, Fusicatenibacter, [Eubacterium]_ventriosum_group, Coprococcus_3 and Lachnospiraceae_UCG-010 were significantly enriched in the HC group. [Eubacterium]_hallii_group, Rothia, Adlercreutzia, Ruminococcaceae_UCG-013 and Peptostreptococcus were significantly higher in the Normo group. The Micro group showed an overrepresentation of [Ruminococcus]_gnavus_group, Lachnospira, Prevotella_6, [Clostridium]_innocuum_group and Ruminococcaceae_UCG-009. The Kruskal-Wallis H test was then used to analyze the top 15 genera with the most significant differences in average abundance between the three groups (Figure 4D). The results revealed decreased abundance of eight bacterial genera (Faecalibacterium, Roseburia, Fusicatenibacter, [Eubacterium]_ruminantium_group, Lachnospiraceae_NK4A136_group, Ruminococcus_1, [Eubacterium]_ventriosum_group and Coprococcus_3) and increased abundance of two bacterial genera ([Ruminococcus]_gnavus_group and Prevotella_7) during the HC-Normo-Micro transition. This suggested the potential relevance of these 10 bacterial genera in the development and progression of T2DM.

3.5 Correlation analysis of gut microbiota, clinical characteristics, and metabolites in healthy controls, T2DM with normoalbuminuria, and T2DM with microalbuminuria

Spearman correlation analysis was performed to assess the relationships between the various differentially bacterial genera (P<0.1), clinical indices and metabolite levels among the three groups (Figure 5). Initially, the metabolites associated with renal function were analyzed. TMA, choline and betaine showed positive correlation with UACR and FBG, while TMAO/TMA ratio showed an inverse relationship. TMA also displayed a positive correlation with Scr (Figure 5A). Next, the differentially bacteria associated with renal function were examined, UACR showed a strong negative correlation with [Eubacterium]_eligens_group, Fusicatenibacter, Faecalibacterium, Lachnospiraceae_NC2004_group, and Coprococcus_3, and a positive correlation with Prevotella_6. Peptostreptococcus demonstrated a strong positive correlation with

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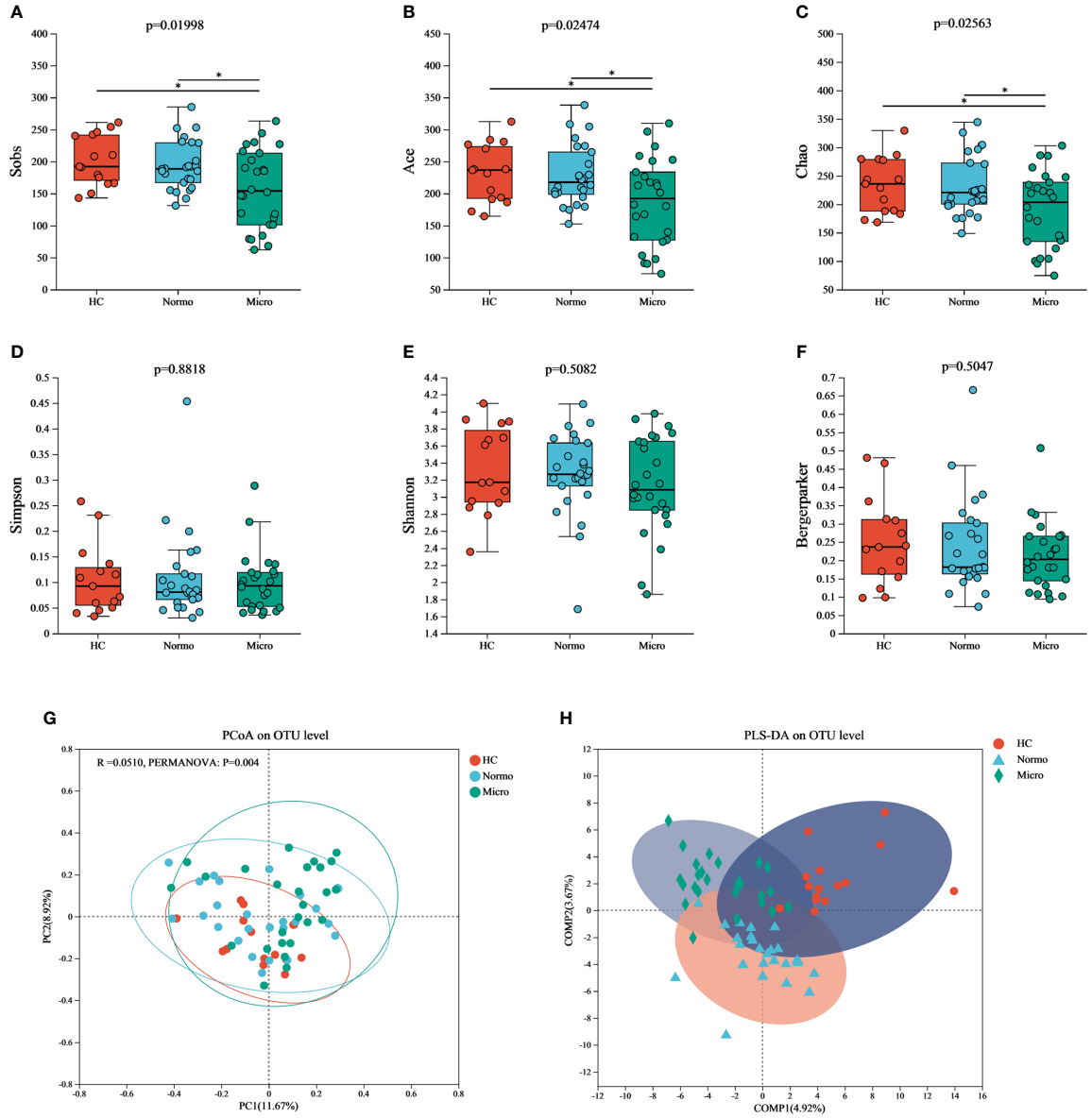

FIGURE 3
α and β diversity of gut microflora among three groups at OTU level. Community richness indices: **(A)** Sobs, **(B)** Ace, and **(C)** Chao; Community diversity indices: **(D)** Simpson, **(E)** Shannon, and **(F)** Bergermaker; β diversity indices: **(G)** PCoA, and **(H)** PLS-DA. *P <0.05; HC, Healthy Controls; Normo, T2DM patients with normoalbuminuria; Micro, T2DM patients with microalbuminuria.

Scr (Figure 5B). Additionally, the correlation between the metabolites and the differentially bacteria was analyzed. TMA and choline levels showed significant negative correlations with 9 bacterial genera ([Eubacterium]_eligens_group, Roseburia, Lachnospira, Lachnospiraceae_NC2004_group, Lachnospiraceae_UCG-010, Ruminococcus_1, [Eubacterium]_ruminantium_group, Lachnospiraceae_UCG-001 and Paraprevotella) and a significant positive correlation with Rothia. The TMAO/TMA ratio demonstrated a strong positive correlation with Roseburia, [Eubacterium]_eligens_group, Lachnospiraceae_NC2004_group, Lachnospira, [Eubacterium_ruminantium_group, Lachnospiraceae_UCG-010 and Coprococcus_3. Betaine showed a strong negative correlation with [Eubacterium]_eligens_group, Lachnospiraceae_NC2004_group, and Faecalibacterium (Figure 5C).

Through the correlation analysis, it was observed that Ruminococcus_1, [Eubacterium]_ruminantium_group, Lachnospiraceae_NC2004_group, Roseburia, [Eubacterium]_eligens_group, Lachnoclostridum, Moryella, Faecalibacterium and Fusicatenibacter were not only significantly correlated with TMA but also exhibited significant correlations with UACR. Furthermore, Coprococcus_3, Ruminococcus_1, [Eubacterium]_ruminantium_group, Lachnospiraceae_NC2004_group, Roseburia and [Eubacterium]_eligens_group showed a positive correlation with TMAO/TMA ratio and a negative correlation with UACR. Lachnospiraceae_NC2004_group, Roseburia, Ruminococcus_1, [Eubacterium]_eligens_group and Faecalibacterium demonstrated significant correlations with betaine and UACR. Based on the findings from Figures 2 and 4,

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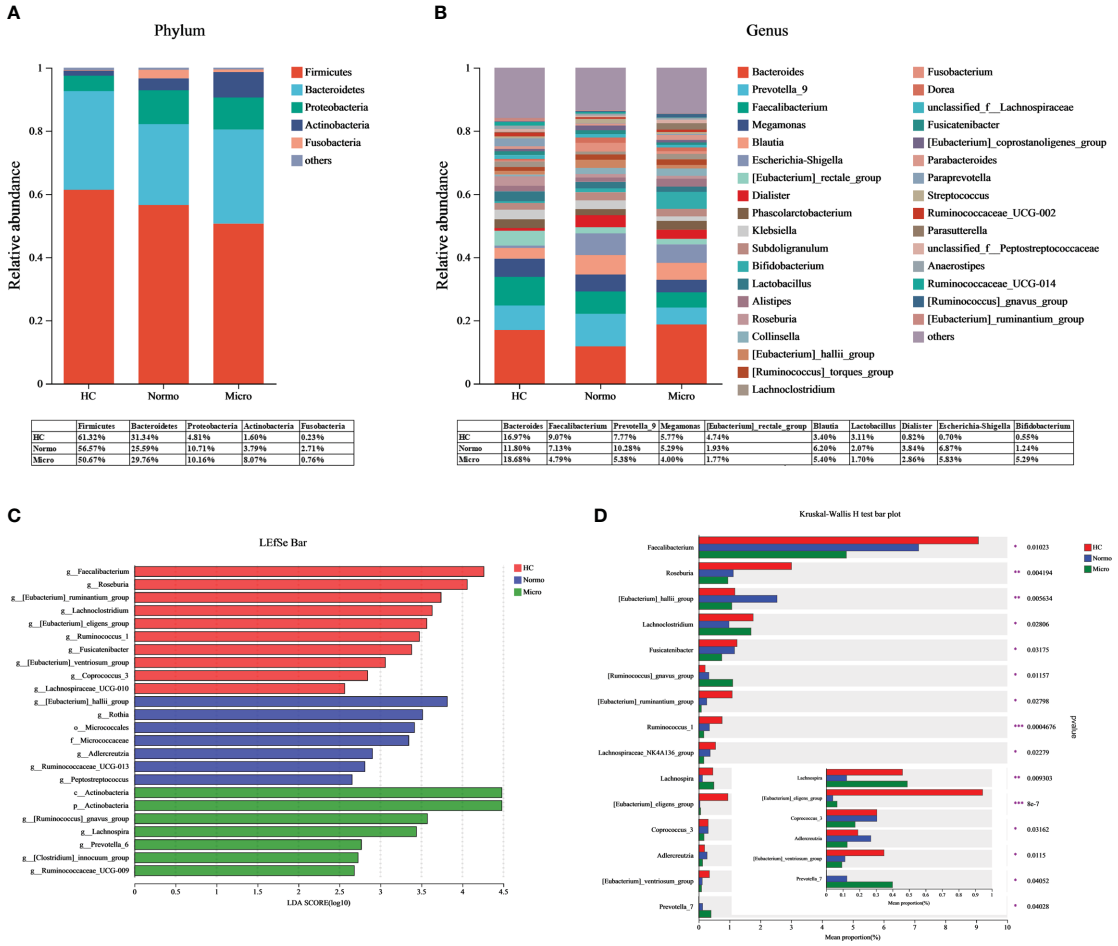

	Firmicutes	Bacteroidetes	Proteobacteria	Actinobacteria	Fusobacteria
HC	61.32%	31.34%	4.81%	1.60%	0.23%
Normo	56.57%	25.59%	10.71%	3.79%	2.71%
Micro	50.67%	29.76%	10.16%	8.07%	0.76%

	Bacteroides	Faecalibacterium	Prevotella_9	Megamonas	[Eubacterium]_rectale_group	Blautia	Lactobacillus	Dialister	Escherichia-Shigella	Bifidobacterium
HC	16.97%	9.07%	7.77%	5.77%	4.74%	3.40%	3.11%	0.82%	0.70%	0.55%
Normo	11.80%	7.13%	10.28%	5.29%	1.93%	6.20%	2.07%	3.84%	6.87%	1.24%
Micro	18.68%	4.79%	5.38%	4.00%	1.77%	5.40%	1.70%	2.86%	5.83%	5.29%

FIGURE 4
Gut microbiota composition between HC, Normo and Micro. **(A)** Gut microbiota composition at the phylum level. **(B)** Gut microbiota composition at the genus level. Bacteria that took up <1% of the microbiota were labeled together as "others". **(C)** Bacterial taxa differences among the three groups using LEfSe analysis, Only taxa meeting the LDA significance thresholds > 2.5 were shown. **(D)** Top 15 differentially bacterial genera in the 3 groups. The comparison of the mean abundance for the bacterial genera between the three groups was based on Kruskal-Wallis H test. *P <0.05, **P <0.01, ***P <0.001; HC, Healthy Controls; Normo, T2DM patients with normoalbuminuria; Micro, T2DM patients with microalbuminuria.

we performed detailed analysis of the differences between the 3 groups and observed that the relative abundances of six bacterial genera, including [Eubacterium]_ruminantium_group, Ruminococcus_1, Roseburia, Faecalibacterium, Fusicatenibacter and Coprococcus_3 were significantly different among the 3 groups (Figure 5D).

4 Discussion

DKD is a severe microvascular complication of diabetes influenced by various factors, including blood pressure, hyperglycemia, body mass index (BMI), and others. Recent studies have shown that gut dysbiosis is associated with T2DM. TMAO is the independent risk factor of DKD. Therefore, in this study, we analyzed urine metabolites, and the composition of the gut microbiota to determine whether the relationship between gut dysbiosis and the early renal complications in patients with T2DM. The results demonstrated dysbiosis of the gut microbiota and increased levels of TMA and its precursors in the urine of T2DM patients with Micro compared to HC and T2DM patients with Normo. Furthermore, six bacterial genera that showed significant differences in the relative abundances between the three groups, and these differentially bacteria were found to be associated with elevated UACR and metabolic levels of TMAO/TMA, TMA, and its precursors.

Previous studies have reported that TMAO, a gut microbiota metabolite, is a risk factor for CVD and stroke (18, 19) and is also linked with CKD progression and all-cause mortality (20). However, our research showed no significant differences in urine TMAO levels between the three groups. This suggested that urine TMAO levels remain relatively stable during the early stages of DKD and are consistent with the findings by Caroline C. Pelletier et al. (21). Further investigation is needed to determine the predictive value of plasma or urinary TMAO levels for early DKD. Interestingly, similar to the previous studies, the levels of TMA and its precursors (choline and betaine) increased sequentially among the three groups, while TMAO/TMA ratio

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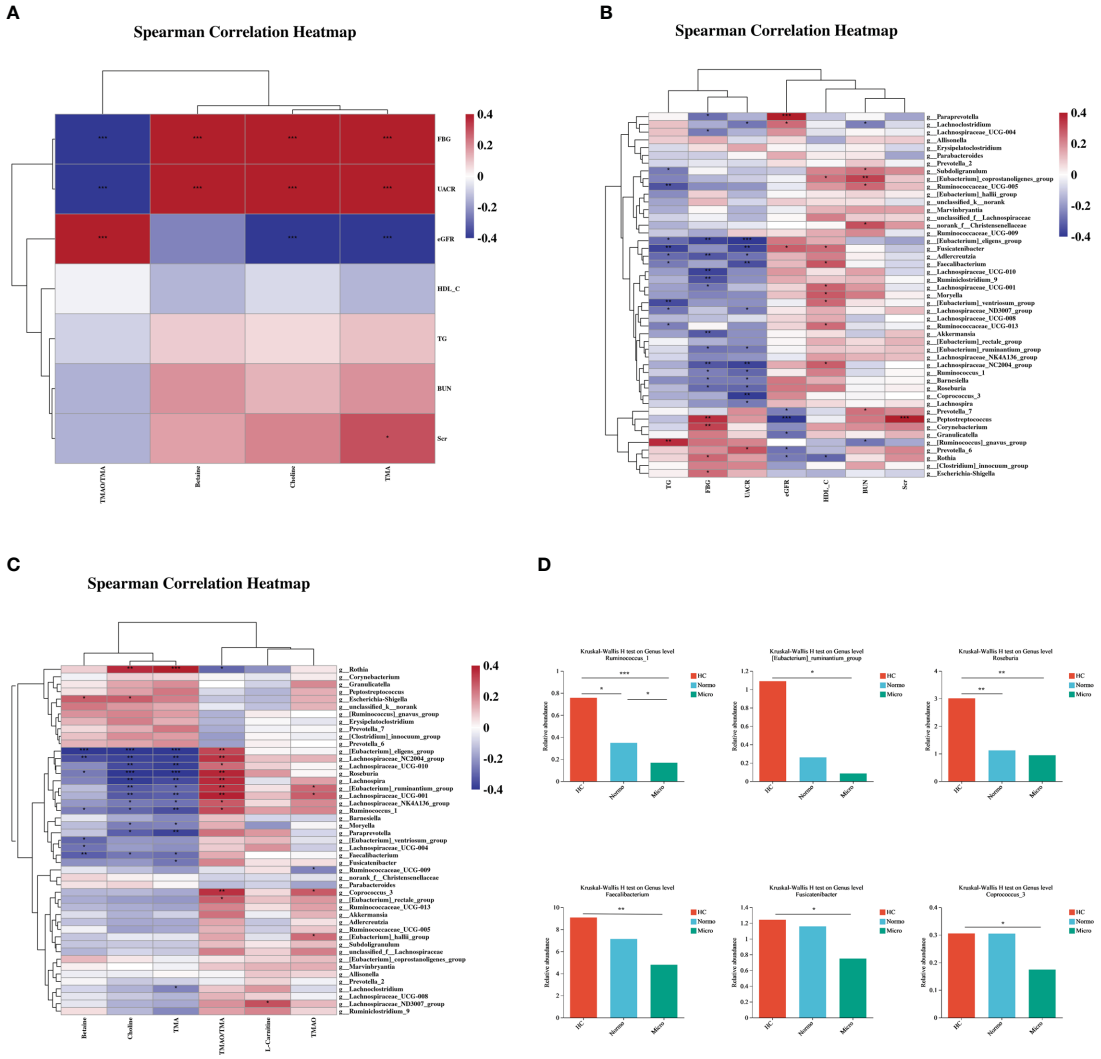

FIGURE 5
Correlations of gut microbiota composition, clinical characteristics, and metabolites between the three groups. **(A)** Correlation of differentially gut microbiota and differential clinical features beween the three groups. **(B)** Correlation of differential metabolites and differential clinical features between the three groups. **(C)** Correlation of differential metabolites and differentially gut microbiota between the three groups. **(D)** Key bacteria associated with clinical features or metabolism in the three groups. *P <0.05, **P <0.01, ***P <0.001; HC, Healthy Controls; Normo, type 2 diabetes patients with normoalbuminuria; Micro, type 2 diabetes patients with microalbuminuria.

decreased sequentially, and these changes were associated with changes in the levels of the renal function biomarkers (22). TMA has long been recognized as a uremic toxin (23), and its accumulation is associated with the increased risk of CVD (18). Therefore, regular monitoring of the TMA levels in the early stage of DKD may be warranted.

T2DM patients with Micro exhibited significantly reduced bacterial richness. These findings were in alignment with a recent systematic review and meta-analysis that reported gut microbiota dysbiosis in DKD (24). Therefore, these data demonstrated the occurrence of gut dysbiosis in the early stages of DKD. Short-chain fatty acid (SCFA), including Eubacterium, are key bacterial metabolites that can influence various physiological and pathological processes such as energy metabolism, blood sugar control, intestinal immunity, and so on (25). Butyrate can improve angiotensin II-mediated renal injury by affecting urinary protein production, glomerulosclerosis, renal fibrosis, and inflammation (26). Faecalibacterium, Fusicatenibacter, Lachnospiraceae_NK4A136_group, Roseburia, Ruminococcus_1 and Coprococcus_3 are beneficial butyrate-producing bacteria, which have been reported to be depleted in patients with DKD, IgA nephropathy, obesity, cardiovascular and other diseases (27–30). The [Eubacterium]_ventriosum_group was noticeably decreased in the gut of the T2DM rat model and was negatively associated with the expression of T2DM-related biomarkers (31). [Ruminococcus]_gnavus_group as a harmful bacteriumis, enriched in individuals with prediabetes and insulin resistance (32). Prevotella is generally associated with a healthy plant-based diet and acts as a “probiotic” in the human body. However, recent human research has demonstrated that increased abundance of Prevotella is associated with local and systemic diseases, including periodontal disease, rheumatoid arthritis, and metabolic disorders

(33, 34). In summary, the research results indicate that dysbiosis of the gut microbiome in the early stage of DKD are characterized by depletion of SCFA-producing bacteria and enrichment of harmful bacteria.

In addition, our findings indicate a strong correlation between SCFA-producing bacterial genera and Eubacterium with the levels of microbiota-derived nephrotoxins, such as TMA and TMAO/TMA ratio in urine. This suggested that SCFA-producing genera and Eubacterium may play a crucial role in suppressing the synthesis of TMA. Previous studies have found that in CKD, two SCFA-producing genera (Pseudobutyrivibrio and Dialister) were inversely correlated with the levels of circulating indoxy sulfonate (35). Furthermore, treatment of CKD mice with Faecalibacterium praussnitzii ameliorated renal dysfunction by attenuating renal inflammation, increasing butyrate levels, and markedly reducing the circulating levels of uremic toxins such as p-cresol sulfate, TMAO, and guanosuccinate (36). Colonization of TMA-producing bacteria alone did not increase TMA levels in the cecum or TMAO leves in the serum. However, when TMA-producing bacteria were colonized along with other intestinal bacterial strains, a significant increase in the relative abundance of TMA producing bacteria in the small gut and the level of TMAO in the serum was observed (37). These findings suggested that the interactions between gut bacteria, particularly SCFA-producing genera and Eubacterium, may influence the production of TMA. However, additional research is required to gain a deeper understanding of the specific mechanisms by which the SCFA-producing genera and Eubacterium influence TMA production.

4.1 Limitations

It is important to acknowledge several limitations of this study. Firstly, the sample size in this study was small. Therefore, larger multicenter studies are required to elucidate the true relationship between gut dysbiosis and the development of DKD. Nonetheless, all participants in this study were residents of Huzhou City, Zhejiang Province, and showed relatively centralized and consistent characteristics and living habits. Secondly, the relative abundances of gut microbiota were based on 16S rRNA gene sequencing. Further analysis based on gut metagenomes is needed to provide more bacterial information and functional genes. Thirdly, the cross-sectional design of our study limits any conclusions about the directionality or causality of the identified microbiota and the metabolite features.

5 Conclusions

In conclusion, the increased TMA metabolism was associated with the imbalance of gut microbiota during the inital stage of DKD. This research provides relevant information that can be used to develop early prevention strategies for DKD. These findings demonstrated the importance of the communication between the gut microbiota and kidney function for the management of DKD. However, further clinical and animal experiments are necessary for confirming the relationship between rebalancing the gut microbiota composition and the levels of the microbial metabolites, including SCFAs and uremia toxins such as TMA.

Data availability statement

The data presented in the study are deposited in the NCBI repository, accession number PRJNA1000711.

Ethics statement

The studies involving humans were reviewed and approved by the Ethics Committee of the Medical Center of the First Affiliated Hospital of Huzhou University. The ethical approval code was 2018KY043. The participants provided their written informed consent to participate in this study. The studies were conducted in accordance with the local legislation and institutional requirements.

Author contributions

LH: Funding acquisition, Methodology, Software, Writing – original draft, Writing – review & editing. HL: Software, Writing – original draft, Writing – review & editing. MZ: Funding acquisition, Investigation, Writing – review & editing. YL: Methodology, Writing – review & editing. LR: Data curation, Funding acquisition, Investigation, Writing – review & editing. JH: Data curation, Investigation, Writing – review & editing. XW: Funding acquisition, Investigation, Project administration, Writing – review & editing.

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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

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Serum VEGF as a predictive marker of glycemic control and diabetic nephropathy in Chinese older adults with type 2 diabetes mellitus

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Objectives: Recent researches have demonstrated good correlation between vascular endothelial growth factor (VEGF) and diabetic nephropathy (DN); however, this relationship seems less clear-cut when VEGF was measured in blood samples. We tended to explore the possible association between serum VEGF and glycemic control and diabetic nephropathy severity in Chinese older adults with type 2 diabetes mellitus (T2DM).

Materials and methods: This study retrospectively enrolled 595 older T2DM adults at random. Participants were clinically grouped across the urine albumin-to-creatinine ratio (UACR) and the HbA1c tertiles by genders. Linear regressions were performed for the correlation of VEGF with HbA1c and UACR and binary logistic regressions for the odds of DN after adjusting for confounders. The receiver operating characteristic (ROC) curves were conducted for the predictive value of VEGF for DN.

Results: Both males and females with DN exhibited higher VEGF levels than non-DN ($P < 0.001$). Furthermore, a positive correlation of VEGF with UACR and HbA1c was presented regardless of adjusting confounding factors ($P < 0.001$). Serum VEGF level and fasting plasma glucose (FPG) were independent risk factors of DN in older adults of both genders ($P < 0.05$), while the risk prediction of DN by HbA1c only reflected in female patients ($P < 0.05$). The ROC curve of VEGF for DN had the area under curve (AUC) of 0.819 for males and 0.793 for females, indicating the clinical value of serum VEGF as a predictive biomarker.

Conclusions: Serum VEGF was strongly associated with UACR and HbA1c in both genders, and could be regarded as a predictive biomarker for glycemic control and diabetic nephropathy in older adults with T2DM.

KEYWORDS

vascular endothelial growth factor, glycemic control, diabetic nephropathy, type 2 diabetes mellitus, older adults

1 Introduction

Human ageing is accompanied by a progressive decline in kidney function. Elderly subjects with type 2 diabetes mellitus (T2DM) are at a greater risk of diabetic nephropathy (DN) (1). The prevalence of diabetes is also higher in older adults. Nearly 60% of patients with T2DM are adults aged ≥60 years, with the highest prevalence in the age range of 75–79 years (2). Furthermore, 30% of patients with T2DM are associated with DN, which progresses to end-stage renal disease (ERSD) with increasing age and duration of T2DM and requires dialysis, thereby significantly burdening the public health system (3, 4). Therefore, early screening and diagnosis of DN is necessary for timely intervention that can significantly delay the progression of DN in older subjects with T2DM.

Vascular endothelial growth factor (VEGF), a major regulator of vascular permeability and angiogenesis, plays a significant role in diabetic albuminuria and in the pathogenetic mechanisms underlying diabetic nephropathy (5). Furthermore, VEGF is associated with adverse effects in subjects with DN and protective effects in the non-DN individuals (6). Therefore, tight regulation of VEGF levels is critical for the maintenance of glomerular filtration and renal health. In the experimental animal models of diabetes, VEGF is significantly elevated in the kidney tissues and blockade of VEGF signaling ameliorates diabetic albuminuria (7, 8). The correlation between VEGF and human diabetic nephropathy is controversial with many studies reporting contradictory findings (5, 6). Several clinical studies have reported that elevated serum VEGF levels are associated with the development of DN (9–12). However, other studies have shown absence of any association or a negative relationship between circulating VEGF levels and diabetic albuminuria (13–15).

Currently, the worldwide prevalence of DN in older adults is gradually increasing with a higher proportion of older individuals developing uremia (4). Therefore, there is greater emphasis in determining the role of VEGF in early DN and its potential as a diagnostic and prognostic biomarker for DN (16–18). Thus, in this study, we investigated the association of VEGF with glycemic control and DN in elderly subjects with T2DM. Furthermore, we estimated the cut-off value for serum VEGF in the early detection of DN among elderly subjects with T2DM.

2 Materials and methods

2.1 Study participants

This retrospective study recruited elderly subjects with T2DM who were treated as inpatients at the Department of Geriatric Endocrinology, the First Affiliated Hospital of Zhengzhou University from June 2021 to June 2023. These participants were diagnosed with diabetes according to the standard diagnostic criteria specified by the American Diabetes Association (ADA) guidelines in 2021 (19).

We then excluded (1) subjects younger than 60 years (n = 15), and (2) subjects missing UACR (n = 35), VEGF (n = 34), and/or HbA1c (n =12) values, and (3) subjects with liver damage (n = 3), coronary heart disease (n = 24), and/or ESRD (n = 113). Finally, we included 595 study subjects, including 313 males and 282 females in this investigation, and categorized them into DN (n = 242) and non-DN groups (n = 353) (Figure 1). DN was defined according to the diagnostic criteria recommended by the Kidney Disease: Improving Global Outcomes (KDIGO) Diabetes Work Group (20). This study was performed according to the Declaration of Helsinki principles and was approved by the Ethics committee of the First Affiliated Hospital of Zhengzhou University. All the participants voluntarily signed informed consent before participating in this study.

2.2 Clinical data

We collected clinical data from the medical records, including gender, age, diabetes duration, height, weight, and blood pressure. The body mass index (BMI, kg/m^2) was calculated by dividing the weight (kg) by the squared value of the height (m). The venous blood samples were collected from the included study subjects after overnight fasting, and the biochemical parameters, including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol were analyzed using an automated biochemical analyzer (Hitachi 7600-020, Japan). The venous blood glucose levels were estimated using the glucose oxidase method. The apolipoprotein levels were estimated by immune transmission turbidimetry. HbA1c levels were estimated using standard high-performance liquid chromatography (HPLC, Bio-Rad, Hercules, CA, USA). We also collected the first urine sample in the morning and analyzed the urinary albumin creatinine ratio (UACR).

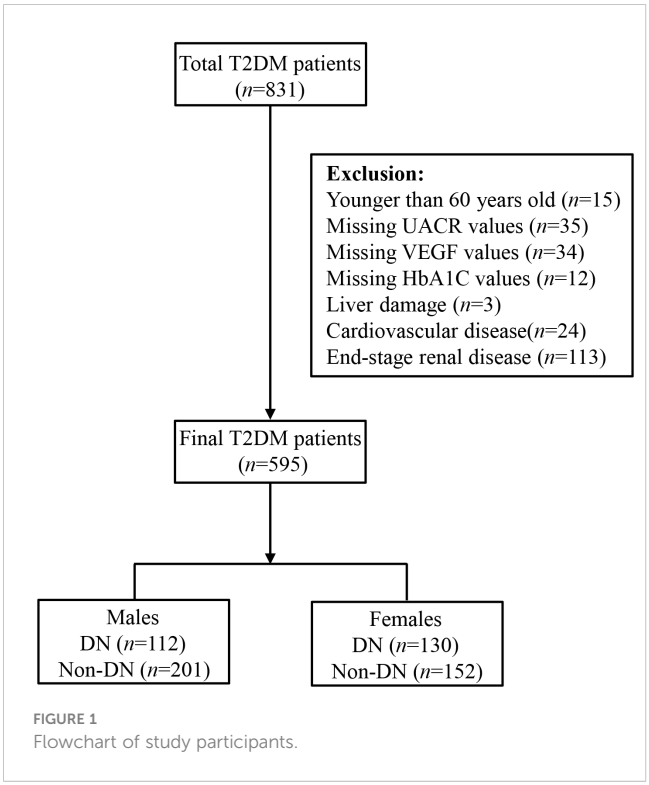

FIGURE 1
Flowchart of study participants.

2.3 ELISA assay

Fresh blood samples were collected in polymer gel chemistry tubes and kept at room temperature for 20 mins. Then, the blood samples were centrifuged at 3000 rpm for 5 mins and the serum was collected. The serum VEGF levels were estimated using a VEGF-specific ELISA kit (Beijing Jianping Venus Biotechnology Co., Ltd., Beijing, China) according to the manufacturer's instructions.

2.4 Statistical analysis

Statistical analysis was performed using the SPSS statistical software 28.0 (IBM Corp, USA). The graphs were generated using the GraphPad Prism 8.0 software (GraphPad Software, California, USA). Normality of data was analyzed using the Shapiro-Wilk test. The data were presented as mean ± standard deviation (SD), median (interquartile range; IQR), or number (percentage). Non-normally distributed data were logarithmically transformed before analysis. Student t test and Mann-Whitney U test were used to estimate the statistical differences between two groups. The analysis of covariance (ANCOVA) was used to compare the natural log (Ln) of serum VEGF levels across the tertiles of HbA1c and clinical groups based on UACR after adjusting for age and BMI. Chi-square test (χ^2) was used to compare categorical variables. Linear regression analysis was used to analyze the correlation of VEGF with HbA1c and UACR. Binary logistic regression analysis was used to identify independent risk factors of DN based on the odds ratio after adjusting for potential confounding factors. The confounding factors were defined as variables with statistical differences in Table 1. Finally, the receiver-operating characteristic (ROC) curves were generated to estimate the clinical performance of serum VEGF for predicting the occurrence of T2DM-induced nephropathy based on gender. The Youden Index was calculated as sensitivity + specificity − 1, and used to determine the cut-off values. P <0.05 was considered as statistically significant.

3 Results

3.1 Demographic information and clinical characteristics of the study subjects

This study included 282 females and 313 males. The prevalence of DN was 46.1% among women and 35.8% among men. The baseline demographic, clinical, and laboratory profiles of the study subjects are shown in Table 1. The median VEGF levels were 253.22 pg/mL (183.19, 328.56) and 140.14 pg/mL (105.07, 178.40) for the male DN and non-DN groups, respectively, and 180.32 pg/mL (132.91, 275.19) and 87.09 pg/mL (62.75, 141.83) for the female DN and non-DN groups, respectively (all $P < 0.001$). Among males, the DN group subjects showed higher BP ($P < 0.05$), longer course of diabetes ($P < 0.001$), higher frequency of diabetic retinopathy ($P < 0.05$), worse renal dysfunction based on higher BUN levels ($P < 0.05$), higher creatinine levels ($P < 0.001$), lower eGFR values ($P < 0.01$), and higher UACR ($P < 0.001$), and higher serum VEGF levels ($P < 0.001$) compared with the non-DN subjects. Among females, the DN group subjects showed higher SBP ($P < 0.01$), longer duration of diabetes ($P < 0.01$), higher BUN levels ($P < 0.001$), higher creatinine levels ($P < 0.05$), higher UACR ($P < 0.001$), lower eGFR ($P < 0.01$), higher serum VEGF levels ($P < 0.001$) and worse glycemic control characterized by higher HbA1c ($P < 0.01$) and higher fasting plasma glucose (FPG) levels ($P < 0.01$) compared with the non-DN subjects.

3.2 Association of serum VEGF with HbA1c and UACR

Linear regression analysis demonstrated that serum VEGF levels were positively associated with HbA1c and UACR in both males and females, before and after adjustment of potential confounding factors (all $P < 0.001$) (Table 2). Furthermore, Ln (VEGF) showed an increasing trend with elevated HbA1c levels and UACR after adjusting for age and BMI in both the genders (all $P < 0.001$) (Figure 2). Stratifying by HbA1c tertiles, there was a significant increase in Ln VEGF from the lowest vs. the highest tertile, while the middle tertile did not show a significant difference with the lowest tertile in males, and this was visually depicted in Figure 2.

3.3 Risk factors for DN in older adults with T2DM

Binary logistic regression analysis was performed to identify the risk factors for DN in older T2DM individuals. In males, ORs for the development of DN were 1.014 (95% CI 1.010, 1.018), 1.147 (95% CI 1.040, 1.266) and 1.006 (95% CI 1.004, 1.009) for every 1 SD increase in serum VEGF, FPG, and UACR, respectively, after adjusting for the confounding factors (all $P < 0.05$). In the females, ORs for the development of DN were 1.009 (95% CI 1.006, 1.012), 1.113 (95% CI 1.008, 1.229) and 1.004 (95% CI 1.002, 1.006) for every 1 SD increase in serum VEGF, FPG, and UACR, respectively, after adjusting for the confounding factors (all $P < 0.05$) (Figure 3). The correlation between VEGF, FPG, UACR and DN were similar in both genders. Furthermore, HbA1c levels showed positive correlation with DN in the female subjects ($P < 0.05$) (Figure 3).

3.4 Predictive value of serum VEGF for DN

Finally, we performed ROC curve analysis to determine the predictive performance of serum VEGF levels for DN in older subjects with T2DM. In the male subjects, the AUC value for serum VEGF was 0.819 (95% CI 0.772 - 0.860) ($P < 0.001$) at the cut-off value of 179.40 pg/mL; in the female subjects, the AUC value for serum VEGF was 0.793 (95% CI 0.741–0.839) ($P < 0.001$) at the cut-off value of 131.57 pg/mL (Figure 4). This demonstrated that serum VEGF was a promising predictive biomarker for DN in older adults with T2DM regardless of the gender.

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TABLE 1 Baseline characteristics of older adults with T2DM.

Variables	Males			Females		
	DN	Non-DN	*P* value	DN	Non-DN	*P* value
N (%)	112 (35.8%)	201 (64.2%)		130 (46.1%)	152 (53.9%)	
Age (year)	69.46 ± 0.48	70.15 ± 0.42	0.292	70.19 ± 0.49	69.57 ± 0.49	0.361
Duration (year)	7.00 (1.00, 13.00)	3.00 (0.46, 8.00)	0.000	7.50 (3.00, 13.25)	5.00 (1.00, 10.00)	0.009
BMI (kg/m^2)	25.65 ± 0.45	25.54 ± 0.27	0.829	24.81 ± 0.32	25.16 ± 0.32	0.447
DBP (mmHg)	87.92 ± 1.24	84.69 ± 0.90	0.034	84.29 ± 1.32	81.10 ± 1.06	0.057
SBP (mmHg)	142.21 ± 1.99	131.75 ± 1.27	0.000	141.86 ± 2.23	133.52 ± 1.65	0.003
Diabetic retinopathy, N (%)	60 (53.57%)	55 (27.36%)	0.000	54 (41.54%)	56 (36.84%)	0.420
UACR (mg/g)	100.03 (35.63, 728.50)	7.90(1.90, 19.45)	0.000	119.03 (47.80, 645.08)	4.16 (1.43, 10.98)	0.000
VEGF (pg/mL)	253.22 (183.19, 328.56)	140.14 (105.07, 178.40)	0.000	180.32 (132.91, 275.19)	87.09 (62.75, 141.83)	0.000
HbA1c (%)	9.88 ± 0.23	10.30 ± 0.17	0.193	10.81 ± 0.23	9.93 ± 0.20	0.004
FPG (mmol/L)	7.82 (6.63, 10.26)	7.59 (6.43, 8.72)	0.059	8.42 (7.25, 9.90)	7.71 (6.69, 9.10)	0.009
TC (mmol/L)	4.49 (3.61, 5.33)	4.54 (3.73, 5.26)	0.669	4.91 (4.00, 5.89)	4.74 (3.67, 5.51)	0.041
TG (mmol/L)	1.43 (0.94, 2.34)	1.65 (1.00, 2.61)	0.113	1.46 (1.00, 2.63)	1.50 (0.99, 2.12)	0.091
HDL (mmol/L)	0.95 (0.82, 1.19)	1.01 (0.85, 1.18)	0.437	1.10 (0.91, 1.31)	1.11 (0.97, 1.34)	0.580
LDL (mmol/L)	2.57 ± 0.08	2.63 ± 0.07	0.605	2.84 ± 0.10	2.74 ± 0.08	0.436
apoA (g/L)	1.45 ± 0.03	1.44 ± 0.02	0.823	1.54 ± 0.31	1.58 ± 0.02	0.253
apoB (g/L)	0.86 ± 0.03	0.86 ± 0.02	0.997	0.95 ± 0.03	0.85 ± 0.02	0.009
ALT (U/L)	18.00 (12.00, 30.00)	18.00 (14.00, 29.00)	0.799	14.00 (9.00, 24.00)	15.00 (11.00, 22.75)	0.301
AST (U/L)	19.50 (15.25, 25.75)	18.00 (15.00, 22.00)	0.151	18.00 (15.00, 25.00)	18.00 (15.00, 24.75)	0.721
ALP (U/L)	77.00 (64.00, 94.00)	79.00 (64.00, 94.00)	0.945	89.00 (70.00, 109.00)	79.00 (64.00, 94.00)	0.006
GGT (U/L)	29.00 (19.00, 42.75)	28.00 (20.00, 43.00)	0.979	20.00 (15.75, 32.00)	21.50 (16.00, 32.00)	0.614
BUN (mmol/L)	5.70 (4.72, 7.38)	5.30 (4.30, 6.60)	0.012	5.40 (4.30, 7.42)	4.75 (3.78, 5.50)	0.000
Cr (umol/L)	71.50 (61.00, 88.75)	66.00 (58.00, 77.00)	0.000	57.00 (48.00, 78.00)	54.00 (46.25,63.75)	0.019
eGFR (mL/min/1.73m2)	116.67 (88.57, 139.70)	128.79 (107.69, 142.13)	0.001	140.86 (107.97, 152.28)	145.70 (134.86, 153.46)	0.009
Uric acid (mmol/L)	297.50 (226.00, 366.75)	278.00 (225.00, 331.50)	0.179	245.00 (197.75, 299.00)	241.00 (186.50, 286.75)	0.388

Data were described as mean ± standard deviation (SD), median and interquartile range (IQR), number (%), as appropriate.
Duration, diabetes duration; BMI, body mass index; UACR, urine albumin/creatinine ratio; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; VEGF, vascular endothelial growth factor; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, glutamyltranspeptidase; apoA, apolipoprotein A; apoB, apolipoprotein B; BUN, blood urea nitrogen; Cr, creatinine; eGFR, estimated glomerular filtration rate.

4 Discussion

This was the first study to investigate the correlation between serum VEGF levels and DN in Chinese T2DM adults older than 60 years. Systemic vascular endothelial homeostasis disorder develops before the signs of microvascular or macrovascular complications are evident in patients with diabetes (21, 22). VEGF plays a vital role in endothelial dysfunction and is associated with both DN and proliferative retinopathy (23). Multiple studies have reported a positive correlation between serum VEGF levels and DN in patients with type 1 and type 2 diabetes (9–12). Our study demonstrated that the serum VEGF was a risk factor for DN in older adults with T2DM and showed positive association with UACR and HbA1c (Table 2; Figures 2, 3). This relationship was independent of SBP, duration of diabetes, TC, ALP, BUN, and creatinine. Yang et al. evaluated 107 T2DM patients with an average age of 49.27 ± 4.26 years and showed an increasing trend for the serum VEGF levels between the normal urinary protein, microproteinuria, and the massive proteinuria groups; moreover, changes in the serum VEGF levels were positively associated with the progression of DN ($r = 0.518$, $P < 0.001$) (12). The results of our study concurred with the findings of Yang et al.

However, the relationship between serum VEGF and DN is controversial. Multiple studies have reported that serum VEGF levels do not associated with progression of DN (13, 15). These differences between studies may be due to variations in the race of the study subjects, sample sizes, or VEGF detection specimens (plasma versus serum (13, 15). In the serum, a large amount of

TABLE 2 Linear regression analysis for the association between VEGF level and indices.

Variables	Ln VEGF (Male)		Ln VEGF (Female)	
	Standardized β (*t*)	*P* value	Standardized β (*t*)	*P* value
HbA1c (%)				
Model1	0.248 (4.511)	0.000	0.296 (5.188)	0.000
Model2	0.291 (4.985)	0.000	0.286 (4.787)	0.000
Model3	0.307 (5.379)	0.000	0.282 (4.725)	0.000
UACR (mg/g)				
Model1	0.472 (9.438)	0.000	0.211 (3.603)	0.000
Model2	0.467 (9.138)	0.000	0.195 (3.330)	0.001
Model3	0.456 (8.215)	0.000	0.160 (2.632)	0.009

Data are expressed as standardized β coefficients and t. VEGF was Ln transformed to correct skewed distribution.
Model 1: Crude model.
Model 2: adjusted for SBP, Duration, TC, ALP.
Model 3: adjusted for SBP, Duration, TC, ALP, BUN, and Cr.

VEGF protein is derived from the activated platelets (10). Hanefeld et al. also confirmed that VEGF-A in the serum was derived mostly from the platelets and better reflected the glycemic burden than the plasma VEGF-A levels (10). Schlingemann et al. reported that β-thromboglobulin, a biomarker for *in vivo* platelet activation, showed positive correlation with proteinuria, thereby confirming the relationship between DN and increased *in vivo* platelet activation (24). VEGF derived from the activated platelets and the podocytes mediates endothelial dysfunction and glomerular damage in patients with diabetes, thereby contributing to the progression of DN (24–26).

Kakizawa et al. evaluated 45 Japanese diabetic individuals aged 26-79 years and did not observe any significant changes in the VEGF levels at various degrees of proteinuria (27). However, this may have been caused by a large age range of the subjects in this study. Our study focused on the elderly subjects above 60 years of age. Moreover, we used a large sample size to achieve consistent data regarding the relationship between VEGF and DN. Thus, our study provides strong evidence for serum VEGF being a promising predictor of DN risk in elderly subjects with T2DM.

Our data also suggested that DN patients with elevated serum VEGF levels were more prone to retinopathy than the non-DN patients (Table 1). This was consistent with previous reports that demonstrated significantly higher serum VEGF levels in diabetic patients with proliferative retinopathy (28).

VEGF-A is a member of the VEGF family of proteins and regulates vascular permeability and angiogenesis (29). VEGF-A is often be referred to as VEGF. The other members of the VEGF family of proteins are VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (PlGF) (30). Mechanistically, podocyte-derived VEGF-A binds to VEGF receptor-2, which is expressed on the surface of the glomerular endothelial cells, and mediates changes in the vascular permeability and vascular endothelial damage in the kidneys (6). Therefore, interventions that suppress VEGFA-VEGFR2 signaling delay the onset of early kidney disease in patients with diabetes (6). VEGF-B is another member of the VEGF family of protein with weak angiogenic effects. VEGF-B is associated with renal dysfunction in patients with T2DM (31, 32). VEGF-C overexpression reduces glomerular permeability and protects against altered VEGF receptor expression, thereby improving glomerular and endothelial barrier functions (33). Therefore, future investigations are necessary to determine the roles of specific VEGF subtypes in the occurrence and progression of DN among the elderly subjects.

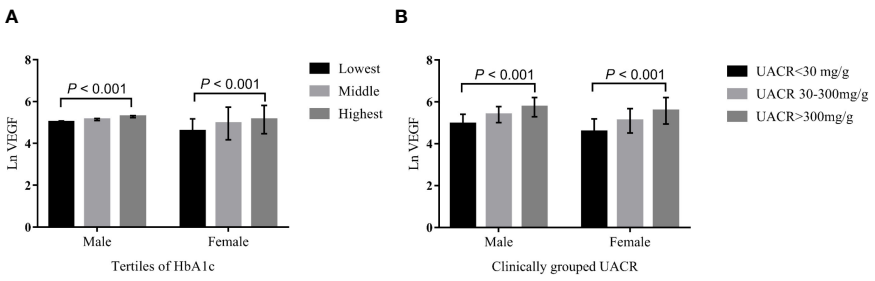

FIGURE 2
The VEGF levels across the HbA1C tertiles **(A)**, and clinically grouped UACR **(B)**. ANCOVA was performed on Ln transformed VEGF, and age and BMI were adjusted. HbA1c (%) were divided into tertiles by genders, male: lowest tertile 4.54-9.00; middle tertile 9.06–11.08; highest tertile11.09-17.69; Female: lowest tertile 4.92-9.03; middle tertile 9.07-11.37; highest tertile 11.43-19.08. Based on the UACR, the patients were divided into three groups by genders: UACR < 30 mg/g, UACR 30–300 mg/g and UACR > 300 mg/g.

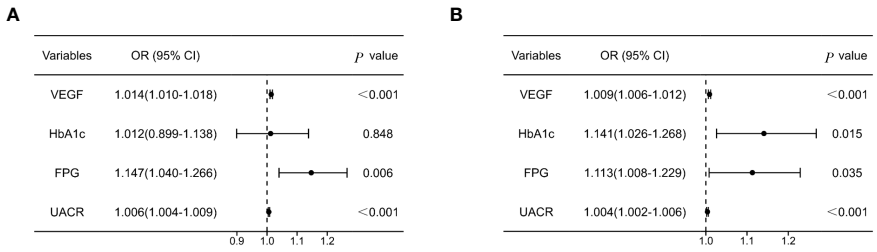

FIGURE 3
Forest plot of logistic regression model investigating risk factors for DN in males **(A)** and females **(B)** with T2DM. Model was adjusted for SBP, Duration, TC, ALP, BUN, Cr. SBP, systolic pressure; TC, total cholesterol; ALP, alkaline phosphatase; BUN, blood urea nitrogen; Cr, creatinine.

Kakizawa et al. reported that plasma VEGF levels were positively associated with FPG and HbA1c; moreover, plasma VEGF levels decreased after comprehensive hypoglycemic treatment with insulin or oral hypoglycemic agents (27). Our results also showed that the serum VEGF levels were associated with glycemic control (HbA1c) in the older adults with T2DM (Table 2). Therefore, VEGF plays a significant role in diabetes. However, analysis of the tertiles of HbA1c against Ln (VEGF) in male subjects showed significant differences only between the lowest tertile and the highest tertile of HbA1c, but the Ln (VEGF) estimates did not show statistical differences between subjects in the lowest and the medium tertiles of HbA1c (Figure 2). This indicated presence of gender-related differences in the relationship between serum VEGF and HbA1c. Kajiwara et al. investigated sex differences in the decline of renal function among Japanese patients with T2DM and reported significant correlation between HbA1c and eGFR decline only in females (34). This may be attributed to poorer metabolic control in females because women experience greater hormonal fluctuations and physical changes than men during their lifetime (35). The elevated VEGF concentration is a sign of poor blood glucose control. Therefore, serum VEGF levels show a better clinical value for predicting the risk of DN. VEGF expression is upregulated in multiple cell types and is indicative of poor glycemic control in the diabetic patients and animal models of diabetes (10, 24, 36). The narrow physiological range of VEGF-A is not only critical for maintaining the optimal kidney function, but also plays a significant role in maintaining the homeostasis and functions of the pancreatic islets in adults 29). Mice with specific down-regulation of VEGF-A in the insulin-producing β cells demonstrated significant reduction in the islet microvascular density and glucose-stimulated insulin secretion (37, 38), whereas overexpression of VEGFA impaired glucose tolerance and β cell mass (39, 40). Therefore, we speculated that hyperglycemia in the older adults with T2DM upregulated VEGF expression. Sustained overexpression of VEGF worsens glycemic control through its effects on the vascular endothelial cells of the islets and the glomerulus, thereby promoting the development of diabetic nephropathy. However, elevated circulating levels of VEGF are not sufficient to determine the corresponding changes in the islet β cells and the glomerulus. Therefore, determination of local VEGF levels may be necessary to establish and confirm the association between higher VEGF levels and dysfunction of the glomerulus and the pancreatic β cells.

The present study has several limitations. Firstly, this was a retrospective observational study. Therefore, we did not analyze the causal relationship between serum VEGF levels and UACR. Moreover, reverse causality cannot be excluded. Secondly, higher

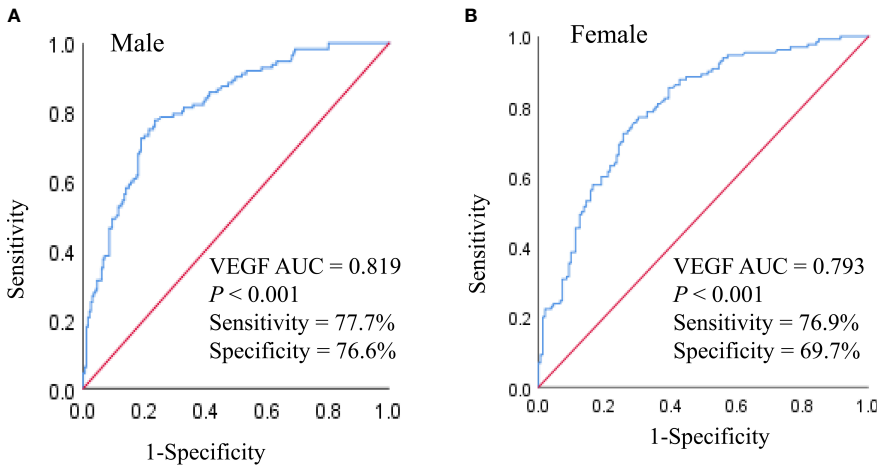

FIGURE 4
ROC analysis of VEGF to indicate DN in older males **(A)** and females **(B)** with T2DM. AUC, Area under the curve.

VEGF levels may be derived from platelet activation during blood collection and may be a confounding factor in this study. Thirdly, the influence of hypoglycemic treatment on the blood glucose control was not analyzed. Finally, this was a single center study. Furthermore, we did not perform any follow-up of the patients. Therefore, in the future, multi-center, large-cohort prospective studies are necessary to confirm our findings.

In conclusion, this study demonstrated that elevated serum VEGF levels were associated with poor glycemic control and progression of DN in older adults with T2DM. Furthermore, serum VEGF is a promising biomarker for the early detection of DN in the diabetic patients and can be used for treating susceptible individuals with effective therapies for better glycemic control to prevent DN and its progression.

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 humans were approved by the First Affiliated Hospital of Zhengzhou University Ethics Review Committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

YJ: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Project administration, Validation, Visualization, Writing – original draft. JL: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft. JZ: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft. SC: Writing – review & editing.

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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

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Changes in serum tumor markers in type 2 diabetes mellitus with microalbuminuria

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Objectives: The objective of this study was to investigate changes in serum tumor markers in type 2 diabetes mellitus (T2DM) with microalbuminuria and analyze the relationship between tumor markers and microalbuminuria.

Methods: A total of 956 T2DM patients aged 40–70 years hospitalized in the Department of Endocrinology, Xinhua Hospital, China, affiliated with Shanghai Jiaotong University School of Medicine, were enrolled from January 2018 to December 2020. The sample comprised 313 T2DM patients with microalbuminuria and 643 T2DM patients with normal urinary microalbumin levels. After assessing the changes in serum tumor markers in T2DM with microalbuminuria, we analyzed the risk of microalbuminuria by the serum tumor marker category using multiple logistic regression analysis.

Results: Serum CEA, CA199, CA125, CA153, CA211, SCC, CA242, and CA50 levels were significantly higher in T2DM patients with microalbuminuria than in those without microalbuminuria, while serum AFP levels were lower in the microalbuminuria group ($P < 0.05$). Following adjustment of confounders, serum CEA, CA211, and SCC were independently associated with microalbuminuria in T2DM. An ROC curve was used to estimate the cutoff point of tumor markers for microalbuminuria. Taking the values under the cutoff points as a reference, values for CEA, CA211, and SCC above the cutoff points indicated a significantly high risk of microalbuminuria. The OR of increased CEA for microalbuminuria was 2.006 (95%CI 1.456–2.765), the OR of increased CA211 for microalbuminuria was 1.505 (95%CI 1.092–2.074), and the OR of increased SCC for microalbuminuria was 1.958 (95%CI 1.407–2.724).

Conclusion: Several serum tumor markers were related to microalbuminuria in T2DM. Serum tumor markers such as CEA, SCC, and CA211 may indicate early diabetic nephropathy, particularly when elevated in combination.

KEYWORDS

tumor markers, microalbuminuria, type 2, diabetes mellitus, UACR

Introduction

Diabetes mellitus is a heterogeneous disease characterized by elevated blood glucose. Various genetic factors and environmental factors can lead to the dysfunction of pancreatic islet beta cells, resulting in a relative or absolute lack of insulin in the body, presenting as a hyperglycemic state (1). The International Diabetes Federation reported that global diabetes prevalence in adults aged 20–79 years reached 10.2% in 2021 and is projected to increase to 12.2% by 2045 (2). In 2021, global diabetes-related health costs were estimated to be 966 billion USD and are expected to reach 1054 billion USD by 2045 (2). China has witnessed one of the most dramatic rises in diabetes prevalence of anywhere in the world (3). It is well established that the prevalence of various cancers is higher in T2DM patients than in the general population (4). Serum tumor markers are widely used for cancer screening in clinical practice. Previous studies have reported connections between T2DM and several tumor markers are elevated in diabetic patients (5). Diabetic kidney disease (DKD) characterized by albuminuria is one of the most common vascular complications of diabetes. The urinary microalbumin-to-creatinine ratio (UACR) is usually recommended to screen and diagnose DKD. Whether a relationship exists between serum tumor markers and diabetic complications, particularly DKD, has rarely been reported. Hence, we conducted a cross-sectional study to explore the relationship between serum tumor markers and UACR and verify whether tumor markers are associated with microalbuminuria in T2DM. We also aimed to identify a new marker of early diabetic nephropathy and explain why tumor markers are increased in T2DM patients in the absence of malignant tumors.

Methods

Subjects

A total of 956 adult T2DM patients without a history of malignant tumor hospitalized in the Department of Endocrinology, Xinhua Hospital, China, affiliated with Shanghai Jiaotong University School of Medicine, were enrolled from January 2018 to December 2020. The sample comprised 313 T2DM patients with microalbuminuria and 643 T2DM patients with normal urinary microalbumin levels. The enrolled subjects were all hospitalized patients who signed the informed consent form for hospitalization when they were admitted and agreed that all their data during hospitalization could be used for future scientific research by our hospital. Extra informed consent was waived by the hospital ethics committee as the research was about hospitalized patients. Patients with a history of kidney disease, including chronic glomerulonephritis, acute nephritis, or urinary tract infection, were excluded, as were those with acute infection and autoimmune disease or those who received a malignant tumor diagnosis while hospitalized.

Anthropometric and biochemical measurements

Anthropometric measurements, including height, weight, and blood pressure, were collected by medical staff. Other data collected included fasting blood glucose (FPG), 2-hour postprandial blood glucose (2hPG), glycosylated hemoglobin (HbA_{1c}), fasting C-peptide (FC-P), 2-hour postprandial C-peptide (2hC-P), fasting insulin (FINS), 2-hour postprandial insulin (2hINS), liver function indexes, kidney function indexes, lipids profiles, tumor markers, and urinary microalbumin-to-creatinine ratio. We used the formulas to calculate the index as follows: BMI was calculated with the formula: weight (kg)/square of height (m^2). HOMA-IR was calculated with the formula: FPG (mmol/L) * FINS (μU/mL)/22.5. Serum tumor markers were determined by the immunoassay method (cobas e 801 analyzer, Roche), while blood glucose, blood lipids, hepatic function, and renal function were determined by an automatic biochemical analyzer (Hitachi LABOSPECT 008 AS, Japan). Blood C-peptide and insulin levels were measured with the chemiluminescence method (BECKMAN COULTER UniCeL Dxl 800 Access immunoassay system, USA). Glycated hemoglobin was determined using high-performance liquid chromatography (Bio-Rad Variant II Turbo, USA). Urine microalbumin was measured by immunoturbidimetry (Siemens automatic protein analyzer, Germany).

Statistical analysis

SPSS 22.0 software (SPSS Inc., Chicago, IL) was used to analyze the data. The data were expressed as mean± standard deviation or median with interquartile range. The comparison between the two groups was performed using the Mann–Whitney U test or independent samples t-test. Spearman correlation analysis and multiple stepwise regression analysis were used to estimate the associations of tumor markers with other variables. Binary logistic regression models were adopted to evaluate the odds ratios (ORs) for microalbuminuria. P values < 0.05 were considered statistically significant. An ROC curve was used to estimate the cutoff point of tumor markers for microalbuminuria.

Results

Clinical characteristics of the two groups

Table 1 shows the basic clinical characteristics of the two groups. There was no difference in sex proportion, ALT, AST, LDL-C, FC-P, 2hC-P,and 2hFINS between the two groups. Compared to the group without microalbuminuria, age, diabetes duration, blood pressure, and BMI, SCr, TC, TG, HbA_{1c}, FPG, 2hPG, FINS, and HOMA-IR were all higher in patients with microalbuminuria. Estimated glomerular filtration rate (eGFR) and HDL-C were lower in patients with microalbuminuria (detailed in Table 1).

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TABLE 1 Clinical characteristics in T2DM with and without microalbuminuria.

Variables	T2DM with microalbuminuria (n=313)	T2DM without microalbuminuria (n=643)	P
Age (y)	61.72 ± 6.20	60.23 ± 6.58	0.001
Male (%)	63%	63%	–
Diabetic duration (y)	11(7~19)	10(3~14)	<0.001
SBP (mmHg)	140(127~153)	131(120~143)	<0.001
DBP (mmHg)	84(76~90)	80(73~88)	<0.001
BMI (kg/m^2)	25.06(23.12~26.87)	24.57(22.48~26.43)	0.014
ALT (U/L)	19(14~28)	19(14~31)	0.361
AST (U/L)	19(15~24)	20(16~25)	0.090
SCr (umol/L)	64(51~81)	60(50~71)	0.001
eGFR ($ml/min^{-1}*1.73m^2$)	101.41(78.89~123.06)	108.87(92.62~127.74)	<0.001
TC (mmol/L)	4.47(3.82~5.23)	4.36(3.73~5.02)	0.037
TG (mmol/L)	1.86(1.27~2.77)	1.51(1.05~2.19)	<0.001
HDL-C (mmol L)	1.11(0.90~1.34)	1.14(0.98~1.37)	0.008
LDL-C (mmol/L)	2.76(2.17~3.37)	2.65(2.06~3.28)	0.118
HbA_{1c} (%)	8.60(7.50~10.20)	8.20(7.00~9.65)	0.002
FPG (mmol/L)	7.43(6.02~9.62)	7.21(5.74~8.90)	0.012
2hPG (mmol/L)	12.91(10.17~15.31)	12.11(9.24~15.08)	0.008
FC-P (nmol/L)	0.63(0.42~0.93)	0.60(0.41~0.82)	0.099
2hC-P (nmol/L)	1.40(0.98~2.20)	1.57(0.94~2.23)	0.213
FINS (pmol/L)	71.85(46.66~110.57)	63.09(41.75~90.29)	<0.001
2hINS (pmol/L)	245.05(182.26~404.14)	247.11(157.53~381.26)	0.176
HOMA-IR	4.02(2.56~7.16)	3.45(2.01~5.25)	<0.001

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; SCr, serum creatinine; eGFR, estimated glomerular filtration rate; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA_{1c}, glycosylated hemoglobin; FPG, fasting blood glucose; 2hPG 2h postprandial glucose; FC-P fasting C-peptide; 2hC-P, 2h postprandial C-peptide; FINS, fasting insulin; 2hINS, 2h postprandial insulin; HOMA-IR, homeostasis model assessment index.

Comparison of tumor markers between the two groups

Data were analyzed using the Mann–Whitney U test (Table 2). There was no statistically significant difference in CA724 and NSE between the two groups. AFP was lower in patients with microalbuminuria (P = 0.033), while serum CEA, CA199, CA125, CA153, CA211, SCC, CA242, and CA50 were all higher (detailed in Table 2).

Variables independently associated with microalbuminuria

As shown in Table 3, following adjustment for all confounders, variables independently associated with microalbuminuria in T2DM patients were duration of T2DM (OR 1.043, 95%CI 1.020–1.066), SBP (OR 1.021, 95%CI 1.013–1.030), HbA_{1c} (OR 1.090, 95% CI 1.003–1.184), BMI (OR 1.053, 95%CI 1.002–1.106), TC (OR 1.221, 95%CI 1.059–1.409), TG (OR 1.220, 95%CI 1.101–1.351), HDL-C (OR 0.524, 95%CI 0.304–0.906), LDL-C (OR 1.218, 95%CI, 1.024–1.449), eGFR (OR 0.993, 95%CI 0.988–0.999), and HOMA-IR (OR 1.056, 95%CI 1.029–1.083). An ROC curve was used to estimate the cutoff point of tumor markers for microalbuminuria. Taking the values under the cutoff points as a reference, values of CEA, CA211, and SCC above the cutoff points indicated a significantly high risk of microalbuminuria. The OR of increased CEA for microalbuminuria was 2.006 (95%CI 1.456–2.765), the OR of increased CA211 for microalbuminuria was 1.505 (95%CI 1.092–2.074), and the OR of increased SCC for microalbuminuria was 1.958 (95%CI 1.407–2.724) (see Table 4).

Variables independently related to CEA, CA211, and SCC

As detailed in Table 5, variables independently associated with CEA were HbA_{1c}, LDL-C, age, and sex. Variables independently

TABLE 2 Tumor markers in T2DM with and without microalbuminuria.

Variables	T2DM with microalbuminuria	T2DM without microalbuminuria	Z	P
AFP (ng/mL)	2.26(1.71~3.29)	2.35(1.80~3.28)	-2.138	0.033
CEA (ng/mL)	3.09(2.03~4.27)	2.36(1.77~3.51)	-4.650	<0.001
CA199 (U/mL)	15.50(10.40~24.69)	12.43(8.72~18.83)	-4.314	<0.001
CA125 (U/mL)	9.84(7.00~13.94)	9.20(7.04~12.02)	-2.586	0.010
CA153 (U/mL)	9.62(7.20~14.28)	9.18(6.60~13.40)	-2.790	0.005
CA724 (U/mL)	2.57(1.68~5.39)	2.78(1.82~5.09)	-0.829	0.407
CA211(ng/mL)	2.40(1.80~3.33)	1.99(1.53~2.67)	-4.853	<0.001
NSE (ng/mL)	15.04(12.4~17.73)	15.60(12.73~18.67)	-1.263	0.207
SCC (ng/mL)	0.90(0.70~1.30)	0.70(0.50~1.00)	-5.439	<0.001
CA242 (U/mL)	6.38(4.20~10.15)	5.35(3.66~8.41)	-2.470	0.014
CA50 (U/mL)	9.87(6.20~15.70)	7.49(4.80~11.75)	-4.742	<0.001

AFP, Alpha-fetoprotein; CEA, Carcinoembryonic antigen; CA199, Carbohydrate antigen 199; CA125, Carbohydrate antigen 125; CA153, Carbohydrate antigen 153; CA724, Carbohydrate antigen 724; CA211, Carbohydrate antigen 211; NSE, Neuron-specific enolase; SCC, Squamous cell carcinoma antigen; CA242, Carbohydrate antigen 242; CA50, Carbohydrate antigen50.

associated with CA211 were HbA_{1c}, LDL-C, age, diabetes duration, SCr, DBP, and FC-P. Variables independently associated with SCC were SCr, age, and FC-P.

Discussion

China is among the countries with the highest prevalence of diabetes worldwide, with the number of patients estimated to exceed 140 million in 2021 and projected to reach over 174 million by 2045 (2). As reported by Zheng et al., diabetes mellitus is the ninth leading cause of mortality globally (6). A previous study that investigated the relationship between T2DM and malignant tumors observed a significant correlation (7). Chronic complications of T2DM lead to increased mortality and morbidity and severely affect the life expectancy and quality of life of patients. DKD is one of the most common vascular complications of diabetes. Clinically, the stage of diabetic nephropathy is primarily determined based on the urinary albumin excretion rate, glomerular filtration rate, creatinine, and total urinary protein. The American Diabetes Association screens and diagnoses DKD according to UACR, defining UACR < 30μg/mg, 30-299μg/mg, and > 300μg/mg as normal, microalbuminuria, and macroalbuminuria, respectively. Gerstein et al. showed that any degree of albuminuria is a risk factor for cardiovascular events in individuals with T2DM and that risk increases progressively with UACR elevation (8). A previous study found that CA153 was negatively related to eGFR and positively related to HbA_{1c} and FPG in patients with T2DM (9). Turgutalp et al. found that the urinary protein excretion rate was correlated with CA125, CA153, and CA199 (10). So far, numerous studies have shown that some serum tumor markers are higher in patients with T2DM than in healthy individuals, but few studies have investigated the relationship between serum tumor markers and microalbuminuria in T2DM patients.

TABLE 3 Variables independently associated with microalbuminuria by logistic analysis.

Variables	β	Exp (β) (95% CI)	P
Diabetic duration (y)	0.042	1.043 (1.020–1.066)	<0.001
SBP (mmHg)	0.021	1.021 (1.013–1.030)	<0.001
HbA_{1c} (%)	0.086	1.090 (1.003–1.184)	0.043
BMI (kg/m^2)	0.052	1.053 (1.002–1.106)	0.040
eGFR ($ml/min^{-1}*1.73m^2$)	-0.007	0.993 (0.988–0.999)	0.015
TC (mmol/L)	0.200	1.221 (1.059–1.409)	0.006
TG (mmol/L)	0.198	1.220 (1.101–1.351)	<0.001
HDL-C (mmol/L)	-0.646	0.524 (0.304–0.906)	0.021
LDL-C (mmol/L)	0.197	1.218 (1.024–1.449)	<0.001
CEA (ng/mL)	0.100	1.172 (1.063–1.293)	0.034
CA211 (ng/mL)	0.263	1.301 (1.123–1.508)	<0.001
SCC (ng/mL)	0.266	1.304 (1.035–1.430)	0.024
HOMA-IR	0.054	1.056(1.029–1.083)	<0.001

Our present study revealed that tumor markers were related to microalbuminuria in T2DM. Most of the serum tumor markers, namely, CEA, CA199, CA125, CA153, CA211, SCC, CA242, and CA50, were increased in the T2DM with microalbuminuria group, while serum AFP was decreased. CA199 has high sensitivity in the diagnosis of pancreatic cancer and, as such, is regarded as the best validated biomarker of pancreatic cancer (11). T2DM is associated with a status of chronic inflammation; pancreatic inflammation leads to impairment of pancreatic exocrine gland function, resulting in increased CA199 levels (12). A previous study indicated that CA199 levels in T2DM patients with microvascular complications including neuropathy, diabetic nephropathy, and retinopathy were significantly increased in comparison with those without microvascular complications (13). It has also been reported that renal impairment could lead to increased serum CA125 levels,

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TABLE 4 Adjusted ORs and 95% CIs for microalbuminuria according to tumor marker categories.

	Adjusted OR (95%CI)		
Variables	Crude OR(95%CI)	Model 1	Model 2
CEA< 2.635 (ng/mL)	1	1	1
CEA≥2.635 (ng/mL)	2.023** (1.532–2.670)	1.975** (1.483–2.629)	2.006** (1.456–2.765)
CA211<2.195 (ng/mL)	1	1	1
CA211≥2.195 (ng/mL)	1.783** (1.354–2.349)	1.673** (1.265–2.213)	1.505* (1.092–2.074)
SCC< 0.750 (ng/mL)	1	1	1
SCC≥0.750 (ng/mL)	2.208** (1.669–2.920)	2.142** (1.609–2.852)	1.958** (1.407–2.724)

Model 1 adjusted for age and sex.
Model 2 further adjusted for age, sex, diabetic duration, blood glucose, HbA_{1c}, BP, BMI, lipid profiles, and FC-P.*p< 0.05, **p < 0.001.

which have been independently correlated with urinary microalbumin (14). Our results are in agreement with this finding. Furthermore, as our studied subjects were T2DM patients, the levels of CA125 were significantly higher in T2DM patients with microalbuminuria than in those without microalbuminuria. In recent years it has been well established that inflammation promotes the occurrence and progression of T2DM, and the activated inflammatory status may play an important role in the occurrence and progression of DKD (15, 16). DKD is an inflammatory disease with increased serum high-sensitivity C-reactive protein levels (17).

CA153 is a glycoprotein that has a proven association with a wide range of cancers (18). CA153 is a product of the Mucin 1 (MUC1) gene, a transmembrane protein expressed on the surface of most epithelial cells (19, 20). Some inflammatory factors such as tumor necrosis factor (TNF), interleukin-1 (IL-1), and interleukin-6 (IL-6) can promote the expression of MUC1 (21), so in an inflammatory state, serum CA153 may be increased. Accordingly, our results showed serum CA153 levels were higher in T2DM patients with microalbuminuria than in those without microalbuminuria. Other researchers have found that serum CA153 levels were negatively correlated with eGFR in diabetic patients (9). CA50, initially reported as a specific antigen expressed on the surface of colorectal cancer cells (22), has also been discovered in other malignant tumors, including lung cancer, pancreatic cancer, liver cancer, gastric cancer, uterine cancer, and bladder cancer (22). Nevertheless, it has been observed to be increased in some benign diseases and notably in patients who suffer from T2DM and pancreatitis. In such cases, the level of serum CA50 decreases after remission of inflammation (22). We suppose that inflammation may be the underlying reason for increased serum CA50 among T2DM patients with microalbuminuria. Our study found that the level of AFP was markedly lower in T2DM patients with microalbuminuria than in patients without microalbuminuria, which is consistent with Turgutalp's conclusions. Turgutalp et al. (10) found that urinary protein excretion was correlated with tumor markers. The possible reasons for the decrease in AFP are loss from urine, increased catabolic rate, decreased synthesis rate, changes in molecular structure, and usage of drugs.

Following adjustment for all confounders, the tumor markers independently associated with microalbuminuria in T2DM patients were CEA, CA211, and SCC. The increased serum CEA levels in T2DM patients with microalbuminuria in our study are consistent with the conclusion of previous research (23). As DKD is more common in

TABLE 5 Variables independently related to CEA, CA211, and SCC by multiple linear regression analysis.

	Variables	β	t	P
CEA (ng/mL)	HbA_{1c} (%)	0.228	6.946	<0.001
	LDL-C (mmol/L)	0.077	2.290	0.022
	Age (y)	0.114	3.403	<0.001
	Sex	-0.233	-6.977	<0.001
CA211 (ng/mL)	DBP (mmHg)	0.109	3.320	<0.001
	FC-P (nmol/L)	0.109	3.157	0.002
	Age (y)	0.101	2.954	0.003
	Duration(y)	0.070	2.039	0.042
	HbA_{1c} (%)	0.101	3.028	0.003
	LDL-C (mmol/L)	0.077	2.290	0.022
	SCr (umol/L)	0.169	4.993	<0.001
SCC (ng/mL)	FC-P (nmol/L)	0.082	2.369	0.018
	Age (y)	0.078	2.292	0.022
	SCr (umol/L)	0.158	4.514	<0.001

patients with poor glycemic control, in the long-term, serum CEA level would be expected to significantly increase due to glucotoxicity damage to the digestive tract. Long-term hyperglycemia could lead to raised levels of advanced glycation end products, resulting in vascular endothelial dysfunction and oxidative stress (23). Another study demonstrated that there exists a positive relationship between serum CEA levels and leukocyte counts in adults; as such, elevated serum CEA levels may reflect a chronic state of inflammation (24). Diabetes mellitus and DKD are chronic inflammatory diseases.

Another reason for increased CEA levels in microalbuminuria in T2DM may be because of the glomerular ultrafiltrate function. According to a previous study, plasma proteins with molecular masses higher than albumin are mostly restricted from passing into the glomerular ultrafiltrate, and thus, only a small proportion can be detected in urine (25). CEA is one of several proteins of this type. In our study, serum levels of SCC and CA211 were also independently associated with microalbuminuria in T2DM. The specific mechanism remains unclear, and we did not find any relevant studies reporting on the relationship between SCC, CA211, and microalbuminuria. The possible reasons may include inflammation, oxidative stress, imbalance of synthesis, catabolism and excretion, and other related factors.

In conclusion, compared to T2DM patients without microalbuminuria, T2DM patients with microalbuminuria were found to have higher levels of CEA, CA199, CA125, CA153, CA211, SCC, CA242, and CA50. Serum CEA, CA211, and SCC were independently correlated with microalbuminuria in T2DM. When serum tumor markers are elevated in diabetic patients, especially when they are increased in combination, it is necessary to screen for diabetic nephropathy in addition to malignant tumors.

Our research has some advantages and limitations. Firstly, the studied population was relatively large. Second, the items of tumor indicators were completely detected, and almost all of the tumor markers were analyzed. Third, our study was the first study to report the relationship between all of the tumor markers and DKD. The limitation of the current study is that it was cross-sectional, and so we cannot ascertain the causality of serum tumor markers for the risk of DKD. As such, further studies are warranted.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics statement

The studies involving humans were approved by Ethics Committee of Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine. The studies were conducted in accordance with the local legislation and institutional requirements. The enrolled subjects are all hospitalized patients who have signed the informed consent form for hospitalization when they were admitted, and have agreed that all their data during hospitalization could be used for future scientific research by our hospital. Extra informed consent form was waived by the hospital ethics committee if the research is about hospitalized patients.

Author contributions

HZ designed the study. LC and SD performed the statistical analysis and drafted the manuscript with assistance from HZ. JZ and LC collected the data. QS and JZ contributed to the specification of the analyses and critically reviewed and edited the manuscript. HZ and JZ are the guarantors of this work, had full access to all the data in the study, and take responsibility for the integrity of the data and the accuracy of the data analysis. 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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Associations of genetic variants contributing to gut microbiota composition in diabetic nephropathy

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Introduction: The gut microbiota is strongly associated with multiple kidney diseases, and since microbial composition is heritable, we hypothesized that genetic variations controlling gut microbiota composition were associated with diabetic nephropathy susceptibility or clinical subphenotypes.

Methods: The genetic variations associated with gut microbiota were retrieved from the genome-wide association study database and analysed in our diabetic nephropathy susceptibility gene screening cohort. Candidate microorganisms with possible genetic associations were identified using the annotation of microbial quantitative trait loci. Finally, the candidate microorganisms were verified by 16S rDNA gene sequencing.

Results: There were 13 genetic variation loci associated with susceptibility to diabetic nephropathy. The TCF7L2 risk genotype was associated with a long duration of diabetes and high diastolic blood pressure, the ZCWPW2 risk genotype was associated with increased glycosylated hemoglobin, and the ZNRF3 risk genotype was associated with an increased urinary microalbumin-to-creatinine ratio. Both the ZNRF3 and SPECC1L risk genotypes were associated with the abundance of Lactococcus. 16S rDNA sequencing confirmed that there was indeed a significant difference in the Lactococcus genus between DN and DM patients.

Conclusions: In this study, we preliminarily confirmed that the gut microbiota of diabetic nephropathy patients is influenced by host genetics and provide a new basis for future accurate diagnosis and treatment.

KEYWORDS

diabetic nephropathy, diabetes mellitus, susceptibility genes, gut microbiota, microbial quantitative trait locus

Introduction

Diabetic nephropathy (DN) is a serious complication of diabetes mellitus (DM) with high morbidity and mortality (1, 2). Approximately 30%-40% of patients with diabetes will develop diabetic nephropathy (3, 4), which has become the most common cause of end-stage renal disease (ESRD) in the world. The pathogenesis of DN is complex and is currently believed to be the result of the comprehensive action of multiple factors. Genetic factors have been found to play an important role (5). Recently, genome-wide association studies (GWAS) on diabetic nephropathy in different ethnic groups have reported some susceptibility sites (6–8).

There are approximately 500-1000 kinds of bacteria in the human gastrointestinal tract. The number of bacteria reaches 10^{14} colony-forming units and are called acquired "organs" (9). They assist the host in maintaining normal physiological functions. At present, a large number of studies have shown that the gut microbiota can participate in the occurrence and development of diseases by regulating host energy metabolism, the systemic inflammatory response, the secretion of enterogenic hormones and other mechanisms (10). The gut microbiota also plays an important role in a variety of kidney diseases, and the endotoxins, proteins and some metabolites produced by them have certain effects on the kidney through the gut-renal axis (11).

Genetic factors and microorganisms can influence the development of a wide range of complex diseases, but how precisely they interact in diabetic nephropathy is unclear. It has been shown that the composition of the gut microbiota is heritable and that host-microbe interactions play a role in the genetic architecture of several disease (12, 13). Therefore, in this study, we explored the role of gut microbiota in the etiology of diabetic nephropathy from the perspective of genetic susceptibility to diabetic nephropathy.

