Diabetes, Metabolic Syndrome and Obesity

Dovepress

open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Association of Serum Tsukushi Levels with Urinary Albumin-Creatinine Ratio in Type 2 Diabetes Patients

Yanyan Li[1], Xia Deng[2], Xunan Wu[2], Ligang Zhou[1], Guoyue Yuan[2]

[1]Department of Endocrinology, Shanghai Pudong Hospital, Fudan University Pudong Medical Center, Pudong, Shanghai, 201399 People's Republic of China; [2]Department of Endocrinology, Affiliated Hospital of Jiangsu University, Zhenjiang, Jiangsu Province, 210031, People's Republic of China

Correspondence: Guoyue Yuan, Department of Endocrinology, Affiliated Hospital of Jiangsu University, No. 438 Jiefang Road, Zhenjiang City, Jiangsu Province, 210031, People's Republic of China, Email yuanguoyue@ujs.edu.cn; Ligang Zhou, Department of Endocrinology, Shanghai Pudong Hospital, Fudan University Pudong Medical Center, 2800 Gongwei Road, Pudong, Shanghai, 201399, People's Republic of China, Email zhouligang@yahoo.com

Objective: Tsukushi is a newly identified hepatokine. Recent studies have shown that it relates to diabetes, lipid metabolism and fibrosis, but there is currently no investigation about whether Tsukushi is associated with diabetic kidney disease. Therefore, this study aimed to investigate the relationship between Tsukushi and diabetic kidney disease by characterizing Tsukushi levels in healthy subjects and type 2 diabetes with urinary albumin-creatinine ratio.

Methods: Serum Tsukushi level was quantified by enzyme-linked immunosorbent assay in 167 normoalbuminuria, 80 microalbuminuria, and 31 macroalbuminuria patients with type 2 diabetes as compared with 53 healthy subjects. The correlation analysis was used to investigate the relationship between urinary albumin-creatinine ratio or Tsukushi level and other metabolic parameters. Multiple linear regression and logistic regression analysis were used to analyze the independent factors for urinary albumin-creatinine ratio and estimated glomerular filtration rate.

Results: The Tsukushi level in the macroalbuminuria group was significantly higher than that in the normoalbuminuria or the microalbuminuria group. Multiple linear regression showed that the significantly independent factors for UACR included high Tsukushi quartile, systolic blood pressure, creatinine, homeostasis model assessment of insulin resistance, low 2-h post-oral glucose tolerance test c-peptide and female. Logistic regression demonstrated that the odds ratio of Tsukushi for glomerular filtration rate ≤90 (mL/min/1.73m^2) was 1.636 (95% CI 1.091–2.452, P=0.017).

Conclusion: The circulating Tsukushi increased in type 2 diabetes patients with albuminuria and was associated with urinary albumin-creatinine ratio, implying that Tsukushi may be involved in the pathogenesis of diabetic kidney disease, which deserves future studies.

Keywords: type 2 diabetes, urinary albumin-creatinine ratio, hepatokine, Tsukushi

Introduction

Diabetic kidney disease (DKD) is the leading cause of chronic kidney disease and one of the most common complications of diabetes, occurring in approximately 40% of patients with diabetes.[1] Past studies revealed that DKD is the result of multiple pathophysiological disturbances, including but not limited to hyperglycemia, hypertension, hyperlipidemia, inflammation, mitochondrial dysfunction, and impaired autophagy, leading to progressive deterioration in kidney structure and function.[2]

Tsukushi (TSK), a novel recognized hepatokine originally isolated and identified in 2004 from the chicken lens, was a member of small leucine-rich proteoglycan family (SLRPs).[3] Current research demonstrated that TSK is involved in modulating both lipid and glucose metabolism.[4,5] Research showed that TSK can affect systemic cholesterol homeostasis[6] and TSK deficiency reduced inflammatory gene expression in macrophages.[7] Clinical research also showed that TSK was independently associated with high insulin and fibroblast growth factor 21 and low total cholesterol in the general participants,[8] and in a study based on obese subjects, TSK was positively associated with BMI, visceral fat area and the deterioration of glucose metabolism.[4] Particularly, our recent study pointed out that TSK increased in newly

Received: 25 April 2024
Accepted: 14 August 2024
Published: 4 September 2024

diagnosed type 2 diabetes (T2D) and had an independent relationship with multiple metabolic characteristics and insulin resistance as well as metabolic syndrome risk.[5,9] It is worth noting that studies have found that other SLRP family members play an important role as extracellular matrix and soluble signaling molecules in regulating various complex biologic processes that are deeply involved in the progression of kidney diseases.[10] However, there is currently no related investigation about TSK associated with DKD.

