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ORIGINAL RESEARCH

Elevated Serum SERPINE2 Levels are Linked to Impaired Renal Function in Patients with Type 2 Diabetes Mellitus

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ABSTRACT

***Introduction*:** Diabetic nephropathy (DN) is the primary complication associated with diabetes mellitus and is increasingly acknowledged as the leading cause of end-stage renal disease worldwide, placing a significant economic burden on society. This study determined how blood serpin peptidase inhibitor clade E member 2 (SERPINE2) levels affect DN in individuals with type 2 diabetes mellitus (T2DM).

***Methods*:** We recruited 292 individuals diagnosed with T2DM and 120 healthy controls for this study. We employed comprehensive and systematic data collection methodologies to gather relevant biomarkers and information on biochemical parameters. We measured serum levels of neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), transforming growth factor-β1 (TGFβ1), connective tissue growth factor (CTGF), and SERPINE2 by the enzyme-linked immunosorbent assay in control subjects and patients with T2DM. We calculated generalized odds ratios (OR) to estimate the risk of developing DN.

***Results*:** Patients with diabetes had significantly higher levels of SERPINE2 (285.64 ± 56.58 pg/mL) than healthy controls (184.84 ± 23.54 pg/mL). Additionally, the multivariate logistic regression analysis indicated that patients with diabetes with DN possessed higher levels of serum SERPINE2 (OR 1.033, 95% confidence interval [CI] 1.013–1.053; P=0.001), along with an increased body mass index (BMI), duration of diabetes, serum creatinine (Scr), NGAL, KIM-1, TGFβ1, and CTGF. Receiver operating characteristic (ROC) curve analysis indicated that patients with T2DM and serum SERPINE2 levels exceeding 278.94 pg/mL had a significantly higher risk of developing DN.

***Conclusion*:** The results showed that patients with diabetes with DN have higher levels of serum SERPINE2. A more extensive population-based prospective study is needed to validate our findings.

Keywords: SERPINE2; Diabetic nephropathy; Type 2 diabetes mellitus; Biomarker; Estimated glomerular filtration rate

Shudan Cao and Qing Tan contributed equally.

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Key Summary Points

Why carry out this study?

The increasing prevalence of diabetes mellitus (DM) has rendered diabetic nephropathy (DN) as the foremost complication associated with diabetes. This condition is now widely acknowledged as the leading cause of end-stage renal disease worldwide, thereby imposing a significant economic burden on society.

In this study, we investigated the influence of blood serpin peptidase inhibitor clade E member 2 (SERPINE2) levels on DN in individuals with type 2 diabetes mellitus (T2DM).

What was learned from the study?

Patients with diabetes exhibited significantly higher levels of SERPINE2 compared to healthy controls.

These findings suggest that patients with T2DM and elevated serum SERPINE2 levels were significantly more likely to develop DN.

INTRODUCTION

The rising incidence of diabetes mellitus has made diabetic nephropathy (DN) the leading complication associated with diabetes [1, 2]. DN is increasingly recognized as the primary cause of end-stage renal disease worldwide, creating a significant economic burden on society [3–5]. Characteristic pathological changes in DN include excessive mesangial matrix production, renal hypertrophy, and fibrosis [6–8]. The causes of DN are complex and multifaceted. Various risk factors, including the buildup of advanced glycation end products (AGEs), renin–angiotensin–aldosterone system (RAAS) activation, and oxidative stress, play critical roles in the onset and progression of DN [9–12]. However, the fundamental mechanisms underlying DN remain largely unclear. As DN advances, patients are frequently diagnosed with glomerular hyperfiltration, microproteinuria, macroproteinuria, and a diminished glomerular filtration rate (GFR) [13]. The quantification of urinary protein (UP) is regarded as the principal parameter for the clinical identification of DN; this encompasses the urinary albumin excretion rate (UAER) in individuals presenting with microproteinuria and the 24-h urinary protein collection for those exhibiting macroproteinuria [14]. Additionally, previous studies have indicated that Smad1 in urine could act as a distinctive marker for predicting future structural damage and could be used to assess the effects of angiotensin II type 1 receptor blockers (ARBs) on DN [15, 16]. In recent decades, only a limited number of pharmacological agents have been developed to reduce the incidence of DN in patients with diabetes. These include RAS inhibitors, finerenone, sodium-glucose co-transporter 2 (SGLT2), and glucagon-like peptide 1 (GLP-1) agonists, which help regulate glucose levels or blood pressure [4, 17, 18]. Thus, therapeutic strategies that directly target DN are still inadequate.

Serpin peptidase inhibitor clade E member 2 (SERPINE2), a protein of 43 kDa initially identified as a neurite-promoting factor in the culture medium derived from glial cells, has subsequently been established as a SERPIN family member exhibiting serine protease inhibitory functionality [19–21]. This protein predominantly resides within the extracellular matrix (ECM). It is secreted by various cell types, including endothelial cells, fibroblasts, macrophages, platelets, smooth muscle cells, chondrocytes, astrocytes, and neoplastic cells [22–26]. SERPINE2 plays a significant role in pathophysiological processes affecting the vascular, renal, nervous, respiratory, and male reproductive systems and in the progression of tumors [27, 28]. Research has further elucidated the potential contributions of SERPINE2 to tumor invasion and metastatic spread of cancer [29, 30]. Elevated expression levels of SERPINE2 have been documented in lung adenocarcinomas (LUAD) compared to both normal lung tissue and squamous cell carcinoma (SCC) [31]. A recent investigation indicated that SERPINE2 may serve as a regulatory factor in response to ionizing radiation (IR) [32]. Currently, there are no documented studies on the effects of diabetes medications on the expression of SERPINE2.

