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Nesfatin-1 and tubulointerstitial damage in diabetic kidney disease: A possible biomarker for the histological severity

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Abstract

Background: Although adipokines are known to contribute to the pathogenesis of diabetic kidney disease (DKD), pathological significance of nesfatin-1, an adipokine in DKD remains unclear. We studied the possible associations between serum nesfatin-1 concentrations and histological renal damages in 56 persons with biopsy-proved DKD.

Methods: The relation between serum nesfatin-1 concentrations, clinical parameters and renal histological damage were cross-sectionally investigated.

Results: Serum nesfatin-1 concentrations showed a significant negative correlation with age, total cholesterol, and high-density lipoprotein cholesterol, but not with other clinical parameters. Persons were divided into the following three groups based on serum nesfatin-1 concentrations (pg/mL): low- (log average: 1.99), normal- (log average: 3.05), and high-group (log average: 3.60). Histological analysis of tubulointerstitial lesions showed higher interstitial fibrosis and tubular atrophy scores and more severe interstitial infiltration in the group with low serum nesfatin-1 concentrations than in the other groups.

Conclusion: Serum nesfatin-1 concentrations showed a strong correlation with diabetic tubulointerstitial damage level, suggesting its clinical utility as a biomarker for histological injury in DKD.

Key words: Nesfatin-1, Tubulointerstitial damage, Diabetic kidney disease, Adipokine, Nucleobindin-2, Type 2 diabetes mellitus

1. Introduction

According to the International Diabetes Federation (IDF), one in ten adults in the world currently has diabetes, and this disease is steadily increasing in prevalence worldwide and has acquired the status of a global epidemic.[1] Under such circumstances, the microvascular complications of diabetes, especially renal dysfunction, are known to reduce the patients' life prognosis and quality of life.[2]

Approximately 40% of persons with diabetes develop diabetic kidney disease (DKD).[3] DKD is a major cause of end-stage kidney disease,[4] making DKD diagnosis and treatment paramount for good outcomes. Adipokines secreted from adipocytes are known to contribute to the pathology of DKD.[5] Previous studies reported the promoting effects of leptin on renal fibrosis through the transforming growth factor-β (TGF-β)[6] and those of visfatin and resistin on inflammation and arteriosclerosis.[7]

Nesfatin-1, a recently identified adipokine, is an anorectic hormone derived from nucleobindin-2 (NUCB2) expressed in rat hypothalamus[8] and contributes to several phenomena, e.g., the control of reproductive and cardiovascular functions, maintenance of glucose homeostasis, and anti-inflammatory and anti-apoptosis effects.[9,10] In addition, the central administration of nesfatin-1 stimulates renal sympathetic nerve activity in rats through the extracellular signal-regulated kinase pathway, leading to the increase in blood pressure.[11] Currently, no specific nesfatin-1 receptor has been identified, but previous studies have reported that G protein-coupled receptors mediate nesfatin-1 actions.[12,13] In addition,

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autoradiography study has confirmed binding to the renal parenchyma,[14] suggesting that kidney may be an important target organ for nesfatin-1. Indeed, several studies showed a positive correlation between serum nesfatin-1 concentrations and urinary protein excretion in DKD,[15-17] though the details remain unknown.

In the present study, we investigated the association between serum nesfatin-1 concentrations and clinical parameters, histological renal damage in persons with DKD, and explored its potential as a biomarker.

2. Materials and methods

Persons with biopsy-proven DKD caused by type 2 diabetes mellitus, who underwent renal biopsy at the Kindai University Hospital between March 2011 and March 2021, were included in this study. The diagnosis of type 2 diabetes mellitus was performed according to the criteria of the Japan Diabetes Society.[18] The exclusion criteria were as follows: aged <20 years at renal biopsy, kidney disease complications, renal transplant or dialysis history, abnormal renal tissue (≤ 8 observable glomeruli), active cancer treatment, active virus or bacterial infection, severe liver dysfunction, or treatment with anticonvulsants that affect serum nesfatin-1 concentrations.[19] All persons provided informed consent. Fifty-six persons were included in this study, which was performed with the approval of the Ethics Committee of the Kindai University Hospital (approval number: R02-092). This study was conducted in accordance with the Helsinki Declaration of the World Medical Association.