Albuminuria is the classic sign of DKD, and microalbuminuria is regarded as the pivotal manifestation of the early stage of DKD.[11] In clinical work, the urinary albumin-creatinine ratio (UACR) is currently widely used and studied as a convenient and accurate marker of early renal injury.[12] The objective of this clinical research was to investigate this relationship between TSK and albuminuria by characterizing TSK levels in healthy subjects and T2D patients with various levels of albuminuria.

Materials and Methods

Study Participants

A total of 331 participants were enrolled in the cross-sectional study, of which 278 were T2D and 53 were healthy controls (Figure 1). All participants were Chinese Hans and recruited in the Department of Endocrinology, Affiliated Hospital of Jiangsu University, from 01.2016 to 11.2020. The diagnosis of T2D was based on 1999 World Health Organization diagnostic criteria. Patients with type 1 diabetes mellitus, urinary infection, other known kidney diseases except diabetic nephropathy, severe heart or liver failure or any other major disease were excluded. We also excluded the patients with severe renal function (serum creatinine ≥3.0 mg/dL or glomerular filtration rate <30 mL/min/1.73) to avoid the possible confounding influence of renal failure on excretion of TSK. All T2D patients were divided into three groups according to the levels of urine albumin-creatinine ratio, normoalbuminuria (UACR < 30 mg/g, n = 167), microalbuminuria (UACR 30–300 mg/g, n = 80), and macroalbuminuria (UACR > 300 mg/g, n = 31). The healthy control subjects had normal glucose tolerance according to oral glucose tolerance test (OGTT) and reported no regular medication use.

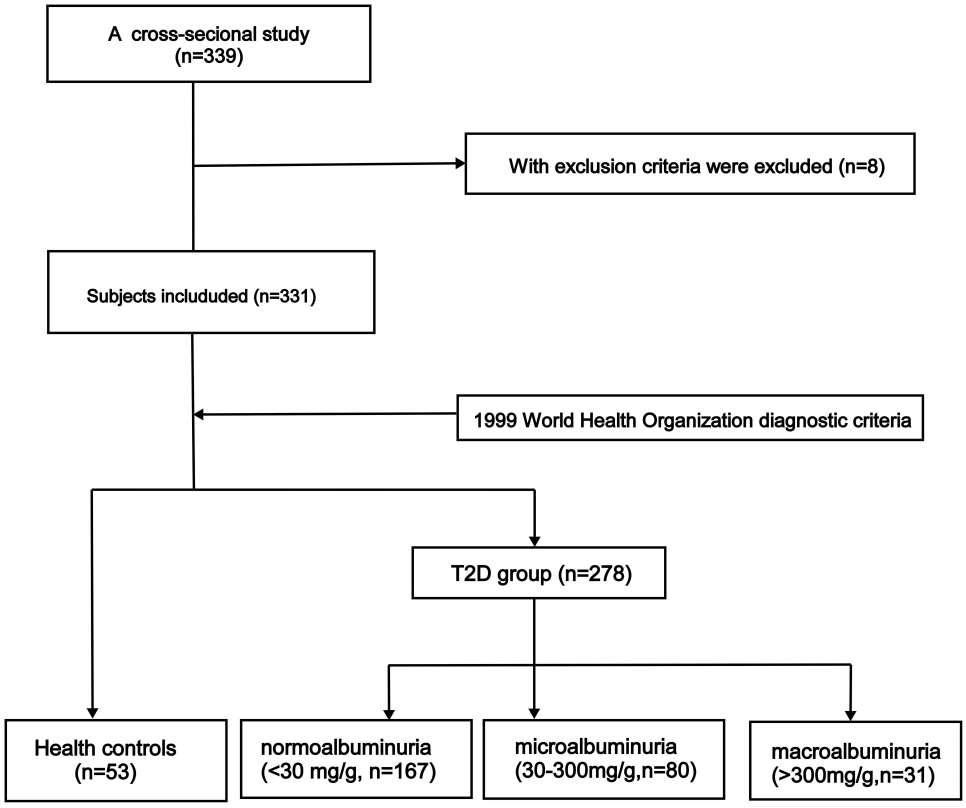

Figure 1 The participant flowchart.

The study complied with the Helsinki Declaration. Ethical approval was obtained from the ethics committee at the Affiliated Hospital of Jiangsu University, and informed consent was obtained from all participants.

