KARBALA JOURNAL OF MEDICINE

Research Article

Sestrin-2 as a Diagnostic Potential Biomarker for Diabetic Kidney Disease in Individuals with Type 2 Diabetes mellitus

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Article information:

Received: 04-08-2025
Accepted: 09-09-2025
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https://doi.org/10.70863/karbalajm.v18i2.4942

Abstract

Background: Diabetic kidney disease (DKD) is a major microvascular complication of type 2 diabetes mellitus (T2DM), contributing significantly to end-stage renal disease. Sestrin-2 (SESN2), a protein induced by stress that plays a role in oxidative stress response, autophagy, and metabolic control, has been identified as a potential biomarker candidate.

Methods: A case-control study involved one hundred and sixty patients who were diagnosed with T2DM. Blood analysis for sugar levels (FBG and HbA1c), lipid profile, renal function test, and albumin-to-creatinine ratio in urine (uACR) were assessed.

Results: The serum sestrin-2 levels were significantly elevated in patients with T2DM compared to healthy controls. Macroalbuminuria patients showed the highest sestrin-2 level (3.28 ± 1.22 ng/ml). However, the serum levels of sestrin-2 in the health group were (2.29 ± 0.59 ng/ml), in the normoalbuminuria group, there was a significant positive correlation between serum sestrin-2 levels with FBG, HbA1c, and High-density lipoprotein (HDL), and a negative correlation between sestrin-2 and glomerular filtration rate (GFR). Moreover, in the microalbuminuria group, a significant positive correlation of a moderate effect size was observed between sestrin-2 level with body mass index (BMI). The area under the receiver operating characteristic curve (AUC) for Sestrin-2 was 0.933 at the cutoff value 3.91, with the sensitivity and specificity for differentiating macroalbuminuria from control being 100% and 75%, respectively.

Conclusions: The serum concentrations of sestrin-2 were markedly elevated in the study groups (normoalbuminuria, microalbuminuria, and macroalbuminuria) compared to the healthy control group, suggesting its involvement in the development of diabetic kidney disease (DKD) as a complication of T2DM. Based on the findings, Sestrin-2 demonstrates a remarkable capacity for diagnosing DKD.

Keywords: Sestrin-2; Type 2 Diabetes Mellitus, Diabetic Kidney Disease.

Introduction

Diabetes mellitus (DM) is a metabolic disorder marked by hyperglycemia. This condition arises from defects in insulin function and production, resulting in abnormal glucose metabolism. Prolonged hyperglycemia impairs the oxidative stress response and cellular autophagy, which subsequently triggers inflammatory responses and coagulation activation, ultimately leading to complications across various organs and systems [1]. In recent years, there has been a significant rise in the global number of patients with diabetes, attributed to an aging population, lifestyle changes, and the growing prevalence of obesity. Type 2 diabetes mellitus (T2DM) is the predominant form of diabetes, representing approximately 90% of all diabetes cases. Diabetes and its associated chronic complications have emerged as significant contributors to disability and mortality among individuals, while also imposing substantial economic burdens on a global scale [2]. Diabetic kidney disease (DKD) is the leading cause of end-stage renal disease (ESRD) worldwide, affecting approximately 40% of diabetic patients and posing a huge burden on public health [3]. DKD is clinically diagnosed in patients demonstrating a decline in their estimated glomerular filtration rate (eGFR) to less than 60 ml/min/1.73m², a sustained rise of albuminuria (urine albumin-to-creatinine ratio (uACR) ≥ 30 mg/g, or both [4]. DKD, sometimes referred to as

diabetic nephropathy, constitutes a significant microvascular consequence of T2DM and is a primary contributor to end-stage renal disease globally. It is marked by chronic albuminuria, including glomerulosclerosis, mesangial enlargement, and tubulointerstitial fibrosis. The pathophysiology, a gradual reduction in glomerular filtration rate, and histological alterations of DKD are multifaceted, encompassing chronic hyperglycemia-induced oxidative stress, inflammation, metabolic dysregulation, and renal cell death [5].

Sestrin-2, a highly conserved protein activated by inflammation and DNA damage, belongs to the sestrin family, which also comprises sestrin-1 and sestrin-3. It maintains cellular integrity under stress via metabolic activities that provide energy and activate the DNA repair mechanism [6]. The expression of sestrin-2 is stimulated by hypoxia and ATP lack [7]. The decrease in intracellular sestrin-2 levels can result in numerous adverse effects, including insulin resistance, oxidative damage, and mitochondrial malfunction [8]. Furthermore, numerous investigations have shown sestrin-2 to be implicated in atherosclerosis [9]. Diabetic nephropathy [10] and diabetic complications [11]. Sestrin-2, a significant member of sestrin family, has recently been identified as a stress-inducible protein prevalent across several animal species. The sestrin-2 gene was initially discovered in human neuroblastoma cells as a gene triggered by hypoxia [12]. Sestrin-2 increases in mammalian cells under diverse pathophysiological conditions, including oxidative stress, endoplasmic reticulum (ER), starvation, radiation, and hypoxia [13]. Investigations on sestrin-2 have predominantly concentrated on metabolic disorders, including obesity, age-associated illnesses, and malignant tumors. The recent study indicates that sestrin-2 has a crucial role in the pathophysiology of cardiovascular, hepatic, pulmonary, renal, and neurological illnesses, while also providing protective benefits to many organs [14]. The complicated mechanism involved mitochondrial function, autophagy, inflammation, metabolism, and regulation of oxidative stress [15]. Sestrin-2, a highly conserved stress-inducible protein, has emerged as a key endogenous regulator with protective effects against the pathophysiological processes underlying DKD. Sestrin-2 exerts its beneficial functions primarily through activation of AMP-activated protein kinase (AMPK), inhibition of the mammalian target of rapamycin complex 1 (mTORC1), and enhancement of antioxidant defense mechanisms mediated by nuclear factor erythroid 2-related factor 2 (NRF2) [16].

