Study of the association of serum level of nesfatin-1 and diabetic kidney disease in patients with type 2 diabetes

Talaat Abd-Elaaty[a], Mohamed M. Rezk[b,c], Hend H. Abdel Moneium[a], Yasmine S. Naga[c], Sara S. Ghoniem[a]

[a]Departments of Diabetes, Metabolism, Internal Medicine, [b]Clinical and Chemical Pathology, [c]Unit of Nephrology, Department of Internal Medicine, Faculty of Medicine, University of Alexandria, Alexandria, Egypt

Correspondence to Talaat Abd-Elaaty, Unit of Diabetes and Metabolism, Department of Internal Medicine, Faculty of Medicine, University of Alexandria, Alexandria, Egypt
Tel: +20 122 749 8562; fax: 03/4864416;
e-mail: talaatabdelaaty@yahoo.com

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Background
Nesfatin-1 is a newly found anorectic neuropeptide with potent metabolic regulatory effects, whose peripheral levels are shown to be elevated in diabetes. It is a newly discovered hypothalamic neuropeptide that regulates appetite. Its discovery has generated great interest in the scientific community because of its implication in energy and glucose homeostasis. Nesfatin-1 is an amino-acid peptide originating from the cleavage of nucleobindin2. It has a molecular weight of 9.8 kDa and the half-life of nucleobindin2 mRNA is ~6 h. Interestingly, nesfatin-1 is also expressed in pancreatic β-cells, where it is localized with insulin in secretion vesicles. The structure of nesfatin-1 is also tripartite; the segment starting from the N-terminal end and going up to 23 amino acids is called N23, the middle segment covering the amino acids from 23 to 53 is called M30, and the segment from the 53rd to 82nd amino acids toward the carboxyl terminus is called C29.

Objective
We compared serum nesfatin-1 in patients with type 2 diabetes with evidence of diabetic kidney disease (DKD) [urinary albumin–creatinine ratio (UACR) >300 mg/day or reduced estimated glomerular filtration rate (eGFR) <60 ml/min] with patients newly diagnosed with type 2 diabetes and who had no evidence of DKD (UACR<30 mg/day) and a control group of healthy nondiabetic individuals.

Patients and methods
Ninety patients attending the outpatient clinics at Alexandria Main University Hospital and Alexandria Police Hospital, Egypt, were enrolled in this cross-sectional study to determine the association of serum level of nesfatin-1 and DKD in patients with type 2 diabetes. They were divided into three groups: group I included 30 type 2 diabetic patients with DKD. Group II included 30 type 2 diabetic patients without DKD. Group III included 30 nondiabetic healthy controls matched for age and sex with group I. Assessment included a thorough assessment of history, complete clinical examination, neurological examination, fundus examination, and laboratory investigations including metabolic profile and plasma nesfatin-1 by enzyme-linked immunosorbent assay.

Results
The study showed a statistically significant difference between the three studied groups in terms of age ($P<0.001$), HbA1c and fetal bovine serum ($P\leq0.001$), fasting insulin level ($P=0.022$), blood urea ($P<0.001$), serum creatinine ($P<0.001$), eGFR ($P<0.001$), and UACR ($P<0.001$). The difference between the three groups studied was not significant in serum nesfatin-1 ($P<0.564$). The mean peripheral concentrations of nesfatin-1 were not significantly higher in patients with diabetes who had evidence of DKD compared with newly diagnosed type 2 diabetic patients who had no evidence of DKD ($P<0.001$).

Conclusion
Serum nesfatin-1 was not significantly higher in albuminuric type 2 diabetic patients compared with normoalbuminuric patients. Serum nesfatin also did not correlate with eGFR and creatinine in the different groups studied. Serum nesfatin-1 may not be useful as an early marker of DKD instead of albuminuria. More studies are needed to identify the role and the significance of nesfatin in diabetic patients.