Clinical Information and Biochemical Measurements

The baseline clinical information of all participants was obtained by trained medical professionals, and biochemical measurements were performed by laboratory medicine as described in a previous published article.[5] To detail, serum creatinine was detected by enzymatic method. Blood urea nitrogen (BUN) was measured by velocity assays, while serum uric acid (SUA) was determined by chemiluminescence method. UACR was tested by immunoturbidimetry on morning midstream urine samples, after the subjects were requested to avoid strenuous exercise within 24 hours. Peripheral venous blood (3–4 mL) was collected from the overnight fasting participants and centrifuged by 10 min at 1500g to separate serum, which was stored at −80°C and thawed at room temperature before use. Serum TSK levels were quantified by a commercially available human enzyme-linked immunosorbent assay kit (Eiaab Science, Wuhan, China; E15159h) with intra- and inter-assay coefficients of variation for <7.4% and <10.1%, respectively. Estimated glomerular filtration rate (eGFR) was calculated by the simplified Modification of Diet in Renal Disease (MDRD) equation.[13] The degrees of insulin resistance and pancreatic beta-cell function were calculated by the homeostasis model:[14] homeostasis model assessment of insulin resistance(HOMA-IR)=FIns * FPG/22.5, and homeostasis model assessment of pancreatic beta-cell function(HOMA-β)= 20 * FIns/(FPG – 3.5).

Statistical Analysis

Data were represented as mean ± standard deviation ($\bar{x}$±SD) for normally distributed continuous variables, as median and interquartile range [M (25th, 75th)] for non-normally distributed continuous variables, or as percentage (n%) for categorical variables alternatively. The log-transformation of skewed distribution variables was taken to approximate normality before analysis. The variable comparisons among the four groups were evaluated by ANOVA, while categorical variables were tested by $\chi 2$ analysis. The correlation coefficients between TSK and UACR with other metabolic variables were calculated by Pearson correlation analysis. The multiple linear regression was performed to evaluate factors influencing UACR and TSK, respectively. Binary logistic regression analyses were taken to assess the odds ratio (OR) of TSK quartile for eGFR ≤90(mL/min/1.73m2) in T2D. Data analyses were performed using SPSS 22. $P < 0.05$ (two-tailed) was considered statistically significant.

Results

Baseline Characteristics of Participants and TSK Levels in Different Groups

The clinical information and biochemical measurements of participants were first summarized in Table 1. The creatinine level of control subjects was higher than that in normoalbuminuric or microalbuminuria group, but lower than that in macroalbuminuria group ($P< 0.05$). On the contrary, the eGFR level of control subjects was lower than that in the normoalbuminuric or the microalbuminuria group, but higher than that in the macroalbuminuria group ($P< 0.05$). What's more, the TSK level in each of the T2D groups was prominently higher than that in the control group. Further, the TSK level in the macroalbuminuria group was significantly higher than that in the normoalbuminuria or the microalbuminuria group.

Correlations Between UACR and TSK with Various Biochemical Parameters

To investigate the relationship between UACR and other metabolic parameters, we used correlation analysis. UACR in T2D was positively correlated with age, duration of T2D, SBP, DBP, BUN, creatinine, SUA, FIns, and TSK and inversely correlated with eGFR, 2hPG, 2hCP, glutamic pyruvic transaminase (ALT), and glutamic oxaloacetic transaminase (AST). We also explored the relationship between TSK and other biochemical parameters, showing that the TSK level in T2D was positively correlated with duration of T2D, TC, and UACR (Table 2).

Table 1 The Anthropometric and Metabolic Parameters in Healthy Controls and in T2D Patients by UACR Categories