The aim of this study is to evaluate the potential of serum sestrin-2 as a diagnostic biomarker for diabetic kidney disease in patients with type 2 diabetes mellitus.

Materials and Methods

Study design

A case-control study was carried out on 160 subjects: 84 males and 76 females. The age for all subjects was between 35 and 70 years. Subjects were divided into a healthy control group (50 non-diabetic individuals) and a case group (110 T2DM), who were age- and sex-matched healthy controls. The current study was done in the chemistry and biochemistry department, College of Medicine, Mustansiriyah University, in collaboration with the National Diabetic Center of Mustansiriyah University. The study was conducted from April to October 2024. Excluded from the study were patients with type 1 diabetes mellitus (T1DM), T2DM accompanied by active infections, systemic or endocrine disorders, liver disease, heart failure, pregnant or lactating women, previous or current kidney replacement therapy, or any other renal or urinary tract disorder confirmed by clinical or laboratory evaluation (Figure 1).

The diabetes patients were subdivided into three groups, depending on the albumin-to-creatinine ratio in urine (uACR); first, group A1: 40 patients with normoalbuminuria (uACR less than 30 mg/g); second, group A2: 40 patients with microalbuminuria (uACR between 30 and 300 mg/g); and finally, group A3: 30 patients with macroalbuminuria (uACR more than 300 mg/g).

Sample collection and measurement

Laboratory investigations were performed in the department of chemistry and biochemistry of the College of Medicine at Mustansiriyah University. The analysis included the albumin-to-creatinine ratio (uACR) (normal value <30 mg/g), fasting blood glucose (FBG) (normal value 70-99 mg/dL), glycated hemoglobin (HbA1c) (normal value <5.7%), serum urea (normal value 15-45 mg/dL), creatinine (normal value 0.6-1.2 mg/dL), total cholesterol (TC) (normal value <200 mg/dL), and triglycerides (TG) (normal value <150 mg/dL). Overnight fasting venous blood samples (7 ml) were collected using disposable sterilized plastic syringes under strict aseptic conditions. 2 ml of blood was transferred into an EDTA tube for HbA1c determination, while the remaining volume was divided into two portions: 5 ml in plain gel tubes and left to clot for 20 minutes at room temperature. After coagulation, sera were separated by centrifugation at 3000

rpm and aliquoted for measurement of creatinine, urea, FBG, and lipid profile parameters. The estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI 2021 equation.

eGFR (ml/min/1.73m^2) = 142 x (S.cr/A) ^{B} x 0.9938^{age} x (1.012 if female) [17].

Females:

- SCr ≤ 0.7 mg/dL, eGFR (mL/min/ 1.73 m^2) = 144 [SCr (mg/dL)/0.7] $^{-0.329}$ (0.993) ^{Age}.
- SCr > 0.7 mg/dL, eGFR (mL/min/1.73 m^2) = 144 [SCr (mg/dL)/0.7] $^{-1.209}$ (0.993) ^{Age}.

Males:

- SCr ≤ 0.9 mg/dL, eGFR (mL/min/1.73 m^2) = 141 [SCr (mg/dL)/0.9] $^{-0.411}$ (0.993) ^{Age}.
- SCr > 0.9 mg/dL, eGFR (mL/min/1.73 m^2) = 141 [SCr (mg/dL)/0.9] $^{-1.209}$ (0.993) ^{Age}.

The remaining serum samples were stored at -20°C for a serum sestrin-2 range value of 0.05-15 ng/ml. The assay was measured using enzyme-linked immunosorbent assay (ELISA) kits (BT-LAB Bioassay Technology Laboratory, Cat. No: E3437Hu, China).

The urine analysis was collected from all participants in sterile containers and centrifuged immediately. The urinary albumin was measured in fresh spot urine samples using the immunoturbidimetric method (Bio system, Spain). Urinary creatinine was determined using the same method applied for serum creatinine following a 50-fold dilution. The measured value was then multiplied by 50 to correct for the dilution factor. The uACR (mg/g) was calculated by dividing the urinary albumin concentration (mg) by the urinary creatinine content (g).

In addition, all participants underwent comprehensive history taking, with emphasis on the duration of diabetes and current medications. Clinical examination was performed, including the calculation of body mass index (BMI) as weight in kilograms divided by the square of height in meters (kg/m^2) (Figure 1).

Ethical issues

The conditional fulfillment of the stated requirements is contingent upon obtaining the requisite approvals from the scientific committee of the College of Medicine of the Mustansiriyah University as well as the Chemistry and Biochemistry Department within the same academic institution. Additionally, approval from the Scientific Committee of the National Diabetes Center of Mustansiriyah University is necessary for proceeding accordingly. Verbal consent agreement from all participants in the study has been taken before the collection of blood samples, after explanation of the purpose, nature, and potential risks of the study. This is based on the document number 2739 on 19 December 2024 issued by the National Diabetes Center of Mustansiriyah University.

Statistical analysis

Data analysis was performed using the statistical package for social sciences (SPSS), version 26.0 for Windows (IBM Corp., Armonk, NY, USA). The GraphPad preferred reporting items for systematic reviews were utilized for data visualization alongside SPSS version 26. The independent student's t-test and analysis of variance were utilized.