Therefore, the impact of SERPINE2 on DN in patients with T2DM remains unclear.

In this study, we explored the hypothesis that serum SERPINE2 might be a potential biomarker for the identification of DN in patients with T2DM. To test our hypothesis, we used an enzyme-linked immunosorbent assay (ELISA) to investigate serum SERPINE2 expression in patients with T2DM and healthy controls. We also explored the correlation between serum SERPINE2 levels and clinical indicators of DN and conducted a logistic regression analysis for the development of DN in patients with T2DM.

METHODS

Study Population

A total of 412 subjects (292 patients with T2DM and 120 healthy controls) from the Department of Endocrinology of Sijing Hospital were enrolled between January 2022 and December 2023. All recruited patients received conventional drugs, including glibenclamide, metformin, and acarbose. The diagnosis of T2DM was based on the following criteria: fasting glucose ≥ 7.0 mmol/L or 2-h postprandial glucose level ≥ 11.1 mmol/L. According to the urinary albumin excretion rate (UAER) in 24 h, patients with T2DM were classified into the normoalbuminuria group (UAER < 30 mg/24 h), microalbuminuria group (UAER 30–300 mg/24 h), and macroalbuminuria group (UAER > 300 mg/24 h) [33]. Blood samples were collected, and sera were isolated by centrifugation and stored at − 80 °C until analysis by ELISA. Urine was collected at 24 h and examined immediately. The exclusion criteria were as follows: (1) subjects with type 1 diabetes, (2) infectious disease, (3) liver cirrhosis, (4) acute and chronic nephritis, (5) cardiovascular disorders, and (6) history of malignancy. Written informed consent was obtained from all study subjects. We also selected 120 subjects who underwent physical examination as a control group. These subjects had no type 1 or type 2 diabetes mellitus or kidney disease and were age- and sex-matched. We excluded individuals with a history of type 1 or type 2 diabetes mellitus and kidney disease. The Ethics Committee of Sijing Hospital approved the study protocol. Figure 1 represents a summary of the inclusion and exclusion of the participants. However, similar methodologies were introduced in earlier research [34].

Measurement of Clinical and Biochemical Data

Complete clinical and laboratory data of the enrolled patients after admission were collected, including age, sex, clinical symptoms, past medical history, T2DM duration, blood pressure, and laboratory test results. BMI is expressed as weight in kilograms divided by height in meters squared (kg/m^2). Serum creatinine (Scr) was measured using the enzyme method, and UACR was measured using immunoturbidimetry. Fasting blood glucose (FBG) was determined using the glucose oxidase method. Glycosylated hemoglobin (HbA1c) was determined using high-performance liquid chromatography (HPLC). The enzymatic method is used to assess the blood lipid profile, including triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C). The estimated glomerular filtration rate (eGFR) was calculated from the simplified formula: (mL/min/1.73 m^2) = 175 × (Scr (μmol/L)/88.4) − 1.154 × (age) − 0.203 × (if female).

Enzyme-Linked Immunosorbent Assay

The collected blood samples were centrifuged (1500×*g*, 4 °C, 20 min); each serum sample was then separated, labelled, and immediately stored in a refrigerator at − 80 °C. Serum biomarker levels in control subjects and patients with T2DM were measured using ELISA kits for NGAL (QK1757, R&D Systems), KIM-1 (DSKM100, R&D Systems), TGFβ1 (DY240, R&D Systems), CTGF (DY9190-05, R&D Systems), and antibodies against SERPINE2 (AF2980, R&D Systems) [35]. The minimum detectable concentration of SERPINE2 was < 10 pg/mL.