Keywords:
diabetic kidney disease, nesfatin-1, type 2 diabetes mellitus, urinary albumin–creatinine ratio

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Introduction

Five hundred and ninety two million individuals worldwide are projected to have diabetes by 2035 according to the International Diabetes Federation [1]. Diabetes complications such as diabetic kidney disease (DKD), diabetic retinopathy, diabetic neuropathy, vasculopathy, and an increased risk for cerebrovascular and cardiovascular diseases have major implications on health resources and medical expenditure. According to the American Diabetes Association, the total estimated cost of diagnosed diabetes in 2012 is $245 billion, of which 18% (about 44 billion) was used to treat diabetes complications [2]. DKD is one of the most common microvascular complications of diabetes mellitus, representing the leading cause of end-stage renal disease (ESRD) [3] worldwide. Therefore, prevention of the disease or at least postponement of its progression has emerged as a key issue. Adverse outcomes of renal failure can be prevented or delayed through early detection and treatment [4].

For quite a long time, the impaired renal function of patients with DKD is mainly reflected by laboratory detection of serum creatinine and blood urea, both of which are not sensitive enough to illustrate early changes of renal function, when active management is important [5].

The general recommendation for patients with diabetes mellitus is to perform kidney function as screening: in type 1 diabetes mellitus 5 years after diagnosis and in type 2 diabetes at the time of diagnosis. One of these markers is the detection of albuminuria using a urinary excretion rate of albumin by 24 h collection of urine (30–300 mg/24 h) or a spot urine albumin to creatinine ratio (30–300 mg/g). Urinary albumin excretion has currently emerged as a sensitive indicator of early renal damage [4].

The routine classical evaluation of DKD includes the appearance of albuminuria, decreased creatinine clearance, and increased serum creatinine [6]. However, it has been reported that a decline in the renal function of patients with diabetes was not always accompanied by an increased urinary albumin–creatinine ratio (UACR). About 20–30% of patients with type 2 diabetes mellitus, accompanied by renal insufficiency, showed normoalbuminuria, which is a condition referred to now as nonproteinuric DKD [7].

Morphological changes are known to start earlier than laboratory abnormalities. Also, some patients with albuminuria have a normal renal structure, whereas some normoalbuminuric diabetics have well-established nephropathic lesions. Also, the albumin excretion rate is a predictor of renal disease in hypertension and cardiovascular diseases; thus, it is not a specific marker for DKD [8].

Measurement of glomerular filtration rate (GFR) is the best functional parameter in renal disease using creatinine clearance. This requires 24 h urine collection and a blood sample, and it involves measurements of creatinine level in blood and urine as well as estimation of urine volume. There are several factors that may interfere with the accuracy of the test such as incomplete collection of urine. Other methods of assessment of GFR are the Cockcroft–Gault formula or the modification of diet in renal disease (MDRD) formula, but they are also not considered accurate methods [9].

Thus, we still need to identify earlier markers of DKD. Also, serum creatinine depends on creatinine production, extrarenal elimination, and tubular handling. Therefore, other biomarkers for the estimation of renal function have been sought.

Nesfatin-1 is a newly discovered hypothalamic neuropeptide that regulates appetite and has generated great interest in the scientific community because of its implications in energy and glucose homeostasis.

It is an 82 amino-acid peptide originating from the cleavage of nucleobindin2 (NUCB2). It has a molecular weight of 9.8 kDa and the half-life of NUCB2 mRNA was ~6 h [10]. Nesfatin-1 is expressed in neurons of various brain areas including hypothalamic nuclei such as para ventricular nucleus (PVN), arcuate nucleus (ARC), and lateral hypothalamic area (LHA) and in the nucleus of the solitary tract (NTS) and dorsal motor nucleus of the vogues (DMNV) at the brainstem level [11]. Interestingly, it is also expressed in pancreatic β-cells, where it is colocalized with insulin in secretion vesicles. Other nesfatins originating from the NUCB2/nuclear EF-hand acidic (nesfatin-2 and nesfatin-3) do not have any anorexigenic activity [12]. Therefore, almost all studies carried out at present have focused on nesfatin-1.