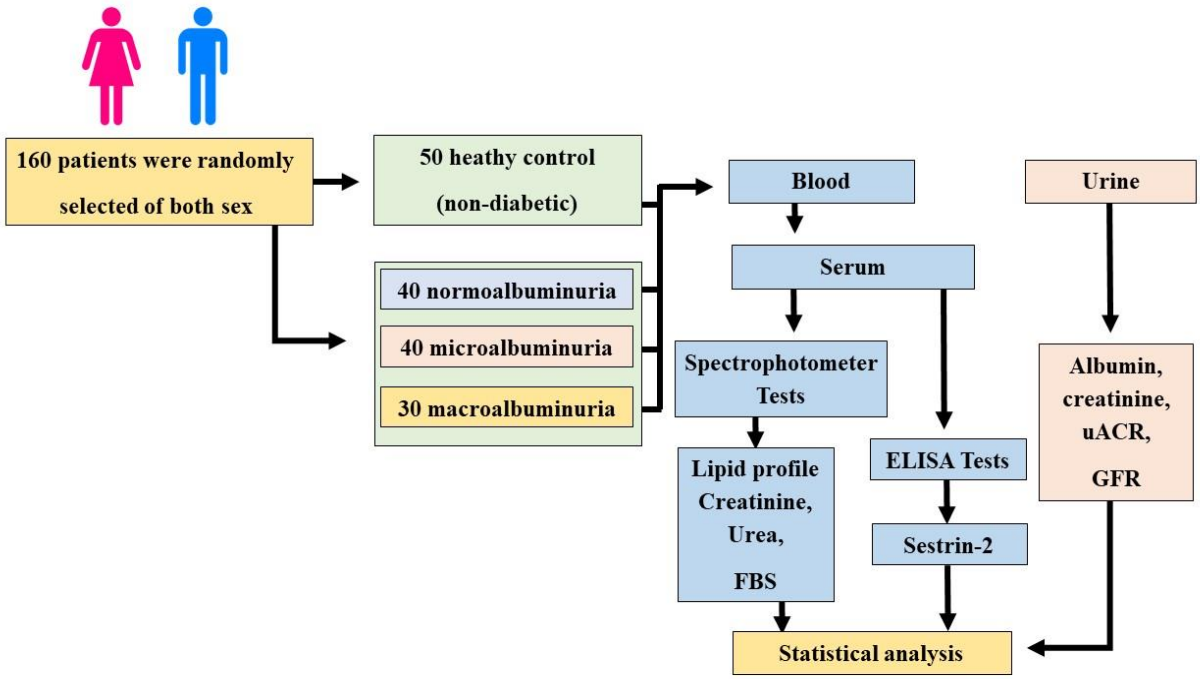

Figure 1: A diagram showing the study's conception

The adjustments were ascertained utilizing Pearson's linear correlation coefficient. The Shapiro-Wilk normality test was used to determine whether the studied parameters followed a Gaussian distribution. Once the data followed a normal distribution by itself, the mean and standard deviation (SD) were employed to display it. Whether group means varied significantly was determined using the analysis of variance. Less than 0.05 was considered statistically significant.

Linear correlation coefficient and regression models were used to assess the linear association between the biomarker sestrin-2 and the other continuous variables. Receiver operating characteristic (ROC) curve analysis was used to determine diagnostic ability, and the cut-off point was calculated according to the Youden index and area under the curve (AUC) of all sestrin-2 values in the study category.

Results

Demographic and clinical characteristics

This study covered 160 subjects, including 84 males (52.5%) and 76 females (47.5%), with an average age of 57.0 years (30-70). The description of the groups' baseline characteristics was summarized in Table 1. In individuals with T2DM, sestrin-2 levels were slightly higher in females (2.71 ng/mL) compared to males (2.43 ng/mL), with no statistically significant difference between the groups ($p = 0.055$). No statistically significant difference in circulating sestrin-2 level was found between males and females with T2DM.

All participants were diagnosed with T2DM, and the patients were classified into normoalbuminuria, microalbuminuria, and macroalbuminuria groups. A total of 50 healthy controls, 40 patients with normoalbuminuria, 40 patients with microalbuminuria, and 30 patients with macroalbuminuria were included in this study. The baseline characteristics of these subgroups are summarized in Table 2.

According to this current study finding, serum sestrin-2 levels were significantly increased in T2DM patients compared with the healthy control group. The mean sestrin-2 level was significantly higher in the macroalbuminuria group (3.28 ± 1.22 ng/ml) compared to both the microalbuminuria (2.97 ± 0.73 ng/ml) and normoalbuminuria (2.78 ± 0.74 ng/ml) groups. Furthermore, the healthy control group (2.29 ± 0.59 ng/ml) exhibited significantly lower sestrin-2 levels than the albuminuria groups (Figure 2). Furthermore, fasting blood glucose (FBG), HbA1c, lipid profile (triglyceride, total cholesterol, HDL, LDL, and VLDL), and renal function tests were assessed (Table 2).

In this study, there were statistical differences in serum sestrin-2 levels between healthy controls and those with normoalbuminuria, microalbuminuria, and macroalbuminuria. Further analysis of the relationship between sestrin-2 and laboratory indexes was performed.