Materials and methods

SNP site selection

The NHGRI GWAS Catalogue (14) database was used to search for genetic variation loci associated with gut microbes until January 1, 2023, and to conduct quality control. The quality control criteria were as follows:

(1) SNP exclusion with loci deletion rate > 5%, (2) SNP loci with minimum allele frequency (MAF) ≤0.01 were excluded, and (3) SNP sites that deviate from Hardy-Weinberg equilibrium test ($P < 0.0001$) were excluded.

Study subjects were included, and clinical indicators were collected.

We included 85 patients with diabetic nephropathy confirmed by renal biopsy and 107 patients with type 2 diabetes for more than 10 years without microvascular disease in Shanxi Provincial People's Hospital from September 2019 to September 2022. In the DN group, the inclusion criteria were as follows: 18-65 y of age; diagnosed with type 2 diabetes; diabetic nephropathy diagnosed based on renal biopsy pathological examination; no evidence of primary renal disease; estimated glomerular filtration rate (eGFR)≥60 ml/min/1.73 m2; and signed informed consent.

In the T2DM group, the inclusion criteria were as follows: 18-65 y of age; the duration of type 2 diabetes was more than 10 years; no diabetic microvascular complications, including diabetic retinopathy and renal damage (eGFR≥60 ml/min/1.73 m2 and urine microalbumin-to-creatinine ratio, UACR<30 mg/g); and signed informed consent.

The exclusion criteria for both groups included the following: severe heart, lung, liver, kidney and other organ dysfunction; malignant tumor, autoimmune disease or psychiatric disorders; and pregnant or lactating women.

Demographic and clinical data, including age, sex, body mass index, duration of diabetes, blood pressure, urinary microalbumin-to-creatinine ratio, 24-hour urinary protein, fasting glucose, serum creatinine, glomerular filtration rate, glycated hemoglobin, and blood uric acid, were collected.

DNA extraction and genotyping

Peripheral blood samples of patients were collected and placed in EDTA anticoagulant tubes. Genomic DNA was extracted by automatic nucleic acid extraction instrument, and genotyping was performed by the CAS-CN1 gene Chip. SNP quality control requirements were as follows: (1) the proportion of sample deletion sites >10% was excluded, (2) the deletion rate of the SNP site was > 10% was excluded, (3) SNP loci with minimum allele frequency (MAF) ≤0.01 were excluded, (4) Hardy-Weinberg balance test (HWE) $P < 0.0001$, excluding deviated indicators, and (5) sex examination.

Fecal DNA extraction and 16S rDNA sequencing

We included 50 patients with diabetic kidney disease confirmed by renal biopsy (DN group) and 50 patients with type 2 diabetes for more than 10 years without microvascular disease (DM group) in Shanxi Provincial People's Hospital from September 2019 to September 2022, with the same inclusion criteria as described above. The exclusion criteria for both groups included severe heart, lung, liver, kidney and other organ dysfunction; malignant tumor, autoimmune disease or gastrointestinal disease; the use of antibiotics, preparations of live bacteria, lactulose or immunosuppressants within nearly a month; and pregnant or lactating women.

Fresh fecal samples were obtained with a sterile fecal collector and the fecal samples were transferred to an ice box at -80°C within 2 hours after sampling. DNA was extracted from the samples using

the QIAamp PowerFecal DNA Kit. Agarose gel electrophoresis was used to analyze DNA integrity. NanoDrop was used to measure the purity, and DNA concentration was accurately quantified with Qubit.

High-fidelity DNA polymerase was used to amplify the V3-V4 variable region of DNA by two-step PCR amplification and the addition of tag and joint sequences. An FC Magnetic Beads Kit (Enlighten) was used to purify and recover the product. Qubit4.0 was used to quantify the purified library. Qsep100 was used to check whether the length of the library was as expected, and each sample was diluted to 4 nM. The hybrid library was prepared and denatured by DNA, and at least a 5% Phix library was added to balance the library polymorphism. Sequencing was carried out on an Illumina MiSeq sequencer using the PE300 strategy.

Ethics statement

All study procedures complied with the ethical guidelines of the Declaration of Helsinki. The studies involving human participants were reviewed and approved by the Biomedical Ethics Committee of Shanxi Provincial People's Hospital (No. 2019-117). The patients/participants provided their written informed consent to participate in this study.

16S rDNA sequencing data processing and analysis

After disembarkation data filtering, the remaining high-quality clean data was obtained for later analysis. The reads were spliced into tags by the overlap between reads. The tags were clustered into OTUs, compared with the database, and species were annotated. Based on OTU and annotation results, sample species complexity analysis and intergroup species difference analysis was performed. Alpha diversity is used to analyze species diversity in a single sample, Beta diversity is used to compare the size of differences in species diversity between different samples. Wilcoxon rank sum tests (two tailed) were conducted to detect differences in relative abundances between the two groups.

Statistical analysis

Plinks-1.07 was used for genetic association analysis (15). The ggpubr R package was used for mapping. The Wilcoxon rank sum test was used for comparisons between two groups, and the Kruskal-Wallis rank sum test was used for comparisons among multiple groups.

Results

General characteristics of the study participants

GWAS analysis was performed on 85 patients with DN and 107 patients with DM. The basic clinical characteristics of the case group and the control group were shown in Table 1. There were no significant differences in gender, body mass index, fasting blood glucose, glycosylated hemoglobin and blood pressure between the two groups. The age of DM group was significantly higher than that of DN group (P=0.007), and the duration of diabetes was longer than that of DN group (P=0.035). Serum creatinine level, urinary microalbumin-to-creatinine level, 24-hour urinary protein level and blood uric acid level in DN group were significantly increased than in DM group.

We included 50 patients with DN, 50 patients with DM to perform 16S rDNA sequence. The median age of the DN group was 51.52 years, and the mean duration of diabetes was 10.36 years. The median age of DM patients was 56.50 years and the mean duration of diabetes was 12.39 years (Table 2).

Single nucleotide polymorphism results of DN GWAS

After quality control, a total of 486,790 SNP loci were obtained. Due to the small sample size included in this GWAS study, no SNP loci reached the significant difference level of genome-wide association studies ($P < 5.0\times 10^{-8}$), but 10 SNPs reached $P < 5\times10^{-5}$ (Table 3).

Gut microbiota related variation in DN genetic susceptibility sites

The NHGRI GWAS Catalogue database was searched for genetic variants associated with the gut microbiota (16, 17), and the searched loci were identified in our DN cohort. No sites were found that reached the level of genome-wide significant association ($P < 5.0\times10^{-8}$). However, we still found 13 sites that were associated with DN, as shown in Table 4.

Correlation between genotypes and clinical subphenotypes of DN

Next, we analyzed the correlation between these 13 DN genetic variation sites related to intestinal microbes and clinical phenotypes and found that the risk genotype of TCF7L2 rs4277044-AG was associated with a longer diabetes duration and higher diastolic

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TABLE 1 General characteristics of participants in DN GWAS study.

	Total	DN	DM	P value
Sex, n (%)				>0.05
Male	140 (73)	62 (73)	78 (73)	
Female	52 (27)	23 (27)	29 (27)	
Age (years)	54.04 ± 8.8	51.19 ± 10.15	56.9 ± 6.08	0.007
BMI (kg/m2)	25.4 ± 3.04	25.87 ± 3.35	24.92 ± 2.66	0.153
Duration of DM (years)	11.87 ± 5.99	10.51 ± 6.23	13.23 ± 5.46	0.035
FBG (mmol/L)	8.16 ± 3.04	8.58 ± 3.75	7.75 ± 2.07	0.521
HbA1C (%)	8.53 ± 1.88	8.73 ± 2.04	8.33 ± 1.7	0.331
Scr (umol/L)	91.15 ± 60.62	114.76 ± 78.07	67.55 ± 14	< 0.001
GFR (ml/min*1.73m2)	94.96 ± 35.71	77.52 ± 35.43	112.4 ± 26.49	< 0.001
UACR (mg/g)	1497.26 ± 2304.45	2951.45 ± 2520.5	43.08 ± 248.59	< 0.001
24-hour urinary protein (g)	2.27 ± 3.31	4.34 ± 3.58	0.19 ± 0.8	< 0.001
Blood uric acid (umol/L)	352.04 ± 85.69	377.66 ± 93.51	326.42 ± 68.98	0.003
SBP (mmHg)	137.46 ± 19.46	142.42 ± 23.46	132.5 ± 12.83	0.063
DBP (mmHg)	82.17 ± 11.24	83.5 ± 12.1	80.83 ± 10.27	0.281

DN, diabetic nephropathy confirmed by renal biopsy; DM, type 2 diabetes mellitus for more than 10 years without kidney damage; BMI, body mass index; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; Scr, serum creatinine; GFR, glomerular filtration rate; UACR, urine microalbumin-to-creatinine ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure.

blood pressure (Figures 1A, B). The risk genotype of ZCWPW2 rs6551253-TC was associated with a higher level of glycosylated hemoglobin (Figure 1C). The risk genotypes of ZNRF3 rs2294239-GG/AG were associated with higher levels of urinary microalbumin-to-creatinine (Figure 1D).

TABLE 2 General characteristics of participants in 16S rDNA sequence study.

	DN (n = 50)	DM (n = 50)	*P* value
Age (years)	51.52 ± 9.45	56.50 ± 6.28	0.018
Sex, n (%)			0.723
Female	14 (28)	16 (32)	
Male	36 (72)	34 (68)	
Duration of DM (years)	10.36 ± 6.49	12.39 ± 5.27	0.143
BMI (kg/m2)	25.94 ± 3.27	24.82 ± 2.79	0.73
Scr (umol/L)	96.34 ± 37.78	67.26 ± 14.02	< 0.001
FBG (mmol/L)	8.16 ± 2.42	7.72 ± 1.91	0.292
HbA1C (%)	9.1 ± 1.73	8.46 ± 1.83	0.934
UACR (mg/g)	2082.01 ± 2295.32	52.78 ± 279.34	< 0.001
GFR (ml/min/1.73m2)	86.33 ± 35.30	112.00 ± 27.07	0.002

DN, diabetic nephropathy confirmed by renal biopsy; DM, type 2 diabetes mellitus for more than 10 years without kidney damage; BMI, body mass index; Scr, serum creatinine; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; UACR, urine microalbumin-to-creatinine ratio; GFR, glomerular filtration rate.

Microbial quantitative trait locus annotation

Data mining of the published microbial quantitative trait loci indicated that the risk genotypes of rs2294239 (ZNRF3) and rs3747113 (SPECC1L) in diabetic nephropathy were correlated with the abundance of Lactococcus, which is a microorganism that is beneficial to the human body (18, 19) (Table 5).

Gut microbiota in patients with diabetic nephropathy and type 2 diabetes

We included 50 patients with diabetic kidney disease confirmed by renal biopsy (DN group) and 50 patients with type 2 diabetes for more than 10 years without microvascular disease (DM group). The alpha diversity of bacterial communities was evaluated according to the Chao and Shannon indices. The Chao index was used to measure microbial species richness in a single sample, and the Shannon index was used to evaluate community diversity in a single sample. The results showed that compared with that in the DM group, the gut microbiota richness in the DN group was decreased (P=5.38×10^{-3}), but there was no significant difference in gut microbiota diversity between the two groups (P=0.13). (Figures 2A, B). β diversity was used to compare the differences in species diversity among different samples, and the results showed that there were significant differences in microbial diversity between the DN and DM groups (P= 1.60×10^{-12}) (Figure 2C).

To verify the clues suggested by the susceptible sites, we observed the differences in bacteria between the DN group and the DM group. We found that the relative abundance of Lactococcus in DN was 6×10^{-6} and that in DM was 1.7×10^{-4}. There was a significant difference between the

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TABLE 3 Ten SNPs reached P< 5×10^{-5} in DN GWAS study.

SNP	Gene symbol	CHR	Minor allele	Major allele	DN(major/minor allele frequency)	DM(major/minor allele frequency)	P value	OR (95% CI)
rs6467788		7	T	C	0.40/0.60	0.72/0.28	6.67E-06	3.90(2.13-7.14)
rs825050	MYBPC1	12	T	G	0.90/0.10	0.61/0.39	5.85E-06	0.18(0.08-0.40)
rs149205645	AKAIN1	18	G	A	0.81/0.19	1.00/0	7.94E-06	
rs251418	PDE8B	5	T	C	0.97/0.03	0.75/0.25	1.30E-05	0.09(0.03-0.33)
rs200888	LOC100289473	20	T	G	0.50/0.50	0.79/0.21	2.39E-05	3.80(2.02-7.17)
rs12029233	LYPLAL1	1	A	G	0.69/0.31	0.93/0.07	2.57E-05	5.78 (2.39-13.96)
rs4676864		3	A	C	0.91/0.09	0.65/0.35	2.64E-05	0.19(0.09-0.44)
rs2066405	USH2A	1	A	G	0.82/0.18	0.54/0.46	2.85E-05	0.25(0.13-0.49)
rs10859525	SOCS2	12	G	A	0.79/0.21	0.51/0.49	4.35E-05	0.27(0.14-0.52)
rs77481693		7	A	C	0.98/0.02	0.78/0.22	4.80E-05	0.08(0.02-0.36)

[a]CHR, chromosome; CI, confidence interval; OR, odds ratio; A, adenine; T, thymine; C, cytosine; G, guanine.

two groups ($P = 0.04$). However, the relative abundance of Lactococcus in the two groups was low, and the comparison of key species between the two groups showed that the abundances of Flavonifractor, Lachnospiracea_incertae_sedis, Eisenbergiella and Prevotella in the DN group were significantly increased compared with those in the DM group (Figure 3). However, no corresponding microbial quantitative trait loci were found in the genetic susceptibility locus of DN.

Discussion

Host genome and gut microbiota composition have influenced the occurrence and development of many human diseases. Genetic variants associated with the microbiome are defined as microbial quantitative trait loci (QTLs). Recently, some studies have identified microbiome QTL in human diseases. For example, in inflammatory bowel disease, 5 functional genetic variants that were shown to be directly involved in gut bacterial processing (12). In IgA nephropathy, LYZL1 and SIPA1L3 risk genotypes are related to the decrease of Dialister and Bacilli and the risk genotypes of PLTP and AL365503.1 were associated with increased abundance of Erysipelotrichaceae and Lachnobacterium (13).

In this study, We searched for microbial QTLs in the GWAS Catalogue database and explored the relationship between the retrieved microbial QTLs and DN susceptibility and clinical subtypes in our DN genetic susceptibility locus screening cohort. In a cohort with 85 patients with DN and 107 patients with DM, we found that 13 loci were associated with DN susceptibility, and the genotypes of these loci

TABLE 4 The thirteen SNPs associated with DN with P values of<$5\times10^{-2\alpha}$.

SNP	CHR	Position (hg19)	Risk allele	RAF in DN (%)	RAF in DM (%)	*P* value	OR (95%CI)	Gene Symbol
rs9600567	13	75871180	T	44.79	18.75	1.07×10^{-4}	3.52(0.33-1.83)	LMO7
rs1422155	5	170892785	G	33.33	54.88	3.83×10^{-3}	0.41(0.31-0.22)	RANBP17
rs17387919	7	24500681	C	6.25	18.75	8.83×10^{-3}	0.29(0.50-0.11)	NPY
rs2140551	2	48595242	G	35.42	54.17	8.99×10^{-3}	0.46(0.30-0.26)	STON1
rs7521798	1	206813581	C	15.62	5.21	1.82×10^{-2}	3.37(0.54-1.17)	IL19
rs9972588	15	88868574	T	13.54	27.08	1.97×10^{-2}	0.42(0.38-0.20)	ACAN
rs4277044	10	113075693	A	7.29	1.04	3.02×10^{-2}	7.47(1.08-0.90)	TCF7L2
rs3746118	19	3762500	T	18.75	32.29	3.14×10^{-2}	0.48(0.34-0.25)	APBA3
rs3747113	22	24321550	A	14.58	27.08	3.30×10^{-2}	0.46(0.37-0.22)	SPECC1L
rs6551253	3	28371475	C	33.33	19.79	3.37×10^{-2}	2.03(0.34-1.05)	ZCWPW2
rs2714053	11	123441355	A	32.29	46.88	3.88×10^{-2}	0.54(0.29-0.30)	GRAMD1B
rs17836935	17	74179810	G	36.46	22.92	4×10^{-2}	1.93(0.32-1.03)	RPL38
rs2294239	22	29053489	G	36.46	51.04	4.17×10^{-2}	0.55(0.29-0.31)	ZNRF3

[a]CHR, chromosome; CI, confidence interval; OR, odds ratio; RAF, risk allele frequency; A, adenine; T, thymine; C, cytosine; G, guanine.

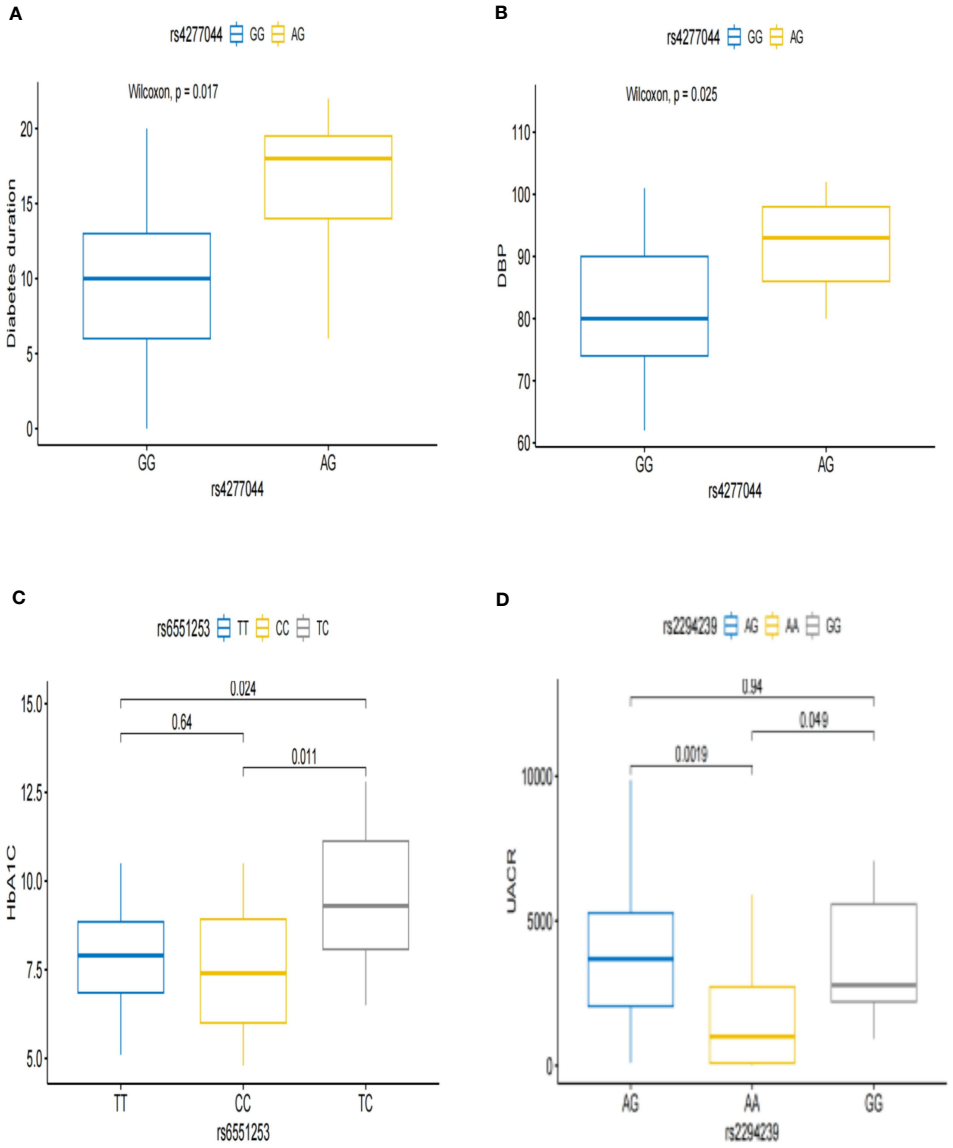

FIGURE 1
Associations between the genotypes and clinical subphenotypes of DN. **(A, B)** Patients with rs4277044-AG genotype had a longer diabetic duration and higher diastolic blood pressure than did patients with the rs4277044-GG genotype. **(C)** Patients with rs6551253-TC genotype had higher glycated hemoglobin than did Patients with the rs6551253-TT/CC genotypes. **(D)** The urine microalbumin-to-creatinine ratio was higher in patients with the rs2294239-GG/AG genotypes than in patients with the rs2294239-AA genotype.

TABLE 5 Microbiome QTL annotations.

Genetic variant/risk allele in present study	Genetic variant/risk allele in GWAS Catalog	Variant annotation	Associated microbiome	*P value*	GWAS Catalog accession no.
rs7521798-C	rs7521798-C	intron_variant		4×10^{-6}	GCST90006995
rs2140551-G	rs2140551-A	synonymous_variant		2×10^{-9}	GCST90032517
rs6551253-C	rs6551253-C	intron_variant		3×10^{-6}	GCST90007005
rs1422155-G	rs1422155-?	intron_variant		4×10^{-6}	GCST90011577
rs17387919-C	rs17387919-?	regulatory_region_variant		2×10^{-6}	GCST90011535

(Continued)

TABLE 5 Continued

Genetic variant/risk allele in present study	Genetic variant/risk allele in GWAS Catalog	Variant annotation	Associated microbiome	*P value*	GWAS Catalog accession no.
rs4277044-A	rs4277044-A	intron_variant		8×10^{-6}	GCST90007003
rs2714053-A	rs2714053-G	intron_variant		5×10^{-8}	GCST90027571
rs9600567-T	rs9600567-T	non_coding_transcript_exon_variant		5×10^{-6}	GCST90006994
rs9972588-T	rs9972588-?	intron_variant		6×10^{-6}	GCST008900
rs17836935-G	rs17836935-?	intergenic_variant		8×10^{-6}	GCST90011571
rs3746118-T	rs3746118-?	upstream_gene_variant		7×10^{-6}	GCST90011351
rs3747113-A	rs3747113-?	synonymous_variant	g_Lactococcus	3×10^{-7}	GCST003221
rs2294239-G	rs2294239-G	intron_variant	g_Lactococcus	3×10^{-6}	GCST003855

[a]g_, genus.

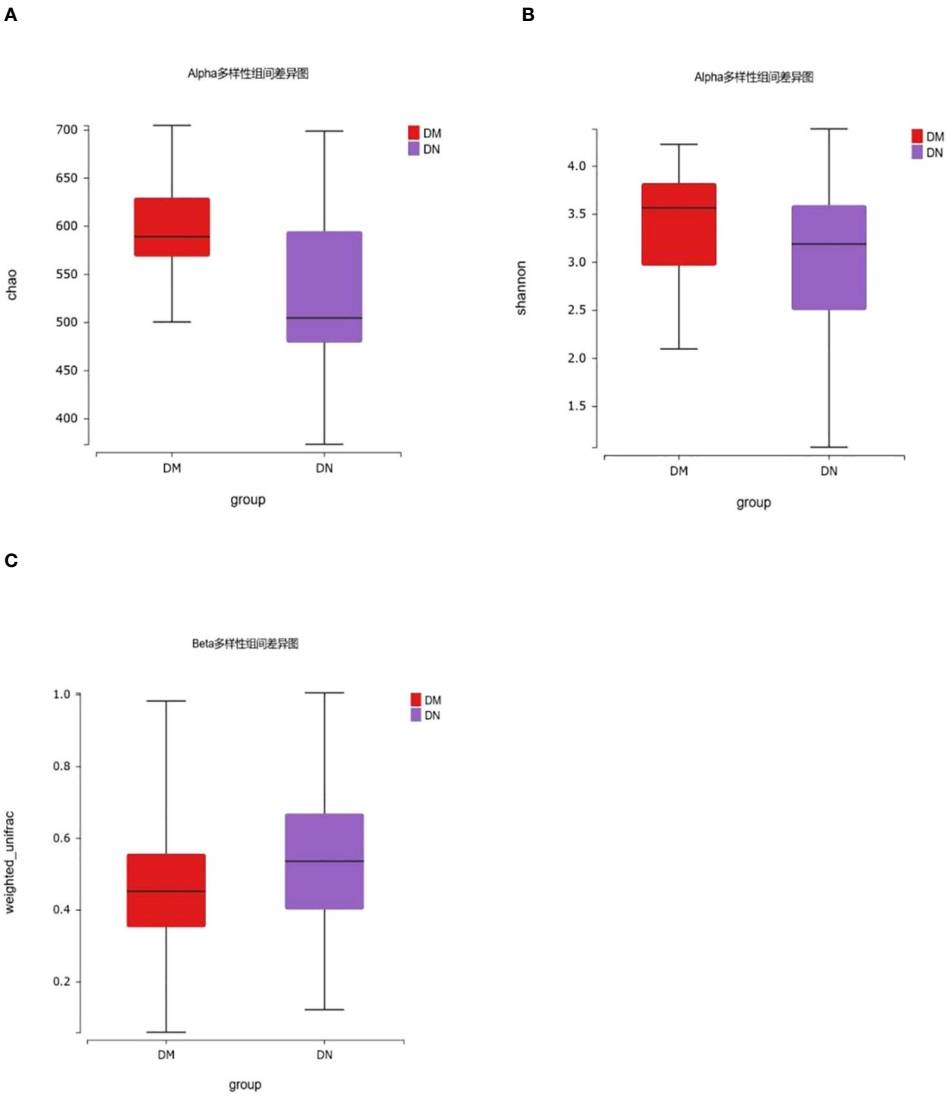

FIGURE 2
16S rDNA gene analysis in DM and DN groups. **(A, B)** The alpha diversity showed that gut microbiota richness of DN patients decreased (P = 5.38×10^{-3}), while there was no significant difference in gut microbiota diversity between the two groups (P = 0.13). **(C)** β diversity showed that there were significant differences in microbial diversity between DN and DM groups (P= 1.60×10^{-12}).

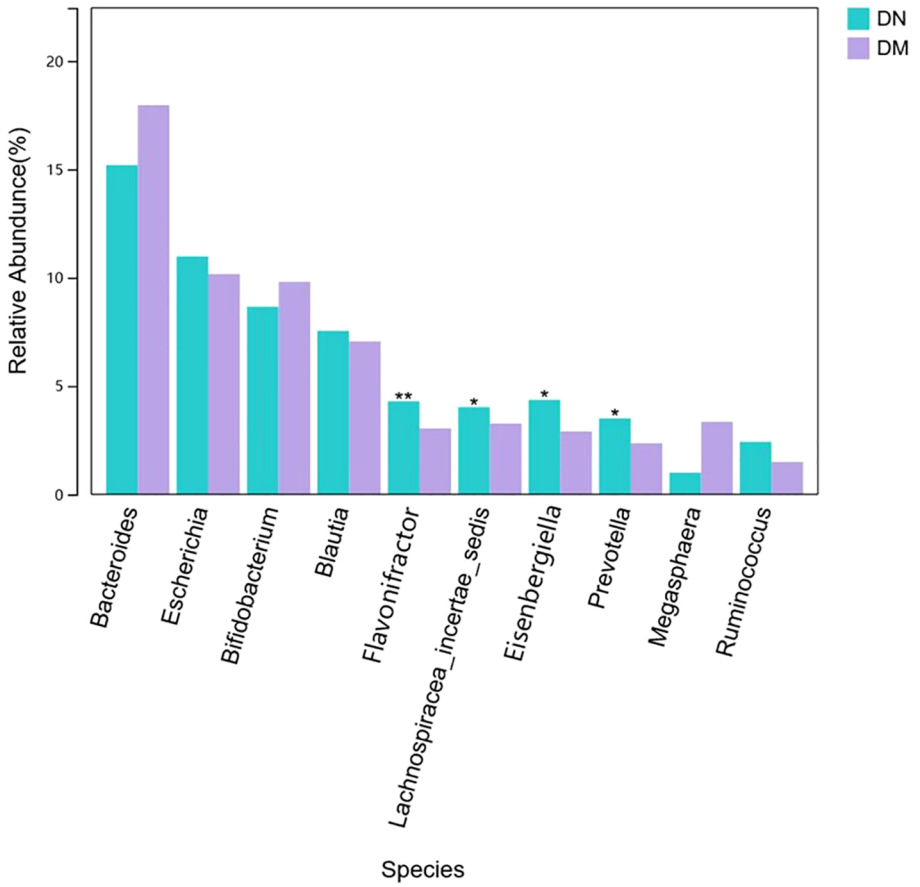

FIGURE 3
Bar chart of difference comparison of key species in gut microbiota (*P<0.05, **P<0.01).

were closely correlated with clinical subphenotypes. For example, the TCF7L2 risk genotype was associated with a long duration of diabetes and high diastolic blood pressure, the ZCWPW2 risk genotype was associated with a high level of HbA1c, and the ZNRF3 risk genotype was associated with an elevated urinary microalbumin-to-serum ratio. Studies of genotypic associations between risk alleles and clinical subphenotypes provide new insights into the etiology and mechanisms of diseases.

Through the study of genetic genes and the annotation of microbial QTLs, we found different microbiota in DN and DM and then identified the structure of the fecal microbial community by 16S rDNA sequencing. The risk genotypes of SPECC1L and ZNRF3 determined by genetic studies were correlated with Lactococcus. 16S rDNA sequencing confirmed that there were obvious differences in Lactococcus between DN and DM patients. Lactococcus is a probiotic, and probiotics play various roles in the human body, such as participating in the formation of the microbial barrier of the digestive tract, substance metabolism, nutrient transformation and biosynthesis, regulation of gastrointestinal immune function, and promotion of human growth and development (20). However, the abundance of Lactococcus in the fecal microbiota of the two groups was low, and the comparison map of the difference in key species between the two groups showed that the abundances of Flavonifractor, Lachnospiracea_incertae_sedis, Eisenbergiella and Prevotella in the DN group were significantly increased compared with those in the DM group. However, since no QTL of these microorganisms was found in our susceptibility genes, it was impossible to determine the influence of genes on these bacteria. In this study, we have tentatively demonstrated that host genetics have an impact on the gut microbiota, which plays an important role in both susceptibility and severity of disease.

Gut microbial composition is not only related to the environment, diet, disease, age, and sex but is also affected by host genetic factors, which will be beneficial to the accurate diagnosis and treatment of diseases (21). First, host genetics can affect the composition of the gut microbiota. Unlike variable factors such as diet, genetic factors are immutable factors. Therefore, we can determine whether the host is susceptible to the influence of DN risk microbiota through genetic analysis. Then, DN risk stratification can be conducted in the population, especially in the high-risk population, through genetic analysis and early intervention can be conducted in the high-risk population. Second, the relationship between genetic factors and the gut microbiota provides new insights into the pathogenesis of DN. In DN, the gut microbiota can interact with the kidney through the gut-renal axis (11), but the exact mechanisms and interactions with genetic factors are still unclear. Finally, this study provides new evidence for precision treatment and gut microbiota intervention in

DN. Future studies integrating host genetics, gut microbiology and microbial metabolomics will be conducive to further elucidating the pathogenesis of DN and making accurate diagnoses and treatments.

There are some limitations to our study. First, the sample size included in our DN susceptibility gene study is small, so some microbiome-related variations may not be found in the DN genetic susceptibility sites. For example, Flavonifractor, Lachnospiracea_incertae_sedis, Eisenbergiella and Prevotella, which have significant differences and high abundance in the two groups, and their related genetic variations may be closely related to DN susceptibility. Second, at present, there is no extensive microbiome-related research on DN and even less research on DN-specific microbial QTLs. Therefore, many DN-related microbial QTLs have not been effectively annotated. Finally, fecal 16S rDNA sequencing has limited accuracy for microbial identification, and some microorganisms may not be effectively identified. In the future, we need to use more advanced technical means, expand the sample size of the study, and conduct multicenter and large-cohort joint studies to verify our results.

In conclusion, our microbial QTL genetic study showed that there were 13 loci closely related to the susceptibility and clinical subphenotypes of DN, and the risk genotype determined by genetic study was related to Lactococcus. Fecal microbial measurement also confirmed that this beneficial bacteria was significantly reduced in DN patients, which benefits the future accurate diagnosis and treatment of DN.

Data availability statement

The data presented in the study are deposited in the Sequence Read Archive (SRA) repository, accession number PRJNA996574.

Ethics statement

The studies involving humans were approved by The Biomedical Ethics Committee of Shanxi Provincial People's Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

XL: Conceptualization, Project administration, Writing – review & editing. JM: Data curation, Methodology, Writing – original draft. LG: Formal analysis, Writing – review & editing. WW: Methodology, Writing – original draft. RL: Project administration, Writing – review & editing.

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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

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Association of systemic immune-inflammation index with diabetic kidney disease in patients with type 2 diabetes: a cross-sectional study in Chinese population

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Objective: Systemic immune-inflammation index (SII), a novel inflammatory marker, has been reported to be associated with diabetic kidney disease (DKD) in the U.S., however, such a close relationship with DKD in other countries, including China, has not been never determined. We aimed to explore the association between SII and DKD in Chinese population.

Methods: A total of 1922 hospitalized patients with type 2 diabetes mellitus (T2DM) included in this cross-sectional study were divided into three groups based on estimated glomerular filtration rate (eGFR) and urinary albumin-to-creatinine ratio (ACR): non-DKD group, DKD stages 1–2 Alb group, and DKD-non-Alb+DKD stage 3 Alb group. The possible association of SII with DKD was investigated by correlation and multivariate logistic regression analysis, and receiver-operating characteristic (ROC) curves analysis.

Results: Moving from the non-DKD group to the DKD-non-Alb+DKD stage 3 Alb group, SII level was gradually increased (*P* for trend <0.01). Partial correlation analysis revealed that SII was positively associated with urinary ACR and prevalence of DKD, and negatively with eGFR (all *P*<0.01). Multivariate logistic regression analysis showed that SII remained independently significantly associated with the presence of DKD after adjustment for all confounding factors [(odds ratio (OR), 2.735; 95% confidence interval (CI), 1.840-4.063; *P* < 0.01)]. Moreover, compared with subjects in the lowest quartile of SII (Q1), the fully adjusted OR for presence of DKD was 1.060 (95% CI 0.773-1.455) in Q2, 1.167 (95% CI 0.995-1.368) in Q3, 1.266 (95% CI 1.129-1.420) in the highest quartile (Q4) (*P* for trend <0.01). Similar results were observed in presence of DKD stages 1–2 Alb or presence

of DKD-non- Alb+DKD stage 3 Alb among SII quartiles. Last, the analysis of ROC curves revealed that the best cutoff values for SII to predict DKD, Alb DKD stages 1- 2, and DKD-non-Alb+ DKD stage 3 Alb were 609.85 (sensitivity: 48.3%; specificity: 72.8%), 601.71 (sensitivity: 43.9%; specificity: 72.3%), and 589.27 (sensitivity: 61.1%; specificity: 71.1%), respectively.

Conclusion: Higher SII is independently associated with an increased risk of the presence and severity of DKD, and SII might be a promising biomarker for DKD and its distinct phenotypes in Chinese population.

KEYWORDS

systemic immune-inflammation index, diabetic kidney disease, distinct phenotypes, Chinese population, biomarker

Introduction

Diabetic kidney disease (DKD) is one of the most common and severe chronic diabetic microvascular complications that is clinically characterized by a gradual decline in renal function, with or without proteinuria (1), affecting approximately 20%–40% of people with type 2 diabetes mellitus (T2DM) (2, 3). Currently, DKD has become the leading cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD) requiring dialysis or transplantation (2, 4), and also is a major risk factor for cardiovascular events and mortality (1, 2), resulting in a significant burden on the public health and economic systems of countries throughout the world. However, DKD is asymptomatic in early stages and its treatment option is limited (3), and thus a complete cure for DKD is an unmet medical need that urgently requires the discovery of novel reliable biomarkers that can guide the early identification and further treatment of DKD.

The systemic immune- inflammation index (SII), a novel inflammatory biomarker integrating three different cells, including neutrophil, lymphocyte, and platelet, was originally used to estimate the prognosis of patients with hepatocellular carcinoma by Hu et al. in 2014 (4, 5), and then has been developed to predict the prognosis in other malignant tumors types, such as colorectal cancer, cervical cancer, lung cancer, esophageal cancer, oropharyngeal cancers, epithelial ovarian cancer, papillary thyroid carcinoma, and melanoma (4, 6, 7). Now, SII is thought to better and more objectively reflect the state of inflammation and immune balance in the body compared with white blood cells and its subtypes (neutrophil and lymphocyte) as well as the neutrophil-to- lymphocyte ratio and platelet-to-lymphocyte (5, 8, 9), and predict the prognosis of certain autoimmune disorders, such as autoimmune encephalitis, systemic lupus erythematosus, and adult-onset Still's disease, and inflammatory diseases, such as acute pancreatitis, ulcerative colitis, and chronic obstructive pulmonary disease (4, 10–15). Recently, growing evidence suggests that SII may be associated with metabolic disorder and its components, such as central obesity, nonalcoholic fatty liver disease, metabolic syndrome, dyslipidemia, and hypertension (15–19), all of which have been reported to be involved in the development and progression of DKD (20). Furthermore, it has been demonstrated that elevated SII levels are associated with subclinical atherosclerosis, and can efficiently predict the development, prognosis and clinical outcomes of various atherosclerotic macrovascular diseases, such as acute coronary syndrome, myocardial infarction, coronary artery disease (CAD), heart failure (HF), stroke, peripheral arterial disease (PAD), and diabetic foot infections (4, 8–10, 13, 15, 16, 18, 21–27), all of which were closely related to DKD (28, 29). Given that chronic inflammation and metabolic disorder are involved in the pathogenesis of DKD, and that atherosclerotic macro- and microvascular diseases share multiple common pathogenetic pathways and risk factors, it is reasonable to hypothesize that T2DM individuals with high SII would have a high risk for DKD. Indeed, only a cross-sectional study from the National Health and Nutrition Examination Survey (NHANES) between 2011 and 2018 in the U.S. showed that higher SII level was associated with DKD among 3937 T2DM patients (4). However, it could not be determined whether there is a graded association between SII quartiles and risk of DKD and its distinct phenotypes, and whether SII could predict the presence of distinct phenotypes of DKD. Moreover, no study thus far has evaluated the associations between SII and DKD in China, where early onset of type 2 diabetes was reported and patients with T2DM have a higher prevalence of DKD, albuminuria, and a faster deterioration of renal function than their Caucasian counterparts in the U.S.

Therefore, to fill this gap in knowledge, we conducted a cross-sectional study to explore the association between SII and DKD and distinct phenotypes of DKD in Chinese adults with T2DM.

Methods

Study population

A total of 3514 adult inpatients with T2DM who had visited the hospital's department of endocrinology between August 2012 and September 2015 were initially selected. T2DM was defined as fasting blood glucose (FBG) ≥7.0 mmol/L, 2-h plasma glucose level on their oral glucose tolerance test (OGTT) ≥11.1 mmol/L, self-reported diagnosis of diabetes by a physician, or use of antidiabetic medications (30). All participants underwent the face-to-face questionnaire interview, systematic physical examinations, blood and urine sample collection, and diabetic complications examinations. The exclusion criteria were as follows: (1) type 1 diabetes mellitus, gestational diabetes, and other specific types of diabetes, acute diabetic complications; (2) non-diabetic kidney disease (such as membranous and IgA nephropathy, systemic lupus erythematosus, ANCA-associated vasculitis), recent history ofdialysis for acute kidney failure or a kidney transplant; (3) liver and gallbladder diseases; (4) inflammatory diseases, infectious disease, presence of stressful conditions (recent surgery, trauma); (5) symptomatic chronic heart failure, acute cardiovascular events (such as hospitalization for heart failure, myocardial infarction, andstroke within three months), severe respiratory failure; (6) autoimmune disease, immunosuppressant, use of systemic glucocorticoid; (7) thromboembolic disease, hematological system diseases; (8) malignant tumours; (9) pregnancy or lactation; and (10) missing baseline data and without available information. Subsequently, 1922 participants (975 men and 947 women) were included in the analysis.

This study was in accordance with the principles of the Declaration of Helsinki, and was approved by the Ethics Committee of the Affiliated Hospital of Southwest Medical University. All patients completed the signing of informed consent form before being enrolled.

General clinical and biochemical measurements

A face-to-face interview was carried out by well-trained interviewers to collect information on demographic data (sex, age), lifestyle factors (smoking status, alcohol consumption, etc.), personal medical history [coronary heart disease (CHD), stroke, and symptomatic PAD], medication history, and family history (diabetes, hypertension, etc.) with a standard questionnaire. Body weight and height were measured following standardized procedures, and body mass index (BMI) was calculated as weight divided by height squared (30, 31). The patients' systolic and diastolic blood pressure (SBP, DBP) were measured three times using a mercury sphygmomanometer while the subject in a sitting position for at least 5 min, and the mean value was recorded (21, 31). Mean arterial pressure (MAP) and pulse pressure (PP) were calculated: PP = SBP − DBP and MAP = DBP + (1/3) PP (32).

Biochemical indicators, including FBG, 2h postprandial blood glucose (PBG), total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein A (apoA), apolipoprotein B (apoB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), serum creatinine (Cr), glycated hemoglobin A1C (HbA1c), neutrophil count, lymphocyte count, hemoglobin (Hb) and platelet (PLT) count, were assayed through venous blood samples obtained in the morning after an overnight fast (≥ 8h). ApoB/A is the ratio between the concentrations of apoB and apoA. The SII was calculated as platelet × neutrophil/lymphocyte counts (4, 5, 9, 17, 21). The glycemic exposure (GE) index was calculated using the following equation: GE index = (HbA1c) $^{1/2}$x (duration of DM in years) $^{1/8}$ (33). Metabolic score for insulin resistance (METS-IR) was calculated as (ln [(2 × FBG) + TG)] × BMI)/(ln [HDL-C]) (FBG, TG, and HDL-C levels expressed as mg/dL and BMI as kg/m^2 in the equation) (34).

Assessment and diagnostic criteria of DKD

The estimated glomerular filtration rate (eGFR; mL/min/1.73 m^2) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation that includes age, sex, and race (4, 17, 31). Urinary albumin-to-creatinine ratio (ACR) was measured by random spot urine for three times with at least two positive results out of three tests (31). DKD was diagnosed with low eGFR (eGFR <60 mL/min/1.73 m^2), albuminuria (urinary ACR ≥30 mg/g), or both in T2DM patients (4, 31, 35).

Definitions of clinical variables

Patients were considered to have overweight/obesity when BMI ≥ 24 kg/m^2 (36). Hypertension was defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg and/or presence of anti-hypertensive drug treatment (8, 18, 36). Glycaemic control was assessed in terms of the HbA1c level and poor glycaemic control was defined as HbA1c ≥7% according to the American Diabetes Association (37). Dyslipidaemia was defined as either incident abnormal lipid laboratory results (comprised of TC >200 mg/dL, TG >150 mg/dL, LDL-C >130 mg/dL, or HDL-C <40 mg/dL) or incident lipid-lowering medications prescriptions (consisting of prescription of statins, bile acid resins, and fibrates) (18, 38). In accordance to the AHA/ACC 2018 cholesterol management guidelines, atherosclerotic cardiovascular disease (ASCVD) consisted of CHD (myocardial infarction, angina, or coronary revascularization), stroke (hemorrhagic and ischemic stroke), and symptomatic PAD (i.e., a history of PAD with claudication, gangrene or ulceration, peripheral artery revascularization, or major amputation secondary to PAD) (39, 40). DR was determined by using fundus photography (Canon Inc., Kanagawa, Japan), which was performed by an ophthalmologist (31).

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) (version 20.0; IBM, Chicago, IL). Continuous variables were expressed as mean ± standard deviation (SD), and compared by Student's t test, Mann-Whitney U, one-way analysis of variance (ANOVA), and Kruskal-Wallis H tests, while categorical variables were described using number (percentage), and compared by chi-squared test. The correlations between SII and other variables with significant differences were determined using Spearman's correlation and partial correlation analysis. The univariate and multivariable logistic regression analyses were conducted to investigate the association of SII and other variables with the risk of presence of DKD, reporting the data as odds ratio (OR) with a 95% confidence interval (CI). All participants were divided into DKD group and non-DKD (eGFR ≥60 mL/min/1.73 m^2 and UACR < 30 mg/g), and then DKD group was categorized into three subgroups: DKD-non-Alb (eGFR < 60 mL/min/1.73 m^2 and urinary ACR < 30 mg/g) subgroup; Alb DKD stage 3 (DKD stage 3 Alb, eGFR < 60 mL/min/1.73 m^2 and urinary ACR ≥ 30 mg/g) subgroup; and Alb DKD stages 1- 2 (DKD stages 1–2 Alb, eGFR ≥60 mL/min/1.73 m2 and urinary ACR ≥ 30 mg/g) subgroup (35, 41). DKD-non-Alb subgroup and DKD stage 3 Alb subgroup were merged into a group called DKD-non-Alb+DKD stage 3 Alb subgroup due to limited sample sizes of DKD-non-Alb subgroup (n=56). A multivariate logistic regression model was used to estimate ORs and 95% CIs for the association of SII as a continuous variable with DKD and different stages of DKD. Then, SII was classified into four quartiles, and the associations between SII quartiles and DKD and different stages of DKD was investigated, with the lowest quartile as the reference group. Last, the receiver operating characteristic (ROC) curves were constructed to evaluate the sensitivity and specificity of SII in predicting DKD, DKD stages 1–2 Alb, and DKD-non-Alb+DKD stage 3 Alb, and area under the curve (AUC) was estimated. A two-sided p-value<0.05 was deemed to be of statistical significance.

Results

Clinical and laboratory characteristics

A total of 1922 participants were enrolled, of whom 1063 (55.31%), 724 (37.67%), and 339 (17.64%) patients had DKD, DKD stages 1–2 Alb, and DKD-non-Alb+DKD stage 3 Alb, respectively. Table 1 and Figure 1 displayed the SII levels and other clinical and laboratory characteristics of the 3 evaluated groups. Among three groups, differences with statistical significance were observed in age, duration of diabetes, family history of diabetes, SBP, MAP, PP, HDL-C, apoA, apoB/A, FBG, PBG, HbA1c, GE index, neutrophil and lymphocyte count, SII, ALT, TBIL, Hb, serum Cr, eGFR, urinary ACR, prevalence of poor glycaemic, hypertension, dyslipidaemia, DR, and ASCVD (P<0.01 or P<0.05). The subgroup with eGFR <60 mL/min/1.73 m^2 (DKD-non-Alb and DKD stage 3 Alb) tended to be older, with a longer duration of diabetes, a higher SBP, PP, apoB/A, FBG, PBG, HbA1c, neutrophil count, SII, serum Cr, urinary ACR, prevalence of poor glycaemic, hypertension, and ASCVD, and a lower lymphocyte count, ALT, TBIL, Hb, and eGFR compared with the DKD stages 1–2 Alb and non-DKD subgroups (P<0.01 or P<0.05). Of note, SII levels in T2DM patients with DKD (DKD stages 1–2 Alb, and DKD-non-Alb and DKD stage 3 Alb) were significantly higher than those in T2DM patients with non-DKD (P<0.01; Figure 1). The DKD-non-Alb and DKD stage 3 Alb subgroup was less likely to have a family history of diabetes, tended to have a higher MAP, GE index, prevalence of dyslipidaemia and DR, and a lower apoA compared with the non-DKD subgroup (P<0.01 or P<0.05). Compared with the non-DKD subgroup, the DKD stages 1–2 Alb subgroup had a lower HDL-C (P<0.05). Supplementary Table 1 displayed the SII levels and other clinical characteristics in T2DM patients with Non-DKD and DKD. Compared with T2DM patients with non-DKD, those with DKD had significantly longer diabetic duration, higher age, SBP, MAP, PP, TG, apoB/A, METS-IR, GE index, neutrophil count, SII, serum Cr, urinary ACR, prevalence of hypertension, dyslipidaemia, DR, and ASCVD, and lower HDL-C, apoA, lymphocyte count, ALT, TBIL, Hb, and eGFR (P<0.01 or P<0.05).

The relationships between SII and DKD-related risk factors

We used Spearman correlation analysis to test the correlation between SII and cardiometabolic risk factors. The results showed that SII was positively associated with age, SBP, PP, apoB/A, FBG, PBG, HbA1c, GE index, neutrophil count, PLT count, serum Cr, urinary ACR, prevalence of low eGFR, albuminuria, DKD, poor glycaemic, and hypertension, and negatively with BMI, drinking, TC, TG, apoA, METS-IR, lymphocyte count, ALT, AST, TBIL, Hb, eGFR, and prevalence of overweight/obesity (P<0.01 or P<0.05; Table 2). Partial correlation analysis controlling for sex, age, BMI, and duration of diabetes demonstrated that SII was positively associated with apoB/A, FBG, PBG, HbA1c, GE index, METS-IR, neutrophil count, PLT count, serum Cr, urinary ACR, prevalence of low eGFR, albuminuria, DKD, and poor glycaemic, and inversely correlated with TC, HDL-C, lymphocyte count, TBIL, Hb, and eGFR (P<0.01 or P<0.05; Table 2).

Univariate and multivariate logistic analysis of factors associated with DKD

Table 3 showed univariable and multivariable analyses of factors associated with DKD. On univariable analysis, age, duration of diabetes, GE index, hypertension, dyslipidaemia, METS-IR, apoB, apoA, apoB/A, ALT, TBIL, Hb, SII, PP, DR, and ASCVD were significantly associated with DKD (P<0.01 or P<0.05). SII remained independently significantly associated with an increased risk of DKD on multivariable analysis (OR = 2.735, 95% CI 1.840-4.063; P<0.01).

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TABLE 1 Baseline characteristics of study participants stratified by DKD phenotype.

Characteristic	Non-DKD (n =859) (44.69%)	DKD stages 1–2 Alb (n =724) (37.67%)	DKD-non-Alb+DKD stage 3 Alb (n =339) (17.64%)	*P*
Male, n (%)	457 (53.20)	353 (48.76)	165 (48.67)	0.150
Age (years)	57.84 ± 10.93	61.18 ± 11.20**	67.06 ± 9.56**##	0.000
BMI (kg/m^2)	24.26 ± 3.46	24.35 ± 4.22	24.65 ± 3.63	0.276
Duration of diabetes (years)	6.41 ± 5.75	8.77 ± 6.34**	11.08 ± 7.25**##	0.000
Family history of diabetes, n (%)	241 (28.06)	192 (26.52)	67 (19.76)*	0.012
Family history of hypertension, n (%)	82 (9.55)	76 (10.50)	32 (9.44)	0.782
Smoking, n (%)	180 (20.95)	164 (22.65)	63 (18.58)	0.311
Drinking, n (%)	149 (17.35)	119 (16.44)	49 (14.45)	0.478
SBP (mmHg)	127.73 ± 19.73	135.87 ± 21.81**	143.37 ± 24.65**##	0.000
DBP (mmHg)	71.76 ± 11.97	73.15 ± 12.56	72.15 ± 14.64	0.069
MAP (mmHg)	90.42 ± 12.70	94.06 ± 13.42**	95.89 ± 16.00**	0.000
PP (mmHg)	55.98 ± 16.99	62.75 ± 19.42**	71.23 ± 20.07**##	0.000
TC (mmol/L)	4.75 ± 1.22	4.77 ± 1.45	4.82 ± 1.63	0.681
TG (mmol/L)	2.21 ± 2.40	2.47 ± 3.01	2.14 ± 1.48	0.061
HDL-C (mmol/L)	1.18 ± 0.35	1.14 ± 0.38*	1.17 ± 0.48	0.010
LDL-C (mmol/L)	2.74 ± 0.92	2.69 ± 1.02	2.81 ± 1.26	0.276
ApoA (g/L)	1.36 ± 0.35	1.26 ± 0.32**	1.26 ± 0.33**	0.000
ApoB (g/L)	0.88 ± 0.25	0.91 ± 0.35	0.92 ± 0.34	0.627
ApoB/A	0.69 ± 0.25	0.76 ± 0.36**	0.77 ± 0.35**##	0.000
FBG (mmol/L)	10.63 ± 4.77	11.13 ± 5.31	10.01 ± 6.24**##	0.000
PBG (mmol/L)	15.89 ± 5.20	16.49 ± 5.21	14.90 ± 4.53**##	0.000
HbA1c (%)	9.32 ± 2.44	9.62 ± 2.57	8.55 ± 2.39**##	0.000
GE index	3.47 ± 0.78	3.80 ± 0.77**	3.72 ± 0.75**	0.000
METS-IR	40.72 ± 8.64	42.06 ± 10.58	41.38 ± 8.81	0.105
Neutrophil (*10^9/L)	4.18 ± 1.89	4.89 ± 2.51**	5.63 ± 3.16**##	0.000
Lymphocyte (*10^9/L)	1.71 ± 0.67	1.61 ± 0.61**	1.38 ± 0.56**##	0.000
PLT (×10^9/L)	194.47 ± 64.27	208.58 ± 87.29	202.96 ± 83.58	0.118
SII	563.07 ± 18.43	807.95 ± 36.19**	1070.36 ± 74.42**##	0.000
ALT (U/L)	25.37 ± 24.25	23.81 ± 27.38**	19.16 ± 14.48**##	0.000
AST (U/L)	22.38 ± 16.68	23.68 ± 23.60	22.47 ± 26.04	0.435
TBIL (μmol/L)	13.21 ± 5.77	11.87 ± 5.98**	9.80 ± 4.62**##	0.000
Hb (g/L)	134.82 ± 17.12	124.99 ± 19.32**	108.41 ± 22.18**##	0.000
Serum Cr (μmol/L)	60.52 ± 15.85	65.26 ± 18.52**	194.81 ± 146.56**##	0.000
eGFR (mL/min/1.73 m^2)	101.25 ± 16.60	94.44 ± 20.15**	37.10 ± 16.27**##	0.000
Urinary ACR (mg/g)	13.34 ± 7.59	37.93 ± 31.00**	153.64 ± 11.30**#	0.000

(Continued)

TABLE 1 Continued

Characteristic	Non-DKD (n =859) (44.69%)	DKD stages 1–2 Alb (n =724) (37.67%)	DKD-non-Alb+DKD stage 3 Alb (n =339) (17.64%)	*P*
Overweight/obesity, n (%)	411 (47.85)	348 (48.07)	154 (45.43)	0.321
Poor glycaemic control, n (%)	707 (82.31)	616 (85.08)	234 (69.03)**##	0.000
Hypertension, n (%)	371 (43.19)	451 (62.29)**	283 (83.48)**##	0.000
Dyslipidaemia, n (%)	578 (67.29)	512 (70.72)	257 (75.81)*	0.016
DR, n (%)	69 (8.03)	116 (16.02)**	67 (19.76)**	0.000
ASCVD, n (%)	211 (24.56)	259 (35.77)**	171 (50.44)**##	0.000

Data are mean ± SD. SD, standard deviation; DKD, Diabetic kidney disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; apoA, apolipoprotein A; apoB, apolipoprotein B; apoB/A, apolipoprotein B-to-apolipoprotein A ratio; FBG, fasting blood glucose; PBG, 2 hour postprandial blood glucose; HbA1c, glycated hemoglobin A1c; GE index, glycemic exposure index; METS-IR, metabolic score for insulin resistance; PLT, platelet; SII, systemic immune-infammation index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TBIL, total bilirubin; Hb, hemoglobin; Cr, creatinine; eGFR, estimated glomerular filtration rate; ACR, albumin- to-creatinine ratio; DR, diabetic retinopathy; ASCVD, atherosclerotic cardiovascular disease. vs. non-DKD: *P< 0.05, **P< 0.01, vs. Alb DKD stages 1- 2: #P< 0.05, ##P< 0.01.

Adjusted ORs and 95% CIs for DKD, DKD stages 1–2 Alb, and DKD-non-Alb+DKD stage 3 Alb according to SII quartiles

Multivariate logistic regression analysis showed that the SII, whether considered as a categorical or continuous variable, remained significant after adjusting for confounders (Table 4). As a continuous variable, SII was associated with a 4.2-fold increased risk of DKD in the partially adjusted regression model 1, and a 2.8-fold increased risk of DKD in fully adjusted model 4. As a categorical variable, compared with subjects in the lowest quartile (Q1), the partially adjusted OR for DKD was 1.134 (95% CI 0.859-1.496) in the second quartile (Q2), 1.233 (95% CI 1.072-1.417) in the third quartile (Q3), and 1.407 (95% CI 1.276-1.553) in the highest quartile (Q4), respectively. The increased risk of DKD from Q1 to Q4 was statistically significant (*P* for trend<0.01). A similar pattern was observed in fully adjusted model 4 (Q2: OR=1.060, 95% CI 0.773-1.455; Q3: OR=1.167, 95% CI 0.995-1.368; Q4: OR=1.266, 95% CI 1.129-1.420; *P* for trend <0.01). Moreover, we further divided DKD group into two subgroups: DKD stages 1–2 Alb subgroup and DKD-non-Alb+DKD stage 3 Alb subgroup, and studied the associations between the SII and different stages of DKD. The result revealed that SII was associated with a 2.3-fold increased risk of DKD stages 1–2 Alb ($P < 0.01$), and there was a graded association with DKD stages 1–2 Alb and increase in SII quartiles infully adjusted model 4 (*P* for trend < 0.01). Participants in the Q4 of SII had a significantly higher risk of DKD stages 1–2 Alb compared with those in the Q1 (OR = 1.211, 95% CI 1.073-1.366). Similarly, SII was associated with a 4.2-fold increased risk of DKD-non-Alb+DKD stage 3 Alb ($P < 0.01$), and the increased risk of DKD-non-Alb+DKD stage 3 Alb from Q1 to Q4 was also statistically significant in fully adjusted model 4 (*P* for trend < 0.01).

Predictive value of SII in screening for the presence of DKD in T2DM patients

To further explore the predictive value of SII for DKD and different stages of DKD, the ROC curve analysis was performed. As

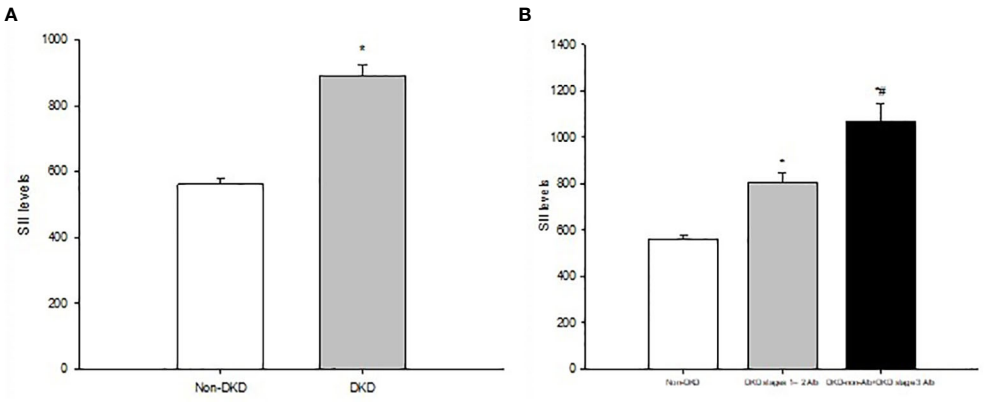

FIGURE 1
(A) Systemic immune-inflammatory index (SII) levels in T2DM patients with non-DKD and DKD. **(B)** SII levels in T2DMpatients with non-DVD Alb DKD stages 1-2, and DKD-non-Alb+DKD stage 3 Alb. Data are expressed as means ± SD. *P<0.01 vs. non-DKD, *P<0.01 vs. Alb DKD stages 1-2.

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TABLE 2 The relationships between SII and DKD- related risk factors.

Variables	*r*	*P*-value	Adjusted *r*	Adjusted *P*-value
Age	0.100	0.000		
Sex	0.041	0.071		
Duration of diabetes	0.020	0.381		
BMI	-0.127	0.000		
Family history of diabetes	-0.020	0.381	0.007	0.772
Family history of hypertension	-0.008	0.726	0.009	0.721
Smoking	-0.013	0.571	-0.009	0.729
Drinking	-0.056	0.014	-0.031	0.229
SBP	0.056	0.015	-0.008	0.746
DBP	-0.035	0.124	-0.023	0.365
MAP	0.011	0.619	-0.006	0.816
PP	0.085	0.000	0.017	0.496
TC	-0.098	0.000	-0.060	0.019
TG	-0.127	0.000	-0.009	0.737
HDL-C	0.002	0.948	-0.109	0.000
LDL-C	-0.004	0.846	-0.011	0.653
ApoA	-0.157	0.000	-0.253	0.000
ApoB	0.005	0.824	0.018	0.475
ApoB/A	0.116	0.000	0.226	0.000
FBG	0.113	0.000	0.165	0.000
PBG	0.103	0.000	0.105	0.000
HbA1c	0.087	0.000	0.119	0.000
GE index	0.084	0.000	0.097	0.000
METS-IR	-0.096	0.000	0.125	0.000
Neutrophil	0.722	0.000	0.705	0.000
Lymphocyte	-0.468	0.000	-0.329	0.000
PLT	0.548	0.000	0.447	0.000
ALT	-0.204	0.000	-0.023	0.366
AST	-0.211	0.000	0.020	0.432
TBIL	-0.191	0.000	-0.075	0.003
Hb	-0.221	0.000	-0.225	0.000
Serum Cr	0.165	0.000	0.104	0.000
eGFR	-0.202	0.000	-0.132	0.000
Urinary ACR	0.226	0.000	0.104	0.000
Low eGFR	0.199	0.000	0.133	0.000
Albuminuria	0.204	0.000	0.157	0.000
DKD	0.220	0.000	0.166	0.000
DR	0.014	0.547	-0.032	0.216

(Continued)

TABLE 2 Continued

Variables	*r*	*P*-value	Adjusted *r*	Adjusted *P*-value
Overweight/obesity	-0.119	0.000	-0.034	0.157
Poor glycaemic control	0.071	0.002	0.091	0.000
Dyslipidaemia	-0.020	0.394	0.004	0.872
Hypertension	0.090	0.000	0.022	0.398
ASCVD	0.035	0.121	0.035	0.168

shown in Figure 2, the best cut-off value of SII was 609.85 for predicting DKD (sensitivity: 48.3%; specificity: 72.8%; and AUC: 0.627; Figure 2A), 601.71 (sensitivity: 43.9%; specificity: 72.3%; and AUC: 0.596; Figure 2B) for predicting DKD stages 1–2 Alb, and 589.27 (sensitivity: 61.1%; specificity: 71.1%; and AUC: 0.695; Figure 2C) for predicting DKD-non-Alb+DKD stage 3 Alb.

Discussion

The current study was the first to investigate the associations of SII, as an indicator of inflammatory marker, with the presence of DKD and its distinct phenotypes in Chinese population with T2DM. We discovered that moving from the non-DKD group to

TABLE 3 Univariate and multivariate logistic analysis of factors associated with DKD.

Variables	Univariate analysis			Multivariate analysis		
	B	OR (95%CI)	*P*-value	B	OR (95%CI)	*P*-value
Female sex	0.179	1.196 (0.999-1.432)	0.051	-0.435	0.648 (0.501-0.837)	0.001
Age	0.043	1.044 (1.035-1.053)	0.000			
BMI	0.012	1.012 (0.987-1.037)	0.338			
Duration of diabetes	0.081	1.084 (1.067-1.101)	0.000	0.035	1.036 (1.010-1.063)	0.007
Family history of diabetes	-0.191	0.826 (0.673-1.013)	0.067			
Family history of hypertension	0.069	1.072 (0.792-1.450)	0.654			
Smoking	0.024	1.024 (0.822-1.277)	0.831			
Drinking	-0.112	0.894 (0.703-1.139)	0.365			
HbA1c	-0.006	0.994 (0.959-1.030)	0.726			
GE index	0.501	1.650 (1.463-1.861)	0.000	0.197	1.218 (1.011-1.467)	0.038
Hypertension	1.077	2.935 (2.433-3.540)	0.000	0.590	1.804 (1.386-2.347)	0.000
Dyslipidaemia	0.245	1.277 (1.045-1.561)	0.017			
METS-IR	0.013	1.013 (1.003-1.024)	0.013	0.023	1.023 (1.009-1.038)	0.001
apoA	-0.912	0.402 (0.298-0.541)	0.000	-1.003	0.367 (0.175-0.770)	0.008
apoB	0.320	1.377 (1.014-1.868)	0.040	1.298	3.661 (1.329-10.086)	0.012
apoB/A	0.908	2.479 (1.772-3.468)	0.000			
ALT	-0.006	0.994 (0.990-0.999)	0.009			
AST	0.002	1.002 (0.998-1.007)	0.353			
TBIL	-0.065	0.937 (0.920-0.954)	0.000	-0.021	0.979 (0.959-0.999)	0.036
Hb	-0.041	0.960 (0.955-0.965)	0.000	-0.036	0.965 (0.957-0.972)	0.000
SII	1.525	4.597 (3.361-6.288)	0.000	1.006	2.735 (1.840-4.063)	0.000
PP	0.027	1.028 (1.023-1.033)	0.000			
DR	0.867	2.381 (1.776-3.192)	0.000	0.573	1.773 (1.247-2.523)	0.001
ASCVD	0.735	2.086 (1.712-2.543)	0.000	0.312	1.366 (1.051-1.774)	0.020

B is the standardized coefficient and measures the influence of each variables on DKD; OR is the odds ratio and refers to the risk of DKD.

TABLE 4 Adjusted ORs and 95% CI for DKD according to SII quartiles.

SII	OR (95% CI)			
	Model 1	Model 2	Model 3	Model 4
DKD				
Continuous	4.234 (3.002-5.973)**	3.389 (2.328-4.933)**	2.681 (1.793-4.010)**	2.780 (1.855-4.166)**
Categories				
Q1	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Q2	1.134 (0.859-1.496)	1.086 (0.809-1.459)	1.076 (0.788-1.470)	1.060 (0.773-1.455)
Q3	1.233 (1.072-1.417)**	1.179 (1.014-1.371)*	1.161 (0.991-1.359)	1.167 (0.995-1.368)
Q4	1.407(1.276-1.553)**	1.342 (1.206-1.494)**	1.263 (1.128-1.414)**	1.266 (1.129-1.420)**
P for trend	0.000	0.000	0.000	0.000
DKD stages 1–2 Alb				
Continuous	3.391 (2.351-4.891)**	2.642 (1.766-3.952)**	2.197 (1.438-3.357)**	2.289 (1.494-3.506)**
Categories				
Q1	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Q2	1.062 (0.793-1.423)	1.013 (0.743-1.382)	1.028 (0.746-1.419)	1.023 (0.738-1.417)
Q3	1.138 (0.980-1.320)	1.095 (0.933-1.286)	1.089 (0.923-1.285)	1.095 (0.927-1.293)
Q4	1.326 (1.195-1.471)**	1.265 (1.129-1.417)**	1.205 (1.070-1.357)**	1.211 (1.073-1.366)**
P for trend	0.000	0.000	0.002	0.002
DKD-non-Alb+DKD stage 3 Alb				
Continuous	9.323 (5.393-16.116)**	7.273 (3.982-13.284)**	3.966 (2.003-7.854)**	4.220 (2.118-8.409)**
Categories				
Q1	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Q2	1.606 (0.965-2.674)	1.664 (0.957-2.895)	1.514 (0.745-3.081)	1.457 (0.709-2.993)
Q3	1.648 (1.293-2.101)**	1.560 (1.194-2.037)**	1.537 (1.124-2.101)**	1.541 (1.125-2.110)**
Q4	1.684 (1.435-1.977)**	1.582 (1.324-1.892)**	1.414 (1.148-1.740)**	1.439 (1.164-1.779)**
P for trend	0.000	0.000	0.001	0.000

Logistic regression analysis with DKD group and each DKD subgroup in contradistinction to non-DKD group to investigate the association between SII quartiles and DKD stages 1–2 Alb or DKD-non-Alb+DKD stage 3 Alb. Data are expressed as OR (95% CI), unless stated otherwise. OR, odds ratio; CI, confidence interval.
Model 1 adjusted for sex, age, BMI, duration of diabetes, family history of diabetes, family history of hypertension, smoking, drinking;
Model 2 adjusted for factors listed in Model 1 plus HbA1c, GE, hypertension, dyslipidaemia, apoA, apoB, apoB/A, METS-IR;
Model 3 adjusted for factors listed in Model 2 plus ALT, AST, TBIL, Hb;
Model 4 adjusted for factors listed in Model 3 plus PP, DR, ASCVD;
*P < 0.05.
**P < 0.01.

the DKD-non-Alb+DKD stage 3 Alb group, SII level was gradually increased, and SII remained independently significantly associated with the presence of DKD after adjustment for confounding factors. Additionally, the risk of DKD and its distinct phenotypes increased progressively across SII quartiles. Last, the ROC curve analysis revealed that SII could predict DKD and its distinct phenotypes. Our finding suggested that SII may be a useful biomarker of DKD and its distinct phenotypes, and high SII may be associated with an increased risk of DKD and its distinct phenotypes in Chinese adults with T2DM.

As mentioned earlier, SII is a relatively novel inflammation biomarker based on peripheral lymphocyte, neutrophil, and platelet counts in clinical applications, which largely reflects three pathways of inflammatory response, thrombus formation and adaptive immune response in the host (5, 22, 27, 42). In the last decade, SII has been demonstrated to predict the prognosis and clinical outcomes of various malignant tumors types, certain inflammatory and autoimmune diseases, atherosclerosis, cardio-cerebrovascular diseases (5, 8–16, 22, 23, 25, 27). Recently, some studies have reported the association between SII and kidney diseases with

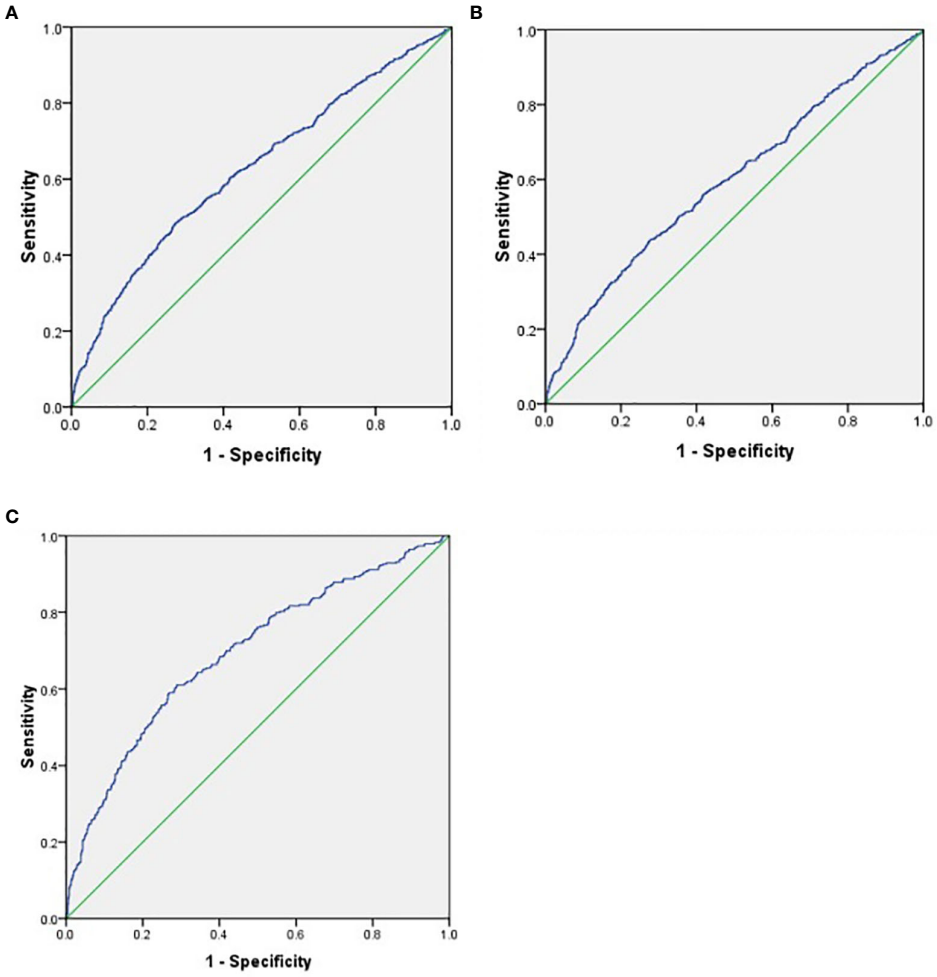

FIGURE 2
(A) ROC analysis of systemic immune-inflammatory index (SII) to indicate DKD. AUC = 0_627; 95% CI: 0.603–0.652; *P*<0.01; identified SII cutoff value = 609.85; Youden index = 0.211; sensitivity: 48.3%; specificity: 72.8%. **(B)** ROC analysis of indicate Alb DVD stages 1-2. AUC = 0.596; CI: 0.568–0.624; *P* <0.01; identified SII cutoff value = 601.71; Youden index = 0.162; sensitivity: 43.9%; specificity: 72.3%. **(C)** ROC analysis of SII to indicate DKD-non-Alb+DKD stage 3 Alb. AUC = 0.695; 95% CI: 0.661–0.729; *P*<0.01; identified SII cutoff value = 589.27; Youden index = 0.322; sensitivity: 61.1%; specificity: 71.1%.

varying epidemiological methods and target populations (5, 43–46). More recently, SII has been reported to be associated with metabolic disorder, such as metabolic syndrome, obesity, hepatic steatosis, dyslipidemia and hypertension, and diabetic vascular complications, such as diabetic retinopathy, diabetic macular edema, peripheral arterial disease, and diabetic foot infections (15–19, 22, 24–27, 47, 48). Considering the role of SII in the development and progression of autoimmune and inflammatory diseases and kidney diseases, and the close relationship between DKD and metabolic disorder, diabetic vascular complications, it seems appropriate that SII may be associated with DKD, and high SII may be an early signal for being at risk for DKD. In the present study, we found that SII levels were significantly. higher in T2DM patients with DKD than those with non-DKD, and SII levels were further increased in T2DM patients with DKD-non-Alb and DKD stage 3 Alb compared to those with DKD stages 1–2 Alb, suggesting that SII might be related to DKD and its distinct phenotypes. Moreover, SII was positively associated with serum Cr, urinary ACR, an early hallmark of DKD, prevalence of low eGFR, albuminuria, and DKD, and inversely with eGFR. In addition, the multivariate logistic regression analysis showed that SII had an independent positive correlation with DKD in T2DM patients, and ROC curve analysis revealed that the SII could effectively predict the presence of DKD. These findings were consistent with a previous cross-sectional study from the NHANES in the U.S. that reported that a high SII level was associated with increased likelihood of DKD among 3937 T2DM patients (4). All the above findings suggested that a higher SII was significantly associated with an increased risk of having DKD, which supported the hypothesis that SII may serve as a promising biomarker for identifying patients at a higher risk of DKD. However, it could not be determined whether there is a graded association between SII quartiles and risk of presence of DKD and its distinct phenotypes, and whether SII could predict the presence of distinct phenotypes of DKD. Our study filled this gap and demonstrated for the first time that the risk of prevalence of DKD increased progressively across SII quartiles,

and participants in the highest quartile was at a significantly increased risk of prevalent DKD compared to those in the lowest quartile, even after adjusting for potential confounding factors. A similar pattern was observed regarding the association of SII quartiles with distinct phenotypes of DKD, including DKD stages 1–2 Alb, and DKD-non-Alb+DKD stage 3 Alb. Besides, SII was found to effectively predict the presence of distinct phenotypes of DKD, especially DKD-non-Alb+DKD stage 3 Alb, with good sensitivity and specificity. Such discoveries suggested that high SII could be linked to an increased risk of DKD and its distinct phenotypes, and SII might be a potential indicator for identifying Chinese patients with T2DM at a higher risk of DKD and its distinct phenotypes.

Growing evidence has implicated the role of chronic inflammation andoxidative stress in the pathogenesis of DKD (49, 50), and inflammation and oxidative stress may be considered as a hub of the different pathogenic pathways that contribute to DKD (51). The imbalance of of several pro-and anti-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), C reactive protein (CRP), neutrophils, lymphocytes, and platelet cells, and pro- and anti-oxidants, such as superoxide dismutase (SOD), malondialdehyde (MDA), and 8-OHdG-8-hydroxy-2′-deoxyguanosine (8-OHdG) have previously been reported to be related to the development of DKD (4, 5, 49, 52, 53). Hb, an iron-containing protein in blood, may serve as a nitricoxide scavenger, and its ability of Hb to bind the main low-molecular-weight thiol of the cell glutathione, both covalently and noncovalently, is not only an important part of the antioxidant protection of red blood cells, but also affects its affinity for oxygen in both cases (54, 55). It has been reported that decreased Hb was correlated with a higher incidence of rapid renal function decline, and could predict the development and progression of DKD (56).

Bilirubin, a byproduct of normal Hb breakdown that plays an important physiologic role as a strong antioxidant and anti-inflammatory molecule through efficiently scavenging of peroxyl radicals and suppression of oxidation, inhibiting platelets, and regulating immunity (57), has been demonstrated to be involved in the development and progression of DKD (58). The present study provided evidence that inflammation and oxidative stress correlated with DKD and its distinct phenotypes, since we found that T2DM patients with DKD had significantly higher neutrophil count, and lower lymphocyte count, TBIL, and Hb than those with non-DKD, and neutrophil count was further increased and lymphocyte count, TBIL, and Hb were further decreased in T2DM patients with DKD-non-Alb and DKD stage 3 Alb compared to those with DKD stages 1–2 Alb, and TBIL and Hb were independently significantly associated with the presence of DKD. In addition, we demonstrated that SII was positively associated with neutrophil and PLT count, and inversely correlated with lymphocyte count, TBIL, and Hb, which was consistent with previous studies that reported that the subjects with higher quartile of SII have significantly higher neutrophil count, PLT count, and lower lymphocyte count, Hb and TBIL than those with lower quartiles, and SII levels were positively related to CRP, erythrocyte sedimentation rate, and 8-OHdG in patients with tumors, hypertension, diabetic foot infections, systemic lupus erythematosus, and alveolar hydatid disease (11, 22, 24, 59–62), suggesting that SII may be associated with chronic inflammation and oxidative stress, and chronic inflammation and oxidative stress might at least partially mediate the potential relationship between SII and DKD; However, the mechanism of action needs to be further investigated.