The middle segment covering the amino acids from 23 to 53, which is called M30, is responsible for the dose-dependent inhibition of food intake. The amino acid sequencing of this segment is similar to that of α-MSH and Agouti-related peptides [13].

Nesfatin-1 has been reported to exert an anti-hyperglycemic effect that is peripheral and time, dose, and insulin dependent [14]. Recent experimental studies have also linked nesfatin-1 to enhanced peripheral and hepatic insulin sensitivity by promoting peripheral glucose uptake and decreasing gluconeogenesis through different pathways [15].

Nesfatin-1 has been studied in several metabolic dysregulations such as diabetes, epilepsy, and inflammation [16–24]. Increased nesfatin-1 levels were significantly associated with impaired glucose tolerance, BMI, HbA1c, fasting blood glucose, and 2-h postprandial plasma glucose [16–22]. Studies have investigated nesfatin-1 as a potential cause of feeding disturbance in patients with CKD, suggesting that nesfatin-1 may have a negative correlation with the total protein intake in these patients [14].

However, at the beginning of the present study, there were no studies describing possible alterations in circulating nesfatin-1 levels in patients with DKD. On the basis of reports of elevated nesfatin-1 levels in diabetics, being an antihyperglycemic and insulin sensitizer neuropeptide, it is biologically plausible for it to be related to the pathogenesis of DKD.

The aim of the present work was to study the changes in the circulating plasma levels of nesfatin-1 in type 2 diabetic patients compared with control participants and to investigate the possible association of nesfatin-1 with some anthropometric and metabolic parameters in such diabetic patients. We also aimed to determine the relationship of circulating nesfatin-1 levels with DKD, as measured by UACR and estimated glomerular filtration rate (eGFR), in type 2 diabetic patients with DKD compared with newly diagnosed type 2 diabetic patients without DKD and to detect the usefulness of using nesfatin-1 as a marker to diagnose DKD and detect the progression of the disease in type 2 diabetes mellitus patients.

Patients and methods

Settings

Patients were recruited from the diabetes outpatient clinic at Alexandria Main University Hospital and Alexandria Police Hospital, Egypt.

Ethical approval

The Faculty of Medicine's Ethics Committee at Alexandria University approved the study. Written consents were obtained from all patients before sampling and after a thorough explanation of the procedure was provided according to the Helsinki Declaration [25].

Eligibility criteria

Inclusion criteria for group I were type 2 diabetic patients with evidence of DKD of any age and sex. Group II included 30 patients with type 2 diabetes without evidence of DKD. Group III included 30 nondiabetic healthy controls matched for age and sex.

Exclusion criteria included patients with nephropathy because of causes other than diabetes, patients with ESRDs as well as severe liver diseases, severe uncontrolled hypertension, connective tissue diseases and vasculitis, hematological disorders or malignancy, chronic inflammatory diseases, and significant infection at time of initiation of the study.

Patients were examined by a dialectologist for inclusion and exclusion criteria, including patient history, clinical examination, and ophthalmoscopy and laboratory investigations. The first 90 consecutive patients who fulfilled our inclusion criteria were selected in this study. No exclusions were made on the basis of age or sex to avoid selection bias.

Methods and techniques

Urine (for UACR) and venous blood samples were obtained after an overnight fasting. All blood samples were divided into two aliquots: the first part was collected in a vacutainer tube containing Na_2-EDTA for the assay of HbA1c; the second was collected in a plain vacutainer tube and centrifuged (3000 rpm) for serum preparation. Serum was used to measure total cholesterol, triglycerides, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, glucose, urea, creatinine, and nesfatin-1. Serum samples for nesfatin-1 assay were kept at −80°C till they were assayed. Serum nesfatin-1 concentration was measured using human enzyme-linked immunosorbent assay kits (Human Nesfatin-1 PicoKine^{TM} ELISA Kit, Boster Biological Technology, Pleasanton CA, USA, and Catalog # EK1138). Serum samples for nesfatin-1 were diluted and assayed according to the manufacturer's instructions. All samples were measured at Alexandria Main University Hospital Laboratory. eGFR was calculated using the MDRD formula.