Table 1: Demographic data for the study groups

Variable	T2DM group		p -value ANOVA test	Healthy control group		p-value ANOVA test
	Males	Females		Males	Females	
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
Age (year)	56 ± 12	59 ± 10	0.219	56 ± 4	49 ± 4	0.001
BMI (kg/m²)	29.57 ± 4.42	31.46 ± 4.57	0.024	28.20 ± 3.48	31.13 ± 3.84	0.010
FBG (mg/dl)	154.71 ± 8.61	155.33 ± 9.19	0.601	96.4 ± 2.22	94.7 ± 1.761	0.117
HbA1c (%)	8.21 ± 1.44	8.24 ± 1.42	0.914	5.61 ± 0.21	5.57 ± 0.23	0.600
TC (mg/dl)	185.43 ± 35.92	196.12 ± 49.15	0.225	149.78 ± 31.0	151.86 ± 27.5	0.814
HDL (mg/dl)	38.11 ± 6.39	38.81 ± 10.65	0.697	47.26 ±7.73	48.83 ± 8.35	0.514
LDL (mg/dl)	84.15 ± 28.55	102.23 ± 38.40	0.010	90.25 ± 26.10	96.69 ± 29.25	0.435
TG (mg/dl)	227.61 ± 28.73	222.42 ± 15.81	0.817	138.81 ± 30.53	126.9 ± 23.29	0.154
VLDL (mg/dl)	45.52 ± 25.75	44.49 ±19.18	0.817	27.77± 6.11	25.38 ± 4.66	0.154
P. creatinine (mg/dl)	0.98 ± 0.32	0.82 ± 0.18	0.002	0.76± 0.20	0.76 ± 0.20	0.953
Urea (mg/dl)	35.62 ± 13.51	35.61 ± 12.56	0.996	31.12 ± 6.96	33.48 ± 7.32	0.271
UACR (mg/g)	61.71 ± 11.07	56.92 ± 8.09	0.802	7.04 ± 2.31	6.68 ± 2.04	0.581
Albumin (mg/l)	54.53 ± 9.24	48.83 ± 8.18	0.748	8.40 ± 3.25	8.03 ±1.59	0.642
Creatinine (mg/dl)	94.75 ± 47.13	86.42 ± 43.30	0.356	97.82 ± 44.10	84.87 ± 29.78	0.265
GFR (mL/min/1.73m2)	86.70 ± 22.96	86.17 ± 21.84	0.904	92.73 ± 19.90	92.35 ±11.12	0.939
Sestrin-2 (ng/ml)	2.43 ± 0.57	2.71 ± 0.82	0.055	2.28 ± 0.71	2.19 ± 0.32	0.019

BMI: Body mass index, FBG: fasting blood glucose, HbA1c: Glycated hemoglobin, TC: Cholesterol, HDL: High density lipoprotein, LDL: Low density lipoprotein, TG: Triglyceride, VLDL: Very Low density lipoprotein, uACR: urine albumin-creatinine ratio, GFR: Glomerular filtration rate, *GFR (mL/min/1.173 m^2) =186∗creatinine (serum) $^{-1.154}$ ∗ age$^{-0.203}$ ∗ 0.742 (if female).

While in the normoalbuminuria group, a significant positive correlation was found between serum sestrin-2 levels with FBG (r_p=0.468, p=0.018), HbA1c (r_p=0.306, p=0.043), and HDL (r_p=0.300, p=0.035) and negatively correlation between sestrin-2 and GFR (r_p=-0.230, p=0.007). Moreover, in the microalbuminuria group, a significant positive correlation of a moderate effect size was observed between sestrin-2 and BMI (r_p=0.323, p=0.032). In the macroalbuminuria group, there was a significant positive correlation between sestrin-2 and FBG (r_p=0.396, p=0.031), HbA1c (r_p=0.355, p=0.042), and uACR (r_p=0.376, p=0.037) and a negative correlation between sestrin-2 and GFR (r_p=-0.365, p=0.034). No other significant correlations were found (Table 3).

Receiver operating characteristic analysis

ROC curve analysis was used to evaluate the diagnostic ability of sestrin-2 to discriminate the presence of various T2DM from healthy control. The area under receiver operating characteristic curve (AUC) for sestrin-2 was 0.723 at the cutoff value of 2.56, the sensitivity and specificity for differentiating normoalbuminuria from control (70.0% and 60.9%, respectively), while the AUC for sestrin-2 was 0.718 at the cutoff value of 2.57, the sensitivity and specificity for differentiating microalbuminuria from control (69.6% and 65.9%, respectively). The AUC for sestrin-2 was 0.933 at the cutoff value of 3.91, with the sensitivity and specificity for differentiating macroalbuminuria from control being 100% and 75.0%, respectively. A value summary comparison of ROC curve analysis criteria was presented in Table 4 and Figure 3.

Discussion

In this study, the levels of serum sestrin-2 were measured in patients with T2DM as well as healthy people. The global incidence and prevalence of diabetes mellitus are rising at an alarming rate, largely driven by the increasing prevalence of metabolic syndrome, obesity, and the progressive westernization of lifestyle [18]. Diabetic kidney disease (DKD) is recognized as one of the most serious and debilitating complications of DM and represents a leading cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD) worldwide [19]. Epidemiological studies estimate that approximately 20-50% of individuals with T2DM will eventually develop DKD, highlighting its substantial contribution to morbidity, mortality, and healthcare expenditures [20].

Sestrin-2 belongs to a highly conserved family of stress-responsive proteins with potent antioxidant properties. Its expression is upregulated in response to various harmful environmental and metabolic stimuli, including oxidative stress, inflammation, and DNA damage, in order to maintain cellular homeostasis and survival. Importantly, sestrin-2 plays a critical role in suppressing the excessive production of reactive oxygen species (ROS), thereby protecting cells from oxidative injury and preserving redox balance [16].