Variables	Control (n = 53)	T2D with Normoalbuminuria (n = 167)	T2D with Microalbuminuria (n = 80)	T2D with Macroalbuminuria (n = 31)	*P* value
Gender, male/female	25/28	96/71	39/41	19/12	0.339
Age (years)	47.57±11.59	55.1±11.91*	60.38±11.71*,†	59.9±10.53*,†	0.000
Duration of T2D§ (months)	/	48(0, 120)	120(12, 180)†	120(72, 216)†	0.000
BMI (kg/m^2)	23.37±2.71	25.24±3.61*	25.36±3*	25.08±3.4*	0.002
SBP (mmHg)	116.70±13.96	129.74±14.25*	139.4±15.52*,†	146.74±24.82*,†,‡	0.000
DBP (mmHg)	69.49±12.02	81.01±9.94*	83.45±9.75*	86.19±12.61*,†	0.000
WHtR	0.51±0.04	0.54±0.06*	0.56±0.05*	0.56±0.05*	0.000
Alcohol use (%)					0.072
Regular drinker	9.4	13.8	17.5	9.7	
Occasional drinker	11.3	4.8	1.3	0	
Never	79.2	81.4	81.3	90.3	
Smoking status (%)					0.356
Current smoker	28.3	28.7	20	22.6	
Former smoker	5.7	3	6.2	0	
Never	66	68.3	73.8	77.4	
Hypertension	/	80(47.9)*	54(67.5)*,†	23(74.2)*,†	0.000
Antihypertensive agent	/	63(37.7)*	45(56.3)*,†	23(74.2)*,†	0.000
ACEI or ARB use	/	42(25.1)*	27(33.8)*	15(48.4)*,†	0.000
Lipid-lowering agents	/	4(2.4)	5(6.3)	3(9.7)	0.040
Hypoglycemic agent	/	32(19.2)*	34(42.5)*,†	13(41.9)*,†	0.000
SGLT2i	/	3(1.8)	3(3.8)	0	0.324
BUN (mmol/L)	4.94±1.09	5.32±1.36	5.56±1.82*	7.34±2.96*,†,‡	0.000
eGFR(mL/min/1.73m^2)	100.68±18.26	122.63±26.31*	120.25±34.11*	84.97±32.67*,†,‡	0.000
Creatinine§ (mg/dl)	0.76(0.67,0.87)	0.65(0.56,0.76)*	0.64(0.52,0.79)*	0.89(0.65,1.3)*,†,‡	0.000
HbA1c (%)	5.75±0.29	9.26±1.94*	9.8±2.13*,†	8.9±2.16*,‡	0.000
FPG (mmol/L)	4.69±0.51	10.16±3.33*	10.51±3.4*	9.46±4.16*	0.000
2hPG (mmol/L)	6.25±0.88	19.05±5.07*	19.5±4.87*	16.59±6.72*,†,‡	0.000
FCP (ng/mL)	2.19±0.83	2.38±1.08	2.23±1.27	2.21±1.09	0.602
2hCP§ (ng/mL)	7.78(5.83,9.74)	5.01(3.29,6.92)*	3.78(2.73,5.97)*,†	4.11(2.92,5.22)*,†	0.000
FIns§ (uIU/mL)	4.66(2.97,6.77)	6.24(3.72,10.38)*	8.23(4.88,14.23)*,†	9.94(4.03,11.62)*	0.000
2hIns§ (uIU/mL)	27.79(18.59,43.83)	29.17(14.26,51.35)	33.41(15.8,57.27)	37.01(21.44,58.24)	0.489
TG§(mmol/L)	1.24(0.93,1.72)	1.93(1.3,2.79)*	1.74(1.38,2.59)*	2.35(1.39,4.2)*	0.000
TC (mmol/L)	4.96±0.81	4.88±1.01	5.05±1.25	5.45±1.36*,†	0.053
HDL-C (mmol/L)	1.46±0.32	1.05±0.29*	1.11±0.31*	1.11±0.29*	0.000
LDL-C (mmol/L)	2.8±0.74	2.8±0.86	2.89±0.98	3±0.91	0.611
ALT§ (U/L)	15.2(9.80,29.80)	22(14.9,35)*	20.55(12.75,35.65)	14.1(8.4,24.7)†,‡	0.006
AST§(U/L)	17.1(14.05,21.80)	17.1(13.1,23.2)	15.8(13.13,25.8)	14.3(10.9,16.8)*,†,‡	0.029
γ-GT§ (U/L)	20(15,29.5)	28(19,46)*	31(21,54)*	26.7(18.8,44)*	0.002
SUA (umol/L)	289.83±82.7	283.02±78.15	285.69±98.15	324.42±84.86†,‡	0.097
HOMAIR§	0.98(0.69,1.50)	3.24(1.61,4.39)*	3.77(2.35,5.5)*,†	3.77(1.79,4.93)*	0.000
HOMA-beta§	72.23(47.83,140.48)	20.16(10.14,45.22)*	23.29(12.11,46.02)*	33.4(20.94,60.17)*,†	0.000
TSK§ (ng/mL)	0.68(0.51,1.18)	1.4(0.88,2.22)*	1.35(0.9,1.83)*	1.95(1.12,3.05)*,†,‡	0.000

Notes: Data are mean ±SD, M (25th, 75th) and n (%), §Log-transformed variable. *P < 0.05 vs healthy subjects, †P < 0.05 vs normoalbuminuria, ‡P < 0.05 vs microalbuminuria.

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WHtR, the waist-to-hip ratio; BUN, blood urea nitrogen; eGFR, Estimated glomerular filtration rate; FPG, fasting plasma glucose; 2hPG, 2-h post-OGTT glucose; HbA1c, glycosylated hemoglobin; FCP, fast c-peptide; 2hCP, 2-h post-OGTT c-peptide; FIns, fast insulin; 2hIns, 2-h post-OGTT insulin; TG, triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol, LDL-C, low density lipoprotein cholesterol; ALT, glutamic pyruvic transaminase; AST, glutamic oxaloacetic transaminase; γ-GT, glutamyl transpeptidase; SUA, serum uric acid; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-beta, homeostasis model assessment of pancreatic beta-cell function; TSK, Tsukushi.