2.1. Clinical and biochemical profile

Body mass index (BMI) for all persons was calculated based on height and weight values obtained at admission for renal biopsy. Blood pressure was measured in the sitting position after a 5-minute rest using an electronic blood pressure monitor (08-G11, Terumo, Tokyo, Japan). Participants' medical and medication history was collected at admission. Venous blood samples were collected after 12-hour nocturnal fasting on the morning of renal biopsy. Serum samples were collected by centrifugation at 3,000 rpm for 20 minutes and stored at -70 °C until analysis. Items of interest included nesfatin-1, hemoglobin A1c (HbA1c), total protein, albumin (Alb), blood urea nitrogen, serum creatine concentration (SCr), estimated glomerular filtration rate (eGFR), uric acid, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels. Urinary protein excretion level was calculated for all persons from morning urine samples collected on the day of renal biopsy.

2.2. Measurement of serum nesfatin-1 concentrations

Serum nesfatin-1 concentrations were measured using a commercially available sensitive enzyme-linked immunosorbent assay (ELISA) kit (Human Nesfatin-1/Nucleobindin-2 Duo Set ELISA [DY5949], R&D Systems, Inc., Minneapolis, MN, USA). The detection range of serum nesfatin-1 concentrations in 10-fold dilution of serum samples was 31.3-2,000 pg/mL. If serum nesfatin-1 concentrations were below the detection limit, serum stock solution was used for the measurement of nesfatin-1 concentrations. Twenty-six samples were re-assayed nesfatin-1 concentration using serum stock solution without dilution.

2.3. DKD histological evaluation

The evaluation of renal histology was performed by four investigators, including two pathologists and two nephrologists of the Kindai University Hospital who were not involved in the study. The evaluation of renal tissue in DKD was performed according to the criteria of the International Renal Pathology Conference (Renal Pathology Society (RPS) classification).[20]

2.4. Statistical analysis

All statistical analyses were performed using EZR version 1.54 (Saitama Medical Centre, Jichi Medical University, Saitama, Japan). P-values of < 0.05 were considered statistically significant. Qualitative data are presented as a percentage. Quantitative data are presented as mean ± standard deviation and median (interquartile range). As nesfatin-1 concentrations were not normally distributed, log-transformed values were used for statistical analysis. T-test, Mann-Whitney U test, Kruskal-Wallis test, and one-way analysis of variance (ANOVA) were used to compare groups classified by kidney histological damage and with or without nephrotic syndrome. The Steel-Dwass multiple comparison test and Tukey-Kramer multiple comparison test were used for multiple comparisons. Pearson correlation or Spearman rank correlation were used to examine the associations among dialysis-related factors.

3. Results

3.1. Baseline participants characteristics

The participants' characteristics were presented in Table 1. All persons had elevated systolic blood pressure, though they were on antihypertensive treatments. Men constituted more than half of the

sample and were younger than women (average age: 62.3 ± 14.3 years; men: 60.7 ± 14.6 years, women: 65.1 ± 13.6 years; men vs. women age, $p = 0.263$: N.S.). Most participants (53.6%) were aged ≥ 65 years. The mean BMI of participants was under thirty. In addition, the average duration of diabetes was relatively long (16.3 ± 10.8 years). The use of dipeptidyl-peptidase-4 (DPP-4) inhibitors (41.1%) and insulin (37.5%) was high in the treatment of diabetes. Participants had moderate to severe renal dysfunction and hypoalbuminemia due to severe urinary protein excretion rate. The log values for the mean fasting serum nesfatin-1 concentrations (log nesfatin-1) were 2.69 ± 1.03 (observed value; 489.8 ± 10.7 pg/mL). More than 80% were diagnosed as having nephrotic syndrome (Table 1). The proportions of persons with Japanese classification of diabetic nephropathy stages 2, 3, and 4 were 3.6%, 48.2%, and 48.2%, respectively. When evaluated by the chronic kidney disease (CKD) stages, the proportions were as follows: stage 1, 2, 3, 4, and 5 were 1.8%, 3.6%, 48.2%, 28.6%, and 17.9%, respectively. Renal histopathological characteristics were presented in Table 2. Tissue damages were observed according to the classification of glomerular lesions or tubulointerstitial lesions (Table 2).