Table 2: Comparison of different groups categorized according to the degree of albuminuria regarding different biological markers

Variable	T2DM groups				p-value ANOVA test
	Normoalbuminuria Mean ± SD	Microalbuminuria Mean ± SD	Macroalbuminuria Mean ± SD	Healthy Control Mean ± SD	
Age (year)	56 ± 10	5 8± 12	61 ± 7	53 ± 5	<0.015*
BMI (kg/m²)	30.00 ± 4.21	31.75 ± 4.89	29.85 ± 4.79	29.48 ± 3.89	0.082
FBG (mg/dl)	151.98 ± 8.30	160.97 ± 9.535	161.84 ± 9.146	94.18 ± 2.073	<0.001*
HbA1c (%)	7.89 ± 1.00	8.37 ± 1.75	8.58 ± 1.35	5.59 ± 0.22	<0.001*
TC (mg/dl)	186.26 ± 34.06	196.92 ± 51.26	194.85 ± 57.95	150.68 ± 29.27	<0.001*
TG (mg/dl)	217.75 ± 110.85	235.96 ± 117.61	205.94 ± 65.26	133.67 ± 27.98	<0.001*
HDL (mg/dl)	38.94 ± 9.29	37.44 ± 8.92	41.72 ± 7.95	47.94 ± 7.95	<0.001*
VLDL (mg/dl)	43.55 ± 22.17	47.19 ± 23.52	41.19 ± 13.05	26.73 ± 5.60	<0.001*
LDL (mg/dl)	105.20 ± 35.36	119.66 ± 36.89	110.57 ± 29.84	93.05 ± 27.39	<0.001*
Plasma creatinine (mg/dl)	0.87 ± 0.23	0.90 ± 0.29	0.95 ± 0.28	0.76 ± 0.20	<0.001*
Urea (mg/dl)	33.75 ± 9.92	37.13 ± 14.95	48.92 ± 17.08	32.14 ± 7.13	<0.001*
Albumin (mg/l)	9.55 ± 3.85	60.1 2 ± 83.34	263.64 ± 71.71	8.24 ± 2.64	<0.001*
Creatinine (mg/dl)	95.81 ± 44.49	84.90 ± 47.89	81.94 ± 27.22	92.19 ± 38.69	<0.001*
uACR (mg/g)	9.99 ± 3.12	63.87 ± 59.36	338.70 ± 60.54	6.88 ± 2.18	<0.001*
GFR (mL/min/1.73m2)	89.5 1 ± 21.45	85.14 ± 13.40	44.18 ± 16.85	95.57 ± 16.50	<0.001*
Sestrin-2 (ng/ml)	2.78 ± 0.74	2.97 ± 0.73	3.28 ± 1.22	2.29 ± 0.59	<0.001*

*Significant correlation (p≤0.05), **high Significant correlation (p≤0.01), BMI: Body mass index, FBG: fasting blood glucose, HbA1c: Glycated hemoglobin, TC: Cholesterol, HDL: High density lipoprotein, LDL: Low density lipoprotein, TG: Triglyceride, VLDL: Very Low density lipoprotein, uACR: urine albumin-creatinine ratio, GFR: Glomerular filtration rate, *GFR (mL/min/1.173 m^2) =186∗creatinine (serum) $^{-1.154}$ ∗ age$^{-0.203}$ ∗ 0.742 (if female).

This study demonstrates that circulating serum sestrin-2 levels were significantly increased in T2DM patients compared with healthy controls, and the results showed a high significant difference in mean for normoalbuminuria and macroalbuminuria compared to microalbuminuria and healthy control groups. Macroalbuminuria patients showed the highest sestrin-2 level, followed by microalbuminuria and normoalbuminuria. This finding was consistent with the finding of MAO *et al.* (2021) [21], and Chung *et al.* (2018) [22] observed that T2DM patients and obese people had higher serum sestrin-2 levels. The sestrin-2 is found to be highly in liver, muscle, and adipose tissues in obesity and T2DM [23]. Sestrin-2 is one of the key factors for B-cell homeostasis and a potential insulin.

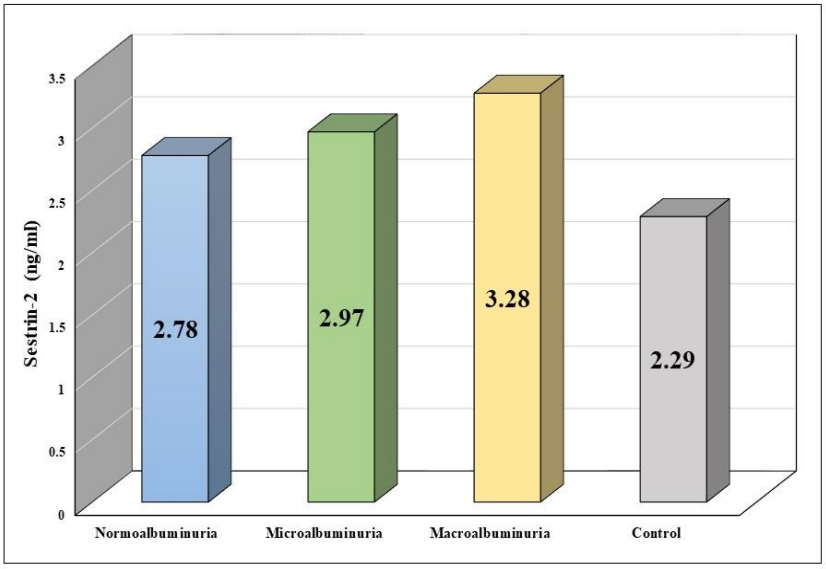

Figure 2: Boxplot of sestrin-2(ng/ml) in the study groups

Table 3: Pearson's correlation for patients with type 2 diabetes mellitus and healthy control