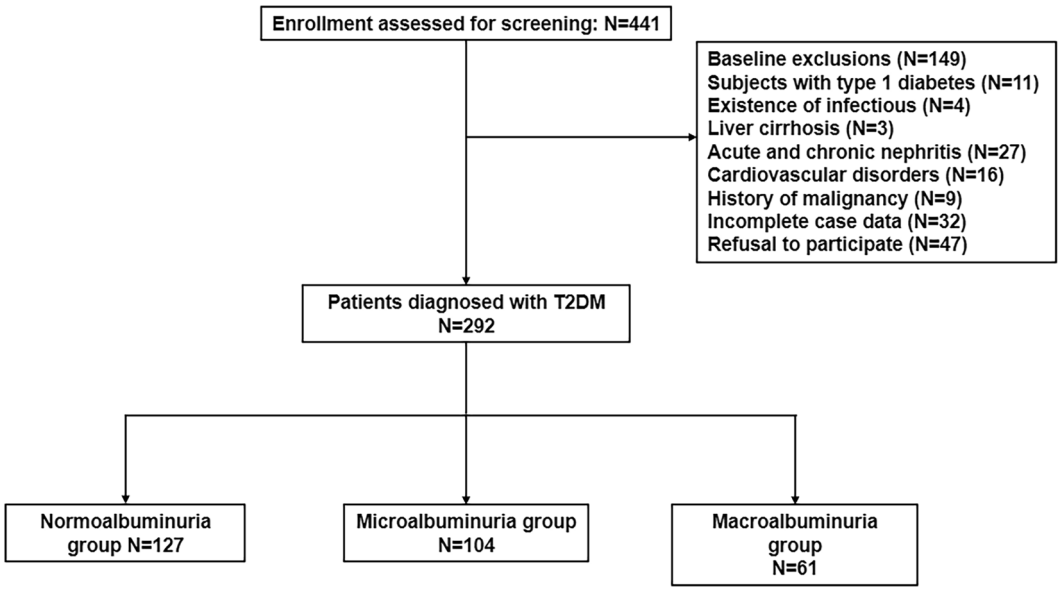

Fig. 1 Flowchart of participant selection. A total of 441 patients with suspected type 2 diabetes mellitus (T2DM) were consecutively enrolled. Eleven patients had type 1 diabetes, 4 had infections, 3 had liver cirrhosis, 27 had acute or chronic nephritis, 16 had cardiovascular disorders, and 9 had a malignancy history were excluded. Thirty-two patients with no complete case data and 47 who refused to participate were also excluded. Finally, this study included 292 patients with T2DM who were divided into the normoalbuminuria group ($n = 127$), microalbuminuria group ($n = 104$), and macroalbuminuria group ($n = 61$) according to the urinary albumin excretion rate (UAER) over 24 h

Statistical Analysis

All statistical analyses were performed using SPSS software 20.0 (IBM, Chicago, USA). Data are presented as mean ± standard deviation (SD) for continuous variables and as frequency (percentage) for categorical variables. Comparisons between two independent groups were analyzed using an unpaired Student *t* test or Kruskal–Wallis test. The normality of the continuous data was evaluated using a Shapiro–Wilk test ($P > 0.05$, indicating a normal distribution) and visualized by a normal *Q*–*Q* plot. More than two groups were compared using one-way analysis of variance (ANOVA) analysis. Pearson's correlation analysis was used to evaluate the correlation between SERPINE2 and other continuous variables. A forward stepwise procedure was applied to the multivariate logistic regression analysis to determine the independent influencing effects of SERPINE2 and other variables, and the odds ratios (OR) and 95% confidence intervals (CI) are shown. The diagnostic value of SERPINE2 was assessed using receiver operating characteristic (ROC) analysis and the area under the ROC curve (AUC). Differences between groups were considered statistically significant if the *P* value was < 0.05.

Ethical Approval

The Ethics Committee of Sijing Hospital granted approval for this study (SJYY202401-YNKT-CSD). The authors adhered to all standard protocols in line with the 1964 Declaration of Helsinki. Informed consent was obtained from all subjects participating in the study.

RESULTS

Baseline Characteristics

This investigation utilized a comprehensive approach to compare patients with T2DM with a control group. The results from Student's *t* test indicated that patients with T2DM exhibited significantly higher levels of several health indicators, including body mass index (BMI), duration of diabetes (years), systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), estimated glomerular filtration rate (eGFR), serum creatinine (Scr), neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), transforming growth factor-β1 (TGFβ1), connective tissue growth factor (CTGF), and serpin peptidase inhibitor clade E member 2 (SERPINE2) (Table 1). However, no significant differences in age or sex were found between the T2DM and control groups ($P<0.05$).

We conducted a comparison of all subgroups of T2DM using one-way ANOVA analysis. The results showed that the macroalbuminuria subgroup had significantly higher BMI, duration of diabetes (years), SBP, DBP, FPG, HbA1c, TC, LDL-C, Scr, NGAL, KIM-1, TGFβ1, CTGF, and SERPINE2 than the microalbuminuria and normoalbuminuria subgroups (Table 2). Conversely, HDL-C levels and eGFR were lower in the macroalbuminuria subgroup. This comprehensive investigation provides a strong foundation for future research and clinical practice.

Serum SERPINE2 Levels in Patients with Type 2 Diabetes Mellitus

Serum levels of SERPINE2 were measured in patients with T2DM ($N=292$) and healthy controls ($N=120$) using ELISA. The results showed that SERPINE2 concentrations were significantly higher in patients with T2DM than in healthy controls (Fig. 2a). Furthermore, we compared serum SERPINE2 levels among groups categorized by albuminuria status: normoalbuminuria ($N=127$), microalbuminuria ($N=104$), and macroalbuminuria ($N=61$). SERPINE2 levels were significantly elevated in both the macroalbuminuria and microalbuminuria groups compared to those in the normoalbuminuria group (Fig. 2b). Notably, the T2DM group had a mean serum SERPINE2 level of 285.64 ± 56.58 pg/mL, which was markedly higher than the healthy control group, which had a mean level of 184.84 ± 23.54 pg/mL (Table 1).

Correlation Between Serum SERPINE2 and Clinical Indicators

In this study involving 292 patients with T2DM, serum levels of SERPINE2 were positively correlated with several factors: age ($r=0.164$, $P=0.005$), BMI ($r=0.297$, $P<0.001$), duration of diabetes ($r=0.529$, $P<0.001$), SBP ($r=0.242$, $P<0.001$), DBP ($r=0.289$, $P<0.001$), FPG ($r=0.373$, $P<0.001$), HbA1c ($r=0.347$, $P<0.001$), TG ($r=0.153$, $P=0.009$), TC ($r=0.237$, $P<0.001$), LDL-C ($r=0.325$, $P<0.001$), Scr ($r=0.647$, $P<0.001$), NGAL ($r=0.628$, $P<0.001$), KIM-1 ($r=0.706$, $P<0.001$), TGFβ1 ($r=0.682$, $P<0.001$), and CTGF ($r=0.703$, $P<0.001$). Conversely, SERPINE2 levels were negatively associated with HDL-C ($r=-0.288$, $P<0.001$) and eGFR ($r=-0.561$, $P<0.001$) (Table 3). Furthermore, a Pearson correlation test revealed that serum levels of SERPINE2 had a positive correlation with Scr, NGAL, KIM-1, TGFβ1, and CTGF, while showing a negative correlation with eGFR (Fig. 3).