Evidence to date has suggested that hyperglycaemia, hypertension, dyslipidaemia, and IR are important risk factors for DKD (49, 63). Hyperglycemia is a widely accepted modifiable risk factor for the initiation and promotion of DKD through triggering three cardinal and inter-related pathways including overproduction of ROS, activation of apoptotic pathway and initiation of autophagy, especially in people with poor glycemic control (64). Elevated blood pressure and dyslipidemia were also identified as other major modifiable risk factors associated with the development and progression of DKD in T2DM individuals (64). Some studies have found that antihypertensive and lipid-lowering therapy can reduce the risk of albuminuria, kidney function decline, and progression to ESRD (35, 65). It has been recognized that IR is closely interrelated with hyperglycaemia, hypertension, and dyslipidaemia, and can increase the hydrostatic pressure of the glomerulus and permeability of the renal vessels, leading to glomerular hyperfiltration and subsequently microalbuminuria and DKD (66, 67). The apoB/apoA ratio has been reported to be significantly associated with IR in certain population including Chinese population, independent of traditional risk factor (68). Chronic glycemic exposure (the degree and duration of plasma hyperglycemia), as reflected by GE index, is thought to be the important modifiable risk covariate for diabetic complications (69). Our study provided further evidence that supported the potential role of hyperglycemia, hypertension, dyslipidaemia, and IR in the pathogenesis of CKD, since we found that the subjects with DKD had significantly higher SBP, MAP, TG, apoB/A, METS-IR, GE index, prevalence of hypertension, and dyslipidaemia, and lower HDL-C and apoA, and GE index, apoB, apoA, hypertension, and METS-IR, a novel inexpensive and reliable surrogate indicator of IR (34), remained independently significantly associated with the presence of DKD after adjustment for confounding factors. Moreover, partial correlation analysis controlling for sex, age, BMI, and duration of diabetes demonstrated that SII was positively associated with apoB/A, FBG, PBG, HbA1c, GE index, poor glycaemic control, METS-IR, and inversely correlated with TC and HDL-C, suggesting that SII might be correlated with metabolic disorders, especially IR, hyperglycemia, and dyslipidaemia, and metabolic disorders might at least partially mediate the relationship between SII and DKD. Our findings were consistent with previous studies that reported the potential relationship between SII and metabolic disorders (70–79). Some studies have demonstrated that the subjects with the highest quartile of SII have significantly higher homeostatic model assessment index of IR (HOMA-IR) than those with the lower quartiles in perimenopausal and postmenopausal women (70, 71), and the U.S. general population (72), and SII was positively associated with HOMA-IR in children with obesity (15). Furthermore, Yang and colleagues also found that CAD patients after coronary intervention with SII ≥ 694.3 had significantly higher glucose

levels and rate of diabetes, and lower lipid profiles than those with SII < 694.3 in Taiwan (73). Similar results were reported in 9107 critically ill patients with HF from the Medical Information Mart for Intensive Care III (MIMIC III) database (74). SII was also considerably higher in patients with low HDL-C in rural areas from the Northeast China Rural Cardiovascular Health Study (NCRCHS) (17). However, some investigations have suggested significant opposite or no association between SII and dyslipidaemia, lipid profiles, and diabetes and its related indexes (FBG, HbA1c) in patients with different pathogenic conditions, such as hypertension, ASCVD, acute coronary syndrome patients with CKD, myocardial infarction, HF, and acute ischemic stroke (74–79). The discrepancies between the above-mentioned studies and ours may be due to the differences in study design and population characteristics, diabetic duration, races, regions, dietary habits, sample size, statistical methods, diagnostic methods for Dyslipidaemia and diabetes, and confounding factors adjusted. Large-scale and multi-center longitudinal studies are warranted to confirm the role of metabolic disorders in the relationship between SII and DKD.

Limitations of our study must be appreciated for an accurate interpretation of the data. First, we cannot infer causal associations between SII and DKD due to the cross-sectional design of this study. Further prospective studies are required to confirm this association. Second, we did not obtain information on educational level, dietary habits, lifestyle (smoking, drinking, and exercise), and consumption of various medicines, such as hypoglycemic agents, antihyperlipidemic drugs, anti-hypertension drugs, and antiplatelet drugs, which may have reduced our ability to explore other risks or protective factors. Third, participants in our study were from a single center and the great majority of them were middle-age or elderly, and hospitalized for relative poor glycemic control, its generalizability should be verified by involving outpatients or community patients in the future. Fourth, DKD-non-Alb subgroup and DKD stage 3 Alb subgroup were combined into a group called DKD-non-Alb+DKD stage 3 Alb group due to limited sample sizes of DKD-non-Alb subgroup. Therefore, prospective studies with larger sample size of DKD and each distinct phenotypes of DKD are still required to clarify the associations between SII and DKD and its distinct phenotypes. Last, the lack of classical inflammation and oxidative stress markers, such as TNF-α, IL-6, CRP, SOD, MDA, and 8-OHdG, make it difficult to further explore the association mechanism of SII and inflammation and oxidative stress in T2DM patients with DKD. Despite these limitations, our analyses also have some noteworthy strength. A key finding was that our study was the first study to assess the association of SII with DKD and its distinct phenotypes in Chinese patients with T2DM, which may provide additional information to identify those at risk for DKD and its distinct phenotypes, and thereby potentially institute earlier therapies. In addition, we recruited a relatively large clinical sample of patients with T2DM and performed this study with a comprehensive and standardized clinical assessment protocol, which can raise the reliability of our findings.

In conclusion, our data delineate that T2DM patients with DKD had significantly higher SII levels, and its levels were gradually increased moving from the non-DKD group to the DKD-non-Alb +DKD stage 3 Alb group. Moreover, SII was independently significantly associated with the presence of DKD and its distinct phenotypes after adjustment for confounding factors. These findings indicate that SII could be potentially used as an easy biomarker to identify those patients at high risk for DKD and its distinct phenotypes that further can help in choosing effective treatment options to delay the development and progression of DKD. However, further research is needed to perform for exploring their exact underlying mechanisms between SII and DKD in Chinese adults with T2DM.

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 humans were approved by the Affiliated Hospital of Southwest Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

PY: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. YY: Data curation, Formal analysis, Investigation, Writing – original draft. XZ: Data curation, Formal analysis, Investigation, Writing – original draft. YZ: Data curation, Formal analysis, Investigation, Writing – original draft. JL: Data curation, Formal Analysis, Investigation, Writing – original draft. ZW: Data curation, Formal analysis, Investigation, Writing – original draft. XD: Data curation, Project administration, Writing – original draft. XW: Data curation, Project administration, Writing – original draft. XC: Data curation, Project administration, Writing – original draft. SL: Data curation, Project administration, Writing – original draft. YX: Data curation, Project administration, Writing – original draft. QW: Data curation, Project administration, Writing – original draft.

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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.2023.1307692/full#supplementary-material

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Association between neutrophil-to-lymphocyte ratio and diabetic kidney disease in type 2 diabetes mellitus patients: a cross-sectional study

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Aims: This investigation examined the possibility of a relationship between neutrophil-to-lymphocyte ratio (NLR) and diabetic kidney disease (DKD) in type 2 diabetes mellitus (T2DM) patients.

Methods: Adults with T2DM who were included in the National Health and Nutrition Examination Survey (NHANES) between 1999 and 2020 were the subjects of the current cross-sectional investigation. Low estimated glomerular filtration rate (eGFR) (< 60 mL/min/1.73 m^2) or albuminuria (urinary albumin-to-creatinine ratio (ACR) ≥ 30 mg/g) in T2DM patients were the diagnostic criteria for DKD. Weighted multivariable logistic regression models and generalized additive models were used to investigate the independent relationships between NLR levels with DKD, albuminuria, and low-eGFR. Additionally, we examined the relationships between DKD, albuminuria, and low-eGFR with other inflammatory markers, such as the aggregate index of systemic inflammation (AISI), systemic immune-inflammation index (SII), system inflammation response index (SIRI), and platelet-to-lymphocyte ratio (PLR) and monocyte-to-lymphocyte ratio (MLR). Their diagnostic capabilities were evaluated and contrasted using receiver operating characteristic (ROC) curves.

Results: 44.65% of the 7,153 participants who were recruited for this study were males. DKD, albuminuria, and low-eGFR were prevalent in 31.76%, 23.08%, and 14.55% of cases, respectively. Positive correlations were seen between the NLR with the prevalences of DKD, albuminuria, and low-eGFR. Subgroup analysis and interaction tests revealed that the associations of NLR with DKD, albuminuria, and low-eGFR were not significantly different across populations. In addition, MLR, SII and SIRI showed positive associations with the prevalence of DKD. ROC analysis discovered that when compared to other inflammatory markers (MLR, PLR, SII, SIRI, and AISI), NLR may

demonstrate more discriminatory power and accuracy in assessing the risk of DKD, albuminuria, and low-eGFR.

Conclusion: Compared to other inflammatory markers (MLR, PLR, SII, SIRI, and AISI), NLR may serve as the more effective potential inflammatory marker for identifying the risk of DKD, albuminuria, and low-eGFR in US T2DM patients. T2DM patients with elevated levels of NLR, MLR, SII, and SIRI should be closely monitored for their potential risk to renal function.

KEYWORDS

neutrophil-to-lymphocyte ratio, type 2 diabetes mellitus, diabetic kidney disease, population-based study, NHANES

1 Introduction

Diabetic kidney disease (DKD), which accounts for more than 50% of all instances of end-stage kidney disease (ESKD), has emerged as the most prevalent chronic kidney disease (CKD) worldwide due to the increased prevalence of type 2 diabetes mellitus (T2DM) associated with obesity (1–3). Even in the early stages of DKD, patients are more susceptible to cardiovascular illness (4). Recent studies have revealed that people with DKD have a greater risk of dying after developing coronavirus disease 2019 (COVID-19) (5). Thus, early intervention is necessary to stop DKD from progressing. Previous studies have identified inflammation, obesity, hypertension, smoking, and sex as significant risk factors for DKD (6–8). Among these, inflammation has drawn interest as a modifiable risk factor that may offer preventative options.

Chronic inflammation is considered a potential mechanism underlying DKD (9–11). However, the use of many inflammatory markers in routine clinical practice has been limited due to their cost and measurement-related technical problems. It is notable that the neutrophil-to-lymphocyte ratio (NLR), a laboratory index that is simple to measure and reasonably priced and is derived from routinely analyzed leukocyte characteristics, integrates the detrimental effects of neutrophils on endothelial damage with the antiatherosclerotic function of lymphocytes (12). In light of this, the NLR has been regarded as a practical biomarker of systemic inflammation (13, 14). The relationship between NLR and DKD has been researched in earlier studies. NLR levels were discovered by Wan et al. to be positively correlated with ACR levels and the prevalence of DKD in Chinese diabetic patients (15). NLR has been reported to be a reliable predictor of early DKD in a prospective study from Egypt (16). In a cohort of Japanese diabetics, higher NLR levels were connected to a higher frequency of albuminuria (17). To our knowledge, no study has examined how NLR is related to DKD in the US diabetic population.

Therefore, this study aims to examine this association between NLR and DKD using data from the National Health and Nutrition Examination Survey (NHANES) in adult T2DM patients in the United States.

2 Methods

2.1 Study population and participants selected

NHANES, conducted by the National Center for Health Statistics (NCHS), provides valuable cross-sectional data for research purposes (18). It is used to assess the physical and nutritional well-being of the non-institutionalized population in the US. Every two years, the NHANES survey data are updated to ensure that it is always up to date. Utilizing a stratified multi-stage probabilistic technique, the NHANES study design produces a sizable representative sample of enrolled individuals. The protocols for the NHANES survey have been approved by the NCHS research ethics review committee, and all study participants have provided informed consent. For more comprehensive information regarding the planning and execution of NHANES, please refer to the official NHANES website.

We utilized data from NHANES spanning the years 1999 to 2020 to select participants for our study. We eliminated those under the age of 20 (n = 48,975), those with cancer (n = 1,158), those in pregnancy (n = 204), and those lacking information on ACR (n = 8,506), eGFR (n = 16,013), and NLR (n = 15,197) after a stringent selection process. Furthermore, those without T2DM (n = 19,339) were not included in the study. After applying these exclusion criteria, our final cohort consisted of 7,153 eligible subjects (Figure 1).

2.2 Definition of NLR

Venous blood samples were collected in the morning after a fasting period to conduct routine clinical chemistry analysis. Using the Coulter counter method, we obtained the neutrophil count (NC) and lymphocyte count (LC) from the whole blood. The ratio of NC to LC was then used to calculate the NLR (19). We also looked at the associations between DKD and other inflammatory markers, such as the platelet-to-lymphocyte ratio (PLR) (PLR=

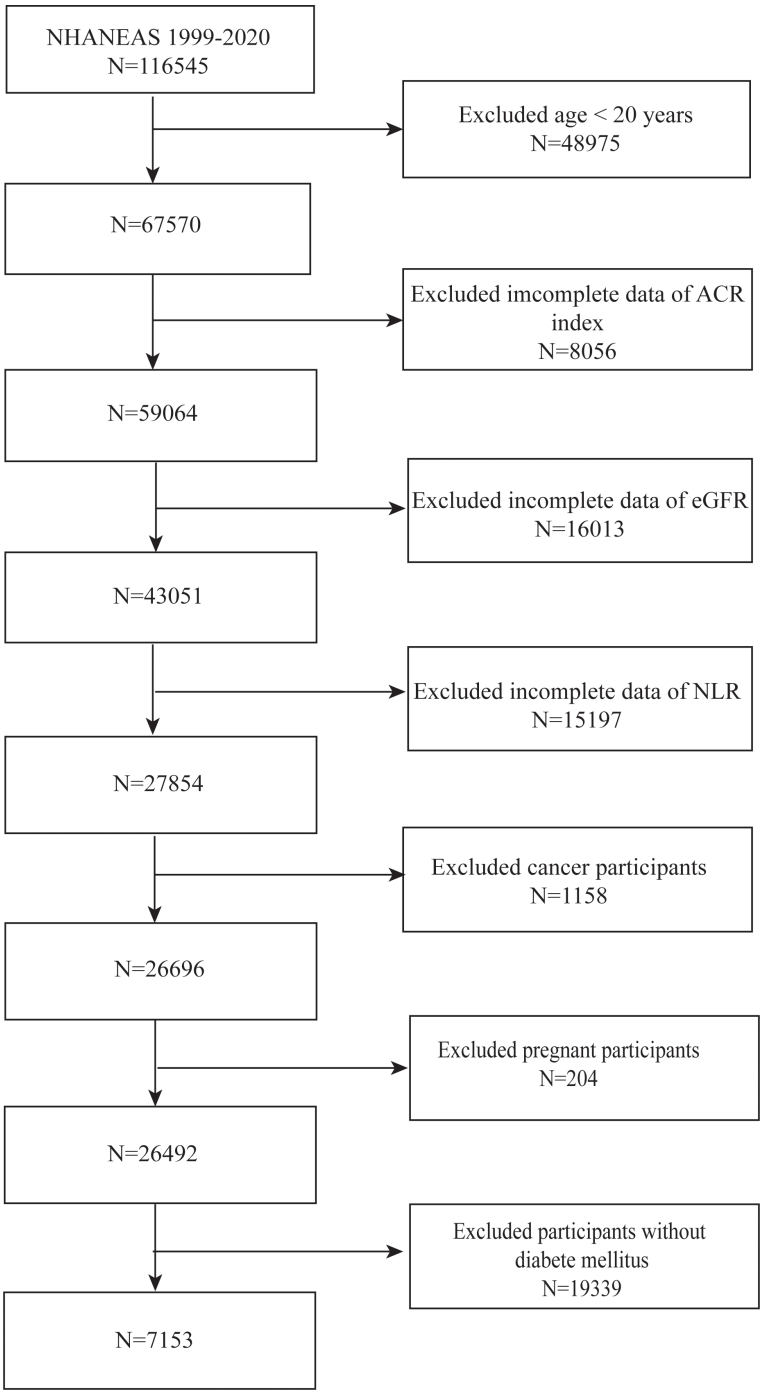

FIGURE 1
Flowchart of the sample selection from NHANES 1999–2020.

platelet count(PC)/LC), monocyte-to-lymphocyte ratio (MLR) (MLR= monocyte count(MC)/LC), systemic immune-inflammation index (SII) (SII=PC * NC/LC), system inflammation response index (SIRI) (SIRI=NC * MC/LC), and aggregate index of systemic inflammation (AISI) (AISI=NC * PC * MC/LC), to fully assess the relationship between NLR and DKD.

2.3 Definition of DKD, low-eGFR, and albuminuria

Self-reported diabetes, the use of insulin or other diabetes drugs, or specific criteria based on fasting glucose (mmol/l) ≥ 7.0 or glycosylated hemoglobin A1c (HbA1c) (%) > 6.5 were all required

for the diagnosis of diabetes. DKD was diagnosed with the low estimated glomerular filtration rate (eGFR) (< 60 mL/min/1.73 m^2) or albuminuria (urinary albumin-to-creatinine ratio (ACR) ≥ 30 mg/g) in T2DM patients (20). The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation for standardized creatinine (21). The main outcome variables in this study were albuminuria, low-eGFR, and DKD.

2.4 Covariates

Demographic parameters included sex, age, race, and education level. Additionally, we considered various anthropometric and laboratory covariates, such as body mass index (BMI)(normal weight, overweight, and obese), smoking status (≥100 cigarettes lifetime/<100 cigarettes lifetime), alcohol consumption (days) (number of days of alcohol consumption in the past year), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), family income to poverty ratio (PIR) (categorized as low income (≤1.3), medium income (>1.3 to 3.5), and high income (>3.5)), cardiovascular diseases (CVD), fasting glucose, glycohemoglobin, and marital status (married/never married/living with a partner/others, including widowed, divorced, or separated). DM-related treatment included insulin use and diabetes drug use.

In addition, we used hypertension in our study to account for variations in health status. A three-part criterion was used to define hypertension. Hypertension was defined as long as one of these criteria was met. Participants were asked to self-report their hypertension in the first segment using the question "Ever told you had hypertension". The second segment is mean systolic blood pressure (SBP) ≥ 130 mmHg and/or mean diastolic blood pressure (DBP) ≥ 80 mmHg (22). The third segment employed the item "taking hypertension prescription" program to identify hypertensive participants Visit the webpage at www.cdc.gov/nchs/nhanes/ to learn more detailed information on these variables.

2.5 Statistical analysis

In all of our statistical analyses, we took into consideration the intricate sample design of a multi-stage cluster survey by the advice of the U.S. Centers for Disease Control and Prevention (CDC) (23). While categorical variables were shown as percentages, continuous variables were summarized using the mean and standard deviation. We used weighted t-tests or chi-square tests to evaluate differences between NLR (tertiles). We used weighted multivariable regression models in three distinct models to examine the relationships between NLR with DKD, albuminuria, and low-eGFR. Model 1 had no covariate adjustments. Model 2 included covariate adjustments for age, race, and sex. In Model 3, we adjusted for several covariates, including sex, age, race, education level, BMI, smoking status, alcohol consumption, TC, LDL-C, HDL-C, AST, ALT, triglycerides, PIR, CVD, hypertension, fasting glucose, glycohemoglobin, insulin use, diabetes drug use, and marital status. We conducted a sensitivity analysis by changing NLR from a continuous variable to a categorical variable (tertiles) to assess the robustness of our findings. Generalized additive models (GAM) and smooth curve fitting were used to address non-linear relationships. By fitting a two-segment linear regression model (segmented regression model) to each interval and comparing them to the one-line model (non-segmented) using the log-likelihood ratio test, we also looked at threshold effects. We utilized a two-step recursive method to find breakpoints. Using stratified multivariable logistic regression models stratified by sex, age, BMI, hypertension, and CVD, subgroup analysis was performed to examine the relationships between NLR with DKD, albuminuria, and low-eGFR. These stratification features were taken into consideration as potential effect modifiers. To examine the heterogeneity of relationships between the subgroups, an interaction term was also included. Furthermore, we used receiver operating characteristic (ROC) curves to evaluate the identifiable power of NLR and other inflammatory markers (MLR, PLR, SII, SIRI, and AISI) for DKD, albuminuria, and low-eGFR and compared the area under the curve (AUC) values. Missing values for categorical variables were handled using mode imputation, whereas missing values for continuous variables were handled by median imputation. We used the Empower software package and R version 4.1.3 to carry out all of our statistical studies. A two-tailed p-value < 0.05 was used to determine statistical significance.

3 Results

3.1 Participants characteristics at baseline

7,153 people in all, with a mean age of 48.91 ± 18.23 years and 44.65% males and 55.35% females, were included in our study. The prevalences of DKD, albuminuria, and low-eGFR were found to be 31.76%, 23.08%, and 14.55%, respectively. The mean NLR observed among the participants was 2.19 ± 1.34. The study found that individuals in the upper tertiles of NLR had higher prevalences of low-eGFR, albuminuria, and DKD than those in the lower tertiles (all p < 0.05) (Table 1).

Additionally, we found significant variations in several variables across the NLR tertiles, including sex, BMI, HDL-C, triglycerides, PIR, fasting glucose, glycohemoglobin, insulin use, diabetes drug use, smoking status, CVD, hypertension, ACR, eGFR, MLR, PLR, SII, SIRI, and AISI (all p < 0.05) (Table 1).

3.2 Association between NLR and DKD

The correlations between NLR and other inflammatory markers with DKD were examined and presented in Table 2. In

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TABLE 1 Baseline characteristics according to NLR tertiles.

NLR	Overall	Tertile 1 (0.09–1.65)	Tertile 2 (1.65–2.25)	Tertile 3 (2.25–24.60)	*P*-value
N	7153	2373	2386	2394	
NLR	2.19 ± 1.34	1.15 ± 0.28	1.93 ± 0.22	3.48 ± 1.58	<0.001
MLR	0.27 ± 0.12	0.19 ± 0.07	0.25 ± 0.07	0.36 ± 0.14	<0.001
PLR	121.75 ± 49.01	97.29 ± 36.29	118.30 ± 37.07	149.44 ± 55.96	<0.001
SII	551.22 ± 394.88	289.27 ± 113.20	481.10 ± 155.28	823.44 ± 516.05	<0.001
SIRI	1.24 ± 0.98	0.69 ± 0.29	1.06 ± 0.40	2.05 ± 1.23	<0.001
AISI	321.81 ± 287.59	162.26 ± 92.55	280.32 ± 145.53	522.34 ± 388.62	<0.001
Age, years					0.072
20-40	2736 (38.25%)	946 (39.87%)	915 (38.35%)	875 (36.55%)	
41-60	2243 (31.36%)	751 (31.65%)	740 (31.01%)	752 (31.41%)	
>60	2174 (30.39%)	676 (28.49%)	731 (30.64%)	767 (32.04%)	
Sex, n (%)					0.001
Male	3194 (44.65%)	1130 (47.62%)	1043 (43.71%)	1021 (42.65%)	
Female	3959 (55.35%)	1243 (52.38%)	1343 (56.29%)	1373 (57.35%)	
Race, n (%)					0.320
Mexican American	1242 (17.36%)	432 (18.20%)	419 (17.56%)	391 (16.33%)	
Other Hispanic	663 (9.27%)	214 (9.02%)	239 (10.02%)	210 (8.77%)	
Non-Hispanic White	3171 (44.33%)	1066 (44.92%)	1020 (42.75%)	1085 (45.32%)	
Non-Hispanic Black	1418 (19.82%)	443 (18.67%)	485 (20.33%)	490 (20.47%)	
Other Races	659 (9.21%)	218 (9.19%)	223 (9.35%)	218 (9.11%)	
Education level, n (%)					0.902
Less than high school	1893 (26.46%)	623 (26.25%)	650 (27.24%)	620 (25.90%)	
High school or GED	1586 (22.17%)	534 (22.50%)	519 (21.75%)	533 (22.26%)	
Above high school	3653 (51.07%)	1211 (51.03%)	1209 (50.67%)	1233 (51.50%)	
Others	21 (0.29%)	5 (0.21%)	8 (0.34%)	8 (0.33%)	
Marital status, n (%)					0.112
Married	3210 (52.73%)	1103 (54.31%)	1052 (51.47%)	1055 (52.41%)	
Never married	1124 (18.46%)	372 (18.32%)	384 (18.79%)	368 (18.28%)	
Living with a partner	477 (7.84%)	171 (8.42%)	147 (7.19%)	159 (7.90%)	
Others	1277 (20.98%)	385 (18.96%)	461 (22.55%)	431 (21.41%)	
BMI, n (%)					0.010
Normal weight	1891 (26.74%)	653 (27.75%)	661 (28.03%)	577 (24.43%)	
Overweight	2188 (30.93%)	746 (31.70%)	716 (30.36%)	726 (30.74%)	
Obese	2994 (42.33%)	954 (40.54%)	981 (41.60%)	1059 (44.83%)	
Smoking status, n (%)					< 0.001
≥100 cigarettes lifetime	3067 (47.05%)	1007 (46.79%)	1001 (46.41%)	1059 (47.92%)	
< 100 cigarettes lifetime	3452 (52.95%)	457 (19.26%)	781 (32.73%)	1034 (43.19%)	
PIR, n (%)					<0.001

(Continued)

TABLE 1 Continued

NLR	Overall	Tertile 1 (0.09–1.65)	Tertile 2 (1.65–2.25)	Tertile 3 (2.25–24.60)	*P*-value
Low income	2708 (40.99%)	449 (20.43%)	531 (24.08%)	582 (26.42%)	
Medium income	2336 (35.36%)	728 (33.12%)	756 (34.29%)	852 (38.67%)	
High income	1562 (23.65%)	1021 (46.45%)	918 (41.63%)	769 (34.91%)	
Insulin use, n (%)					<0.001
Yes	1360 (21.97%)	372 (18.34%)	428 (20.79%)	560 (26.64%)	
No	4829 (78.03%)	1656 (81.66%)	1631 (79.21%)	1542 (73.36%)	
Diabetes drug use, n (%)					0.007
Yes	3810 (69.56%)	1252 (70.10%)	1308 (71.67%)	1250 (66.99%)	
No	1667 (30.44%)	534 (29.90%)	517 (28.33%)	616 (33.01%)	
CVD, n (%)	643 (8.99%)	141 (5.94%)	210 (8.80%)	292 (12.20%)	< 0.001
Alcohol consumption, days	4.09 ± 8.85	3.81 ± 3.75	4.42 ± 13.62	4.04 ± 5.61	0.346
Hypertension, n (%)	4423 (61.83%)	1396 (58.83%)	1460 (61.19%)	1567 (65.46%)	< 0.001
Fasting glucose, mmol/L	8.91 ± 3.62	8.73 ± 3.62	9.14 ± 3.81	8.86 ± 3.43	0.022
glycohemoglobin, %	7.44 ± 1.81	7.46 ± 1.83	7.53 ± 1.83	7.34 ± 1.75	0.001
TC, mg/dL	182.81 ± 43.78	183.39 ± 43.78	182.46 ± 42.51	182.59 ± 45.02	0.732
HDL-C, mg/dL	52.11 ± 15.93	53.20 ± 15.72	51.30 ± 15.37	51.83 ± 16.62	<0.001
LDL-C, mg/dL	105.25 ± 35.46	106.38 ± 36.71	104.44 ± 33.88	104.89 ± 35.71	0.228
ALT, U/L	24.39 ± 19.38	24.83 ± 20.81	24.28 ± 18.74	24.06 ± 18.51	0.408
AST, U/L	24.94 ± 17.39	25.34 ± 23.31	24.77 ± 12.83	24.72 ± 14.12	0.437
Triglyceride, mg/dL	121.94 ± 114.70	115.33 ± 118.14	126.01 ± 122.55	124.66 ± 101.56	0.009
ACR, mg/g	90.98 ± 488.64	54.06 ± 337.31	97.52 ± 473.45	121.07 ± 612.47	<0.001
Albuminuria, n (%)	1651 (23.08%)	321 (13.53%)	585 (24.52%)	745 (31.12%)	< 0.001
eGFR, mL/min/1.73 m2	90.55 ± 29.84	94.51 ± 24.80	90.18 ± 29.79	87.00 ± 33.75	<0.001
Low-eGFR, n (%)	1041 (14.55%)	216 (9.10%)	346 (14.50%)	479 (20.01%)	< 0.001
DKD, n (%)	2272 (31.76%)	457 (19.26%)	781 (32.73%)	1034 (43.19%)	< 0.001

NLR, neutrophil-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SII, systemic immune-inflammation index; SIRI, system inflammation response index; AISI, aggregate index of systemic inflammation; GED, general educational development; BMI, body mass index; PIR, family income to poverty ratio; CVD, cardiovascular diseases; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein cholesterol; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ACR, urinary albumin-to-creatinine ratio; eGFR, urinary albumin-to-creatinine ratio; DKD, diabetic kidney disease.

Model 3, NLR, MLR, SII, and SIRI were significantly positively correlated with DKD (NLR: OR = 2.90; 95% CI: 1.51, 5.58; MLR: OR = 6.93; 95% CI: 2.37, 20.31; SII: OR = 1.00; 95% CI: 1.00, 1.01; SIRI: OR = 3.01; 95% CI: 1.18, 7.68). Significant associations remained even when the inflammatory markers were categorized into tertiles. Individuals in the highest tertiles of NLR, MLR, and SIRI exhibited higher prevalences of DKD compared to those in the lowest tertile (all p for trend < 0.05).

The threshold effect of the nonlinear relationship between MLR and DKD was found using GAM and smooth curve fitting, and it was shown to have a breakpoint of 0.43 (logarithmic likelihood ratio test P-value <0.05) (Table 3). The relationship between NLR and DKD was not shown to be nonlinear (Figure 2).

3.3 Association between NLR and albuminuria

The study revealed that elevated levels of NLR and MLR were linked to an increased prevalence of albuminuria (Table 2). Model 3

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TABLE 2 Associations between NLR and other inflammatory markers with DKD, albuminuria, and low-eGFR.

Index	Outcome	Continuous or categories	Model 1[3]		Model 2[4]		Model 3[5]	
			OR[1] (95%CI[2])	*P*- value	OR (95%CI)	*P*- value	OR (95%CI)	*P*- value
NLR	**DKD**	NLR as continuous variable	1.45 (1.38, 1.52)	<0.0001	1.44 (1.37, 1.51)	<0.0001	2.90 (1.51, 5.58)	0.0014
		Tertile 1	Reference		Reference		Reference	
		Tertile 2	2.04 (1.79, 2.33)	<0.0001	2.01 (1.76, 2.30)	<0.0001	2.16 (0.56, 8.30)	0.2632
		Tertile 3	3.19 (2.80, 3.63)	<0.0001	3.12 (2.74, 3.56)	<0.0001	6.59 (1.72, 25.25)	0.0059
		P for trend	<0.0001		<0.0001		0.0057	
	Albuminuria	NLR as continuous variable	1.36 (1.29, 1.43)	<0.0001	1.36 (1.30, 1.43)	<0.0001	2.01 (1.10, 3.68)	0.0224
		Tertile 1	Reference		Reference		Reference	
		Tertile 2	2.08 (1.79, 2.41)	<0.0001	2.09 (1.80, 2.43)	<0.0001	7.13 (1.18, 43.14)	0.0324
		Tertile 3	2.89 (2.50, 3.34)	<0.0001	2.92 (2.52, 3.38)	<0.0001	7.31 (1.43, 37.34)	0.0169
		P for trend	<0.0001		<0.0001		0.0275	
	Low-eGFR	NLR as continuous variable	1.25 (1.18, 1.31)	<0.0001	1.23 (1.17, 1.30)	<0.0001	2.50 (1.05, 5.99)	0.0390
		Tertile 1	Reference		Reference		Reference	
		Tertile 2	1.69 (1.41, 2.03)	<0.0001	1.63 (1.35, 1.96)	<0.0001	0.50 (0.08, 3.20)	0.4622
		Tertile 3	2.50 (2.10, 2.97)	<0.0001	2.36 (1.98, 2.82)	<0.0001	2.31 (0.49, 10.88)	0.2899
		P for trend	<0.0001		<0.0001		0.2920	
MLR	**DKD**	MLR as continuous variable	9.45 (6.14, 14.54)	<0.0001	8.84 (5.73, 13.65)	<0.0001	6.93 (2.37, 20.31)	0.0004
		Tertile 1	Reference		Reference		Reference	
		Tertile 2	1.57 (1.38, 1.79)	<0.0001	1.56 (1.37, 1.78)	<0.0001	3.17 (0.91, 11.01)	0.0688
		Tertile 3	2.17 (1.92, 2.46)	<0.0001	2.13 (1.88, 2.41)	<0.0001	5.73 (1.46, 22.50)	0.0124
		P for trend	<0.0001		<0.0001		0.0127	
	Albuminuria	MLR as continuous variable	6.96 (4.44, 10.90)	<0.0001	7.14 (4.55, 11.19)	<0.0001	5.75 (1.76, 18.76)	0.0038
		Tertile 1	Reference		Reference		Reference	
		Tertile 2	1.47 (1.27, 1.69)	<0.0001	1.47 (1.28, 1.70)	<0.0001	2.11 (0.45, 9.95)	0.3435
		Tertile 3	1.90 (1.65, 2.18)	<0.0001	1.91 (1.67, 2.20)	<0.0001	7.96 (1.33, 47.76)	0.0232
		P for trend	<0.0001		<0.0001		0.0212	
	Low-eGFR	MLR as continuous variable	4.47 (2.69, 7.41)	<0.0001	3.86 (2.28, 6.55)	<0.0001	2.99 (0.76, 11.83)	0.1177
		Tertile 1	Reference		Reference		Reference	
		Tertile 2	1.41 (1.19, 1.68)	<0.0001	1.38 (1.16, 1.65)	0.0004	2.54 (0.42, 15.21)	0.3074
		Tertile 3	1.86 (1.58, 2.20)	<0.0001	1.76 (1.49, 2.09)	<0.0001	2.84 (0.45, 17.99)	0.2665
		P for trend	<0.0001		<0.0001		0.3008	

(Continued)

TABLE 2 Continued

Index	Outcome	Continuous or categories	Model 1[3]		Model 2[4]		Model 3[5]	
			OR[1] (95%CI[2])	*P*- value	OR (95%CI)	*P*- value	OR (95%CI)	*P*- value
PLR	**DKD**	PLR as continuous variable	1.00 (1.00, 1.01)	<0.0001	1.00 (1.00, 1.01)	<0.0001	1.01 (0.99, 1.02)	0.2460
		Tertile 1	Reference		Reference		Reference	
		Tertile 2	1.26 (1.11, 1.42)	0.0004	1.26 (1.11, 1.43)	0.0004	4.46 (1.19, 16.71)	0.0267
		Tertile 3	1.58 (1.40, 1.79)	<0.0001	1.58 (1.40, 1.79)	<0.0001	2.40 (0.67, 8.67)	0.1807
		P for trend	<0.0001		<0.0001		0.2829	
	Albuminuria	PLR as continuous variable	1.00 (1.00, 1.01)	<0.0001	1.00 (1.00, 1.01)	<0.0001	1.01 (0.99, 1.03)	0.1251
		Tertile 1	Reference		Reference		Reference	
		Tertile 2	1.21 (1.05, 1.39)	0.0078	1.21 (1.05, 1.39)	0.0073	2.36 (0.42, 13.18)	0.3271
		Tertile 3	1.46 (1.27, 1.67)	<0.0001	1.46 (1.27, 1.67)	<0.0001	2.54 (0.50, 12.93)	0.2599
		P for trend	<0.0001		<0.0001		0.3157	
	Low-eGFR	PLR as continuous variable	1.00 (1.00, 1.01)	0.0006	1.00 (1.00, 1.01)	0.0002	1.01 (0.99, 1.02)	0.4864
		Tertile 1	Reference		Reference		Reference	
		Tertile 2	1.24 (1.05, 1.46)	0.0126	1.25 (1.05, 1.49)	0.0121	5.64 (0.77, 41.39)	0.0890
		Tertile 3	1.49 (1.27, 1.76)	<0.0001	1.51 (1.27, 1.78)	<0.0001	2.07 (0.27, 15.68)	0.4817
		P for trend	<0.0001		<0.0001		0.6258	
SII	**DKD**	SII as continuous variable	1.00 (1.00, 1.01)	<0.0001	1.00 (1.00, 1.01)	<0.0001	1.00 (1.00, 1.01)	0.0426
		Tertile 1	Reference		Reference		Reference	
		Tertile 2	1.69 (1.49, 1.92)	<0.0001	1.68 (1.48, 1.91)	<0.0001	1.17 (0.37, 3.75)	0.7860
		Tertile 3	2.00 (1.76, 2.27)	<0.0001	1.98 (1.74, 2.25)	<0.0001	1.65 (0.47, 5.80)	0.4330
		P for trend	<0.0001		<0.0001		0.4279	
	Albuminuria	SII as continuous variable	1.00 (1.00, 1.01)	<0.0001	1.00 (1.00, 1.01)	<0.0001	1.00 (0.99, 1.00)	0.1171
		Tertile 1	Reference		Reference		Reference	
		Tertile 2	1.65 (1.44, 1.90)	<0.0001	1.66 (1.44, 1.91)	<0.0001	1.20 (0.27, 5.45)	0.8112
		Tertile 3	1.83 (1.60, 2.11)	<0.0001	1.85 (1.60, 2.12)	<0.0001	1.51 (0.32, 7.11)	0.6030
		P for trend	<0.0001		<0.0001		0.6030	
	Low-eGFR	SII as continuous variable	1.00 (1.00, 1.01)	<0.0001	1.00 (1.00, 1.01)	<0.0001	1.00 (0.99, 1.01)	0.0925
		Tertile 1	Reference		Reference		Reference	
		Tertile 2	1.56 (1.32, 1.86)	<0.0001	1.54 (1.29, 1.84)	<0.0001	2.05 (0.35, 11.94)	0.4261
		Tertile 3	1.79 (1.52, 2.12)	<0.0001	1.76 (1.48, 2.09)	<0.0001	1.11 (0.18, 6.79)	0.9081
		P for trend	<0.0001		<0.0001		0.9967	
SIRI	**DKD**	SIRI as continuous variable	1.31 (1.23, 1.39)	<0.0001	1.30 (1.22, 1.38)	<0.0001	3.01 (1.18, 7.68)	0.0213
		Tertile 1	Reference		Reference		Reference	
		Tertile 2	1.76 (1.55, 2.01)	<0.0001	1.74 (1.53, 1.98)	<0.0001	2.40 (0.63, 9.16)	0.1984

(Continued)

TABLE 2 Continued

Index	Outcome	Continuous or categories	Model 1[3]		Model 2[4]		Model 3[5]	
			OR[1] (95%CI[2])	*P*- value	OR (95%CI)	*P*- value	OR (95%CI)	*P*- value
		Tertile 3	2.26 (1.99, 2.57)	<0.0001	2.20 (1.94, 2.50)	<0.0001	4.41 (1.13, 17.18)	0.0325
		P for trend	<0.0001		<0.0001		0.0378	
	Albuminuria	SIRI as continuous variable	1.25 (1.17, 1.32)	<0.0001	1.25 (1.18, 1.33)	<0.0001	2.45 (0.92, 6.57)	0.0742
		Tertile 1	Reference		Reference		Reference	
		Tertile 2	1.76 (1.53, 2.03)	<0.0001	1.77 (1.53, 2.04)	<0.0001	1.82 (0.36, 9.06)	0.4663
		Tertile 3	2.11 (1.83, 2.43)	<0.0001	2.13 (1.85, 2.46)	<0.0001	3.84 (0.87, 17.02)	0.0767
		P for trend	<0.0001		<0.0001		0.0764	
	Low-eGFR	SIRI as continuous variable	1.17 (1.10, 1.25)	<0.0001	1.14 (1.07, 1.22)	<0.0001	2.17 (0.61, 7.77)	0.2320
		Tertile 1	Reference		Reference		Reference	
		Tertile 2	1.45 (1.22, 1.72)	<0.0001	1.40 (1.17, 1.68)	0.0002	1.29 (0.19, 8.93)	0.7985
		Tertile 3	1.82 (1.54, 2.15)	<0.0001	1.68 (1.42, 2.00)	<0.0001	1.20 (0.21, 7.01)	0.8357
		P for trend	<0.0001		<0.0001		0.8698	
AISI	**DKD**	AISI as continuous variable	1.00 (1.00, 1.01)	<0.0001	1.00 (1.00, 1.01)	<0.0001	1.00 (0.99, 1.01)	0.1370
		Tertile 1	Reference		Reference		Reference	
		Tertile 2	1.49 (1.32, 1.69)	<0.0001	1.47 (1.29, 1.66)	<0.0001	0.86 (0.25, 3.01)	0.8196
		Tertile 3	1.55 (1.37, 1.75)	<0.0001	1.51 (1.34, 1.72)	<0.0001	1.96 (0.54, 7.08)	0.3032
		P for trend	<0.0001		<0.0001		0.2432	
	Albuminuria	AISI as continuous variable	1.00 (1.00, 1.01)	<0.0001	1.00 (1.00, 1.01)	<0.0001	1.00 (0.99, 1.01)	0.1744
		Tertile 1	Reference		Reference		Reference	
		Tertile 2	1.43 (1.25, 1.64)	<0.0001	1.44 (1.25, 1.65)	<0.0001	1.31 (0.29, 5.92)	0.7254
		Tertile 3	1.50 (1.30, 1.72)	<0.0001	1.50 (1.31, 1.73)	<0.0001	2.00 (0.46, 8.72)	0.3547
		P for trend	<0.0001		<0.0001		0.3493	
	Low-eGFR	AISI as continuous variable	1.00 (1.00, 1.01)	0.0081	1.00 (1.00, 1.01)	0.0353	1.00 (0.99, 1.01)	0.3983
		Tertile 1	Reference		Reference		Reference	
		Tertile 2	1.37 (1.16, 1.62)	0.0002	1.32 (1.12, 1.57)	0.0013	0.47 (0.06, 3.59)	0.4705
		Tertile 3	1.40 (1.19, 1.65)	<0.0001	1.32 (1.11, 1.56)	0.0016	0.57 (0.08, 4.10)	0.5780
		P for trend	<0.0001		0.0070		0.6994	

In sensitivity analysis, NLR, MLR, PLR, SII, SIRI, and AISI were converted from continuous variables to categorical variables (tertiles).
[1]OR: Odd ratio.
[2]95% CI: 95% confidence interval.
[3]Model 1: No covariates were adjusted.
[4]Model 2: Adjusted for age, sex, and race.
[5]Model 3: Adjusted for sex, age, race, education level, BMI, smoking status, alcohol consumption, TC, LDL-C, HDL-C, AST, ALT, triglycerides, PIR, CVD, hypertension, fasting glucose, glycohemoglobin, insulin use, diabetes drug use, and marital status.

demonstrated that each unit increase in NLR and MLR corresponded to a 1.01-fold and 4.75-fold rise in albuminuria prevalence, respectively (NLR: OR = 2.01; 95% CI: 1.10, 3.68; MLR: OR = 5.75; 95% CI: 1.76, 18.76). To further investigate the relationships, sensitivity analysis was conducted by categorizing inflammatory markers into tertiles. Participants who were in the higher tertiles of NLR showed a higher prevalence of albuminuria than those in the lower tertiles (p for trend < 0.05).

Additionally, through the use of GAM and smooth curve fitting, no significant nonlinear connections were discovered between NLR and other inflammatory markers with albuminuria (Figure 2; Table 3).

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TABLE 3 Threshold effect analysis of NLR and other inflammatory markers on DKD, albuminuria, and low-eGFR using a two-piecewise linear regression model in Model 3.

	DKD		Albuminuria		Low-eGFR	
	OR[1] (95%CI[2])	*P*- value	OR (95%CI)	*P*- value	OR (95%CI)	*P*- value
NLR						
Fitting by standard linear model	2.90 (1.51, 5.58)	0.0014	2.01 (1.10, 3.68)	0.0224	2.50 (1.05, 5.99)	0.0390
Fitting by two-piecewise linear model						
Breakpoint (K)	3.43		3.22		1.88	
OR1(< K)	4.30 (1.77, 10.48)	0.0013	3.28 (1.30, 8.31)	0.0121	0.27 (0.04, 1.73)	0.1685
OR2(> K)	0.88 (0.14, 5.74)	0.8961	0.60 (0.10, 3.71)	0.5863	24.02 (2.72, 212.30)	0.0042
OR2/OR1	0.21 (0.02, 2.08)	0.1799	0.18 (0.02, 1.98)	0.1631	88.13 (2.72, 285.20)	0.0116
Logarithmic likelihood ratio test P-value	0.162		0.136		0.003	
MLR						
Fitting by standard linear model	6.93 (2.37, 20.31)	0.0004	5.75 (1.76, 18.76)	0.0038	2.99 (0.76, 11.83)	0.1177
Fitting by two-piecewise linear model						
Breakpoint (K)	0.43		0.29		0.21	
OR1(< K)	28.87 (6.52, 127.78)	<0.0001	55.20 (3.81, 799.07)	0.0033	72.06 (0.60, 455.06)	0.0747
OR2(> K)	0.22 (0.01, 4.60)	0.3281	1.47 (0.21, 10.20)	0.6974	1.34 (0.22, 8.33)	0.7512
OR2/OR1	0.01 (0.01, 0.34)	0.0115	0.03 (0.01, 1.25)	0.0647	0.01 (0.01, 5.49)	0.1468
Logarithmic likelihood ratio test P-value	0.006		0.060		0.138	
PLR						
Fitting by standard linear model	1.01 (0.99, 1.02)	0.2460	1.01 (0.99, 1.03)	0.1251	1.01 (0.99, 1.02)	0.4864
Fitting by two-piecewise linear model						
Breakpoint (K)	113.64		193.57		66.14	
OR1(< K)	1.03 (0.99, 1.06)	0.0770	1.00 (0.99, 1.02)	0.7448	14.14 (0.01, 52.10)	0.7752
OR2(> K)	1.00 (0.98, 1.01)	0.6753	1.06 (1.00, 1.12)	0.0337	0.99 (0.98, 1.01)	0.5739
OR2/OR1	0.97 (0.93, 1.01)	0.1409	1.06 (0.99, 1.13)	0.0714	0.01 (0.01, 2.74)	0.7750
Logarithmic likelihood ratio test P-value	0.129		0.058		0.003	
SII						
Fitting by standard linear model	1.00 (1.00, 1.01)	0.0426	1.00 (0.99, 1.00)	0.1171	1.00 (0.99, 1.01)	0.0925
Fitting by two-piecewise linear model						
Breakpoint (K)	149.52		850		245	
OR1(< K)	0.97 (0.92, 1.02)	0.2072	1.00 (0.99, 1.01)	0.1983	0.98 (0.96, 1.00)	0.0597
OR2(> K)	1.00 (1.00, 1.01)	0.0218	1.00 (0.99, 1.01)	0.7228	1.00 (1.00, 1.01)	0.0213
OR2/OR1	1.04 (0.98, 1.09)	0.1844	1.00 (0.98, 1.00)	0.6001	1.03 (1.00, 1.05)	0.0389
Logarithmic likelihood ratio test P-value	0.182		0.599		0.031	
SIRI						
Fitting by standard linear model	3.01 (1.18, 7.68)	0.0213	2.45 (0.92, 6.57)	0.0742	2.17 (0.61, 7.77)	0.2320
Fitting by two-piecewise linear model						
Breakpoint (K)	1.96		1.03		0.87	
OR1(< K)	4.88 (1.34, 17.81)	0.0165	17.66 (1.09, 286.46)	0.0434	0.02 (0.01, 2.56)	0.1176

(Continued)

TABLE 3 Continued

	DKD		Albuminuria		Low-eGFR	
	OR[1] (95%CI[2])	*P*- value	OR (95%CI)	*P*- value	OR (95%CI)	*P*- value
OR2(> K)	0.68 (0.04, 12.59)	0.7940	0.97 (0.20, 4.68)	0.9721	12.02 (1.27, 113.42)	0.0299
OR2/OR1	0.14 (0.01, 5.03)	0.2811	0.06 (0.01, 2.38)	0.1312	94.21 (0.91, 374.26)	0.0537
Logarithmic likelihood ratio test P-value	0.265		0.121		0.039	
AISI						
Fitting by standard linear model	1.00 (0.99, 1.01)	0.1370	1.00 (0.99, 1.01)	0.1744	1.00 (0.99, 1.01)	0.3983
Fitting by two-piecewise linear model						
Breakpoint (K)	252.08		143.92		264.44	
OR1(< K)	1.00 (0.99, 1.00)	0.3813	0.99 (0.97, 1.02)	0.5628	0.99 (0.98, 1.00)	0.1627
OR2(> K)	1.00 (1.00, 1.01)	0.0427	1.00 (1.00, 1.01)	0.1238	1.01 (1.00, 1.01)	0.0572
OR2/OR1	1.01 (0.99, 1.02)	0.1642	1.01 (0.98, 1.04)	0.4599	1.01 (1.00, 1.03)	0.0810
Logarithmic likelihood ratio test P-value	0.156		0.460		0.070	

Adjusted for sex, age, race, education level, BMI, smoking status, alcohol consumption, TC, LDL-C, HDL-C, AST, ALT, triglycerides, PIR, CVD, hypertension, fasting glucose, glycohemoglobin, insulin use, diabetes drug use, and marital status.
[1]OR: Odd ratio.
[2]95% CI: 95% confidence interval.

3.4 Association between NLR and low-eGFR

Three distinct models were used in the study to examine the connections between NLR and other inflammatory markers with low-eGFR (Table 2). In Model 3, a positive correlation remained only between NLR and low-eGFR, indicating that as each unit increased in NLR, the prevalence of low-eGFR increased by 1.50-fold (OR = 2.50; 95% CI:1.05, 5.99).

A nonlinear association with a calculated breakpoint of 1.88 was identified between NLR and low-eGFR through GAM and smooth curve fitting (logarithmic likelihood ratio test *P*-value < 0.05) (Figure 2). When NLR > 1.88, NLR displayed a positive connection with low-eGFR. There was no significant connection seen on the left side of the breakpoint, nevertheless (Table 3).

3.5 Subgroup analysis

Our findings imply that there was an inconsistent association between NLR and other inflammatory markers with DKD (Figure 3). Significant associations were found between NLR and DKD in all subgroups stratified by sex (all $p < 0.05$). In the 41-60 years old, normal weight, nonhypertensive, and nonCVD population, positive but nonsignificant associations were observed. The results of the interaction tests indicated that in each subgroup, the relationship between NLR and DKD was not significantly impacted by age, sex, BMI, hypertension, or CVD (all p for interaction > 0.05). Additionally, the association between MLR and DKD was dependent on age, particularly applicable to individuals over 60 years of age.

Regarding the associations between NLR and PLR with albuminuria, there was no substantial association observed across different population subgroups, indicating a consistent association across populations (all p for interaction > 0.05) (Figure 3).

Age, sex, BMI, hypertension, and CVD were found to have no significant impact on the correlations between low-eGFR with NLR, MLR, PLR, SII, SIRI, and AISI, according to the interaction tests (all p for interaction > 0.05) (Figure 3).

3.6 ROC analysis

The AUC values were calculated to assess the predictive accuracy of NLR and other inflammatory markers (MLR, PLR, SII, SIRI, and AISI) in predicting DKD, albuminuria, and low-eGFR (Figure 4). We observed that NLR had the highest AUC value among all inflammatory markers in predicting DKD, albuminuria, and low-eGFR. Table 4 shows that there was a statistically significant difference in AUC values between NLR and other inflammatory markers (all $p < 0.05$). This suggests that when it comes to assessing the risk of DKD, albuminuria, and low-eGFR, NLR may be more accurate and discriminative than other inflammatory markers (MLR, PLR, SII, SIRI, and AISI).

4 Discussion

The prevalences of DKD, albuminuria, and low-eGFR with NLR were positively correlated in this cross-sectional research of 7,153 US adult T2DM patients. Subgroup analysis and interaction tests revealed that their associations were not significantly different

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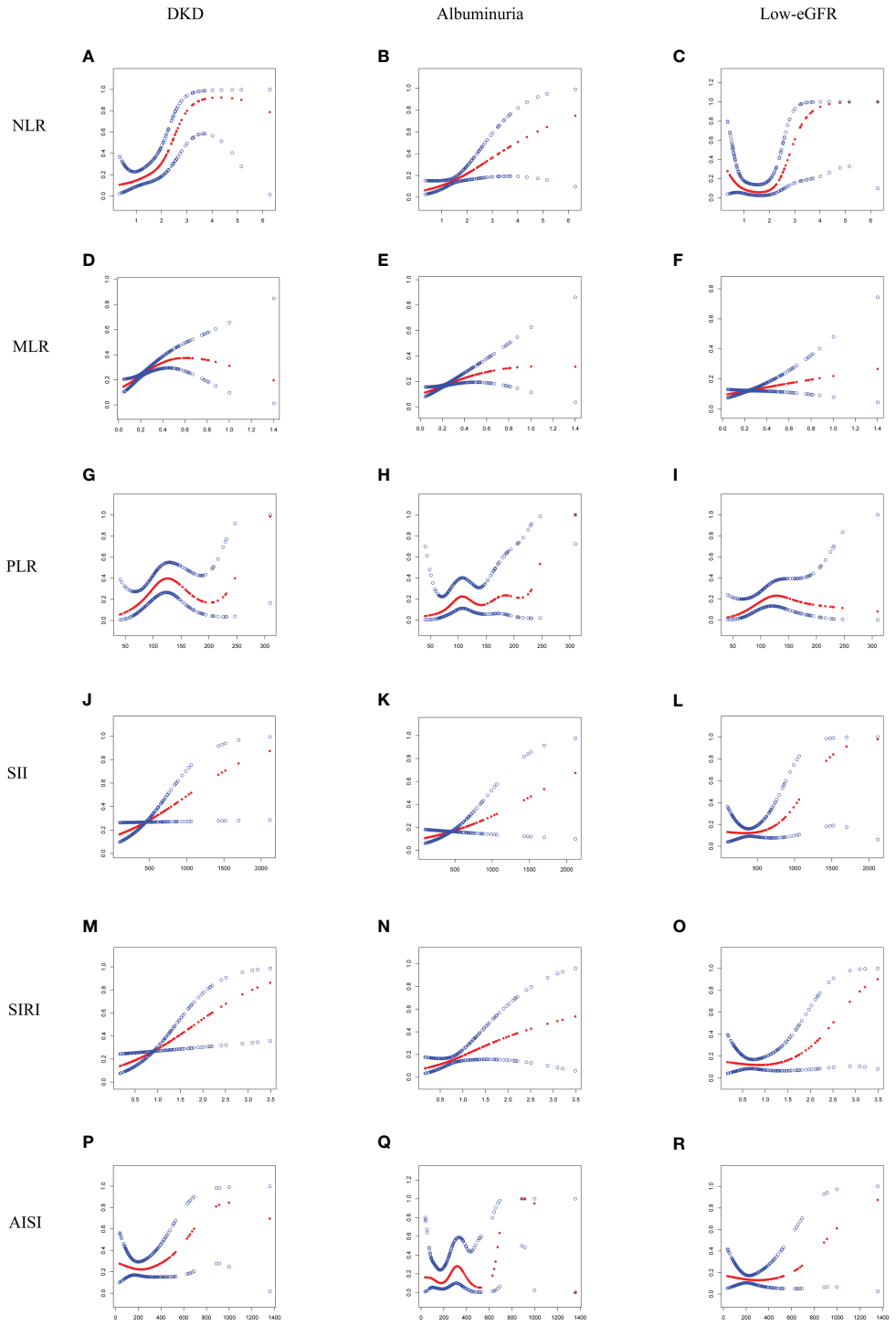

FIGURE 2
Smooth curve fitting for NLR and other inflammatory markers with DKD, albuminuria, and low-eGFR. **(A)** NLR and DKD; **(B)** NLR and albuminuria; **(C)** NLR and low-eGFR; **(D)** MLR and DKD; **(E)** MLR and albuminuria; **(F)** MLR and low-eGFR; **(G)** PLR and DKD; **(H)** PLR and albuminuria; **(I)** PLR and low-eGFR; **(J)** SII and DKD; **(K)** SII and albuminuria; **(L)** SII and low-eGFR; **(M)** SIRI and DKD; **(N)** SIRI and albuminuria; **(O)** SIRI and low-eGFR; **(P)** AISI and DKD; **(Q)** AISI and albuminuria; **(R)** AISI and low-eGFR.

across populations. ROC analysis showed that compared with other inflammatory markers (MLR, PLR, SII, SIRI, and AISI), NLR may have better discriminative ability and accuracy in identifying the risk of DKD, albuminuria, and low-eGFR. To sum up, we must emphasize the significance of NLR levels in evaluating kidney health in the US diabetic population.

The relationships between inflammatory markers and DKD have been investigated in a few earlier research. In a study by Wan

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FIGURE 3
Subgroup analysis for the associations of NLR and other inflammatory markers with DKD, albuminuria, and low-eGFR. **(A)** NLR and DKD; **(B)** NLR and albuminuria; **(C)** NLR and low-eGFR; **(D)** MLR and DKD; **(E)** MLR and albuminuria; **(F)** MLR and low-eGFR; **(G)** PLR and DKD; **(H)** PLR and albuminuria; **(I)** PLR and low-eGFR; **(J)** SII and DKD; **(K)** SII and albuminuria; **(L)** SII and low-eGFR; **(M)** SIRI and DKD; **(N)** SIRI and albuminuria; **(O)** SIRI and low-eGFR; **(P)** AISI and DKD; **(Q)** AISI and albuminuria; **(R)** AISI and low-eGFR.

et al., it was discovered that NLR levels were positively correlated with ACR levels and the prevalence of DKD in Chinese diabetes patients (15). The prevalence of albuminuria increased with increasing NLR levels in the Japanese diabetic population (17). NLR and PLR were revealed to be significant risk factors for predicting albuminuria in a study of diabetic individuals from Syria (24). Furthermore, the predictive usefulness of NLR for early DKD was discovered by a prospective Egyptian investigation (16). Our study has some benefits as compared to earlier research. First, we provided new evidence for the association between NLR and DKD in US diabetic patients, while previous studies mainly focused on Asian and African populations. Second, to our knowledge, no research has examined the connections between low-eGFR, DKD, and albuminuria with NLR and other inflammatory markers (MLR, PLR, SII, SIRI, and AISI) in the same diabetic population. Lastly, we used ROC analysis to

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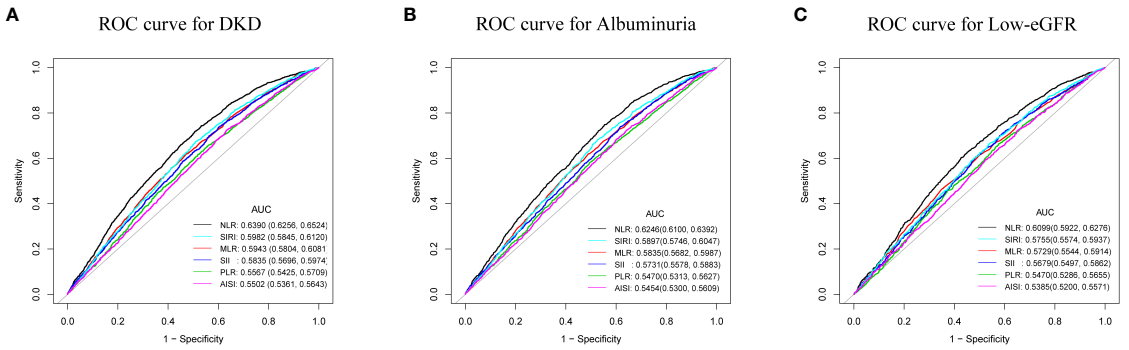

FIGURE 4
ROC curves and the AUC values of the six inflammatory markers (NLR, MLR, PLR, SII, SIRI, and AISI) in diagnosing DKD, albuminuria and low-eGFR. **(A)** Six inflammatory markers were assessed to identify DKD. **(B)** Six inflammatory markers were assessed to identify albuminuria. **(C)** Six inflammatory markers were assessed to identify low-eGFR.

evaluate the predictive power of NLR and other inflammatory markers on DKD, albuminuria, and low-eGFR. This was an essential difference between our study and previous studies.

Our research delved into the relationships between NLR and other inflammatory markers with renal function in T2DM patients. Firstly, our findings demonstrated the positive correlation between MLR and DKD. Previous studies have come to similar conclusions (25). Notably, our study introduces a novel dimension by highlighting a nonlinear link between them. When MLR < 0.43, MLR displayed a positive connection with DKD. There was no

TABLE 4 Comparison of AUC values between NLR and other inflammatory markers.

Test	AUC[1]	95%CI[2] low	95%CI upp	Best threshold	Specificity	Sensitivity	*P* for different in AUC
DKD							
NLR	0.6390	0.6256	0.6524	1.3521	0.4913	0.7201	Reference
MLR	0.5943	0.5804	0.6081	0.2198	0.5269	0.6202	<0.0001
PLR	0.5567	0.5425	0.5709	109.1833	0.4811	0.6162	<0.0001
SII	0.5835	0.5696	0.5974	327.8316	0.3986	0.7377	<0.0001
SIRI	0.5982	0.5845	0.6120	0.7214	0.4911	0.6725	<0.0001
AISI	0.5502	0.5361	0.5643	161.9677	0.3650	0.7258	<0.0001
Albuminuria							
NLR	0.6246	0.6100	0.6392	1.3037	0.4457	0.7486	Reference
MLR	0.5835	0.5682	0.5987	0.2211	0.5105	0.6196	<0.0001
PLR	0.5470	0.5313	0.5627	112.4500	0.5046	0.5821	<0.0001
SII	0.5731	0.5578	0.5883	327.8316	0.3837	0.7396	<0.0001
SIRI	0.5897	0.5746	0.6047	0.7214	0.4749	0.6802	<0.0001
AISI	0.5454	0.5300	0.5609	162.7960	0.3585	0.7244	<0.0001
Low-eGFR							
NLR	0.6099	0.5922	0.6276	1.6172	0.5730	0.6033	Reference
MLR	0.5729	0.5544	0.5914	0.2347	0.5574	0.5591	<0.0001
PLR	0.5470	0.5286	0.5655	105.3033	0.4230	0.6638	<0.0001
SII	0.5679	0.5497	0.5862	403.1517	0.5153	0.6061	<0.0001
SIRI	0.5755	0.5574	0.5937	0.7236	0.4588	0.6667	<0.0001
AISI	0.5385	0.5200	0.5571	161.9677	0.3466	0.7253	<0.0001

[1]AUC: area under the curve.
[2]95% CI: 95% confidence interval.

significant connection seen on the right side of the breakpoint, nevertheless. Furthermore, a 4.75-fold rise in the prevalence of albuminuria was also observed in our study for every unit increase in MLR. We also found that the prevalence of DKD increased with the SII level, in line with earlier research (26, 27). However, our investigation did not reveal a substantial connection between PLR and renal function, which differed from earlier research findings (24, 28). The reasons for these inconsistent results include differences in sample size, eGFR calculation method, population, race, and geography.

To our knowledge, no research has looked into how SIRI and AISI relate to renal function in T2DM patients. Past research primarily focused on the robust correlation between SIRI levels with CVD and peripheral arterial disease (PAD) in diabetic patients (29, 30). In our study, we demonstrated that for every one-unit increase in SIRI, the prevalence of DKD increased by 2.01-fold. According to earlier research, the increased neutrophils and monocytes and decreased lymphocytes all contribute to the onset of DKD (31–33). This might elucidate the link between SIRI and renal function. More further comprehensive prospective studies are imperative to validate and consolidate these outcomes.

The primary outcome of our investigation reveals a positive correlation between NLR and DKD in US T2DM patients. That is, the prevalence of DKD increased 1.90 times for every unit rise in NLR. Paralleling earlier research underscored the significant connection between NLR levels and albuminuria among diabetic patients (16, 17, 24). Similarly, we found that the higher the level of NLR, the higher the prevalence of albuminuria. In addition, we observed a positive and nonlinear association between NLR and low-eGFR. And a breakpoint of the threshold effect was calculated to be 1.88. When NLR > 1.88, the prevalence of low-eGFR increased 23.02 times for each unit increase in NLR. There was no proof of a meaningful relationship on the left side of the breakpoint. The previous research similarly points to the potential of NLR in forecasting deteriorating renal function in diabetic patients (34). In conclusion, the American T2DM population with higher NLR levels should be aware of kidney health. This might be because innate immunity (mediated by neutrophils) and adaptive immunity (mediated by lymphocytes) are both reflected in the easily accessible and inexpensive NLR (15). In addition, the stability of NLR is better and less affected by physiological and pathological status. The superiority of NLR has been supported by prior studies. The ROC value of NLR was significantly better than PLR in diagnosing DKD (24, 28). This conclusion was supported by our study, where ROC analysis revealed that, when compared to the other five inflammatory markers (MLR, PLR, SII, SIRI, and AISI), NLR may have the better discriminatory power and accuracy in assessing the risk of DKD, albuminuria, and low-eGFR. In conclusion, the NLR offers a great deal of potential for assessing renal function in T2DM patients in the United States as a straightforward, affordable, and frequently used inflammatory marker.

Our subgroup analysis indicated that the prevalence of DKD was significantly higher in males than in females for each unit increase in NLR. This result is in line with earlier research (35). This could be explained by either the detrimental effect of testosterone or the protective property of estrogen (36). Additionally, we discovered that the associations between NLR with DKD, albuminuria, and low-eGFR was not significantly impacted by age, sex, BMI, hypertension, or CVD. These relationships may be valid for different populations. These results confirm and add to the evidence that NLR is a risk factor threatening renal function in the community of Americans with T2DM.

Research is still ongoing to determine the possible mechanism underlying the relationship between DKD and NLR. We think this finding might have something to do with the inflammatory state associated with DKD. Research has indicated a direct relationship between systemic and renal inflammation and the pathological process of DKD. Nod-like receptor protein 3 (NLRP3) inflammasome and several inflammatory cytokines, such as interleukins and tumor necrosis factors, cause pathological changes in kidney structure through a variety of inflammatory pathways (37). They worsen renal fibrosis, tubular damage, and glomerular sclerosis in addition to raising urine albumin excretion. Traditional markers of inflammation, neutrophils are a crucial part of the innate immune response (38, 39). Renal cellular stress brought on by ongoing hyperglycemia in the early stages of diabetes triggers an innate immune response and draws leukocytes to the kidney (40). Additionally helpful in the early and advanced phases of diabetic nephropathy are macrophages and lymphocytes (41). As a result, the progression of DKD is accelerated and renal damage occurs.

Our study has several advantages. We used information from the NHANES, a comprehensive, population-based survey with stringent research procedures and quality assurance checks. The reliability and representativeness of our findings are increased by the size of our sample and the adjustment for relevant confounders. NLR is a promising tool for therapeutic usage because it is a commonly used, non-invasive, simple to use, and affordable technique. Our study does, however, have certain shortcomings. Due to the cross-sectional design of the study, we were unable to establish a causal relationship between NLR and DKD. We took a lot of significant factors into account, but it is impossible to completely rule out the impact of additional unmeasured confounders. Due to the cross-sectional survey of the US population, our results may not apply to other populations or ethnic groups.

5 Conclusion

Compared to other inflammatory markers (MLR, PLR, SII, SIRI, and AISI), NLR may serve as the more effective potential inflammatory marker for identifying the risk of DKD, albuminuria, and low-eGFR in US T2DM patients. T2DM patients with elevated levels of NLR, MLR, SII, and SIRI should be closely monitored for their potential risk to renal function. Nevertheless, additional thorough prospective research is required to confirm and validate these 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.

Ethics statement

The studies involving humans were approved by National Center for Health Statistics Research Ethics Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

XL: Writing – original draft. LW: Writing – original draft, Writing – review & editing. HX: Writing – review & editing. HZ: Writing – review & editing. ML: Data curation, Supervision.

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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

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Defining the threshold: triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio's non-linear impact on tubular atrophy in primary membranous nephropathy

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Background: Hyperlipidemia is common in primary membranous nephropathy (PMN) patients, and tubular atrophy (TA) is an unfavorable prognostic factor. However, the correlation between the triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio and TA is controversial. Therefore, our study aimed to investigate the association between the TG/HDL-C ratio and TA in PMN patients.

Methods: We conducted a cross-sectional study and collected data from 363 PMN patients at Shenzhen Second People's Hospital from January 2008 to April 2023. The primary objective was to evaluate the independent correlation between the TG/HDL-C ratio and TA using binary logistic regression model. We used a generalized additive model along with smooth curve fitting and multiple sensitivity analyses to explore the relationship between these variables. Additionally, subgroup analyses were conducted to delve deeper into the results.

Results: Of the 363 PMN patients, 75 had TA (20.66%). The study population had a mean age of 46.598 ± 14.462 years, with 217 (59.78%) being male. After adjusting for sex, age, BMI, hypertension, history of diabetes, smoking, alcohol consumption, UPRO, eGFR, HB, FPG, and ALB, we found that the TG/HDL-C ratio was an independent risk factor for TA in PMN patients (OR=1.29, 95% CI: 1.04, 1.61, P=0.0213). A non-linear correlation was observed between the TG/HDL-C ratio and TA, with an inflection point at 4.25. The odds ratios (OR) on the left and right sides of this inflection point were 1.56 (95% CI: 1.17, 2.07) and 0.25 (95% CI: 0.04, 1.54), respectively. Sensitivity analysis confirmed these results. Subgroup analysis showed a consistent association between the TG/HDL-C ratio and TA, implying that factors such as gender, BMI, age, UPRO, ALB, hypertension and severe nephrotic syndrome had negligible effects on the link between the TG/HDL-C ratio and TA.

Conclusion: Our study demonstrates a non-linear positive correlation between the TG/HDL-C ratio and the risk of TA in PMN patients, independent of other factors. Specifically, the association is more pronounced when the ratio falls below 4.25. Based on our findings, it would be advisable to decrease the TG/HDL-C ratio below the inflection point in PMN patients as part of treatment strategies.

KEYWORDS

triglyceride to high-density lipoprotein cholesterol ratio, tubular atrophy, primary membranous nephropathy, non-linear, cross-sectional study

Background

Primary membranous nephropathy (PMN) is the leading pathological subtype of nephrotic syndrome (NS) in adults, characterized by proteinuria, hypoalbuminemia, edema, and hyperlipidemia (1). PMN is an immune-complex-mediated disorder, with 60-80% of patients exhibiting NS, where dyslipidemia is a defining feature, marked by high cholesterol and triglyceride (TG) levels (2). In PMN, significant proteinuria triggers hepatic overproduction of proteins and lipoproteins. The lipid and lipoprotein abnormalities in NS are mainly due to decreased clearance, as NS causes deficits in lipoprotein lipase, hepatic lipase, and the very-low-density lipoprotein (VLDL) receptor, while increasing cholesteryl ester transfer protein and the low-density lipoprotein (LDL) receptor-related protein. Additionally, alterations in lipoprotein structure hinder their receptor binding, activation of lipolytic enzymes, and exchange with high-density lipoprotein cholesterol (HDL-C) for lipid and apoprotein transfer (3). Hyperlipidemia results in lipid accumulation in kidney tubules, prompting an inflammatory response, oxidative stress, and subsequent cell damage, a process termed lipotoxicity. Tubular lipotoxicity contributes to a cascade of renal injuries, including oxidative stress, endoplasmic reticulum stress, tubular epithelial cell apoptosis, tubulointerstitial fibrosis, mitochondrial dysfunction, and inflammation (4). Tubular atrophy (TA) is a well-documented factor in the progression of renal disease (5).

In a rat model of diabetic nephropathy, there was a discovered link between TG accumulation in the kidneys and interstitial fibrosis in the tubulointerstitium (6). Additionally, pravastatin was shown to have a positive impact on reducing tubulointerstitial fibrosis in another rat model of cyclosporine-induced nephropathy (7). These findings emphasize the potential importance of lipid management in attenuating tubulointerstitial lesions in individuals with PMN.

TG levels, which are greatly influenced by feeding status, making it less reliable as a biomarker for prediction (8). The predictive value of high-density lipoprotein cholesterol (HDL-C) for cardiovascular disease (CVD) or mortality prediction remains a contentious issue, as evidenced by the ongoing debate in the medical literature (9). To address this uncertainty, researchers have suggested the use of the triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio as a more practical indicator for evaluating atherogenicity and insulin resistance (10, 11). The ratio has garnered attention for its enhanced predictive ability for CVD such as all-cause mortality, cardiac death, non-fatal myocardial infarction, stroke, or coronary revascularization procedures (10, 12, 13), as well as its importance in prognosing conditions like peritoneal dialysis and chronic kidney disease (CKD) (14).

According to a recent literature report, patients with IgA nephropathy who had a higher ratio of TG/HDL-C were observed to display more pronounced pathological lesions, such as interstitial fibrosis and TA (15). However, limited research has explored the potential link between the TG/HDL-C ratio and TA in patients diagnosed with PMN. Existing studies on dyslipidemia in PMN have mainly focused on its correlation with proteinuria, rather than its direct effect on renal outcomes (16).

Consequently, PMN presents a unique opportunity to investigate the TG/HDL-C ratio as a potential indicator of renal damage within a homogenous population where the primary disease process is well-defined, as opposed to a more heterogeneous group with varying causes of NS. To scrutinize this hypothesis, a retrospective cross-sectional study was carried out with 363 PMN participants, specifically examining the link between TG/HDL-C ratio levels and TA. Logistic regression modeling, smooth curve fitting, and a comprehensive suite of sensitivity analyses were employed to evaluate the relationship between the TG/HDL-C ratio and TA.

Subjects and methods

Study design

This cross-sectional study was conducted at a single center. In this particular research, the TG/HDL-C ratio was considered as the independent variable, while the dependent variable was TA (dichotomous variable: TA, non-TA).

Study population

The researchers collected initial data for the study from kidney biopsy samples obtained during the hospitalization of qualified participants. Patients were screened based on clearly delineated inclusion and exclusion criteria to ensure their eligibility and suitability for the study sample. Obtaining biopsy data in this manner allowed the researchers to establish a baseline for key measures that would be tracked over the course of the study.

This cross-sectional study obtained data from individuals admitted to Shenzhen Second People's Hospital from January 2008 to April 2023. The inclusion criteria consisted of patients diagnosed with membranous nephropathy via renal biopsy, and data collection followed a non-selective and consecutive approach. The researchers accessed the electronic case system and pathology reports to gather the essential information. It is crucial to highlight that the study strictly adhered to the principles outlined in the Declaration of Helsinki and obtained approval from the Medical Ethics Committee of Shenzhen Second People's Hospital (ethical approval document number: 20210620213357018-FS01-02PJ).

The initial enrollment of the cross-sectional study included 445 patients aged >14 years who were diagnosed with membranous nephropathy through renal biopsy. Patients were excluded if they met any of the following criteria: (1) renal pathology lacking TA description (1 patient); (2) cases of atypical membranous nephropathy, secondary membranous nephropathy, viral hepatitis, or malignancy (51 patients); (3) participants with missing data on HDL-c and TG levels (15 patients); (4) individuals with outliers in the TG/HDL-C ratio beyond the range of mean ± three standard deviations (13 patients); (5) patients with end-stage renal disease (ESRD) at the time of renal biopsy (2 patients). Consequently, 82 patients were excluded, leaving a final sample size of 363 patients with primary membranous nephropathy (PMN) who entered the cross-sectional study (see Figure 1). The patients were further divided into two groups based on the presence of TA.

Baseline data for the study were obtained from renal biopsies performed during hospitalization, ensuring that the patients satisfied the defined inclusion and exclusion criteria.

Clinical data

During the initial renal biopsy, pertinent demographic data such as age, gender, blood pressure, hypertension, diabetes history, smoking and alcohol status, as well as body mass index, were carefully recorded. Moreover, essential laboratory measurements comprising serum creatinine, estimated glomerular filtration rate (eGFR), hemoglobin (HB), albumin (ALB), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), HDL, triglycerides (TG), TG/HDL-C ratio, uric acid (UA), fasting plasma glucose (FPG) levels, 24-hour urine protein quantification (UPRO), and renal biopsy results were meticulously collected. The eGFR was evaluated by the CKD-EPI equation, providing valuable insights into renal function (17).

Pathological data

The renal biopsy specimens underwent standard processing and examination techniques, including light microscopy, immunofluorescence, and electron microscopy. Pathological staging was conducted following the Ehrenreich-Churg system, which categorized stages into five distinct categories (18). Light microscopy utilized staining methods such as hematoxylin-eosin (HE), periodic acid-Schiff (PAS), hexosamine silver (PASM), and Masson staining. Various factors were assessed, including glomerular count, presence of spherical sclerosis, segmental sclerosis, crescent formation, mesangial proliferation, interstitial infiltration, and TA. Immunofluorescence was employed to observe deposits' location and extent, such as IgG, IgA, IgM, C3, C1q, and PLA2R, using direct methods. Electron microscopy provided detailed insight into the ultrastructure, including the glomerular basement membrane, podocyte pedicles, and electron-dense material deposition. Renal pathology results were independently interpreted by two pathologists at the Guangzhou Jinyu Medical Laboratory Center.

Variables

Triglyceride to high-density lipoprotein cholesterol ratio

The TG/HDL-C ratio was considered a continuous variable and calculated as follows: TG/HDL-C ratio = triglycerides divided by high-density lipoprotein cholesterol.

Tubular atrophy

In this study, the main focus was on the outcome variable TA, which was defined as a dichotomous variable categorized as 1 for TA and 0 for Non-TA. To assess TA, participants were diagnosed based on the criterion that the percentage of affected tubules ≥10% (19).

Covariates

Based on our clinical expertise and thorough review of relevant literature, we meticulously selected covariates for our study. These covariates, consisting of both continuous and categorical variables, have been previously identified in studies (5, 20) The continuous variables included age, BMI, 24-hour urine UPRO, eGFR, HB, FPG, and ALB. The categorical variables encompassed sex, hypertension, history of smoking, diabetes, and alcohol consumption. In order to ensure accuracy, we used the automated analyzer from Abbott AxSYM following standard protocols to assess the biochemical values. A physician conducted a comprehensive health habit inventory to gather pertinent information. BMI was calculated using weight in kilograms divided by the square of height in

meters (kg/m^2). We determined eGFR using the CKD-EPI equation (17) which takes into account age, gender, and creatinine levels. Data collection was carried out meticulously under standardized conditions, adhering to uniform procedures. Impaired kidney function was clinically defined as an estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73m^2 (21), while hypertension was determined by either documented history of hypertension or baseline systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥90 mmHg.

Statistical analysis

The participants were categorized based on the presence of TA. For continuous variables, we reported the mean ± standard deviation (SD) for those with a Gaussian distribution and the median (interquartile ranges) for those with a skewed distribution. As for categorical variables, we presented frequencies and percentages. We employed different statistical tests to compare the TA and non-TA groups. The $\chi 2$ test was used for categorical variables, independent samples t-test for variables with a normal distribution, and the Mann-Whitney test for variables with a skewed distribution. In our study, we encountered missing data points. Specifically, BMI data was missing for 33 participants (9.1%), TC data for 1 participant (0.27%), UPRO data for 3 participants (0.826%), history of alcohol data for 5 participants (1.38%), FBG data for 28 participants (7.71%), eGFR data for 1 participant (0.27%), and HB data for 11 participants (3.03%). To address these missing values, we performed multiple imputations (22). The imputation model included age, gender, DBP, SBP, BMI, hypertension, TC, UPRO, ALB, LDL-C, UA, FPG, eGFR, HB, history of alcohol, smoking, and diabetes. We conducted a missing data analysis assuming missing-at-random (MAR) conditions (23, 24).

The linear association between TG/HDL-c ratio and TA

After conducting a collinearity screening process, we employed both univariate and multivariate binary logistic regression models in accordance with the guidelines outlined by the STROBE statement (24). These models enabled us to generate three unique variations: a non-adjusted model (referred to as the Crude model), a minimally-adjusted model (Model I), and a fully-adjusted model (Model II).