Table 2 The Correlation Analysis of Variables Associated with TSK and UACR Levels

Varies	UACR§				TSK§			
	Control		T2D		Control		T2D	
	r	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value
Age (years)	0.032	0.822	0.223	0.000	−0.139	0.321	0.054	0.371
Duration of T2D§ (months)	/	/	0.298	0.000	/	/	0.182	0.002
BMI (kg/m^2)	0.169	0.226	0.002	0.973	0.230	0.097	−0.031	0.609
SBP (mmHg)	−0.042	0.765	0.374	0.000	0.258	0.062	0.096	0.109
DBP (mmHg)	0.086	0.539	0.187	0.002	0.010	0.942	0.040	0.510
BUN (mmol/L)	−0.155	0.268	0.270	0.000	0.049	0.730	0.019	0.758
eGFR(mL/min/1.73m^2)	0.370	0.006	−0.295	0.000	0.135	0.334	−0.094	0.116
Creatinine§(mg/dl)	−0.559	0.000	0.275	0.000	0.051	0.717	0.053	0.378
SUA (umol/L)	−0.251	0.069	0.128	0.033	0.169	0.225	−0.044	0.461
HbA1c (%)	−0.009	0.947	0.036	0.550	−0.065	0.645	−0.076	0.207
FPG (mmol/L)	−0.137	0.328	−0.016	0.793	0.079	0.574	−0.107	0.076
2hPG (mmol/L)	−0.074	0.601	−0.121	0.044	0.026	0.855	−0.091	0.128
FCP (ng/mL)	−0.022	0.875	−0.079	0.191	0.058	0.678	−0.054	0.370
2hCP§ (ng/mL)	−0.068	0.627	−0.180	0.003	0.004	0.976	−0.034	0.576
FIns§ (uIU/mL)	0.034	0.808	0.133	0.027	0.168	0.230	0.038	0.524
2hIns§ (uIU/mL)	0.077	0.586	0.069	0.250	0.020	0.887	0.077	0.203
TG§ (mmol/L)	0.025	0.856	0.026	0.661	0.089	0.528	−0.072	0.233
TC (mmol/L)	0.074	0.597	0.114	0.058	−0.213	0.127	0.145	0.015
HDL-C (mmol/L)	0.201	0.150	0.104	0.083	−0.179	0.200	0.033	0.580
LDL-C (mmol/L)	−0.031	0.828	0.049	0.418	−0.171	0.220	0.094	0.117
ALT§ (U/L)	0.149	0.288	−0.189	0.002	0.155	0.268	−0.021	0.724
AST§ (U/L)	0.082	0.561	−0.147	0.014	−0.041	0.773	−0.091	0.130
γ-GT§ (U/L)	−0.149	0.286	−0.018	0.764	0.054	0.701	−0.029	0.631
HOMA-IR§	0.009	0.947	0.114	0.057	0.169	0.227	−0.012	0.844
HOMA-beta§	0.151	0.280	0.110	0.067	0.103	0.465	0.081	0.178
TSK§	0.078	0.576	0.124	0.040	/	/	/	/
UACR§	/	/	/	/	0.078	0.576	0.124	0.040

Note: §Log-transformed variable.

Multiple Linear Regression Analysis for UACR According to the Quartile of TSK in T2D

To further elaborate the independent associations, the multiple linear regression was performed. TSK in T2D was divided into four groups, that is Q1(<0.819, n = 69), Q2(0.819~1.324, n = 70), Q3(1.324~2.078, n = 70), and Q4(≥2.078, n = 69), according to the quartile of TSK level. As shown in Table 3, the quartile of TSK was a significant determining factor for UACR in both Model 1 (unadjusted) and Model 2 (adjusted age and gender) ($P< 0.01$). When the regression was further adjusted in Model 3 (adjusted model 2 and duration of T2D, SBP, DBP, WHtR, hypertension, BUN, creatinine, eGFR, HbA1c, 2hPG, 2hCP, TC, LDL-C, ALT, AST, SUA, HOMA-IR, HOMA-beta, smoking, drinking, and medication use), the results showed that high TSK quartile, SBP, creatinine, HOMA-IR, low 2hCP, and female were the significantly independent factors for UACR ($P< 0.05$) (Table 3).