Table 1. Baseline clinical characteristics of the study participants.

Clinical characteristics	DKD (n=56)
Age (years)	62.3±14.3
Sex (men %)	35 (62.5%)
Duration of diabetes (years)	16.3±10.8
BMI (kg/m²)	25.9±5.3
Sbp (mmHg)	155.6±20.6
Dbp (mmHg)	78.0±14.0
HbA1c (% (mmol/moL))	6.8±1.3 (50 ± 14)
CRP (mg/L)	3.1±6.3
Blood urea nitrogen (mmol/L)	10.2±4.9
SCr (umol/L)	200.7±143.2
eGFR (ml/min/1.73m²)	32.4±22.0
Total protein (g/L)	60.8±10.0
Albumin (g/L)	29.6±7.9
Uric acid (umol/L)	414.6±115.4
Triglyceride (mmol/L)	2.1±1.3
TC(mmol/L)	5.6±1.1
HDL-C (mmol/L)	1.4±0.4
LDL-C (mmol/L)	3.2±1.1
Urinary protein excretion (g/gCr)	9.13[4.28-12.12]
Nesfatin-1 (pg/mL)	489.8±10.7
log Nesfatin-1	2.69±1.03
Coexisting retinopathy n (%)	31 (62.0%)
Coexisting nephrotic syndrome n (%)	46 (82.1%)
Use of statin n (%)	19 (33.9%)
Use of fibrate n (%)	19 (33.9%)

Continuous variables were expressed as mean ± standard deviation or median (interquartile range). Categorical variables were expressed as frequency (percent).
DKD, diabetic kidney disease; BMI, body mass index; Sbp, systolic blood pressure; Dbp, diastolic blood pressure; HbA1c, glycated haemoglobin; CRP, C-reactive protein; SCr, serum creatine concentration; eGFR, estimated glomerular filtration rate; TC, total cholesterol; HDL-C, high-density lipoproteins cholesterol; LDL-C, low-density lipoproteins cholesterol.

Table 2. Baseline characteristics of kidney histological evaluation.

RPS classification		DKD (n=56)
Glomerular classification n (%)	Mesangial expansion	10 (17.9)
	Nodular sclerosis	21 (37.5)
	Global glomerular sclerosis	25 (44.6)
IFTA n (%)	<25%	11 (19.6)
	25~50%	21 (37.5)
	>50%	24 (42.9)
Interstitial inflammation n (%)	Infiltration only in relation to IFTA	47 (83.9)
	Infiltration in areas without IFTA	9 (16.1)
Arteriolar hyalinosis n (%)	Absent	4 (7.1)
	At least one area of arteriolar hyalinosis	1 (1.8)
	More than one area of arteriolar hyalinosis	51 (91.1)
Arteriosclerosis n (%)	No intimal thickening	51 (91.1)
	Less than thickness of media	9 (16.1)
	Greater than thickness of media	45 (80.4)

Categorical variables were expressed as frequency (percent).
RPS, Renal Pathology Society; IFTA, interstitial fibrosis and tubular atrophy.

3.2. Relation between nesfatin-1 concentrations and clinical parameters

Log nesfatin-1 showed a significant negative correlation with age (r = -0.363, p = 0.006), TC (r = -0.274, p = 0.041), and HDL-C (r = -0.280, p = 0.042), but not blood pressure, LDL-C, duration of diabetes, BMI (r = 0.199, p = 0.141), HbA1c (r = 0.073, p = 0.592), SCr, eGFR, or urinary protein excretion rate (Figure 1).