Statistical analysis

Data were fed to the computer using IBM SPSS software package, version 20.0. Qualitative data were described using number and percent. Comparison of categorical variables between different groups was performed using the χ^2-test. When more than 20%

of the cells had an expected count less than 5, correction for χ^2 was performed using Fisher's exact test. The distributions of quantitative variables were tested for normality using the Shapiro–Wilk test and D'Agstino test; also, a histogram and a QQ plot were used for a vision test. If it showed a normal data distribution, parametric tests were applied. If the data were abnormally distributed, nonparametric tests were used.

Quantitative data were described as mean and SD for normally distributed data, whereas abnormally distributed data were expressed as median, minimum, and maximum.

For normally distributed data, comparisons between two independent populations were performed using an independent *t*-test. Correlations between two quantitative variables were assessed using the Pearson coefficient.

For abnormally distributed data, the Mann–Whitney test (for data distribution that was significantly deviated from normal) was used to analyze two independent populations. Correlations between two quantitative variables were assessed using the Spearman coefficient.

Significance test results are quoted as two-tailed probabilities. The significance of the results obtained was judged at the 5% level.

Results

There were 23 men in group I, representing 76.7% of the sample, 30 men in group II, representing 100% of the sample, and 25 men in group III, representing 83.3% of the sample. There were seven women in group I, representing 23.3% of the sample, and five men in group III, representing 16.7% of the sample. The age of the patients in group I ranged from 35.0 to 75.0 years, with a mean of 55.10±10.18 years, the age of the patients in group II ranged from 32.0 to 52.0 years, with a mean age of 42.50±5.76 years, the age of the patients in group III ranged from 30.0 to 80.0 years, with a mean age of 54.73±12.09 years.

There was a statistically significant difference in the demographic data (sex, age) between the three groups at *P* values of 0.015 and 0.001, respectively. The BMI in group I ranged from 22.0 to 32.0, with a mean of 27.27±2.02, the BMI in group II ranged from 24.0 to 37.0, with a mean of 29.27±3.64, and the BMI in group III ranged from 21.0 to 28.10, with a mean of 24.47±1.38.

The waist circumference in group I ranged from 79.0 to 130.0, with a mean of 98.27±9.43, that in group II ranged from 84.0 to 110.0, with a mean of 97.83±7.42, and that in group III, it ranged from 60.0 to 99.0, with a mean of 87.10±9.37.

The waist hip ratio in diabetic group I ranged from 0.78 to 1.05, with a mean of 0.94±0.06, the waist hip ratio in group II ranged from 0.81 to 1.12, with a mean of 0.96±0.06, and the waist hip ratio in group III ranged from 0.56 to 1.0, with a mean 0.85±0.10.

There was a significant difference in anthropometrics (BMI, waist circumference, waist hip ratio) between the three groups at *P* values less than 0.001, 0.001, and 0.001, respectively.

Fundus examinations were normal in two cases in group I, representing 6.7% of the sample, non-proliferative diabetic retinopathy (NPDR) was found in 15 cases in group I, representing 50.0 % of the sample, and proliferative diabetic retinopathy (PDR) was found in 13 cases in group I, representing 43.3% of the sample.

Fundus examination was normal in 24 cases in group II, representing 80.0% of the sample, NPDR was found in six cases in group II, representing 20.0 % of the sample, and PDR was found in 0 cases. Fundus examination was normal in 30 cases in group III, representing 100% of the sample.

There was a significant difference in fundus examination between the three groups as the *P* value was less than 0.001 (Table 1).