Regression Analysis for TSK and eGFR in T2D

Besides, to explore the independent factors for TSK in T2D, the multiple regression was executed again, which has demonstrated that TC ($P< 0.01$) and 2hPG ($P< 0.05$) were independent of TSK. In addition, we estimated the odds ratio of TSK quartile for eGFR ≤90(mL/min/1.73m^2) by binary logistic regression analyses. The results showed that the quartile of TSK was a factor for eGFR ≤90(mL/min/1.73m^2) in unadjusted model [OR = 1.394 (95% CI 1.044–1.861), P=0.024]. Following multiple adjustment with various characteristics (age, gender, duration, BMI, SBP, DBP, WHtR, HbA1c, 2hPG,

Table 3 The Multiple Linear Regression Analysis with UACR as the Dependent Variable in T2D

Model	Variable	Standardized Coefficients (SE.)	95.0% CI	*P* value
1	TSK quartile	0.17(0.037)	0.033~0.178	0.004
2	Age	0.213(0.003)	0.006~0.019	0.000
	TSK quartile	0.157(0.036)	0.026~0.169	0.007
3	SBP	0.307(0.002)	0.008~0.017	0.000
	Creatinine	0.383(0.164)	0.76~1.405	0.000
	Gender^	−0.24(0.08)	−0.493~-0.177	0.000
	2hCP	−0.148(0.011)	−0.055~-0.011	0.004
	TSK quartile	0.107(0.032)	0.004~0.129	0.037
	HOMA-IR	0.101(0.009)	0~0.035	0.048

Notes: ^Gender: Female=0, Male=1. Model 1: crude; Model 2: adjusted for age, gender; Model 3: adjusted for age, gender duration of T2D, SBP, DBP, WHtR, hypertension, BUN, creatinine, eGFR, HbA1c, 2hPG, 2hCP, TC, LDL-C, ALT, AST, UA, HOMA-IR, HOMA-beta, smoking, drinking and medication use.

2hCP, TC, LDL-C, ALT, AST, γ-GT, SUA, HOMA-IR, HOMA-beta, smoking, drinking, medication use, and three UACR groups), we observed that the OR of TSK for eGFR ≤90(mL/min/1.73m^2) was 1.636 (95% CI 1.091–2.452, *P*=0.017).

Discussion

In this study, we showed that TSK increased in T2D with normoalbuminuria, microalbuminuria or macroalbuminuria compared with the control group, especially in macroalbuminuria group. In addition, TSK in T2D was positively correlated with the duration of DM, TC, and UACR. Furthermore, the quartile of TSK was a significant determining factor for UACR and an important factor for eGFR ≤90(mL/min/1.73m^2), even after adjusting for various characteristics. The above results implied that TSK may be involved in DKD development, which has not elaborated in the existing research.

As a novel hepatokine, TSK was demonstrated to be capable of regulating metabolic processes, including lipid and glucose metabolism. This study showed that serum TSK was higher in T2D than that in the healthy group and was positively correlated with TC. This result was consistent with the result of our previous study on a newly diagnosed T2D group.[5] Nevertheless, our findings in the control group did not show the correlation, which did not accord with the research performed by Masato et al, in which plasma TSK was associated with low TC in the general subjects.[8] The disparities may derive from the differences in sample size or sample testing. This suggests that larger sample sizes or cohort studies in future studies should be considered. Although results from different clinical research were partly divergent, they collectively suggested that TSK may be involved in lipid metabolism disorder. What's more, multiple linear regression analysis had demonstrated that TSK quartile was the significantly independent factor for UACR and high TSK was a risk factor for eGFR ≤90(mL/min/1.73m^2), which hints that TSK may participate in DKD development.

Although the mechanism by which TSK contributes to DKD progression remains unclear, previous studies offer some hints. A study about the hair cycle revealed that TSK deletion induces the down expression of TGF-β1 and TGF-β2, and then inhibits the expression of downstream phosphorylated Smad2/3.[15] Additionally, immunoprecipitation analysis indicates that TSK directly binds to TGF-β1. It is worth noting that TGFβ1-Smad2/3 pathway plays a crucial part in profibrotic transcription process in DKD.[16] Smad3 null mice are protected from renal fibrosis, including glomerular basement membrane thickening and ECM overproduction in STZ-induced diabetes, and they prevent renal fibrosis and inflammation in db/db mice.[17,18] Interestingly, previous studies have shown that other SRLP families, such as biglycan, lumican, decorin, and fibromodulin, place a special emphasis on DKD.[10] Studies have shown that the increased renal biglycan, caused by elevated TGF-beta in diabetes and hypercholesterolemia, may lead to the renal LDL accumulation, which finally contributes to the development of

glomerular injury.[19] Biglycan in STZ mice can trigger chemoattractant in macrophages, leading to Th1 and Th17 cell recruitment into the kidney, which induces renal fibrosis. Besides, the recent results from proteomics have shown lumican increase in urinary excretion in early T1DM, which may involve the early kidney response to hyperglycemia.[20] In contrast, studies have demonstrated that decorin deficiency in STZ-induced diabetic mice causes the aggravation of nephropathy, implying that decorin is a protector for DKD, which also causes the upregulation of biglycan,[21] and increased decorin may neutralize the hypertrophic and prosclerotic function of high TGF-beta in diabetic kidney.[22] Moreover, fibromodulin shows anti-fibrotic function by modulating TGF-beta.[23] Altogether, these findings suggest that SLRPs are related to DKD by varied mechanisms, and they may have a direct or indirect mutual effect on different members of SLRPs, such as decorin and biglycan. In addition, some researchers have proposed that the dichotomy (pro- or anti-inflammatory) of SLRP signaling allows for fine-tuning the inflammatory response, which is crucial for the outcome of kidney diseases.[24] Our study preliminarily describes the relationship between TSK and UACR from a clinical perspective. Presumably, as a valued member of SLRPs, TSK, especially the circulating TSK, may also play a part in the pathogenesis of DKD, which deserves further attention, especially in the basic research on metabolic disorders, inflammatory and fibrotic renal diseases.