3.3. Impact of nephrotic syndrome on nesfatin-1 concentrations

Serum nesfatin-1 concentrations (and its log value) were slightly lower in persons with nephrotic syndrome than those without; 1245.5 ± 25.8 (2.6 ± 0.9) pg/mL vs. 400.5 ± 8.5 (3.1 ± 1.4) pg/mL (p = 0.174: N.S., Figure 2). Presence of nephrotic syndrome had no effect on the relation between nesfatin-1 concentrations and clinical parameters.

Parameter	Correlation coefficient	p value
Duration of diabetes (years)	-0.060	0.671
Age (years)	-0.363	0.006*
BMI (kg/m²)	0.199	0.141
HbA1c (% (mmol/moL))	0.073	0.592
Creatinine (umol/L)	-0.047	0.729
eGFR (ml/min/1.73m²)	0.122	0.369
Sbp (mmHg)	-0.218	0.107
Dbp (mmHg)	0.011	0.938
Total cholesterol (mmol/L)	-0.274	0.041*
HDL-C (mmol/L)	-0.280	0.042*
LDL-C (mmol/L)	-0.228	0.104
Triglyceride (mmol/L)	0.122	0.372
UAE (g/gCre)	-0.135	0.325
Uric acid (umol/L)	0.054	0.695

Figure 1. Bivariate correlations of fasting serum nesfatin-1 with (A) age, (B) TC, (C) HDL-C levels.
There was a significant negative correlation between fasting serum nesfatin-1 concentrations and (A) age, (B) TC and (C) HDL-C levels in person with biopsy-proven DKD.
*Denotes significant correlations (p < 0.05).

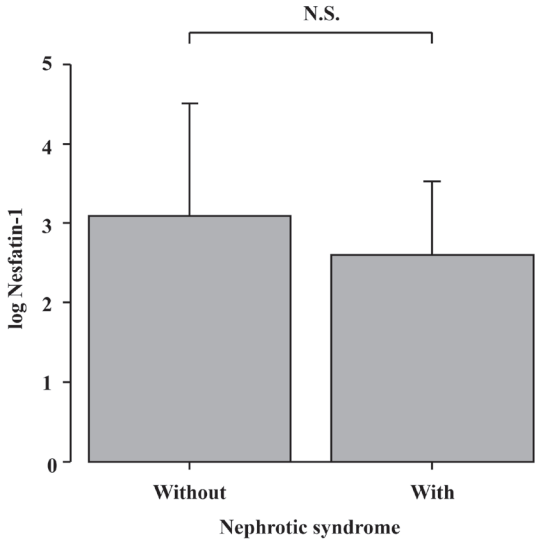

Figure 2. Fasting serum nesfatin-1 concentrations with and without nephrotic syndrome.
Without nephrotic syndrome vs with nephrotic syndrome (p = 0.174, N.S. : Not Significant).
Fasting serum nesfatin-1 concentrations were tended to be lower in persons with nephrotic syndrome than those without.

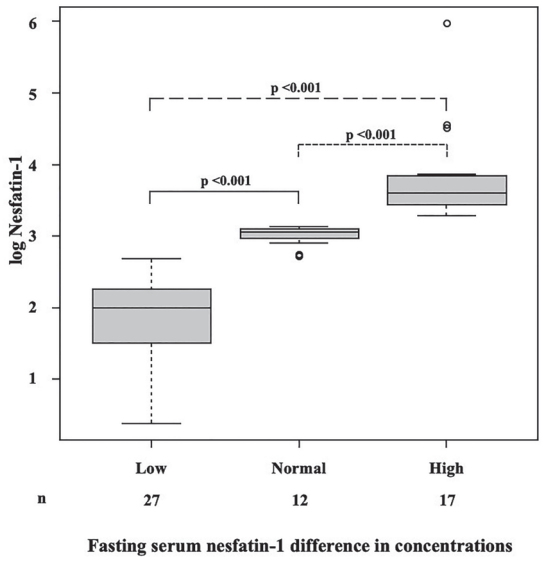

Figure 3. Distribution of fasting serum nesfatin-1 concentrations (log nesfatin-1) in diabetic kidney disease.
There was a significant difference between all groups (p < 0.001).
Range of each serum nesfatin-1 concentrations (observed value (pg/mL) and log value): Low; <500 pg/mL (log: <2.70), Normal; 500-1500 pg/mL (log: 2.70-3.18), High; 1500 pg/m≤ (log: 3.18≤).