The fetal bovine serum (FBS) in group I ranged from 110.0 to 260.0, with a mean of 156.47±32.98, the FBS in group II ranged from 71.0 to 370.0, with a mean of 176.00±75.66, and the FBS in group III ranged from 89.0 to 111.0, with a mean of 97.60±5.04. The HBA1c % in group I ranged from 6.20 to 10.0, with a mean of 7.86±1.08, the HBA1c% in group II ranged from 5.90 to 11.80, with a mean of 8.03±1.85, and the HBA1c% in group III ranged from 5.0 to 5.90, with a mean of 5.59±0.29. There was a statistically significant difference in the glycemic profile between the three groups as *P* value less than 0.001 and 0.001, respectively.

The total cholesterol in group I ranged from 127.0 to 290.0, with a mean of 207.57±43.90, the total cholesterol in group II ranged from 112.0 to 272.0, with a mean of 185.97±44.64, and the total

Table 1 Comparison between the study groups according to demographic and clinical data

	Group I (*n*=30)	Group II (*n*=30)	Group III (*n*=30)	*P*
Sex				
Male	23 (76.6)	30 (100)	25 (83.3)	0.015*
Female	7 (23.3)	0	5 (16.7)	
Age (years)	55.10±10.18	42.50±5.76	54.73±12.09	<0.001*
BMI (kg/m^2)	27.27±2.02	29.27±3.64	24.47±1.38	<0.001*
Waist circumference (cm)	98.27±9.43	97.83±7.42	87.10±9.37	<0.001*
W/H ratio	0.94±0.06	0.96±0.06	0.85±0.10	<0.001*
Fundus examination				
Normal	2 (6.7%)	24 (80%)	30 (100%)	
NPDR	15 (50%)	6 (20%)	0	<0.001*
PDR	13 (43.3%)	0	0	

Values was expressed as mean±SD for normally distributed parameters, median (minimum–maximum) for abnormally distributed parameters and percentage for qualitative values. W/H ratio, waist/hip ratio. *Statistically significant at $P \leq 0.05$.

cholesterol in group III ranged from 130.0 to 206.0, with a mean of 184.63±19.58. The LDL in group I ranged from 50.0 to 188.0, with a mean of 122.03± 41.93, the LDL in group II ranged from 36.0 to 161.0, with a mean of 102.63±34.56, and the LDL in group III ranged from 40.0 to 130.0, with a mean of 72.33±22.99. The HDL in group I ranged from 32.0 to 80.0, with a mean of 51.77±11.97, the HDL in group II ranged from 25.0 to 80.0, with a mean of 46.43±11.79, and the HDL in group III ranged from 40.0 to 141.0, with a mean of 55.07± 18.45. The triglycerides in group I ranged from 62.0 to 290.0, with a mean of 168.50±57.61, the triglycerides in group II ranged from 61.0 to 310.0, with a mean of 178.17±62.46, and the triglycerides in group III ranged from 46.0 to 200.0, with a mean of 128.13±40.65.

There was a statistically significant difference in the lipid profile (total cholesterol, LDL, triglycerides) between the three groups as *P* value 0.036, less than 0.001, and 0.001, respectively, and there was no a statistically significant difference in HDL between the three groups at *P* value 0.103.

The urea in group I ranged from 27.0 to 100.0, with a mean of 48.73±16.50, the urea in group II ranged from 20.0 to 38.0, with a mean of 28.67±4.37, and the urea in group III ranged from 13.0 to 45.0, with a mean of 26.87±7.96. The creatinine in group I ranged from 1.0 to 3.50, with a mean of 1.50±0.46, the creatinine in group II ranged from 0.6 to 1.0, with a mean of 0.87±0.10, and the creatinine in group III ranged from 0.70 to 1.10, with a mean of 0.86±0.11. The eGFR in group I ranged from 18.20 to 60.0, with a mean of 48.61±10.64, the eGFR in group II ranged from 90.0 to 160.0, with a mean of 101.87±15.82, and the eGFR in group III ranged from 80.0 to 119.0, with a mean of 96.60±8.84. The UACR in group I ranged from 303.0 to 1205.0, with a mean of 809.30±246.83, the UACR in group II ranged from 33.0 to 280.0, with a mean of 145.53±64.64, and the UACR in group III ranged from 5.0 to 28.0, with a mean of 17.10±5.95.