Logistic Regression Analysis for Developing Diabetic Nephropathy

Multivariate logistic regression analyses were conducted to identify the independent risk factors associated with DN. The results revealed several significant variables, including BMI (OR 0.692, 95% CI 0.486–0.985; $P=0.041$), duration of diabetes (OR 1.806, 95% CI 1.174–2.777; $P=0.007$), Scr (OR 1.096, 95% CI 1.028–1.169; $P=0.005$), NGAL (OR 1.042, 95% CI 1.009–1.076; $P=0.012$), KIM-1 (OR 1.312, 95% CI 1.046–1.645; $P=0.019$), TGFβ1 (OR 1.694, 95% CI 1.106–2.593; $P=0.015$),

Table 1 Baseline characteristics of the included subjects

Characteristics	Control ($N=120$)	T2DM ($N=292$)	*P* value
Age (years)	58.80 ± 10.41	57.03 ± 9.29	0.090
Sex (male, %)	63 (52.5%)	157 (53.8%)	0.815
BMI (kg/m^2)	23.43 ± 1.38	24.40 ± 1.68	< 0.001
Duration (years)	0	9.16 ± 1.65	< 0.001
SBP (mmHg)	123.44 ± 11.21	139.42 ± 18.59	< 0.001
DBP (mmHg)	78.09 ± 7.23	88.42 ± 11.45	< 0.001
FPG (mmol/L)	4.78 ± 1.43	7.63 ± 1.24	< 0.001
HbA1c (%)	5.13 ± 0.41	7.93 ± 1.22	< 0.001
TG (mmol/L)	1.60 ± 0.62	1.89 ± 0.65	< 0.001
TC (mmol/L)	4.95 ± 0.46	5.43 ± 0.63	< 0.001
LDL-C (mmol/L)	3.04 ± 0.46	3.49 ± 0.57	< 0.001
HDL-C (mmol/L)	1.50 ± 0.20	1.21 ± 0.19	< 0.001
eGFR (mL/min/1.73 m^2)	102.60 ± 12.08	97.71 ± 18.07	0.002
Scr (μmol/L)	67.91 ± 10.40	85.26 ± 15.12	< 0.001
NGAL (μg/mL)	82.40 ± 16.31	128.88 ± 26.06	< 0.001
KIM-1 (ng/mL)	12.30 ± 2.15	23.00 ± 5.29	< 0.001
TGFβ1 (ng/mL)	7.28 ± 1.26	11.24 ± 2.76	< 0.001
CTGF (ng/mL)	3.21 ± 0.37	6.75 ± 1.49	< 0.001
SERPINE2 (pg/mL)	184.84 ± 23.54	285.64 ± 56.58	< 0.001

Continuous variables are expressed as mean ± SD, and analyzed using *t* test or Wilcoxon–Mann–Whitney test. Categorical variables are expressed as frequency (percentage), and analyzed using the chi-square test

T2DM type 2 diabetes mellitus, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *FPG* fasting plasma glucose, *HbA1c* glycated hemoglobin, *TG* triglyceride, *TC* total cholesterol, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *eGFR* estimated glomerular filtration rate, *Scr* serum creatinine, *NGAL* neutrophil gelatinase-associated lipocalin, *KIM-1* kidney injury molecule 1, *TGFβ1* transforming growth factor-β1, *CTGF* connective tissue growth factor, *SERPINE2* serpin peptidase inhibitor clade E member 2

CTGF (OR 3.696, 95% CI 1.594–8.570; $P=0.002$), and SERPINE2 (OR 1.033, 95% CI 1.013–1.053; $P=0.001$) (Table 4). Additionally, the ROC curve analysis indicated that the optimal cutoff value for serum SERPINE2 was 278.94 pg/mL, with a sensitivity of 75.8% and a specificity of 90.6%, resulting in an area under the curve (AUC) of 0.904 (Fig. 4). Furthermore, patients with T2DM and serum SERPINE2 levels exceeding 278.94 pg/mL had a significantly higher risk of developing DN.

Molecular Mechanism of How SERPINE2 Regulates Development of Diabetic Nephropathy

Figure 5 outlines the molecular mechanism by which SERPINE2, a key player, regulates the development of DN. Elevated levels of SERPINE2 in patients with T2DM and renal dysfunction lead to podocyte damage and increased albuminuria, which is a crucial

Table 2 Baseline characteristics of patients with type 2 diabetes mellitus (T2DM) according to albuminuria status