3.4. Distribution of nesfatin-1 concentrations

Serum nesfatin-1 concentrations were graded into three groups (low, normal, high serum nesfatin-1) based on its reported normal range (500-1,500 pg/mL).[17,21,22] The value of each group showed a significant difference (low vs. normal group, $p < 0.001$; low vs. high group, $p < 0.001$; and normal vs. high group, $p < 0.001$) (Figure 3). All measured parameters, including BMI, sex and duration of diabetes mellitus, which reportedly affect nesfatin-1 concentration, were compared in the three groups divided by nesfatin-1 concentration, but no significant differences were found except for age.

3.5. Nesfatin-1 and renal tissue damage

Glomerular injury and vascular damages were not associated with serum nesfatin-1 concentrations. On the other hand, there was a significant negative correlation between histological severity levels and serum nesfatin-1 concentrations in tubulointerstitial injury and interstitial infiltration. As shown in Figure 4, serum nesfatin-1 concentrations were significantly ($p < 0.05$) different depending on the severity of the interstitial fibrosis and tubular atrophy (IFTA), indicating that the low serum nesfatin-1 concentrations may be a possible predictor for severe IFTA. Higher interstitial infiltration levels were also associated with lower serum nesfatin-1 concentrations ($p < 0.05$).

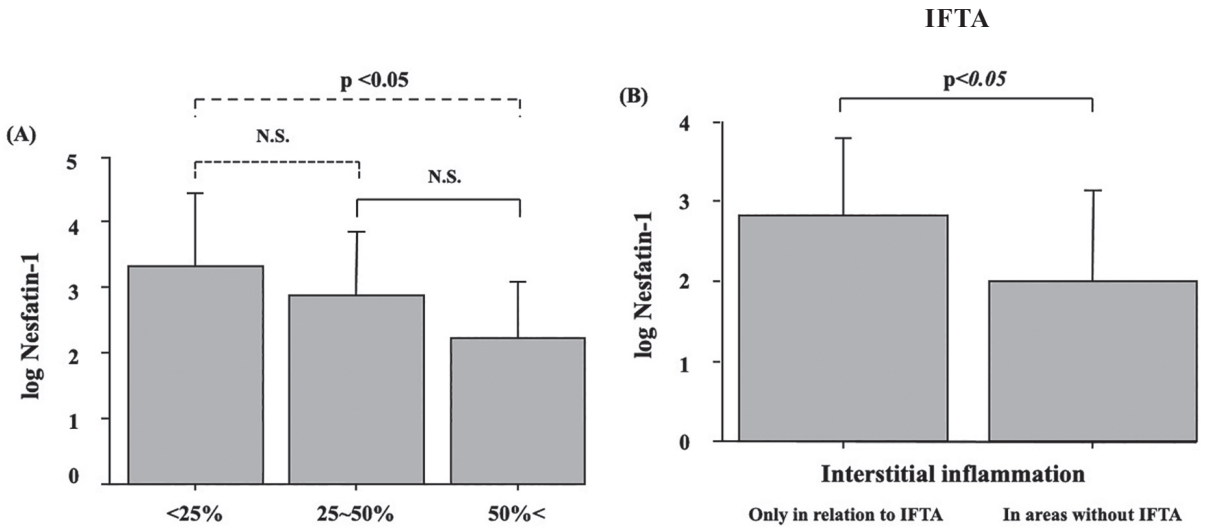

Figure 4. Fasting serum nesfatin-1 concentrations (log nesfatin-1) classified by the severity of interstitial each interstitial disorders (A: IFTA level, B: Interstitial inflammation).