The Crude model did not involve any covariate adjustments. For Model I, we made adjustments solely for sociodemographic variables, including age, hypertension, sex, BMI, history of diabetes, smoking, and alcohol consumption. In Model II, we further adjusted for a comprehensive range of covariates presented in Table 1, such as age, hypertension, sex, BMI, history of diabetes, smoking, alcohol consumption, UPRO, eGFR, HB, FPG, and ALB. We recorded effect sizes (OR) alongside their corresponding 95% confidence intervals. If the introduction of covariates resulted in a change of 10% or more in the odds ratio (OR), we made necessary adjustments to ensure accuracy (24). These adjustments were informed by the outcomes derived from the collinearity screening process.

TABLE 1 Relationship between TG/HDL-c ratio and TA in different models.

Variable	Crude model (OR,95%CI, P)	Model I (OR,95% CI, *P*)	Model II (OR,95% CI, *P*)
TG/HDL-c ratio	1.34 (1.10, 1.64) 0.0034	1.30 (1.05, 1.61) 0.0156	1.29 (1.04, 1.61) 0.0213
TG/HDL-c ratio (Quintile)			
Q1	Ref.	Ref.	Ref.
Q2	2.27 (1.11, 4.67) 0.0255	1.92 (0.90, 4.12) 0.0921	1.71 (0.78, 3.76) 0.1800
Q3	3.52 (1.76, 7.05) 0.0004	3.20 (1.53, 6.70) 0.0021	2.99 (1.40, 6.36) 0.0046
P for trend	0.0003	0.0017	0.0035

Crude model: we did not adjust other covariants Model I: we adjusted age, hypertension, sex, BMI, history of diabetes, smoke, alcohol, Model II: we adjusted age, hypertension, sex, BMI, history of diabetes, smoke, alcohol, UPRO, eGFR, HB, FPG, ALB. OR, odds ratios; CI, confidence; Ref, reference.

The non–linear association between TG/HDL-c ratio and TA

To address concerns about the adequacy of binary logistic regression models for handling nonlinear relationships, we utilized generalized additive models (GAM) and smooth curve fitting (penalized spline method) to examine the correlation between the TG/HDL-C ratio and TA. In case nonlinearity was detected, a recursive algorithm was applied to determine the inflection point. Subsequently, a two-piece binary logistic regression model was created for each side of the inflection point (25). A log-likelihood ratio test was then conducted to identify the most suitable model for describing the relationship between the TG/HDL-C ratio and TA. Furthermore, a generalized additive model (GAM) and smooth curve fitting were utilized to evaluate the non-linear relationship between the TG/HDL-C ratio and the incidence of TA in patients with normal kidney function. These methods enabled us to comprehensively explore and identify nuanced connections between these variables.

Subgroup analysis

We conducted subgroup analyses using a stratified binary logistic regression model across various subgroups, including gender, hypertension, age, BMI, ALB, UPRO and severe nephrotic syndrome.

Firstly, we converted continuous variables such as age (<50, ≥50 years), BMI (<24, ≥24 kg/m^2), ALB (<30, ≥30 g/L), UPRO (<3500, ≥3500 mg/24h) (26, 27), severe nephrotic syndrome ((edema, UPRO > 3500 mg/24h, ALB <25 g/L, hypertriglyceridemia) Yes, No) into categorical variables using established clinical thresholds.

Secondly, in addition to the stratification factor itself, we adjusted for all relevant factors, including age, hypertension, sex, BMI, history of diabetes, smoking, alcohol consumption, UPRO, eGFR, HB, FPG, and ALB. Finally, we conducted interaction tests

using the likelihood ratio test to compare models with and without interaction terms (28, 29).

Sensitivity analysis

To ensure the credibility of our findings, we undertook a comprehensive set of sensitivity analyses (30). Initially, we stratified the TG/HDL-c ratio into tertiles and performed trend tests to validate its use as a continuous variable while also investigating potential non-linear associations. Recognizing that individuals with impaired kidney function may face an elevated risk of TA (5), we conducted additional sensitivity analyses by exclusively including participants with normal kidney function to explore the linkage between the TG/HDL-c ratio and TA. Furthermore, we employed E-values (31) to assess the plausibility of unmeasured confounding factors influencing the relationship between the TG/HDL-c ratio and the risk of TA.

Results

Characteristics of patients

Table 2 displays the demographic and clinical profiles of the participants included in this study. A total of 363 adults were analyzed, with 217 (59.78%) males, and an average age of 46.59 ± 14.46 years. The median TG/HDL-c ratio was 1.373 (0.907, 2.312), and the average BMI was 24.49 ± 4.0 kg/m^2. The median UPRO was 3711.17 (2029.385, 6854) mg/24h and the mean eGFR was 103.23 ± 27.12 mL/min/1.73 m^2. Among the participants, 75 patients (20.66%) were diagnosed with TA. Individuals with TA tended to be older, with higher systolic and diastolic blood pressure, TG levels, TG/HDL-C ratio, and lower eGFR. However, there were no significant differences between the two groups in relation to smoking status, diabetes history, alcohol consumption, gender, age, BMI, HB, FPG, ALB, uric acid, cholesterol, LDL, or HDL-C.

Results of a binary logistic regression model used in univariate analyses

The univariate analyses revealed negative correlation between eGFR (OR = 0.980, 95% CI: 0.97-0.99), HDL-c 0.53 (OR = 0.980, 95% CI: 0.28-1.00) and TA, while positive correlations were observed with age (OR = 1.03, 95% CI: 1.01-1.05), DBP (OR = 1.04, 95% CI: 1.02-1.06), SBP (OR = 1.02, 95% CI: 1.01-1.04), TG/HDL-C ratio (OR = 1.34, 95% CI: 1.10-1.64), and TG (OR = 1.27, 95% CI: 1.06-1.52) (all $P < 0.05$; refer to Table 3). However, there was no significant associations between TA and gender, ALB, UA, UPRO, HB, FPG, TC, and LDL-C (all $P > 0.05$).

Analysis of multivariate data using binary logistic regression

The authors employed a binary logistic regression model to examine the correlation between the TG/HDL-C ratio and TA by constructing three distinct models. The findings indicated a significant association between the TG/HDL-C ratio and TA in the

TABLE 2 The baseline characteristics of patients.

TA	Non- TA	TA	*P*-value
N	288 (52.06%)	75(47.93%)	
GENDER			0.582
Male	167 (57.99%)	50 (66.67%)	
Female	121 (42.01%)	25 (33.33%)	
AGE(years)	45.35 ± 14.60	51.39 ± 12.91	0.001
BMI(kg/m^2)	24.36 ± 4.30	25.02 ± 2.66	0.207
DBP(mmHg)	81.12 ± 11.44	87.69 ± 15.14	<0.001
SBP(mmHg)	129.22 ± 18.84	137.56 ± 19.67	0.011
ALB(g/L)	27.47 ± 7.55	27.01 ± 6.75	0.634
TC(mmol/L)	7.10 ± 2.26	6.58 ± 2.05	0.069
HDL.C(mmol/L)	1.52 ± 0.75	1.52 ± 0.75	0.072
TG(mmol/L)	1.83 (1.33-2.65)	2.31 (1.66-3.25)	0.002
LDL.c(mmol/L)	4.72 ± 1.84	4.46 ± 1.54	0.261
TG/HDL-c ratio	1.28 (0.81-2.16)	1.88 (1.20-2.80)	<0.001
UA(umol/L)	385.06 ± 100.46	401.68 ± 90.44	0.194

(Continued)

TABLE 2 Continued

TA	Non- TA	TA	*P*-value
UPRO(mg/24h)	3670.73 (2057.38-6557.84)	4201.66 (1873.34-7311.55)	0.596
FPG(mmol/L)	4.90 ± 0.91	5.02 ± 1.12	0.327
eGFR (mL/min/1.73 m^2)	106.79 ± 25.83	89.53 ± 27.75	<0.001
HB(g/L)	132.30 ± 19.67	130.51 ± 23.06	0.499
Smoke history			0.582
NO	231 (80.21%)	58 (77.33%)	
YES	57 (19.79%)	17 (22.67%)	
Alcohol history			0.432
NO	243 (84.38%)	66 (88.00%)	
YES	45 (15.62%)	9 (12.00%)	
Diabetes history			0.459
NO	265 (92.01%)	67 (89.33%)	
YES	23 (7.99%)	9 (12.00%)	
Hypertension			<0.001
NO	162 (56.25%)	23 (30.67%)	
YES	126 (43.75%)	52 (69.33%)	

BMI, Body mass index; DBP, Diastolic blood pressure; SBP, Systolic blood pressure; ALB, albumin; TC, Total cholesterol; HDL-C, High-density lipoprotein cholesterol; TG, Triglyceride; LDL-C, Low-density lipid cholesterol l; TG/HDL-c ratio; Triglyceride to High-Density Lipoprotein Cholesterol ratio; UA, uric acid; UPRO, 24 h urine protein; FPG, Fasting plasma glucose; eGFR, evaluated glomerular filtration rate; HB, hemoglobin.

Crude model (OR=1.34, 95% CI: 1.1 - 1.64, P=0.0034). It was observed that each 1 unit increase in the TG/HDL-C ratio corresponded to a 34% increase in TA. In Model I, where only demographic variables such as age, hypertension, sex, BMI, history of diabetes, smoking, and alcohol consumption were adjusted for, each additional unit increase in the TG/HDL-C ratio was linked to a 30% elevated risk of TA (OR = 1.30, 95% CI: 1.05 to 1.61). Furthermore, in the fully adjusted Model II, which considered age, hypertension, sex, BMI, history of diabetes, smoking, alcohol consumption, UPRO, eGFR, HB, FPG, and ALB, each additional unit increase in the TG/HDL-C ratio was associated with a 29% increased risk of TA (OR = 1.29, 95% CI: 1.04 to 1.61). The confidence intervals of the results supported the reliability of the association between the TG/HDL-C ratio and the risk of TA as obtained from the model (Table 1).

Sensitivity analysis

We performed a series of sensitivity analyses to validate the robustness of our findings. Firstly, we categorized the TG/HDL-C ratio into tertiles, transforming it from a continuous variable to a categorical one. This categorization was then reintroduced into the model. The results demonstrated that after this transformation, the effect sizes in different groups showed a consistent pattern, and the P-value for this trend remained in line with the results obtained when the TG/HDL-C ratio was treated as a continuous variable (Tables 1, 4). Additionally, the authors calculated an E-value to evaluate the impact of unmeasured confounders on the results. The determined E-value was 1.53, surpassing both the relative risk associated with unmeasured confounders and the TG/HDL-C ratio. This suggests that the link between the TG/HDL-C ratio and the risk of TA remains largely unaffected by unknown or unmeasured confounding factors.

Furthermore, another sensitivity analysis was conducted where participants with impaired kidney function were excluded. Out of all the participants, 23 (6.3%) were identified as having impaired kidney function. The findings indicated that even after controlling for confounding factors, there still existed a positive association between the TG/HDL-C ratio and the risk of TA (OR = 1.33, 95% CI: 1.06 to 1.67) (Table 4).

The nonlinearity addressed by the generalized additive model

Through the use of GAM and smooth curve fitting, we have discovered a nonlinear association between the TG/HDL-C ratio and TA (Figure 2). Employing a recursive algorithm, we accurately determined the inflection point to be at 4.25. Subsequently, our investigation applied a two-piece binary logistic regression model to calculate the effect size and establish the confidence intervals surrounding the inflection point. Notably, on the left side of the inflection point, the effect size and corresponding 95% CI values were 1.56 (1.17, 2.07), respectively. Conversely, on the right side of this pivotal point, the effect size and 95% CI values were 0.25 (0.04, 1.54), respectively (Table 5).

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TABLE 3 The results of univariate analysis.

Variable	Statistics	OR (95%CI)	*P*-value
Gender			
Male	217 (59.780%)	Ref.	
Female	146 (40.220%)	0.69 (0.40, 1.18)	0.1734
Age, years	46.60 ± 14.46	1.03 (1.01, 1.05)	0.0015
BMI(kg/m^2)	24.494 ± 4.020	1.04 (0.98, 1.11)	0.2075
DBP(mmHg)	82.482 ± 12.561	1.04 (1.02, 1.06)	0.0002
SBP,mmHg	130.939 ± 19.284	1.02 (1.01, 1.04)	0.0011
Smoke history			
NO	289 (79.61%)	Ref.	
YES	74 (20.386%)	1.19 (0.64, 2.19)	0.5823
Alcohol history			
NO	309 (85.12%)	Ref.	
YES	54 (14.876%)	0.74 (0.34, 1.58)	0.4334
Diabetes history			
NO	332 (91.46%)	Ref.	
YES	31 (8.540%)	1.38 (0.59, 3.21)	0.4609
Hypertension			
NO	185 (50.96%)	Ref.	
YES	178 (49.04%)	2.91 (1.69, 5.00)	0.0001
ALB(g/L)	27.373 ± 7.385	0.99 (0.96, 1.03)	0.633
UA, umol/L	388.50 ± 98.59	1.00 (1.00, 1.00)	0.194
eGFR (mL/min/ 1.73 m^2)	103.226 ± 27.120	0.98 (0.97, 0.99)	<0.0001
UPRO(mg/24h)	5087.214 ± 4506.857	1.000 (1.000, 1.000)	0.3578
HB, g/L	131.932 ± 20.395	1.00 (0.98, 1.01)	0.4976
FPG(mmol/L)	4.923 ± 0.960	1.13 (0.88, 1.46)	0.3279
TG/HDL-c ratio	1.753 ± 1.200	1.34 (1.10, 1.64)	0.0034
TC(mmol/L)	6.993 ± 2.225	.89 (0.79, 1.01)	0.0695
TG, mmol/L	2.254 ± 1.287	1.27 (1.06, 1.52)	0.0112
HDL-c(mmol/L)	1.487 ± 0.696	0.53 (0.28, 1.00)	0.0486
LDL-c(mmol/L)	4.663 ± 1.782	0.92 (0.79, 1.06)	0.2604

Values are n (%) or mean ± SD or median (quartile) BMI, Body mass index; DBP, Diastolic blood pressure; SBP, Systolic blood pressure; ALB, albumin; UA, uric acid; eGFR, evaluated glomerular filtration rate; UPRO, 24 h urine protein; HB, hemoglobin; FPG, Fasting plasma glucose; TG/HDL-c ratio; Triglyceride to High-Density Lipoprotein Cholesterol ratio; TC, Total cholesterol; TG, Triglyceride; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipid cholesterol.

Furthermore, an additional sensitivity analysis was exclusively conducted on participants with normal kidney function. After adjusting for various confounding variables including age, hypertension, sex, BMI, history of diabetes, smoking, and alcohol consumption, a comprehensive two-stage linear regression analysis revealed a non-linear relationship between the TG/HDL-C ratio and the risk of TA in PMN patients. The investigation effectively identified the inflection point for the TG/HDL-C ratio, precisely pinpointing it at 4.25. On the left side of this critical point, the odds ratio (OR) and its associated 95% CI were determined to be 1.65 (1.22, 2.23), respectively. On the opposite side, specifically the right side of the inflection point, the OR and corresponding 95% CI values were found to be 0.23 (0.03, 1.47), respectively (refer to Table 5; Figure 3).

TABLE 4 Relationship between TG/HDL-c ratio and TA in patients with normal kidney function.

Variable	Crude model (OR,95%CI, *P*)	Model I (OR,95% CI, *P*)	Model II (OR,95% CI, *P*)
TG/HDL-c ratio	1.42 (1.16, 1.74) 0.0009	1.34 (1.08, 1.67) 0.0092	1.33 (1.06, 1.67) 0.0145
TG/HDL-c ratio (Quintile)			
Q1	Ref.	Ref.	Ref.
Q2	2.22 (0.98, 5.01) 0.0558	1.92 (0.82, 4.51) 0.1355	1.71 (0.72, 4.09) 0.2273
Q3	4.35 (2.03, 9.33) 0.0002	3.75 (1.67, 8.42) 0.0013	3.44 (1.50, 7.86) 0.0035
P for trend	<0.0001	0.0008	0.0022

Table 4 was sensitivity analysis in participants with ml/min/1.73m^2(n= 340). Crude model: we did not adjust other covariants Model I: we adjusted age, hypertension, sex, BMI, history of diabetes, smoke, alcohol, Model II: we adjusted age, hypertension, sex, BMI, history of diabetes, smoke, alcohol, UPRO, eGFR, HB, FPG, ALB. OR, odds ratios; CI, confidence; Ref, reference.

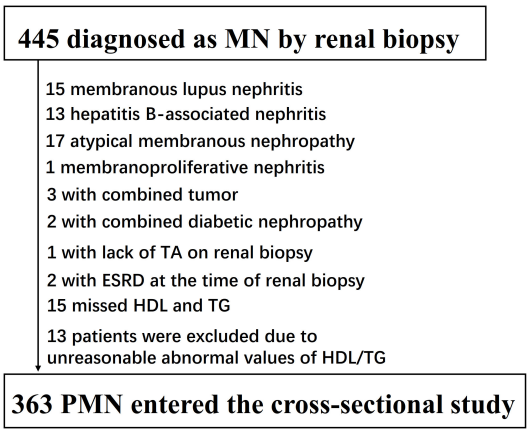

FIGURE 1
Flowchart of study participants. Figure 1 showed the inclusion of participants. 445 participants were assessed for eligibility in the original study. We excluded patients with missing values of lupus nephritis (n=15), hepatitis B-associated nephritis (n=13), atypical membranous nephropathy (n=17), membranoproliferative nephritis (n=1), combined tumor (n=3), combined tumor (n=1), lack of TA on renal biopsy (n=1), missed HDL and TG (n=15), unreasonable abnormal values of TG/HDL-C (n=13), ESRD at the time of renal biopsy (n=2). The final analysis included 363subjects in the present study.

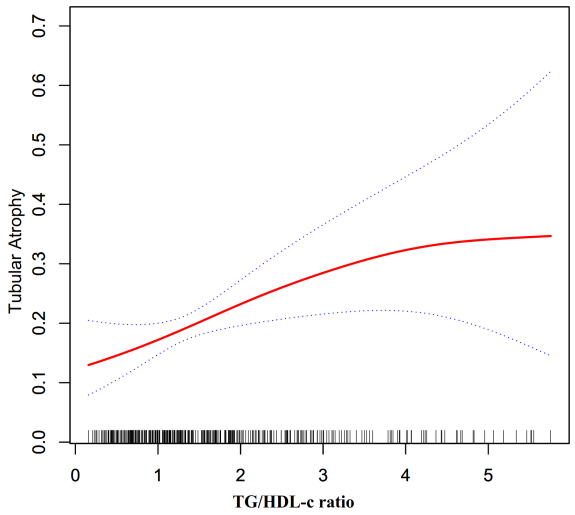

FIGURE 2
The non-linear relationship between TG/HDL-c ratio and the risk of TA.

The results of subgroup analyses

The authors conducted a comprehensive subgroup analysis to explore the potential influence of various confounding factors, including hypertension, BMI, UPRO, and age, on the association between the TG/HDL-C ratio and TA risk. Stratification variables, namely gender, age, hypertension, ALB, BMI, and UPRO, and severe nephrotic syndrome were utilized to detect any identifiable patterns in effect sizes across these variables (Table 6). Interestingly, results from Table 6 indicate that gender, age, hypertension, ALB, BMI, UPRO, and severe nephrotic syndrome do not significantly affect the relationship between the TG/HDL-C ratio and TA risk (All P for interaction >0.05).

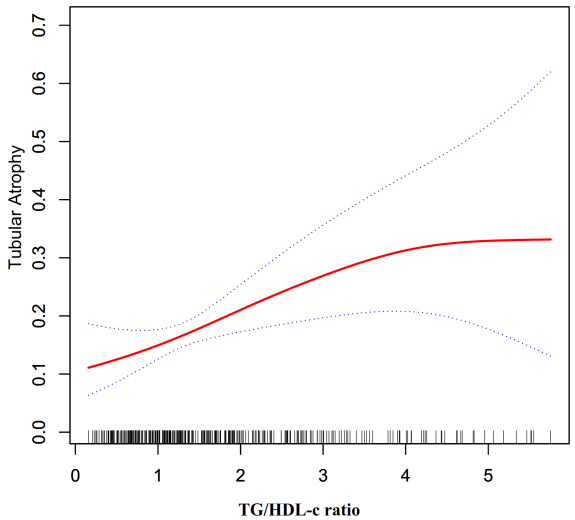

FIGURE 3
The non-linear relationship between TG/HDL-c ratio and the risk of TA in participants with normal kidney function.

These findings suggest that the connection between the TG/HDL-C ratio and TA remains consistent and unchanged.

Discussion

The objective of this cross-sectional study was to investigate the correlation between the TG/HDL-C ratio and TA among PMN patients. The findings indicated an independent association between the TG/HDL-C ratio and the risk of TA in this specific patient population. Notably, a threshold effect curve was observed, suggesting varying associations between the TG/HDL-C ratio and TA on either side of the inflection point among PMN patients, which was determined to be 4.25. Additional support for the robustness and consistency of these results was provided by sensitivity and subgroup analyses. Overall, these findings suggest that the TG/HDL-C ratio may serve as a valuable reference for the primary prevention of TA in PMN patients.

Among the 363 PMN patients included in this study, 75 (20.66%) were diagnosed with TA. These findings deviated from previous studies which reported a proportion of renal TA ranging from 52.6% to 66% in patients with PMN (5, 32, 33). The observed disparities may be attributed to several possible reasons. Firstly, differences in racial backgrounds among the study populations could have played a role. Secondly, inconsistencies in the criteria used to define tubular atrophy may have contributed to the variations in results. Importantly, it is noteworthy that the prevalence of TA was found to be high. Therefore, it remains crucial to actively explore additional risk factors associated with the development of TA. Our analysis revealed that patients with TA tended to be older, with hypertension, elevated TG levels, higher TG/HDL-C ratio, and lower eGFR.

After conducting a literature search, there was rare literature investigating the relationship between the TG/HDL-C ratio and TA risk. However, a recent retrospective cohort study at the West China Hospital of Sichuan University investigated this topic specifically in patients diagnosed with IgA nephropathy. The study included 1146 subjects with IgA nephropathy, divided into two groups based on their TG/HDL-C ratio at the time of renal biopsy: a high TG/HDL group (TG/HDL ≥ 1.495, N=382) and a low TG/HDL group (TG/HDL-C < 1.495, N=764). The study's findings indicated that individuals with a higher TG/HDL-C ratio exhibited more severe pathological lesions with tubular atrophy/interstitial fibrosis (odds ratio [OR] 1.610, 95% confidence interval [CI] (1.203-2.154, P=0.001) (15).

In our recent cross-sectional study, we observed a higher TG/HDL ratio among individuals with TA. To investigate the potential association between TG/HDL-C ratio and TA risk, we conducted logistic regression analysis, carefully adjusting for numerous factors, including age, hypertension, sex, BMI, history of diabetes, smoking, alcohol consumption, UPRO, eGFR, HB, FPG, and ALB. The results revealed a significant positive relationship between TG/HDL-C ratio and TA risk (OR=1.29, 95% CI: 1.04, 1.61, P=0.0213). These findings are consistent with the earlier mentioned study and contribute to the existing literature by demonstrating that an elevated TG/HDL-C ratio increases the risk of TA, regardless of participants' eGFR levels.

TABLE 5 The result of the two-piecewise Cox regression model.

Incident TA	Model I (OR,95%CI, *P*)	Model II (OR,95%CI, *P*)
Fitting model by standard Cox regression	1.29 (1.04, 1.61) 0.0213	1.33 (1.06, 1.67) 0.0145
Fitting model by two-piecewise Cox regression		
Inflection point of TG/HDL-c ratio	4.25	4.25
≤Inflection point	1.56 (1.17, 2.07) 0.0023	1.65 (1.22, 2.23) 0.0013
>Inflection point,	0.25 (0.04, 1.54) 0.1356	0.23 (0.03, 1.47) 0.1193
P for log-likelihood ratio test	0.043	0.033

Model I: All participants; Model II: Participants with eGFR ≥ 60 ml/min/1.73m2; OR, odds ratios; CI, confidence; Ref, reference.
We adjusted age, hypertension, sex, BMI, history of diabetes, smoke, alcohol, Model II: we adjusted age, hypertension, sex, BMI, history of diabetes, smoke, alcohol, UPRO, eGFR, HB, FPG, ALB.
We adjusted age, SBP, sex, BMI, SBP, history of diabetes, hypertension, smoke and alcohol, UPRO, eGFR, HB, FPG, ALB. HR, Hazard ratios; CI, confidence; Ref, reference.

Importantly, our study stands out from previous research in several key aspects. Firstly, we meticulously adjusted for multiple confounding variables, a factor that the previous study overlooked as it solely relied on univariate logistic regression models. Secondly, our study specifically examined patients with a distinct kidney condition, namely individuals with PMN, thereby advancing the understanding of the relationship between the TG/HDL-C ratio and TA across different pathological types of kidney diseases. Moreover, through subgroup and sensitivity analyses, we found that this relationship remained consistent even among participants with normal kidney function. These comprehensive efforts have further strengthened the stability and reliability of the association between the TG/HDL-C ratio and TA risk. In summary, our study offers valuable insights into the correlation between the TG/HDL-C ratio

TABLE 6 Effect size of TG/HDL-c ratio on TA in prespecified and exploratory subgroups.

Characteristic	No of participants	HR (95%CI)	P-value	P for interacion
Gender				0.5155
Male	217	1.22 (0.92, 1.62)	0.1664	
Female	146	1.43 (0.96, 2.14)	0.0762	
BMI (kg/m^2)				0.6062
≥24	1462	0.970 (0.932, 1.009)	0.1292	
<24				
Age (years)				0.5868
≥50	153	1.33 (0.97, 1.82)	0.0769	
<50	210	1.17 (0.85, 1.62)	0.3319	
UPRO (mg/24h)				0.7299
≥3500	195	1.22 (0.90, 1.65)	0.2049	
<3500	168	1.32 (0.95, 1.84)	0.1019	
ALB (g/L)				0.9679
≥30	132	1.31 (0.91, 1.89)	0.1451	
<30	231	1.33 (1.00, 1.75)	0.0473	
Hypertension				0.4625
NO	185	1.16 (0.78, 1.73)	0.4678	
YES	178	1.39 (1.05, 1.85)	0.0208	
Heavy nephrosis				0.4774
NO	288	1.436 (1.107, 1.861)	0.0063	
YES	75	1.107 (0.569, 2.155)	0.7653	

Note 1: Above model adjusted for age, hypertension, sex, BMI, history of diabetes, smoke, alcohol, UPRO, eGFR, HB, FPG, ALB.
Note 2: In each case, the model is not adjusted for the stratification variable
HR, Hazard ratios; CI, confidence; Ref, reference.

and TA risk. The findings highlight the significance of clinical intervention targeted at managing TG/HDL-C ratio levels to mitigate the risk of TA in patients with PMN.

Additionally, in comparison to existing medical literature, our cross-sectional study has provided novel insights into the relationship between the TG/HDL-C ratio and TA risk. Notably, this study is the first to observe a non-linear association between these variables. To examine this complex relationship, our research team utilized a two-piecewise logistic regression model. Through meticulous adjustment for confounding factors, we determined the inflection point to be at 4.25. Intriguingly, our findings indicated that when the TG/HDL-C ratio was below 4.25, a 1-unit increase in the TG/HDL-C ratio level was accompanied by a significant 56% increase in the adjusted odds ratio (OR) of TA risk (OR = 1.56, 95% CI: 1.17–2.07). Conversely, when the TG/HDL-C ratio exceeded 4.25, no statistically significant correlation between the TG/HDL-C ratio and TA was observed.

The identification of a 4.25 TG/HDL-C ratio inflection point is indeed a novel finding. Lipid metabolism dysfunction may substantially impact renal health. In PMN patients specifically, intrinsic disease processes disrupt lipid homeostasis, potentially increasing TG and decreasing HDL-C plasma levels, thereby affecting the TG/HDL-C ratio. The non-linear relationship between the TG/HDL-C ratio and the risk of TA in PMN patients can be elucidated by several mechanisms. (1). Lipid toxicity (4, 34): At a certain threshold, the TG/HDL-C ratio may reflect a critical level of dyslipidemia that leads to lipid toxicity, this can result in the accumulation of free fatty acids and their toxic metabolites, causing oxidative stress and inflammation, which ultimately leads to tubular epithelial cell injury and tubulointerstitial damage. (2). Glomerular filtration of lipids (35): An increased TG/HDL-C ratio may cause lipids to be filtered by the glomeruli and subsequently taken up by tubular cells, inducing cellular stress, apoptosis, and contributing to tubulointerstitial changes. (3). Threshold effect: Our investigation reveals a TG/HDL-C ratio threshold 4.25, below this ratio, TA susceptibility rises sharply, while above, risk plateaus. This non-linear pattern likely stems from multiple factors. Foremost, rigorous statistical analysis determined the inflection point, controlling confounders that could impact the TG/HDL-C ratio and TA risk. Such findings imply that beyond a certain lipid-induced renal impairment level, ancillary factors may come into play in the injury's progression. We found individuals with ratios ≤4.25 were predominantly female, without hypertension, and had lower BMIs, DBPs, UAs and UPROs compared to those with higher ratios (Supplementary Table S1). Although these features strongly associate with TA risk (5, 33, 36–38), their collective impact enhances among those with ratios >4.25, reducing the TG/HDL-C ratio's effect. In contrast, with fewer impactful risk factors present below 4.25, the ratio's influence is amplified—likely contributing to the nonlinear relationship. Recent studies have shown a nonlinear correlation between TG/HDL-C ratios and the risk of CKD in American adults, pinpointing the curve's inflection point at a TG/HDL-C ratio of 6.68 (39). Furthermore, our literature review indicates that this ratio also exhibits a nonlinear relationship with the risk of other conditions, such as in-hospital mortality from acute type B aortic dissection, arterial stiffness, the incidence of type 2 diabetes, and the onset of pre-diabetes (40–43). These studies consistently reveal that as TG/HDL-C ratios increase, the risk of disease rises but then plateaus after exceeding a certain threshold, suggesting a saturation effect. This implies that beyond a certain point, further increases in the TG/HDL-C ratio do not proportionally escalate the risk of disease. The exact factors and mechanisms underlying this pattern warrant further exploration. Another possible explanation is that high TG/HDL-C ratios often lead clinicians to initiate lipid-lowering therapy, which could mitigate TG/HDL-C related damage to renal tubules through medical intervention (7).

Therefore, a non-linear relationship means changes in one variable do not consistently correspond to changes in another-their interrelation is complex, virtually absent and unpredictable, unlike linear relationships. However, non-linear entities can be related to each other in ways that are fairly predictable, but simply more complex than in a linear relationship. Given the intricate association between the TG/HDL-C ratio and TA risk, identifying this non-linear relationship brings us closer to elucidating their true connection. Pinpointing a TG/HDL-C threshold ratio of 4.25 holds major clinical implications: (1). Risk Stratification: The TG/HDL-C ratio serves to assess TA risk in PMN patients. Recognizing 4.25 as a potential inflection point for renal damage assists clinician risk appraisal and therapeutic timing. (2). Therapeutic Intervention: This non-linear relationship finding enables tailored lipid-lowering treatments. For example, statins/fibrates could be used more aggressively in patients exceeding this threshold to mitigate TA risk. (3). Monitoring and Management: Consistent tracking of the TG/HDL-C ratio in PMN patients can reveal the success of treatments and signal when adjustments in dyslipidemia management are necessary.

After conducting a thorough subgroup analysis, the authors found that age, BMI, hypertension, UPRO, ALB, and severe nephrotic syndrome do not function as effect modifiers in influencing the association between the TG/HDL-C ratio and TA. Irrespective of factors such as gender, age, BMI, blood pressure, albumin level, the presence of massive proteinuria or severe nephrotic syndrome, effectively controlling the TG/HDL-C ratio demonstrated a significant decrease in the risk of TA.

The potential mechanisms underlying renal damage caused by the TG/HDL-C ratio can be explained as follows. Firstly, the reabsorption of phospholipids and cholesterol in renal tubular epithelial cells leads to the release of inflammatory factors and tissue damage (44–46). Secondly, the accumulation of lipoproteins in the glomerular mesangium stimulates the production of inflammatory cytokines, activating macrophages and ultimately resulting in glomerulosclerosis (44). Thirdly, a high TG/HDL-C ratio is a risk factor for both atherosclerosis (47) and CKD (48). Additionally, the TG/HDL-C ratio proves to be a reliable indicator of insulin resistance (49), which in turn induces oxidative stress (50). This oxidative stress

impairs the activation of nuclear factor erythroid-2-related factor-2, which is a protective mechanism against kidney tissue injury (51).

Study strengths and limitations

Our study possesses several strengths. Firstly, we employed both categorical and continuous TG/HDL-C ratios as independent variables to assess their correlation with TA risk. This approach effectively minimizes information loss and allows for the quantification of their relationship. Secondly, we tackled missing data by utilizing multiple imputations, a method that enhances the statistical power and mitigates potential bias arising from missing covariate information. Thirdly, our study represents a significant advancement in the understanding of nonlinearity compared to prior research. Moreover, we have revealed a non-linear relationship between the TG/HDL-C ratio and the risk of TA in patients with PMN, thereby enhancing our understanding in this domain.

However, it is important to consider several limitations in our study. Firstly, our study focused exclusively on PMN patients, and therefore further validation is necessary to generalize these findings to other types of glomerulonephritis. In future studies, efforts should be made to validate the correlation between the TG/HDL-C ratio and TA in different types of glomerulonephritis. Secondly, we only examined the TG/HDL-C ratio at baseline, without considering any subsequent changes over time. Additionally, certain indicators related to TA, such as medication history, were not included in the original data. To address these limitations, we can adjust our study design or collaborate with other researchers to gather additional data points, including information on the dynamic changes of the TG/HDL-C ratio during follow-up. Thirdly, as is customary in observational research, there may be uncontrolled or unmeasured confounding factors that persist, such as the use of antihypertensive, lipid-lowering, uric acid-lowering, antidiabetic medications, immunosuppression or RAS inhibition, even after accounting for known potential confounders like blood pressure and FPG. Nonetheless, we have calculated the E-value to evaluate the potential impact of these unmeasured confounders, and the results suggest that they are unlikely to explain the outcomes. Furthermore, in future research, it would be beneficial to expand the range of variables collected, including antihypertensive, lipid-lowering, SUA-lowering, antidiabetic agents, immunosuppression or RAS inhibition among others. Fourthly, our center started testing blood PLA2R in 2018, while the study population we included started from 2008, so 10 years of PLA2R results are missing. As PLA2R antibody results were missing for 228 of the 363 patients, comprising over 60% of the intended study sample, we didn't analysis the associations between anti-PLA2R antibody with TG/HDL-C ratio. We will prioritize consistent PLA2R monitoring moving forward in our longitudinal cohort. Once a more robust dataset is available, we will be well positioned to conduct exploratory analyses on relationships of anti-PLA2R levels with TG/HDL-C ratio and other relevant clinical parameters. Finally, it is crucial to acknowledge that this study employed a cross-sectional design, which limits our ability to establish a definitive causal relationship.

Conclusion

This study provides evidence supporting the TG/HDL-C ratio as an independent risk factor for TA in patients with PMN. Additionally, it reveals a non-linear correlation between the TG/HDL-C ratio and TA risk. Notably, there is a strong association between the TG/HDL-C ratio and TA risk, particularly when the ratio is below 4.25. These findings suggest that reducing the level of the TG/HDL-C ratio could be a sensible approach to mitigating TA risk, regardless of kidney function status. Therefore, it is crucial to prioritize TG management in PMN patients to effectively minimize the risk of TA. Further studies should also explore the mechanisms by which TG/HDL-C ratio influences TA risk and whether interventions aimed at altering this ratio can lead to a tangible reduction in the incidence of TA among PMN patients.

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

The studies involving humans were approved by Medical Ethics Committee of Shenzhen Second People's Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

MG: Writing – original draft, Methodology, Resources. LW: Methodology, Writing – review & editing. YC: Investigation, Writing – review & editing. DQ: Investigation, Writing – review & editing. JC: Investigation, Writing – review & editing. HS: Writing – review & editing, Methodology, Formal analysis. HH: Writing – review & editing, Investigation, Methodology, Software. QW: Writing – review & editing, Funding acquisition, Project administration, Supervision.

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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.2024.1322646/full#supplementary-material

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Network pharmacology combined with Mendelian randomization analysis to identify the key targets of renin-angiotensin-aldosterone system inhibitors in the treatment of diabetic nephropathy

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Background: Diabetic Nephropathy (DN) is one of the microvascular complications of diabetes. The potential targets of renin-angiotensin-aldosterone system (RAAS) inhibitors for the treatment of DN need to be explored.

Methods: The GSE96804 and GSE1009 datasets, 729 RAAS inhibitors-related targets and 6,039 DN-related genes were derived from the public database and overlapped with the differentially expressed genes (DN vs. normal) in GSE96804 to obtain the candidate targets. Next, key targets were screened via the Mendelian randomization analysis and expression analysis. The diagnostic nomogram was constructed and assessed in GSE96804. Additionally, enrichment analysis was conducted and a 'core active ingredient-key target-disease pathway' network was established. Finally, molecular docking was performed.

Results: In total, 60 candidate targets were derived, in which *CTSC* and *PDE5A* were screened as the key targets and had a causal association with DN as the protective factors ($P < 0.05$, OR < 1). Further, a nomogram exhibited pretty prediction efficiency. It is indicated that Benadryl hydrochloride might play a role in the DN by affecting the pathways of 'cytokine cytokine receptor interaction', etc. targeting the *CTSC*. Moreover, *PDE5A* might be involved in 'ECM receptor interaction', etc. for the effect of NSAID, captopril, chlordiazepoxide on DN. Molecular docking analysis showed a good binding ability of benadryl hydrochloride and *CTSC*, NSAID and *PDE5A*. *PTGS2*, *ITGA4*, and *ANPEP* are causally associated with acute kidney injury.

Conclusion: *CTSC* and *PDE5A* were identified as key targets for RAAS inhibitors in the treatment of DN, which might provide some clinical significance in helping to diagnose and treat DN. Among the targets of RAAS inhibitors, PTGS2, ITGA4 and ANPEP have a causal relationship with acute kidney injury, which is worthy of further clinical research.

KEYWORDS

diabetic nephropathy, GEO, network pharmacology, mendelian randomization, acute kidney injury

Introduction

Diabetic nephropathy is a disease characterized by a persistent increase in proteinuria and progressive elevation of blood pressure (1), and is also one of the most serious chronic microvascular complications of diabetes mellitus (2). About 50% of patients with DN eventually develop end-stage renal disease (ESRD) (3), and with the global diabetes epidemic, DN has gradually replaced other kidney diseases as the leading cause of ESRD (2, 4). DN has an insidious onset and early symptoms are not obvious (5), so it cannot be diagnosed by simple clinical signs (6). If DN progresses to ESRD, the only effective treatments for patients are dialysis and kidney transplantation (7), but these two treatments do not improve the survival prognosis of patients (8, 9). Therefore more and more researches are devoted to finding new therapeutic targets and diagnostic sites (10–12). The mechanisms of DN progression are complex and diverse, involving multiple pathways and mediators (13). Traditionally, the mechanism of development of DN is the result of abnormalities in body homeostasis, including hemodynamic abnormalities, metabolic disturbances, and imbalances in hormone synthesis, such as angiotensin II (Ang-II) (14). Although the exact pathogenesis of DN cannot be fully elucidated, studies have suggested that the renin-angiotensin-aldosterone system (RAAS), oxidative stress, and TGF-β are relatively common pathogenic mechanisms in the complex pathogenesis of DN (15, 16), and that a more comprehensive blockade of the RAAS may have more clinical benefits for DN (17, 18).

The RAAS is a complex network of multiple proteases and short peptides that regulate cardiovascular and renal function (19). RAAS inhibitors include angiotensin-converting enzyme inhibitors (ACEi), angiotensin II receptor antagonists (ARBs), aldosterone antagonists, and direct renin inhibitors (20). Recent studies have found that RAAS inhibitors significantly slow the progression of a wide range of diseases, including hypertension, myocardial remodeling after acute myocardial infarction, acute and chronic heart failure, and renal insufficiency, improving the prognosis of patients (21, 22). RAAS inhibitors dilate the glomerular outgoing and incoming small arteries to different degrees, and reduce the glomerular intraglomerular pressure leading to a decrease in urinary protein (23). Although RAAS inhibitors provide the rationale for current renoprotective therapies, there are limited data on whether early targeting of RAAS prevents kidney disease (24). Besides, nonproteinuric DN and DN without retinopathy in type 2 DM patients affects the detection of persistent albuminuria, so that RAAS inhibitors that target blocking albuminuria cannot be applied in time (25). ARBs reduce cardiovascular and renal complications in patients with DN, but the therapeutic effect may vary from patient to patient, and the exact regulatory mechanisms remain unclear (26). Previous studies involving ACEi have demonstrated beneficial effects on proteinuria, but it has not been demonstrated that blockade of the renin-angiotensin system is superior to non-blockade forms of therapy in slowing the progression of end-stage renal disease (27). Theoretically, the combination of ACEi and ARBs reduces proteinuria, but actually increases the risk of Acute kidney injury (AKI) and acute electrolyte disturbances (28). Combining the renin inhibitor aliskiren with an ACEI or ARB significantly increased the risk of hyperkalemia and did not reduce the risk of cardiovascular disease or renal failure (29). Therefore, there is an urgent need to further explore about the target mechanism of RAAS inhibitors acting in DN.

Mendelian randomization (MR) is a combination of the instrumental variables (IVs) method and Mendel's laws of inheritance (30), which breaks through the limitations of traditional randomized controlled studies and avoids the interference of confounding factors (31). With the current development of basic Mendelian theory and the increase in practical applications, drug-targeted MR analysis is emerging as an effective tool for inferring the effects of drugs, antagonists, agonists, activators, or inhibitors targeting protein-coding genes on disease risk (32). In contrast to conventional MR analysis, drug-targeted MR analysis utilizes genetic variants in DNA sequences located within or near genes to predict the effect of the corresponding drug (33). Network pharmacology is an analysis method to visualize the correlation between drug components, targets and diseases (34). There are currently no studies on the causal relationship between the key targets of RAAS inhibitors and DN, and thus there is an urgent need for rational approaches to

further elucidate the potential nature and significance of the above relationship.

In this study, we combined network pharmacology and drug-target MR for the first time to estimate the causal relationship between key targets of RAAS inhibitors and DN. Based on the DN-related data in the Gene Expression Omnibus (GEO) database and other public databases, we screened two key targets in the treatment of DN by RAAS inhibitors. The study further constructed a diagnostic nomogram, enrichment analysis and subcellular localization analysis of the key targets. Networks were constructed through network pharmacology, such as the Transcription Factors (TF) -Key target regulatory network and the competitive endogenous RNA (ceRNA) regulatory network. Finally, molecular docking was performed (Figure 1 **Graphical Abstract**).

Materials and methods

Data source

The GSE96804 dataset, which included 41 type 2 DN samples (female) and 20 samples of unaffected parts of tumor nephrectomy, was applied as the training set (35). The GSE1009 dataset, which involved 3 DN samples and 3 normal samples, was considered as the validation set. All samples were derived from human renal glomerular tissues. The GSE96804 and GSE1009 datasets were downloaded from GEO database (https://www.ncbi.nlm.nih.gov/geo/). The components of RAAS inhibitors included aliskiren, enalapril, captopril, cilazapril, benazepril hydrochloride, enalapril maleate, valsartan, losartan, irbesartan, telmisartan, olmesartan, spironolactone, eplerenone, and fennelenone. RAAS inhibitors-related targets were identified by using the Swiss Target Prediction database. DN-related genes (DRGs) were screened via the GeneCards database and Online Mendelian Inheritance in Man (OMIM) database. The final DRGs were derived from the summary of the gene data that retrieved from the two databases and the removal of duplicate targets.

Differential expression analysis

Differential expression analysis was carried out between normal and DN groups in the GSE96804 dataset via 'Limma' package (version 3.54.1) to obtain differentially expressed genes (DEGs) (36). Screening conditions were $|\log2FC| > 0.5$ and $P < 0.05$. Candidate targets were derived from the intersection of DEGs, RAAS inhibitors-related targets and DRGs.

Functional enrichment analysis and protein-protein interaction (PPI) network construction

In order to better explain the potential biological role of DEGs and candidate targets, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was carried out via 'clusterProfiler' (version 4.0) in the GSE96804 dataset (37), and screening condition was $P < 0.05$. Moreover, in order to determine the interaction between candidate targets, a PPI network was established with confidence score > 0.4 via search tool for the retrieval of interacting genes (STRING) database (https://string-db.org/).

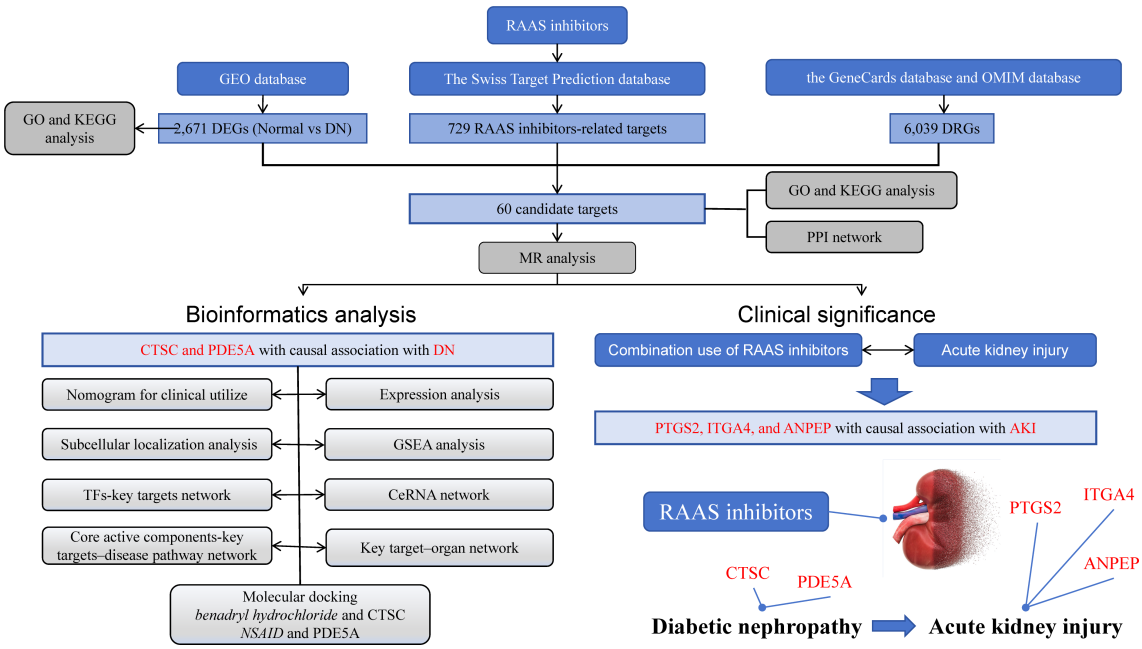

FIGURE 1
Graphical Abstract. The research process is illustrated in the diagram. The upper part represents the data included in the study, the bottom left corner represents the analysis involved in the research, and the bottom right corner represents the research findings.

Identification of key targets

Candidate targets that was causally associated with DN were screened via the MR analysis. The screening conditions were $P < 0.05$ for IVW method and $P > 0.05$ for Horizontal pleiotropy analysis. Next, the above targets were included in the GSE96804 and GSE1009 datasets for expression level analysis, and the targets with significant expression levels and consistent expression trends in two datasets were considered as key targets.

MR analysis

In order to explore whether there was a causal relationship between key targets and DN, the key targets were considered as exposures, and DN was considered as the outcome for MR analysis. Considering the potential impact of RAAS inhibitors on acute renal injury, it is further proposed to use key targets as exposure factors and AKI as the outcome. The single nucleotide polymorphisms (SNPs), which had a significant link with exposures, were selected as IVs ($P < 5 \times 10^{-8}$) via 'TwoSampleMR' package in R (version 0.5.6) (38). Subsequently, the IVs with strong linkage disequilibrium (LD) were removed ($r^2 < 0.001$, kb = 10000). The F-statistic values of SNPs were displayed in Supplementary Table S1. The effect alleles and effect quantities were unified via the R package 'TwoSampleMR' mv_harmonise_data function, and the mv_lasso_feature_selection function was devoted to eliminate the collinearity screening variables. Various MR approaches were used to confirm the causal associations between the key target genes and DN, containing the inverse variance weighted (IVW) (39), Mendelian randomization-Egger (MR-Egger) (40), weighted median (WM) (41), simple mode (42) and weighted mode methods (43), with IVW method predominating. $P < 0.05$ for IVW method was considered suggestive for the potential causal association. Furthermore, the odds ratios (ORs) were calculated. The value was greater than 1 being the risk factor and less than 1 being the protective factor. The scatter plot, forest plot and funnel plot were devoted to exhibit the results. Thereafter, the sensitivity analysis was devoted to estimate the reliability of the MR results via the Heterogeneity, Horizontal pleiotropy and Leave-One-Out (LOO) analysis. Moreover, the heterogeneity test was carried out and $P > 0.05$ demonstrated that there was no heterogeneity. $P > 0.05$ indicated that there was no horizontal pleiotropy in the horizontal pleiotropy test. LOO analysis was implemented by removing SNPs which were outliers.

Construction of nomogram

In order to predict the prevalence rate of DN patients, a diagnostic nomogram was constructed in the GSE96804 dataset via the 'RMS' package (version 6.6-0) (44) based on key targets. The ability of the nomogram to predict DN was assessed via the calibration curves and decision curve analysis (DCA). Moreover, receiver operator characteristic (ROC) curve was plotted via the R package 'pROC' (version 4.0.5) (45) to assessed the prediction effect of the nomogram.

Construction of a key target–organ network, subcellular localization analysis and enrichment analysis

In order to clarify the expression of key targets in the various organs and tissues, the expression of key targets in different organs was derived from the BioGPS database. The expression of the second abundant tissue was no more than one-third as a screening condition. The key target-organ network was constructed via the Cytoscape software (version 3.8.2) (46). Subcellular localization analysis of key targets was performed via 'mRNALocater' database, and protein sequences of key targets were downloaded from NCBI database. When the key targets were set as the objective genes, the correlation coefficients of the expression levels of all genes and the objective genes were calculated as the ranking criteria. Furthermore, Gene Set Enrichment Analysis (GSEA) was performed to explore the fuction of key targets via the 'ClusterProfiler' package (version 4.0) in the GSE96804 dataset (37). The screening condition was $P.adj < 0.05$. The most significant TOP5 pathway was selected for display.

Constrution of 'core active ingredient-key target-disease pathway', TF-key targets and ceRNA network

In order to explore the relationship between 'core active ingredient-key target-disease pathway', active ingredients targeting key targets were selected as core active ingredients. The disease pathway were derive from the TOP5 KEGG pathway enriched by the key targets. TFs associated with key targets were predicted via the ChEA3 database. Moreover, the miRNAs associated with key targets were forecasted by Starbase database (https://starbase.sysu.edu.cn/index.php), and the screening criterion was clipExpNum > 8. The lncRNAs associated with miRNAs were predicted by Starbase database, and the screening criterion was clipExpNum > 13. The 'Cytoscape' software (version 3.8.2) was devoted to establish the 'core active ingredient-key target-disease pathway', 'TF-key targets' network and 'lncRNA-miRNA-mRNA' network (46).

Molecular docking

In order to determine the binding ability between the core active components and key targets, molecular docking was conducted via the AutoDock Vina (version 4.2) (47). Briefly, the docking was as follows. 1) The 3D structure of the key target protein was retrieved from the RSCB PDB database and downloaded with the PDB file

format, and then non-polar hydrogen was added to the three-dimensional structure using AutoDockTools software to calculate the charge and saved it as a PDBQT file as a pair of receptors. 2) The SDF format file of the 2D structure of the core active ingredient was retrieved and downloaded from PubChem, and then converted into the mol3 format file of the 3D structure via the ChemBio2D software. Then the ligand mol2 file was integrated into AutoDockTools, and the file was output to the PDBQT format file as a docking ligand. 3) Molecular docking of key targets and core active components was conducted via the 'AutoDock Vina', and the free binding energy was evaluated. 4) PyMOL and Discovery Studio software were used to visualize the molecular docking results.

Statistical analysis

Statistical tests were carried out via the R software (version 4.2.2). Statistical significance was defined as $P < 0.05$.

Results

Identification of DEGs and functional enrichment analysis in the GSE96804 dataset

A number of 2,671 DEGs were discovered between normal and DN groups in GSE96804 dataset, which included 1,278 up-regulated genes and 1,393 down-regulated genes. Volcano plot and heatmap were plotted to visualize these DEGs (Figures 2A, B). In order to better explain the potential biological role of DEGs, GO and KEGG enrichment analysis were conducted. GO results showed that DEGs were mainly involved in the 'small molecule catabolic process' and 'alpha−amino acid metabolic process' (Figure 2C). KEGG enrichment analysis revealed that DEGs mainly involved in 'Fatty acid degradation' and 'AGE−RAGE signaling pathway in diabetic complications' (Figure 2D).

Identification of candidate genes and exploration of potential biological functions

A total of 729 RAAS inhibitors-related targets were identified via the Swiss Target Prediction database. A number of 6,039 DRGs were discovered. A number of 60 candidate genes were derived by intersecting DEGs, RAAS inhibitors-related targets and DRGs (Figure 3A; Supplementary Table S2). In addition, GO results showed that candidate genes were involved in 'leukocyte migration' and 'response to oxidative stress' (Figure 3B). KEGG result revealed that candidate genesmainly involved in 'Renin −angiotensin system' and 'PPAR signaling pathway' (Figure 3C). A PPI network, which contained 57 nodes and 216 edges was constructed, in which *CASP3*, *ITGA5* and *MMP14* were more important (Figure 3D).

CTSC and *PDE5A* were viewed as protective factors for DN

A total of six candidate targets that was causally associated with DN, which were *ALPL*, *CETP*, *CTSC*, *FOS*, *ITGA5* and *PDE5A* were screened with $P < 0.05$ for IVW method and $P > 0.05$ for Horizontal pleiotropy analysis (Table 1). Furthermore, *CTSC* and *PDE5A* were selected as the key targets. The expression level of *CTSC* and *PDE5A* were significantly high in the CCA group (Figure 4A). *CTSC* and *PDE5A* were causally associated with DN by IVW approach, and they were the protective factors for DN ($P < 0.05$, OR < 1) (Table 2). The scatter plot revealed that *CTSC* and *PDE5A* were negatively correlated with DN (slope < 0) (Figures 4B, C). In the forest plot, the MR effect size was less than 0, indicating that *CTSC* and *PDE5A* were the protective factors for DN (Figures 4D, E). The funnel plot of two genes exhibited that the MR analysis conformed to the random grouping of Mendel's second law (Figures 4F, G). In order to evaluate the reliability of MR results, the sensitivity analysis was carried out. The *P* value of the Cochrane's Q test was greater than 0.05, indicating that there was no heterogeneity between the two sample datasets of exposures and outcome (Table 3). Meanwhile, the *P* value of the horizontal pleiotropy test was greater than 0.05, indicating that there was no interference of confounding factors (Table 4). LOO analysis revealed that there was no significant deviation in the effect value of the IVs (Figures 4H, I).

Diagnostic value of *CTSC* and *PDE5A* in DN

In order to predict the prevalence rate of DN patients, the diagnostic nomogram was established on the basis of key targets in the GSE96804 dataset (Figure 5A). In the GSE96804, the AUC value of ROC curve for normogram was greater than 0.7, indicating that predictive accuracy of nomogram was high (Figure 5B). The slope of the calibration curve was close to 1, and it demonstrated that nomogram had pretty prediction efficiency (Figure 5C). Moreover, the results of DCA showed that the net income of the nomogram was higher than a single factor. It also reflected the pretty prediction effect of the nomogram (Figure 5D).

Exploring potential binding sites for *CTSC* and *PDE5A*

The mRNA levels of the *CTSC* and *PDE5A* were evaluated. In total, 12 organs or tissues were associated with *CTSC*, including the lung, smooth muscle and CD56+_NK Cells. A number of 11 organs or tissues were associated with *PDE5A*, containing adrenal gland, heart and liver (Figure S1). Subcellular localization analysis showed that *CTSC* had the highest proportion in cytoplasm and *PDE5A* had the highest proportion in nucleus (Figure S2). *CTSC* was significantly involved in 'cytokine cytokine receptor interaction', 'ribosome', 'ECM_receptor_interaction', 'focal_adhesion' and 'oxidative phosphorylation' (Figure 6A). Benadryl hydrochloride might play a role in the DN by affecting these pathways through the

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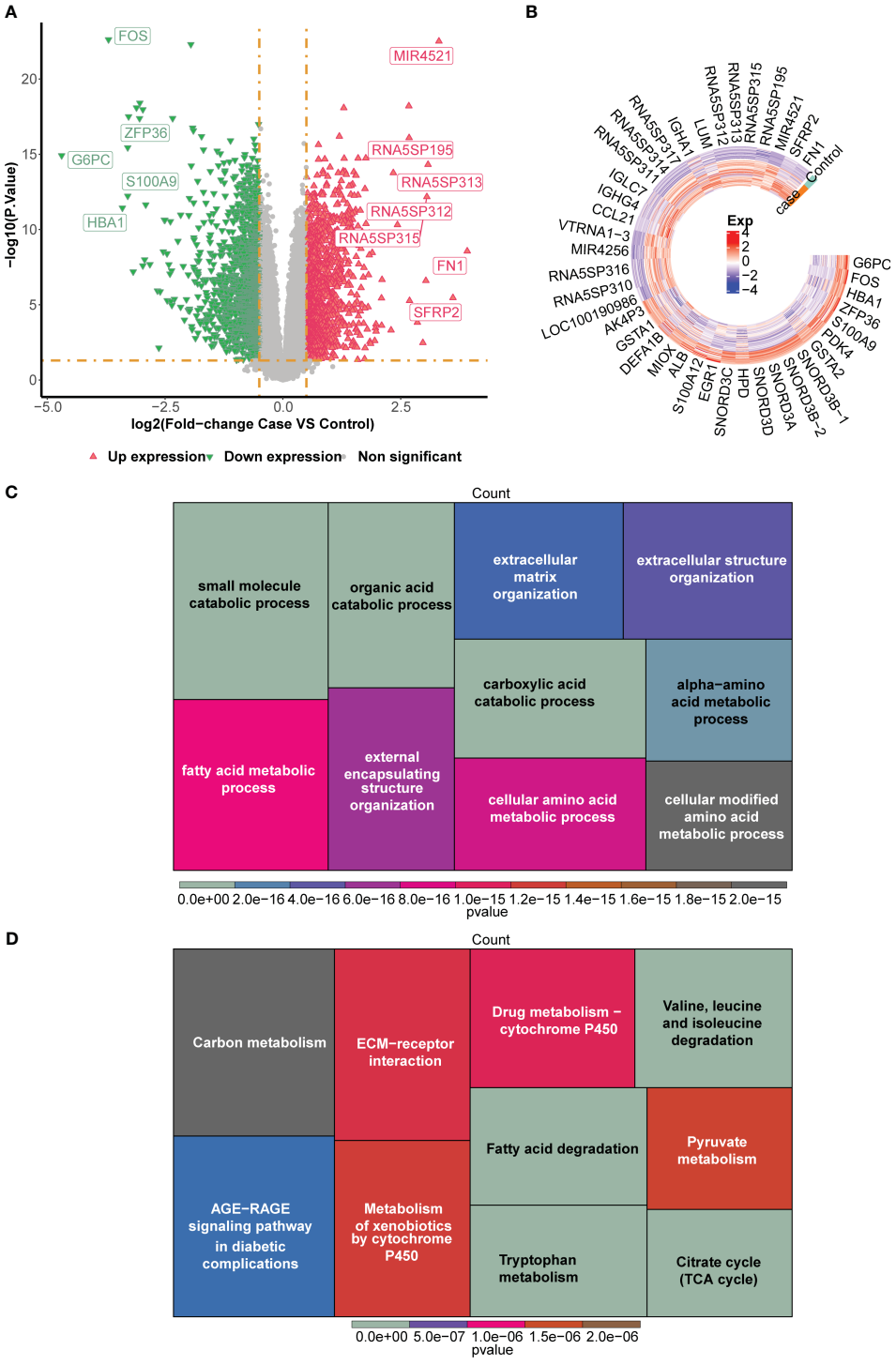

FIGURE 2
Screening of differentially expressed genes (DEGs) between normal and DN groups in the GSE96804 dataset. **(A)** The volcano plot and **(B)** heatmap for the expression patterns of the DEGs. The heatmap for the enriched **(C)** Gene Ontology (GO) terms and **(D)** Kyoto Encyclopedia of Genes and Genomes (KEGG) terms by the DEGs.

CTSC. Moreover, *PDE5A* was significantly involved in 'parkinsons disease', 'peroxisome', 'huntingtons disease', 'ECM receptor interaction' and 'oxidative phosphorylation' (Figure 6B). NSAID, captopril, chlordiazepoxide, Enalapril maleate, cilazapril, valsartan and Inaquillon might play roles in the DN by affecting these pathways through the *PDE5A* (Figure 6C).

Investigation of regulatory mechanisms between key targets and other types of molecules

To further explore the potential mechanism of *CTSC* and *PDE5A*, we predicted their targeted TFs, miRNAs, and lncRNAs by online

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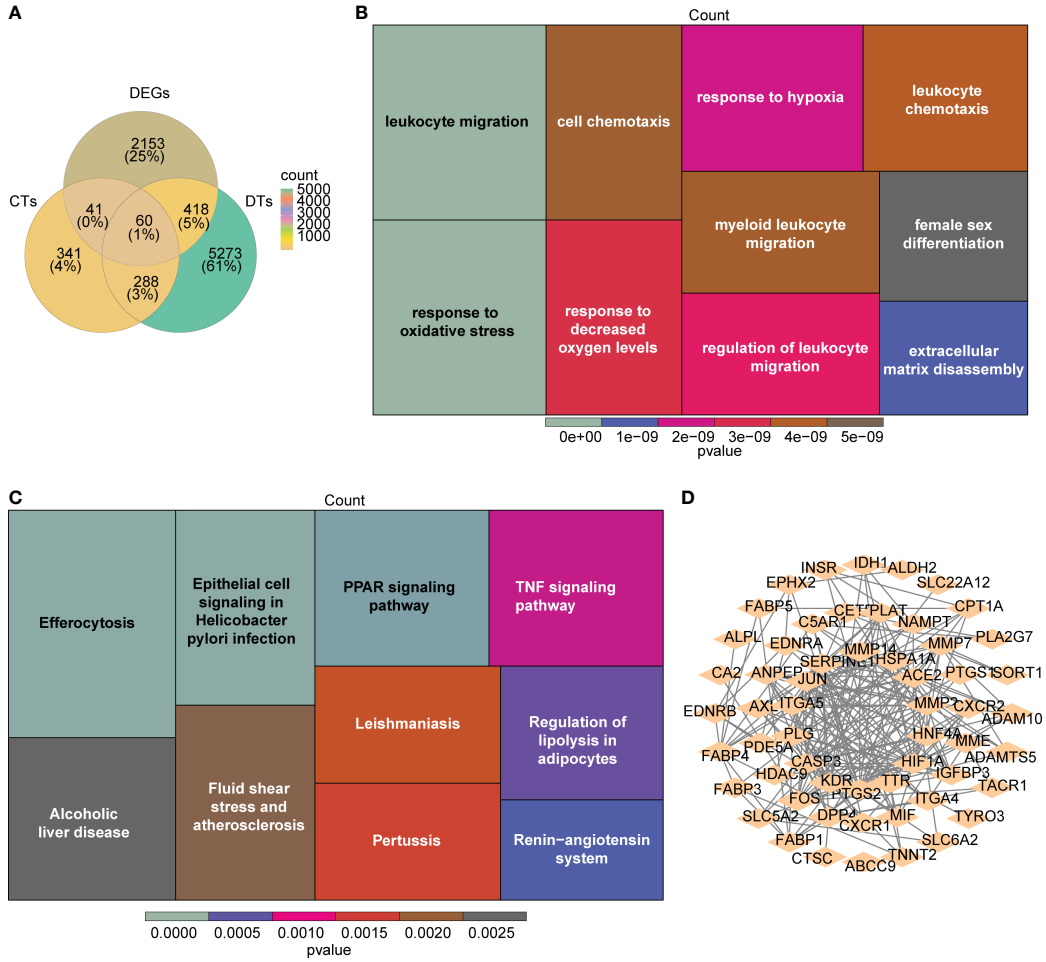

FIGURE 3
Identification and interaction of potential candidate genes in DN. **(A)** The venn plots of the intersection for DEGs, renin-angiotensin-aldosterone system (RAAS) inhibitors-related targets, and DN-related genes (DRGs). **(B, C)** The heatmap for the functional enrichment analysis (B:GO; C:KEGG) of the 60 candidate genes. **(D)** The protein-protein interaction (PPI) network for interaction of candidate genes with confidence score > 0.4.

databases and constructed regulatory networks. In total, 44 TFs that associated with *CTSC* were prediceted, a number of 11 TFs that associated with *PDE5A*, in which *CTSC* and *PDE5A* were regulated by E2F6, MAZ, CTCF and TCF12 (Figure 7A). Moreover, a ceRNA network, that contained 12 miRNAs, 45 lncRNAs, *CTSC* and *PDE5A* was established, in which SNHG5 regulated *CTSC* by hsa-miR-216a-5p, SNHG5 regulated *PDE5A* by hsa-miR-181a-5p (Figure 7B).

Prediction of active ingredient-target binding capacity by molecular docking

In order to determine the binding ability between the core active components and key targets, molecular docking was carried out. Molecular docking analysis showed that the docking affinity between core active ingredients benadryl hydrochloride and *CTSC*

TABLE 1 Information for six candidate targets causally associated with diabetic nephropathy (DN).

id.exposure	id.outcome	method	nsnp	pval	pleio_pval
eqtl-a-ENSG00000162551	ebi-a-GCST90018832	IVW	5	0.017	0.762
eqtl-a-ENSG00000087237			5	0.015	0.823
eqtl-a-ENSG00000109861			12	0.041	0.693
eqtl-a-ENSG00000170345			3	0.003	0.661
eqtl-a-ENSG00000161638			5	0.000	0.667
eqtl-a-ENSG00000138735			9	0.018	0.746

IVW, Inverse variance weighted.

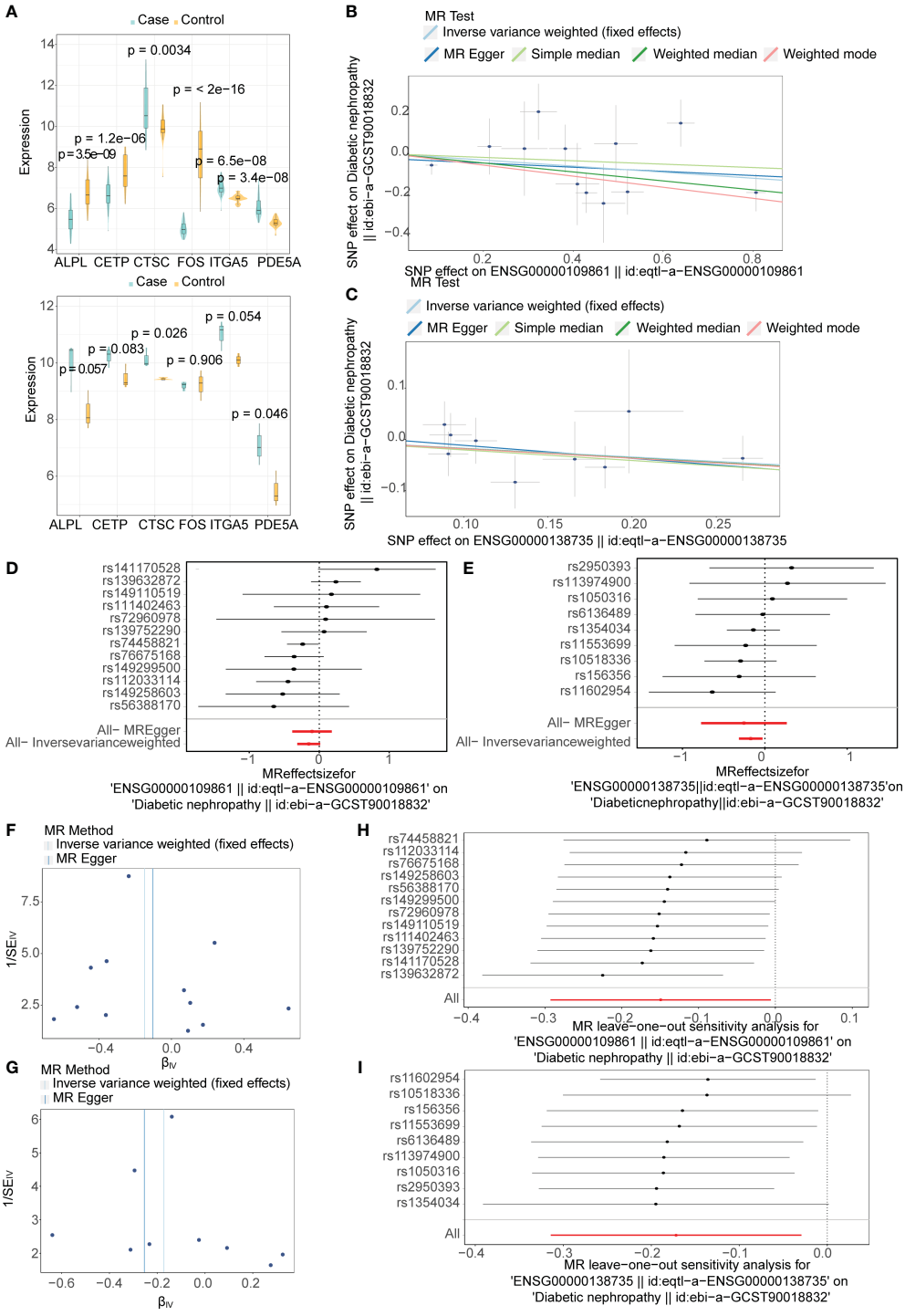

FIGURE 4
Mendelian randomization (MR) analysis and expression analysis for selecting two key targets in DN. **(A)** Boxplots for the expressions levels of six candidate targets with potential causality on DN in the GSE96804 (top) and GSE1009 (bottom) datasets. **(B, C)** The scatter plot of the Mendelian randomization (MR) analysis for relationship of two key targets (B:*CTSC*; C:*PDE5A*) and DN. **(D, E)** Forest plots of the MR analysis for diagnostic significance of two key targets (D:*CTSC*; E:*PDE5A*) on DN. **(F, G)** Funnel plots of the MR analysis for two key targets (**F**:*CTSC*; **G**:*PDE5A*) on DN. **(H, I)** Leave-one-out analysis of the MR analysis for sensitivity analyses of two key targets (H:*CTSC*; I:*PDE5A*) on DN.

TABLE 2 Mendelian randomization (MR) analysis for causal relationship of two key targets (*CTSC* and *PDE5A*) and DN.

outcome	exposure	Method	Pvalue	OR
ebi-a-GCST90018832	eqtl-a-ENSG00000109861 (CTSC)	MR Egger	0.492	0.902
		IVW	0.041	0.861
		Weighted median	0.025	0.802
		Simple mode	0.680	1.090
		Weighted mode	0.024	0.761
	eqtl-a-ENSG00000138735 (PDE5A)	MR Egger	0.371	0.776
		IVW	0.018	0.842
		Weighted median	0.171	0.835
		Simple mode	0.331	0.814
		Weighted mode	0.231	0.832

IVW, Inverse variance weighted; MR-Egger, Mendelian randomization-Egger; WM, weighted median.

was - 5.3 kcal/moL, indicating that the binding ability was good (Figure 8A). The core active ingredients NSAID formed a covalent bond with the *PDE5A*. The docking affinity between NSAID and *PDE5A* was - 5.32 kcal/moL, indicating that the binding ability was good (Figure 8B).

PTGS2, ITGA4, and ANPEP are causally associated with AKI.

To investigate potential factors contributing to renal injury observed in clinical coadministration of RAAS inhibitors, we employed Mendelian Randomization analysis of the core targets of RAAS inhibitors on AKI. Our findings indicate a causal relationship between PTGS2, ITGA4, and ANPEP and AKI (Table 5; Supplementary Information 2).

Discussion

Clinicians have long been accustomed to treating DN with RAAS inhibitors to reduce urinary protein leakage in patients (48, 49). RAAS inhibitors are known to modulate DN perfusion by dilating small glomerular arterioles, which is why they reduce proteinuria. The combination of renin inhibitors, ARBs and ACEi can inhibit Ang II faster and more comprehensively (50), so blocking the multiple pathways of action of RAAS is expected to increase the efficacy of DN. However, excessive inhibition of Ang II will cause the glomerular outflow arterioles to dilate more than the inlet arterioles, thus increasing the risk of renal damage. This may be one of the explanations for the increased risk of acute renal failure found with RAAS inhibitor combination in several large clinical studies (28, 29). Then, the molecular mechanism of the RAAS inhibitor co-administration process in renal tissues remains unknown, and it is still debatable whether there are unknown targets involved in the regulatory process of DN. On the other hand, although the use of RAAS inhibitors did effectively reduce cardiovascular events and inhibit the progression of DN, there is no evidence that RAAS inhibitors reduce renal endpoint events (51). Drug-targeted Mendelian studies are mostly modeling the therapeutic effect of a single drug on a particular disease, making it difficult to break through to how a combination of drugs affects the disease (52). The method of combining drug-targeted Mendelian analysis through network pharmacology may be an effective means to end this problem.

Through our study, We firstly observed that CTSC and PDE5A were causally associated with DN. According to network analysis, both targets were core targets of RAAS inhibitors acting on DN. We analyzed the expression of CTSC, PDE5A in the GSE96804 and GSE1009 between DN and normal groups. The results showed significant and consistent expression trends of CTSC and PDE5A. Besides, the PTGS2, ITGA4, and ANPEP with causal association with AKI were investigated, providing more theoretical perspectives for the combined use of RAAS inhibitors to promote the risk of AKI.

TABLE 3 Results for heterogeneity test.

outcome	exposure	method	Q	Q_df	Q_pval
ebi-a-GCST90018832	eqtl-a-ENSG00000109861 (CTSC)	MR Egger	14.049	10	0.171
		Inverse variance weighted	14.280	11	0.218
	eqtl-a-ENSG00000138735 (PDE5A)	MR Egger	3.689	7	0.815
		Inverse variance weighted	3.803	8	0.874

TABLE 4 Results for horizontal pleiotropic test.

	exposure	egger_intercept	se	pval
ebi-a-GCST90018832	eqtl-a-ENSG00000109861 (CTSC)	-0.024	0.059	0.693
	eqtl-a-ENSG00000138735 (PDE5A)	0.014	0.041	0.746

CTSC encodes for a lysosomal cysteine protease. Rare mutations in the gene cause autosomal-recessive PapillonLefèvre syndrome (53). It is well known that kidney damage does not occur in all diabetics. This suggests that neither the genetic variant itself nor hyperglycemia is sufficient to cause the typical proteinuric kidney damage in DN (54). So what exactly causes DN to occur in diabetics, and the increased risk of renal failure? A previous genome-wide association studies (GWAS) and meta-analysis (comprising 20 studies, 54,450 participants, 2,191,945 SNPs) has confirmed that RAB38/CTSC and HS6ST1 were human DN urinary protein genes (55). Study has indicated that RAB38/CTSC and HS6ST1 are involved in renal regulation of albumin and are associated with proteinuria in diabetic patients. It is worth mentioning that exposure to the environment is also important in diabetic patients, and the above feature (proteinuria in diabetic patients) is more significantly observed when environmental exposure and genetic susceptibility variants occur together (54). But due to the intergenic index SNP mapped upstream of RAB38 and downstream of CTSC and was associated with transcript levels of both genes in whole blood (55). Thus, the exact molecular mechanism and causality of the RAB38/CTSC variants associated with human proteinuria remain unproven. Considering the effectiveness of RAAS inhibitors in clinical control of urinary protein in patients with DN. It is reasonable to speculate that CTSC may indeed be involved in human urinary protein regulation, which provides a theoretical basis for the previous question.

Subcellular localization analysis revealed that CTSC had the highest percentage in Cytoplasm. Enrichment analysis indicated that CTSC was significantly involved in 'cytokine cytokine receptor interaction', 'ribosome', 'ECM_receptor_interaction', 'focal_adhesion' and 'oxidative phosphorylation'. We used molecular docking to explore the interaction of RAAS inhibitor actives with CTSC at the molecular level, and found that Benadryl hydrochloride interacts with CTSC (molecular binding energy for both is -5.3 kcal/mol). This suggests that Benadryl hydrochloride may play a role in DN by influencing the above pathways through

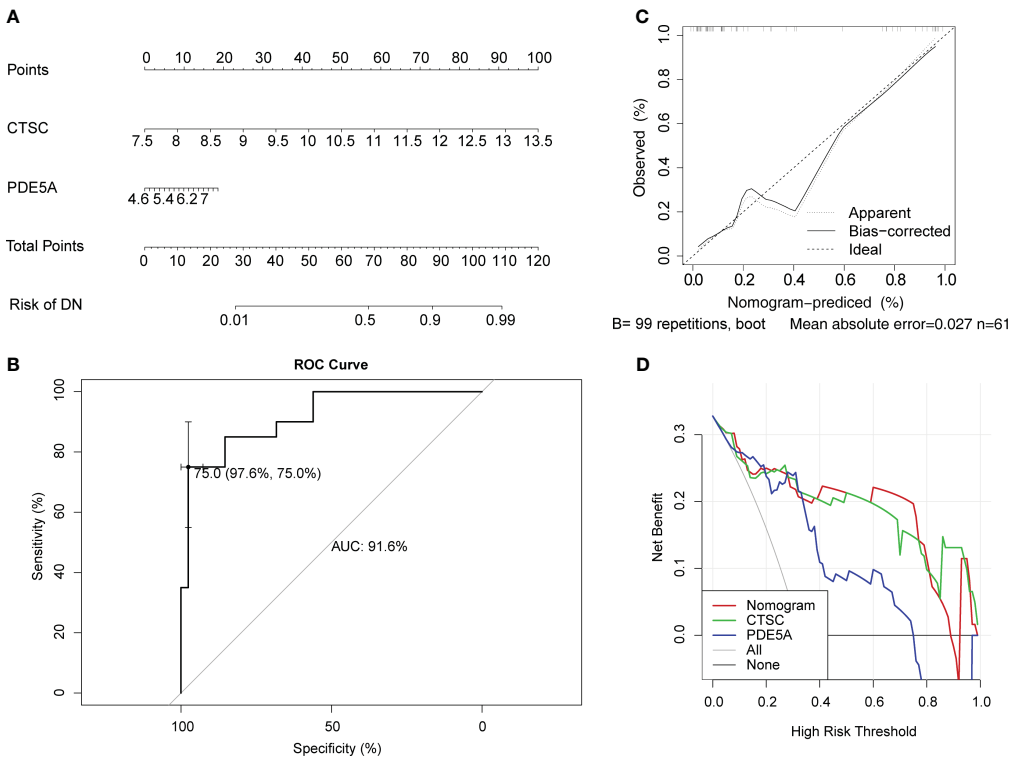

FIGURE 5
Evaluation for clinical utilize of *CTSC* and *PDE5A* via a nomogram. **(A)** Nomogram for predicting the risk of DN. **(B)** Receiver operator characteristic (ROC) curve evaluating the predictive accuracy of nomogram in GSE96804. **(C)** Calibration curves of nomogram for comparing predicted (the horizontal coordinate) and actual (the vertical coordinate) probability of DN. The 45-degree line represents the ideal prediction. **(D)** Decision curve analysis (DCA) for the GSE96804 dataset.