Characteristics	Normoalbuminuria ($N = 127$)	Microalbuminuria ($N = 104$)	Macroalbuminuria ($N = 61$)	*P* value
Age (years)	56.41 ± 9.24	57.18 ± 9.27	58.05 ± 9.48	0.516
Sex (male, %)	65 (51.2%)	57 (54.8%)	35 (57.4%)	0.702
BMI (kg/m^2)	23.93 ± 1.65	24.64 ± 1.61**	24.98 ± 1.59***	< 0.001
Duration (years)	8.19 ± 1.22	9.51 ± 1.37***	10.57 ± 1.61***###	< 0.001
SBP (mmHg)	135.70 ± 16.40	140.31 ± 20.00	145.64 ± 18.83**	0.002
DBP (mmHg)	84.32 ± 11.00	90.64 ± 11.32***	93.16 ± 9.73***	< 0.001
FPG (mmol/L)	7.22 ± 1.21	7.74 ± 1.14**	8.31 ± 1.16***##	< 0.001
HbA1c (%)	7.46 ± 0.99	8.12 ± 1.38***	8.56 ± 0.99***#	< 0.001
TG (mmol/L)	1.81 ± 0.52	1.93 ± 0.83	1.98 ± 0.52	0.210
TC (mmol/L)	5.29 ± 0.56	5.51 ± 0.61**	5.57 ± 0.75**	0.004
LDL-C (mmol/L)	3.31 ± 0.47	3.58 ± 0.59***	3.70 ± 0.62***	< 0.001
HDL-C (mmol/L)	1.27 ± 0.18	1.19 ± 0.17**	1.14 ± 0.19***	< 0.001
eGFR (mL/min/1.73 m^2)	106.79 ± 17.49	99.93 ± 11.12***	76.72 ± 10.20***###	< 0.001
Scr (μmol/L)	75.48 ± 9.25	86.58 ± 9.31***	103.37 ± 15.54***###	< 0.001
NGAL (μg/mL)	112.41 ± 17.60	134.24 ± 21.14***	154.06 ± 24.67***###	< 0.001
KIM-1 (ng/mL)	19.39 ± 2.76	22.82 ± 2.71***	30.81 ± 4.18***###	< 0.001
TGFβ1 (ng/mL)	9.10 ± 1.50	12.00 ± 2.02***	14.41 ± 2.06***###	< 0.001
CTGF (ng/mL)	5.54 ± 0.88	7.18 ± 0.91***	8.56 ± 0.98***###	< 0.001
SERPINE2 (pg/mL)	243.70 ± 28.15	294.05 ± 37.95***	358.60 ± 46.15***###	< 0.001

BMI body mass index, *SBP* systolic blood pressure, *DPB* diastolic blood pressure, *FPG* fasting plasma glucose, *HbA1c* glycated hemoglobin, *TG* triglycerides, *TC* total cholesterol, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *eGFR* estimated glomerular filtration rate, *Scr* serum creatinine, *NGAL* neutrophil gelatinase-associated lipocalin, *KIM-1* kidney injury molecule 1, *TGFβ1* transforming growth factor-β1, *CTGF* connective tissue growth factor, *SERPINE2* serum serpin peptidase inhibitor clade E member 2

**$P < 0.01$, ***$P < 0.001$, vs normoalbuminuria group; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$, vs microalbuminuria group

aspect of our research. Additionally, SERPINE2 contributes to renal fibrosis by enhancing the production of fibrosis-related cytokines, such as TGFβ1 and CTGF. Consequently, the rise in SERPINE2 levels worsens injury to glomerular and tubular cells, exacerbated by hyperglycemia and AGEs.

DISCUSSION

Our investigation explored the influence of serum SERPINE2 concentration on the progression of DN in individuals diagnosed with T2DM. The results showed that serum SERPINE2 levels were significantly higher in patients with T2DM than in healthy controls. Additionally, our analysis found a positive correlation between serum SERPINE2 levels and several factors, including

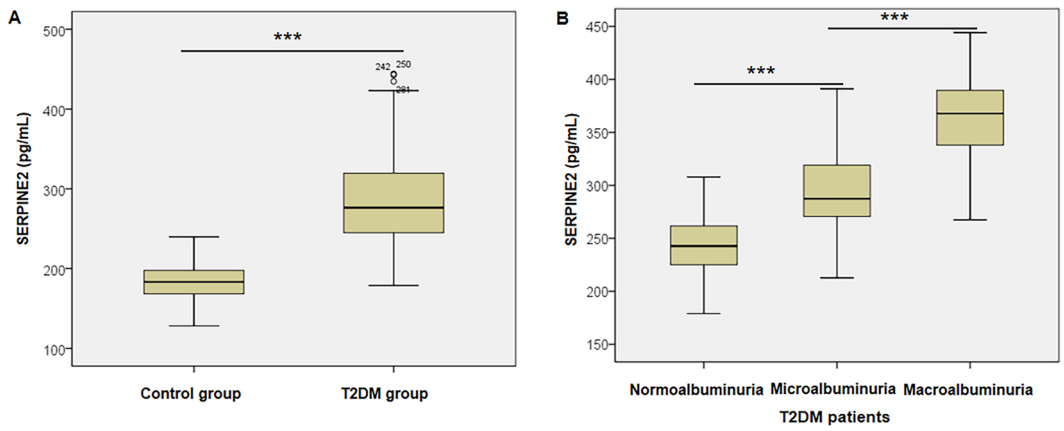

Fig. 2 Serum serpin peptidase inhibitor clade E member 2 (SERPINE2) levels were compared between control subjects and patients with type 2 diabetes mellitus (T2DM). **a** Serum SERPINE2 levels in control subjects and patients with T2DM were detected by enzyme-linked immunosorbent assay (ELISA). The serum SERPINE2 concentration in patients with T2DM ($n = 292$) was significantly higher than that in control subjects ($n = 120$). Student t test was applied. **b** Comparison of serum SERPINE2 levels in patients with T2DM with normoalbuminuria, microalbuminuria, and macroalbuminuria. ANOVA was applied. $^{***}P < 0.001$

age, BMI, duration of diabetes, SBP, DBP, FPG, HbA1c, TG, TC, LDL-C, Scr, NGAL, KIM-1, TGFβ1, and CTGF. In contrast, HDL-C and eGFR were negatively correlated with serum SERPINE2 concentrations. Significantly higher risks of DN were observed in patients with T2DM with elevated serum SERPINE2 levels (>278.94 pg/mL). These findings suggest that blood SERPINE2 might act as a possible marker for the early detection of DN in individuals with T2DM.