There was a statistically significant difference in renal function (urea, creatinine, eGFR, UACR) between the three groups as *P* value less than 0.001, 0.001, 0.001, and 0.001, respectively.

The fasting insulin level in group I ranged from 6.70 to 25.0, with a mean of 15.26±5.73, the fasting insulin level in group III ranged from 2.50 to 75.0, with a mean of 19.96±20.23, and the fasting insulin level in group III ranged from 3.25 to 75.0, with a mean of 13.34± 14.43.

There was a statistically significant difference in the fasting insulin level between the three groups at *P* value 0.022.

The nesfatin-1 in group I ranged from 0.07 to 4.65, with a mean of 0.43±0.86, the nesfatin-1 in group II ranged from 0.05 to 5.23, with a mean of 0.41±0.94, and the nesfatin-1 in group III ranged from 0.02 to 4.29, with a mean of 0.34±0.78 (Table 2).

There was no statistically significant difference in nesfatin-1 levels between the three groups at *P* value 0.564.

The study showed a statistically significant difference between the three groups studied in terms of sex and age (P=0.015, P<0.001), HbA1c and FBS ($P \leq 0.001$), fasting insulin level (P=0.022), blood urea (P<0.001), serum creatinine (P<0.001), eGFR (P<0.001), and UACR (P<0.001).

Table 2 Comparison between the groups studied according to laboratory investigations

	Group I (*n*=30)	Group II (*n*=30)	Group III (*n*=30)	*P*
FBG (mg/dl)	156.47±32.98	176.0±75.66	97.60±5.04	<0.001*
HBA1c (%)	7.86±1.08	8.03±1.85	5.59±0.29	<0.001*
Total cholesterol (mg/dl)	207.57±43.90	185.97±44.64	184.63±19.58	0.036*
LDL-c (mg/dl)	122.03±41.93	102.63±34.56	72.33±22.99	<0.001*
HDL-c (mg/dl)	51.77±11.97	46.43±11.79	55.07±18.45	0.103
Triglycerides (mg/dl)	168.50±57.61	178.17±62.46	128.13±40.65	<0.001*
Urea (mg/dl)	48.73±16.50	28.67±4.37	26.87±7.96	<0.001*
Creatinine (mg/dl)	1.50±0.46	0.87±0.10	0.86±0.11	<0.001*
eGFR (ml/min/1.73 m^2)	48.61±10.64	101.87±15.82	96.60±8.84	<0.001*
UACR (mg/mg)	809.30±246.83	145.53±64.64	17.10±5.95	<0.001*
Fasting insulin level	15.26±5.73	19.96±20.23	13.34±14.43	0.022*
Nesfatin-1 level (pg/ml)	0.43±0.86	0.41±0.94	0.34±0.78	0.564

Values was expressed as mean±SD for normally distributed parameters or median (minimum–maximum) for abnormally distributed parameters. eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; UACR, urinary albumin–creatinine ratio. *Statistically significant at $P \leq 0.05$.

The difference between the three groups studied was not significant in terms of serum nesfatin-1 ($P<0.564$).

Discussion

In our study, there was a statistically significance difference between the patient groups and the control group in terms of blood urea ($P<0.001$). Blood urea is not an accurate marker for measuring renal function. It is filtered by the glomerulus and reabsorbed by the renal tubule. The concentration of urea in serum could vary with diet; also, it may increase in dehydration in association with poor glycemic control that causes polyuria.

Serum creatinine ranged from 1.0 to 3.50 in group I, with a mean of 1.50±0.46, ranged from 0.6 to 1.0 in group II, with a mean of 0.87±0.10, and ranged from 0.70 to 1.10 in group III, with a mean of 0.86±0.11. Therefore, in our study, there was a statistically significant difference between the patient groups and the control group at *P* value less than 0.001.