A few issues from this study warrant additional discussion. The results showed that the control group had higher creatinine and lower eGFR than the normoalbuminuric or the microalbuminuria group, whereas it had lower creatinine and higher eGFR than the macroalbuminuria group. This discrepancy may arise from the affection of glomerular hyperfiltration in early diabetic nephropathy,[25] and results showed that the trend of TSK level in albuminuria was unaffected by it. Apart from that, recent results from Sum Lam et al showed that serum TSK levels increase in subjects with NAFLD and reflect the severity of liver fibrosis independent of T2DM,[26] which also provide valuable insights for future research and the treatment of TSK in fibrosis.

Several limitations should be considered in this study. To begin with, the cross-sectional nature was the major limitation of this research, which limited our ability to illuminate the causality and specific mechanisms. Then, we exclude the patients GFR <30 mL/min/1.73m^2 to avoid the possible confounding influence on excretion of TSK level, but also to hinder exploration of the relationship between TSK and UACR and between TSK and GFR in renal failure. Apart from these, the potential bias from single-center research and the nubilous influence of medication usage on TSK level should also not be overlooked, such as the effect of drugs on metabolism and fibrosis. The lack of NAFLD data in this study may have affected the results and their interpretation. Therefore, future research should consider those limitations. However, the implications of this study remain important even with these limitations.

Conclusion

Altogether, the findings of our study pointed out that the circulating TSK may be associated with UACR in T2DM and could be a novel potential predictive or therapeutic target in DKD, which would provide important clinical hint for future studies. Future clinical research should expand the sample or employ a cohort study approach to investigate the relationship between TSK and various kidney diseases. Moreover, the underlying mechanisms of TSK in DKD development require further studies, especially in the context of metabolic disorders, inflammation and renal fibrosis.

Data Sharing Statement

Primary datasets are available from the corresponding author on reasonable request.

Acknowledgments

We thank all participants for their work in this study. We appreciated the opportunity to present our preliminary results of this study as a poster at the 2023 American Diabetes Association conference.[27]