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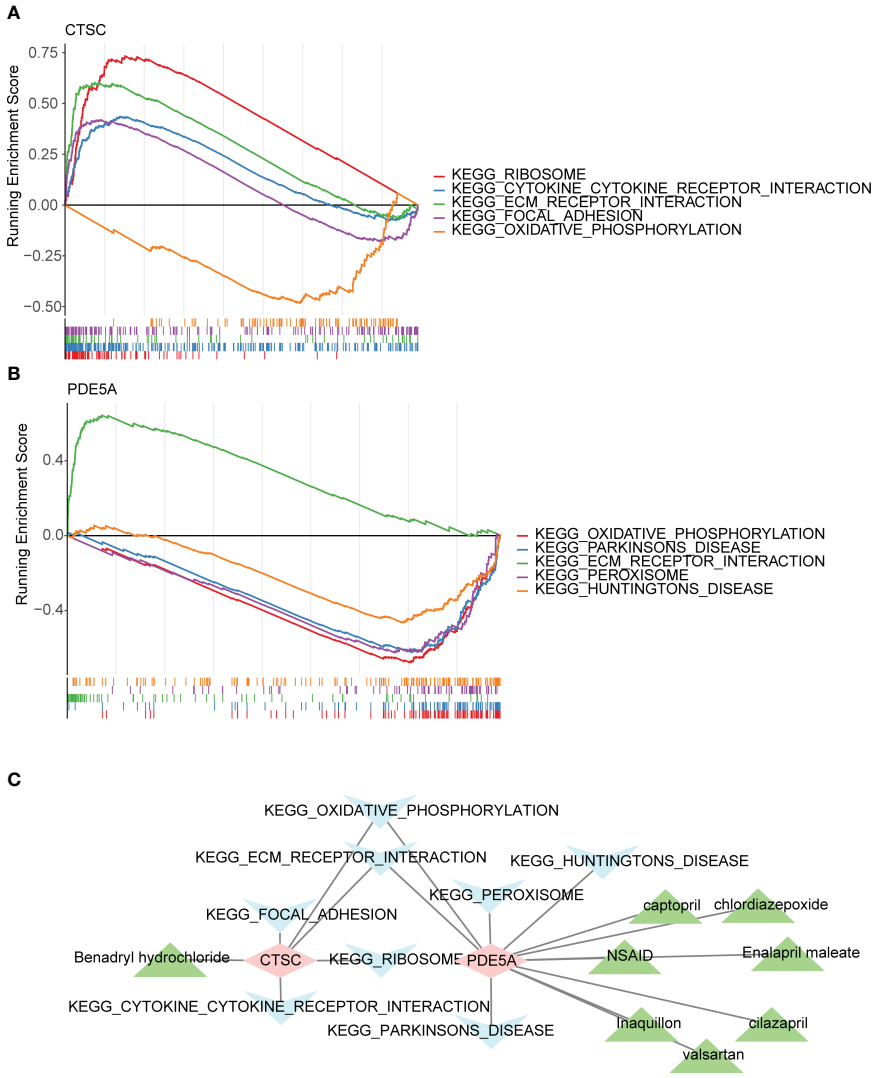

FIGURE 6
Functionality, active ingredients and disease pathway targeting two key targets. Gene set enrichment analysis (GSEA) of **(A)** *CTSC* and **(B)** *PDE5A*. **(C)** The core active ingredient-key target-disease pathway network. Pink is the key target gene, blue is the disease pathway, and green is the core active ingredient.

CTSC, whereas the research of Benadryl hydrochloride in the treatment of DN is still relatively limited. Likewise, there is few studies on the functional mechanism between CTSC and DN, various challenges need to be overcome in combination with the predicted functionality-related clues, and the sufficient clinical samples and the suitable animal models need to be further collected and analyzed.

Phosphodiesterases (PDE) are a superfamily of enzymes (PDE1-PDE11) that hydrolyze cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Therefore, PDE play key roles in intracellular signaling (56). PDE5A belongs to the PDE family, and targeted regulation of PDE5A is considered an effective target for the treatment of cardiovascular disease (57). Indeed, the PDE5 inhibitor sildenafil, which has been approved for clinical use, has been shown to ameliorate diabetic renal podocyte injury, proteinuria, and renal fibrosis (58). Targeted regulation of PDE5 further modulates miR-22 and BMP7 to improve renal hemodynamics and function in DN mice (59). Recent study has suggested that the combination of PDE5 inhibitors with irbesartan is recommended for the treatment of DN because targeted modulation of PDE5 shows anti-renal fibrillary capacity and nephroprotection (58). The current study confirmed some DN therapeutic effects through PDE5 inhibitors, but the mechanism was reported to be related to the reduction of cGMP catabolism or maintenance of cGMP concentration (60, 61). However, in our study, we observed that RAAS inhibitors targeting modulation of PDE5A was a protective factor for DN, which seems to be different from the results of previous studies. As mentioned before, PDE5A is also involved in renal hemodynamic alterations, and excessive reduction of renal entry arterial blood by the RAAS rather increases the risk of renal damage (59). Combined with the fact thatRAAS inhibitors can act on PDE5A in the present study. Therefore, the effect of RAAS inhibitors on PDE5A modulation on DN deserves to be investigated in depth.

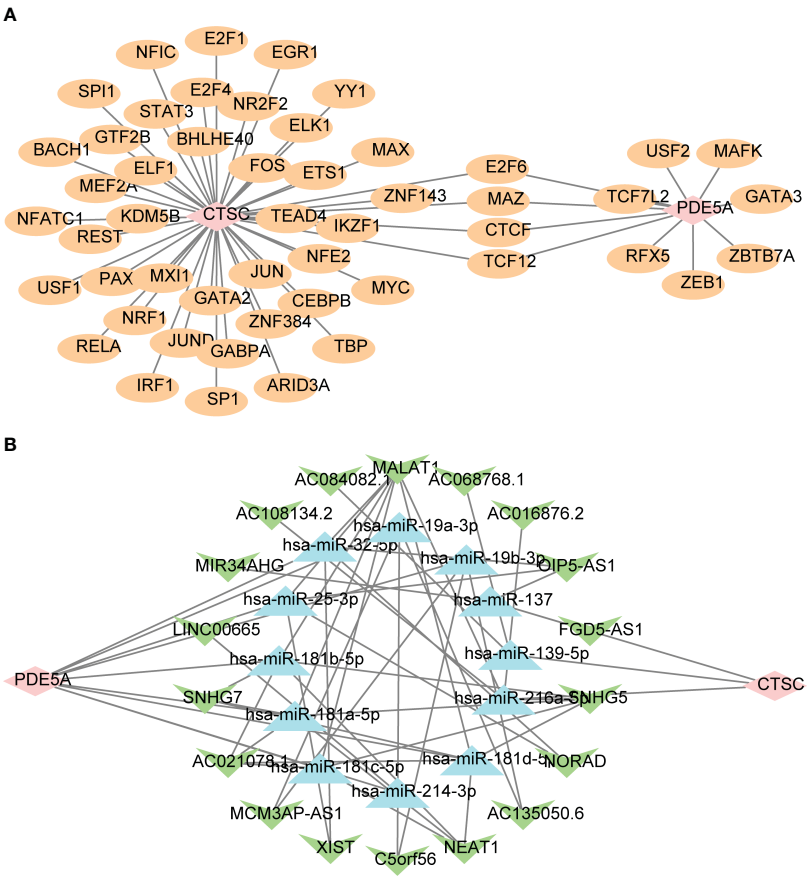

FIGURE 7
Regulatory networks of two key targets. **(A)** The transcription factors (TF)-key targets network. Pink is the target gene, and yellow is TF. **(B)** The competitive endogenous RNA (ceRNA) network targeting key targets. Pink is the key target gene, green is lncRNA, and blue is miRNA.

Enrichment analyses showed that PDE5A was clearly involved in 'ECM receptor interaction', 'Parkinson's disease', 'peroxisome', 'Huntington's disease' and 'oxidative phosphorylation'(Figure 6B). NSAID, captopril, chlordiazepoxide, enalapril maleate, cilazapril, valsartan, and Inaquillon might play roles in DN by affecting these pathways through PDE5A. Both CTSC and PDE5A are associated with oxidative stress and phosphorylation. Whereas oxidative stress is a central factor in the development of DN (62). Phosphorylation is involved in the metabolism of glycosylation end products and thus affects DN (63). Therefore, it is worthwhile to pay attention about the target regulation of CTSC and PDE5A in DN.

As we have discussed in the background section, the current diagnosis of DN is also a major clinical challenge. Earlier clinical symptoms of DN were so mild that leakage of urinary microalbumin did not alert patients to early intervention, especially in patients with type 2 DN. Renal puncture is the gold standard for clarifying the presence of DN pathologic changes, but it is not practical to perform renal puncture in every diabetic patient (64). In addition, for patients with DN who already have urinary protein, intensive glycemic control is not effective in reversing proteinuria but rather increases the risk of severe hypoglycemia (65). The results of the expression of CTSC and PDE5A in DN patients significantly differed from that in the control group showed that the expression level of CTSC and PDE5A was closely related to disease progression. Monitoring the expression of CTSC and PDE5A may be helpful in the diagnosis of type 2 DN. The nomogram prediction model is a common and effective tool by inverting the expression into a total score to clarify the association between predictors and the risk of disease, and can enhance the practicality of gene expression monitoring for clinical decision-making. The diagnostic value of key targets for DN was evaluated in our study by constructing diagnostic nomogram. Combined with the results, CTSC and PDE5A can be used as potential diagnostic targets for DN. This provides new ideas and evidence for clinical DN diagnosis.

Currently, it is believed that ACEi/ARB medications have a greater dilation effect on the arterioles that carry blood away from the glomerulus compared to the arterioles that carry blood towards the glomerulus. When used concomitantly, this may cause a decrease in glomerular filtration pressure, resulting in insufficient pressure within the glomerulus and reduced blood flow, ultimately leading to impaired kidney function. However, further evidence is required to fully explain the heightened risk of developing acute kidney injuries when renin inhibitors are combined with ACEi/ARB medications. Theoretically, combining RAAS inhibitors offers better control of urinary protein in patients with DN, but empirical data suggests that there are more intricate changes at play that need to be investigated. In our study, we discovered that

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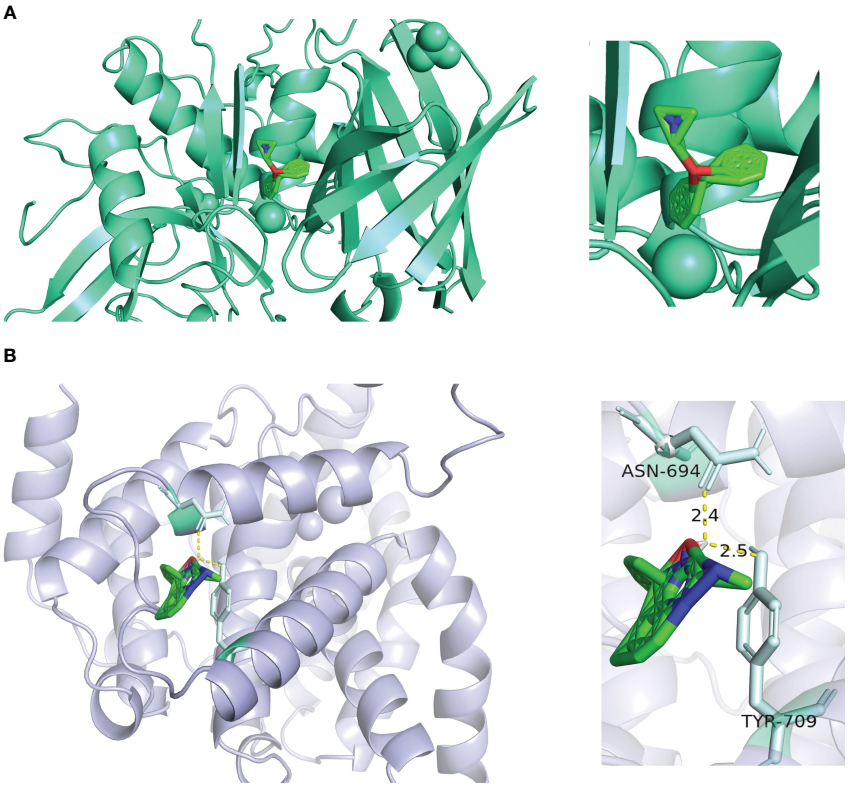

FIGURE 8
Molecular docking results of two key targets. **(A)** *CTSC*. **(B)** *PDE5A*.

TABLE 5 Mendelian randomization (MR) analysis for causal relationship of three key targets (PTGS2, ITGA4, and ANPEP) and AKI.

outcome	exposure	Method	Pvalue	OR
ukb-b-4963	eqtl-a-ENSG00000166825 (ANPEP)	MR Egger	0.000	0.000
		IVW	0.000	3.82E-07
		Weighted median	0.000	-4.78E-06
		Simple mode	0.000	0.001
		Weighted mode	0.000	4.44E-06
	eqtl-a-ENSG00000115232 (IGTA4)	MR Egger	0.637	0.9998
		IVW	0.000	0.9997
		Weighted median	0.128	0.9997
		Simple mode	0.350	0.9995
		Weighted mode	0.320	0.9997
	eqtl-a-ENSG00000073756 (PTGS2)	MR Egger	0.320	1.001
		IVW	0.004	1.001
		Weighted median	0.007	1.001
		Simple mode	0.169	1.001
		Weighted mode	0.081	1.001

out of the 60 targets affected by RAAS inhibitors in DN, three (PTGS2, ITGA4, ANPEP) had a significant causal relationship with acute renal failure. These findings may provide additional insights into the current therapeutic challenges associated with RAAS inhibitors in DN.

PTGS2, also referred to as cyclooxygenase-2 (COX-2), is an enzyme implicated in the inflammatory response. Its primary role is to facilitate the conversion of arachidonic acid to prostaglandin H2, which serves as a catalyst for an inflammatory response (66). PTGS2 expression is influenced by a range of factors, with stress being a significant regulatory element (67). Enzymes associated with PTGS2 are present in various sites within the mammalian kidney, including dense plaques, medullary interstitial cells, arteriolar endothelium, and glomerular podocytes (68). Consequently, targeted adjustment of PTGS2 could be a viable approach for treating renal diseases. A PTGS2 inhibitor (celecoxib) was previously linked to acute kidney damage and substantial urinary protein loss in a study (69). Another study reported celecoxib as a cause of acute renal failure and hyperkalemia, with recovery observed upon discontinuation of the drug (70). In mammalian kidneys, the expression of PTGS2 enzyme increases when extracellular fluid volume is reduced, and tachyzoites prompt the expression of PTGS2 in dense spots (71). PTGS2 induces prostaglandins to mitigate the constriction of small glomerular arteries related to filtration in a paracrine manner (72). COX-2 inhibitors may diminish this protective mechanism that sustains glomerular perfusion, leading to prolonged constriction of small glomerular arteries and inadequate renal perfusion. This helps elucidate the connection between PTGS2 and renal function. On one hand, excessive PTGS2 expression exacerbates the inflammatory response, causing harm to the kidneys, while on the other hand, excessive suppression of PTGS2 may elevate the risk of hyperkalemia and renal failure. Therefore, based on the findings of the current study, it is suggested that RAAS inhibitors modifying the level of PTGS2 expression in various tissues of DN kidneys may be associated with acute kidney damage, although the precise mechanism of action remains unclear. The only definite conclusion drawn is that PTGS2 may heighten the risk of acute renal failure when RAAS inhibitors are active in DN conditions.

The impact of diabetes on the gene expression of integrin subunits is widely acknowledged, affecting various cell types and tissues including monocytes, arterial endothelial cells, glomerular cells, and the retina (73). The ITGA4 gene, which codes for α4 integrin, has been demonstrated in recent research to play a role in the PI3K-AKT signaling pathway associated with nephroprotective effects (74). This aligns with our discovery that ITGA4 exhibits a protective effect against acute renal failure in the presence of RAAS inhibitors in DN. ANPEP, also known as aminopeptidase N or CD13, is a multifunctional membrane-bound zinc-dependent metalloprotease that is widely present in renal tissue (75). Current studies did not identify any link between ANPEP and kidney failure. According to our findings, ANPEP serves as a protective factor against acute renal failure in DN patients treated with RAAS inhibitors. This sets the stage for further comprehensive exploration of RAAS inhibitors. In addition, we predicted the TFs, miRNAs and lncRNAs targeting CTSC and PDE5A, and constructed their interaction networks. The construction of ceRNA networks can help us to deeply study the mechanism of gene regulation, reveal the laws of DN occurrence and development, and provide new ideas and strategies for the treatment of DN. However, the mechanism of CTSC and PDE5A in DN needs to be further studied and verified.

Conclusion

The MR approach utilizes IVs as exposures to examine correlations with outcomes, enhancing the persuasiveness of the results by minimizing confounding factors. In this study, we employed network pharmacology combined with MR to screen the key targets of RAAS inhibitors currently employed in clinical practice for DN. Our findings establish a causal relationship between CTSC, PDE5A, and DN. These findings warrant further investigation into the following three primary aspects. Firstly, previous studies have lacked sufficient evidence establishing a causal relationship between RAB38/CTSC and human urinary protein. However, our study observed a causative link between CTSC and DN, considering the notable protein-lowering ability of RAAS inhibitors in the clinical setting. It is reasonable to hypothesize that CTSC, as the core target of RAAS inhibitors, plays a role in human urinary protein. Deeper studies are necessary to explore the specific mechanisms and potential involvement of RAB38. Secondly, the impact of RAAS inhibitors on PDE5A has been found to be causally associated with DN. Several studies have concluded that current PDE5A inhibitors possess renoprotective effects and are linked to altered renal hemodynamics, with excessive dilation or inhibition having detrimental renal effects. The role of current medications in regulating the balance of PDE5A in different kidney tissues remains unknown. Further studies focused on this target may uncover additional clinical benefits. Thirdly, CTSC and PDE5A contribute to the diagnosis of DN. Thus, there is an opportunity to combine CTSC and PDE5A screening with urine protein, urine protein/creatinine ratio, 24-hour urinary protein quantitation, and urinary protein excretion rate to predict the risk of DN in diabetic patients. The finding that PTGS2, ITGA4, and ANPEP may control acute kidney failure in the presence of RAAS inhibitors in DN is supported by the fact that all three are impacted by RAAS inhibitors and causal analysis using drug-target Mendelian randomization has demonstrated their link to acute kidney failure. Specifically, PTGS2 is identified as a risk factor, while the other two are regarded as protective factors. This discovery provides new evidence for the potential reduction of kidney damage with concurrent clinical use of RAAS inhibitors in treating DN.

Limitation

In this study we used strict analytical criteria (e.g., $P < 5 \times 10^{-8}$, $r2 < 0.001$, $kb = 10000$) in the drug-target Mendelian analysis considering the accuracy and rigor of the results. Thus there may be other RAAS inhibitor-related targets that have a causal relationship with DN yet to be discovered. Due to sample limitations,

stratification by disease type and patient gender was not performed, which resulted in certain biases. Vasopressin is associated with the activation of the RAAS system. In our study, we have not yet included vasopressin in the analysis, but we will further explore this direction in the future. Clinical applications of key targets need to be supported by data from more samples. However, we will continue to focus on the role of these key targets.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

DZ: Writing – original draft, Writing – review & editing. TZ: Methodology, Software, Writing – review & editing. ST: Writing – review & editing. QL: Writing – review & editing. WL: Writing – review & editing. GG: Software, Writing – review & editing. ML: Writing – review & editing. QC: Writing – original draft, Writing – review & editing.

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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.

The reviewer DX declared a shared affiliation, with no collaboration, with several of the authors TZ, QL, WL, QC to the handling editor at the time of the review.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2024.1354950/full#supplementary-material

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Unveiling the pathogenesis and therapeutic approaches for diabetic nephropathy: insights from panvascular diseases

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Diabetic nephropathy (DN) represents a significant microvascular complication in diabetes, entailing intricate molecular pathways and mechanisms associated with cardiorenal vascular diseases. Prolonged hyperglycemia induces renal endothelial dysfunction and damage via metabolic abnormalities, inflammation, and oxidative stress, thereby compromising hemodynamics. Concurrently, fibrotic and sclerotic alterations exacerbate glomerular and tubular injuries. At a macro level, reciprocal communication between the renal microvasculature and systemic circulation establishes a pernicious cycle propelling disease progression. The current management approach emphasizes rigorous control of glycemic levels and blood pressure, with renin-angiotensin system blockade conferring renoprotection. Novel antidiabetic agents exhibit renoprotective effects, potentially mediated through endothelial modulation. Nonetheless, emerging therapies present novel avenues for enhancing patient outcomes and alleviating the disease burden. A precision-based approach, coupled with a comprehensive strategy addressing global vascular risk, will be pivotal in mitigating the cardiorenal burden associated with diabetes.

KEYWORDS

diabetic nephropathy, panvascular disease, endothelial dysfunction, renin-angiotensin system, novel antidiabetic drugs, individualized therapy

Introduction

According to the International Diabetes Federation's report, over 530 million people worldwide have diabetes (1). About one-third of diabetic patients develop diabetic nephropathy (DN) after the incubation period, which may last several years (2). Diabetic nephropathy, as a common complication of diabetes, has drawn widespread attention

globally. With the increasing number of diabetes patients and lifestyle changes, DN is rising. DN is characterized by structural and functional kidney damage due to prolonged high blood glucose levels, resulting in a gradual decline in glomerular filtration rate, proteinuria, and progressive renal function impairment (3). It is one of the leading causes of chronic kidney disease (CKD) and a major contributing factor to end-stage renal disease (ESRD) (4).

Additionally, diabetes patients often risk panvascular diseases (5), which involve the entire vascular system, including atherosclerosis, cardiovascular diseases, and cerebrovascular disorders. Due to alterations in the vascular wall structure and function in diabetes patients, they are more prone to developing atherosclerosis. Factors such as endothelial dysfunction, inflammation, oxidative stress, and platelet activation play important roles in promoting the progression of atherosclerosis in diabetes patients (6). The treatment strategies for DN and panvascular diseases include controlling blood glucose levels, managing blood pressure, restricting protein intake, and using renal protective agents. Furthermore, addressing atherosclerosis and cardiovascular diseases is crucial, involving lipid management, antiplatelet therapy, and cardiovascular protection measures (7, 8).

DN often has varying degrees of systemic vascular damage, making DN's development more complex and severe. On the one hand, panvascular diseases accelerate the progression of DN, leading to a further decline in glomerular filtration rate, worsening proteinuria, and impaired renal function. On the other hand, DN is an independent risk factor for panvascular diseases, increasing the risk of cardiovascular events and all-cause mortality. The connection between DN and panvascular diseases is significant for preventing, diagnosing, and treating these conditions. While investigating the relationship between DN and panvascular diseases, several key questions deserve further exploration. Firstly, it is important to understand the common pathological mechanisms between DN and panvascular diseases. Factors such as high blood glucose, inflammation, and oxidative stress play crucial roles in both diseases. Secondly, it is necessary to explore how panvascular diseases affect the development and prognosis of DN. Further understanding the interplay between the two conditions can aid in developing more precise treatment strategies and preventive measures.

While further investigating the connection between DN and panvascular diseases, this perspective article addresses the following key questions:

Common pathological mechanisms

High blood glucose, inflammation, and oxidative stress are important in DN and panvascular diseases. It is crucial to delve into these shared pathological mechanisms and explore how they interact to exacerbate disease progression.

Impact of panvascular diseases on DN

How do panvascular diseases affect the development and prognosis of DN? Does it increase the risk of cardiovascular events and all-cause mortality? Exploring these key questions can enhance our understanding of the overall risk in DN patients and guide relevant interventions.

Treatment strategies

More precise treatment strategies can be developed based on a thorough understanding of the connection between DN and panvascular diseases. Identifying common treatment targets and developing targeted therapeutic drugs may improve patients' prognosis and quality of life.

Molecular pathogenesis of DN: insights from panvascular

DN is one of the most common vascular complications of diabetes (9). In the early stages, abnormalities in the glomerular filtration membrane arise, characterized by thickening of the glomerular basement membrane and proliferation of mesangial cells, resulting in altered glomerular filtration rate (GFR). Concurrently, endothelial cell injury within the glomerulus and dysfunction of tubular epithelial cells manifest. The progression of disease leads to glomerulosclerosis, which is marked by substantial glomerular injury and proteinuria (10). Glomerulosclerosis and mesangial proliferation further exacerbate the decline in GFR. The advancement of DN may also induce interstitial fibrosis and tubular atrophy, impairing tubular function, including urine concentration ability and acid-base balance. In the late stages of DN, a gradual reduction in GFR ensues, intensifying glomerular dysfunction and aggravating glomerulosclerosis. Consequently, interstitial fibrosis, arterial sclerosis, and renal artery lesions may manifest in the kidney (11). The pathogenesis involves multiple molecular and cellular abnormalities. High blood glucose levels represent a primary risk factor for DN development in diabetic patients. Direct injury of renal vascular endothelial cells and podocytes by hyperglycemia plays a pivotal role in DN progression (12). Inflammation and oxidative stress significantly affect DN by activating inflammatory signaling pathways and induction of cellular apoptosis, thereby contributing to renal pathological changes (13). Additionally, aberrant activation of the renal renin-angiotensin system (RAS) and other signaling cascades promotes DN advancement (14). At the cellular level, prolonged hyperglycemic exposure elicits mitochondrial dysfunction, cellular metabolic derangements and oxidative stress in renal cells. This triggers the emission of damage-associated molecular patterns that stimulate innate immune cascades, leading to glomerular and tubulointerstitial inflammation (15). Pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) exacerbate podocyte and endothelial injuries via multiple mechanisms involving caspases, SMAD pathways and Rho-associated protein kinase. Concomitantly, hyperglycemia-induced advanced glycation end products (AGEs) formation and their engagement with renal receptors for AGEs amplify inflammation and fibrosis through diverse intracellular signaling molecules including protein kinase C, transforming growth factor-β

and nuclear factor-κB (NF-κB) (16, 17). The intrarenal RAS is concurrently activated through Ang II and further propagates oxidative stress, inflammation, and extracellular matrix accumulation in DN progression via hemodynamic and non-hemodynamic effects (18, 19). These multi-factorial and inter-related pathophysiological processes synergistically promote renal structural and functional impairments characteristic of DN.

Here, we discuss the key pathophysiological processes at the cellular and molecular levels based on current evidence:

Formation of advanced glycation end products and renal injury

The formation of advanced glycation end products (AGEs) is a complex process in hyperglycemia. Under hyperglycemia, non-enzymatic glycation of proteins/lipids leads to excessive AGEs accumulation, a hallmark of diabetic dysmetabolism (20). The binding of AGEs to receptors such as RAGE on renal endothelial cells activates pro-oxidative and pro-inflammatory pathways including NADPH oxidase, MAPK and NF-κB, inducing oxidative stress and inflammation (21, 22). Several studies have demonstrated the detrimental effects of AGEs and RAGE activation in the pathogenesis of diabetic nephropathy (23). For example, blocking RAGE activation has been shown to alleviate renal injury and reduce inflammation in experimental models of diabetes (24). Additionally, inhibition of the NADPH oxidase system, which is upregulated by RAGE signaling, has been found to attenuate oxidative stress and improve renal function in DN (25). Furthermore, inhibition of MAPK and NF-κB pathways has shown promising results in reducing inflammation and fibrosis in diabetic kidney disease (26, 27).

Increased oxidative stress and inflammation

Mitochondrial dysfunction driven by persistent hyperglycemia plays a pivotal role in diabetes-induced oxidative stress and kidney inflammation. Sustained hyperglycemia leads to impaired ATP generation in renal cells by disrupting mitochondrial function (28). It has been shown that hyperglycemia decreases mitochondrial electron transport chain complexes I and IV activities, compromising the balance between superoxide production and dismutation in renal cells (29, 30). This triggers an overproduction of reactive oxygen species (ROS), such as superoxide anions.

Concurrently, hyperglycemia activates inflammatory pathways by upregulating proinflammatory mediators and accumulating immune cells in the kidneys. Hyperglycemia stimulates NF-κB and MAPK signaling pathways, leading to increased expression of cytokines, including IL-1β, TNF-α, and MCP-1 (31, 32). These inflammatory cytokines amplify local inflammation by recruiting monocytes that differentiate into resident proinflammatory M1 macrophages in the renal interstitium (33). Renal tubular epithelial cells also secrete chemokines that perpetuate inflammation (34).

Elevated ROS production results in oxidative damage to lipids and proteins, impairing cell membrane integrity and function (35). Inflammatory cytokines can activate hormonal systems to induce renal tubular cell apoptosis (36). Abnormal extracellular matrix remodeling involving increased collagen deposition and osteopontin contributes to renal interstitial fibrosis (37). In summary, mitochondrial dysfunction-induced oxidative stress and inflammation form a vicious cycle that cooperatively drives the pathogenesis of diabetic kidney disease.

Endothelial dysfunction and increased permeability

Hyperglycemia elicits direct damage to endothelial cells lining the renal vasculature and glomeruli, manifesting as impaired endothelium-dependent vasorelaxation (38, 39), weakened homeostatic control of vascular tone and permeability (40, 41), and deregulated integrity of the glomerular filtration barrier (42). Chronic hyperglycemic conditions trigger excessive production of reactive oxygen species and proinflammatory signaling, which promote endothelial activation and a proinflammatory phenotype.

Activated endothelial cells exhibit disturbances in barrier function, increasing vascular permeability at the glomerular (43) and tubular (44) vascular beds. This allows plasma proteins such as albumin to leak into the urine, presenting clinically as proteinuria - a hallmark of DN (45). At the molecular level, hyperglycemia enhances endothelial expression of adhesion molecules that recruit leukocytes (46), decreasing the synthesis of antiproteinuric factors (47). The combined effects of direct hyperglycemic toxicity, oxidative stress and chronic low-grade inflammation thus converge to induce endothelial dysfunction in the renal vasculature. Figure 1 shows the relevant mechanisms and possible approaches.

The common relationship between DN and panvascular disease

The common pathological processes of DN and panvascular disease include vascular injury, inflammatory response, oxidative stress and fibrosis (48). At the same time, patients with DN are at risk for systemic vasculopathy. It has been found that diabetic patients are often accompanied by systemic vascular lesions such as abnormal vascular function, atherosclerosis, and platelet activation (49). These vasculopathies are somewhat similar to the development of DN, in which vascular endothelial cell injury, inflammatory response and oxidative stress are common pathological features (50, 51). Thus, DN and panvascular disease may share common molecular mechanisms that further exacerbate the development of DN.

Inflammatory response and oxidative stress are important common links. The inflammatory response is activated in both

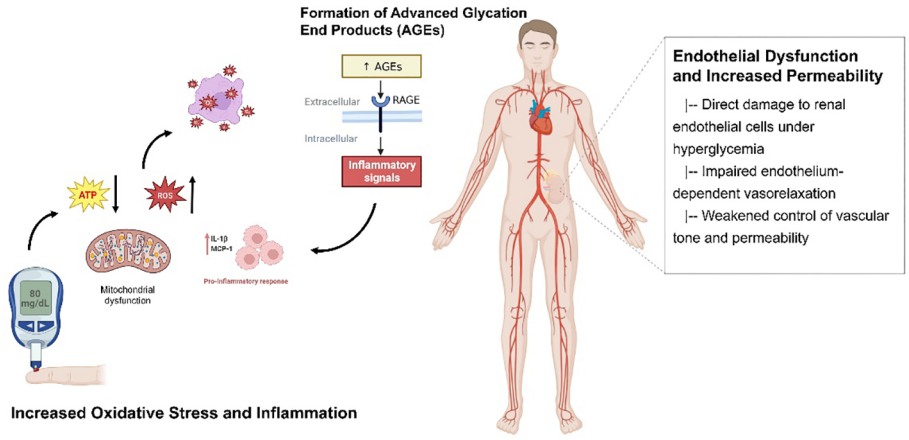

FIGURE 1
The relevant mechanisms and possible approaches for DN. The figure illustrates the critical mechanisms involved in renal injury in diabetes mellitus. Increased oxidative stress and inflammation, mitochondrial dysfunction driven by persistent hyperglycemia, and the formation of advanced glycation end products (AGEs) contribute to renal damage. Additionally, endothelial dysfunction and increased permeability further exacerbate the pathogenesis of diabetic nephropathy.

diseases, leading to increased production of inflammatory cytokines (52). These cytokines include tumor necrosis factor-α (TNF-α) (53), interleukin-1β (IL-1β) (54), and interleukin-6 (IL-6) (55), among others, which are involved in the inflammatory response of the vascular wall and damage to vascular endothelial cells (56). The increase in inflammatory cytokines further activates the NF-κB signaling pathway, promoting the inflammatory response's continuation.

Under a hyperglycemic state, excessive glucose metabolism generates many oxygen free radicals, disrupting the intracellular redox balance. In addition, oxidative stress in diabetic patients can be caused by mitochondrial dysfunction and accumulation of glycosylation end products. Excessive production of oxygen free radicals and imbalance of the antioxidant system leads to increased intracellular oxidative stress, further damaging renal and vascular cells (57).

In addition to inflammatory responses and oxidative stress, several important molecular pathways and signaling molecules are involved in developing DN and panvascular disease (58). For example, transforming growth factor-β (TGF-β) (59) is important in both diseases. TGF-β is involved in thickening and fibrosis of the glomerular basement membrane, disrupting the glomerular filtration barrier. In addition, angiotensin II (Ang II) has been implicated as a co-regulatory molecule in DN and panvascular disease, exacerbating the progression of both diseases through mechanisms that promote vasoconstriction and increase inflammatory responses and oxidative stress (60, 61).

A close interaction exists between renal vascular abnormalities in DN and systemic vascular complications. Persistent hyperglycemia can exacerbate systemic vascular injuries through renal endothelial dysfunction, inflammation and the spill over of vascular reactive species and cytokines. Meanwhile, panvascular diseases such as atherosclerosis can directly impair renal hemodynamics by narrowing intrarenal arterioles and compromising renal blood supply and pressure profiles, undermining glomerular filtration function. Furthermore, advanced glycation end products and platelet hyperreactivity may precipitate thrombotic occlusions obstructing renal microcirculation, augmenting ischemic injuries to the glomeruli. These reciprocal effects between renal and systemic vessels collectively form a vicious cycle that reinforces the progression of DN and panvascular comorbidities in diabetes.

Treatment strategies for DN

Tight glycemic and blood pressure control are recognized as cornerstone therapies for DN (62). Mounting evidence suggests that stringent control of hyperglycemia effectively mitigates renal complications (63). Clinical studies (64, 65) have validated that achieving near-normal glycemia through a combinatorial pharmacotherapy approach, dietary modification and exercise substantially delays DN progression and lowers risks of renal adverse outcomes. Hypertension is also a key driver of DN pathogenesis (66, 67). Effectively managing blood pressure alleviates hemodynamic overload on the kidneys and hampers disease progression, as corroborated by numerous trials (68, 69). Given individual variations in disease severity and responses, glycemic and blood pressure control treatment targets should be personalized. Close monitoring with timely adjustments is pivotal to minimizing clinical deterioration and organ damage over the long term. With a treatment paradigm centered around intensive management of the two metabolic abnormalities, multidisciplinary care integrating medical, lifestyle and educational elements can help optimize renal protection in this high-risk population. Achieving recommended targets demands relentless efforts from patients and healthcare providers alike.

Chronic inflammation and oxidative stress play pivotal roles in the pathogenesis and progression of DN. Targeting these pathogenic processes represents a promising therapeutic strategy.

Preclinical evidence (70) suggests anti-inflammatory interventions, including non-steroidal anti-inflammatory drugs, glucocorticoids, and anti-cytokine therapies, may attenuate renal inflammation and fibrosis in DN to a certain extent (71). Based on the 2024 latest research finding (72) by the American Diabetes Association Professional Practice Committee, cardiovascular event risk reduction in DN patients is recommended through finerenone, a non-steroidal selective mineralocorticoid receptor antagonist. This medication has been clinically proven to reduce cardiovascular events and the progression of chronic kidney disease (73). Recently, guidelines (74–77) reflect growing recognition of finerenone's clinical benefits and increasingly emphasize the need for earlier intervention strategies that concurrently target cardiorenal protection in patients with coexisting diabetes, kidney disease and cardiovascular illness.

Similarly, antioxidative agents such as vitamins E and C and glutathione have demonstrated renoprotective effects by ameliorating oxidative insults and preserving renal and vascular cellular integrity from radical-mediated damage (78–80). Pentoxifylline (PTF) is a methylxanthine derivative and a phosphodiesterase inhibitor that can inhibit the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha and interleukin-1 beta. It reduces inflammation and has a significant therapeutic effect on DN (81). PTF exerts its anti-proteinuric effects through various mechanisms, including improving renal microcirculation, inhibiting oxidative stress, and reducing collagen deposition (82). Clinical studies (83, 84) have shown that PTF can significantly decrease proteinuria in DN patients and improve CRP and TNF-α. Therefore, PTF has become an important adjunctive therapy for DN. However, larger and longer clinical outcome trials are still warranted to definitively establish the efficacy and safety profile of anti-inflammatory and antioxidative therapies in DN management. Unresolved questions around optimal drug selection, dosing regimen, duration of intervention and long-term benefits need to be addressed to inform clinical recommendations. Nonetheless, given their mechanistic rationale targeting the underlying chronic pro-oxidative and pro-inflammatory milieu driving DN progression, further exploring these therapeutic avenues through well-designed studies remains an active area of research interest. Combinatorial regimens harnessing multiple protective mechanisms may also hold promise.

The renin-angiotensin-aldosterone system (RAAS) axis plays a pivotal role in the pathogenesis of DN. RAAS blockade with ACE inhibitors (ACEi) and angiotensin receptor blockers (ARB) are well-established therapeutic interventions for DN. Angiotensin II receptor antagonist losartan can effectively reduce the progression of nephropathy in patients with type 2 diabetes. Compared to conventional antihypertensive treatment, losartan can lower the risk of renal function deterioration, end-stage renal disease, and death (85). These agents retard DN progression by lowering intraglomerular pressure and mitigating renal inflammation. However, their use requires close monitoring due to potential adverse effects such as worsening kidney function and hyperkalemia in some patients. Sodium-glucose cotransporter 2 (SGLT2) inhibitors are emerging antidiabetic agents. They promote glucosuria and lower blood glucose levels by inhibiting SGLT2-mediated glucose reabsorption in proximal tubules (86). Recent clinical trials (87, 88) demonstrated their renoprotective benefits in DN, including reductions in proteinuria and slower eGFR decline. They also confer cardiovascular protection.

Glucagon-like peptide-1 (GLP-1) receptor agonists stimulate insulin secretion and inhibit intestinal glucose absorption. Emerging evidence (89, 90) indicates their treatment may confer renal benefits in DN, such as decreasing proteinuria, improving glomerular filtration rates and attenuating fibrosis.

In summary, while RAAS blockade forms the mainstay of pharmacotherapy for DN, SGLT2 inhibitors and GLP-1RAs hold promise as adjunctive therapies given their additional reno- and cardio-protective effects observed in recent landmark outcome trials. Their integration into routine clinical care warrants further investigation.

Emerging treatments and future perspectives

Epigenetics and metabolic memory

Individualized therapy and precision medicine also hold great potential in managing DN. Recent findings (91, 92) have elucidated the roles of epigenetics and metabolic memory in linking genetic and environmental risk factors. Mechanisms, including DNA methylation, histone modification, and non-coding RNA regulation, are involved, promoting the development of metabolic memory and leading to a poor prognosis in DN (93, 94). Long non-coding RNAs (lncRNAs) help regulate these epigenetic changes and drive gene expression changes in DN pathogenesis. While our mechanistic understanding has progressed, effective therapies still need to be improved. A precision medicine approach integrating multi-omics profiling with clinical characteristics holds promise to precisely tailor individualized treatment for DN. Targeting disease-relevant lncRNAs may uncover new opportunities for genomic medicine to treat DN.

NETosis and neutrophil extracellular traps

NETosis, the formation of neutrophil extracellular traps (NETs), plays a significant role in the pathogenesis of DN (95). While inflammation and oxidative stress are known contributors to DN, the involvement of neutrophils has been largely overlooked. Elevated glucose levels increase PKC activity, NADPH-oxidase overstimulation, and oxidative burst, irrespective of diabetes type (96). This oxidative burst is crucial for NET formation. Inflammatory cytokines and free fatty acids hinder insulin signaling, activating inflammatory pathway mediators such as IKKβ and JNK1. This results in the translocation of NFκβ to the

nucleus, triggering the activation of proinflammatory genes necessary for priming. Additionally, high extracellular glucose promotes a proinflammatory M1 phenotype in macrophages. The interaction between NETs and M1 macrophages exacerbates the proinflammatory response, leading to apoptosis and the release of extracellular DNA. This NET-mediated process contributes to an increased burden of free DNA and disease progression in DN. Understanding the complex pathomechanisms of DN highlights the notable role of NETosis, presenting an opportunity to target NETosis as an emerging therapeutic approach for DN.

Natural products and therapeutic approaches

Natural products have gained increasing attention as potential therapeutic agents for DN. Recent studies (97–99) have identified comprehensive therapeutic approaches, including natural products, that may provide potential treatment strategies for DN. One example of a natural product with therapeutic potential is colchicine. A recent clinical (100) investigation explored the beneficial effects of low-dose colchicine on neutrophil-related chronic inflammation in DN patients. The findings demonstrated that low-dose colchicine effectively and safely attenuated neutrophil-related chronic inflammation in DN patients with concomitant microalbuminuria in type 2 diabetes. Plant-derived compounds, such as resveratrol (101), curcumin (102), and quercetin (103, 104), have exhibited anti-inflammatory, antioxidant, and renoprotective effects, potentially mitigating the development and progression of DN. Further research is needed to elucidate the underlying mechanisms of action and optimize the therapeutic potential of these natural products. This includes investigating their effects on key molecular pathways involved in DN, such as oxidative stress, inflammation, apoptosis, and fibrosis. Additionally, studies exploring the synergistic effects of natural product combinations or their interaction with conventional therapies could provide valuable insights into their clinical utility.

Timely focus on the risk of DN complications

Studies (105, 106) have shown a higher risk of urinary tract infections (UTIs) events in individuals with DN, supported by ample epidemiological evidence. Regional studies (107) indicate a UTI prevalence of 25.3% among individuals with diabetes, with females accounting for 41.1% of cases. Elevated blood glucose levels create a favorable environment for bacterial growth, increasing susceptibility to infection by promoting colonization of uropathogens and compromising the immune system. Untreated UTIs in T2DM are closely associated with the risk of DN. They can worsen the pro-inflammatory state by releasing cytokines and inflammatory mediators that damage the kidneys and promote DN progression (108). Numerous studies (109, 110) have explored the potential of uromodulin (UMOD) in preventing UTIs and preserving kidney function. UMOD, also known as Tamm-Horsfall protein, is predominantly produced by cells in the kidney's thick ascending limb of the loop of Henle (111). It is the most abundant protein in urine and exhibits antibacterial properties. UMOD is believed to play a vital role in UTI defense by acting as a barrier. It binds to bacterial pathogens in the urinary tract, impeding their adherence to the urothelium and subsequent colonization (110). This mechanism reduces the risk of UTIs and restricts infection spread to the kidneys. Given UMOD's antibacterial effects and potential protective role, it presents an appealing therapeutic target for preventing UTIs and preserving kidney function in individuals with DN. Future research can focus on elucidating the underlying mechanisms of UMOD in UTI prevention, exploring its potential as a diagnostic or prognostic marker for UTIs in DN, and developing interventions to enhance UMOD expression or function. In addition, since UTIs are associated with the possibility of affecting fetal development (112), the resulting targeting of content related to urinary tract infections emphasizes attention to the risk of DN complications.

Conclusion

In conclusion, DN often co-occurs with cardiovascular complications including hypertension, coronary artery disease and cerebrovascular diseases (113). A comprehensive approach integrating the management of these comorbidities is imperative. Tackling the global vascular burden may lower mortality and enhance quality of life. Future research should further elucidate DN pathogenesis and discover novel treatment paradigms. Targeting inflammation, oxidative stress and vascular dysfunction with drugs and interventions represents major opportunities. Advancing individualized and precision approaches through technological evolution will transform DN care. Promoting an integrated vascular risk reduction strategy also benefits long-term outcomes. In summary, DN management is evolving towards personalized, precise, holistic models. Novel mechanism-based therapies, especially those targeting pathogenic pathways, combined with individualized/precision regimens and comprehensive comorbidity control, will optimize therapeutic strategies for better patient outcomes.

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

XQZ: Conceptualization, Formal Analysis, Writing – original draft, Writing – review & editing. JZ: Conceptualization, Formal

Analysis, Writing – original draft, Writing – review & editing. YR: Formal Analysis, Writing – original draft, Writing – review & editing. RS: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. XZ: Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing.

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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

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Secretory leukocyte protease inhibitor as a novel predictive biomarker in patients with diabetic kidney disease

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Background: Secretory leukocyte protease inhibitor (SLPI) is a multifunctional protein involved in the chronic inflammatory process, implicated in the pathogenesis of diabetic kidney disease (DKD). However, its potential as a diagnostic and prognostic biomarker of DKD has yet to be evaluated. This study explored the clinical utility of SLPI in the diagnosis and prognosis of renal endpoint events in patients with DKD.

Methods: A multi-center cross-sectional study comprised of 266 patients with DKD and a predictive cohort study comprised of 120 patients with stage IV DKD conducted between December 2016 and January 2022. The clinical parameters were collected for statistical analysis, a multivariate Cox proportional hazards model was used to evaluate the independent risk factors for renal endpoints.

Results: Serum SLPI levels gradually increased with DKD progression ($p<0.01$). A significant correlation was observed between serum SLPI levels and renal function in patients with DKD. The mean follow-up duration in this cohort study was 2.32 ± 1.30 years. Multivariate Cox regression analysis showed SLPI levels$\geq$51.61ng/mL (HR=2.95, 95% CI[1.55, 5.60], $p<0.01$), 24h urinary protein levels$\geq$3500 mg/24h (HR=3.02, 95% CI[1.66, 5.52], $p<0.01$), Alb levels<30g/l (HR=2.19, 95% CI[1.12, 4.28], $p<0.05$), HGB levels<13g/dl (HR=3.18, 95% CI[1.49, 6.80], $p<0.01$), and urea levels$\geq$7.1 mmol/L (HR=8.27, 95% CI[1.96, 34.93], $p<0.01$) were the independent risk factors for renal endpoint events in DKD patients.

Conclusions: Serum SLPI levels increased with DKD progression and were associated with clinical parameters of DKD. Moreover, elevated SLPI levels showed potential prognostic value for renal endpoint events in individuals with DKD. These findings validate the results of previous studies on SLPI in patients with DKD and provide new insights into the role of SLPI as a biomarker for the diagnosis and prognosis of DKD that require validation.

KEYWORDS

diabetic kidney disease, secretory leucocyte protease inhibitor, prognosis, renal clinical endpoint events, biomarkers

1 Introduction

The incidence of diabetic kidney disease (DKD) is increasing annually and has become the main cause of end-stage renal disease (ESRD) worldwide, posing a serious threat to the lives and health of patients (1). Numerous studies have indicated that the processes leading to DKD progression are heterogeneous and include inflammation, metabolic stress, and morphological kidney lesions, making it difficult to identify high-risk individuals who are more likely to develop DKD and ultimately progress to ESRD (2). In the past decade, several studies have reported associations between the occurrence and progression of DKD and proinflammatory and profibrotic markers (3–5). However, biomarkers for predicting the onset and progression of kidney disease in diabetes patients remain inefficient. Hence, extensive efforts are underway to identify and confirm new diagnostic and prognostic biomarkers that can lead to a better understanding of DKD.

Secretory leukocyte protein inhibitor (SLPI), a non-glycosylated cationic protein produced by mucous membrane epithelial cells, neutrophils, and macrophages, controls the excess release of host-secreted serine proteases in response to injurious stimuli (6). In addition to its antiprotease activity, SLPI has anti-inflammatory, antimicrobial, antiviral, and immunomodulatory properties (7). This inhibitor has emerged as a protective agent regulating the inflammatory cascade (8). SLPI was first discovered and isolated from the secretions of patients with chronic obstructive pulmonary disease and cystic fibrosis in the 1970 (9). Subsequently, SLPI was found to be expressed on several mucosal surfaces, including those of the respiratory, digestive, and reproductive tracts (10–13). In 2001, Ohlsson et al. (14) first observed the distinct expression of SLPI mRNA and protein in distal renal tubular cells. In contrast, few studies exist on the correlation between SLPI levels and kidney disease, with the literature mainly focusing on acute kidney injury after surgery and the evaluation of the condition before and after renal transplantation (15, 16). Recently, circulating SLPI has been reported to correlate with metabolic and inflammatory parameters in metabolic diseases (17). Given the common inflammatory and metabolic mechanisms in DKD, we speculated that SLPI may play a crucial role in the initiation and progression of DKD.

Our previous study on serum biomarker concentrations in patients with DKD showed a significant correlation between SLPI and renal function at various stages of DKD (18). Therefore, we expanded the sample size of this cross-sectional study to investigate the performance of SLPI as a complementary diagnostic and prognostic biomarker of DKD.

2 Materials and methods

2.1 Cross-sectional study

2.1.1 Patients

A total of 266 patients with DKD aged 18-75 years participated in this study. This multi-center cross-sectional study was conducted from December 2016 to January 2022 at six hospitals in Beijing: Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing Hospital of Integrated Traditional Chinese and Western Medicine, Beijing Hospital of Traditional Chinese Medicine, Xiyuan Hospital, China Academy of Chinese Medical Sciences, and the Guang'anmen Hospital, China Academy of Chinese Medical Sciences. In addition, 20 healthy individuals and 20 patients with diabetes t without a diagnosis of DKD were selected as controls. The inclusion criteria were as follows: (1) early stage, urinary microalbumin excretion rate (UAER) of 30-300 mg/24h or urinary albumin-to-creatinine ratio (UACR) of 30-300 mg/g; (2) established stage, UAER>300 mg/24h or UACR>300 mg/g, or 24h urinary total protein (24h UTP)>0.5g and eGFR≥60mL/min/1.73m^2; (3)advanced stage, 30≤eGFR<60mL/min/1.73 m^2. The demographic and clinical data were recorded. Subjects meeting any of the following criteria were excluded: (1) severe infection, moderate and severe anemia, electrolyte disturbance, and acute complications of diabetes occurring within four weeks; (2) patients with severe diseases of the heart, brain, liver, and hematopoietic system and those on glucocorticoid or immunosuppressants in the last three months before admission; (3) oliguria, anuria, serious pleural effusion or ascites, severe edema, or mental illness; (4) patients who received a kidney transplant or dialysis treatment; (5) patients who were pregnant or preparing for pregnancy or lactation; (6) participants in other interventional clinical trials; (7) participants who did not provide signed informed consent.

2.1.2 Clinical and laboratory measurements

Serum samples were collected and immediately frozen at −80 °C. The routine clinical parameters of patients with DKD included age, sex, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), serum creatinine (Scr), estimated glomerular filtration rate (eGFR), urea, blood uric acid (UA), albumin (ALB), 24h UTP, low-density lipoprotein (LDL), serum cholesterol (CHO), and triglyceride (TG) levels. The SLPI levels were measured using a Luminex liquid suspension chip (cat. LXSAHM; R&D Systems), according to the manufacturer's instructions(Wayen Biotechnologies).

2.1.3 Statistical analysis

Statistical analyses were performed using SPSS statistics software 24 (SPSS, Inc.). Enumeration data were expressed as percentages, and measurement data were expressed as the mean ± standard deviation (SD). Data showing a skewed distribution were expressed as the median and interquartile range (IQR). Categorical data were assessed using the chi-squared test. Continuous variables were compared using an independent group t-test or one-way ANOVA for normally distributed data.

Otherwise, the Mann–Whitney U test or Kruskal-Wallis H test was used for skewed distributions. Correlations between SLPI and clinical characteristics were analyzed using Pearson's correlation. The performance of the SLPI as a diagnostic biomarker was tested using receiver operating characteristic (ROC) curve analysis, and the Youden index was used to determine the cutoff value. The area under the ROC curve (AUC) was calculated, with AUC values

between 0.7~0.9 indicating a degree of accuracy for diagnosis. Statistical significance was set at $p< 0.05$.

2.2 Predictive cohort study

2.2.1 Patients

In this predictive cohort study, we enrolled 120 non-dialysis patients aged 18–75 yearsbetween December 2016 and January 2022. This cohort study was comprised of subjects from Dongzhimen Hospital, Beijing University of Chinese Medicine. The exclusion criteria were identical tothose used in the cross-sectional study. The criteria for stage IV DKD were as follows: (1) UAER>300 mg/24 h or UACR>300 mg/g; (2) 24h UTP>0.5g, and (3) eGFR≥15 mL/min/1.73 m^2.

2.2.2 Data collection

Patients were followed up at three-month intervals until the end of the study period or the occurrence of primary endpoint events. The collected data were the same as those used in the cross-sectional study. The primary outcome was disease progression, which was defined as progression to ESRD (sustained eGFR<15mL/min/1.73 m^2), having received renal replacement, or death from a variety of causes associated with kidney diseases.

2.2.3 Statistical analysis

The prognostic value of these variables was further tested using univariate and multivariate Cox proportional hazards regression analyses. Kaplan-Meier survival curves were plotted and differences in survival between groups were estimated using the log-rank test. To calculate the ORs for DKD according to the quartiles of SLPI levels, and a trend test was conducted. Statistical significance was set at $p \leq 0.05$. Kaplan-Meier analysis was performed using GraphPad Prism 8.0.

3 Results

3.1 Cross-sectional study

3.1.1 Baseline characteristics

A total of 266 patients with DKD were enrolled in the multi-center cross-sectional study, including 175 males and 91 females with a mean age of 57.94 ± 8.60 years. Statistically significant differences were observed in age, male-to-female ratio, duration of diabetes, and duration of proteinuria among the three groups ($p<0.01$). Statistically significant differences were also observed in the levels of SBP, HGB, urea, Scr, UA, eGFR, 24h UTP, Alb, CHO, TG, and LDL (Table 1). Trends in clinical laboratory parameters were in accordance with the course of DKD.

TABLE 1 Baseline characteristics of the study population in different stages of DKD.

	Early	Established	Advanced	*P*
Cases(Male/Female)	92 (43/49)	92 (74/18)	82 (58/24)	<0.001
Age(years)	59.43 ± 8.42	55.07 ± 8.98	59.48 ± 7.58	<0.001
Duration of diabetes(years)	12.50 ± 10.00	15.00 ± 8.75	17.00 ± 10.00[A]	0.006
Duration of proteinuria (months)	12.00 ± 23.00	24.00 ± 52.75	36.00 ± 48.50	<0.001
SBP(mm Hg)	130.00 ± 15.50	136.00 ± 10.00	136.00 ± 16.75	<0.001
DBP(mm Hg)	79.00 ± 10.00	80.00 ± 9.00	78.00 ± 15.00	0.077
BMI(kg/m^2)	25.10 ± 6.18	25.27 ± 3.54	25.59 ± 4.94	0.456
HGB(g/dl)	13.75 ± 3.05	13.30 ± 2.75	11.60 ± 2.32	<0.001
GLU(mmol/L)	8.04 ± 3.51	8.47 ± 4.28	7.27 ± 4.12	0.087
HbA1c(%)	6.90 ± 1.60	6.97 ± 1.58	6.80 ± 1.40	0.462
Urea(mmol/L)	5.46 ± 1.68	6.81 ± 3.51	11.25 ± 4.66	<0.001
Scr(μmol/L)	60.30 ± 23.47	80.50 ± 30.85	150.00 ± 79.70	<0.001
UA(μmol/L)	326.60 ± 123.80	378.55 ± 102.60	403.50 ± 131.65	<0.001
eGFR(mL/min/1.73m^2)	121.86 ± 55.23	88.51 ± 48.81	40.59 ± 18.51	<0.001
24h UTP(mg/24h)	220.84 ± 200.50	1583.00 ± 2499.75	3448.00 ± 3517.25	<0.001
Alb(g/L)	43.16 ± 6.88	40.1 ± 7.83	36.50 ± 8.53	<0.001
CHO(mmol/L)	4.42 ± 1.38	4.96 ± 2.02	4.76 ± 2.30	0.001
TG(mmol/L)	1.66 ± 1.10	1.82 ± 1.49	1.92 ± 1.87	0.004
LDL(mmol/L)	2.53 ± 1.09	2.87 ± 1.16	2.72 ± 1.26	0.045

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; HGB, haemoglobin; GLU, Glucose; HbA1c, hemoglobin A1c; eGFR, estimated glomerular filtration rate; Scr, serum creatinine; UA, uric acid; 24h UTP, 24 hours urinary total protein; Alb, albumin; CHO, serum cholesterol; TG, triglyceride; LDL, low-density lipoprotein.

3.1.2 SLPI and decline of kidney function

3.1.2.1 Serum SLPI levels at different stages of DKD

As shown in Figure 1, serum SLPI levels increased among healthy controls, the diabetes group, and the early, established and advanced stages of DKD, with statistically significant differences observed among the five groups (H = 84.26, p<0.01). In the comparison between the two groups, the serum SLPI levels were significantly higher in the established group and advanced group than in the healthy controls (p<0.05, p<0.01, respectively); however, no statistically significant difference was observed in the SLPI levels between the diabetes group and early group compared to the healthy group. Additionally, patients in the advanced group has significantly higher SLPI levels than those in the other groups (p<0.01).

3.1.2.2 Correlation between serum SLPI levels and routine clinical parameters in patients with DKD

We evaluated the correlation between serum SLPI levels and baseline parameters in patients with DKD. As shown in Table 2, the serum SLPI levels were negatively correlated with eGFR (r= -0.564, p<0.01) and positively correlated with urea (r=0.532, p<0.01) and Scr (r=0.604, p<0.01). Moreover, serum SLPI levels were positively correlated with 24h UTP (r = 0.372, p<0.01) and the UA levels (r = 0.233, p<0.01). In addition, serum SLPI levels were negatively correlated with the HGB (r = -0.158, p<0.01) and Alb (r = -0.126, p<0.05) levels, whereas they were positively correlated with SBP (r = 0.161, p<0.01) and the duration of proteinuria(r = 0.233, p<0.01). These results suggest that the serum SLPI levels in patients with DKD are positively correlated with disease progression.

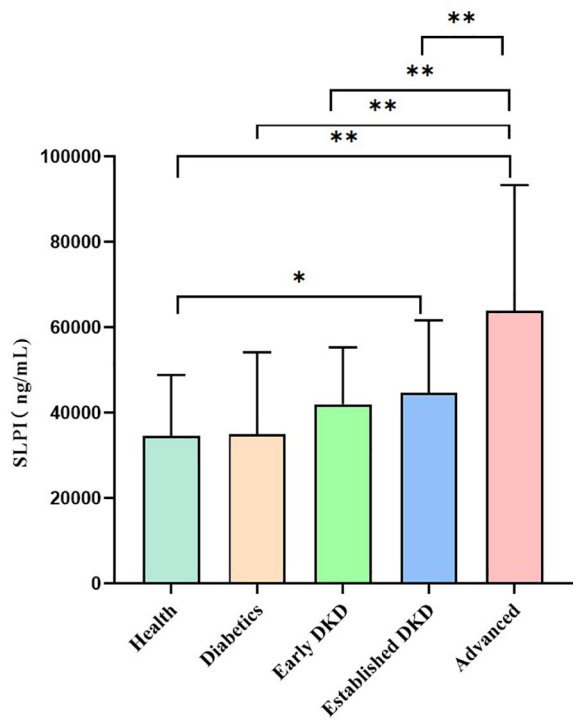

FIGURE 1
Serum SLPI levels among groups. Notes: Healthy group versus other groups (*p<0.05); healthy group versus other groups (**p<0.01); diabetes group versus other groups (**p<0.01); early group versus other groups (**p<0.01); established group versus other groups (**p<0.01).

3.1.2.3 Evaluation of the role of SLPI level in DKD diagnosis by ROC curve analysis

All subjects were grouped into DKD and non-DKD groups, and the role of serum SLPI level as a predictor was tested using ROC curve analysis. ROC analysis showed that an SLPI cutoff level of 39.11ng/ml offered optimal differentiation between patients with and without DKD (73.31% sensitivity and 69.23% specificity). The ROC analysis indicated that the serum SLPI level tended to offer diagnostic value for DKD (AUC=0.76, 95% CI [0.69, 0.83], p<0.01) (Figure 2). Therefore, the results revealed that serum SLPI levels may be a factor in the diagnosis of DKD.

3.2 Predictive cohort study

3.2.1 Comparison of participant data in low and high SLPI expression groups

A total of 120 patients with stage IV DKD were included in the predictive cohort study. Patients with DKD were divided into low and high SLPI expression groups based on the median serum SLPI level (51.61 ng/mL). Table 3 shows the clinical and laboratory characteristics of the study population. No significant differences were observed betweenthe groups in term of age, sex, duration of diabetes, or duration of proteinuria. However, patients with higher SLPI levels had a lower eGFR and a higher frequency of renal endpoint eventsthan patients with lower SLPI levels (p<0.01).

3.2.2 Prognostic value of serum SLPI level on renal endpoint events

The mean follow-up duration for the cohort study was 2.32 ± 1.30 years. As shown in Figure 3, elevated SLPI levels (≥51.61 ng/mL) were associated with an increased risk of renal endpoint events (log-rank p=0.001). Furthermore, an increased risk of developing renal endpoint events was observed in patients with 24h UTP≥3.5g (log-rank p<0.01). In addition, a decrease in HGB levels (<13g/dl) was associated with a higher risk of developing renal endpoints (log-rank p=0.007), as well as reduced ALB levels(< 30g/L) (log-rank p=0.019). Therefore, Kaplan-Meier analysis showed a significantly faster progression to renal clinical endpoint events in subjects with SLPI ≥51.61 ng/mL, 24 h UTP≥3.5 g, HGB <13 g/dl, and ALB <30 g/L.

Next, we performed univariate and multivariate analyses to identify risk factors associated with renal endpoint events in patients with DKD. In the univariate analysis, variables associated with renal endpoint events were high expression SLPI level (HR= 2.95, p=0.001), 24h UTP≥3500mg (HR = 3.85, p<0.001), HGB<13g (HR = 2.65, p=0.010), Alb<30g (HR = 2.19, p=0.022) and urea≥7.1 mmol/L (HR = 9.59, p=0.002). Risk factors for renal clinical endpoint events were further analyzed using a multivariate regression model to determine the most important factors. Based on the multivariate regression model, only serum SLPI levels ≥ 51.61 ng/mL (HR = 2.95, 95% CI[1.55, 5.60], p=0.001), 24h

TABLE 2 Correlation between serum SLPI and clinical parameters.

Parameters	Serum SLPI
	Correlation coefficients(r_s)
eGFR(mL/min/1.73m^2)	-0.564**
24h UTP(mg/24h)	0.372**
Urea(mmol/L)	0.532**
Scr(μmol/L)	0.604**
UA(μmol/L)	0.233**
HbA1c(%)	-0.132*
Alb(g/L)	-0.126*
CHO(mmol/L)	0.135*
HGB(g/dl)	-0.158**
SBP(mmHg)	0.161**
Duration of proteinuria(months)	0.233**

*P<0.05; **P<0.01.

UTP≥3.5g (HR = 3.02, 95% CI[1.66, 5.52], p<0.001), HGB<13g (HR = 3.18, 95% CI[1.49, 6.80], p=0.003), Alb<30g (HR = 2.19, 95% CI [1.12, 4.28], p=0.021) and urea≥7.1 mmol/L (HR = 8.27, 95% CI [1.96, 34.93], p=0.004) were independently associated with renal endpoint events in DKD patients. Survival prediction analysis indicated that higher SLPI levels, massive proteinuria, increased urea levels, and lower ALB and HGB levels increased the probability of renal endpoint events in patients with DKD (Table 4).

In addition, we further grouped SLPI into quartiles to observe trends in disease progression in thedifferent SLPI subgroups, as well as to analyze the relationship between SLPI levels and disease progression in DKD. As shown in Table 5, the disease progression rate of DKD progressively increased from 16.7% (5/30) to 53.3% (16/30) across the SLPI quartiles. Similar trends were observed in each model. With an increase in the SLPI levels, the incidence of endpoint events in DKD patients also increased (all p<0.05).

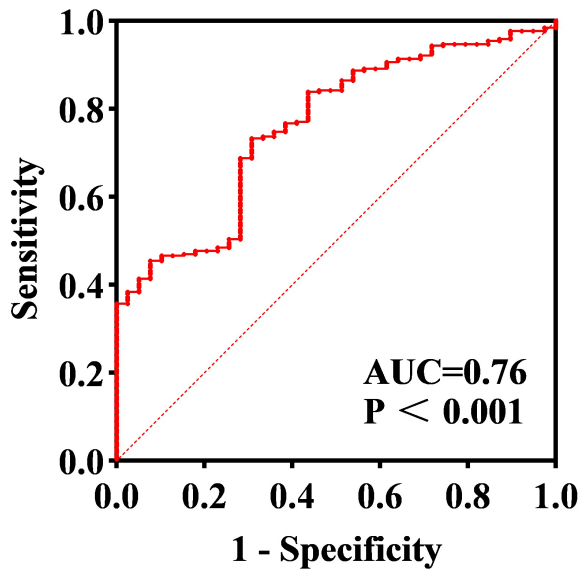

FIGURE 2
ROC curve of serum SLPI for predicting DKD. Cut-off SLPI level of 39.11 ng/ml offered optimal differentiation between patients with and without DKD (AUC=0.76, 95% CI=[0.69–0.83]).

4 Discussion

The discovery of novel biomarkers is extremely important for the early diagnosis and prediction of adverse outcomes of DKD. As shown in Figure 4 this cross-sectional study evaluated the diagnostic and prognostic value of serum SLPI levels in patients with DKD. Serum SLPI levels were found to gradually increase during the occurrence and progression of DKD. Furthermore, serum SLPI levels in patients with DKD were positively correlated with aggravation of the disease, and act as an important reference value for the diagnosis of DKD. In this predictive cohort study, we further observed that elevated SLPI levels, 24 h UTP≥3.5 g, HGB <13 g/dl, and ALB < 30 g/L were independent risk factors and increased the probability of renal endpoint events in patients with DKD. In addition, we further divided the SLPI into quartiles, observed trends in disease progression across different SLPI subgroups, and analyzed the relationship between the SLPI and DKD progression. The results showed that an increase in the SLPI level was a risk factor for DKD progression. After adjusting for a range of relevant factors, SLPI remained an independent risk factor for DKD progression. These results highlighted the diagnostic and prognostic potential of serum SLPI levels in DKD.

We investigated the association between serum SLPI levels and renal function at different stages of DKD in a large multi-center cross-sectional study. For these 266 patients, the results showed that serum SLPI was expressed at higher levels in the early-to-moderate stages of DKD and gradually increased with the progression of DKD, suggesting that SLPI plays a role in the initiation and progression of DKD. Moreover, SLPI levels were highly correlated with renal dysfunction in patients with DKD. This validates the previous observation of a strong correlation between the SLPI and DKD in a smaller cross-sectional study (18). Circulating SLPI levels consistently increase with the progression of metabolic dysfunction and are independently associated with metabolic and inflammatory markers in a prospective cross-sectional study (17, 19). This result is consistent with the increased expression of SLPI in DKD, a well-known chronic inflammatory state.

We also found that elevated SLPI levels were positively correlated with renal dysfunction in patients with DKD. This suggests that SLPI, as an alarm reactant, increases upon stimulation with renal injury and reflects the severity of the inflammatory situation during DKD. The mechanism of SLPI expression level in the initiation and progression of DKD may be related to counterbalance the pro-inflammatory condition that increases the risk for DKD by attenuating neutrophil recruitment and phagocytosis, inhibiting the monocyte/macrophage response to endotoxins and suppressing the activation of transcription factor NF-κB (20, 21). Additionally, a positive correlation was observed between SLPI and hyperglycemia, hypertension, and

TABLE 3 Baseline characteristics of participants in the low and high SLPI expression groups.

Characteristics	Low SLPI expression group(n=60)	High SLPI expression group (n =60)	Statistics	P
Age (years)	57.28 ± 8.17	56.25 ± 8.8	T = 0.67	0.51
Male, n (%)	42(70.00)	48(80.00)	χ^2 = 1.60	0.21
Duration of diabetes (years)	14.82 ± 6.08	16.4 ± 7.09	t = -1.32	0.19
Duration of Proteinuria (months)	34.02 ± 36.24	45.65 ± 52.14	t = -1.42	0.16
24hUTP (mg/24h)	3195.62 ± 3443.50	3591.41 ± 3187.63	Z = -1.74	0.081
eGFR (mL/min/1.73m^2)	87.77 ± 54.57	54.80 ± 28.99	Z = -4.91	<0.01
HbA1c (%)	7.03 ± 1.08	6.61 ± 1.03	t = 2.204	0.029
Renal endpoint events, n (%)	14(23.33)	32(53.33)**	χ^2=11.42	<0.01

*P<0.05; **P<0.01.

hyperlipidemia, which are common risk factors for DKD. Given the increased chronic stress and inflammation in DKD, it is tempting to speculate that the upregulation of circulating SLPI may be associated with metabolic disorders to meet the demands of anti-stress and anti-inflammatory activities (22, 23).

We analyzed the diagnostic value of the serum SLPI in patients with DKD using ROC curves. The results showed that serum SLPI levels had moderate diagnostic value for DKD, which validated the diagnostic potential of SLPI as a biomarker, previously identified in a large cross-sectional study (18). Similarly, a prospective observational study implicated SLPI in the pathogenesis of kidney diseases and identified it as a postoperative biomarker for the early diagnosis of acute kidney disease (15). This may be considered one of the best predictors of DKD progression. However, given that SLPI may widely interfere with kidney injury, the measurement of serum SLPI elevation alone is not sufficient to diagnose DKD. Further studies are needed to compare SLPI levels between patients with and without DKD. Current clinical methods used to predict the initiation and progression of DKD are usually an elevated urinary albumin-to-creatinine ratio (UACR) or microalbuminuria of 30~300 mg/24h (24). However, high interindividual variability in the mild-to-moderate stages of DKD is a major limitation for

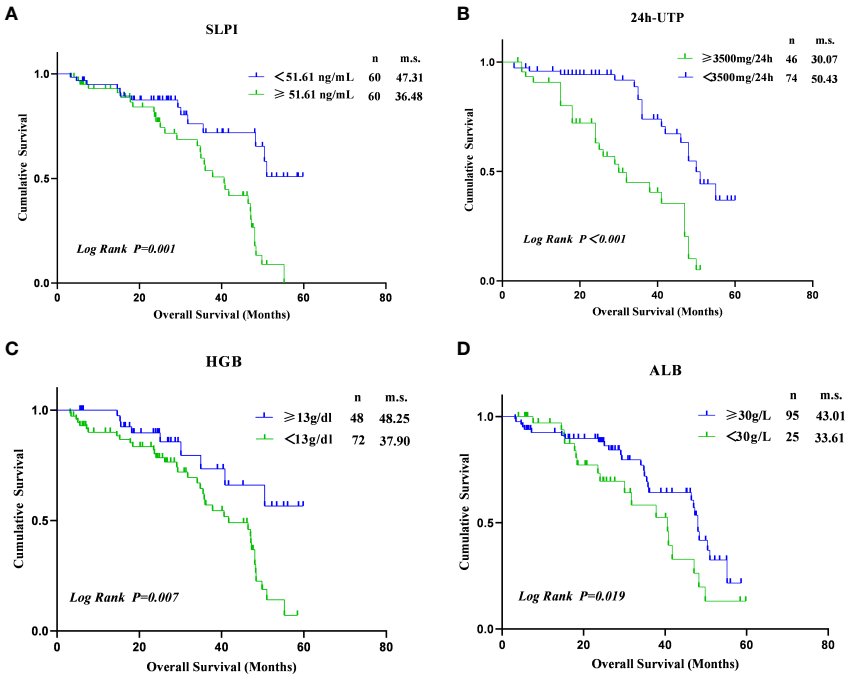

FIGURE 3
Kaplan–Meier estimates of survival during follow-up in DKD. **(A)** Between SLPI≥51.61 ng/mL and SLPI<51.61ng/ml (log-rank p=0.001). **(B)** Between 24h UTP≥3500mg/24h and 24h UTP<3500mg/24h (log-rank p<0.001). **(C)** Between HGB<13g/dl and HGB≥13g/dl (log-rank p=0.007); **(D)** Between ALB<30g/L and ALB≥30g/L (log-rank p=0.019).

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TABLE 4 Cox regression analysis for outcomes in DKD.

Factors	Univariable		Multivariable model [a]	
	HR(95% *CI*)	*P value*	*HR*(95% *CI*)	*P value*
Sex				
male	1.0		1.0	
female	0.83(0.41,1.69)	0.614	0.57(0.27,1.17)	0.124
Age(years)				
<65	1.0		1.0	
≥65	1.15(0.74,2.92)	0.270	1.51(0.76,2.99)	0.243
Serum SLPI(ng/mL)				
low expression group	1.0		1.0	
high expression group	2.95(1.55, 5.62)	0.001	2.95(1.55, 5.60)	0.001
24h UTP(mg/24h)				
<3500mg	1.0		1.0	
≥3500mg	3.85(2.09, 7.06)	<0.001	3.02(1.66, 5.52)	<0.001
HGB(g/dl)				
≥13g	1.0		1.0	
<13g	2.65(1.27,5.53)	0.010	3.18(1.49, 6.80)	0.003
Alb(g/L)				
≥30g	1.0		1.0	
<30g	2.19(1.12,4.29)	0.022	2.19(1.12, 4.28)	0.021
Urea(mmol/L)				
<7.1	1.0		1.0	
≥7.1	9.59(2.29, 40.26)	0.002	8.27(1.96, 34.93)	0.004

[a]Each risk factor (age, serum SLPI, 24h UTP, HGB, Alb, Urea) has been adjusted for the other factors.

accurate early diagnosis and prognosis (25, 26). In addition, other biomarkers reported in recent studies, including renal injury(such as KIM-1, β2-MG, Cys-C, and YKL-40) and proinflammatory factors (such as TNF receptor-1/2, suPAR, and MCP-1) showed a relatively lower sensitivity and specificity for predicting kidney injury associated with diabetes (4, 5, 27). Thus, it is more feasible to establish routine diagnostic biomarkers for kidney injury in DKD. This study revealed that its combination with microalbuminuria and other biomarkers may be more accurate for the early diagnosis and prognosis of DKD. Moreover, higher serum SLPI levels may be used to identify individuals with diabetes who are at a risk of eGFR decline.

At present, the prognostic value of SLPI in DKD has not yet been evaluated in detail. In our cohort study, patients with stage IV DKD were selected as the study subjects because of their relatively rapid rate of progression and because it is easy to observe renal clinical endpoint events. We found that a higher serum SLPI concentration was independently associated with an elevated risk of renal endpoint events among individuals with stage IV DKD, even after adjusting for clinical risk factors. To date, because of SLPI's ability to protect against tissue repair, studies investigating SLPI and kidney disease in humans have mostly focused on acute kidney injury (15, 16). To the best of our knowledge, The present study is the first to describe the association between elevated serum SLPI concentrations and adverse renal outcomes in patients with DKD. These results are consistent with previous findings that high SLPI expression is associated with poor prognosis in cancers (28–30). However, the cause-and-effect relationship between increased SLPI expression and disease progression has not yet been studied, and most studies have indicated that the upregulation of SLPI is associated with proinflammatory process. In addition, whether elevated serum SLPI levels lead to renal damage by limiting the organism's ability to counter overactivated inflammatory and metabolic stress during DKD has not yet been ruled out (31, 32). Thus, additional studies on the exact biological contribution of serum SLPI to DKD progression are required. Additionally, we also

TABLE 5 Odds ratios and 95% confidence interval for DKD and its individual components according to quartile of SLPI.

	Quartile of SLPI				*P* Value for Trend
	Q1	Q2	Q3	Q4	
	≤40.93	40.94-51.61	51.62-67.32	>67.32	
Patients, n	30	31	29	30	
Disease Progression, n	5	10	15	16	0.001
Model 1	1.00	3.361(1.141-9.899)	5.236(1.840-14.897)	5.397(1.965-14.822)	0.012
P Values		0.028	0.002	0.001	
Model 2	1.00	3.338(1.134-9.826)	5.344(1.850-15.434)	5.724(2.071-15.821)	0.027
P Values		0.029	0.002	0.001	
Model 3	1.00	4.180(1.283-13.622)	4.843(1.502-15.616)	4.815(1.510-15.360)	0.020
P Values		0.018	0.008	0.008	

Model 1: crude, no adjustment; Model 2: adjusting for age, gender, duration of diabetes, and duration of proteinuria; Model 3: adjusting for age, gender, duration of diabetes, duration of proteinuria, Urea, GLU, HbA1c, UA, and ALB.

found that DKD patients with 24h UTP≥3.5g, HGB < 13g/dl, and ALB < 30g/L showed a significantly faster progression to renal clinical endpoints, which were consistent with previous studies in DKD patients. Albuminuria is an established risk factor of kidney failure. Previous studies have indicated that albuminuria is a risk factor for renal prognosis in patients with type 2 diabetes (33). In a previous prospective, multicenter cohort study of 1138 pre-dialysis CKD, decreased serum albumin and HGB were identified as risk factors for renal endpoints (34). Moreover, measurements of urea levels revealed a valuable prognostic capacity to predict progression to renal endpoint events. Taken together, these results indicated that SLPI levels≥51.61ng/ml in combination with 24h UTP≥ 3.5g, HGB < 13 g/dl, ALB < 30 g/L and urea ≥ 7.1mmol/L may be the optimal thresholds for predicting and identifying high-risk populations.