Variables	Sestrin-2 in T2DM groups					
	Normoalbuminuria		Microalbuminuria		Macroalbuminuria	
	r_p	p-value	r_p	P-value	r_p	p-value
DM Duration	0.058	0.687	-0.017	0.914	0.304	0.465
Age (year)	0.070	0.628	-0.221	0.150	-0.416	0.305
BMI (kg/m²)	-0.115	0.425	0.323*	0.032	0.453	0.260
FBG (mg/dl)	0.468*	0.018	0.099	0.524	0.396*	0.031
HbA1c (%)	0.306*	0.043	-0.125	0.419	0.355*	0.042
TC (mg/dl)	-0.184	0.201	-0.071	0.646	-0.481	0.228
TG (mg/dl)	-0.056	0.565	-0.272	0.071	-0.467	0.240
HDL (mg/dl)	0.300*	0.035	-0.004	0.979	0.127	0.765
LDL (mg/dl)	-0.076	0.599	0.151	0.328	-0.231	0.582
VLDL(mg/dl)	-0.065	0.655	-0.273	0.073	-0.469	0.241
P. creatinine (mg/dl)	-0.126	0.383	-0.180	0.242	0.572	0.139
Urea (mg/dl)	-0.160	0.268	-0.056	0.717	-0.348	0.398
uACR (mg/g)	-0.134	0.354	-0.195	0.205	0.376*	0.037
Albumin (mg/l)	-0.079	0.585	-0.090	0.560	0.417	0.304
Creatinine (mg/dl)	-0.012	0.931	0.051	0.740	0.342	0.407
GFR (mL/min/1.73m^2)	-0.230*	0.007	-0.052	0.739	-0.365*	0.034

*Significant correlation ($p \leq 0.05$), **high Significant correlation ($p \leq 0.01$), BMI: Body mass index, FBG: fasting blood glucose, HbA1c: Glycated hemoglobin, TC: Cholesterol, HDL: High density lipoprotein, LDL: Low density lipoprotein, TG: Triglyceride, VLDL: Very Low density lipoprotein, uACR: urine albumin-creatinine ratio, GFR: Glomerular filtration rate, *GFR (mL/min/1.173 m^2) =186$*$creatinine (serum) $^{-1.154}$ $*$ age$^{-0.203}$ $*$ 0.742 (if female).

Table 4: ROC analysis criteria of selected markers as differentiating between diabetic patient's subgroups

Combination	Variable	Cutoff	SE	SP	AUC	95% CI	p-value
normoalbuminuria Versus Control	Sestrin-2 (ng/ml)	2.56	70.0%	60.9%	0.723	0.623 to 0.823	<0.0001
microalbuminuria Versus Control		2.57	69.6%	65.9%	0.718	0.607 to 0.828	<0.0001
macroalbuminuria Versus Control		3.91	100%	75.0%	0.933	0.809 to 1.0	<0.0001

SE: Sensitivity, SP: Specificity, AUC: area under curves, 95% CI: 95% Confidence Interval

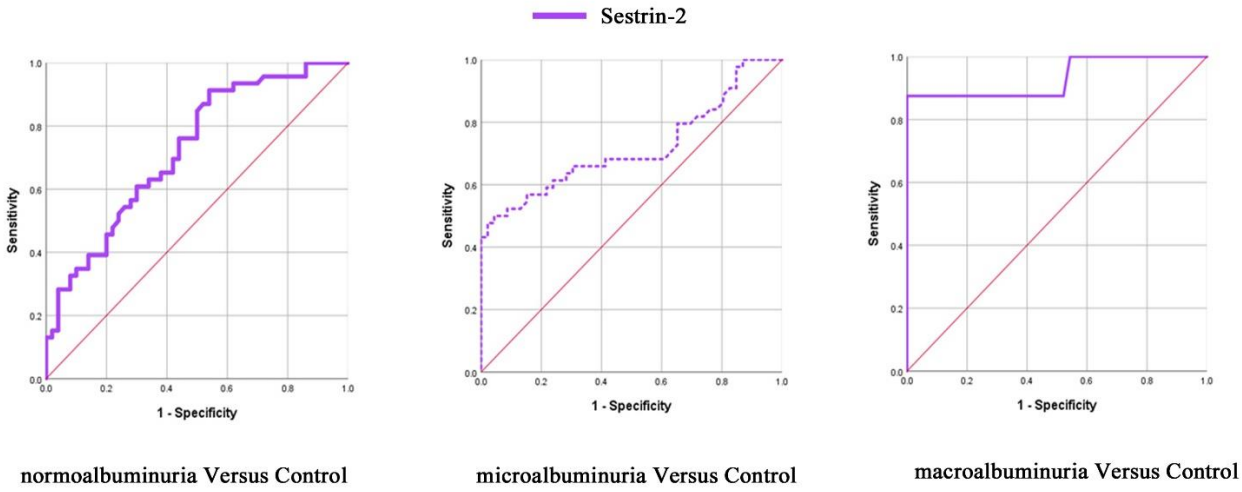

Figure 3: Receiver operating characteristics curve analysis of sestrin-2

The deficiency and dysfunction of sestrin-2 may result in insulin resistance and development of diabetes [24] Sestrin-2 is an evolutionary, stress-inducing protein that plays a role in various cellular functions in metabolic diseases, including obesity and diabetes. It is inducible through oxidative stress and is a recently discovered antioxidant molecule [25].