DN is the primary cause of end-stage renal disease (ESRD) and a microvascular consequence of diabetes. The key features of DN include a gradual decline in GFR and persistent albuminuria, defined as an albumin excretion rate exceeding 300 mg/day, evaluated at least twice over a span of 3–6 months [36]. Microalbuminuria, macroalbuminuria, ESRD, and early glomerular hyperfiltration are the clinical phases of diabetic kidney disease, with albuminuria being one of the most distinctive clinical indications [37]. Our findings demonstrated that patients with T2DM serum SERPINE2 concentrations were significantly higher than those in control subjects, and serum SERPINE2 levels increased from normoalbuminuria to microalbuminuria and then to macroalbuminuria in patients with T2DM. This indicates that SERPINE2 is associated with the microvasculature of the glomerulus and albuminuria. SERPINE2 can also modulate extracellular matrix degradation and vascular remodeling, and is involved in the development of vascular lesions [38].

The renal parenchyma mainly consists of renal tubules (90%) and tubulointerstitial tissues (10%). In early DN, tubular cells exhibit hypertrophy and thickening of the basement membrane. With the progression of DN, interstitial fibrosis and tubular atrophy are observed [39]. Our study showed positive correlations between serum SERPINE2 concentrations and NGAL, a biomarker of kidney injury that is released after tubular damage. SERPINE2 is highly expressed in renal cell carcinoma tissues and expressed at low levels in para-tumor tissues or HK-2, a human renal tubular cell line [40]. This indicates that SERPINE2 can activate the epithelial–mesenchymal transition pathway. Whether SERPINE2 is highly expressed in the tubular tissue of patients with DN remains unknown and deserves further investigation.

Podocytes are specialized glomerular cells that wrap around capillaries and filter blood, forming a glomerular filtration barrier to prevent plasma proteins from entering the urine filtrate. Podocytes are involved in the early onset of DN.

Table 3 Correlation between serum serpin peptidase inhibitor clade E member 2 (SERPINE2) and clinical indicators

Parameters	All subjects ($N = 292$)	
	r	*P* value
Age (years)	0.164	0.005
BMI (kg/m^2)	0.297	< 0.001
Duration (years)	0.529	< 0.001
SBP (mmHg)	0.242	< 0.001
DBP (mmHg)	0.289	< 0.001
FPG (mmol/L)	0.373	< 0.001
HbA1c (%)	0.347	< 0.001
TG (mmol/L)	0.153	0.009
TC (mmol/L)	0.237	< 0.001
LDL-C (mmol/L)	0.325	< 0.001
HDL-C (mmol/L)	− 0.288	< 0.001
eGFR (mL/min/1.73 m^2)	− 0.561	< 0.001
Scr (μmol/L)	0.647	< 0.001
NGAL (μg/mL)	0.628	< 0.001
KIM-1 (ng/mL)	0.706	< 0.001
TGFβ1 (ng/mL)	0.682	< 0.001
CTGF (ng/mL)	0.703	< 0.001

The correlation between serum SERPINE2 and other continuous variables was analyzed using the Pearson correlation test

BMI body mass index, *SBP* systolic blood pressure, *DPB* diastolic blood pressure, *FPG* fasting plasma glucose, *HbA1c* glycated hemoglobin, *TG* triglycerides, *TC* total cholesterol, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *eGFR* estimated glomerular filtration rate, *Scr* serum creatinine, *NGAL* neutrophil gelatinase-associated lipocalin, *KIM-1* kidney injury molecule 1, *TGFβ1* transforming growth factor-β1, *CTGF* connective tissue growth factor

The number of podocytes in the glomerulus of patients with DN with proteinuria is reduced and has become a predictor of DN progression [41]. Podocyte injury occurs in the early stage of DN and is mainly manifested by the fusion or disappearance of foot processes, abnormal expression of podocyte-related proteins, and decrease in podocyte density and number [42]. Our study showed positive correlations between serum SERPINE2 concentrations and KIM-1, a biomarker of podocyte injury. This evidence indicates that SERPINE2 can promote damage to podocytes.

Our study suggests that SERPINE2 may be an important therapeutic target for DN, which is consistent with a recently published paper showing the role of SERPINE2 in glomerulosclerosis in the pathogenesis of DN. SERPINE2 expression is upregulated in mesangial cells of diabetic mice [43]. However, the role of SERPINE2 in renal tubular cells and podocytes remains unclear. Our study provides evidence that SERPINE2 may be related to the injury of renal tubular cells and podocytes. In addition to positive correlations with NGAL and KIM-1, SERPINE2 also showed a positive correlation with TGFβ1 and CTGF. Serum TGFβ1 and CTGF are elevated in patients with DN and are associated with a high risk of DN [44]. They also constitute a signaling pathway for enhancing interstitial fibrosis in DN [45]. Therefore, we proposed a hypothesis on how SERPINE2 promotes the development of DN (Fig. 5). Increased SERPINE2 levels in patients with T2DM and renal dysfunction promote podocyte injury and albuminuria. SERPINE2 also enhanced renal fibrosis by promoting the expression of fibrosis-related cytokines (TGFβ1 and CTGF). Therefore, an increase in SERPINE2 levels plays an aggravating role in the injury of glomerular and tubular cells caused by hyperglycemia and AGEs.