The mechanisms underlying the protective role of SLPI in this process are diverse. DKD is a chronic inflammatory disease, triggered by metabolic disturbances, hemodynamic changes, increased oxidative stress, and profibrotic mediators (35). SLPI has been identified as a potent inflammation-suppressing protein that is triggered in proportion to the degree of inflammation, playing a role in suppressing the inflammatory environment. Higher expression of SLPI can antagonize the activation of the inflammatory transcription factor NF-κB and counteract the effect

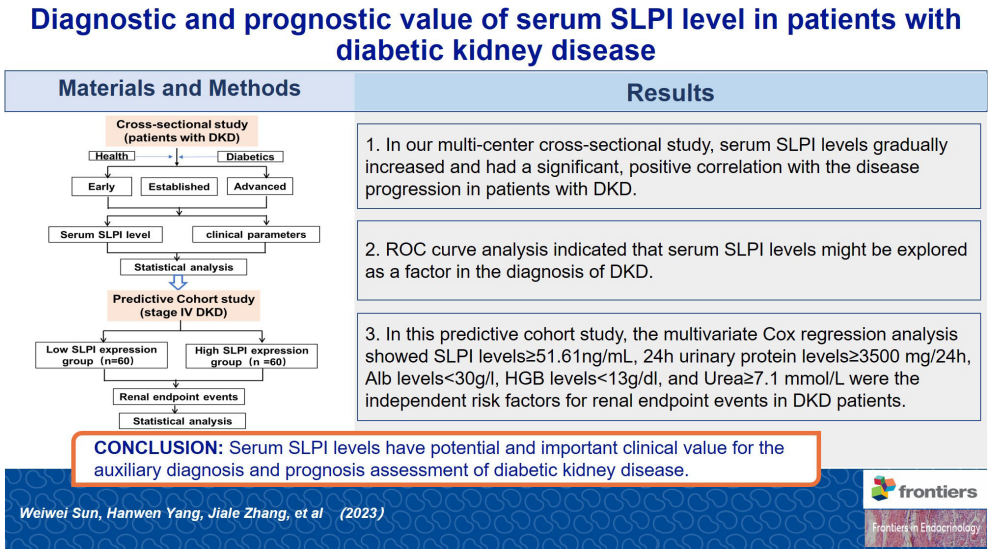

FIGURE 4
Graphical abstract.

of proinflammatory cytokines, thus alleviating kidney damage and promoting proximal tubular cell regeneration in a diabetic state (23). Moreover, SLPI is recognized as a molecule that favors the host via its immunomodulatory, anti-proteolytic, and anti-microbial properties (6). SLPI regulates the pro-immunogenic function of neutrophil extracellular traps and inhibits the activity of several proteolytic enzymes, making it a component of the defense mechanisms in the kidney (22). However, to date, the specific role of SLPI in DKD pathogenesis remains unclear and requires further examination in experimental studies.

To the best of our knowledge, this study is the first to comprehensively evaluate the potential clinical value of serum SLPI levels for the combined diagnosis and prognosis of DKD. However, several limitations and potential drawbacks of this study should beconsidered. First, the sample sizes of both the cross-sectional and predictive cohort studies were relatively small. Furthermore, the study's limitations to Beijing hospitals may limit the applicability of the findings to a larger population. Among these, the external validity of the study could be improved by replicating it with a nationwide multi-center clinical research with a larger sample size. Second, wewere unable to directly establish a causal relationship between elevated SLPI levels and DKD progression. Whether an elevated SLPI level is the cause of the occurrence and progression of DKD or an accompanying factor of DKD will be further verified through subsequent large-sample clinical trials and precise experimental studies. in addition, the segment of the study that examined future possibilities had a limited timeframe, potentially not capturing a comprehensive picture over an extended period. In future studies, we will increase the follow-up period, which may provide a deeper understanding of the long-term predictive usefulness of the SLPI. Another limitationis that we only observed patients with stage IV DKD in this predictive cohort study, which could not provide the risk of clinical renal endpoints for patients with other stages of DKD. Furthermore, the exclusion criteria, targeting patients with specific conditions, may introduce selection bias, limiting the applicability of the findings to a broader DKD patient population with diverse comorbidities. The prognostic utility of SLPI levels in other study populations is warranted to further investigate under the setting of DKD. Third, potential confounding factors, such as medications that may affect SLPI levels and the course of DKD, were not sufficiently addressed in this study. Therefore, we will further explore the available data on the effect of medications on SLPI levels during the course of DKD to provide a comprehensive analysis. Moreover, the absence of information on the ethnic or racial composition of the study population is notable because such differences can influence disease progression and biomarker performance. Thus, larger studies using genetic data, serum SLPI levels, and a greater number of DKD events are required to elucidate whether genetically mediated SLPI levels are associated with DKD.

In conclusion, we demonstrated that the measurement of serum SLPI levels had auxiliary diagnostic and prognostic value in patients with DKD. In future studies, we will expand the sample size to verify its predictive value in the diagnosis and prognosis of DKD and explore the specific functional properties of SLPI in DKD through animal and cell experiments.

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 humans were approved by the Medical Ethics Committee of Dongzhimen Hospital Affiliated to Beijing University of Chinese Medicine (DZMEC-KY-2016-95). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

WS: Investigation, Methodology, Project administration, Writing – original draft. HY: Investigation, Methodology, Writing – original draft. JZ: Data curation, Investigation, Software, Writing – review & editing. SW: Data curation, Investigation, Writing – review & editing. QW: Investigation, Writing – review & editing. CC: Data curation, Investigation, Writing – review & editing. JY: Data curation, Investigation, Writing – review & editing. ZC: Data curation, Formal analysis, Project administration, Writing – review & editing. HZ: Data curation, Project administration, Supervision, Writing – review & editing. YW: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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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

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Association between lactate dehydrogenase and the risk of diabetic kidney disease in patients with type 2 diabetes

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Objective: This study aims to investigate the association between lactate dehydrogenase (LDH) and the risk of diabetic kidney disease (DKD) in patients with type 2 diabetes (T2D).

Methods: The study enrolled patients with diagnosis of T2D between 2009 and 2018 from the National Nutrition and Health Examination Survey (NHANES) database. Demographic information, laboratory test, and diagnostic data were collected. Restricted cubic spline (RCS) plots were used to assess the dose-effect relationship between LDH levels and the risk of DKD in patients with T2D. Based on LDH levels, individuals were divided into higher and lower groups using dichotomy, and multivariate logistic regression analysis was conducted to explore the relationship between different LDH levels and the risk of DKD in T2D patients. Stratified analysis was performed to assess the consistency of the result.

Results: A total of 4888 patients were included in the study, with 2976 (60.9%) patients without DKD and 1912 (39.1%) patients with DKD. RCS plots showed that the risk of DKD increased with increasing LDH levels. Multifactorial logistic regression analysis revealed that T2D patients with higher LDH levels had a 45% increased risk of DKD compared to those with lower LDH levels (OR=1.45; 95% CI: 1.11-1.89). Furthermore, each standard deviation increase in LDH level was associated with a 24% increase in DKD incidence among T2D patients (OR=1.24; 95% CI: 1.07-1.44). Stratified analysis consistently supported these findings.

Conclusions: LDH can serve as a valuable biomarker for screening DKD in patients with T2D.

KEYWORDS

type 2 diabetes mellitus, diabetic kidney disease, database research, NHANES, risk factors

1 Introduction

Diabetes mellitus (DM) is a chronic metabolic disease characterized by impaired insulin secretion and function. It was considered irreversible and has become one of the leading causes of death worldwide (1, 2). The International Diabetes Federation (IDF) predicts that the number of diabetic patients will increase from 240 million in 2007 to 380 million in 2025, further rising to 439 million in 2030 (3). Diabetic kidney disease (DKD), found in approximately 40% of diabetic patients, was the main cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD) globally (4, 5). This condition imposes a significant social and economic burden, highlighting the importance of early identification of DKD.

LDH is an enzyme belonging to the family of 2-hydroxy acid oxidoreductases. It plays a crucial role in both anaerobic and aerobic glycolysis, converting pyruvate into lactic acid and nicotinamide adenine dinucleotide (NADH), thereby affecting metabolism (6). LDH was commonly used for diagnosing such as myocardial infarction, vascular injury, tissue injury, advanced sarcoma, and other mesenchymal tumors (7). It has now been found that LDH can be used as a biomarker for the prognosis of diseases such as tumors, metabolism-associated fatty liver disease, and malaria (8–10), and it can also be used as a biomarker for predicting the pathological status of the lungs (11) and for the possibility of DM (12).

Lactate dehydrogenase (LDH) is an enzyme composed of four subtypes, namely LDH-A, LDH-B, LDH-C, and LDH-D. LDH-A is mainly expressed in muscle and heart tissue, and is involved in the production and dehydrogenation of lactate. It promotes glycolysis to produce ATP under aerobic conditions, while converting lactate to pyruvate under hypoxic conditions to maintain intracellular acid-base balance. LDH-B mainly expressed in the liver and kidneys, involved in lactate metabolism and clearance. which participates in the gluconeogenesis pathway by converting lactate into glucose in the liver, and helps clear excess lactate in the kidneys. LDH-C and LDH-D are expressed in embryonic and germ cells, and their functions are not fully understood, but they may be related to cell proliferation and differentiation. Their proportion in the blood is usually 25-35% for LDH-A (LDH-1), 30-40% for LDH-B (LDH-2), 20-30% for LDH-C (LDH-3), and 5-10% for LDH-D. These proportions may have slight variations, and the specific proportions may also be influenced by different laboratories and measurement methods (13). More and more studies found that LDH may be associated with the development of DKD. Al-Rubeaan K et al. (14)found that the progression of DKD was associated with elevated levels of LDH, and elevated uric acid and LDH were associated with microalbuminuria and an increased risk of ESRD. Mohammadi-Karakani et al (15) observed significantly elevated urinary LDH and microalbuminuria levels in diabetic patients compared to healthy individuals. Lee DY et al. (16) found that elevated levels of LDHA were associated with renal insufficiency in DKD patients. LDHA was expressed at high levels in both glomerular and tubular epithelial cells of renal tissues in DKD patients. The decrease in glomerular filtration rate was associated with increased urinary lactate levels and LDHA expression as well as increased fasting blood glucose and glycosylated hemoglobin levels. In addition, LDH has considerable potential value in early warning of rejection in renal transplant recipients, screening for renal disease, and detection of renal injury secondary to hypertension, diabetes mellitus and rheumatoid arthritis (17).

Therefore, LDH activity and expression levels were elevated in DKD and may be involved in the onset and progression of DKD. However, there were no studies that clearly show the relationship between LDH levels and the risk of DKD in Type 2 diabetes (T2D) patients. Therefore, the aim of this study was to explore the relationship between LDH and the risk of DKD in T2D patients.

2 Methods

2.1 Study design and participants

The data for this study were obtained from the National Nutrition and Health Survey (NHANES) database from 2009 to 2018, which includes information on patients with T2D. NHANES aims to explore the individual-level demographic, health, and nutritional information through personal interviews and standardized physical exams at specialized Mobile Examination Centers (MECs), as well as evaluate the health and nutritional status of non-hospitalized civilians in the United States (18).

In this study, a total of 4,888 patients were included, consisting of 2976 (60.9%) T2D patients without DKD and 1912 (39.1%) T2D patients with DKD. To ensure the accuracy of the study, specific diagnostic criteria were employed to determine the presence of diabetes and DKD. The diagnostic criteria for diabetes included: 1) doctor diagnosis of diabetes, 2) glycated hemoglobin (HbA1c) level > 6.5%, 3) fasting blood glucose level ≥ 7.0 mmol/L, 4) random blood glucose level ≥ 11.1 mmol/L, or 5) random blood glucose level after a two-hour oral glucose tolerance test (OGTT) ≥ 11.1 mmol/L, meet any one of the above (19). Antidiabetic drugs administration was obtained through a questionnaire.

The diagnostic criteria for DKD were based on the diagnosis of diabetes and met the guidelines for CKD established by the working group for kidney disease improving global outcomes (KDIGO). These criteria included a urinary albumin-to-creatinine ratio (ACR) exceeding 30 mg/g or an estimated glomerular filtration rate (eGFR) below 60 ml/min/1.73m^2 (20). Exclusion criteria for the study were: 1) age under 18 years, 2) pregnancy, 3) type 1 diabetes mellitus, 4) missing lactate dehydrogenase (LDH) data or abnormally high LDH values, and 5) missing diagnoses of T2D or CKD. NHANES is a survey project conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC) and approved by the National Center for Health Statistics Institutional Review Board. The study protocol conformed to the ethical standards of the 1964 Declaration of Helsinki and its subsequent amendments and was approved by the National Ethics Review Board for Health Statistics Research. All participants had signed an informed consent form (19).

2.2 Statistical analysis

Following the guidelines of the US Centers for Disease Control and Prevention, the study utilized weighted samples and employed

stratification and clustering techniques to generate estimates representative of the overall US population. Continuous variables were summarized using means and standard errors, while classified variables were expressed as percentages and standard errors. To assess differences between different groups, weighted t-tests and chi-square tests were used for continuous variables and categorical variables, respectively.

The relationship between LDH levels and the risk of DKD in T2D patients was evaluated using a restricted cubic spline (RCS) plot of complex sampling. Individuals were classified into two groups, higher LDH group and lower LDH group, through dichotomy. Additionally, a complex sampling multivariate linear regression model was established to evaluate the correlation between LDH and clinical variables after adjusting for multiple factors. Furthermore, a weighted multivariate Cox regression model analysis was performed to identify risk factors for DKD in T2D patients, and the consistency of the results was evaluated through subgroup analysis. Weights were calculated by determining the smallest subset of variables based on their inclusion in the study and selecting the corresponding weights. Finally, the weights were combined over the years. All statistical analyses were performed with R version 4.3.1 software. A significance level of $P<0.05$ was used for all statistical analyses.

3 Results

3.1 Baseline characteristics

In our study, we utilized the data of 49,693 individuals registered in the NHANES database from 2009 to 2018. In this dataset, we included 4,888 patients diagnosed with T2D (Figure 1). The patients enrolled were both male and female, the percentage of males was 51.58. And the average age of T2D patients was 59.59 years. The mean level of LDH was 138.85 U/L. When comparing T2D patients without DKD, we observed that those with DKD were older and had a higher proportion of hypertension and smoking, as well as a lower proportion of drinkers (All $P<0.05$). Additionally, T2D patients with DKD showed lower eGFR, higher levels of LDH, uric acid, triglyceride, glycosylated hemoglobin, and ACR, and lower levels of hemoglobin and serum albumin (All $P<0.05$) (Table 1). Among the use of antidiabetic drugs, 43.73% of the patients used the oral hypoglycemic agents, 10.87% of the patients used insulin while 6.04% of the patients used each of them. Furthermore, compared with patients with lower LDH, patients with higher LDH were older, had a higher prevalence of women and hypertension, had lower rates of alcohol use and smoking, higher levels of total cholesterol and high-density lipoprotein (HDL), and lower levels of hemoglobin, eGFR, and triglycerides. (all $P<0.05$) (Table 2).

3.2 Association of LDH with clinical characteristics

After adjusting for age, sex, race, alcohol use ("Yes" or "No"), smoking ("Yes" or "No"), multivariate linear regression model analysis showed that LDH significantly impacted serum albumin, ACR, and eGFR. The level of LDH was positively correlated with urinary ACR ($\beta=1.48$, $P<0.001$) and negatively correlated with serum albumin ($\beta=-0.01$, $P<0.001$) and eGFR ($\beta=-0.06$, $P<0.001$) (Table 3).

3.3 Association between LDH and risk of DKD

In the 4888 T2D patients, 1912 (39.1%) of T2D patients without DKD, whereas 2976 (60.9%) of T2D patients with DKD. The patients were grouped into a lower LDH level group (<134 U/L) (n=2452) and a higher LDH level group (≥134 U/L) (n=2436) based on dichotomization. The results of RCS plots showed a linear relationship between LDH and the risk of DKD in T2D, with the risk of DKD increased in patients with T2D as LDH was elevated (Figure 2). Compared with T2D patients with lower LDH, the risk of DKD in T2D with higher LDH ($134 < LDH \leq 367$)(U/L) was 39% higher (OR: 1.39; 95% CI, 1.15-1.68, $P < 0.001$) (Figure 3). After adjusting for baseline age, sex, and race, Model 1[a] revealed that the risk of DKD in T2D patients with higher LDH was 48% higher compared to those with lower LDH (OR = 1.48; 95% CI, 1.23-1.77, $P<0.001$). Model 2[b], which included adjustments for covariates from Model 1[a] as well as alcohol use ("Yes" or "No") and smoking ("Yes" or "No"), demonstrated that the risk of DKD in T2D patients with higher LDH was 43% higher than those with lower LDH (OR = 1.43; 95% CI, 1.18-1.74, $P<0.001$). Model 3[c] was adjusted for covariates from Model 2[b] as well as hypertension ("Yes" or "No"), hemoglobin, serum albumin, and uric acid. The

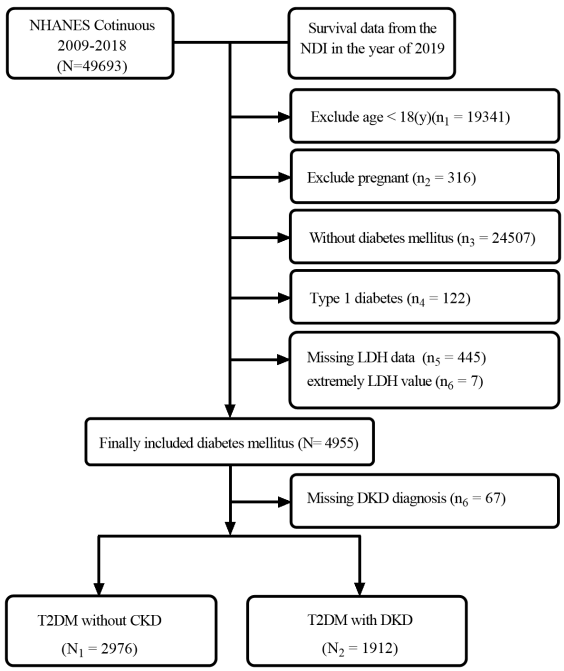

FIGURE 1
Flowchart of included individuals in this study.

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TABLE 1 Baseline clinical features of enrolled T2D patients without and with CKD.

Variable	Total	T2DM without CKD (N1 = 2976)	T2DM with CKD (N2 = 1909)	P-value
LDH (U/L)	138.85 (0.92)	135.01 (0.92)	145.74 (1.54)	< 0.001
Age (years)	59.59 (0.32)	56.52 (0.37)	65.10 (0.45)	< 0.001
BMI (kg/m^2)	33.16 (0.18)	33.21 (0.21)	33.05 (0.29)	0.62
Hemoglobin (g/dL)	14.00 (0.03)	14.17 (0.04)	13.69 (0.06)	< 0.001
Serum albumin (g/L)	41.38 (0.08)	41.77 (0.10)	40.67 (0.13)	< 0.001
Uric_acid (μmol/L)	341.41 (2.06)	327.10 (2.21)	367.06 (2.98)	< 0.001
Total cholesterol (mmol/L)	4.76 (0.02)	4.78 (0.03)	4.71 (0.03)	0.08
Triglyceride (mmol/L)	2.22 (0.04)	2.15 (0.06)	2.34 (0.05)	0.01
HDL (mmol/L)	1.23 (0.01)	1.22 (0.01)	1.24 (0.01)	0.48
LDL (mmol/L)	2.72 (0.03)	2.76 (0.04)	2.65 (0.04)	0.06
CRP	0.57 (0.04)	0.55 (0.05)	0.61 (0.05)	0.39
eGFR (ml/min/1.73m^2)	83.60 (0.44)	92.15 (0.45)	68.26 (0.96)	< 0.001
ACR (mg/g)	110.28 (8.45)	10.05 (0.14)	295.64 (24.44)	< 0.001
HbA1c (%)	7.08 (0.03)	6.91 (0.04)	7.38 (0.05)	< 0.001
Sex (%)				0.62
Female	48.42 (0.02)	48.02 (1.55)	49.13 (1.58)	
Male	51.58 (0.02)	51.98 (1.55)	50.87 (1.58)	
Race (%)				0.11
Mexican American	10.30 (0.01)	10.65 (1.28)	9.68 (1.29)	
Non-Hispanic Black	13.62 (0.01)	13.36 (1.19)	14.08 (1.25)	
Non-Hispanic White	60.15 (0.03)	59.34 (2.01)	61.60 (2.00)	
Other Hispanic	6.23 (0.01)	6.82 (0.70)	5.18 (0.70)	
Other Race - Including Multi-Racial	9.70 (0.01)	9.83 (0.81)	9.46 (0.91)	
Hypertension (%)				< 0.001
No	29.73 (0.01)	35.92 (1.36)	18.64 (1.30)	
Yes	70.27 (0.02)	64.08 (1.36)	81.36 (1.30)	
Alcohol user (%)				< 0.001
No	13.18 (0.01)	13.21 (0.78)	17.76 (1.27)	
Yes	75.82 (0.03)	86.79 (0.78)	82.24 (1.27)	
Smoke (%)				0.04
No	50.00 (0.02)	51.79 (1.36)	47.07 (1.64)	
Yes	49.81 (0.02)	48.21 (1.36)	52.93 (1.64)	
Antidiabetic drugs (%)				< 0.001
OHAS	43.73 (0.02)	76.64 (1.53	67.14 (1.47)	
Insulin	10.87 (0.01)	16.46 (1.35)	19.53 (1.39)	
OHAS + Insulin	6.04 (0.00)	6.90 (0.90)	13.32 (1.18)	

BMI, Body Mass Index; ACR, albumin-creatinine ratio; eGFR, estimated glomerular filtration rate; CRP, C-reaction protein; LDH, lactate dehydrogenase; LDL, low density lipoprotein; HDL, high density lipoprotein; OHAS, Oral hypoglycaemic agents; CKD, chronic kidney disease; T2D, type 2 diabetes.

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TABLE 2 Baseline clinical features of enrolled T2D patients with various LDH levels.

Variable	Total	Lower LDH (<134U/L) (N1 = 2452)	Higer LDH (≥134U/L) (N2 = 2436)	P-value
LDH (U/L)	138.38 (0.89)	114.74 (0.41)	163.15 (1.09)	< 0.001
Age (years)	59.59 (0.32)	58.32 (0.38)	60.93 (0.46)	< 0.001
BMI (kg/m^2)	33.16 (0.18)	32.35 (0.20)	34.00 (0.29)	< 0.001
Hemoglobin (g/dL)	14.00 (0.03)	14.07 (0.04)	13.92 (0.05)	0.01
Serum albumin (g/L)	41.38 (0.08)	41.80 (0.09)	40.93 (0.14)	< 0.001
Uric acid (μmol/L)	341.41 (2.06)	336.93 (2.41)	346.10 (3.14)	0.02
Total cholesterol (mmol/L)	4.76 (0.02)	4.72 (0.03)	4.79 (0.04)	0.16
Triglyceride (mmol/L)	2.22 (0.04)	2.26 (0.05)	2.18 (0.07)	0.35
HDL (mmol/L)	1.23 (0.01)	1.19 (0.01)	1.27 (0.01)	< 0.001
LDL (mmol/L)	2.72 (0.03)	2.73 (0.04)	2.71 (0.04)	0.75
CRP	0.57 (0.04)	0.56 (0.04)	0.58 (0.06)	0.58
eGFR (ml/min/1.73m (2))	83.60 (0.44)	86.89 (0.59)	80.15 (0.65)	< 0.001
ACR (mg/g)	110.28 (8.45)	80.19 (10.97)	142.07 (12.68)	< 0.001
HbA1c (%)	7.08 (0.03)	7.08 (0.05)	7.08 (0.04)	0.93
Sex (%)				< 0.001
Female	48.43 (0.02)	45.10 (1.49)	51.92 (1.47)	
Male	51.57 (0.02)	54.90 (1.49)	48.08 (1.47)	
Race (%)				< 0.001
Mexican American	10.30 (0.01)	11.47 (1.55)	9.08 (1.08)	
Non-Hispanic Black	13.61 (0.01)	11.18 (1.01)	16.16 (1.45)	
Non-Hispanic White	60.17 (0.03)	60.97 (2.18)	59.33 (2.01)	
Other Hispanic	6.24 (0.01)	6.86 (0.81)	5.59 (0.64)	
Other Race - Including Multi-Racial	9.67 (0.01)	9.51 (0.91)	9.84 (0.87)	
Hypertension (%)				< 0.001
No	29.70 (0.01)	33.96 (1.51)	25.24 (1.17)	
Yes	70.30 (0.02)	66.04 (1.51)	74.76 (1.17)	
Alcohol user (%)				0.17
No	13.19 (0.01)	13.95 (1.01)	15.79 (0.95)	
Yes	75.81 (0.03)	86.05 (1.01)	84.21 (0.95)	
Smoke (%)				0.22
No	50.04 (0.02)	48.95 (1.25)	51.37 (1.50)	
Yes	49.78 (0.02)	51.05 (1.25)	48.63 (1.50)	
Antidiabetic drugs (%)				< 0.001
OHAS	43.73 (0.02)	76.64 (1.53)	67.14 (1.47)	
Insulin	10.87 (0.01)	16.46 (1.35)	19.53 (1.39)	
OHAS + Insulin	6.04 (0.00)	6.90 (0.90)	13.32 (1.18)	

BMI, Body Mass Index; ACR, albumin-creatinine ratio; eGFR, estimated glomerular filtration rate; CRP, C-reaction protein; LDH, lactate dehydrogenase; LDL, low density lipoprotein; HDL, high density lipoprotein; OHAS, Oral hypoglycaemic agents; CKD, chronic kidney disease; T2D, type 2 diabetes.

TABLE 3 Relationship between LDH and clinical indicators in T2D patients.

Variables	Adjusted		
	β	95%CI	p value
Hemoglobin	0.00	0.00 (0.00, 0.00)	0.78
Serum albumin	-0.01	(-0.02, -0.01)	<0.001
Uric acid	0.10	(-0.03, 0.22)	0.14
Total cholesterol	0.00	(0.00, 0.00)	0.01
Triglyceride	0.00	(0.00, 0.01)	0.52
HDL	0.00	(0.00, 0.00)	<0.001
LDL	0.00	(0.00, 0.00)	0.30
CRP	0.00	(0.00, 0.00)	0.13
ACR	1.48	(0.77, 2.18)	<0.001
eGFR	-0.06	(-0.08,-0.03)	<0.001

Adjust for age (<65,≥65), sex ('Female', 'Male'), race, BMI. ACR, albumin-creatinine ratio; eGFR, estimated glomerular filtration rate; BMI, Body Mass Index;LDL, low density lipoprotein; HDL, high density lipoprotein; CRP, C-reaction protein, T2D, type 2 diabetes.

results indicated that the risk of DKD in T2D patients with higher LDH was 45% higher than those with lower LDH (OR: 1.45; 95% CI, 1.11-1.89, P=0.01) (Figure 3; Supplementary Tables 1, 2). Furthermore, each standard deviation (SD) increase in LDH was associated with a 24% increase in the risk of DKD (OR=1.24; 95% CI, 1.07-1.44, P=0.005) (Figure 3; Supplementary Tables 1, 2). Stratified analyses showed that the association between LDH and the risk of DKD in patients with T2D varied by age ('<60','≥60' years), sex ('male,' 'female'), serum albumin ('<35','≥35' g/L), anemia ('yes'. 'No'), and HbA1c ('<7.0', '≥7.0') were consistent (all P>0.05) (Figure 4).

4 Discussion

In this study, we found that LDH was associated with the conventional markers of kidney injury, ACR and eGFR. With increasing levels of LDH, the risk of DKD in T2D patients increased, and higher levels of LDH were an independent risk factor for the risk of DKD in T2D patients.

LDH is an enzyme involved in glycolysis, and there are four isozymes in the human genome: LDHA, LDHB, LDHC, and LDHD. Among these isozymes, LDHA, LDHB and LDHC are L isomers, whereas LDHD is a D isomer (6). The expression of LDH increases with age, and it plays a crucial role in promoting glycolysis by converting pyruvate to lactate and NADH to NAD (21). Numerous studies showed that LDH is closely related to the occurrence, development and prognosis of various tumors. For example, it has been found that elevated expression of LDHA was positively correlated with tumor size, clinical stage, and histological grade, and that its elevated expression was associated with poor prognosis in cancer patients (22, 23). In addition, LDH, as a target of many oncogenes and tumor factors, has shown potential as a therapeutic target for cancer (24).

Moreover, the role of LDH in kidney disease-related mortality has also been reported. Ryu SY et al (25) showed that LDH levels above 280 U/L were positively associated with increased all-cause mortality and cardiovascular mortality in hemodialysis patients, while levels below 240 U/L were associated with improved survival. Shen J et al. (26) found that elevated serum LDH levels were associated to shorter overall survival in renal cell carcinoma patients, suggesting LDH as a valuable biomarker for monitoring prognosis. Zhang D et al (27) found an independent association between LDH levels and in-hospital mortality in patients with acute kidney injury (AKI), where mortality increased with higher LDH levels. Similarly, Xiao X et al. found that high LDH levels were associated with an increased risk of cardiovascular mortality in patients with DKD (28).

Some studies have also found associations between LDH and disease-induced kidney involvement. Cai X et al. (29) demonstrated a positive correlation between LDH and albuminuria severity in hypertensive patients, highlighting high LDH levels as an early marker for increased risk of kidney involvement. Zager RA et al. (30) demonstrated that LDH was released from cells during injury, making it a potential marker for renal injury. In addition, LDH has

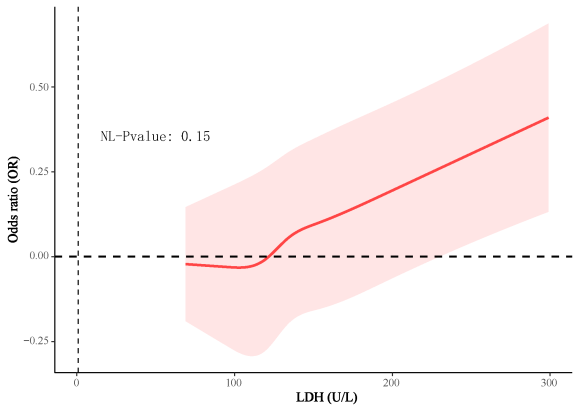

FIGURE 2
The dose-effect relationship between LDH levels and the risk of DKD in patients with T2D.

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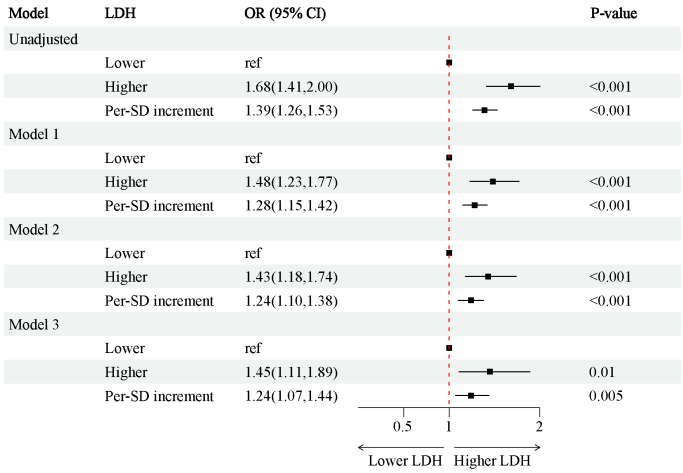

FIGURE 3
Associations between SII and the risk of CKD in individuals. Model 1[a] adjusted for baseline age, gender, race; Model 2[b] adjusted for covariates in model 1 plus alcohol user ('yes' or 'no'), smoke ('yes' or 'no'). Model 3[c] adjusted for adjusted for covariates in model 2 plus hypertension ('yes' or 'no'), hemoglobin, serum albumin, uric acid. OR, odds ratio; CI, Confidence interval; BMI, Body Mass Index; CRP, C-reaction protein; LDH, lactate dehydrogenase; LDL, low density lipoprotein; HDL, high density lipoprotein; CKD, chronic kidney disease; T2D, type 2 diabetes.

been considered as a biomarker to predict acute kidney injury in patients with rhabdomyolysis, sickle cell anemia and non-Hodgkin's lymphoma (31–34). These findings are consistent with that of our study, which showed a significant association between LDH and proteinuria as well as eGFR in patients with T2DM.

In the status of DM, serum LDH levels can be used as a reference marker for short-term glucose monitoring. Changes in serum glycated albumin (GA) and abnormal elevations in LDH levels often occurred simultaneously with the glycemic changes. Therefore, the use of LDH as a biomarker can be more convenient and rapid in assessing glycemic changes in patients with T2D (35). In addition, studies have shown a close relationship between LDH and DKD. Azushima K et al. (36) found that LDH isoenzymes, especially LDHA and LDHB isoenzymes, were increased in patients

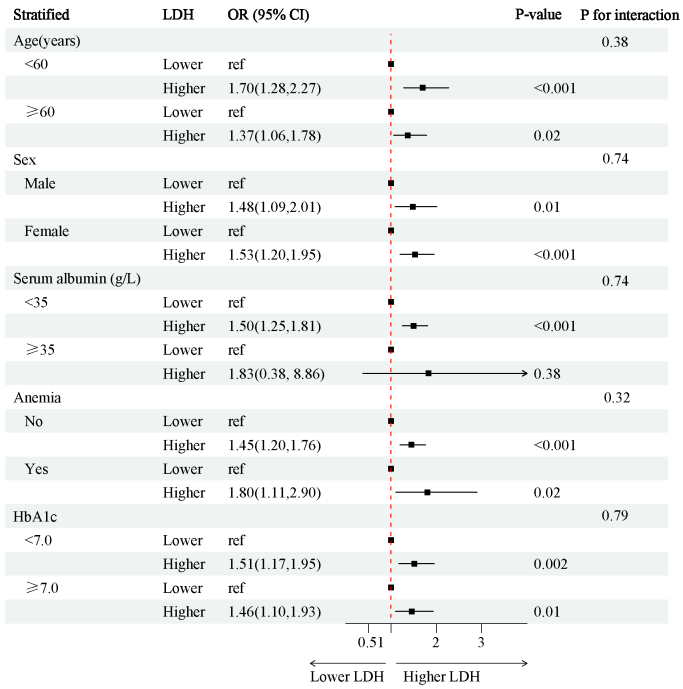

FIGURE 4
Stratified analysis of the risk of the CKD in individuals with T2D. Adjust for age, sex ('Female', 'Male'), race, BMI. OR, odds ratio; CI, confidence interval; BMI, Body Mass Index; T2D, type 2 diabetes.

with DKD. Uslu S et al. (37) found that a significant positive correlation between urinary LDH activity and serum creatinine (Cr) levels, and when creatinine clearance (CCr) decreased, urinary LDH activity began to increase, which suggested that measurement of urinary LDH activity could be used as a screening marker for glomerular and tubular dysfunction in diabetic patients during follow-up. And Jung K et al. (38) found that renal tubular dysfunction can be used as an early indicator of early DKD. These suggested that high LDH levels are associated with early renal function decline in diabetic patients. Xiao X et al (28) found that the risk of progression to ESRD was higher in DKD patients with high levels of LDH. However, few studies have investigated the association between LDH and the risk of DKD in patients with T2D. The association was evidenced in our study, where the risk of DKD in adult T2D patients in the United States increased with elevated LDH, and higher LDH levels were an independent risk factor for the risk of DKD in patients with T2D.

However, why the risk of DKD development is higher in T2D patients with higher LDH needs to be further explored. Firstly, LDH is a catalytic enzyme for lactate formation, and lactate production can contribute to the progression of kidney disease. Lee DY et al. (16) suggested that LDHA-mediated lactic acidosis may be associated with renal failure and fibrosis. Azushima K et al. (36) also conducted targeted metabolomics analysis in a mouse model of DKD and found that LDHA and LDHB isoforms in the DKD mouse was significantly increased in kidneys, and elevated lactate levels and impaired energy metabolism may lead to renal injury in mice. Secondly, Yu SL et al. (39) found that high serum LDH levels were associated with systemic inflammatory responses, and the development of DKD involves inflammatory processes (40). Finally, LDH may influence the lactylation of proteins and consequently the progression of kidney disease (18). Zhang D et al. (41) inhibited lactate production by suppressing LDH activity, and found that intracellular lactate levels and histone lactylation were reduced, and that LDHA-deficient cells had decreased levels of both lactate production and histone lactation levels. Li X et al. (42) found that high lactate concentration and lactylation levels affect energy metabolism, which in turn serves as a predictor of renal injury.

In addition, some of the studies showed that the use of antidiabetic drugs may also affect LDH levels (43). Metformin was shown to decrease LDH activity in cardiomyocytes as well as serum LDH levels (44). In addition, cagliflozin decreased serum LDH levels in diabetic myocardial injury mice. Similar results were observed for other SGLT2i (45, 46). GLP-1/GIP dual agonist has also been shown to decrease serum LDH levels (47). These findings suggest that glucose-lowering drugs may effect LDH levels, but the mechanism is not clear. However, we additionally corrected for the variable of glucose-lowering drug classification in a multivariate logstic regression analysis, which showed that glucose-lowering drug classification did not affect the predictive value of LDH for the risk of CKD in patients with T2DM. Moreover, besides SGLT2i, some studies showed that other renoprotective drugs may also affect LDH (48). Perindopril decreased LDH in serum of mice with diabetic myocardial injury (48). Zahler et al. (49) also found that angiotensin-converting enzyme inhibition (ACEI) attenuated myocardial injury as well as serum levels of LDH. In addition, Ibrahim MA et al. (50) also showed that ACEI and angiotensin AT (1)-receptor antagonism in ameliorating adriamycin-induced cardiotoxicity and nephrotoxicity decreased the level of LDH. However, non-selective saline corticotropin receptor antagonists have not yet been reported to have an effect on LDH. In view of the fact that all these nephroprotective drugs can lower LDH, it is also suggested that LDH plays an important role in kidney injury and hence it can be used to screen patients with DKD in T2DM.

However, we also need to be aware of some limitations of the study. Firstly, due to cross-sectional design, we were unable to establish a cause-effect relationship between LDH levels and the risk of DKD in patients with T2D. We recommend future longer-term follow-up studies to determine the predictive ability of LDH for the occurrence of DKD. Secondly, there was some selection bias due to the retrospective character of the study. Thirdly, although we controlled for some factors that may have influenced the results, there are still some other factors that may have had an impact on the results. Finally, further research is needed on the possible relationship between variations in LDH concentrations and the development of DKD in patients with T2D.

In conclusion, our findings suggest that higher LDH levels were associated with the risk of DKD in T2D patients. It will be necessary for clinicians to monitor LDH levels in patients with T2D, which can assist in screening for DKD in patients with T2D.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The study protocol conformed to the ethical standards of the 1964 Declaration of Helsinki and its subsequent amendments and was approved by the National Ethics Review Board for Health Statistics Research. All participants had signed an informed consent form. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

XX: Writing – review & editing, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization. LT: Writing – original draft, Formal analysis, Resources, Software, Validation. QY:

Writing – original draft, Conceptualization, Data curation, Methodology. RM: Writing – original draft. PZ: Writing – original draft. CP: Writing – original draft. CX: Writing – original draft. QL: Writing – original draft. TW: Writing – original draft. WG: Writing – original draft. HY: Writing – original draft. GD: Writing – original draft. ZD: Writing – original draft. NM: Writing – original draft, Supervision, Validation.

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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.2024.1369968/full#supplementary-material

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Diagnostic value of retinol-binding protein 4 in diabetic nephropathy: a systematic review and meta-analysis

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Objective: Diabetic nephropathy (DN) is a major microvascular complication of diabetes and the leading cause of end-stage renal disease. Early detection and prevention of DN are important. Retinol-binding protein 4 (RBP4) has been considered as a single diagnostic marker for the detection of renal impairment. However, the results have been inconsistent. The present meta-analysis aimed to determine the diagnostic potential of RBP4 in patients in type 2 diabetes mellitus (T2DM) with DN.

Methods: We searched PubMed, Web of Science, Embase, Wanfang and CNKI databases from inception until January 2024. The meta-analysis was performed by Stata version 15.0, and sensitivity, specificity, positive and negative likelihood ratios (PLR and NLR), diagnostic odds ratio (DOR) and area under the curve (AUC) were pooled. The Quality Assessment of Diagnostic Accuracy Studies-2 tool was utilized to assess the quality of each included study. In addition, heterogeneity and publication bias were evaluated.

Results: Twenty-nine studies were included in the meta-analysis. The pooled sensitivity and specificity were 0.76 [95% confidence interval (CI), 0.71–0.80] and 0.81 (95% CI, 0.76–0.85), respectively. The results showed a pooled PLR of 4.06 (95% CI, 3.16–5.21), NLR of 0.29 (95% CI, 0.24–0.36) and DOR of 13.76 (95% CI, 9.29–20.37). The area under the summarized receiver operating characteristic curve was given a value of 0.85 (95% CI, 0.82–0.88). No obvious publication bias existed in the Deeks' funnel plot asymmetry test.

Conclusion: Our findings suggest that RBP4 has a promising diagnostic value with good sensitivity and specificity for patients with T2DM with DN.

KEYWORDS

retinol-binding protein 4, diabetic nephropathy, diagnosis, biomarkers, meta-analysis

Introduction

Diabetic nephropathy (DN) is a leading cause of morbidity and mortality among patients with type 2 diabetes mellitus (T2DM). It is characterized by increased glomerular filtration rate (GFR) with intraglomerular hypertension and clinically progressive albuminuria, followed by eventual loss of renal function (1). Changes in GFR or albuminuria are currently considered hallmarks of onset or progression of DN. However, the levels of estimated GFR (eGFR) or urinary albumin are in the normal range in some patients with early stage DN, which suggests that eGFR or albuminuria is not a suitable marker for early diagnosis of DN. This has motivated researchers to consider potential novel diagnostic biomarkers (2).

Retinol-binding protein 4 (RBP4) is an adipokine that belongs to the lipocalin superfamily, binds specifically to vitamin A, transports small hydrophobic molecules and is generated mainly in the liver and mature fat cells (20%–40%) (3). Several studies have shown that RBP4 is closely associated with obesity in diabetic patients, insulin resistance (IR), renal impairment and cardiometabolic indices (4, 5). Previous research has indicated that RBP4 influences insulin-responsive glucose transporter-4 in adipocytes, which is related to insulin sensitivity (6, 7). Elevated serum RBP4 levels are high in patients with T2DM, IR and impaired glucose tolerance (8, 9). Serum RBP4 concentrations are also correlated with changes in eGFR and serum creatinine, demonstrating its correlation with renal function (10). As a result of the low molecular weight (21 kDa) of RBP4, it is freely filtered through the glomeruli and then almost entirely reabsorbed in the proximal tubules, making urinary RBP4 an effective marker of small changes in proximal tubule function (11, 12). RBP4 is present before the increase of other markers such as proteinuria and serum creatinine (4, 13). Most previous studies have revealed a positive relationship between RBP4 and renal dysfunction markers such as albuminuria (4, 14, 15). However, the results remain inconsistent (16). Thus, our meta-analysis aimed to assess the diagnostic value of RBP4 as a biomarker for early detection of DN in patients with T2DM.

Materials and methods

Literature search

Two independent reviewers (TJ and WH) searched PubMed, Web of Science, Embase, Wanfang and CNKI databases from inception until January 2024. The study type was not restricted. The terms of our search were as follows: ("Diabetic Nephropathy" OR "Diabetic Kidney Disease" OR "Diabetic Nephropathies" OR "Diabetes Mellitus" OR "Type 2 Diabetic" and "Nephropathy", then combined these items using AND with "Retinol-binding protein 4" OR "RBP4" AND ("diagnosis" OR "classification" OR "discriminate") AND ("accuracy" OR "sensitivity" OR "specificity" OR "area under the curve"). This meta-analysis followed the PRISMA statement of preferred reporting items for systematic evaluation and meta-analysis.

Criteria for study inclusion and exclusion

The study inclusion criteria were as follows: (1) diagnostic study; (2) T2DM patients with or without DN; (3) availability of indexes containing true positive (TP), false positive (FP), false negative (FN) and true negative (TN) values; and (4) inclusion of diagnostic cut-off values for RBP4. Exclusion criteria were: (1) reviews, letters, conference abstracts or animal studies; (2) studies with duplicate data; and (3) failure to extract four-cell table data. XC and JW selected the studies independently according to the above criteria. If there were disagreements among the reviewers, a joint consultation was held with a third reviewer (BS) for verification.

Literature quality assessment

Two independent researchers (BS and RW) completed the quality assessment of included studies using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2). Items assessed contained two categories of risk of bias and applicability concerns. Patient selection, index test, reference standard, flow and timing were assessed for risk of bias, and the first three items were also assessed for applicability concerns. According to the answers to the landmark issues included in each section of yes, no or uncertain, the bias risk was judged as low, high or uncertain.

Data extraction

Key variables from each study included: first author, publication year, country of origin sample source, number of participants, TP, FP, FN, TN, cut-off values and diagnostic criteria for DN. The sources of heterogeneity were discovered by meta-regression analysis with sample source (serum or urine), region (China or not), diagnostic criteria [albumin/creatinine ratio (ACR) or others], bias risk for index test (bias or no bias), study design (cross-sectional or case–control study) and sample size (>200 or ≤200) as independent variables. Data extraction was accomplished independently by two investigators (XC and JW). Disagreements were discussed and resolved by consensus.

Statistical analysis

Data from the selected studies were reconstructed in 2 × 2 tables (TP, FN, FP, TN), and their sensitivity and specificity were calculated. The diagnostic meta-analyses were conducted using Stata version 15.0 software, with pooled effect sizes containing specificity, sensitivity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR) and area under the curve (AUC) with their 95% confidence intervals (CIs). The "MIDAS" module was used for synthesizing the data to explore the combined sensitivity and specificity and their 95% CI. The summary ROC (SROC) was used for calculating the AUC of the

diagnostic value. Heterogeneity was evaluated statistically by the Cochran Q test and I^2 statistics. If P was <0.05 or I^2>50%, the data were analyzed in a random-effects model. Otherwise, a fixed-effects model was used. The sources of heterogeneity were analyzed using meta-regression. Sensitivity analysis was conducted to assess the robustness of the meta-analysis. Fagan's nomogram was performed to further estimate the diagnostic efficacy of RBP4. The publication bias was assessed using Deeks' funnel plot asymmetry test, and P<0.05 was considered statistically significant.

Results

Characteristics of the included studies

The search strategy yielded 336 publications according to the eligibility criteria, among which 97 were duplicates. After screening title or abstracts, we excluded 99 because they were reviews or covered irrelevant topics. Of the 140 remaining articles, 111 were excluded after full-text evaluation, including 79 without sensitivity and specificity, 11 without available groups, 12 without cut-off values and nine animal studies. Finally, 29 articles were included, providing data on 2849 samples in the DN group and 2700 controls. The detailed screening process is shown in Figure 1. Among the 29 articles, two were published in English (4, 14) and the other 27 in Chinese (17–43). Serum or urine samples were collected from patients for RBP4 detection. The included patients were diagnosed with DN according to estimated glomerular filtration rate (eGFR), ACR and albumin excretion rate (AER) values. The main characteristics of the articles included in the meta-analysis are listed in Table 1.

QUADAS–2 scores

The bias risk assessment of the included studies is described in Figure 2. In terms of reference standards and flow and timing, all the included studies had a low risk of bias. However, there were several case–control comparative studies and the corresponding bias risk was high. The bias risk of 16 enrolled studies for index test was judged as high because the threshold was not prespecified. With regard to applicability concerns, the matching degree of all studies and evaluation questions were high.

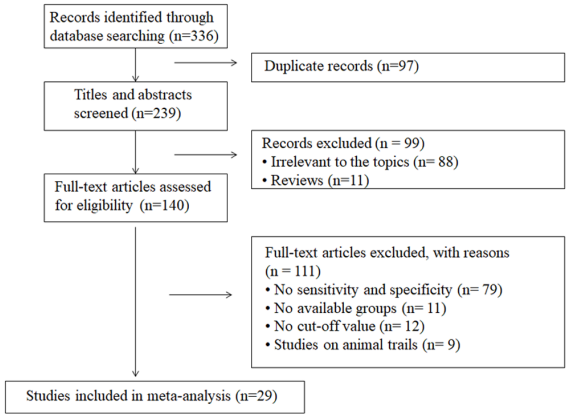

FIGURE 1
Flow diagram of literature selection.

Meta–analysis

The pooled diagnostic accuracy demonstrated the diagnostic value of RBP4 in T2DM with DN. The pooled sensitivity and specificity were 0.76 (95% CI, 0.71–0.80) and 0.81 (95% CI, 0.76–0.85), respectively (Figure 3). The heterogeneity was significant in the pooled analysis of sensitivity (I^2 = 88.41, P<0.001) and specificity (I^2 =84.77, P<0.001). The pooled PLR was 4.06 (95% CI, 3.16–5.21) with significant heterogeneity (I^2 = 80.60, P<0.001), and the pooled NLR was 0.29 (95% CI, 0.24–0.36) with significant heterogeneity (I^2 = 89.74, P<0.001) (Figure 4). The pooled DOR was 13.76 (95% CI, 9.29–20.37), with significant heterogeneity (I^2 = 100, P<0.001) (Figure 5). Additionally, the pooled summarized receiver operating characteristic (SROC) curve was calculated by sensitivity against (1 – specificity), and the AUC was 0.85 (95% CI, 0.82–0.88), revealing a high overall accuracy of RBP4 for T2DM with DN (Figure 6). The high diagnostic efficacy of RBP4 was confirmed by Fagan's nomogram, with 50% and 7% for positive and negative post-test probability, respectively, when the pretest probability was set at 0.2 (Figure 7).

Meta regression and sensitivity analyses

We performed meta-regression analysis with sample source, region, diagnostic criteria, bias risk for index test, study design and sample size as independent variables to explore the sources of heterogeneity (Figure 8). For sensitivity, six independent variables, sample source, region, diagnostic criteria, bias risk for index test, study design and sample size were statistically significant. For specificity, four independent variables, diagnostic criteria, bias risk for index test, study design and sample size were statistically significant. The results indicated that sample source, region, diagnostic criteria, bias risk for index test, study design and sample size were sources of heterogeneity.

The results of the sensitivity analysis are shown in Figure 9. The goodness of fit (Figure 9A) and bivariate normality (Figure 9B) indicated that the random-effects model was applicable. Influence analysis showed that studies of Lu et al. (29) and Qiu et al. (39) were the most dominant studies in weight (Figure 9C). Outlier detection illustrated that heterogeneity might be attributed to the related data of Lu et al. (29) and Qiu et al. (39) (Figure 9D). After excluding the two outlier studies, the I^2 value of heterogeneity was reduced by 1.2% and 4.57% in sensitivity and specificity, respectively. There was no significant change in the pooled results for diagnostic efficacy (Table 2).

Publication bias

No obvious publication bias existed in the Deeks' funnel plot asymmetry test (P=0.06) (Figure 10).

TABLE 1 Characteristics of the included studies.

Author	Year	Region	Sample	Case	Control	TP	FP	FN	TN	Cut-off	Reference for GFR/ACR/AER
Zhao	2024	China	urine	130	97	106	25	24	72	0.7mg/L	ACR
Xu	2023	China	urine	89	98	62	34	27	64	58mg/L	ACR
Lin	2023	China	serum	50	50	39	1	11	49	39.62mg/L	ACR
Qiu	2022	China	urine	56	48	55	24	1	24	2.49mg/L	ACR
Wu	2022	China	serum	42	68	36	13	6	55	70mg/L	AER
Chang	2022	China	serum	199	657	103	100	96	557	50.5 mg/L	ACR
Yang	2022	China	serum	48	50	35	7	13	43	55.97 mg/L	AER
Zeng	2022	China	serum	87	60	63	12	24	48	58.42mg/L	ACR
Gao	2021	China	serum	242	87	142	19	100	68	50mg/L	AER
Tao	2021	China	serum	42	58	33	12	9	46	50mg/mL	AER
Xiang	2020	China	serum	63	65	45	13	18	52	53.88mg/L	ACR
Wang	2020	China	serum	90	90	74	12	16	78	54.28mg/L	ACR
Li	2020	China	urine	46	66	35	13	11	53	70mg/L	AER
Gao	2020	China	serum	99	102	45	20	54	82	45.95mg/L	ACR
Abbasi	2020	Iran	serum	89	44	75	17	14	27	46.1 ng/mg	GFR
Li	2019	China	serum	64	60	43	4	21	56	70mg/L	AER
Wang	2019	China	serum	165	81	120	24	45	57	70mg/L	GFR
Yang	2018	China	serum	127	41	89	10	38	31	70mg/L	AER
Lu	2018	China	urine	150	74	125	0	25	74	3.0mg/L	AER
Shen	2018	China	serum	370	370	223	73	147	297	70mg/L	NR
Chen	2018	China	serum	40	40	34	9	6	31	30.1mg/L	GFR
Kong	2017	China	urine	89	35	76	6	13	29	0.32mg/L	AER
Huang	2017	China	serum	101	47	87	5	14	42	64.2mg/L	ACR
Li	2016	China	urine	32	43	26	15	6	28	0.7mg/L	AER
Mahfouz	2016	Saudi Arabia	serum	100	50	84	5	16	45	24.5 ng/ml	ACR
Xie	2015	China	serum	38	44	24	8	14	36	57.9mg/L	AER
Li	2015	China	serum	95	60	77	3	18	57	40.95mmol/L	ACR
Qiu	2013	China	urine	60	60	30	27	30	33	1.5mg/L	AER
Zhang	2012	China	serum	46	55	41	18	5	37	51mg/L	AER

TP, true positive; FP, false positive; FN, false negative; TN, true negative; NR, not reported; GFR, glomerular filtration rate; ACR, albumin/creatinine ratio; AER, albumin excretion rate.

Discussion

Early detection and prevention of DN, which is the major microvascular complication of DM and the main cause of end-stage renal disease (ESRD), are important (44). RBP4 has been considered as a single diagnostic marker for the detection of renal impairment (4, 14). Several studies have evaluated the relationship between RBP4 levels and early DN in patients with T2DM. Some studies have indicated an increase in serum RBP4 concentrations in T2DM patients with DN (4, 14, 15), and others have found similar RBP4 levels and a correlation with renal function and early DN in T2DM (16). Zhang et al. (45) conducted a meta-analysis to investigate the associations between RBP4 concentration and clinical indices of renal function and albuminuria in patients with T2DM. They demonstrated that RBP4 levels in the micro +macroalbuminuria group were significantly higher than those in the normal albuminuria group of patients with T2DM. The concentration of circulating RBP4 was positively correlated with ACR but negatively with eGFR. To our knowledge, a meta-analysis has not yet been conducted to explore the accuracy of the role of

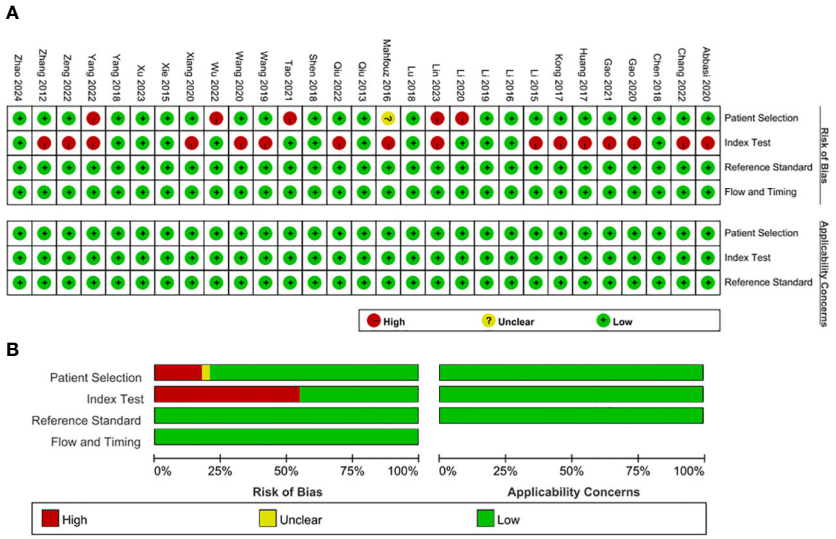

FIGURE 2
Bias risk assessment by Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS–2). **(A)** QUADAS–2 summary plot **(B)** QUADAS–2 bar plot.

RBP-4 in diagnosis of DN in T2DM patients. Hence, we performed this study to evaluate the diagnostic value of RBP4 for early kidney damage in T2DM patients.

This meta-analysis included 29 original articles (5549 patients) with sufficient data for an investigation of the diagnostic accuracy of RBP4 in DN. The pooled sensitivity and specificity of RBP4 were 0.76 (95% CI, 0.71–0.80) and 0.81 (95% CI, 0.76–0.85), respectively. The likelihood ratio was useful for assessing the diagnostic value of the detection method. PLR>10 and NLR<0.1 demonstrated convincing diagnostic potential. The pooled PLR and NLR of RBP4 were 4.06 (95% CI, 3.16–5.21) and 0.29 (95% CI, 0.24–0.36), respectively, indicating that the diagnostic efficacy of RBP4 for DN was still limited. DOR, which combines sensitivity, specificity, PLR and NLR, is used as an independent indicator to determine diagnostic performance. The higher the DOR value, the better the discriminant effect of diagnostic indices. The pooled DOR in this meta-analysis was 13.76 (95% CI, 9.29–20.37), indicating good overall accuracy. The AUC of SROC for RBP4 was 0.85 (95% CI, 0.82–0.88), suggesting that RBP4 has a promising diagnostic accuracy for DN.

Xu et al. (46) investigated the association of serum RBP4 with impaired glucose regulation and microalbuminuria in Chinese adults aged ≥40 years. The results illustrated that serum RBP4

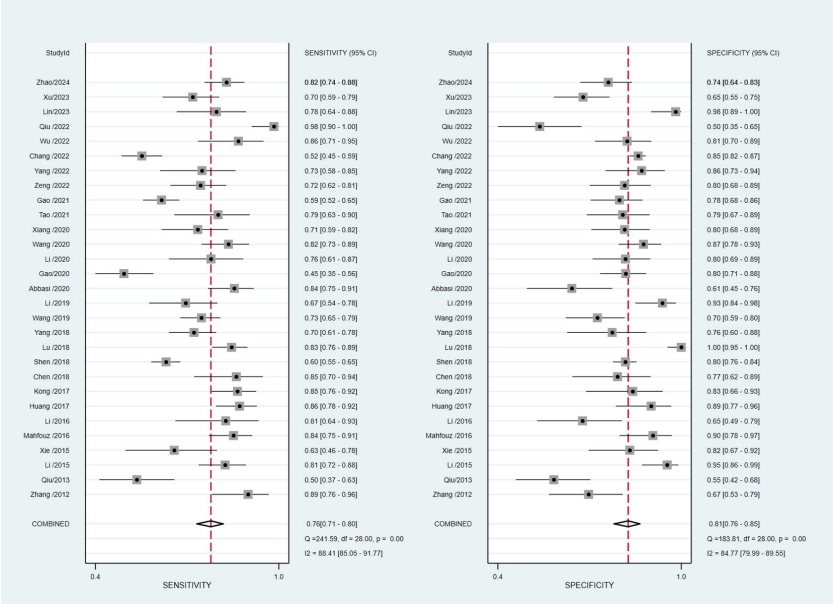

FIGURE 3
Forest plot of pooled sensitivity and specificity.

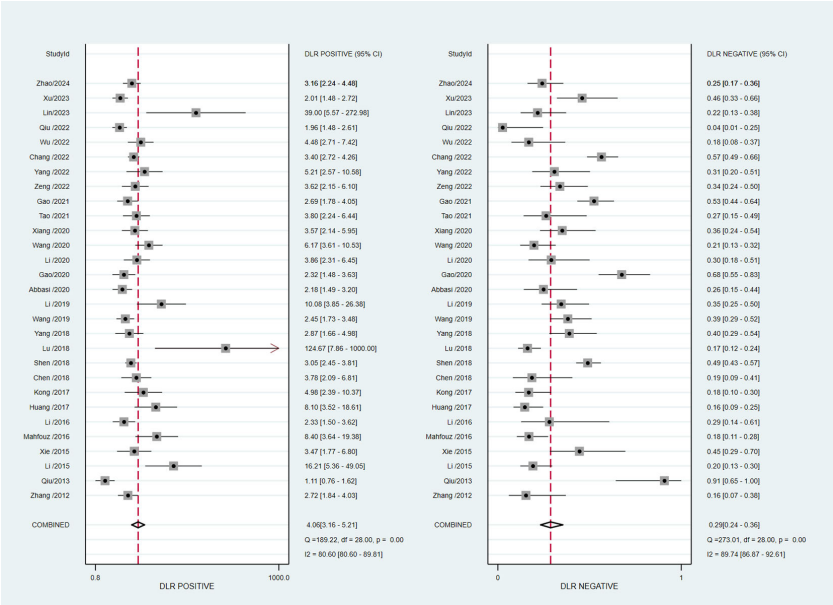

FIGURE 4
Forest plot of pooled positive likelihood ratio (PLR) and negative likelihood ratio (NLR).

level was closely related with impaired glucose regulation and an independent risk factor for microalbuminuria. Chang et al. (47) indicated that serum RBP4 in patients with DM was positively associated with ACR and uric acid but negatively related with eGFR. Multiple stepwise linear regression analysis showed that uric acid and eGFR remained significantly correlated with serum RBP4. Mohamed et al. (14) performed a comparison between the output data of ROC curves for RBP4 and ACR to assess whether RBP4 was more sensitive and specific than ACR. A serum level of RBP4 >24.5 ng/mL predicted the presence of nephropathy with 84% sensitivity, 90% specificity, and AUC=0.912 with 86% accuracy; and urinary ACR >37.5 mg/g creatinine predicted the presence of nephropathy with 89% sensitivity, 72% specificity, and AUC=0.819 with 83.3% accuracy. These studies demonstrated a positive correlation between serum RBP4 and urine ACR and indicated that RBP4 was more specific than ACR for early prediction of DN.

The pathogenic mechanism explaining the differences in RBP4 levels in DM patients with and without renal dysfunction might be

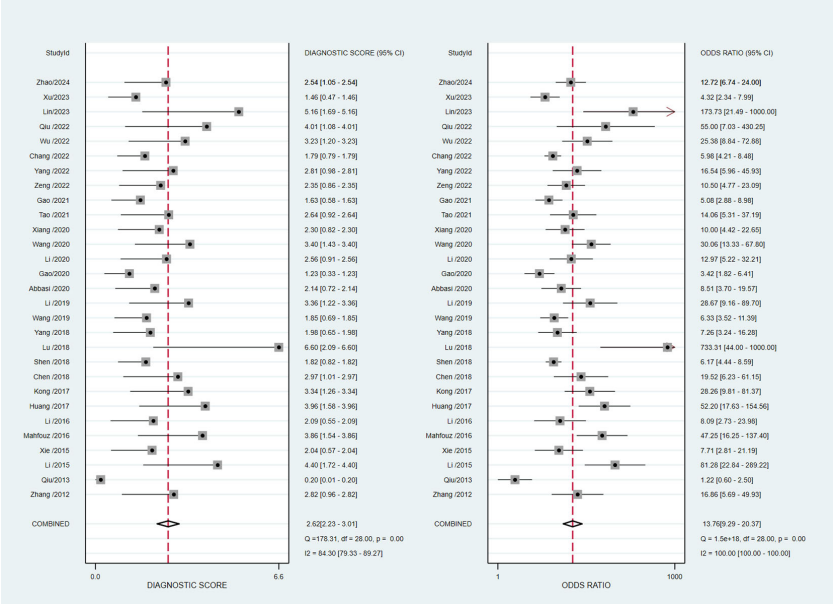

FIGURE 5
Forest plot of pooled diagnostic score and diagnostic odds ratio (DOR).

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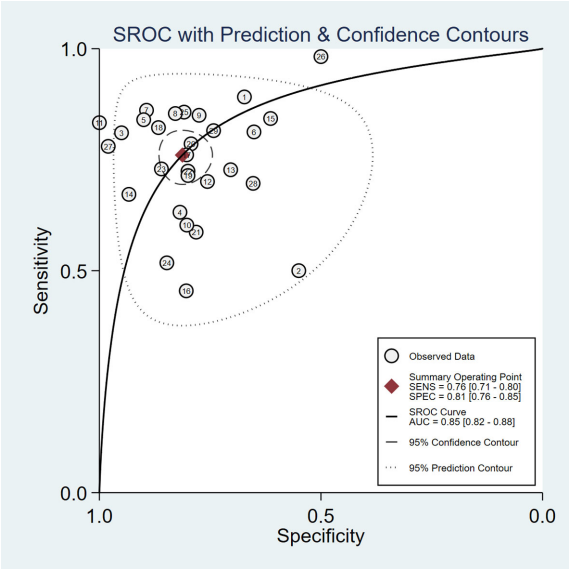

FIGURE 6
Summary receiver operating characteristic (SROC) plots.

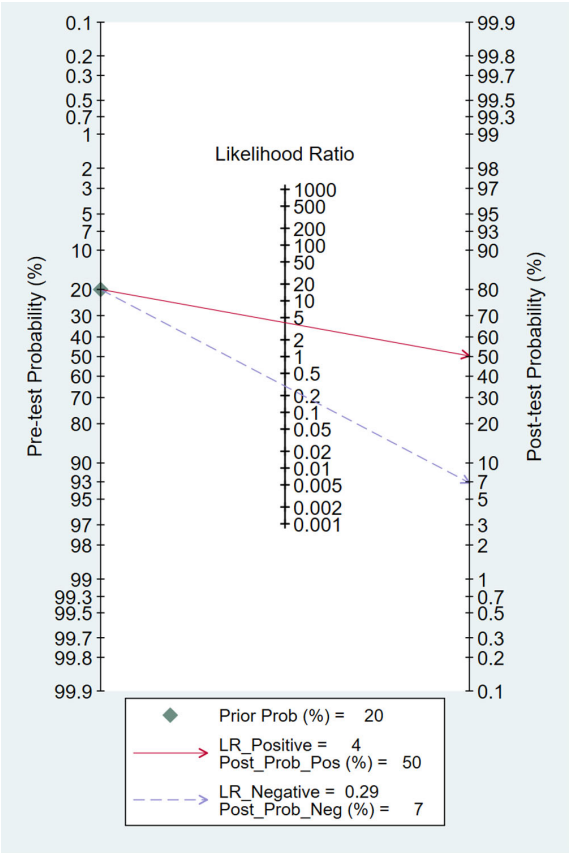

FIGURE 7
Fagan nomogram of retinol-binding protein 4 for the diagnosis of diabetic nephropathy.

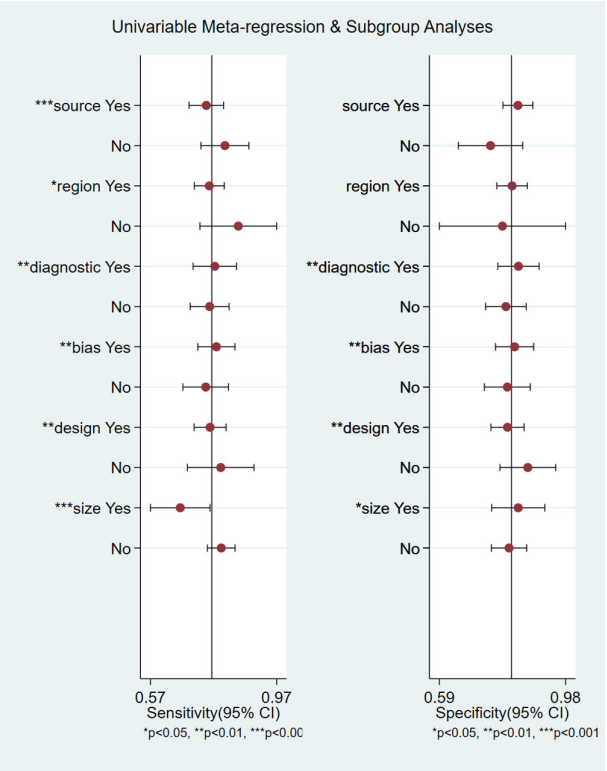

FIGURE 8
Meta-regression analysis for examining sensitivity and specificity of retinol-binding protein 4 for the diagnosis of diabetic nephropathy. *P<0.05, **P<0.01, ***P<0.001.

TABLE 2 Diagnostic performance of RBP4 in DN.

Analysis	Overall	Outliers excluded
No. of studies	29	27
Sen (95% CI)	0.76 (0.71–0.80)	0.74 (0.70–0.79)
Spe (95% CI)	0.81 (0.76–0.85)	0.81 (0.77–0.84)
PLR (95% CI)	4.10 (3.20–5.20)	3.80 (3.10–4.70)
NLR (95% CI)	0.29 (0.24–0.36)	0.32 (0.26–0.38)
DOR (95% CI)	14.0 (9.0–20.0)	12.0 (8.0–17.0)
AUC (95% CI)	0.85 (0.82–0.88)	0.85 (0.81–0.87)

associated with reduced catabolism and IR. First, the kidneys play a critical role in maintenance of retinol homeostasis throughout the body, which is regulated by glomerular filtration and subsequent reabsorption of RBP4 into the proximal tubular tissues (48). Thus, disorder of renal function leads to accumulation of RBP4 in the plasma and hence to higher concentration in patients with DN than in T2DM patients without kidney disease (10). Second, RBP4 is a novel adipokine and increased circulating levels might be associated with deterioration of IR in patients with DN (6). This could result from increased expression of the gluconeogenic enzyme in live cells (mainly phosphoenolpyruvate carboxykinase), inhibition of insulin signaling, impairment of glucose uptake in skeletal muscle, resulting in higher glucose generation by the liver (49, 50).

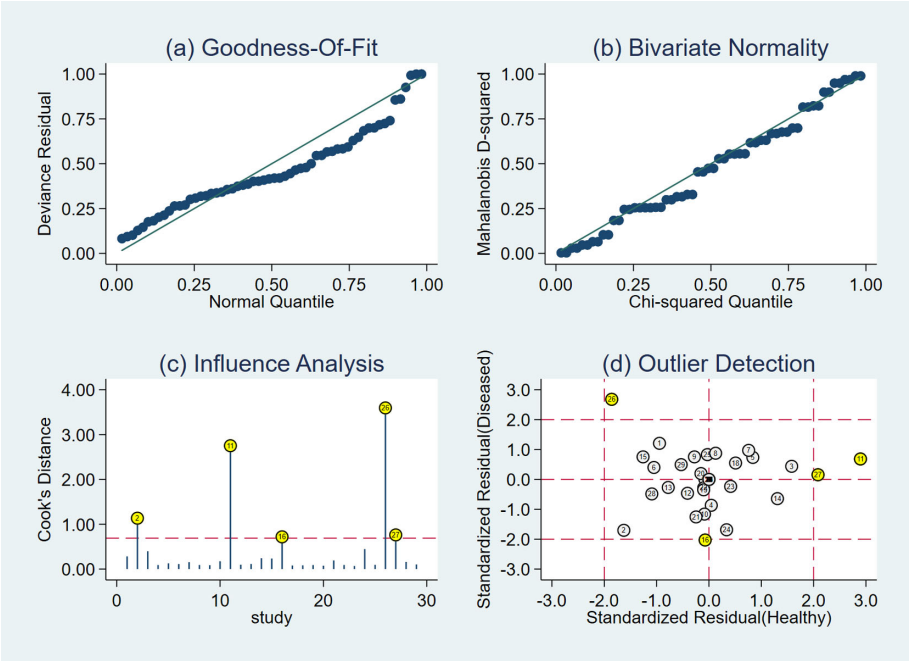

FIGURE 9
Diagram of sensitivity analysis showing **(A)** goodness-of-fit; **(B)** bivariate normality; **(C)** influence analysis; **(D)** outlier detection.

Meta regression analyses suggested that the sample source, region, diagnostic criteria, bias risk for index test, study design and sample size might be the sources of heterogeneity. Higher sensitivity was found in the groups with urine samples, non-Chinese, ACR for detection of DN, bias risk for index test, case–control studies, and sample size ≤200 than in the corresponding groups. There was no significant difference in specificity between studies from serum and urine samples, China and other countries. Publication bias indicated that the findings were stable and reliable.

There were some limitations to the meta-analysis that need to be addressed when interpreting the results. Firstly, although we conducted an extensive literature search, there were no related studies from Europe or America. Secondly, information such as randomization and blindness were not stated in some studies. Thirdly, the heterogeneity in the present meta-analysis was obvious. In addition, some important factors, such as cut-off value and staging of DN were inconsistent among the studies. Therefore, investigation of the diagnostic value of RBP4 as a biomarker for early detection of DN needs a large sample, with blinding and randomization, using a unified detection method for DN staging, so that the authenticity and reliability of the analysis are more clinically meaningful.

In summary, this meta-analysis showed that RBP4 has promising diagnostic value with good sensitivity and specificity for patients with T2DM with DN. Considering the limitations of the present study, more high-quality research is needed to confirm the diagnostic potential of RBP4 in patients with DN.

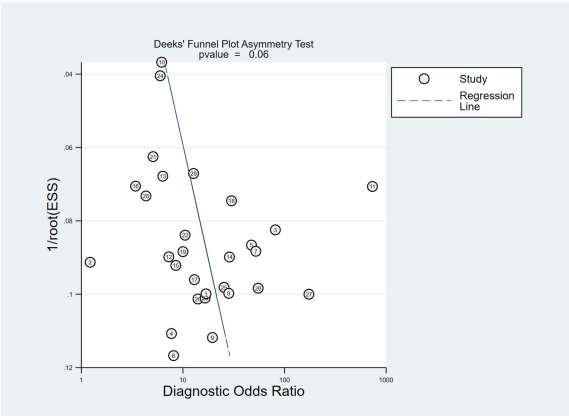

FIGURE 10
Deeks' funnel plot asymmetry test for publication bias.

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.

Author contributions

XC: Writing – original draft, Software, Funding acquisition, Conceptualization. GZ: Writing – review & editing. TJ: Writing – original draft, Investigation. WH: Writing – review & editing, Investigation. JW: Writing – review & editing, Data curation. BS: Writing – review & editing, Methodology. RW: Writing – review & editing, Methodology.

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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.2024.1356131/full#supplementary-material

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Early detection of diabetic neuropathy based on health belief model: a scoping review

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Introduction: Uncontrolled blood sugar levels may result in complications, namely diabetic neuropathy. Diabetic neuropathy is a nerve disorder that causes symptoms of numbness, foot deformity, dry skin, and thickening of the feet. The severity of diabetic neuropathy carries the risk of developing diabetic ulcers and amputation. Early detection of diabetic neuropathy can prevent the risk of diabetic ulcers. The purpose: to identify early detection of diabetic neuropathy based on the health belief model.

Method: This research searched for articles in 6 databases via Scopus, Ebsco, Pubmed, Sage journal, Science Direct, and SpringerLink with the keywords "screening Neuropathy" AND "Detection Neuropathy" AND "Scoring Neuropathy" AND "Diabetic" published in 2019-2023. In this study, articles were identified based on PICO analysis. Researchers used rayyan.AI in the literature selection process and PRISMA Flow-Chart 2020 to record the article filtering process. To identify the risk of bias, researchers used the JBI checklist for diagnostic test accuracy.

Results: This research identified articles through PRISMA Flow-Chart 2020, obtaining 20 articles that discussed early detection of diabetic neuropathy.

Conclusion: This review reports on the importance of early detection of neuropathy for diagnosing neuropathy and determining appropriate management. Neuropathy patients who receive appropriate treatment can prevent the occurrence of diabetic ulcers. The most frequently used neuropathy instruments are the vibration perception threshold (VPT) and questionnaire Michigan Neuropathy Screening Instrument (MNSI). Health workers can combine neuropathy instruments to accurately diagnose neuropathy.

KEYWORDS

diabetes mellitus, neuropathy, early detection of neuropathy, neuropathy instrument, neuropathy examination

Introduction

Diabetes mellitus may cause neuropathy, retinopathy, and nephrotic complications. The increase in the number of diabetes mellitus cases that occur if not managed properly can cause complications, some complications that occur in diabetes mellitus sufferers that occur can significantly affect the decline in the quality of life of diabetes patients so that low quality of life can affect the physical and mental well-being of diabetes patients (1). On the other hand, diabetes mellitus over a long period may be a factor that worsens the condition of heart failure patients (2). For diabetes mellitus patients, diabetic neuropathy is the most common complication in type 2 diabetes mellitus patients. Diabetic neuropathy results in decreased function of the sensory (decreased sensitivity), motor (deformity), and autonomic (callus) nerves (3). The majority of diabetics experience small wounds on the feet that lose sensitivity and develop into diabetic ulcers. Diabetic ulcers can cause infection and foot amputation (4). The health belief model estimates patient attitudes in preventing diabetic neuropathy. The health belief model includes vulnerability, benefits, obstacles, the seriousness of illness, and support received (5).

The incidence of neuropathy in the world reaches 2.4% of the human population, and the prevalence of neuropathy cases increases in old age by 8.0%. Globally, the highest prevalence of neuropathy occurs in the Asian continent. A higher incidence of neuropathy can be found in countries on the Southeast Asian continent, namely Malaysia (54.3%), the Philippines (58.0%) and Indonesia (58.0%) (6). A study showed that 50% of patients aged > 60 years experience neuropathy in the early stages of type 2 diabetes (7). Diabetic who experience complications from diabetic neuropathy in Indonesia reach 54% (8).

Early detection of neuropathy is to establish an early diagnosis of neuropathy and determine patient care. Proper treatment for neuropathy patients can prevent diabetic ulcers (9). Nurses can carry out early detection of neuropathy using neuropathy instruments before the emergence of neuropathy symptoms. Patients who are aware of the signs of neuropathy and carry out appropriate foot care can prevent diabetic ulcers (10). In fact, patients are willing to undergo a neuropathy examination if the patient feels the severity of neuropathy symptoms. Health workers make a diagnosis of neuropathy after clinical signs of neuropathy appear (11).

Based on the explanation above, early detection of neuropathy is carried out to confirm the diagnosis and prevent diabetic ulcers. This research aimed to determine early detection of diabetic neuropathy based on the health belief model.

Methodology

This research used a scoping review approach. The initial stage of this research was identifying problems based on existing phenomena. Next, the researcher determined inclusion and exclusion criteria in literature screening. The researcher compiled the final results based on the literature included in the screening process. Researchers used the PRISMA Flow chart 2020 diagram to document the literature selection process. Researchers conducted literature searches based on 6 databases, namely PubMed, Scopus, Science Direct, Sage Journal, Ebsco and SpringerLink. At the literature search stage, researchers used a combination of the keywords "Screening Neuropathy" AND "Detection Neuropathy" AND "Scoring Neuropathy" AND "Diabetic" in literature published in the last 5 years (2019-2023). Based on the results of the literature search, the researcher downloaded the articles and carried out filtering. Researchers excluded review articles, letters to the editor, subchapters from books, and articles that were incomplete. Researchers carried out literature screening analysis that was explained in the inclusion and exclusion criteria. The literature selection process used Rayyan. AI by inputting literature search results on the website. In the initial stage of literature selection, researchers remove duplicate literature that was detected. Next, select articles based on title, abstract, full text. Documentation of the literature selection process using the PRISMA Flow chart 2020 diagram in Figure 1. Data extraction based on the results of the literature selection, the researcher carried out data extraction including the following: 1. Author and year, 2. Study design, 3. Sample, 4. Variables, 5. Instrument, 6. Intervention, 7. Analysis, 8. Results. Researchers recorded all instruments used in early examination of diabetic neuropathy. The risk of bias assessment in this review uses a critical appraisal checklist that is available from the Joanna Brings Institute (JBI). Researchers used the JBI diagnostic test accuracy checklist to assess the risk of bias across the literature. The JBI diagnostic test accuracy checklist can be used in literature assessments with cross-sectional and case study research designs. Risk bias if an assessment of ≥50% is considered to meet critical assessment criteria (12). The risk of bias results can be seen in Table 1.

Results

In this study, there were 1,061 pieces of literature that were included in the screening process. The researcher identified duplicate literature and removed them. Next, the literature was selected based analysis to obtain the final results of the literature to be reviewed. Based on the results of the selection of literature included in this review, there were 20 pieces of literature. The literature research design was divided into 2 types, namely 18 literatures with a cross sectional study design and 2 literatures with a case control study design. The results of the selection of literature to be reviewed can be seen in the PRISMA Flow Chart 2020 diagram at Figure 1 (32).

Based on the final results of the literature screening, showed that early detection of neuropathy can be done using several methods that will be described as follows Tables 2, 3.