In individuals with T2DM, mean serum sestrin-2 levels were slightly higher in females compared to males; however, this difference did not reach statistical significance. This agrees with a study by Emara *et al.* (2024) that did not report sex-specific sestrin-2 levels, implying sex was not a variable found to influence results significantly [26].

Analysis of fasting blood glucose (FBG) revealed highly significant differences among the study groups. Patients in the macroalbuminuria group exhibited significantly higher FBG levels compared with both the normoalbuminuria and microalbuminuria groups. In addition, FBG levels in the microalbuminuria group were significantly elevated relative to the normoalbuminuria group. This result was agreed upon by Choi *et al.* (2022) [27].

The lipid profile demonstrates statistically significant discrepancies in cholesterol, TG, HDL, LDL, and VLDL levels among the groups studies. Dyslipidemia is a common metabolic modification associated with diabetes. It is characterized by a wide range of lipid abnormalities that are collectively known as diabetic dyslipidemia. These abnormalities include high levels of TG, low levels of HDL cholesterol, and a shift toward small, dense LDL cholesterol. These findings are reinforced by Abdulfattah *et al.* (2025) [28]. This pathogenen is commonly seen in diabetes mellitus patients [29].

According to the present results, in the patients' group (normo-, micro-, and macroalbuminuria), serum sestrin-2 demonstrated a significant positive correlation with both FBG and HbA1c. Notably, within the normoalbuminuria subgroup, higher sestrin-2 levels were associated with poorer glycemic control. The positive correlation with HDL suggests a complex metabolic link, as HDL is often protective in diabetes contexts. The negative correlation with eGFR indicates that higher sestrin-2 levels are associated with reduced kidney function, even in patients classified as normoalbuminuric, potentially signaling early kidney impairment. These findings are consistent with those of Mao *et al.* (2021) [21].

In the microalbuminuria group, a significant positive correlation of moderate effect size was observed between serum sestrin-2 levels and body mass index (BMI). This suggests that higher-level sestrin-2 levels are associated with greater adiposity in patients with microalbuminuria, a condition representing early kidney damage in diabetes. Several studies have reported positive correlations of serum sestrin-2 with BMI in diabetic patients, indicating sestrin-2 may be involved in metabolic regulation related to obesity and diabetes [30]. While in the macroalbuminuria group, serum sestrin-2 strongly correlates with markers of glycemic control and kidney damage, underscoring its role in the metabolic and renal pathophysiology of advanced diabetic nephropathy. In the macroalbuminuria group, serum sestrin-2 showed significant positive correlations with fasting blood glucose (FBG), HbA1c, and urinary albumin-to-creatinine ratio (uACR). There is also a significant negative correlation between sestrin-2 and glomerular filtration rate (GFR). These relationships indicate that higher serum sestrin-2 levels are associated with poor glycemic control, which increased kidney damage and reduced kidney function in patients with macroalbuminuria, an advanced stage of diabetic nephropathy. This aligns with the known role of sestrin-2

in metabolic regulation and kidney disease progression in diabetes [31].

The main strength of the study is that the results provided new insights into the possible role of serum sestrin-2 in the pathophysiology of DKD in T2DM, while the limitations of the study include that the sample size was not sufficiently large to achieve definitive conclusions. The single-center design may restrict the generalizability of the findings, and the absence of mechanistic studies to validate and elucidate the observed correlations between serum sestrin-2 and diabetic kidney disease. The difference between other research and our findings on the levels of sestrin-2 may be caused by various immunoassays, sample size, or subject differences, particularly in terms of BMI and diabetes mellitus.

Conclusions

Serum sestrin-2 levels are increased in patients with T2DM. It may be a novel modulatory factor for metabolic disturbances in diabetes complications. Further analysis is needed to validate the present findings and reveal the underlying mechanism of sestrin-2 on DKD pathophysiology.

Funding: There is no funding for this research.

Conflict of interest: The authors state that there is no conflict of interest.

Contribution authors: Conceptualization, E.A.A. and A.M.R.; Methodology, E.A.A. and A.M.R.; Formal analysis, E.A.A. and A.M.R.; Investigation, E.A.A. and A.M.R.; Supervision, A.J.T.; Resource, K.F.S., Writing, A.M.M.