However, the dynamics of SERPINE2 expression in relation to diabetic kidney disease progression have not been fully elucidated. Some studies have indicated that SERPINE2 levels may fluctuate in response to acute kidney injury or transient metabolic changes, which could limit its reliability for continuous monitoring [46]. However, the peak levels of SERPINE2 might provide more valuable diagnostic or predictive information regarding impaired renal function [47]. These peak levels could potentially be correlated with significant events in disease progression or acute exacerbations. Furthermore, implementing SERPINE2 in routine clinical practice

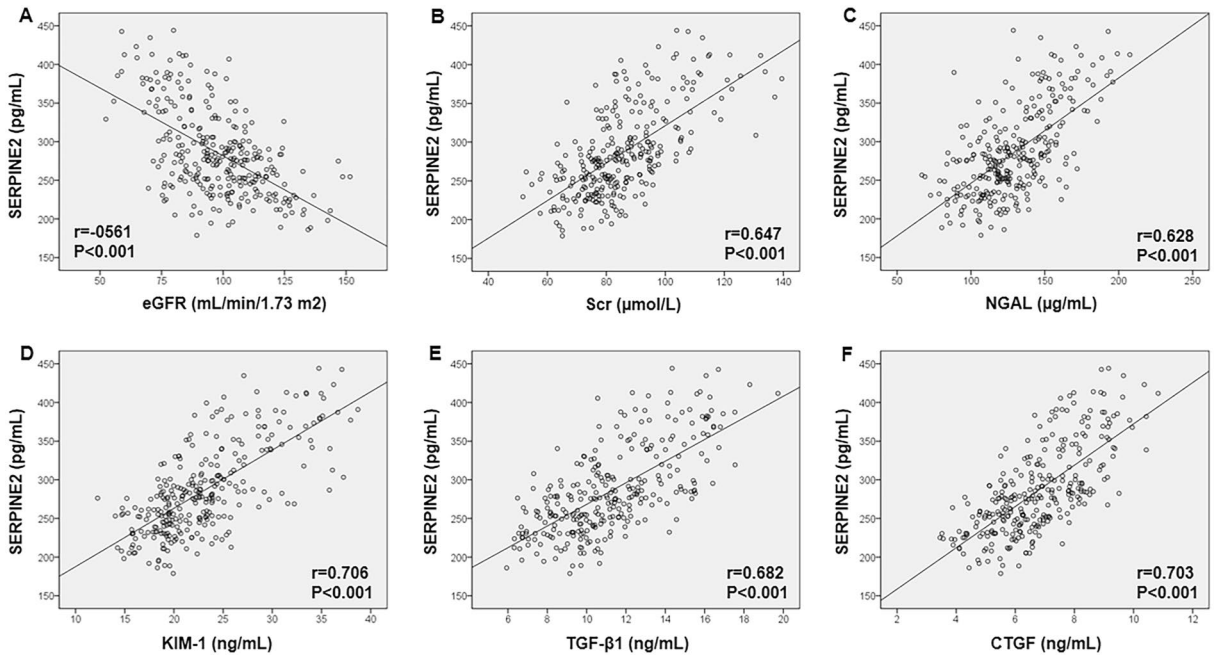

Fig. 3 Correlation between serum serpin peptidase inhibitor clade E member 2 (SERPINE2) and clinical indicators. Pearson correlation test was performed between SERPINE2 with **a** estimated glomerular filtration rate (eGFR), **b** serum creatinine (Scr), **c** neutrophil gelatinase-associated lipocalin (NGAL), **d** kidney injury molecule 1 (KIM-1), **e** transforming growth factor-β1 (TGFβ1), and **f** connective tissue growth factor (CTGF) in all patients with type 2 diabetes mellitus (T2DM)

Table 4 Logistic multivariate regression for developing diabetic nephropathy (DN)

Characteristics	Odds ratio	95% confidence interval	*P* value
BMI (kg/m^2)	0.692	0.486–0.985	0.041
Duration (years)	1.806	1.174–2.777	0.007
Scr (μmol/L)	1.096	1.028–1.169	0.005
NGAL (μg/mL)	1.042	1.009–1.076	0.012
KIM-1 (ng/mL)	1.312	1.046–1.645	0.019
TGFβ1 (ng/mL)	1.694	1.106–2.593	0.015
CTGF (ng/mL)	3.696	1.594–8.570	0.002
SERPINE2 (pg/mL)	1.033	1.013–1.053	0.001

BMI body mass index, *Scr* serum creatinine, *NGAL* neutrophil gelatinase-associated lipocalin, *KIM-1* kidney injury molecule 1, *TGFβ1* transforming growth factor-β1, *CTGF* connective tissue growth factor, *SERPINE2* serum serpin peptidase inhibitor clade E member 2

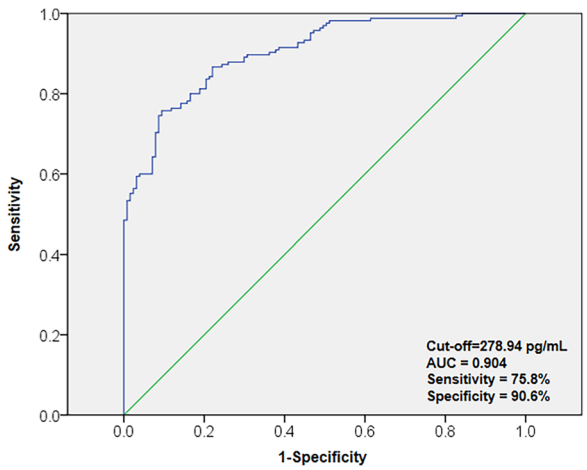

Fig. 4 Receiver operating characteristic (ROC) curve was used to obtain the optimal cutoff value of serum serpin peptidase inhibitor clade E member 2 (SERPINE2) (278.94 pg/mL) that distinguishes the patients with type 2 diabetes mellitus (T2DM) with and without albuminuria. *AUC* area under the curve