Discussion

The final results of this review were 20 pieces of literature that discussed early detection of neuropathy in diabetes patients.

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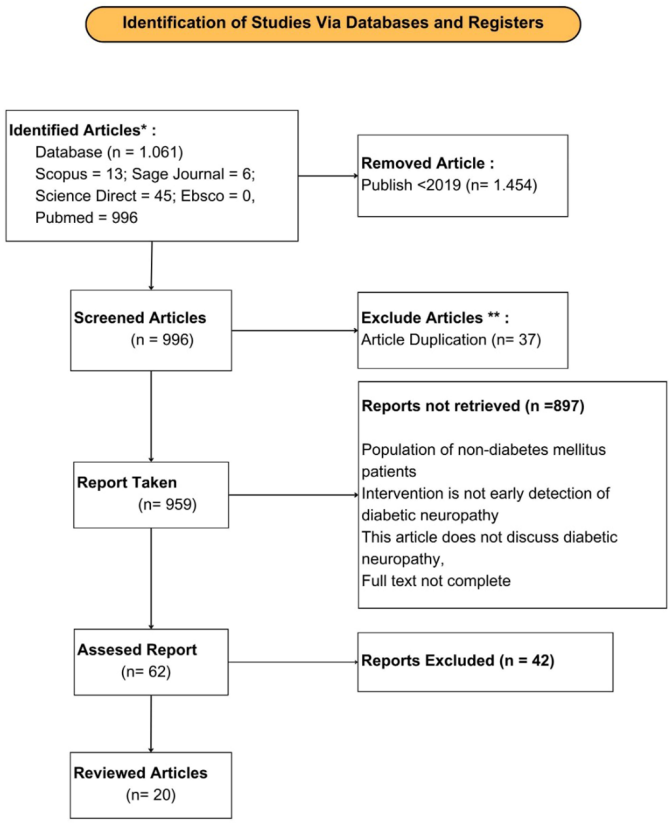

FIGURE 1
PRISMA flow chart 2020 diagram.

TABLE 1 Critical appraisal of eligible diagnostic test accuracy.

Citation	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Total	Note
(13)	1	1	0	0	0	1	1	1	1	1	6	Eligible
(14)	1	1	0	0	0	1	0	1	1	1	6	Eligible
(15)	1	1	0	1	0	0	1	1	0	1	6	Eligible
(16)	0	1	1	1	1	1	1	0	0	1	7	Eligible
(17)	0	1	0	1	1	1	1	1	1	1	8	Eligible
(11)	1	1	1	0	1	1	0	0	1	1	7	Eligible
(18)	1	1	0	0	1	1	1	1	0	1	7	Eligible
(19)	1	1	0	1	1	1	1	0	1	0	7	Eligible
(20)	1	1	0	0	0	1	0	1	1	1	6	Eligible
(21)	1	1	1	1	1	0	1	0	0	1	7	Eligible
(4)	1	0	0	1	1	1	0	1	1	1	6	Eligible
(22)	1	1	1	0	0	1	0	0	1	1	6	Eligible
(23)	1	1	0	1	0	1	1	0	1	1	7	Eligible
(24)	1	0	0	1	1	1	1	1	1	1	8	Eligible
(25)	0	1	1	1	1	0	1	1	1	0	7	Eligible
(26)	1	1	0	1	0	1	0	1	0	1	6	Eligible

(Continued)

TABLE 1 Continued

Citation	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Total	Note
(27)	1	1	0	0	1	1	1	1	1	1	8	Eligible
(28)	1	1	0	1	1	1	1	0	0	1	7	Eligible
(29)	1	1	0	0	1	1	1	1	1	1	8	Eligible
(30)	0	1	0	1	0	1	1	0	1	1	6	Eligible
Percentage	60.0	70.0	15.0	60.0	60.0	85.0	65.0	55.0	65.0	75.0		

(31).

TABLE 2 Journal review.

Author	Study design	Sample	Variable	Instrument	Intervention	Analysis	Results
(13)	Cross-sectional study	34 patients	Independent: Toronto clinical neuropathy score (TCNS) and modified Toronto clinical neuropathy score (m TCNS) Spanish version Dependents: Diabetic polyneuropathy	Toronto clinical neuropathy score Spanish version and modified Toronto clinical neuropathy score Spanish version	Neuropathy examination using Toronto Clinical Neuropathy Score (TCNS) Spanish version.	Cronbach's alpha	The validity test used Cronbach's alpha with a TCNS result of 0.83 and m TCNS of 0.85. P showed that the Spanish version of the TCNS and mTCNS instruments was valid and reliable for use as an instrument for examining diabetic neuropathy.
(14)	Cross-sectional study	625 patients	Independent: Accuracy and Cost-effectiveness of the Diabetic Foot Screen Proforma Dependents: *Diabetic Neuropathy* Detection	Biothesimeter and Diabetic Foot Screen	Measurement of vibration perception threshold (VPT) using biothesimeter and early detection of diabetic foot complications using the Diabetic Foot Screen (DFS)	System 15.0. From ROC analysis and Youden's index	Vibration perception threshold check (VPT) using DFS was ≥1.5 (sensitivity 62%; specificity 76%), indicating diabetic neuropathy. During the examination, the results were obtained: 74.76% (95% CI: 70.46%-79.06%) of patients experienced diabetic neuropathy. It showed that the use of the DFS DNA biothesimeter can detect diabetic neuropathy early and can be applied to health services
(15)	Cross -sectional study	144 orang	Independent: Michigan Neuropathy Screening Instrument Dependents: Diabetic Peripheral Neuropathy Screening	Michigan Neuropathy Screening Instrument (MNSI), SUDOSCAN, 10-g monofilament test.	Diabetic neuropathy examination using the Michigan Neuropathy Screening Instrument (MNSI), SUDOSCAN, 10 g monofilament test.	Mann–Whitney U test: chi-square test, Spearman.	This combination of instruments can be used for optimal examination of diabetic neuropathy
(16)	Cross-sectional study	10.180 patients	Independent: Machine Learning Michigan Neuropathy Screening Instrument Dependents: Diabetic	*Machine Learning* Michigan Neuropathy Screening Instrument based on Machine Learning	Neuropathy detection using MNSI Machine Learning	performance test: ML Algorithms Correlation: Pearson's correlation Significant: ANOVA test Correlation Observations	Michigan Machine Learning-based Machine Learning can be used to measure diabetic neuropathy. MNSI machine learning ranks in the 10th Class of diabetic neuropathy screening

(Continued)

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TABLE 2 Continued

Author	Study design	Sample	Variable	Instrument	Intervention	Analysis	Results
			Sensorimotor Polyneuropathy			and predictions: Cohen's kappa	
(17)	cross-sectional study	156 patients	Independent: Ultrasonography (USG) Dependents: Peripheral neuropathy in type 2 diabetes	ultrasonography, Neuropathy Total Score (TNS), Modified Toronto Clinical Neuropathy Screening (MTCNS)	Examination based on ultrasound results and Neuropathy Total Score (TNS), Modified Toronto Clinical Neuropathy Screening (MTCNS)	Shapiro–Wilk test	Ultrasonography (USG) can examine diabetic neuropathy on peripheral nerve ultrasound.
(11)	cross-sectional survey	574 dokter	Independent: Screening and diagnostics Dependents: diabetic polyneuropathy	tripartite questionnaire.	Neuropathy examination with a tripartite questionnaire.	encompassed descriptive	In health care practice in Germany. Patients can use the tripartite questionnaire to screen for diabetic neuropathy
(18)	Cross-sectional study	69 patients	Independent: Frequency Vibration Perception Thresholds Dependents: Diabetic Neuropathy	Customized vibration exciter	Provides vibration to the 1st metatarsal (MTH1) at a frequency of 30 Hz and the heel at a frequency of 200 Hz	Spearman and Pearson, ANOVA	Custom vibration exciters can be used to examine diabetic neuropathy by measuring the vibration perception threshold (VPT) on the metatarsals and heels
(19)	Cross-sectional study	277 patients	Independent: Small and large fiber sensory polyneuropathy Dependents: neuropathy subtypes	128Hz tuning fork, reflex hammer, and pinprick	Vibration perception threshold (VPT) examination uses a 128Hz tuning fork, ankle reflexes were tested with a hammer reflex, and hypoalgesia and hyperalgesia were tested using a pinprick.	Clopper Pearson method	Diabetic neuropathy can be classified into three types, namely, small fiber neuropathy (SFN), large fiber neuropathy (LFN), and mixed fiber neuropathy (MFN).
(20)	Cross-sectional observational study	48 patients	Independent: Conventional Nerve Conduction Studies Dependent: Sensorimotor Polyneuropathy	Biothesiometer, semmes weinstein monofilament (SWMF), nerve conduction studies (NCS), and Michigan Neuropathy Screening Instrument (MNSI)	Neuropathy measurements using a biothesiometer, Semmes Weinstein monofilament SWMF, nerve conduction studies (NCS), and the Michigan Neuropathy Screening Instrument (MNSI)	Independent t-test/ Wilcoxon Rank -sum test	Measuring neuropathy using biothesiometry, SRA waves can be done to diagnose neuropathy in a shorter time.
(21)	Cross-sectional study	31 patients	Independent: Conduction nerve interdigital sensory Dependents: Initial diagnosis of Diabetic Neuropathy	Electrode diagnostic	Physical neuropathy examination using diagnostic electrodes was carried out on the sensory nerves consisting of the dorsal nerve, medial plantar nerve, and toes I, II, and III. The filter was set at 2 Hz – 10 kHz, with a speed of 2 sweeps and a sensitivity of 10–20 μV	Mann Whitney U test And the Kolmogorov-Smirnov test	The results showed nine respondents experienced nerve conduction study (NCS) disorders, and 22 subjects were normal. interdigital nerve examination results were abnormal in 17 of 22 patients, whereas nerve conduction studies (NCS) were normal
(4)	Cross-sectional study	104 patients	Independent: Shear wave elastography (SWE) and the	shear wave elastography (SWE) and the Toronto	SWE examination on peripheral nerve examination with Ultrasonography and	Evaluated: Mann–Whitney U test	Shear wave elastography (SWE) is an effective tool used to diagnose neuropathy. The combined use of SWE with

(Continued)

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TABLE 2 Continued

Author	Study design	Sample	Variable	Instrument	Intervention	Analysis	Results
			Toronto clinical scoring system (TCSS) Dependents: Diabetic peripheral neuropathy	clinical scoring system (TCSS)	Toronto Clinical Scoring System (TCSS)	Compare: Wilcoxon signed-rank test w Correlation: Spearman correlation	TCSS is an effective parameter for neuropathy screening
(22)	Cross-sectional study	389 patients	Independent: Diagnosis of neuropathy Dependents: Diabetic neuropathy	The Michigan Neuropathy Screening Instrument (MNSI) and Toronto Clinical Neuropathy Scoring System (TCNS) use the 128 HZ tuning fork tool, biothesimeter, and monofilament thread.	Neuropathy examination used the Michigan (MNSI), Toronto Clinical Neuropathy Scoring System (TCNS), a 128 HZ tuning fork, a biothesimeter, and a monofilament thread.	Spearman's rank-order correlation	Early neuropathy examination results were obtained using a questionnaire, and more clinical symptoms of neuropathy were brought compared to neuropathy examinations using only a questionnaire
(23)	Cross-sectional study	153 patients	Independent: Clinical Tools for Peripheral Neuropathy Dependents: Diabetic neuropathy	Neurothesiometer, 10 g semmes-weinstein monofilament, Ipswich touch, DPN Check, Neuropathy Disability Score	Assessment of significant nerve fiber function with neurothesiometer, 10 G Semmes-Weinstein monofilament, Ipswich touch, DPN examination, neuropathy disability score (DNS)	Colmogorov -Smirnov test	Slight nerve fiber function examination with negative results of 97%, sensitivity of 89%, and specificity of 73%. In a study using the vibration perception threshold, a pessimistic prediction of 91%, sensitivity of 62%, and specificity of 75% were obtained.
(24)	cross-sectional study.	83 patients	Independent: Turkish version of the Michigan Neuropathy Screening Instrument Dependents: Diabetic peripheral neuropathy	Michigan Neuropathy Screening Instrument Turkish version and Toronto clinical scoring system	Pemeriksaan neuropati menggunakan Michigan *Neuropathy Screening Instrument* version Turki dan Sistem penilaian klinis Toronto	intraclass correlation coefficient, Cronbach's alpha	The Turkish version of the Michigan Neuropathy Screening Instrument (MNSI) can be used to measure neuropathy symptoms
(25)	Cross sectional study	5088 patients	Independent: Predicting Diabetic Neuropathy Dependents: Artificial Neural Networks and Clinical Parameters	Neurothesimeter	Vibration perception threshold (VPT) measurement using a neurothesimeter	Neural network toolbox on the MATLAB platform	Evaluation of the risk of diabetic neuropathy was carried out using a neurothesimeter and recording the risk factors experienced by the patient. Neurothesimeter examination was categorized into three risks: low at 0-20.99 Volts, medium at 21-30.99 Volts, and high at ≥31 Volts
(26)	Cross sectional study	518 patients	Independent: Artificial intelligence Dependents: Diagnosis of peripheral neuropathy	Artificial intelligence (AI)	Neuropathy examination using Figure cornea identified with AI	Cohen's κ score	The use of artificial intelligence (AI) to detect neuropathy in people with diabetes by examining the cornea can be done to see neuropathy early.
(27)	Cross sectional study	421 patients	Independent: Vibration perception threshold Dependents: Diabetic polyneuropathy	Neurothesimeter	Vibration perception threshold (VPT) measurement using a neurothesimeter	Mann Whitney	The neurothesimeter can be used to examine diabetic neuropathy by measuring the vibration perception threshold (VPT)

(Continued)

TABLE 2 Continued

Author	Study design	Sample	Variable	Instrument	Intervention	Analysis	Results
(28)	Cross sectional study	221 patients	Independent: Detection of peripheral neuropathy Dependents: Type 2 diabetes mellitus patient	Michigan Neuropathy Screening Instrument (MNSI) and electrochemical skin conductance (ESC)	Diabetic neuropathy was measured using the Michigan Neuropathy Screening Instrument (MNSI) and electrochemical skin conductance (ESC) on the patient's hands and feet.	ANOVA test	MNSI and electrochemical skin conductance (ESC) can detect neuropathy in small fiber neuropathy.
(29)	Case control study	60 patients	Independent: Corneal Nerve Plexus Dependents: Diabetic Peripheral Neuropathy	Inspection of early neuropathy diabetes with subbasal nerve plexus (SNP). Inspection done with the method see Rostock Cornea Module (HRT-RCM) and Eye Guidance module (EG) for subbasal nerve plexus (SNP), which indicates neuropathy diabetes.	Rostock Cornea Module (HRT-RCM) and EyeGuidance module (EG)	Mann–Whitney test	Diabetes examination is divided into three categories: corneal nerve fiber length (CNFL; mm/mm2), corneal nerve fiber density (CNFD; no./mm2), corneal nerve branch density (CNBD; no./mm2). Based on this, it showed that in assessing diabetic neuropathy using SNP at an early stage, there were no differences in neuropathy in diabetes mellitus patients.
(30)	Case control study	341 patients	Independent: Neuropathy screening tool Dependents: Diabetic sensorimotor polyneuropathy	Toronto Clinical Neuropathy Score (TCNS)	Neuropathy examination with the Toronto Clinical Neuropathy Score (TCNS)	ANOVA tests	Patient assessment using the Toronto Clinical Neuropathy Score (TCNS). Screening by examining the hand cold detection threshold (CDT), hand warm detection threshold (WDT), foot CDT, and foot WDT. Early detection neuropathy more accurate by clinical symptoms.

Researchers used diabetic neuropathy instruments to carry out early detection of neuropathy. Of the 20 literatures, there were 20 literatures that showed good results in diabetic neuropathy examination. The results of JBI's critical appraisal risk of bias, show that the journals included in this research meet the critical appraisal requirements with an assessment reaching ≥50%. However, in question 3, the assessment was <50%, 3 studies did not include exclusions for samples included in the study, and 3 articles excluded samples because the sample data was empty.

Diabetes Mellitus is a very important health problem in society, the incidence and number of cases of Diabetes Mellitus sufferers has always increased over the past few years (34, 35). Diabetes Mellitus (DM) or diabetes is a heterogeneous group of disorders with typical signs of increased blood glucose levels or hyperglycemia (36). Diabetic patients experience blood glucose resistance for a long time resulting in neuropathy complications. Prolonged high blood glucose levels will result in damage to the blood vessels walls (37). In this condition, the patient's body cannot use the glucose in the blood to convert it into energy due to the accumulation of glucose in the blood (38). Neuropathy may cause damage to sensory, motor, and autonomic nerves. The clinical symptoms felt by diabetes patients are based on the damaged nerves, for example motor neuropathy (deformity), sensory neuropathy (decreased sensitivity), and autonomic neuropathy (callus). To confirm the diagnosis of neuropathy, health workers can carry out early detection of neuropathy (3).

Delay in early diagnosis of neuropathy may cause the severity of neuropathy and development of diabetic ulcers. The length of time a person living with diabetes can provide an idea of the course of the disease and also the person's severity (37). Examination results showed severe neuropathy identify the risk of diabetic ulcers (39). Patients suffering from neuropathy will experience decrease in quality of life because they experience symptoms of neuropathy such as pain, deformity and callus (3).

Diabetic neuropathy examination can be carried out using instruments that are available in health services. However, most neuropathy instruments can only detect after the patient has symptoms of neuropathy. For instance, monofilament instruments can detect neuropathy that has decreased sensitivity in the feet (40), the MNSI questionnaire can identify neuropathy based on signs of neuropathy symptoms felt by diabetics (41).

Vibration perception threshold (VPT): there are 7 studies using different instruments in the vibration perception threshold measurement method, namely biotesimeter (14, 20), neurotesimeter (23) (25) (27), (18) vibratip, 128 Hz tuning fork (19). During the VPT examination, researchers provided vibrations at certain points to detect vibration sensations in the feet of diabetic patients. The vibration range given during the VPT examination was from 1-50V

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TABLE 3 Neuropathy detection based on review.

Type Instrument	Author	Number	Methods examination
Vibration perception threshold or Biothesimeter	(18), (23), (25), (27), (14), (20), (22)	7 articles	VPT is a vibration activated under controlled pressure, there is pressure monitoring and the elasticity of the vibration is electrically controlled in both directions with five indicator lights. VPT adopted this new technology biothesiometer to assess VPT trends in subjects without sensorimotor distal symmetric polyneuropathy and identify age-specific normality thresholds. The voltage is given starting from 0.5 volts. Patients are considered to have neuropathy if they do not feel a voltage of ≥ 25 mV
Michigan Neuropathy Screening Instrument	(33), (15), (20), (22), (24), (28)	6	Interview with questions on the questionnaire with 15 questions about sensory perception. The result if the patient neuropathy, will answer ≥ 7 questions.
Toronto clinical neuropathy score (TCNS)	(30), (13), (17), (4), (22), (24), (30)	7	TCNS is to know level severity with check symptoms and sensitivity in the patient's feet. The tool use Questionnaire Toronto, reflex examination and sensory test score. Questionnaire consist 6 symptoms: Pain, Numbness, tingling, weakness, ataxia, upper limb symptom. Ask patient about present (score 1) or absence (score 0) of symptom. After that reflex examination to knee and ankle reflex result Absence: score 2, Reduce: score 1, Normal: score 0). Sensory Test Score include pinprick, temp, light touch, vibration, position. The result sensory Abnormal (score 1), Normal (score 0). Conclusion TCNS: No neuropathy 0-5 points, Mild neuropathy 6-8 points, Moderate neuropathy 9-11 points, Severe neuropathy 12+ points.
tripartite questionnaire.	(11)	1	This questionnaire is divided into 3 parts: the first part contains participant data, the second part contains the neuropathy examination procedures, and the third part contains questions regarding the examination of pain, sensitivity, and temperature sensation.
128Hz tuning fork, reflex hammer, and pinprick	(19)	1	Inspection done on the instep, Inspect Vibration with 128 Hz tuning fork, sensation temperature cold with tuning fork, sensation puncture needle with monofilament test, Achilles tendon reflex with use patellar hammer.
Electrode diagnostic	(21)	1	The examination uses an electrode with a current of <25 mA with a distance between the electrode and the stimulus depending on the size of the foot of 8-10 cm. Nerve action potential (NAP) was considered absent if it was not recorded at >20 mA indicating neuropathy
shear wave elastography (SWE	(4)	1	Shear wave elastography (SWE) detects neuropathy by looking at images of the nerves in the tibial area which indicates neuropathy if the results show nerve stiffness in the tibial area
10 g semmes-weinstein monofilament	(15), (20), (22), (23)	4	Push monofilament 10 gr thread on point- point specifically on the feet. Ask the patient to close his eyes, The nurse explains that they will check the feet in several places, say "yes" if the patient feels it or if the patient does not feel it. Hold the monofilament to the skin perpendicularly, bending it, and then holding it back perpendicularly for about 1.5 seconds. Examine the plantar toes 1, 3, 5, metatarsal heads of toes 1, 3, 5, medial and lateral arches, heel and dorsum of the foot
Artificial intelligence (AI)	(26)	1	Neuropathy examination with AI using the Heidelberg Retina Tomograph III using the Rostock Corneal Module (RCM) to view the cornea in diabetes patients
electrochemical skin conductance (ESC)	(28)	1	Electrochemical skin conductance (ESC) detects neuropathy using electrodes connected to a computer. Electrodes are attached to the feet and hands, and then connected to a computer. This tool measures the response of skin conductance to electric current given through an electrode and then connects the results to a computer
Rostock Cornea Module (HRT-RCM) and EyeGuidance module (EG)	(29)	1	The examination includes patient demographic data, subsequent examination using an ophthalmological slit lamp and ophthalmoscopy to determine retinopathy, then

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TABLE 3 Continued

Type Instrument	Author	Number	Methods examination
			examination using the Heidelberg Retina Tomograph II equipped with the Rostock Cornea Module (HRT-RCM) and Eye Guidance (EG) to result corneal confocal microscopy (CCM) in quantifying nerve fiber abnormalities in diabetic neuropathy
SUDOSCAN	(15)	1	SUDOSCAN is a test that provides an accurate evaluation of sweat function. The test focuses on small nerve fibers within the peripheral nervous system innervating the sweat glands. The device consists of a computer and 4 electrodes on which patients place their hands and bare feet. In less than 3 minutes, SUDOSCAN offers a stimulation of the sweat glands that assess nerve C fibers.
Neuropathy Total Score (TNS)	(17)	1	In the TNS examination, there are 8 parts: sensory and motor symptoms, pricking sensation, vibration sensation, strength examination, deep tendon reflexes, sural sensory amplitude, and tibial motor amplitude. TNS assessment score 1-4 with a total score of 32. Examination results are categorized into 4: level 0: 0-1, level 1: 2-8, level 2: 9-16, level 3: 17-24, level 4: 25- 32.
Neuropathy Disability Score	(23)	1	NDS examination of small nerve fibers uses pricking sensation and temperature sensation. This examination is considered positive neuropathy if there is damage to one of the 2 examination points in the lower extremity
DPN Check	(23)	1	DPN-Check is used for automatic sural nerve conduction examination. examination results show neuropathy if the amplitude is 4 or the conduction velocity is 40m/s in 1 of the 2 lower extremities
Ipswich touch	(23)	1	Ipswich touch examination of the feet with pressure using the index finger on the 1st, 3rd, and 5th toes of the lower extremities. The results show neuropathy if you don't feel two touches

with the result categories being mild neuropathy, moderate neuropathy and severe neuropathy.

Michigan neuropathy screening instrument (MNSI): there are 7 studies using the MNSI in early screening for diabetic neuropathy. The MNSI questionnaire consists of 11 questions regarding signs and symptoms of neuropathy in diabetes mellitus patients. Researchers have developed the MNSI questionnaire, there is a research that has developed the MNSI in the form of machine learning, the MNSI is available in various versions such as the Turkish version of the MNSI (33).

Toronto clinical neuropathy score (TCNS): there are 7 studies that use TCNS in the examination of diabetic neuropathy. Researchers translated the TCNS into Spanish (13). In another study, TCNS was modified into the modified Toronto clinical neuropathy score (m-TCNS) instrument (m-TCNS) (17).

Other examinations: Diabetic neuropathy examination, apart from using the above instruments, can also use ultrasonography (USG), tripartite questionnaire, electrode diagnostic, shear wave elastography artificial neural network and artificial intelligence, cornea module. The results based on neuropathy examination using this instrument were normal and neuropathic. However, this instrument is rarely used in health services.

The research used a combination of early detection methods for neuropathy: other research used a combination of the early detection instruments mentioned above and combined using other instruments such as monofilament, sudoscan, electrochemical skin conductance, Ipswich touch, neuropathy disability score and Hammer reflex. Instruments for early detection of neuropathy in each study can also be seen in detail in Table 2.

Over time, many researchers have developed instruments for early detection of diabetic neuropathy. The development of this instrument can make it easier for health workers to detect neuropathy early and determine appropriate treatment so as to prevent the occurrence of diabetic ulcers. For example, researchers translated the Turkish version of the MNSI questionnaire so that it can be used by Turkish health workers in detecting neuropathy (24). Based on existing research, examination of diabetic neuropathy can use artificial intelligence instruments by looking at images of the cornea in diabetes mellitus patients (26).

The results of the diabetic neuropathy examination are stated to be in accordance with the instrument used. There is an instrument that describes the results of neuropathy with 3 classifications, namely mild neuropathy, moderate neuropathy and severe neuropathy (41). Furthermore, there are instruments that identify neuropathy with normal results and neuropathy. Health workers can combine instruments for early detection of neuropathy so that examination results are more accurate (42).

A study using the Toronto Clinical Neuropathy Score (TCNS), hand cold Detection Threshold (CDT), Hand Warm Detection Threshold (WDT), Foot CDT, and Foot WDT instruments in diagnosing neuropathy did not show accurate results. Early diagnosis of neuropathy is more accurate through the patient's clinical symptoms. Based on this, the doctor confirms the diagnosis of neuropathy after the patient feels clinical symptoms (30).

Neuropathy examination in diabetes patients using an early neuropathy detection tool. Various neuropathy screening tools are available with different assessment methods. The neuropathy questionnaire instrument detects neuropathy through clinical symptoms. Questionnaire questions cover patient symptoms such as pain, deformity, and decreased sensitivity. Physical examination of neuropathy using a monofilament instrument and a tuning fork.

Based on the articles included in this study, it discusses the sensitivity and specificity of neuropathy instruments. The sensitivity and specificity of the instrument show the accuracy of the instrument in diagnosing neuropathy. Validity measurements in articles use different methods including validity tests using Cronbach's alpha and ROC/AUC assessments. We found vibration perception threshold examination (biothesimener/neurothesimeter/vibratip) is the most frequently used physical examination instrument for neuropathy detection with a sensitivity value of 62%; and specificity of 76%). Vibrations of 1-50V are given to the patient's feet at several examination points, indicating neuropathy if they feel vibrations ≥25V and no neuropathy if they feel <25V. The vibration perception threshold instrument has become the gold standard for detecting neuropathy and ulcer risk (14).

Apart from physical examination instruments using the vibration perception threshold, the MNSI questionnaire is also the most frequently used in the early detection of neuropathy. This questionnaire consists of 15 questions regarding neuropathy symptoms with the results identifying neuropathy into 3 categories, namely low, moderate, and severe (15). Several studies combine the MNSI questionnaire with physical examination tools such as monofilaments and tuning forks. The MNSI questionnaire has been adapted and translated into various languages including Indonesian, Arabic, and Thai.

Meanwhile, neuropathy instruments such as the Toronto Clinical Neuropathy Score (TCNS, ultrasonography (USG), tripartite questionnaire, diagnostic electrodes, artificial neural network shear wave elastography, and artificial intelligence, cornea modules are still rarely used in diabetic neuropathy examination. Research also combines instruments of Early detection to get accurate results.

Conclusion

This review reports on the importance of early detection of neuropathy for diagnosing neuropathy and determining appropriate management. Neuropathy patients who receive appropriate treatment can prevent the occurrence of diabetic ulcers. The most frequently used neuropathy instruments are the vibration perception threshold (VPT) and questionnaire Michigan Neuropathy Screening Instrument (MNSI). Health workers can combine neuropathy instruments to accurately diagnose neuropathy.

Author contributions

OP: Conceptualization, Investigation, Methodology, Resources, Software, Visualization, Writing – original draft, Writing – review & editing. N: Supervision, Validation, Writing – original draft, Writing – review & editing, Methodology. MP: Supervision, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

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The association between statin use and diabetic nephropathy in US adults: data from NHANES 2005 - 2018

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Background: A serious consequence of diabetes is diabetic nephropathy (DN), which is commonly treated by statins. Studies evaluating the effects of statin medication have yielded inconsistent results regarding the potential association with diabetic nephropathy. To manage diabetic nephropathy's onset and improve the quality of life of patients, it is imperative to gain a comprehensive understanding of its contributing factors.

Data and methods: Our study was conducted using the National Health and Nutrition Examination Survey (NHANES) as well as weighted multivariate logistic regression models to determine the odds ratio (OR) and 95% confidence intervals (95%CI) for diabetic nephropathy. We conducted stratified analyses to examine the impact of statins and the duration of their usage on diabetic nephropathy in different subgroups. A nomogram model and the receiver operating characteristic (ROC) curve were also developed to predict DN risk.

Results: Statin use significantly increased the incidence of DN (OR=1.405, 95%CI (1.199,1.647), p<0.001). Individuals who used statins for 5 to 7 years were more likely to develop diabetic nephropathy (OR=1.472, 95%CI (1.057,2.048), p=0.022) compared to those who used statins for 1-3 years (OR=1.334, 95%CI (1.058,1.682), p=0.015) or <1 year (OR=1.266, 95%CI (1.054,1.522), p = 0.012). Simvastatin has a greater incidence of diabetic nephropathy (OR=1.448, 95%CI (1.177, 1.78), P < 0.001).

Conclusion: Taking statins long-term increases the risk of DN. Statin use is associated with an increased risk of DN. Caution should be exercised when prescribing atorvastatin and simvastatin for long-term statin therapy.

KEYWORDS

diabetic nephropathy, statin use, NHANES, diabetes, association

Abbreviations: NHANES, the National Health and Nutrition Examination Survey; BMI, Body Mass Index; HDL, High Density Lipoprotein; ALB, Albumin; ALT, Alanine Aminotransferase; AST, Aspartate Transaminase; ALP, Alkaline Phosphatase; LDL, Low Density Lipoprotein; GLB, Globulin; ACR, Urinary microalbumin creatinine ratio; BUN, Blood Urea Nitrogen; SCR, Serum Creatinines.

1 Introduction

An underlying metabolic disorder characterized by chronic hyperglycemia is diabetes mellitus (1). By 2030, 643 million adults will have diabetes, and by 2045, 783 million will, according to current projections (2). Regrettably, numerous endeavors aimed at managing and mitigating diabetes have proven ineffective owing to the dynamic nature of its consequences. One of the severe complications of diabetes mellitus, diabetic nephropathy (DN) has the potential to develop into end-stage renal disease (3). Additionally, in the early phases of DN, the presence of the condition may increase the risk of cardiovascular disease in diabetic patients, according to one study (4). From 1997 to 2017, DN accounted for one-third of all disability-adjusted life years for individuals with chronic kidney disease worldwide, according to a new report (5). Presently, the primary emphasis of clinical therapies for DN lies in the management of risk factors, including hyperglycemia, hypertension, and proteinuria. These interventions aim to alleviate symptoms and impede the advancement of DN, albeit with restricted effectiveness (6, 7). In this way, a deeper understanding of DN and its associated factors can make a positive difference in its prevention and management, thus improving the prognosis and quality of life of diabetics.

Diabetic patients exhibit a heightened incidence of lipid abnormalities (8). A genus of lipid-lowering medications commonly prescribed for the prevention of atherosclerotic cardiovascular disease (ASCVD) are statins (9, 10). Contradictory results have emerged from clinical trials of statin therapy regarding the potential risk of DN (11–13). Notably, the dosage of statin therapy may play a crucial role in modulating the progression of DN. For instance, an analysis of clinical studies indicated that the utilization of rosuvastatin was linked to a rise in proteinuria and microhaematuria that increased in proportion to the dosage. However, this effect was not observed with other statins (14). In a major insurance claims database analysis, Dormuth et al. discovered that individuals commencing high-potency statins had a 34% higher risk of acute renal damage hospitalization when compared to those on low-potency statins(High-potency statins were defined as atorvastatin ≥20 mg, simvastatin ≥40 mg, or rosuvastatin ≥10 mg; all other statins were defined as low-potency.) (15). Recent research indicates that a 50-week duration of statin use causes lipid deposition and worsens DN (16). Additionally, a recent meta-analysis revealed that the administration of statins did not appear to have a beneficial impact on the occurrence of kidney failure (17, 18).

Nevertheless, several studies appeared to establish a positive correlation between the use of statins and a decreased incidence of DN. For instance, several studies showed that statins have a time-dependent effect on renal function and are more effective in patients with type 2 diabetic nephropathy (19, 20). A study suggests that statins may have a positive effect on COVID-19-associated acute kidney injury (21). Adding atorvastatin to irbesartan provides extra kidney protection in patients with early diabetic nephropathy (22). Potentially attributable to sample variation, these studies' contradictory findings regarding the correlation between statin use and DN. The benefit of statin use in the treatment of DN remains unknown. Additionally, different statins might contribute to these conflicts.

As a result, our objective is to examine the correlation between statin usage and DN via the seven cycles (2005-2018) of NHANES screening diabetic patients to elucidate the precise impacts of various commonly prescribed statins.

2 Methods

2.1 Study population

The NHANES is a research initiative that evaluates the health and nutritional condition of individuals residing in the United States. The participants in our study were collected from 7 cycles that occurred between 2005 and 2018. We categorized all participants into two groups: those with diabetes and those without diabetes, based on the widely accepted international diagnostic criteria for diabetes. This study encompassed all individuals diagnosed with diabetes. The criteria for exclusion were as follows: (1) Individuals who are younger than 18 years old or older than or equal to 85 years old, (2) Individuals who are pregnant, (3) Participants who do not have data on plasma creatinine, urinary albumin, urinary creatinine, and statin use.

2.2 Data collection

The population's fundamental data was gathered by proficient individuals, and all experimental measurements were rigorously conducted throughout the procedure by experts in compliance with the technical requirements given on NHANES' official website. The NHANES website provides the option to download all data and experimental procedures. The experiments were conducted at a laboratory located in Minnesota.

Data was collected on various demographic factors including age, gender, race, education, finance, marital status, smoking habits, alcohol consumption, physical activity levels. Blood pressure and body mass index(BMI), results from biochemical tests such as high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), glycosylated hemoglobin (Hba1c), alanine aminotransferase(ALT), aspartate transaminase(AST), alkaline phosphatase(ALP), albumin(ALB), globulin(GLB), total cholesterol and triglyceride were also included. Individuals who used any form of statin, either on their own or in conjunction with other medications, were classified as statin users.

2.3 Definition of diabetic nephropathy

Health measurements are performed in specially-designed and equipped mobile centers, which travel to locations throughout the country. DN is identified at this point in time, according to participants' urine and blood samples which were collected in the NHANES's mobile examination centers (MECs). Patients with an estimated glomerular filtration rate (eGFR) below 60 mL × min^{-1} ×

1.73 m^{-2}, as determined by the Modification of Diet in Renal Disease (MDRD) Study equation, or patients with a urine albumin-creatinine ratio(ACR) equal to or more than 30 mg/g, were diagnosed with DN. The MDRD estimate of kidney function was derived using the formula: 175 × plasma creatinine$^{-1.154}$ × age$^{-0.203}$ (× 0.742 if female; × 1.21 if black) (23).

2.4 Definition of statin users

A survey was conducted during the in-home interview to evaluate the utilization of prescribed drugs. Individuals who indicated in the questionnaire that they had previously used statins were categorized as statin users. The duration of statin usage was categorized based on the specific number of days as follows: less than one year (<365 days), one to three years (≥365 days and <1095 days), three to five years (≥1095 days and <1825 days), five to seven years (≥1825 days and <2555 days), seven to ten years (≥2555 days and <3650 days), and more than ten years (≥3650 days).

2.5 Diagnosis of diabetes mellitus

Diabetic patients were determined to have diabetes based on self-reports of diabetes, hypoglycemic drugs, or meeting the American Diabetes Association diagnostic criteria (glycosylated hemoglobin ≥ 6.5% or fasting blood glucose > 7.0mmol/L) (24, 25).

2.6 Definition of hypertension

We calculated the mean blood pressure from three successive readings taken while the individual was in a relaxed state. The term "hypertension" was defined in the following manner: (1) Mean systolic blood pressure equal to or more than 140 mmHg, (2) Mean diastolic blood pressure equal to or greater than 90 mmHg, (3) Self-reported hypertension, (4) Antihypertensive drug users. The 140/90 mmHg criterion is based on recommendations made by the International Society of Hypertension (26).

2.7 Covariable screening

Demographics encompass factors such as age, gender, race, educational level, financial situation, and marital status. Race was classified as Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black, and Other Race. The education level was categorized into three groups: low (less than a high school diploma), middle (high school graduate or equivalent), and high (college or above). The financial position was categorized into three groups: low, middle, and high, based on the poverty income ratio (PIR ≤ 1, 1<PIR<4, PIR≥4). The marital status was categorized as accompanied, separated, or never married. The body mass index is calculated by dividing an individual's weight (in kilograms) by the square of their height (in square meters) (27).

The Healthy Lifestyle encompassed data regarding cigarette smoking, alcohol intake, and physical activity. Participants were categorized into two groups based on their self-reported smoking history: Individuals who self-reported having smoked a minimum of 100 cigarettes during their lives, as well as those who smoked fewer than 100 cigarettes. Annual alcohol consumption was classified as "12 cups or more" or "less than 12 cups." "Average daily physical activity," "vigorous recreational activity," and "moderate recreational activity" were utilized to classify physical activity in the questionnaire as either active or inactive. Individuals exhibiting HDL (high-density lipoprotein) levels below 40 mg/dL, total cholesterol levels equal to or beyond 240 mg/dL, triglyceride levels surpassing 200 mg/dL, or LDL levels at or above 160 mg/dL were categorized as having dyslipidemia (28).

The biochemical indicators comprise BUN, Hba1c, HDL, ALB, ALT, AST, ALP, BUN, SCR, Total cholesterol, Triglyceride, LDL, and GLB. Subsequently, all quantities were measured and expressed about internationally recognized standard units.

2.8 Statistical methods

Data processing and analysis were conducted using R version 4.3.0 (2023-04-21), in conjunction with the Storm Statistical Platform (www.medsta.cn/software). As shown in Prevention (2023) (29), the NHANES reporting requirements adjust and recognize the NHANES sample weights.

Statistical significance was determined for P values less than 0.05. Continuous variables are depicted through comprehensive sample descriptions accompanied by a 95% confidence interval. Discrete counts and weighted proportions represent categorical variables. The atypical distribution is depicted by the median and the interquartile range (Q1-Q3). The participants were categorized into two groups: the DN group and the non-DN group, based on whether their ACR exceeded 30 or their eGFR was below 60. We employed the "multiple imputation" technique to address the issue of missing covariates, mitigating the potential selection bias that could arise from eliminating participants with incomplete data. We aimed to ascertain whether the presence of DN was influenced by the utilization of statins, in comparison to not using them.

Following the construction of the weighted single-factor and weighted multifactor logistic regression models, four distinct models were generated in accordance with the variable types. Model 1, which is the single-factor logical regression model, does not involve any variable adjustments. Model 2 was modified to account for variables such as gender, race, BMI, and finance. Model 3 incorporated the variables from model 2, along with adjustments for smoking, drinking, physical activity, and dyslipidemia. Model 4 included additional variables compared to model 3, namely Hba1c, HDL, ALB, AST, ALT, ALP, LDL, Triglyceride, Total cholesterol, and GLB.

We conduct a more detailed analysis in the group of patients with diabetes to investigate the impact of different types of statins and the length of treatment on DN. In order to evaluate the strength of the results, weighted stratified logistic regression and subgroup analyses were conducted on each of the subgroups. The duration of

statin usage was subsequently converted to a categorical variable. Furthermore, to examine for interaction terms between subgroups during effect correction tests, likelihood ratio tests were applied. The nomogram model was constructed using R. Using receiver operating characteristic curve (ROC), the discriminatory authority of the nomogram model in DN risk detection was assessed.

3 Result

3.1 Basic information

After undergoing screening by the aforementioned stringent criteria (Figure 1), a total of 6483 diabetic patients (mean age: 61.14 ± 13.67 years) were selected from the NHANES 2005–2018 population (n = 70,190). The aforementioned groups comprised over 200 million adults residing in the United States. The baseline characteristics are specified in Supplementary Table 1. Individuals who presented with DN were found to be older (67.00 (58.00 - 76.00) years), male (53.83%), non-Hispanic white (37.83%), accompanied (87.69%), alcoholics (46.83%), hypertensive (89.83%), possess a high level of education (38.19%), have a moderate financial situation (58.07%), engage in inactive physical activity (74.31%), and utilize statins (53.94%). The glycosylated hemoglobin (7.00 (6.30, 8.20) %, GLB (3.10 (2.80, 3.40) g/dL), ALP (74.00 (60.00, 94.00) g/L), BUN (18.00 (13.00, 25.00) mg/dL), SCR (1.10 (0.83, 1.40) mg/dL), and triglyceride (132.00 (92.00 - 197.00) mg/dL) of patients with DN were all significantly higher than those of patients without DN. The levels of BMI and AST did not differ significantly between the two groups. We also found that in this study, 3,116 participants used statin medication, while 3,367 did not (Supplementary Table 1). There were significant differences between the two groups in terms of age, gender, race, marital status, economic status, hypertension, physical activity, diabetic nephropathy, and several biochemical indicators (P<0.05). Specifically, statin users had a higher incidence of diabetic nephropathy (45.03% vs 35.58%, P<0.001).

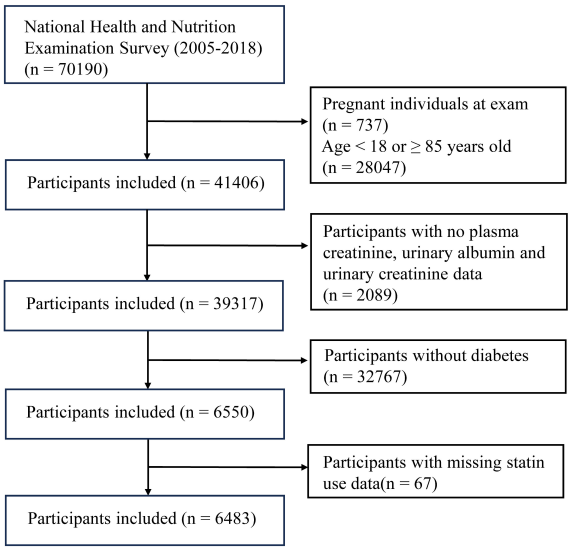

FIGURE 1
Flowchart of participant selection.

3.2 Univariate regression analysis

The results of univariate logistic regression analysis (Table 1) indicated that the following factors were associated with DN: educational situation, finance, smoking, drinking, physical activity, dyslipidemia, GGT, statin use, Hba1c, BUN, SCR, ALP, GLB, and ALB. Individuals with a high level of education or financial resources are at a reduced risk of developing DN compared to those low or middle. Non-smokers have a reduced risk of developing DN in comparison to smokers. Conversely, individuals who consume alcohol, are physically inactive or have dyslipidemia are at an elevated risk of developing DN. A negative correlation was observed between age, Hba1c, BUN, SCR, ALP, and GLB with the incidence of DN. Conversely, there existed a positive correlation between ALB and the incidence of DN. Furthermore, pharmacological indicators (specifically, statin use) demonstrated a significant association with the exacerbation of DN symptoms (OR=1.32, 95%CI (1.147,1.52), p<0.001) in comparison to those who did not use statins. This association carries a 32% increased risk of developing DN.

3.3 Multivariate regression analysis

Utilizing weighted multivariate logistic regression, the relationship between statin use and DN was investigated. Table 2 details the relationship between statin use and DN, and four logistic regression models were developed, with the effect value expressed as odds ratio and 95% confidence interval. Depending on the magnitude of the effect size, patients treated with statins may have a relatively increased risk of developing DN. The unadjusted analysis (model 1) revealed a statistically significant association between statin use and DN (OR 1.32; p<0.001). This association corresponds to a 32% escalation in the risk of DN among patients who were prescribed statins. The effect value in model 2, which was marginally adjusted, was (OR=1.349, 95%CI (1.168,1.558), p<0.001). This value indicated that patients taking statins had a 34.9% increased risk of developing DN. The effect value in further adjusted model 3, denoted as (OR=1.351, 95%CI (1.165,1.566), p<0.001), suggested that patients undergoing statin treatment faced a 35.1% increased risk of developing DN. The effect value in fully adjusted model 4 was (OR=1.405, 95%CI (1.199,1.647), p<0.001), indicating that the use of statins significantly increased the risk of DN by 40.5% in comparison to those who did not use statins.

3.4 Subgroup analysis and the nomogram model

To ensure the results' stability, subgroup and sensitivity analyses were performed. We examined whether DN and statin

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TABLE 1 Univariate analysis of association between factors of statin use and diabetic nephropathy.

Variable	Diabetic nephropathy	
	OR (95%CI)	P-value
Age	1.042(1.037,1.048)	<0.001
Gender		
Male	1	
Female	0.985(0.856,1.132)	0.829
Race		
Mexican American	1	
Other Hispanic	0.901(0.705,1.151)	0.404
Non-Hispanic White	1.076(0.904,1.281)	0.41
Non-Hispanic Black	1.034(0.868,1.232)	0.706
Other Race - Including Multi-Racial	1.008(0.785,1.296)	0.949
NA	2.138(0.58,7.88)	0.254
BMI	1(0.991,1.008)	0.914
Finance		
Low	1	
Medium	0.895(0.761,1.053)	0.18
High	0.592(0.476,0.736)	<0.001
NA	0.764(0.589,0.991)	0.043
Cigarette smoking		
No	1	
Yes	1.183(1.028,1.361)	0.019
NA	1.764(0.531,5.858)	0.354
Alcohol drinking		
No	1	
Yes	0.732(0.626,0.857)	<0.001
NA	0.732(0.6,0.894)	0.002
Physical activity		
No	1	
Yes	0.732(0.627,0.855)	<0.001
NA	0.64(0.211,1.94)	0.43
Dyslipidemia		
No	1	
Yes	1.303(1.129,1.504)	<0.001
Statin use		
No		
Yes	1.32(1.147,1.52)	<0.001
Hba1c	1.148(1.105,1.194)	<0.001
HDL	0.998(0.992,1.003)	0.44
ALB	0.929(0.911,0.948)	<0.001
AST	1.001(0.998,1.004)	0.504
ALT	0.998(0.992,1.004)	0.452
ALP	1.006(1.002,1.009)	<0.001
Total cholesterol	0.999(0.998,1.001)	0.461
Triglyceride	1(1,1)	0.252
LDL	1(0.999,1.002)	0.77
GLB	1.758(1.528,2.023)	<0.001

(Continued)

TABLE 1 Continued

NA, Missing; BMI, Body Mass Index; HDL, High Density Lipoprotein; ALB, Albumin; ALT, Alanine Aminotransferase; AST, Aspartate Transaminase; ALP, Alkaline Phosphatase; LDL, Low Density Lipoprotein; GLB, Globulin.

use differed with regard to age, gender, race and finance. The findings indicated that the association between statin use and DN remained consistent across all subgroups (Figure 2), and no interaction was observed (P > 0.05).

Upon adjusting for model 4, individuals who had used statins for 5 to 7 years had a significantly higher and more pronounced risk (OR=1.472, 95%CI (1.057,2.048), p=0.022) of developing DN compared to those who had used them for < 1 year (OR=1.266, 95%CI (1.054,1.522), p = 0.012), 1 to 3 years (OR=1.334, 95%CI (1.058,1.682), p=0.015) or 3 to 5 years of statin use (OR=1). However, as the duration of use increases (7 to 10 years, p = 0.852; or > 10 years, p = 0.077), this association gradually weakens and becomes statistically insignificant after more than 7 years of use. According to NHANES, the five most frequently prescribed statins in the entire sample from 2005 to 2018 (Supplementary Table 1) were simvastatin, atorvastatin, pravastatin, rosuvastatin, and lovastatin. A significant risk of developing DN was associated with atorvastatin (OR=1.443, 95%CI (1.168,1.784), p = 0.001). Furthermore, simvastatin, the most frequently prescribed statin, exhibited noteworthy and even the most pronounced ascending effects on the development of DN (OR=1.448, 95%CI (1.177, 1.78), P<0.001) (Table 2). We selected variables (age, statin use, alcohol drinking, cigarette smoking, Hba1c, ALB, GLB, ALP) that were more clinically and statistically significant to construct a nomogram model (Figure 3). The discriminatory capacity of the nomogram model developed in this study was confirmed by the area under the curve (AUC) of 68.4% (95% CI: 65.3%–71.5%) as shown in the ROC curve (testing) results (Figure 4).

4 Discussion

The majority of scientists believe that statins protect DN, according to an extensive body of clinical trials and fundamental research. A meta-analysis revealed that statins can enhance renal function indicators in DN therapy, lowering inflammation and preserving the kidney (30). According to a randomized clinical

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TABLE 2 Multivariate analysis of statin use and related factors of diabetic nephropathy.

Variable	Model 1		Model 2		Model 3		Model 4	
	OR (95%CI)	P-value	OR (95%CI)	P-value	OR (95%CI)	P-value	OR (95%CI)	P-value
Statin use	1.32(1.147,1.52)	p<0.001	1.349(1.168,1.558)	p<0.001	1.351(1.165,1.566)	p<0.001	1.405(1.199,1.647)	p<0.001
P for trend	p<0.001		p<0.001		p<0.001		p<0.001	
Statin types								
ATORVASTATIN	1.347(1.109,1.636)	0.003	1.383(1.136,1.683)	0.001	1.407(1.152,1.719)	0.001	1.443(1.168,1.784)	0.001
FLUVASTATIN	1.225(0.203,7.38)	0.825	1.011(0.154,6.644)	0.991	1.233(0.196,7.757)	0.823	1.407(0.194,10.208)	0.736
LOVASTATIN	1.335(0.902,1.976)	0.148	1.341(0.901,1.998)	0.148	1.358(0.905,2.038)	0.139	1.428(0.955,2.134)	0.082
SIMVASTATIN	1.354(1.122,1.634)	0.002	1.382(1.14,1.674)	0.001	1.365(1.122,1.661)	0.002	1.448(1.177,1.78)	p<0.001
PITAVASTATIN	1		1		1		1	
PRAVASTATIN	1.218(0.882,1.682)	0.23	1.229(0.889,1.699)	0.211	1.221(0.882,1.689)	0.229	1.253(0.896,1.752)	0.187
ROSUVASTATIN	1.295(0.901,1.862)	0.162	1.327(0.925,1.902)	0.124	1.331(0.928,1.908)	0.12	1.385(0.946,2.027)	0.094
None	1		1		1		1	
Duration								
<1 year	1.194(1.004,1.418)	0.045	1.225(1.029,1.459)	0.022	1.218(1.021,1.453)	0.028	1.266(1.054,1.522)	0.012
1 to 3 years	1.261(1.017,1.562)	0.034	1.298(1.045,1.611)	0.018	1.305(1.047,1.626)	0.018	1.334(1.058,1.682)	0.015
3 to 5 years	1		1		1		1	
5 to 7 years	1.484(1.096,2.01)	0.011	1.509(1.108,2.056)	0.009	1.523(1.112,2.086)	0.009	1.472(1.057,2.048)	0.022
7 to 10 years	0.893(0.555,1.437)	0.642	0.901(0.553,1.468)	0.677	0.886(0.537,1.462)	0.637	0.951(0.56,1.615)	0.852
>10 years	1.481(0.877,2.501)	0.142	1.457(0.865,2.454)	0.157	1.519(0.894,2.581)	0.122	1.609(0.951,2.722)	0.077
None	1		1		1		1	

Model 1: Non-adjusted.
Model 2: Gender, Race, BMI, Finance.
Model 3: Model 2+ Smoking, Drinking, Physical activity, Dyslipidemia.
Model 4: Model 3 + Hba1c, HDL, ALB, AST, ALT, ALP, LDL, Triglyceride, Total cholesterol, GLB.
The reference for statin types was participants without statin use (None).

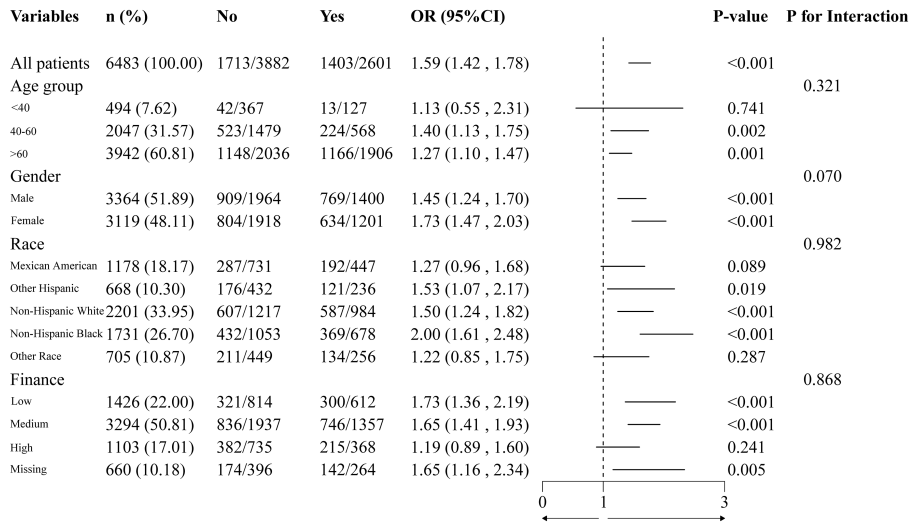

FIGURE 2
Forest plot of statin use and diabetic nephropathy.

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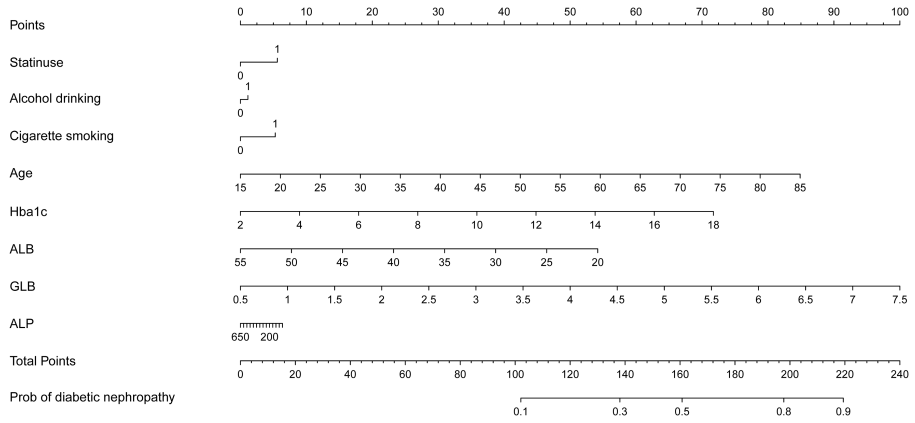

FIGURE 3
Nomogram model established for predicting the risk of diabetic nephropathy.

trial, atorvastatin appears to have stronger renoprotective effects in the chronic renal disease group (31). However, it is crucial to highlight that the duration of all trials and fundamental research was brief. Furthermore, there is a lack of data regarding the long-term administration of statins for diabetes from trials that have lasted over a decade (32).

As of yet, the impact of statins on the progression of DN is contentious. The available experimental and clinical data indicate that dyslipidemia has the potential to accelerate the advancement of chronic kidney disease (CKD) by impacting the renal microvasculature and promoting the activation of intracellular signaling pathways associated with inflammation and fibrosis (33). Nevertheless, the impact of lipid-lowering drugs on the advancement of CKD remains uncertain. Numerous studies have documented in recent years that statins can impair glucose metabolism and exacerbate insulin resistance; users of statins have an increased risk of developing type 2 diabetes at the commencement of the disease (34). The potential adverse effects of statin therapy cast doubt on the efficacy of statin use in the treatment of diabetes (35). Recent research has elicited apprehension on the potential renal adverse effects associated with some statins, particularly when administered at elevated dosages (36).

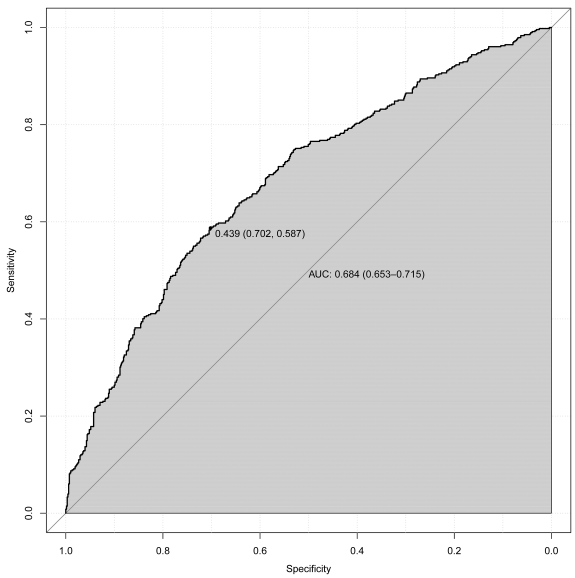

FIGURE 4
ROC curve for evaluating the diagnostic power of the nomogram model in this study.

Recent years have seen the confirmation of a number of large-scale randomized controlled trials (RCTs) that statin therapy does not inhibit the progression of kidney disease within five years in a wide range of patients with CKD (37). Moreover, certain case studies have demonstrated that statins like Rosuvastatin has the potential to cause interstitial nephritis (38). More significantly, the use of statins that have a high efficacy in lowering CHOL levels may elevate the likelihood of developing severe renal failure (39). These findings serve as a reminder that the extent to which the beneficial effects of prolonged statin use on DN outweigh its detrimental effects remains uncertain.

We first compared and contrasted the effects of statins in patients with DN. Among the 6483 participants who did not take statins, we discovered that statin use was significantly associated with an increased risk of developing DN. We devised four logical regression models to examine the correlation between the use of statins and DN to account for potential confounding variables The effect value in model 4 is 1.405 (1.199,1.647) ($p<0.001$). This indicates that statin-treated patients have a 1.32-fold increased risk of developing DN compared to non-statin-treated patients. Concurrently, we examined the correlation between the duration of statin use and DN and categorized statin users according to this criterion. The results indicated that individuals who had been taking statins for a longer period (5 to 7 years) had a significantly increased risk of developing DN (Table 2). But this association gradually weakens and becomes statistically insignificant after more than 7 years of use (7 to 10 years, $p = 0.852$ or > 10 years, $p = 0.077$). Furthermore, upon validating the findings across age, gender, race, and finance subgroups, we observed that they remained constant without interaction across all subgroups (Figure 2). Simvastatin and atorvastatin were associated with a significantly increased risk of

DN. The red points show an example in Supplementary Figure 1 that, for 80-year-old diabetic participant with statin use, alcohol drinking, cigarette smoking, Hba1c (8%), ALB (35g/L), GLB (3g/dL) and ALP (100IU/L), the risk of DN increased by 73%.

Several studies may provide a partial explanation for the mechanisms underlying statin use and DN. Statins, a class of drugs, act as competitive and potent inhibitors of HMGCR, a microsomal enzyme that facilitates the rate-determining conversion of HMG-CoA to mevalonate in LDL cholesterol, thereby reducing cholesterol levels in the blood (40). Various statins exhibit comparable effectiveness in reducing LDL cholesterol levels in relation to their primary purpose of mitigating the likelihood of atherosclerotic cardiovascular events. This result offers compelling evidence that the main impact of statins is the reduction of low-density lipoprotein cholesterol (41). However, it remains uncertain whether there are variations among statins in terms of their effects on the kidneys. A growing body of evidence suggests that statins may cause disruptions in insulin resistance; these findings suggest the possibility of therapeutic adverse effects for diabetes (42). An experiment indicated that inflammatory stress enhanced resistance to statins and boosted intracellular CHOL production by improving HMG-CoA-R activity (43). Chronic inflammation caused by stress triggers the production of cholesterol within cells, which interferes with the normal control of HMG-CoA-R in the kidneys through SCAP. This disruption results in a condition known as "renal statin resistance" (44). An animal experiment demonstrated that the administration of statins over a prolonged period in mice resulted in a significant upregulation of CD36 expression in the kidneys (16). CD36 is a multifunctional receptor that facilitates the internalization of oxidized lipids and long-chain fatty acids. Lipid accumulation, inflammatory signaling, energy reprogramming, apoptosis, and renal fibrosis are among the many functions it performs (45). It could be quite some time before these effects become apparent. Hence, it partially facilitates the development of DN. Therefore, it is hypothesized that diabetic patients may face an elevated risk to their renal function as a result of prolonged statin use. This potential risk highlights the importance of close monitoring and management of renal function in diabetic patients who are prescribed statins.

The limited sample size of participants in this study who used statins for more than 7 years may explain the results showing a loss of statistical significance after more than 7 years of use. Further prospective research is warranted to validate and generalize these findings to a larger and more diverse population. It is essential to fully understand the impact of statin use on renal function in diabetic patients in order to optimize their treatment and improve their overall health outcomes.

5 Limitations

Several limitations apply to this investigation. NHANES datasets do not contain information regarding the dosage of statins, even though this is a critical variable that can significantly impact their efficacy or adverse effects. Since a portion of the data was collected via questionnaire and memory, the survey may be subject to recall bias. Statin users and non-users may have potential confounding factors at baseline, such as differences in the risk of cardiovascular disease. We recognize that the adjustments made to the model may still be insufficient to completely eliminate confounding bias. The NHANES database's inclusion was constrained in our study by its exclusive focus on normal populations, thereby excluding certain special populations and other countries.

6 Conclusion

In conclusion, the use of statins was associated with an increased risk of DN in adults in the United States; furthermore, the risk of DN is increased with long-term statin use. Atorvastatin and simvastatin should be used with caution in long-term treatment with statins.

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 humans were approved by National Center for Health Statistics Ethics Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JG: Conceptualization, Data curation, Software, Writing – original draft, Writing – review & editing. ZJ: Data curation, Writing – review & editing. YX: Software, Writing – review & editing. HW: Software, Writing – review & editing. QT: Funding acquisition, Investigation, Methodology, Project administration, Writing – review & editing. BM: Methodology, Writing – review & editing.

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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.2024.1381746/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Nomogram model based on the significant factors screened by logistic regression.

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Gut microbiota microbial metabolites in diabetic nephropathy patients: far to go

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Diabetic nephropathy (DN) is one of the main complications of diabetes and a major cause of end-stage renal disease, which has a severe impact on the quality of life of patients. Strict control of blood sugar and blood pressure, including the use of renin–angiotensin–aldosterone system inhibitors, can delay the progression of diabetic nephropathy but cannot prevent it from eventually developing into end-stage renal disease. In recent years, many studies have shown a close relationship between gut microbiota imbalance and the occurrence and development of DN. This review discusses the latest research findings on the correlation between gut microbiota and microbial metabolites in DN, including the manifestations of the gut microbiota and microbial metabolites in DN patients, the application of the gut microbiota and microbial metabolites in the diagnosis of DN, their role in disease progression, and so on, to elucidate the role of the gut microbiota and microbial metabolites in the occurrence and prevention of DN and provide a theoretical basis and methods for clinical diagnosis and treatment.

KEYWORDS

diabetic nephropathy, gut microbiota, microbial metabolites, diagnosis and treatment, therapeutic strategies

1 Introduction

Diabetes is one of the most common chronic diseases worldwide, with prevalence and incidence rates increasing annually (Liu et al., 2021). It is estimated that by 2045, the absolute number of diabetes patients will increase by 46% (Sun H. et al., 2022). Diabetes can cause various serious and some life-threatening complications (Popoviciu et al., 2023).

Abbreviations: DN, diabetic nephropathy; FMT, Fecal microbiota transplantation; ESRD, end-stage renal disease; GFR, glomerular filtration rate; UAE, urinary albumin excretion; T2DM, type 2 diabetes; CKD, chronic kidney disease; LPS, lipopolysaccharides; HC, health control; SCFAs, short-chain fatty acids; AUC, the receiver operating characteristic curve; PS, phenyl sulfate; DM, diabetes mellitus; ACR, Urinary Albumin To Creatinine Ratio; STZ, streptozotocin; GLP-1, glucagon like peptide-1; FOS, Fructooligosaccharide; T1DM, type 1 diabetes mellitus; RS, Resistant starch; IL, interleukin.

Diabetic nephropathy (DN) is one of the common microvascular complications, characterized by structural and functional damage to the kidneys (Wu and Huang, 2023). Clinical manifestations include massive proteinuria, hypertension, and edema, and it is one of the main causes of end-stage renal disease (ESRD) (Wu et al., 2023). At present, the diagnosis of DN depends on a decreased glomerular filtration rate (GFR) or increased urinary albumin excretion (UAE), but these changes are not unique to DN, and the diagnostic sensitivity and specificity in the preclinical stage of diabetic kidney damage are also limited (Oshima et al., 2021). At present, the treatment of DN mainly involves lifestyle guidance, metabolic therapy, and hypoglycemic and antihypertensive drugs to help patients slow down disease progression, thereby improving their quality of life (Liu P. et al., 2023). However, due to the complex pathogenesis of DN, no breakthrough progress has been made in the treatment of DN. Therefore, there is an urgency to search for new biomarkers generated by the pathogenesis of this disease to assist in its diagnosis, follow-up, treatment, and prognosis.

The human intestine harbors a variety of microorganisms, such as bacteria, fungi, and viruses, that are involved in the digestion of food, synthesis of essential vitamins and amino acids, elimination of pathogens, and clearance of toxins (Fernandes et al., 2022; Xiang et al., 2023). Through metagenomic sequencing analysis of human fecal samples, intestinal flora such as Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Verrucomicrobia, Cyanobacteria, and Spirochaetes have been identified (de Vos et al., 2022; Chen et al., 2023). Many studies have shown that changes in the abundance, diversity, and colonization location of the gut microbiota and alterations in serum metabolites can lead to DN, diabetic retinopathy, diabetic cardiovascular disease, and other complications (Lv et al., 2022; Xu et al., 2022). However, the specific role of the gut microbiota in DN is not yet fully understood. The recent emergence of the gut–kidney axis theory has gradually revealed the correlation between gut microbiota and kidney diseases (Paul et al., 2022). The gut microbiota of DN patients is significantly different from that of healthy individuals, with a decrease in beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*, and an increase in the number of pathogenic bacteria, such as *Enterobacter* (Hu et al., 2020; Castillo-Rodriguez et al., 2018). An imbalance of the gut microbiota can lead to intestinal barrier damage, increased intestinal permeability, and accelerated transfer of microbial metabolites (such as indoxyl sulfate and p-cresyl sulfate) into the bloodstream, exacerbating kidney damage (Chen et al., 2023). Imbalance of the gut microbiota leads to metabolic endotoxemia, which induces chronic inflammation, short-chain fatty acid (SCFA) metabolism, oxidative stress, and other factors that affect the development of DN (Li J. et al., 2022). Correcting the imbalance of the gut microbiota may be a new target for treating DN.

We summarize the characteristics of the gut microbiota and metabolism in DN patients and discuss the application of gut microbiota and metabolism as biomarkers in DN, the role of the gut microbiota and metabolism in disease occurrence and development, and the application of microbial targeted therapy in DN.

2 Gut microbiota in DN patients

2.1 Alteration of gut microbiota in DN Patients

The stability of the gut microbiota is closely related to host health and disease (Gebrayel et al., 2022). Normal gut microbiota contains a large number of bacteria such as *Bacteroides*, *Bifidobacterium*, and *Lactobacillus*. DN patients have imbalances in gut microbial composition, abundance, and diversity (Kikuchi et al., 2019). In 20 patients with type 2 diabetes (T2DM) and chronic kidney disease (CKD), the gut microbiota showed significantly higher levels of Proteobacteria, Verrucomicrobia, and Fusobacteria, which can produce lipopolysaccharides (LPS), compared with a health control group (Salguero et al., 2019). Tao et al. (2019) also found a high abundance of Proteobacteria in 14 confirmed cases of DN. Cai et al. (2022) also found high abundance of Proteobacteria in nondialysis-dependent DN patients. In addition, compared with healthy controls, the relative abundance of Ruminococcaceae, *Butyricicoccus*, and Lachnospiraceae, which produce SCFAs, was reduced in 31 nondialysis-dependent patients. Shang et al. (2022) found that the gut microbiota of 180 DN patients was enriched in Proteobacteria, Actinobacteriota, Synergistota, Euryarchaeota, Patescibacteria, Verrucomicrobiota, and Cyanobacteria, compared with healthy controls, while Bacteroidota and Bacteria unclassified were depleted. Compared with healthy controls, there was a decrease in the abundance of Firmicutes in 20 patients with DN, while Corynebacteriales and *Eisenbergiella*, as well as *Ralstonia*, were enriched (Song et al., 2021). In a study involving 60 patients with DN, there was no significant difference in the relative abundance of Actinobacteria and Firmicutes between the DN and healthy control group (Chen et al., 2021). That study confirmed that *Alistipes*, *Bacteroides*, *Subdoligranulum*, *Lachnoclostridium*, and *Ruminococcus torques* were detrimental factors in the development of DN (Chen et al., 2021). Compared with healthy controls, the gut microbiota of 43 patients diagnosed with stage 3 or 4 DN was enriched in *Haemophilus*, *Escherichia–Shigella*, *Megalococcus*, *Veillonella*, and *Anaerostipes* (Du et al., 2021). Butyrate-producing bacteria (*Clostridium*, *Ruminococcus*, and *Eubacterium*) and potential probiotics (*Lactobacillus* and *Bifidobacterium*) were significantly reduced in T2DM and DN patients (Zhang L. et al., 2022). Compared with T2DM patients without kidney damage for >10 years, 35 confirmed cases of DN showed a significant increase in the abundance of *Christensenella*, *Clostridium-XIVa*, *Eisenbergiella*, *Flavonifractor*, and *Clostridium-XVIII*, while the abundance of butyrate-producing bacteria, *Bacillus*, *Enterobacter*, *Trichospira*, and *Roseburia* was significantly reduced (Lu et al., 2023). Whole-genome analysis showed enrichment of seven bacterial species in the feces of 15 DN patients, including *Alistipes shahii*, *Alistipes communis*, *Alistipes onderdonkii*, *Bacteroides intestinalis*, *Ruminococcus* sp. *strain JE7A12*, and *Odoribacter splanchnicus* (Kim et al., 2023). However, whole-genome analysis of European women showed that *A. shahii* was higher in the healthy control group than in the diabetes group (Dwiyanto et al., 2021), which may be due to racial, dietary, and geographical differences

(Gaulke and Sharpton, 2018). Differences in lifestyle, diet, race, and medical conditions may be the main factors leading to differences in gut microbiota expression in DN (Dwiyanto et al., 2021). Therefore, long-term, multicenter research is still needed to help us better understand the relationship between the gut microbiota and DN (Table 1).

2.2 The diagnostic and early warning value of microbiota in DN patients

The gut microbiota composition in DN patients undergoes significant changes, which can serve as biomarkers to differentiate clinical diagnosis or confirm DN through biopsy. For patients who are contraindicated for renal biopsy, gut microbiota testing may be a crucial alternative solution (Shang et al., 2020). Among the 14 DN patients confirmed by biopsy in Sichuan, China, the genus *Prevotella_9* accurately distinguished DM patients from healthy controls, with an area under the receiver operating characteristic curve (AUC) of 0.900. *Escherichia–Shigella* and *Prevotella_9* also

TABLE 1 Alteration of gut microbiota in DN.

Studies	Subjects	The variety of Gut microbiota	Research method
Song et al. (2021)	DN patients	**Increased:** **At the genus level:** *Eisenbergiella*, *Ralstonia*, *Intestinimonas*, *Eubacterium_fissicatena_group* **Decreased:** **At the phylum levels:** Firmicutes	High-throughput sequencing
Chen et al. (2021)	DN patients	**Increased:** **At the genus level:** *Alistipes*, *Bacteroides*, *Subdoligranulum*, *Lachnoclostridium*, *Parabacteroides* **Decreased:** *Klebsiella*	High-throughput sequencing
Salguero et al. (2019)	DN patients	**Increased:** **At the phylum levels:** Proteobacteria, Verrucomicrobi, Fusobacteria **Decreased:** **At the phylum levels:** Firmicutes	16sRNA
Tao et al. (2019)	DN patients	**Increased:** **At the phylum levels:** Proteobacteria **At the genus level:** *Coriobacteriaceae*, *Escherichia-Shigella* **Decreased:** **At the genus level:** *Prevotella_9*	16sRNA
Du et al. (2021)	DN patients	**Increased:** **At the phylum levels:** Actinobacteria **At the class levels:** Actinobacteria, Bacilli, Coriobacteriia, Negativicutes **At the order levels:** Betaproteobacteriales, Bifidobacteriales, Coriobacteriales, Lactobacillales, Selenomonadales	16S rDNA
		At the family level: Atopobiaceae, Bifidobacteriaceae, Burkholderiaceae, Lactobacillaceae, Streptococcaceae, Tannerellaceae, Veillonellaceae **At the genus level:** *Acidaminococcus*,Lactobacillus, *Megasphaera, Mitsuokella, Olsenella, Prevotella_7, Sutterella* **Decreased**: **At the class levels:** Alphaproteobacteria, Clostridia **At the order levels:** Chitinophagales, Clostridiales, Rhizobiales, Xanthomonadales **At the family level:** Chitinophagaceae, Lachnospiraceae, Rhodanobacteraceae **At the genus level:** *Lachnoclostridium, Roseburia, Tyzzerella_3*	
Zhang L. et al. (2022)	DN patients	**Increased:** **At the genus level:***Bacteroides, Bacteroides stercoris, Prevotella* sp. *MSX73, Barnesiella, Alistipes ihumii, Bacteroides stercoris CAG_120, Tannerella* sp. *CAG_51, Parabacteroides* sp. *20_3* **At the species level**:*Bacteroides stercoris, Bacteroides_eggerthii* **Decreased:** **At the genus level:***Prevotella, Lachnospira, oseburia intestinalis, Bacteroides plebeius CAG_211, Clostridium* sp. *CAG_768, Fusobacterium varium, Clostridium* sp. *26_22, Eubacterium* sp. *AF22_9, Roseburia* sp. *AM23_20* **At the species level**: *Bacteroides fragilis*	Metagenomic sequencing
Lu et al. (2023)	DN patients	**Increased:** **At the genus level**:*Christensenella, Clostridium-XIVa, Eisenbergiella, Flavonifractor, Clostridium-XVIII* **Decreased:** **At the genus level**:*butyric-producing bacteria, Bacillus, Enterobacter, Trichospira, Rosacella*	16S rDNA
Kim et al. (2023)	DN patients	**Increased:** **At the species level**:*Alistipes onderdonkii, Alistipes shahii, Alistipes communis, Ruminococcus* sp. strain JE7A12, *Bacteroides intestinalis*, and *Odoribacter splanchnicus*	Metagenomic sequencing
Cai et al. (2022)	DN patients	**Increased:** **At the phylum levels:** Proteobacteria **At the class levels:** δ-proteobacteria, γ-probacteria, **At the order levels:**	16S rDNA

(Continued)

TABLE 1 Continued

Studies	Subje-cts	The variety of Gut microbiota	Research method
		Pseudomonadales, Desulfovibrionales **At the family levels:** Moraxellaceae, Desulfovibrionaceae **At the genus levels:** *Acinetobacter*, *Desulfovibrio*, *Erysipelatoclostridium*, *Hungatella*, **Decreased:** **At the phylum levels:** Firmicutes **At the class levels:** Clostridia **At the order levels:** Clostridiales **At the family levels:** Ruminococcaceae, Lachnospiraceae **At the genus levels:** *Ruminococcaceae_UCG_013*, *Lachnospira*, *Ruminococcaceae_UCG_014*, *Ruminococcaceae_UCG_003*, *Butyricicoccus*, *Lachnospiraceae_NK4A136_group*, *Eubacterium*	
Zhang B. et al. (2022)	DN rats	**Increased:** **At the phylum levels:** Actinobacteriota **At the class levels:** Bacilli, Bacteroidia **At the order levels:** Lactobacillales, Erysipelotrichales **At the family levels:** *Lactobacillaceae* **At the genus levels:** *NK4A214_group* **Decreased:** **At the phylum levels:** Firmicutes **At the class levels:** Clostridia **At the order levels:** Clostridiales, Clostridia UCG-014 **At the genus levels:** *Lachnospiraceae_NK4A136_group*, *Romboutsia*	16S rRNA
Wu et al. (2022)	DN rats	**Increased:** **At the genus levels:** *Negativibacillus*, *Rikenella* **Decreased:** **At the genus levels:** *Akkermansia*, *Candidatus*, *Erysipelatoclostridium*, *Ileibacterium*	16s rDNA

accurately differentiated DN patients confirmed by biopsy from DM patients, with an AUC of 0.860, which aided in the diagnosis of DN (Tao et al., 2019). However, Lu et al. found different results in 35 cases of DN confirmed by biopsy in Shanxi, China, where *Flavonifractor* (AUC=0.909) or *Eisenbergiella* (AUC=0.886) accurately identified DN and DM patients (Lu et al., 2023), which may be related to differences in northern and southern regions and dietary habits. *Clostridium* sp. CAG_768 (AUC=0.941), *Bacteroides propionicifaciens* (AUC=0.905), and *Clostridium* sp. CAG_715 (AUC=0.908) effectively differentiated DN patients from the healthy control group. Multiple linear regression analysis showed that the combined detection of *Fusobacterium varium*, Pseudomonadales, and *Prevotella* sp. MSX73 (AUC=0.889) distinguished T2DM from DN, and the AUC of bacterial biomarkers for T2DM and DN was higher than urinary albumin to creatinine ratio (ACR), albumin, and urinary creatinine ratio (Zhang L. et al., 2022). A random forest model constructed from the 25 least correlated microbial genera had an AUC of 0.972, indicating a high predictive ability of gut microbiota for DN (Du et al., 2021). These results suggest that the gut microbiota may be promising candidates for diagnosing DN. However, current research shows that the biomarkers of gut microbiota used for diagnosing DN vary among regions and races (Gaulke and Sharpton, 2018). Therefore, more clinical research is needed to explore the value of gut microbiota in DN diseases.