References

1. Ge X, Wang L, Fei A, Ye S, Zhang Q. Research progress on the relationship between autophagy and chronic complications of diabetes. Frontiers in Physiology. 2022; 13: 956344.
2. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Research and Clinical Practice. 2022;183: 109119.
3. Chesnaye NC, Carrero JJ, Hecking M, Jager KJ. Differences in the epidemiology, management and outcomes of kidney disease in men and women. Nature Reviews Nephrology. 2024;20(1):7-20.
4. de Boer IH, Khunti K, Sadusky T, Tuttle KR, Neumiller JJ, Rhee CM, et al. Diabetes management in chronic kidney disease: a consensus report by the American Diabetes Association (ADA) and Kidney Disease: Improving Global Outcomes (KDIGO). Diabetes Care. 2022;45(12):3075-90.
5. Forbes JM, Cooper ME. Mechanisms of diabetic complications. Physiological Reviews. 2013;93(1):137-88.
6. Kim M, Sujkowski A, Namkoong S, Gu B, Cobb T, Kim B, et al. Sestrins are evolutionarily conserved mediators of exercise benefits. Nature Communications. 2020;11(1):190.
7. Sun W, Wang Y, Zheng Y, Quan N. The emerging role of Sestrin2 in cell metabolism, and cardiovascular and age-related diseases. Aging and Disease. 2020;11(1):154.
8. Fan X, Zeng Y, Song W, Li J, Ai S, Yang D, et al. The role of Sestrins in the regulation of the aging process. Mechanisms of Ageing and Development. 2020;188:111251.
9. Sundararajan S, Jayachandran I, Subramanian S, Anjana R, Balasubramanyam M, Mohan V, et al. Decreased Sestrin levels in patients with type 2 diabetes and dyslipidemia and their association with the severity of atherogenic index. Journal of Endocrinological Investigation. 2021;44(7):1395-405.
10. Mohany KM, Al Rugaie O. Association of serum sestrin 2 and betatrophin with serum neutrophil gelatinase associated lipocalin levels in type 2 diabetic patients with diabetic nephropathy. Journal of Diabetes & Metabolic Disorders. 2020;19(1):249-56.
11. Sun X, Han F, Lu Q, Li X, Ren D, Zhang J, et al. Empagliflozin ameliorates obesity-related cardiac dysfunction by regulating Sestrin2-mediated AMPK-mTOR signaling and redox homeostasis in high-fat diet–induced obese mice. Diabetes. 2020;69(6):1292-305.
12. Budanov AV, Lee JH, Karin M. Stressin'Sestrins take an aging fight. EMBO Molecular Medicine. 2010;2(10):388-400.
13. Park H-J, Yang S-G, Koo D-B. SESN2/NRF2 signaling activates as a direct downstream regulator of the PERK pathway against endoplasmic reticulum stress to improve the *in vitro* maturation of porcine oocytes. Free Radical Biology and Medicine. 2022;178:413-27.
14. Ala M, Eftekhar SP. Target Sestrin2 to rescue the damaged organ: mechanistic insight into its function. Oxidative medicine and cellular longevity. 2021;2021(1):8790369.
15. Pasha M, Eid AH, Eid AA, Gorin Y, Munusamy S. Sestrin2 as a novel biomarker and therapeutic target for various diseases. Oxidative Medicine and Cellular longevity. 2017;2017(1):3296294.
16. Lin Q, Ma Y, Chen Z, Hu J, Chen C, Fan Y, et al. Sestrin-2 regulates podocyte mitochondrial dysfunction and apoptosis under high-glucose conditions via AMPK. International Journal of Molecular Medicine. 2020;45(5):1361-72.
17. Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al. New creatinine-and cystatin C–based equations to estimate GFR without race. New England Journal of Medicine. 2021;385(19):1737-49.
18. Organization WH. Tracking universal health coverage: 2023 global monitoring report: World Health Organization; 2023.
19. Rossing P, Caramori ML, Chan JC, Heerspink HJ, Hurst C, Khunti K, et al. KDIGO 2022 clinical practice guideline for diabetes management in chronic kidney disease. Kidney International. 2022;102(5):S1-S127.
20. Thomas MC, Brownlee M, Susztak K, Sharma K, Jandeleit-Dahm KA, Zoungas S, et al. Diabetic kidney disease. Nature Reviews Disease Primers. 2015;1(1):1-20.
21. Mao E, Cheng X, Li W, Kan C, Huang N, Wang H, et al. Association between serum Sestrin2 level and diabetic peripheral neuropathy in type 2 diabetic patients. World Journal of Clinical Cases. 2021;9(36):11156.

22. Chung HS, Hwang H, Hwang SY, Kim NH, Seo JA, Kim SG, et al. Association of serum Sestrin2 level with metabolic risk factors in newly diagnosed drug-naïve type 2 diabetes. Diabetes Research and Clinical Practice. 2018;144:34-41.
23. Kowalsky AH, Namkoong S, Mettetal E, Park H, Kazyken D, Fingar DC, et al. The GATOR2–mTORC2 axis mediates Sestrin2-induced AKT Ser/Thr kinase activation. Journal of Biological Chemistry. 2020;295(7):1769-80.
24. Gong L, Wang Z, Wang Z, Zhang Z. Sestrin2 as a potential target for regulating metabolic-related diseases. Frontiers in Endocrinology. 2021;12:751020.
25. Kumar A, Dhiman D, Shaha C. Sestrins: Darkhorse in the regulation of mitochondrial health and metabolism. Molecular Biology Reports. 2020;47(10):8049-60.
26. Emara AM, El Bendary AS, Ahmed LM, Okda HI. Evaluation of serum levels of sestrin 2 and betatrophin in type 2 diabetic patients with diabetic nephropathy. BMC Nephrology. 2024;25(1):231.
27. Choi JW, Lee MH, Fujii T. Insulin Resistance and microalbuminuria in patients with impaired fasting glucose versus hemoglobin A1c-Defined prediabetes. Annals of Clinical & Laboratory Science. 2022;52(5):802-10.
28. Abdulfattah SW, Abdulsattar SA, Rahmah AM. Kidney dysfunction prediction by serum adropin in type 2 diabetes Iraqi patients. Mustansiriyah Medical Journal. 2025:10.4103.
29. Piccirillo F, Mastroberardino S, Nusca A, Frau L, Guarino L, Napoli N, et al. Novel antidiabetic agents and their effects on lipid profile: A single shot for several cardiovascular targets. International J Molecular Sciences. 2023;24(12):10164.
30. Zahid MA, Abdelsalam SS, Raïq H, Abunada HH, Parray A, Agouni A. Association of plasma levels of sestrin2 with adiposity and metabolic function indices in healthy and diabetic subjects from Qatar Biobank. Frontiers in Endocrinology. 2025;16:1518388.
31. Zhang X, Luo Z, Li J, Lin Y, Li Y, Li W. Sestrin2 in diabetes and diabetic complications. Frontiers in Endocrinology. 2023;14:1274686.