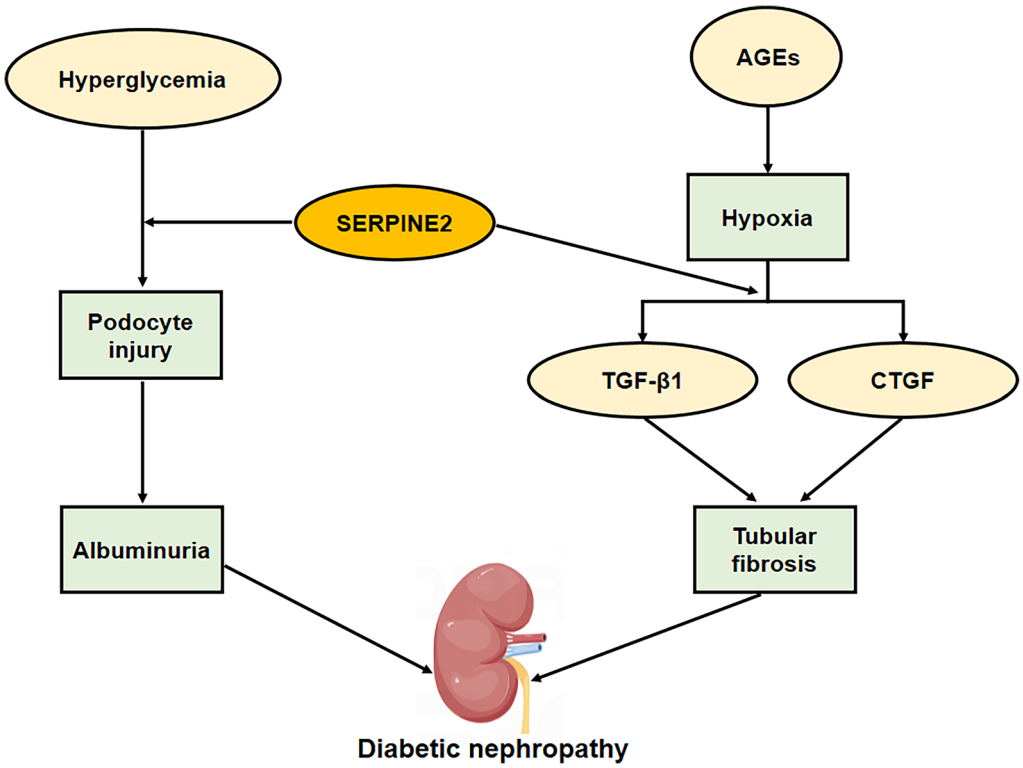

Fig. 5 Schematic diagram of serpin peptidase inhibitor clade E member 2 (SERPINE2) in the development of diabetic nephropathy. Increased SERPINE2 in patients with type 2 diabetes mellitus (T2DM) with renal dysfunction promotes podocyte injury and albuminuria. SERPINE2 also enhances renal fibrosis by promoting fibrosis-related cytokines, such as transforming growth factor-β1 (TGFβ1) and connective tissue growth factor (CTGF). Therefore, the increase in SERPINE2 levels plays an aggravating role in injury of glomerular and tubular cells caused by hyperglycemia and advanced glycation end products (AGEs)

may lead to economic challenges, owing to the potential costs associated with testing, equipment, and personnel training. The financial burden could be particularly significant for healthcare systems with limited resources, potentially creating disparities in access to this diagnostic or therapeutic tool across regions and settings.

Limitations

Several study had several limitations. (1) We did not use a power calculation to determine the required sample size for the study. In the future, we will conduct a power calculation to select the appropriate sample size. (2) Caution should be exercised when interpreting our results owing to the relatively modest size of our research sample. (3) The serum concentration of SERPINE2 and renal function of the patient were assessed at a single time point. In future investigations, we will determine the serum concentrations and renal function of the patient at different time points. (4) As a result of the unavailability of frozen urine samples, we measured the serum KIM-1 protein levels. In subsequent studies, we intend to measure KIM-1 in urine samples. (5) The study design was not intended to be a prospective longitudinal analysis; instead, it was structured as a cross-sectional investigation. The prognostic significance of serum SERPINE2 levels requires additional validation. (6) This research offers critical insights into the irregularities of SERPINE2 that contribute to the pathophysiology

of diabetes as observed in patients diagnosed with DN.

CONCLUSIONS

Serum SERPINE2 levels were higher in patients with diabetes and DN than in healthy controls. This observation suggests that patients with T2DM and elevated serum SERPINE2 levels were significantly more likely to develop DN. A more comprehensive population-based prospective study is required to validate our findings.

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Author Contribution. Shudan Cao and Qing Tan: Conceptualization, Data curation, Investigation, Methodology, Writing-original draft Preparation. Lijuan Yang: Writing -review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation.

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Data Availability. The datasets used/analyzed during the present study are available from the corresponding author upon reasonable request.

Declarations

Conflict of Interest. The authors Shudan Cao, Qing Tan, and Lijuan Yang have nothing to disclose.

Ethical Approval. The Ethics Committee of Sijing Hospital granted approval for this study (SJYY202401-YNKT-CSD). The authors adhered to all standard protocols in line with the 1964 Declaration of Helsinki. Informed consent was obtained from all subjects participating in the study.

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