2.3 Gut microbiota associated with occurrence and development of DN

Many studies have shown significant changes in the gut microbiota of patients with DN. Dysbiosis of the gut microbiota in DN patients is associated with endotoxemia, inflammation (Zhang et al., 2021), intestinal barrier dysfunction (Xiong et al., 2019; Sun X. et al., 2022; Xu et al., 2022), and a decrease in beneficial bacteria that produce SCFAs (Sabatino et al., 2017). Pathogenic bacteria, such as *Clostridium*, *Bacteroides*, and *Prevotella*, can increase intestinal barrier permeability by producing toxins (Das et al., 2021). Increased intestinal permeability promotes the reabsorption of ammonia, and toxins produced by microbial metabolism (such as indoxyl sulfate and p-cresyl sulfate) are transferred into the blood, exacerbating kidney damage (Lv et al., 2022). Microbial dysbiosis, mainly characterized by an overgrowth of *Proteus*, is associated with increased inflammation in DN patients and a decrease in SCFA-producing bacteria, which is a key factor in the pathogenesis of DN (Salguero et al., 2019; Stavropoulou et al., 2021). In a DM rat model, excess acetate produced by dysbiosis of the gut microbiota induced early kidney damage by activating the renal renin–angiotensin system (Lu et al., 2020). In experimental models of diabetes, microbiota-derived phenyl sulfate (PS) is associated with the progression of albuminuria (Kikuchi et al., 2019). Several recent studies have shown that regulating gut microbiota dysbiosis and improving intestinal barrier function can effectively reduce uremic toxin levels and serum proinflammatory mediators [such as tumor necrosis factor-α, interleukin (IL)-1β, and IL-18], thereby delaying the progression of DN (Han et al., 2023; Shi et al., 2023; Wang et al., 2023; Wu et al., 2023). These studies indicate that gut microbiota disorders play an essential role in the development of DN, and further exploration is needed to diagnose or treat DN by targeting the composition of gut microbiota (Figure 1).

3 Microbial metabolites in DN patients

3.1 Alteration of metabolites in DN patients

The interaction between gut microbiota and the host is mainly achieved through the production of metabolites, which play a key

role in the pathogenesis of DN by producing a large number of metabolites (Zhang B. et al., 2022). Zhu et al. (2022) have shown that amino acid metabolism may play an important role in the progression of DM and DN. N-Acetylaspartic acid, L-valine, betaine, isoleucine, asparagine, and L-methionine are upregulated in patients with T2DM and DN, with a more significant increase in the latter. High levels of L-leucine and isoleucine are significantly correlated with rapid estimated GFR decline. Compared with healthy controls, DN patients have elevated levels of stearic acid, glutaric acid, 2-Amino-3-methylimidazo(4,5-f) quinoline, and L-proline, and decreased levels of 1,3,7-trimethyluric acid, homocarnosine, epinephrine, N-acetylputrescine, linoleic acid, and ephedrine (Chen et al., 2023). In addition, the abundance of SCFA metabolites, valerate, and caproate, are significantly decreased in the serum of DN patients (Zhong et al., 2022). Compared with healthy controls, 11 DN patients had significantly higher levels of leucine, isoleucine, methionine, valeric acid, and phenylacetate, and lower levels of acetate (Kim et al., 2023). Li Y. et al. (2022) have also found decreased levels of acetate in DN patients. Acetate is one of the main components of SCFAs, and the levels of other SCFAs components, propionate, and butyrate, are lower in DN patients compared with DM patients and healthy controls. This may be related to the decrease in SCFA-producing bacteria such as Ruminococcaceae, Lachnospiraceae, and Bacteroidaceae in the gut microbiota of DN patients (Chen T. et al., 2022). However, the construction of DN rat models showed that serum acetate levels increase in DM rats, accompanied by increased proteinuria, and *in vitro* experiments have confirmed that excessive acetate can cause tubulointerstitial damage (Hu X. et al., 2020; Lu et al., 2020). This difference may be related to different research subjects and diseases, and multicenter and cross-racial studies are needed to confirm the role of SCFAs in DN. Gut microbiota metabolites, such as PS and trimethylamine-N-oxide, are typical uremic toxins associated with podocyte injury (Fernandes et al., 2019) (Table 2).

3.2 The diagnostic and early warning value of metabolites in DN patients

Enrichment analysis has confirmed the involvement of the urea cycle, TCA cycle, glycolysis, and amino acid metabolism in the pathogenesis of DN. Meta-analysis of existing studies on DN identified lactate, hippuric acid, urea (in urine), and glutamine (in blood) as the most important noninvasive early diagnostic biomarkers (Roointan et al., 2021). Random forest model analysis showed that methionine and branched-chain amino acids (AUC=0.832) were among the most significant features, second only to estimated GFR and proteinuria, for distinguishing between DN patients and healthy controls (Kim et al., 2023). Zhu et al. (2022) confirmed that high levels of L-leucine (AUC=0.834) and isoleucine (AUC=0.932) have high diagnostic ability in distinguishing between DN and T2DM. Two oligopeptides, Asn-Met-Cys-Ser and Asn-Cys-Pro-Pro, were correlated with the severity of proteinuria, with AUC values of 0.8857 and 0.9963, respectively, making them potential biomarkers for differentiating the severity of DN (Peng et al., 2022). Through UHPLC-QTOF-ESI-MS analysis of serum and urine from 90 DN patients, arginine (AUC=0.500), L-acetylcarnitine (AUC=0.600), hippuric acid (AUC=0.700), indoxyl sulfate (AUC=0.600), butenoyl carnitine (AUC=0.600), and sorbitol (AUC=0.500) in serum, and p-cresylsulfate (AUC=0.800) in urine may serve as biomarkers for early DN (Balint et al., 2023). In the rat diabetic model constructed by Kikuchi et al. (2019), high levels of phenyl PS were correlated with the severity of glomerular lesions, and

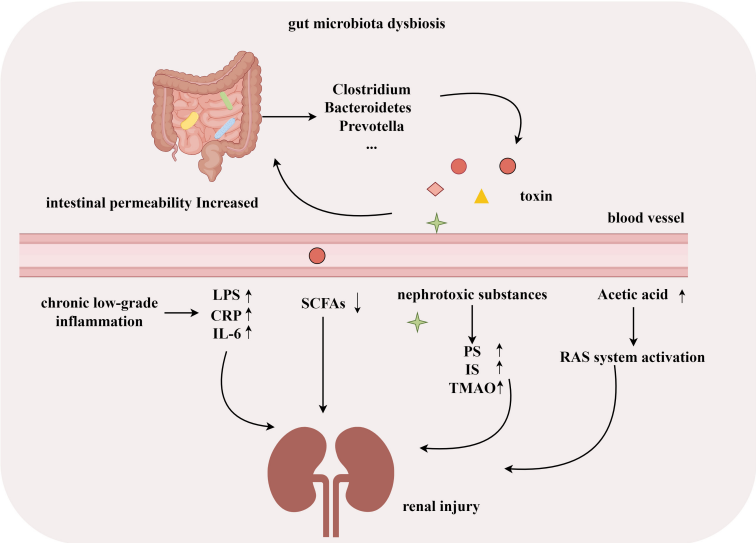

FIGURE 1
Gut microbiota associated with development of DN. (By Figdraw).

TABLE 2 Alteration of metabolic Changes in DN.

Studies	Subjects	The variety of Gut microbiota	Research method
Zhong et al. (2022)	DN patients	**Decreased:** valerate, caproate	GC–MS
Balint et al. (2023)	DN patients	**Increased:** indoxyl sulfate, Butenoylcarnitine, Sorbitol, Dimethyl Arginine **Decreased:** arginine, hippuric acid	UHPLC-QTOF-ESI-MS Analysis
Chen et al. (2023)	DN patients	**Increased:** Stearic acid, Glutaric acid, 2-Amino-3-methylimidazo [4,5-f]quinoline, L-Proline **Decreased:** 1,3,7-Trimethyluric acid, Homocarnosine, Epinephrine, N-Acetylputrescine, Linoleic acid, Ephedrine	UPLC-MS/MS
Peng et al. (2022)	DN patients	**Increased:** L-homocys, 3-sulfinylpyruvate, 2,3-Diketo-5-methythiopentyl-1-phosphate, dehydroalanine, L-cysteine, s-adenosyl-L-methionine, s-methyl-5-thio-D-ribose 1-phosphate, sn-Met-Cys-Ser, Asn-Cys-Pro-Pro **Decreased:** Mercaptopyruvate,	untargeted LC/MS
Kim et al. (2023)	DN patients	**Increased:** valine, isoleucine, methionine, valerate, phenylacetate **Decreased:** acetate	NMR spectroscopy
Shi et al. (2023)	DN patients	**Increased:** urinary metabolites propionic acid, oxoadipic acid, leucine, isovaleric acid, isobutyric acid, and indole-3-carboxylic acid	UPLC-MS/MS
Zhang B. et al. (2022)	DN rats	**Increased:** isomaltose, D-mannose, galactonic acid, citramalic acid, prostaglandin B2 **Decreased:** 3-(2-Hydroxyethyl) indole, 3-methylindole, indoleacrylic acid	UHPLC-QE-MS
Trifonova et al. (2022)	DN patients	**Increased:** L-arginine, L-proline, L-cysteine, citrulline, 4-guanidinobutanamide, N2-succinyl-L-ornithine, creatinine, citrulline, phosphoglycolic, 2-oxo-3-hydroxy-4-phosphobutanoic acids **Decreased:** creatine, thiosulfate, thiocysteine, 3-sulfinylpyruvic acid	MS/MS
Zhu et al. (2022)	DN patients	**Increased:** N-acetylaspartic acid, L-valine, isoleucine, asparagine, betaine, L-methionine	LC–MS
Winther et al. (2020)	DN patients	**Increased:** indoxyl sulphate, L-citrulline. Homocitrulline, L-kynurenine **Decreased:** tryptophan	HPLC MS/MS
Wu et al. (2022)	DN rats	**Increased:** D-arabinose 5-phosphate, estrone 3-sulfate, L-theanine, 3′-aenylic acid, adenosine 5′-monophosphat **Decreased:** aurohyocholic acid sodium salt, calcium phosphorylcholine chloride, tauro-alpha-muricholic, sodium salt, galactinol, phosphocholine	LC-MS

(Continued)

a significant correlation between PS levels and ACR was subsequently demonstrated in human plasma. Receiver operating characteristic curve analysis showed that the combined use of PS with known factors increased the AUC from 0.713 to 0.751. These results indicate that the detection of metabolites is helpful for the early diagnosis of DN and assessment of disease severity, and can be used as a disease marker of DN and a target for future treatment.

3.3 Metabolism associated with occurrence and development of DN

Disturbance of the gut microbiota in DN patients can disrupt intestinal epithelial function, reduce beneficial SCFA production, and release gut-derived toxins (indoxyl sulfate) and inflammatory factors that can damage the kidneys (Meijers and Evenepoel, 2011). Zhong et al. (2022) confirmed that the decreased levels of gut microbiota metabolites valerate and caproate in DN patients are independently related to the progression of DN and can predict the progression of DN to ESRD (Zhong et al., 2022). Urinary metabolomics analysis revealed an increase in urinary myo-inositol concentration with progression of DN. It showed an additive effect in predicting the progression of ESRD in terms of serum creatinine and urinary protein-to-creatinine ratio (Kwon et al., 2023). In the pathways of cysteine and methionine metabolism, serum L-homocysteine and 3-sulfinyl pyruvic acid, as well as 2,3-diketomethylthiobutyryl-1-phosphate, were elevated in the DN group and increased with the progression of DN proteinuria, while mercapto-pyruvate was decreased in the DN group and further decreased in the heavy proteinuria group (Peng et al., 2022). The level of butyrate was decreased in DN patients, and supplementation with sodium butyrate increased autophagy by activating the AMPK/mTOR pathway in DN rats and improving kidney injury (Cai et al., 2022) (Figure 2). Tang et al. (2022) also found a decrease in butyrate levels in DN patients. In db/db mice, supplementation with butyrate can improve intestinal barrier function, activate the PI3K/Akt/mTOR pathway, suppress oxidative stress, and improve muscle atrophy caused by DN. However, some SCFAs have damaging effects on the kidneys. Lu et al. (2020) demonstrated that acetate derived from the gut microbiota activated G-protein-coupled receptor 43, which inhibits AMPKα activity, leading to dysregulation of cholesterol

homeostasis and insulin signaling, and progression of DN. Hu Z. et al. (2020) also reached similar conclusions. These results indicate that the metabolites produced by DN patients in different metabolic pathways and different sample types will have different changes, and the role of various types of SCFAs in DN is still controversial. Therefore, more clinical and animal trials are needed to confirm the mechanism of metabolites in DN.

4 Gut microbiota and microbial metabolites as therapeutic strategies in treatment of DN

4.1 FMT

FMT is the transfer of gut microbiota from healthy individuals to patients with gut microbiota disorders, achieving the goal of rebuilding the homeostasis and diversity of the gut microbiota (Bian et al., 2022). In recent years, FMT has shown specific therapeutic effects in diseases such as migraine (Kappéter et al., 2023), CKD (Liu et al., 2022), and *Clostridium difficile* infection (Wei et al., 2022). After FMT, DN mice showed significant relief of glomerulosclerosis and fibrosis, glomerular injury, basement membrane thickening, and mesangial proliferation, indicating that reconstruction of normal gut microbiota can alleviate DN. In addition, the levels of microbial-derived uremic solutes such as hippuric acid and cholic acid significantly decreased after FMT, indicating that FMT can affect the metabolism of DN mice by regulating microorganisms (Shang et al., 2022). FMT can reduce the destruction of cholesterol homeostasis, thereby improving the damage of renal tubulointerstitium in diabetic rats, suggesting that FMT may be a new strategy for the prevention and treatment of DN (Hu Z. et al., 2020). Another study showed that FMT improved the glomerular injury of streptozotocin (STZ)-induced diabetes in rats (Lu et al., 2021). In a T2DM mouse model, FMT reduced blood sugar, improved glucose tolerance and insulin resistance, and alleviated pancreatic island damage (Wang et al., 2020). These results indicate that FMT may be a new strategy for preventing and treating DN. Although FMT has some potential in the treatment of DN, it is mostly used in animal research, and more clinical trials are needed to confirm its therapeutic efficacy in DN patients, as well as the potential risks.

4.2 Diet

A high-fiber diet contributes to the reconstruction of intestinal microorganisms. After the induction of diabetes by a high-fiber diet and STZ, mice had reduced intestinal Firmicutes, increased Bacteroides, and increased Prevotella and Bifidobacterium, which produce SCFAs. This led to increase in concentration of SCFAs in serum and feces, preventing DN through the key pathways and genes involved in innate immunity, inflammation, and macrophage recruitment (Li et al., 2020). It also caused the generation of probiotics and a significant increase in *Akkermansia muciniphila*. A low carbohydrate diet can cause an increase in the abundance of SCFA-producing bacteria (*Roseburis*) and *Ruminococcus* (Liu K. et al., 2023). Intermittent fasting can improve metabolic diseases such as diabetes and cardiovascular disease by improving the composition of gut microbiota (Liu et al., 2020). Dietary polyphenols can stimulate the secretion of glucagon like peptide-1 (GLP-1) by intestinal L cells to improve glucose homeostasis (Wang et al., 2021). Dietary fiber can promote the production of SCFAs by intestinal bacteria, thereby enhancing insulin sensitivity and GLP-1 secretion (Mazhar et al., 2023). These results indicate that adjusting diet can prevent or delay DN by improving gut microbiota and related metabolites, which is worth further exploration.

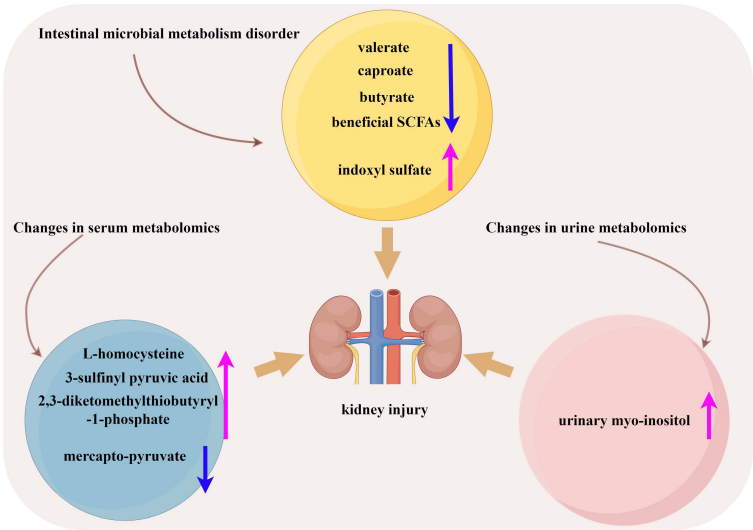

FIGURE 2
Metabolism associated with development of DN. (By Figdraw).

4.3 Probiotics and postbiotics

Probiotics can promote human health by improving intestinal inflammation, regulating gut microbiota homeostasis, repairing cell damage, and regulating immunity, which is important in treating and preventing diseases (Staniszewski and Kordowska-Wiater, 2021; Wolfe et al., 2023). A randomized, double-blind, placebo-controlled trial showed that the intake of probiotics can reduce symptomatic factors by producing SCFAs in the intestine and reducing the production of hydrogen peroxide free radicals, thereby reducing kidney inflammation and fibrosis (Ross, 2022). *Lactobacillus reuteri* GMNL-263 can reduce hemoglobin A1c and blood glucose levels in rats with STZ-induced diabetes, and inhibit renal fibrosis caused by hyperglycemia (Lu et al., 2010). In a randomized controlled clinical trial, DN patients who consumed soy milk containing *Lactobacillus plantarum* A7 for 8 weeks showed significantly lower levels of cystatin C and inflammatory adipokine progranulin than in the soy milk group (Miraghajani et al., 2019). Supplementing probiotic *Lactobacillus casei* Zhang can improve SCFAs and nicotinamide metabolism, reduce renal injury, and delay renal function decline (Zhu et al., 2021). New compound probiotics (*L. plantarum* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) can serve as adjuncts for metformin by increasing the production of butyrate, enhancing glucose metabolism in patients (Liang et al., 2023). In a mouse model of chronic renal failure induced by hyperglycemia, supplementing probiotics (including TYCA06, BLI-02, and VDD088) can alleviate deterioration of renal function in mice (Kuo et al., 2023). These studies suggest that probiotic supplementation is a potential therapy to improve kidney disease caused by diabetes-related metabolism.

Postbiotics come from metabolites or fragments of microorganisms (such as vitamins, lipids, secondary bile acids, bacteriocins, enzymes, extracellular polysaccharides, and SCFAs), and can also regulate gut microbiota without living microorganisms, resulting in lower intake risk (Gao J. et al., 2019; Żółkiewicz et al., 2020). *Bifidobacterium longum* 35624 can produce an extracellular polysaccharide, which prevents bacterial inflammation and promotes barrier function (Schiavi et al., 2016). When there is a sufficient amount of SCFAs in postbiotic formulations, it can improve epithelial barrier function and protect the body from damage induced by lipopolysaccharides (Feng et al., 2018). In a T2DM rat model treated with postbiotics, heat-inactivated *Streptococcus thermophilus* reduced fasting blood glucose levels, glucose tolerance, and insulin resistance, and increased the abundance of beneficial bacteria such as Ruminococcaceae and *Veillonella* (Gao X. et al., 2019). In a randomized double-blind parallel clinical trial, compared with the placebo group, oral pasteurization of *Lactobacillus griffii* CP2305 significantly increased the content of bifidobacteria in the intestines of the experimental group (Sugawara et al., 2016). The mechanism of action of postbiotics in intestinal diseases has not been fully elucidated, and more clinical trials are needed to verify their effectiveness.

4.4 Prebiotics and synbiotics

Prebiotics can regulate glucose metabolism by changing intestinal flora, thus slowing the progress of diabetic complications (Bock et al., 2021). Fructooligosaccharide (FOS) is a common prebiotic. FOS supplementation can improve the renal-related pathological changes caused by diabetes (Pengrattanachot et al., 2022). Similarly, FOS has a protective effect on the kidneys of rats with STZ-induced type 1 diabetes mellitus (T1DM) and improves diabetes-related metabolic abnormalities (Gobinath et al., 2010). Inulin type fructan regulates the gut microbiota of db/db mice, inducing bacterial enrichment that produces SCFAs, leading to an increase in acetate concentration that can improve glomerular injury and renal fibrosis (Luo et al., 2022). Prebiotic supplements can significantly reduce the concentration of uremic toxin cresol sulfate in patients with CKD (Chen L. et al., 2022), increase the level of SCFAs, improve intestinal permeability, and alleviate inflammation (Snelson et al., 2021). Resistant starch (RS) is a prebiotic that promotes the proliferation of beneficial bacteria, such as bifidobacteria and lactobacilli, leading to an increase in SCFA production and a decrease in uremic solutes produced by the microbial community (Snelson et al., 2019). In addition, RS can also alleviate polyuria symptoms and disruption of vitamin D homeostasis in rats with STZ-treated T1DM (Koh et al., 2014).

Synbiotics are a combination of prebiotics and probiotics. Supplementing synbiotics can improve the composition of intestinal microorganisms and delay the progression of diabetic complications (Jiang et al., 2022). Oral administration of synbiotics (containing *Bifidobacterium lactis* HN019, *Lactobacillus rhamnosus HN001*, and oligofructose) can increase the abundance of beneficial bacteria in the intestine, such as *Clostridium sensu stricto 1*, *Bifidobacterium*, *Lactobacillus*, and *Collinsella* (Li et al., 2023), as well as inhibitory effects on pathogens, increased production of SCFAs, and optimized colon function (Rinninella et al., 2019). In a T2DM model, an increase in SCFA-producing bacteria was observed in rats treated with synbiotics (Mangiferin and *L. reuteri* 1-12) (Meng et al., 2023). However, Liu F. et al. (2023) found that synbiotics cannot reduce serum creatinine levels in nondialysis patients, which may be related to different research subjects and pathogenic factors of kidney disease. At present, there is limited research on synbiotics in DN, and a large number of clinical studies are still needed to confirm their effects (Figure 3).

5 Conclusion and prospects

In conclusion, we have summarized the composition of gut microbiota and serum and urine metabolites in patients with DN, elucidating the application of microbiota and microbial metabolites in diagnosing DN and their role in disease progression. Kidney damage in DN patients can lead to dysbiosis of the gut microbiota, and disruption of the microbiota can further impair kidney function by producing numerous metabolites, even causing irreversible lesions. Improving the stability of gut microbiota, enhancing

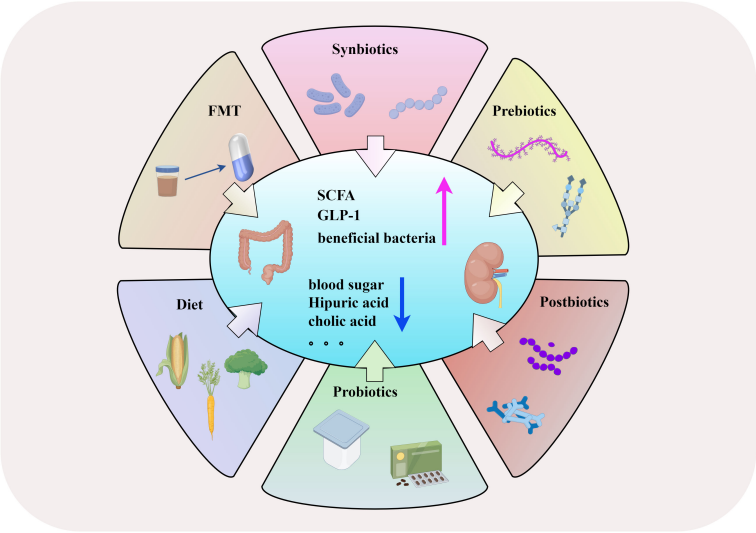

FIGURE 3
The management and therapeutic strategies of DN based on gut microbiota. (By Figdraw).

glucose metabolism, and reducing the production of uremic toxins by adjusting the structure of the diet, FMT, and oral intake of probiotics/prebiotics can delay the progression of DN.

Despite numerous studies, our understanding of the relationship between DN and gut microbiota and metabolism is still in its early stages. Gut microbiota and microbial metabolites show different patterns in different stages of DN, and the underlying mechanisms are poorly understood. Currently, large-scale clinical studies are not conducted in multiple centers, both domestically and internationally. Evaluating gut microbiota and microbial metabolites as therapeutic strategies in the treatment of DN still requires extensive clinical research for validation. Future research should clarify the specific targets of the impact of gut microbiota and related metabolites on DN, providing new insights for diagnosing and treating DN.

Author contributions

J-XY: Writing – original draft. XinC: Writing – original draft. S-GZ: Writing – original draft. XiC: Writing – original draft. Y-YW: Writing – original draft. L-PW: Writing – review & editing. S-HX: Writing – review & editing.

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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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Association between oxidative balance score and diabetic kidney disease, low estimated glomerular filtration rate and albuminuria in type 2 diabetes mellitus patients: a cross-sectional study

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Objective: The oxidative balance score (OBS) is a comprehensive concept that includes 20 oxidative stressors and can be used to assess individual pro-oxidant versus antioxidant exposure, and the aim of the present study was to investigate the association between OBS and the risk of diabetic kidney disease (DKD), low estimated glomerular filtration rate (low-eGFR) and albuminuria in patients with diabetes mellitus (DM).

Methods: This cross-sectional study included nationally representative consecutive National Health and Nutrition Examination Survey DM patients aged 18 years and older from 2003-2018. The continuous variable OBS was converted into categorical variables by quartiles, and weighted multiple logistic regression analyses and restricted triple spline models were used to explore the relationships. We also performed subgroup analyses and interaction tests to verify the stability of the results.

Results: A total of 5389 participants were included, representing 23.6 million non-institutionalized US residents. The results from both multivariate logistic regression analysis and restricted cubic spline models indicated that OBS and dietary OBS levels were negatively associated with the risk of DKD, low-eGFR, and albuminuria, without finding a significant correlation between lifestyle OBS and these clinical outcomes. Compared to the lowest OBS quartile group, the prevalence risk of DKD (OR = 0.61, 95% CI: 0.46-0.80), low-eGFR (OR = 0.46, 95% CI: 0.33-0.64) and albuminuria (OR = 0.68, 95% CI: 0.51-0.92) decreased by 39%, 54% and 32%, respectively, in the highest OBS quartile group. The results remained stable in subgroup analyses and no interaction between subgroups was found.

Conclusion: Higher levels of OBS and dietary OBS were associated with a lower risk of DKD, low-eGFR, and albuminuria. These findings provided preliminary evidence for the importance of adhering to an antioxidant-rich diet and lifestyle among individuals with diabetes.

KEYWORDS

diabetic kidney disease, oxidative balance score, type 2 diabetes mellitus, NHANES, cross-sectional study

1 Introduction

Diabetes Mellitus (DM) is a major disease that endangers human health. According to the International Diabetes Federation, it is estimated that the number of people affected by DM will reach 1.09 billion by 2045 (1), leading to a corresponding increase in the prevalence of diabetic kidney disease (DKD). DKD is responsible for 30% to 50% of end-stage renal disease cases worldwide (2). The burden of DKD is significant, leading to reduced quality of life, increased incapacity, premature death (3), and higher healthcare costs (4). Therefore, it is still imperative to address the urgent issues of early prevention and slowing down the progression of DKD, as well as gaining a comprehensive understanding and effectively controlling the risk factors for its development.

The pathogenesis of DKD is complex (5), and oxidative stress is one of the important triggers (6). Oxidative stress is characterized by an overproduction of reactive oxygen species (ROS) that surpasses the body's antioxidant defense system, resulting in an imbalance in the oxidative system. Hyperglycemia can result in the formation of advanced glycation end-products and ROS. These, in turn, activate intercellular signaling pathways that lead to pro-inflammatory and pro-fibrotic gene expression, causing tissue and cellular damage (7, 8). It is critical to maintain the balance of the body's redox system to slow the progression of DKD (9). These findings have also sparked exploration into whether the intake of antioxidants can reduce the risk of DKD. However, the results are not entirely consistent (10–12), which is associated with the presence of various antioxidant and pro-oxidant factors in diet and lifestyle. Conclusions drawn from a sole evaluation of a specific factor may be one-sided.

The oxidative balance score (OBS) is a comprehensive indicator that considers various dietary components and lifestyle factors to assess an individual's exposure to pro-oxidants and antioxidants. A higher OBS indicates a stronger antioxidant capacity. Since oxidative stress is an important pathogenic mechanism in DKD, managing and improving oxidative balance may be a crucial strategy for the prevention and treatment of DKD. This includes consuming an adequate amount of antioxidants in the diet and maintaining a healthy lifestyle. Although the OBS has been widely used in numerous studies and a higher OBS has been associated with a lower prevalence and incidence of chronic kidney disease (CKD) (13, 14), as well as various diseases such as chronic obstructive pulmonary disease (15), kidney stones, cardiovascular disease (CVD) (16), and depression (17), research on the relationship between OBS and DKD is currently lacking. Given that individuals with diabetes are among the most susceptible populations to kidney damage, the presence of DKD complicates diabetes management, increases the risk of CVD, and mortality. The primary objective of this study is to utilize data from the National Health and Nutrition Examination Survey (NHANES) to conduct a cross-sectional analysis among the diabetic population to explore the association between OBS and DKD. The aim is to provide new insights and strategies for the prevention and treatment of DKD.

2 Materials and methods

2.1 Study population

This investigation is a nationwide cross-sectional study, utilizing data from the NHANES 2003-2018 survey cycle. NHANES is administered by the National Center for Health Statistics, a division of the Centers for Disease Control and Prevention, and has been approved by an ethics review board. The NHANES sample design incorporates a multi-year, stratified, clustered four-stage sampling approach, with data released in 2-year cycles (adjustments to the sampling methods for each cycle can be accessed via the following URL: https://wwwn.cdc.gov/nchs/nhanes/analyticguidelines.aspx#sample-design). Investigators gather comprehensive information from participants through home interviews and laboratory testing to assess the health and nutritional status of the non-institutionalized civilian population across the United States (18, 19), and all participants have provided informed consent.

Participants in this study underwent screening based on the following criteria (Figure 1): individuals were excluded if (1) <18 years; (2) Items comprising the OBS <16 (16); (3) incomplete data of urinary albumin-to-creatinine ratio (ACR); (4) incomplete data

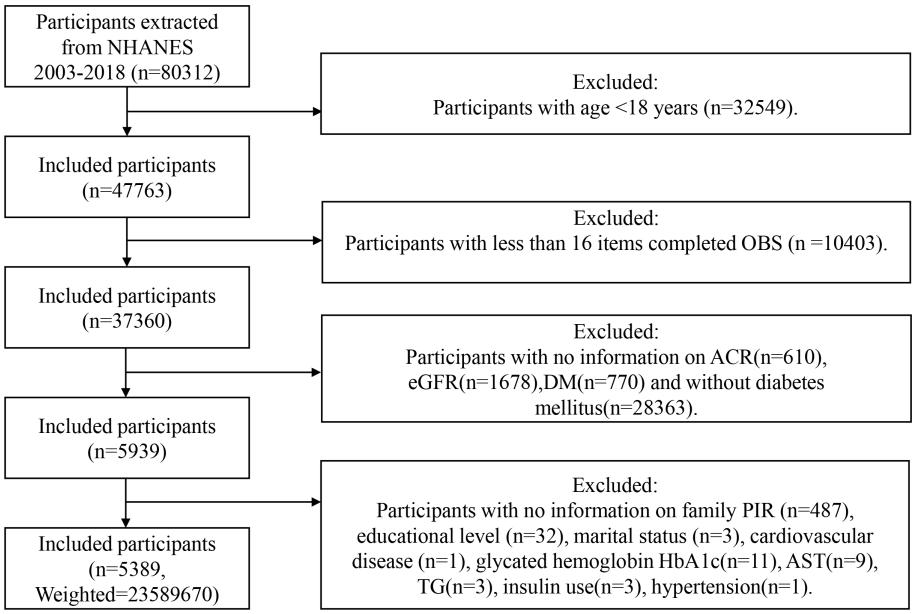

FIGURE 1
Flowchart of the sample selection from NHANES 2003–2018. NHANES, National Health and Nutrition Examination Survey; OBS, oxidative balance score; family PIR, family poverty income ratio; ACR, urinary albumin-to-creatinine ratio; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; AST, aspartate aminotransferase; TG, triglycerides.

of estimated glomerular filtration rate (eGFR); (5) without DM; (6) pregnant; (7) incomplete data of covariates.

2.2 Definition of the oxidative balance score

The OBS is based on 16 dietary nutrients and 4 lifestyle factors. The 16 dietary nutrients comprise of dietary fiber, carotenoids, riboflavin, niacin, vitamin B6, total folate, vitamin B12, vitamin C, vitamin E, calcium, magnesium, zinc, copper, selenium, total fat, and iron. The 4 lifestyle factors include physical activity, body mass index (BMI), alcohol consumption, and smoking status. The study identified pro-oxidant factors, including total fat, total iron intake, smoking status, alcohol consumption status, and BMI. The remaining factors were classified as antioxidants (20). Food type and quantity intake were obtained by two 24-hour dietary recall interviews (the first assessment was administered at the Mobile Examination Center, followed by a telephone follow-up for the second assessment. The mean value of the data from both assessments was calculated, in the event of missing data from the second interview, the results from the first interview were considered as the default.), and based on the Food Intake Analysis System of the University of Texas and the United States Department of Agriculture Survey Nutritional Database. The amount of physical activity was calculated as the total metabolic equivalent of task (MET) level of all exercises performed in a week (metabolic equivalent per physical activity × frequency of physical activity × duration). The smoking level was determined by the cotinine level. Alcohol consumption was categorized into three groups based on gender: non-drinkers, moderate drinkers (0-15 g/d for women and 0-30 g/d for men), and heavy drinkers (≥15 g/d for women and ≥30 g/d for men). Obesity scores were assigned based on weight status, obesity: BMI≥30 kg per square meter (kg/m^2), overweight: 25≤BMI<30 kg/m^2, and normal weight: BMI<25 kg/m^2. The OBS was calculated as follows (refer to Supplementary Table 1): non-drinkers, moderate drinkers, and heavy drinkers were scored 2, 1, and 0, respectively; obesity, overweight, and normal weight were scored 0, 1, and 2, respectively. The other components were grouped in tertiles by gender, with antioxidants in groups 1 - 3 being assigned a score of 0-2 and pro-oxidant factors in groups 1-3 being assigned a score of 2-0. Taking fiber intake as an example in men, daily fiber intake of ≤12.7g is assigned 0 point, between 12.7 and 19.85g is 1 point, and >19.85g is 2 points. Each component contributes an equal weight, and the sum of the scores yields the OBS. A higher OBS indicates a stronger antioxidant profile.

2.3 Definition of DKD, low-eGFR, and albuminuria

The diagnosis of diabetes (21): the use of diabetes medication or insulin, a diagnosis by a doctor, glycated hemoglobin HbA1c ≥6.5%, fasting glucose ≥7 mmol/L, 2-h oral glucose tolerance test with blood glucose ≥11.1 mmol/L. The DKD was defined as the diabetes combined with albuminuria (ACR≥30 mg/g) and/or low-eGFR (eGFR<60 mL/min/1.73 m^2) according to the KDIGO 2021 Guidelines (22). For eGFR calculation, we applied the CKD-Epidemiology Collaboration equation as follows (23): GFR = 141·min [Scr/κ,1]α × max [Scr/κ, 1] − 1.209 × 0.993 Age × 1.018 [if women] ×1.159 [if black]); κ was 0.7(women) or 0.9(men), a was

−0.329 (women) or −0.411(men), and min/max indicate the minimum/maximum of Scr/κ or 1.

2.4 Covariates assessment

Based on previous research (24, 25), we included a number of covariates that may influenced the results of the study. Demographic factors included age, gender, marital status (divorced/separated/widowed, married/living with a partner, never married), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American and other), education level (less than high school, high school diploma, more than high school), the family poverty income ratio (PIR) (<1.3, 1.3-3.5, >3.5); laboratory indicators including aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), triglycerides (TG), albumin (ALB), fasting glucose, glycated hemoglobin HbA1c; Additionally, we considered a number of chronic diseases: hypertension (taking blood pressure-lowering medication, diagnosed by a physician as having a high systolic blood pressure ≥140mmhg or diastolic blood pressure ≥90mmhg) (26), hyperlipidemia (taking cholesterol-lowering drugs, total cholesterol ≥200 mg/dL, triglyceride ≥150 mg/dL, low-density lipoprotein ≥130 mg/dL, or high-density lipoprotein ≤50 mg/dL for women and ≤40 mg/dL for men) (27), CVD (coronary heart disease, congestive heart failure, heart attack, stroke, angina) and metabolic syndrome(Mets), additionally insulin use.

2.5 Statistical analyses

In light of the stratified and complex multistage sampling design employed by NHANES, we have weighted the data using the sample weight calculation method recommended by NHANES. This involved pooling data from eight cycles spanning the period from 2003 to 2018, where the weights for 16 years are calculated to be one-eighth of the weights for 2 years. This weighting approach corrects for the imbalance in the samples, allowing for a more accurate reflection of the characteristics of the overall population. Categorical variables are presented as weighted percentages, while continuous variables are described using mean ± standard deviations. The Shapiro-Wilk statistical test was employed to confirm the normal distribution of continuous variables. Variables with skewed distributions are represented by medians and quartiles. We converted the continuous variable OBS into categorical variables by quartiles (Q1: ≤P25; Q2: P25-P50; Q3: P50-P75; Q4: >P75) and assessed differences between OBS (quantile) groups using weighted t-tests or chi-square tests. Multiple logistic regression is suitable for analyzing the relationship between multiple predictor variables and a binary outcome variable, allowing for the control of other potential confounding variables. Weighted multivariate logistic regression analysis was employed to evaluate the correlation between OBS, dietary OBS, lifestyle OBS, and DKD, low-eGFR, as well as albuminuria across three models. The results were reported as odds ratios (ORs) with their 95% confidence intervals (95% CIs), *p*-values, and trend *p*-values. Collinearity among variables was assessed using the variance inflation factor (VIF). The VIF quantifies the increased variance of an estimated regression coefficient due to collinearity. A common rule of thumb is that VIF values greater than 5 indicate a problematic level of collinearity (28), which can lead to instability in regression results and weaken predictive capabilities. In Model 1, adjustments were made for gender, age, marital status, race/ethnicity, education level, and family PIR. Model 2 adjusted for hypertension, hyperlipidemia, CVD, Mets, and insulin use based on Model 1. Model 3 adjusted for all covariates. A multivariate-adjusted restricted cubic spline model was employed to more accurately capture the relationship between continuous variables and the outcome. The model established OR curves at three knot points to explore whether there exists a nonlinear dose-response association between OBS and DKD, low-eGFR, and albuminuria. To determine the potential effect moderators, subgroup analyses were performed based on subjects' age, sex, and whether they had hypertension, hyperlipidemia, CVD, and Mets, and interaction analyses were performed to check the heterogeneity of the relationship between subgroups. A two-sided *P* value of <0.05 was considered a statistically significant difference. R software (R version 4.3.3) was used for all statistical analyses in this study.

3 Results

3.1 Baseline characteristics

The study included 5389 participants, representing 23.6 million non-institutionalized residents of the United States. The mean age of all subjects was 59.57, with 50.37% males and 39.63% females. Among DM patients, the weighted prevalence of DKD, low-eGFR, and albuminuria was 36.57%, 18.38%, and 25.82%, respectively. Furthermore, we discovered significant differences ($p < 0.05$) in race, education level, marital status, family PIR, ALT, ALB, prevalence of hypertension, CVD and Mets, and insulin use among the OBS groups. Table 1 presents the baseline characteristics of the study participants, categorized by their OBS quartiles.

3.2 Association between OBS, dietary OBS, lifestyle OBS with DKD, low-eGFR and albuminuria

As shown in Supplementary Table 2, all covariates exhibit VIF values less than 5, suggesting that collinearity has a minimal impact on the results.

As depicted in Tables 2–4, weighted multivariate logistic regression was employed to examine the association between OBS and the risk of DKD, low-eGFR, and albuminuria. The results revealed that the risk of DKD, low-eGFR, and albuminuria decreased with increasing quartiles across all models (*p* for trend <0.05). In model 3, which was fully adjusted, compared to the lowest quartile of OBS, the highest quartile was associated with a

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TABLE 1 Characteristics of participants by quartiles of the OBS in the NHANES 2003–2018 cycles.

Variables	Total	Q1, [3, 13]	Q2, (13,18]	Q3, (18,24]	Q4, (24,35]	*p* value
	n = 23589670	n = 5424960	n = 5207344	n = 6636816	n = 6320550	
Age, years	59.57 ± 13.58	60.12 ± 13.52	60.32 ± 13.86	50.48 ± 13.92	58.56 ± 12.97	0.0626
Sex						0.5359
Male	50.37	52.57	49.19	50.92	48.89	
Female	49.63	47.43	50.81	49.08	51.11	
Race and ethnicity						< 0.0001
Non-Hispanic White	64.95	59.67	62.90	65.38	70.72	
Non-Hispanic Black	13.49	20.10	15.59	11.18	8.49	
Mexican American	8.79	8.05	9.57	9.27	8.28	
Other	12.77	12.18	11.94	14.16	12.50	
Educational level						< 0.0001
Less than high school	22.57	34.14	24.16	20.28	13.74	
High school diploma	26.20	27.79	28.99	23.25	25.62	
More than high school	51.23	38.06	46.85	56.46	60.64	
Marriage status						0.0144
Divorced/separated/widowed	27.60	31.10	28.84	28.29	22.84	
Married/living with a partner	64.02	59.04	62.82	63.59	69.73	
Never married	8.38	9.85	8.34	8.12	7.43	
Family PIR						< 0.0001
<1.3	24.85	36.39	28.07	21.15	16.19	
1.3-3.5	39.91	39.12	44.55	39.58	37.10	
≥3.5	35.24	24.49	27.38	39.27	46.71	
Hyperlipidemia						0.738
Yes	88.93	88.85	88.9	88.16	89.82	
No	11.07	11.15	11.10	11.84	10.18	
Hypertension						0.0107
Yes	70.75	73.73	74.72	68.47	67.31	
No	29.25	26.27	25.28	31.53	32.69	
Cardiovascular disease						< 0.0001
Yes	24.97	31.77	24.67	24.02	20.38	
No	75.03	68.23	75.33	75.98	79.62	
Metabolic syndrome						0.0184
Yes	76.86	76.30	79.37	79.14	72.86	
No	23.14	23.70	20.63	20.86	27.14	
Insulin use						0.0431
Yes	19.17	18.89	23.06	17.00	18.46	
No	80.83	81.11	76.94	83.00	81.54	
Glycohemoglobin, %	7.06 ± 1.60	7.11 ± 1.71	7.12 ± 1.58	7.01 ± 1.56	7.03 ± 1.56	0.3577
Fasting glucose, mmol/L	8.00 ± 3.56	8.02 ± 3.74	8.16 ± 3.60	7.95 ± 3.40	7.90 ± 3.51	0.6039

(Continued)

TABLE 1 Continued

Variables	Total	Q1, [3, 13]	Q2, (13,18]	Q3, (18,24]	Q4, (24,35]	*p* value
	n = 23589670	n = 5424960	n = 5207344	n = 6636816	n = 6320550	
AST, U/L	23(19, 29)	23(19, 28)	23(19, 29)	23(19, 29)	23(19, 29)	0.2665
ALT, U/L	23(17, 31)	21(16, 30)	23(17, 32)	23(18, 31)	24(18, 31)	0.0005
TC, mmol/L	4.88 ± 1.24	4.95 ± 1.34	4.91 ± 1.24	4.87 ± 1.22	4.80 ± 1.19	0.2921
TG, mmol/L	1.81(1.22, 2.62)	1.74(1.23, 2.63)	1.83(1.22, 2.57)	1.91 (1.24, 2.69)	1.74(1.20, 2.62)	0.2162
ALB, g/dL	4.15 ± 0.33	4.09 ± 0.34	4.14 ± 0.33	4.18 ± 0.33	4.18 ± 0.32	0.0001
ACR, mg/g	11.30(6.28, 31.50)	13.74(7.09, 50.00)	11.39(6.55,30.40)	11.20(5.98, 26.88)	10.00(5.77,23.29)	<0.0001
Albuminuria						<0.0001
Yes	25.82	34.26	25.35	23.53	21.36	
No	74.18	65.74	74.65	76.47	78.64	
eGFR, mL/min/1.73 m^2	82.93 ± 24.19	79.62 ± 26.55	81.11 ± 25.47	84.07 ± 23.11	86.06 ± 21.47	<0.0001
Low-eGFR						<0.0001
Yes	18.38	23.52	23.40	16.59	11.73	
No	81.62	76.48	76.60	83.41	88.27	
DKD						<0.0001
Yes	36.57	45.20	40.05	34.19	28.80	
No	63.43	54.80	59.95	65.81	71.20	

OBS, oxidative balance score; Q, quartiles; NHANES: National Health and Nutrition Examination Survey; DKD, diabetic kidney disease; Family PIR, family poverty income ratio; TC, total cholesterol; TG, triglycerides; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin; ACR, urinary albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate.
Data presented as weighted percentage for categorical variables and mean+ SD for continuous variables, and skewed distribution variables as medians and quartiles.

39% reduction in the risk of DKD (OR = 0.61, 95% CI: 0.46-0.80), a 54% reduction in the risk of low-eGFR (OR = 0.46, 95% CI: 0.33-0.64), and a 32% reduction in the risk of albuminuria (OR = 0.68, 95% CI: 0.51-0.92).

Moreover, weighted multivariate logistic regression was also used to investigate the impact of dietary OBS and lifestyle OBS on the risk of DKD, low-eGFR, and albuminuria. The results indicated that both dietary OBS and lifestyle OBS were considered protective factors, although there was no statistically significant difference between the highest and lowest quartiles of lifestyle OBS.

3.3 Analysis of restricted cubic spline regression

Based on the results of the multivariate regression analysis, restricted cubic spline regression was employed to examine the relationship between OBS, dietary OBS, and the outcomes. After adjusting for all covariates, it was found that OBS was linearly associated with DKD, low-eGFR, and albuminuria. Figures 2A–C respectively display the trend of OR values for DKD, low-eGFR, and albuminuria decreasing with increasing OBS. Notably, although dietary OBS exhibited linear associations with DKD and low-eGFR (Figures 2D, E), it showed a non-linear association with albuminuria (p for non-linearity = 0.021, p for overall = 0.004). Specifically, Figure 2F illustrates that as dietary OBS increased, the risk of albuminuria initially decreased and then increased.

3.4 Analysis of subgroup

In the subgroup analysis, we observed inconsistent associations. In the CVD and Mets subgroups, OBS was negatively correlated with DKD. However, in other subgroups, the correlation between OBS and DKD was only observed in individuals aged ≥60 years, males, those with hyperlipidemia or hypertension (Figure 3). A negative correlation between OBS and low-eGFR was present in all subgroups (Figure 4). The correlation between OBS and albuminuria was confined to subgroups of individuals aged ≥60 years, males, and those with hyperlipidemia, hypertension, CVD, or Mets (Figure 5). No interaction was found among the subgroups.

4 Discussion

We conducted a cross-sectional analysis of 5,389 participants from the NHANES, representing an estimated 23.6 million noninstitutionalized residents of the United States. To the best of our knowledge, this is the first study to explore the relationship between OBS and DKD. The results showed that OBS, as well as

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TABLE 2 Weighted logistic regression analysis models showing the associations between OBS and DKD.

Variable	OBS levels quartile						*p* value	*p* for trend
	Q1	Q2	*p* value	Q3	*p* value	Q4		
	OR (95%CI)	OR (95%CI)		OR (95%CI)		OR (95%CI)		
OBS								
Unadjusted	1.00(ref)	0.81(0.65-1.01)	0.065	0.63(0.52-0.77)	<0.001	0.49(0.38-0.63)	<0.001	<0.001
Model 1	1.00(ref)	0.82(0.65-1.05)	0.112	0.68(0.55-0.85)	<0.001	0.58(0.44-0.76)	<0.001	<0.001
Model 2	1.00(ref)	0.80(0.62-1.04)	0.09	0.70(0.55-0.88)	0.003	0.59(0.45-0.78)	<0.001	<0.001
Model 3	1.00(ref)	0.81(0.63-1.05)	0.116	0.71(0.56-0.91)	0.006	0.61(0.46-0.80)	<0.001	<0.001
Dietary OBS								
Unadjusted	1.00(ref)	0.72(0.58-0.89)	0.003	0.65(0.53-0.81)	<0.001	0.49(0.39-0.62)	<0.001	<0.001
Model 1	1.00(ref)	0.73(0.58-0.92)	0.007	0.71(0.56-0.90)	0.004	0.60(0.46-0.77)	<0.001	<0.001
Model 2	1.00(ref)	0.71(0.56-0.90)	0.005	0.72(0.56-0.92)	0.008	0.60(0.46-0.77)	<0.001	<0.001
Model 3	1.00(ref)	0.73(0.57-0.92)	0.009	0.74(0.57-0.95)	0.017	0.61(0.47-0.79)	<0.001	<0.001
Lifestyle OBS								
Unadjusted	1.00(ref)	1.03(0.82-1.30)	0.77	0.75(0.62-0.92)	0.006	0.69(0.49-0.97)	0.033	<0.001
Model 1	1.00(ref)	0.95(0.74-1.22)	0.687	0.68(0.55-0.85)	<0.001	0.57(0.38-0.85)	0.006	<0.001
Model 2	1.00(ref)	0.95(0.74-1.23)	0.71	0.74(0.60-0.93)	0.009	0.68(0.44-1.05)	0.081	0.004
Model 3	1.00(ref)	0.96(0.75-1.23)	0.744	0.79(0.64-0.99)	0.04	0.74(0.47-1.15)	0.174	0.027

Model 1: Adjusted for age, sex, race, marriage status, education and family PIR.
Model 2: Adjusted for model 1 + hypertension, hyperlipidemia, cardiovascular disease, metabolic syndrome, insulin use.
Model 3: Adjusted for model 2 + AST, ALT, TC, TG, glycohemoglobin, fasting glucose, ALB.
OBS, oxidative balance score; DKD, diabetic kidney disease. Q, quartiles; OR, odds ratio; 95% CI, 95% confidence interval.

TABLE 3 Weighted logistic regression analysis models showing the associations between OBS and low-eGFR.

Variable	OBS levels quartile						*p* value	*p* for trend
	Q1	Q2	*p* value	Q3	*p* value	Q4		
	OR (95%CI)	OR (95%CI)		OR (95%CI)		OR (95%CI)		
OBS								
Unadjusted	1.00(ref)	0.99(0.77-1.28)	0.959	0.65(0.51-0.82)	<0.001	0.43(0.34-0.56)	<0.001	<0.001
Model 1	1.00(ref)	0.94(0.72-1.24)	0.67	0.60(0.45-0.79)	<0.001	0.43(0.31-0.59)	<0.001	<0.001
Model 2	1.00(ref)	0.94(0.70-1.25)	0.664	0.62(0.45-0.85)	0.003	0.44(0.32-0.62)	<0.001	<0.001
Model 3	1.00(ref)	0.97(0.72-1.31)	0.831	0.64(0.47-0.86)	0.004	0.46(0.33-0.64)	<0.001	<0.001
Dietary OBS								
Unadjusted	1.00(ref)	0.91(0.70-1.18)	0.48	0.69(0.54-0.89)	0.005	0.44(0.33-0.57)	<0.001	<0.001
Model 1	1.00(ref)	0.88(0.65-1.18)	0.378	0.68(0.50-0.92)	0.014	0.48(0.35-0.66)	<0.001	<0.001
Model 2	1.00(ref)	0.87(0.64-1.17)	0.347	0.68(0.49-0.94)	0.021	0.48(0.34-0.67)	<0.001	<0.001
Model 3	1.00(ref)	0.91(0.67-1.24)	0.549	0.69(0.49-0.96)	0.027	0.49(0.35-0.68)	<0.001	<0.001
Life OBS								
Unadjusted	1.00(ref)	1.36(1.04-1.77)	0.024	1.03(0.78-1.35)	0.85	0.98(0.68-1.40)	0.901	0.63
Model 1	1.00(ref)	1.03(0.78-1.37)	0.821	0.73(0.55-0.97)	0.031	0.56(0.37-0.87)	0.01	0.001

(Continued)

TABLE 3 Continued

Variable	OBS levels quartile						*p* value	*p* for trend
	Q1	Q2	*p* value	Q3	*p* value	Q4		
	OR (95%CI)	OR (95%CI)		OR (95%CI)		OR (95%CI)		
Life OBS								
Model 2	1.00(ref)	1.04(0.79-1.38)	0.763	0.84(0.63-1.14)	0.262	0.76(0.48-1.19)	0.225	0.114
Model 3	1.00(ref)	1.02(0.77-1.36)	0.885	0.91(0.68-1.23)	0.536	0.85(0.53-1.37)	0.508	0.386

Model 1: Adjusted for age, sex, race, marriage status, education and family PIR.
Model 2: Adjusted for model 1 + hypertension, hyperlipidemia, cardiovascular disease, metabolic syndrome, insulin use.
Model 3: Adjusted for model 2 + AST, ALT, TC, TG, glycohemoglobin, fasting glucose, ALB.
OBS, oxidative balance score; DKD, diabetic kidney disease. Q, quartiles; OR, odds ratio; 95% CI, 95% confidence interval.

dietary OBS, was negatively associated with the risk of DKD, low-eGFR, and albuminuria, but no significant correlation was found between lifestyle OBS and these clinical outcomes. In the subgroup analysis, OBS was associated with DKD in both the CVD and Mets subgroups, in other subgroups, the association was observed only in individuals aged ≥60 years, males, those with hyperlipidemia or hypertension. Collectively, our study provides preliminary evidence for the relationship between OBS and DKD and offers new insights for future clinical and basic research.

In this study, we investigated the relationship between 20 oxidative stressors and DKD. Most of these oxidative stressors have been shown to prevent the development of DKD by modulating redox homeostasis. A cross-sectional study based on the MHANES database similarly found that maintaining an adequate antioxidant diet, as reflected in higher composite dietary antioxidant index (CDAI) levels, may lower the risk of DKD and mortality in diabetic individuals (29). This further supports our findings, yet differently, the OBS incorporates a more comprehensive range of antioxidant dietary factors while also considering lifestyle factors, which may provide a broader perspective for the comprehensive management of diabetic patients. Another study assessing the correlation between OBS, CDAI, and diabetic retinopathy (DR) found a negative association between OBS and DR after adjusting for potential confounders, but

TABLE 4 Weighted logistic regression analysis models showing the associations between OBS and albuminuria.

Variable	OBS levels quartile						*p* value	*p* for trend
	Q1	Q2	*p* value	Q3	*p* value	Q4		
	OR (95%CI)	OR (95%CI)		OR (95%CI)		OR (95%CI)		
OBS								
Unadjusted	1.00(ref)	0.65(0.51-0.82)	<0.001	0.59(0.47-0.74)	<0.001	0.52(0.40-0.68)	<0.001	<0.001
Model 1	1.00(ref)	0.69(0.54-0.88)	0.003	0.67(0.53-0.85)	0.001	0.65(0.49-0.87)	0.004	0.004
Model 2	1.00(ref)	0.66(0.51-0.85)	0.002	0.69(0.54-0.88)	0.003	0.66(0.49-0.89)	0.006	0.01
Model 3	1.00(ref)	0.66(0.51-0.86)	0.002	0.70(0.55-0.90)	0.005	0.68(0.51-0.92)	0.012	0.021
Dietary OBS								
Unadjusted	1.00(ref)	0.58(0.45-0.74)	<0.001	0.60(0.48-0.77)	<0.001	0.53(0.41-0.69)	<0.001	<0.001
Model 1	1.00(ref)	0.62(0.48-0.80)	<0.001	0.68(0.53-0.87)	0.003	0.67(0.52-0.87)	0.003	0.01
Model 2	1.00(ref)	0.60(0.46-0.78)	<0.001	0.69(0.53-0.89)	0.005	0.67(0.51-0.88)	0.004	0.016
Model 3	1.00(ref)	0.61(0.47-0.78)	<0.001	0.71(0.55-0.93)	0.012	0.69(0.52-0.91)	0.009	0.032
Life OBS								
Unadjusted	1.00(ref)	0.86(0.68-1.09)	0.206	0.67(0.55-0.83)	<0.001	0.58(0.41-0.82)	0.002	<0.001
Model 1	1.00(ref)	0.85(0.66-1.09)	0.193	0.67(0.53-0.84)	<0.001	0.56(0.38-0.82)	0.003	<0.001
Model 2	1.00(ref)	0.84(0.65-1.09)	0.186	0.71(0.57-0.90)	0.004	0.65(0.43-0.97)	0.037	0.002
Model 3	1.00(ref)	0.86(0.67-1.10)	0.227	0.77(0.61-0.96)	0.022	0.69(0.46-1.05)	0.086	0.015

Model 1: Adjusted for age, sex, race, marriage status, education and family PIR.
Model 2: Adjusted for model 1 + hypertension, hyperlipidemia, cardiovascular disease, metabolic syndrome, insulin use.
Model 3: Adjusted for model 2 + AST, ALT, TC, TG, glycohemoglobin, fasting glucose, ALB.
OBS, oxidative balance score; DKD, diabetic kidney disease. Q, quartiles; OR, odds ratio; 95% CI, 95% confidence interval.

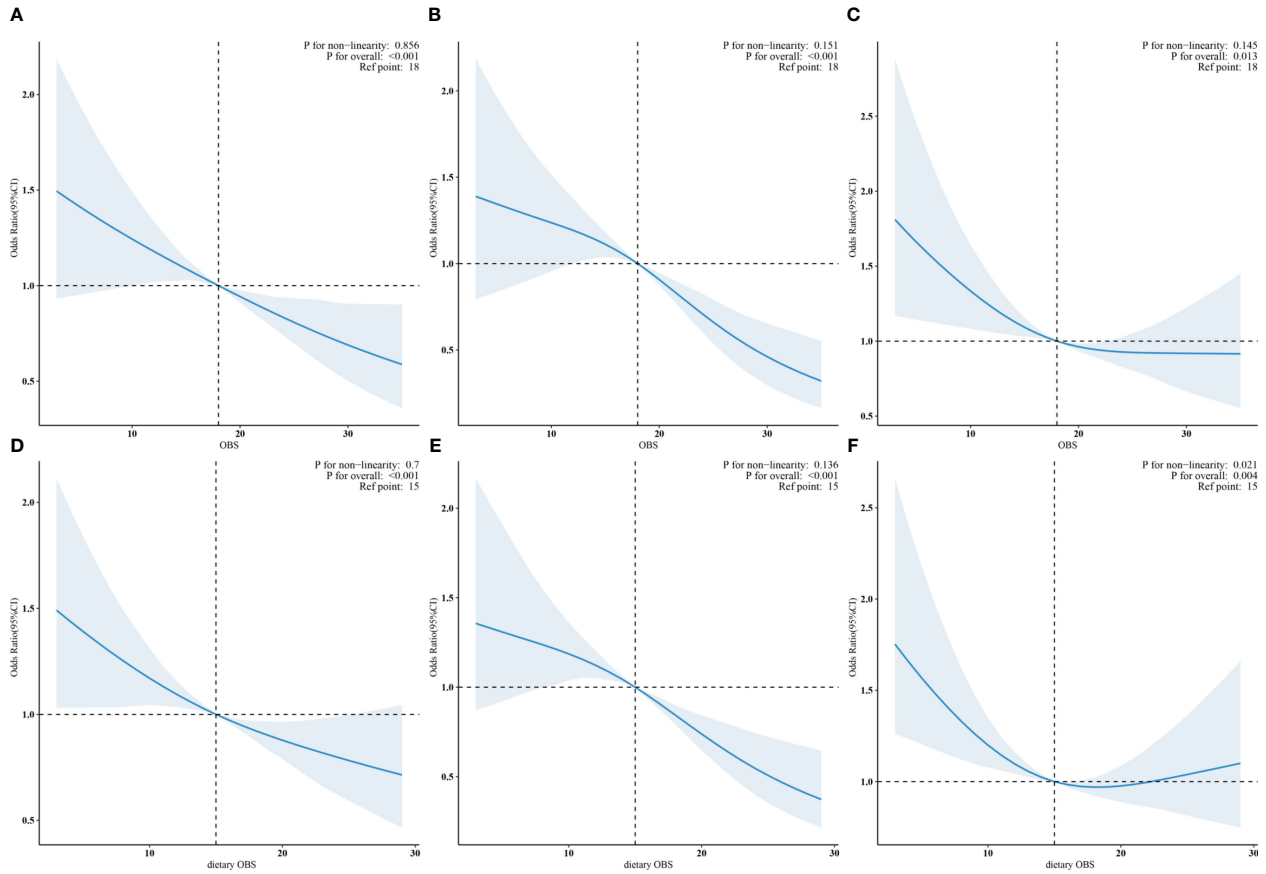

FIGURE 2
(A–C): Linear associations of OBS with DKD, low-eGFR, and albuminuria. **(D, E)**: Linear trends in dietary OBS related to DKD and low-eGFR. **(F)**: Non-linear relation of dietary OBS to albuminuria.

no such association was found in CDAI (30). It is well-known that both DR and DKD are microvascular complications of diabetes, with a shared pathogenesis involving endothelial dysfunction, and oxidative stress being one of the key pathological pathways (31). Additionally, some studies have focused on the impact of single factors on DKD, with varying results. In terms of dietary factors, an animal experiment (32) have suggested that crocin (water-soluble carotenoids) may reduce proteinuria levels by its antioxidant properties, enhancing the host's antioxidant defense system, inhibiting inflammation and fibrosis. Ozcelik et al. (33, 34) found that zinc sulfate supplementation in diabetic rats can reduce kidney damage by activating metallothionein, which interact with Zn and iron to reduce ROS. A study (35) has demonstrated that vitamin C and E supplementation, as well as a combination of magnesium, zinc, and vitamins C and E, can decrease urinary albumin excretion levels and improve glomerular function in type 2 diabetes patients. Selenium supplementation for 12 weeks in patients with DKD has been shown to be beneficial for plasma glutathione peroxidase (GPx) and serum insulin levels (36). Folic acid, betaine, vitamins B6 and B12 have also been reported to delay the development of T2 DM by methylating degraded homocysteine (37). However, a meta-analysis suggests that neither vitamin B alone nor in combination improved renal function or blood pressure in diabetic patients. Due to the limited number and lower quality of included studies, these findings require further confirmation (38). In terms of lifestyle, exercise training has been shown to alleviate oxidative stress and inflammation in type 2 diabetic rats (39). Moreover, a lack of physical activity is more likely to cause obesity, exacerbating kidney damage. Smoking can lead to insulin resistance, induce advanced glycation end products, and increase kidney vascular permeability. Excessive alcohol consumption leads to the production of large amounts of ROS and reduces the antioxidant activity of GPx (40). Most of the above studies focusing on the association of a single factor with DKD are consistent with our results considering multiple factors together, suggesting that OBS may improve DKD by modulating oxidative homeostasis.

In the present study, restricted cubic spline regression showed that the OR of albuminuria showed a trend of decreasing followed by a slight increase with increasing dietary OBS, but overall the risk of albuminuria was still significantly lower in the higher dietary OBS group compared with the lower dietary OBS group, which may be related to the threshold effect of some antioxidants and interactions between antioxidants (41), beyond which antioxidants may exhibit pro-oxidant or depleting antioxidant properties. For example, the antioxidant properties of carotenoids depend on their interactions with vitamins E and C (42), and carotenoids may lose their antioxidant properties at high concentrations or high partial pressures of oxygen. Copper may also be pro-oxidant in certain environments, and vitamin E may play an inhibitory role in copper-dependent low density lipoprotein

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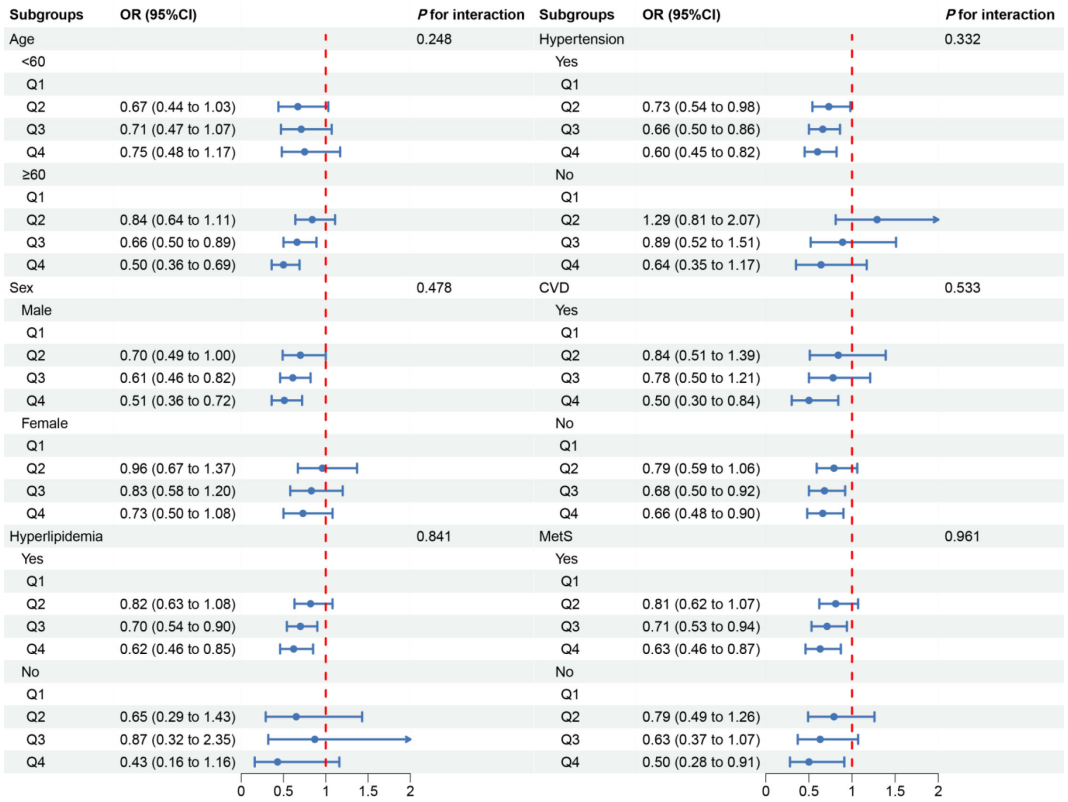

FIGURE 3
Associations between OBS and DKD in different subgroups. OR, odds ratio; 95% CI, 95% confidence interval; CVD, cardiovascular disease; Mets, metabolic syndrome. Except for the stratification component itself, each stratification factor was adjusted for all covariates.

oxidation. In addition, lifestyle factors such as adequate physical activity, smoking cessation, alcohol restriction and weight control have beneficial antioxidant effects in a variety of diseases, including DKD. However, no direct relationship between lifestyle OBS and DKD, low-eGFR and albuminuria was found in the present study, which may be due to the insufficient degree of lifestyle variability in the study population or the possibility that lifestyle factors may influence DKD by interacting with other variables. Studies have reported clear benefits of an antioxidant lifestyle in reducing blood pressure and improving insulin resistance (43, 44). A cohort study conducted in China (45) explored the relationship between the triglyceride-glucose (TyG) index, a biomarker associated with insulin resistance, and the risk of CKD in hypertensive patients with abnormal glucose metabolism. The study found that a higher TyG index was associated with an increased risk of CKD, suggesting that an antioxidant lifestyle may indirectly influence the progression of DKD by affecting metabolic parameters such as the TyG index (46–48). For this reason, the present study also prefers to use the overall OBS to systematically assess the redox status of the organism and to avoid considering the effect of a single factor while ignoring the complex correlations and interactions between antioxidants.

In subgroup analyses, OBS was associated with DKD in both the CVD and Mets subgroups, in other subgroups, the association was present only in individuals aged 60 years or older, males and those with hyperlipidemia or hypertension. A cross-sectional study suggests that OBS is inversely associated with accelerated phenotypic ageing (49). Oxidative damage is considered to contribute to the progression of ageing and fundamental components of pathological pathways, which are thought to drive various age-related diseases (50), such as DKD. Hypertension, hyperlipidemia, and type 2 diabetes are common comorbidities. Compared to non-diabetic individuals, diabetic patients have twice the incidence of hypertension (51), and the specific pathogenesis is related to oxidative stress. Hyperglycemia, hypertension, and hyperlipidemia can all lead to increased vascular ROS generation, and oxidative stress activation promotes post-translational oxidation of proteins, mitochondrial dysfunction, etc., thereby causing cellular damage and vascular dysfunction (52), and leading to kidney injury. Furthermore, it has been reported that gender differences may also be one of the key factors affecting the progression of DKD (53). In animal models, estrogens can counteract renal fibrosis and apoptosis (54), while testosterone promotes inflammatory, apoptotic, and fibrotic processes (55), which may explain the significant association between OBS and DKD in male populations. However, these findings differ from human-based studies, which indicate that oral contraceptives and estrogen replacement therapy are associated with an increased risk of microalbuminuria and declining renal function (56, 57). Therefore, the different mechanisms of gender differences in DKD

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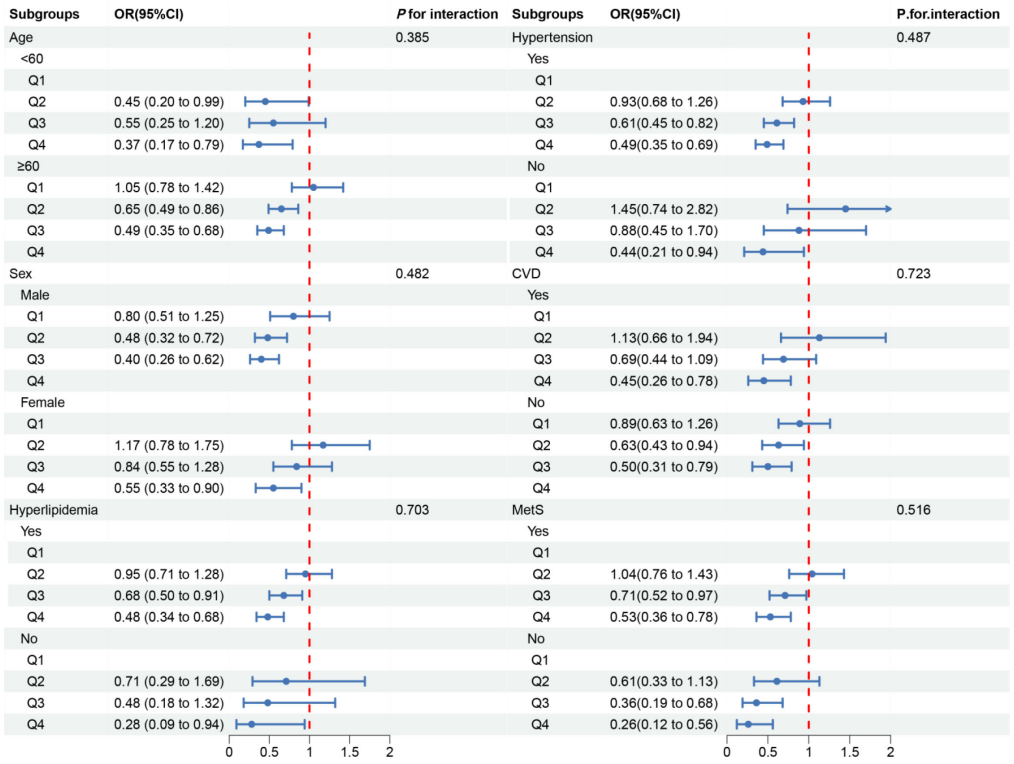

FIGURE 4
Associations between OBS and low-eGFR in different subgroups. OR, odds ratio; 95% CI, 95% confidence interval; CVD, cardiovascular disease; Mets, metabolic syndrome. Except for the stratification component itself, each stratification factor was adjusted for all covariates.

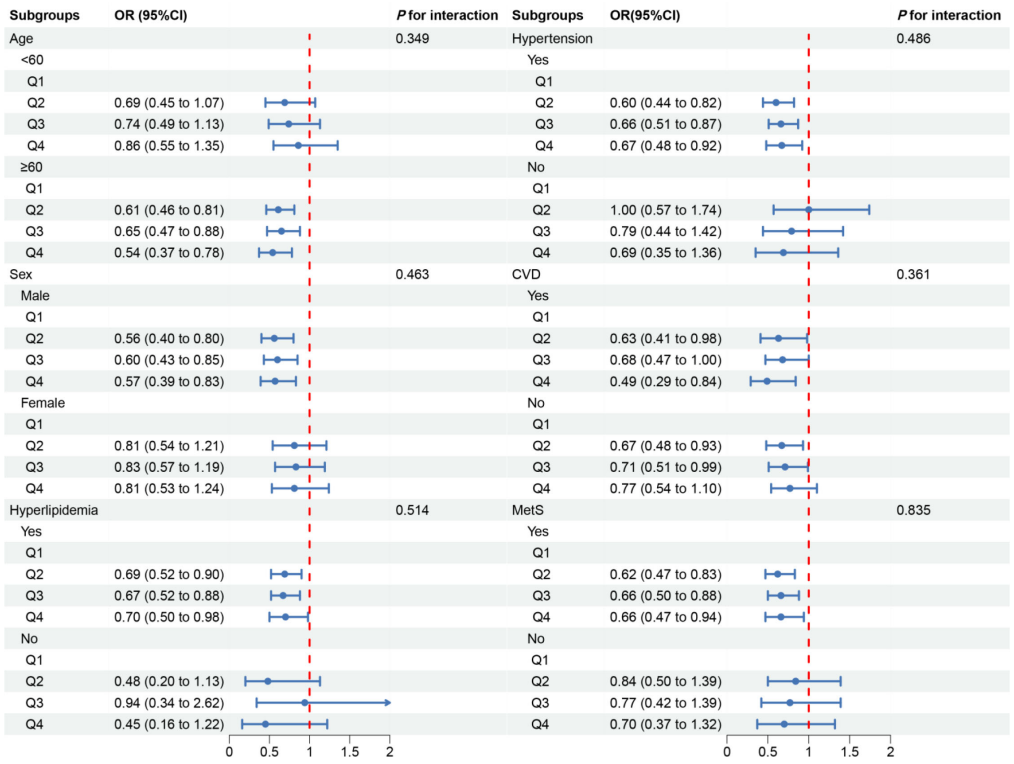

FIGURE 5
Associations between OBS and albuminuria in different subgroups. OR, odds ratio; 95% CI, 95% confidence interval; CVD, cardiovascular disease; Mets, metabolic syndrome. Except for the stratification component itself, each stratification factor was adjusted for all covariates.

warrant further exploration. Furthermore, considering the possibility of multiple comparisons and type I errors, the results of subgroup analyses still need to be validated in future studies.

This study has several strengths. Firstly, it is based on the NHANES database, which employs a multistage complex probability sampling design and weighted adjustments, enhancing the representativeness and reliability of the study results—a crucial factor for improving the generalizability of our findings within the American context. Secondly, our study provides preliminary evidence of the correlation between OBS and DKD, with information on OBS being obtained solely through questionnaires and incorporating the comprehensive impact of 20 oxidative stress factors, which enables OBS to have the potential as a tool for diabetes risk stratification. This integration offers new insights for the development of personalized and more effective preventive strategies. Furthermore, our research paves the way for future longitudinal study designs and the exploration of the mechanisms by which OBS affects DKD. Confirmation and expansion of these findings will assist in the formulation or adjustment of public health policies, such as recommending specific antioxidant dietary and lifestyle practices for diabetes patients, which will contribute to the broader goal of reducing the global burden of DKD.

This study, despite employing weighted multivariate logistic regression models for adjustment and validating the stability of results through subgroup analyses, still has certain limitations. Firstly, the inherent nature of cross-sectional study restricts the establishment of a causal relationship between OBS and DKD. Acknowledging this limitation, we recommend that future research on the relationship between OBS and DKD should employ a longitudinal design. This would enable researchers to determine whether OBS precedes the development of DKD, thereby providing more compelling evidence for a potential causal relationship. Secondly, despite the stratified and multistage sampling methods used in NHANES aiming to produce a nationally representative sample, there may still be some potential selection biases, such as non-response bias, self-selection bias, and three-stage sampling bias. These biases could result in the study findings differing from the true situation in the overall population. Although we used the weighted methods recommended by NHANES guidelines to analyze the data, which may have corrected this bias to some extent, caution should still be exercised when interpreting the results. Additionally, the OBS data in this study were collected through a 24-hour food recall questionnaire, a convenient method but potentially subject to recall bias and selection bias. In future studies, we will work towards identifying relevant objective biological markers to reduce this bias. Thirdly, the study sample was limited to the US population, hence the generalizability of the results to other racial backgrounds requires further validation in diverse racial populations.

5 Conclusion

Our study findings indicated that higher levels of OBS and dietary OBS were associated with a reduced risk of DKD, low-eGFR, and albuminuria. These results provided preliminary evidence for the importance of adhering to an antioxidant-rich diet and lifestyle among diabetic patients and highlighted the potential utility in formulating feasible dietary and lifestyle recommendations for them. Consequently, the integration of OBS into clinical practice may represent an innovative strategy to mitigate the burden of DKD in diabetic patients. However, further large-scale prospective studies are necessary to validate and expand upon our observations.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: The data employed in this study can be accessed through the NHANES website: https://www.cdc.gov/nchs/nhanes/.

Ethics statement

The studies involving humans were approved by National Center for Health Statistics. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

CL: Data curation, Formal analysis, Methodology, Software, Validation, Writing – original draft, Writing – review & editing. JY: Conceptualization, Data curation, Formal analysis, Methodology, Writing – review & editing. HL: Conceptualization, Methodology, Visualization, Writing – review & editing. YD: Formal analysis, Software, Visualization, Writing – review & editing. PH: Conceptualization, Methodology, Validation, Visualization, Writing – review & editing. JZ: Validation, Writing – review & editing. MZ: Data curation, Supervision, Visualization, Writing – review & editing.

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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.2024.1412823/full#supplementary-material

SUPPLEMENTARY TABLE 1
Components of the oxidative balance score. OBS, oxidative balance score; A, antioxidant; P, prooxidant; RE, retinol equivalent; ATE, alpha-tocopherol equivalent; MET, metabolic equivalent.

SUPPLEMENTARY TABLE 2
The collinearity assessment outcomes. Family PIR, family poverty income ratio; TC, total cholesterol; TG, triglycerides; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALB, albumin.

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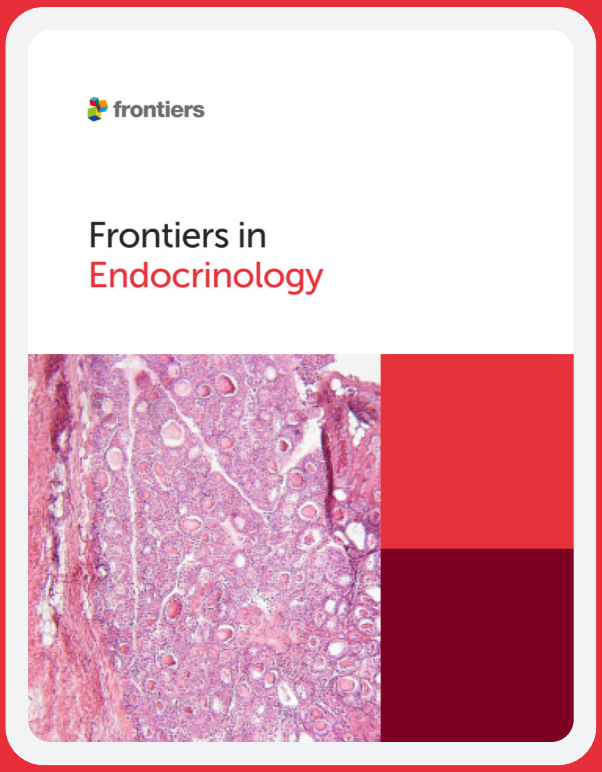

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