In our study, neither serum creatinine nor blood urea had a significant correlation with serum nesfatin-1.

In our study, we used the MDRD formula to calculate the eGFR. There was a significant difference between the patient groups and the control group ($P<0.001$).

In our study, there was a statistically significant difference between the patient group and the control group in albumin–creatinine ratio ($P<0.001$). Classical evaluation of DKD includes the detection of albuminuria, decreased creatinine clearance, and increased serum creatinine.

Albuminuria expresses underlying endothelial dysfunction as the presence of chronic hyperglycemia causes disruption of the endothelial permeability through the production and activation of mediators such as reactive oxygen species (ROS), vascular endothelial growth factor, and proinflammatory cytokines. This disturbance of endothelial cell podocyte communication contributes toward and amplifies the endothelial lesions, leading to albuminuria.

With more progression, there is an increase in urinary albumin excretion because of an underlying inflammatory process with excess production of growth factor, deposition of extra cellular membrane (ECM) with subsequent interstitial fibrosis, and glomerulosclerosis leading to renal function deterioration. In contrast to the control group, with a subsequently lower or insignificant inflammatory process in the kidney, the level of albumin/creatinine ratio (ACR) did not correlate significantly with renal function.

In our study, there was a positive nonsignificant correlation between serum nesfatin-1 and HbA1c in the three groups (P_I=0.342, P_{II}=0.138, P_{III}=0.073).

In our study, there was no correlation between serum nesfatin-1 and ACR in the patient group and the control group (P_I=0.157, P_{II}=0.314, P_{III}=0.646).

The current Kidney Disease Outcomes Quality Initiative guidelines advocate the use of creatinine-based equations for estimation of GFR to identify patients with potential kidney disease and to classify them into different stages on the basis of these results. These stages also include individuals with normal or near-normal GFR. Such a stratification requires an

accurate and precise measurement of GFR that is inexpensive, reliable, and widely available.

In our study, there was a nonsignificant correlation between eGFR and serum nesfatin-1 (P_I=0.601, P_{II}=0.341, P_{III}=0.249).

In general, unlike healthy individuals, diabetic patients are continuously exposed to the various metabolic and hemodynamic risks associated with this disease. Recent studies have mainly focused on tubular damage, which is known to correlate with acute kidney injury in patients with DKD [26]. Some cross-sectional studies have reported that several tubular markers increase more in diabetic patients than in healthy controls, and this correlated with the severity of albuminuria [26].

Increased level of HbA1c and poor glycemic control act as an inflammatory milieu in the kidney, and stimulate protein kinase C (PKC) and oxidative stress, which increases the expression of cytokines that attract more inflammatory cells to the kidney, leading to endothelial dysfunction and albuminuria. More progression of the inflammatory state leads to structural changes in glomerular basement membrane (GBM) and to greater loss of proteins and increased urinary albumin excretion rate.

In our study, there was no statistically significant difference in the nesfatin-1 levels between the three groups as *P* value was 0.564.

Zhang *et al.* [14] confirmed a positive correlation between nesfatin-1 with fasting plasma insulin and homeostasis model assessment of insulin resistance. Conversely, nesfatin-1 and HbA1c were negatively correlated in hypothyroid patients [27]. However, the side effects of underlying diseases such as hypothyroidism and polycystic ovary syndrome are suggested to affect nesfatin-1 concentrations [27]. Nesfatin-1 was found to be significantly correlated with serum uric acid. Serum uric acid causes endothelial dysfunction [28] and is associated with the presence of microalbuminuria in type 2 diabetic patients [29].

Although we did not find an association between glycemic control and DKD, other authors have found that plasma nesfatin-1 was increased in patients with diabetes [14]; conflicting results have refuted the presence of this association [21]. Currently, the reasons for this discrepancy are unclear.