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically

reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

YYL is supported by the Talents Training Program of Shanghai Pudong Hospital (YQ202101). GYY has financial support from the National Natural Science Foundation of China (81870548), the Social Development Project of Jiangsu Province (BE2018692 and BE2023757), the Natural Science Foundation of Jiangsu Province (BK20191222), the "169-Phase V" Science Research Project of Zhenjiang, and the sixth phase 333 second level talent training project of Jiangsu Province (tackling bottleneck technology) (BRA2022008).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Alicic Radica Z, Rooney Michele T, Tuttle Katherine R. Diabetic kidney disease: challenges, progress, and possibilities. *Clin J Am Soc Nephrol*. 2017;12:2032–2045. doi:10.2215/CJN.11491116
2. Chan-Young J, Tae-Hyun Y. Pathophysiologic mechanisms and potential biomarkers in diabetic kidney disease. *Diabet Metabol J*. 2022;46:181–197. doi:10.4093/dmj.2021.0329
3. Xia D, Yanyan L, Chang G, et al. Novel roles of Tsukushi in signaling pathways and multiple disease processes. *BioFactors*. 2021;47:512–521. doi:10.1002/biof.1723
4. Y. L, Jin L, Yan J, et al. Tsukushi and TSKU genotype in obesity and related metabolic disorders. *J Endocrinol Invest*. 2021;44:2645–2654. doi:10.1007/s40618-021-01572-x
5. Yan-Yan L, Xu-Nan W, Xia D, et al. Serum Tsukushi levels are elevated in newly diagnosed type 2 diabetic patients. *Diabet Res Clin Pract*. 2021;178:108987. doi:10.1016/j.diabres.2021.108987
6. Mouchiroud M, É C, Aldow M, et al. The hepatokine Tsukushi is released in response to NAFLD and impacts cholesterol homeostasis. *JCI Insight*. 2019;4:e129492.
7. Qiuyu W, Sharma Vishal P, Hong S, et al. The hepatokine Tsukushi gates energy expenditure via brown fat sympathetic innervation. *Nat Metab*. 2019;1:251–260. doi:10.1038/s42255-018-0020-9
8. Masato F, Yukimura H, Akiko S, et al. Plasma Tsukushi concentration is associated with high levels of insulin and FGF21 and low level of total cholesterol in a general population without medication. *Metabolites*. 2022;12:237. doi:10.3390/metabo12030237
9. Yanyan L, Xia D, Xunan W, et al. Association of serum Tsukushi level with metabolic syndrome and its components. *Endocrine*. 2023;79:469–476. doi:10.1007/s12020-022-03285-4
10. Liliana S. Small leucine-rich proteoglycans in kidney disease. *J Am Soc Nephrol*. 2011;22:1200–1207. doi:10.1681/ASN.2010050570
11. Mogensen CE, Christensen CK, Vittinghus E. The stages in diabetic renal disease. With emphasis on the stage of incipient diabetic nephropathy. *Diabetes*. 1983;32 Suppl 2:64–78. doi:10.2337/diab.32.2.S64
12. Yue Z, Binqi L, Yang L, et al. Association between metabolic phenotype and urinary albumin-creatinine ratio in Chinese community adults: a cross-sectional study. *J Diabetes*. 2022;14:541–550. doi:10.1111/1753-0407.13302
13. Ying-Chun M, Zuo L, Jiang-Hua C, et al. Modified glomerular filtration rate estimating equation for Chinese patients with chronic kidney disease. *J Am Soc Nephrol*. 2006;17:2937–2944. doi:10.1681/ASN.2006040368
14. R. MD, P. HJ, S. RA, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412–419. doi:10.1007/BF00280883
15. Daisuke N, Rie K, Athary F, et al. Tsukushi controls the hair cycle by regulating TGF-β1 signaling. *Dev Biol*. 2012;372:81–87. doi:10.1016/j.ydbio.2012.08.030
16. Wang L, Hong-Lian W, Tong-Tong L, et al. TGF-beta as a master regulator of diabetic nephropathy. *Int J Mol Sci*. 2021;22:7881. doi:10.3390/ijms22157881
17. Masaki F, Yoshiro M, Koutaro Y, et al. Mice lacking Smad3 are protected against streptozotocin-induced diabetic glomerulopathy. *Biochem Biophys Res Commun*. 2003;305:1002–1007. doi:10.1016/S0006-291X(03)00885-4
18. Bi-Hua X, Jingyi S, Yong-Ke Y, et al. Deletion of Smad3 prevents renal fibrosis and inflammation in type 2 diabetic nephropathy. *Metabolism*. 2020;103:154013. doi:10.1016/j.metabol.2019.154013
19. Thompson J, Wilson P, Brandewie K, et al. Renal accumulation of biglycan and lipid retention accelerates diabetic nephropathy. *Am J Pathol*. 2011;179:1179–1187. doi:10.1016/j.ajpath.2011.05.016
20. Van JAD, Clotet-Freixas S, C. HA, et al. Urinary proteomics links keratan sulfate degradation and lysosomal enzymes to early type 1 diabetes. *PLoS One*. 2020;15:e0233639.
21. Merline R, Lazaroski S, Babelova A, et al. Decorin deficiency in diabetic mice: aggravation of nephropathy due to overexpression of profibrotic factors, enhanced apoptosis and mononuclear cell infiltration. *J Physiol Pharmacol*. 2009;60(4):5–13.
22. Mogyorosi A, Ziyadeh FN. Increased decorin mRNA in diabetic mouse kidney and in mesangial and tubular cells cultured in high glucose. *Am J Physiol*. 1998;275:F827–832.
23. F. JM, Biglari A, Mazloomzadeh S, et al. Recombinant fibromodulin has therapeutic effects on diabetic nephropathy by down-regulating transforming growth factor-β1 in streptozotocin-induced diabetic rat model. *Iran J Basic Med Sci*. 2016;19:265–271.

24. V. NM, Janicova A, Roedig H, et al. Small leucine-rich proteoglycans in renal inflammation: two sides of the coin. *J Histochem Cytochem*. 2018;66:261–272. doi:10.1369/0022155417738752
25. Volker V, Thomson Scott C. Renal function in diabetic disease models: the tubular system in the pathophysiology of the diabetic kidney. *Annu Rev Physiol*. 2012;74:351–375. doi:10.1146/annurev-physiol-020911-153333
26. Sum L, Chi-Ho L, Y. FCH, et al. Serum Tsukushi level is associated with the severity of liver fibrosis independent of type 2 diabetes. *J Clin Endocrinol Metab*. 2023;109:e1048–e1054.
27. Li Y. 15-LB: serum tsukushi is associated with urinary albumin excretion in type 2 diabetes. *Diabetes*. 2023;72(Supplement_1):15–LB. doi:10.2337/db23-15-LB