For instance, the possible association between nesfatin-1 level and insulin has not been completely understood thus far; nesfatin-1 stimulates the excretion of insulin in pancreatic β-cells [30,31], insulin contributes directly toward increased nesfatin-1 [32,33], and nesfatin-1 enhances the actions of insulin by increasing peripheral and hepatic insulin sensitivity, which in turn leads to decreased gluconeogenesis and enhanced peripheral glucose uptake *in vivo* [34,35].

Recently, a research paper reported on the upregulation of glucagon release following the administration of nesfatin-1 in isolated mouse islets or interestingly, nesfatin-1 (INS-1) (832/13) cells *in vitro* [36]. To explain the increase in serum nesfatin-1 in patients with diabetic nephropathy, possible inflammatory mechanisms should be considered. Albuminuria actively contributes toward endothelial dysfunction, which is predominantly characterized by a chronic and low state of systemic inflammation. A proportion of nesfatin-1 neurons at the level of the hypothalamus and the brainstem are highly sensitive to peripheral inflammatory stimuli such as proinflammatory cytokines interleukin-1 (IL-1), IL-6, and IL-8 [29,30]. In addition, insulin exerts proatherogenic and antiatherogenic actions on the vasculature. Under insulin-resistant conditions, pathway-specific impairment in phosphatidylinositol 3-kinase-dependent signaling potentially causes an imbalance between the production of nitric oxide and the secretion of endothelin-1 to promote endothelial dysfunction [37]. Interestingly, nesfatin-1 may play a central role in the pathogenesis of insulin resistance [38].

Elevated serum nesfatin-1 is also secondary to the activation of defensive mechanisms against DKD-mediated metabolic and inflammatory disturbance. Consistently, Jiang *et al.* [39] reported the potency of nesfatin-1 as a potential therapeutic agent in renal ischemic–reperfusion injury by suppressing the oxidative stress and cell apoptosis by its antioxidant, anti-inflammatory, and antiapoptotic features. Nesfatin-1 has also been reported to be an anti-inflammatory biomolecule, especially in brain damage [40,41].

Recent histopathologic studies have also reported overexpressed levels of NUCB2/nesfatin-1 and binding sites localized to the renal tissue cells at both mRNA and protein levels in the absence of inflammation [42,43]. These reports suggest that the increase in plasma NUCB2/nesfatin-1 is derived from renal tubules in DKD to act as one of the renal protective

factors or as a possible response against sympathetic nerve stimuli (i.e. regulation of increased blood pressure from central nesfatin-1 activity).

To further explore the noninflammatory increase in nesfatin-1 in DKD, a common link through leptin and satiety is perceived. Ob/ob mice, which are leptin deficient, hardly develop renal disease. In comparison, db/db mice that are hyperleptinemic develop microalbuminuria similar to that observed in human diabetic nephropathy [44]. Serum leptin has also been shown to increase in diabetic nephropathy and promote glomerular sclerosis [45,46] as a further indication of the potential association between leptin and nesfatin-1. Paraventricular 2 NUCB2/nesfatin-1 has been recognized to be a target site of leptin in leptin-induced anorexia [47].

This study has several important limitations. First, our research had a cross-sectional design and the causal associations could not be addressed. Second, gold-standard method used to diagnose DKD is renal biopsy, whereas we used the ACR classification of microalbuminuria as a surrogate marker of the diagnostic gold standard. Third, we depended on history for the exclusion of other systemic disease, hyperthyroidism, lung cancer, and coronary artery disease. Fourth, the relatively small sample size of patient included in this study may preclude the generalizability of the present study findings.

Conclusion

Serum nesfatin-1 was not significantly higher in albuminuric type 2 diabetic patients compared with normoalbuminuric patients. Serum nesfatin also did not correlate with eGFR and creatinine in the different groups studied. Serum nesfatin-1 may not be useful as an early marker of DKD instead of albuminuria. More studies are needed to identify the role and the significance of nesfatin in diabetic patients.

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Conflicts of interest

There are no conflicts of interest.

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