A) There was a significant difference in the serum nesfatin-1 concentrations between the IFTA<25% and 50%< groups ($p<0.05$). The higher the IFTA level, the lower the serum nesfatin-1 concentrations. N.S.: Not Significant.

B) Serum concentrations of nesfatin-1 was tended to be lower in the persons with wide interstitial inflammation than in the group without it ($p<0.05$).

4. Discussion

In the present study, we found a strong correlation between serum nesfatin-1 concentrations and severity of tubulointerstitial but not glomerular injury in persons with DKD. Thus, serum nesfatin-1 concentrations may be available as a novel biomarker for tubulointerstitial histological injury in DKD.

It has been reported that nesfatin-1 enhances glucose-induced insulin secretion.[23,24] In addition, it also suppresses gluconeogenesis in the liver via the mammalian target of rapamycin (mTOR) and signal transducer and activator of transcription 3 (STAT3) pathway.[25] Therefore, nesfatin-1 play a great important role in the maintenance of plasma glucose homeostasis. However, its pathological significance in DKD is so far scarcely none.

The present findings show a wide variation in serum nesfatin-1 concentrations and a negative correlation between serum nesfatin-1 concentrations and age, TC, and HDL-C in persons with biopsy-proven DKD.

Several studies have reported an increase in serum nesfatin-1 concentrations in persons with type 2 diabetes,[26,27] whereas others reported their decrease.[17,28] The cause of this discrepancy was thought to be differences in the race, study design and patients background such as BMI and insulin resistance.[17,27] A meta-analysis of seven studies that adjusted for these factors showed that the duration of diabetes and presence or absence of treatment for diabetes affect nesfatin-1 concentrations,[21] though it was not the case in the present study (serum nesfatin-1 concentrations showed no significant correlation with the duration of diabetes or presence or absence of treatment for diabetes). On the other hand, we found a significant negative correlation with age (Figure 1), though *Li et al*.[17] reported a positive correlation. The inconsistency may be due to protein-energy wasting (PEW) in our persons with renal insufficiency. PEW is characterised by reduced muscle and adipose tissue mass in persons with renal insufficiency due to dietary restriction or chronic inflammation.[29] Indeed, the prevalence of PEW has been estimated in the range of 11% to 54% in persons with stage 3, 4, or 5 CKD,[30] and the risk of PEW is known to increases with age.[31] In addition, serum nesfatin-1 concentrations has been reported to be positively correlated with fat weight.[32, 33] It may be that most persons studied in the present study have PEW because 94.7% of them are diagnosed as stage 3 to 5 CKD. PEW due to aging and the progression of CKD may have led to reduced adipose tissue mass and secretion of adipokines from adipocytes.

Several studies have reported serum nesfatin-1 concentrations correlate with BMI in persons with diabetes[27,34] but not in persons with DKD.[15-17] This is consistent with our results that serum nesfatin-1 concentrations significantly correlate with age but not BMI. The reason for the disappearance the correlation may be due to DKD-related increase in body fluid volume. It is well-known that BMI does not

accurately reflect the body composition of persons with renal insufficiency and that measurement using bioelectrical impedance analysis or the dual energy X-ray absorptiometry method are required to accurately measure of body composition. in persons with CKD.[35]

Serum nesfatin-1 showed a weak but significant correlation with TC and HDL-C, suggesting a possible role of nesfatin-1 in the lipid metabolism in persons with DKD. It has been reported that intravenous administration of nesfatin-1 activates AMPK-activated protein kinase (AMPK) resulting in a decrease in free fatty acids (FFA) in Streptozotocin-induced type 2 diabetic mouse.[36] Thus, it is possible that serum nesfatin-1 contributes lipid metabolism via AMPK activation in diabetes.

In the present study, serum nesfatin-1 concentrations were extremely high in some DKD persons. Renal fibrosis and infiltration of inflammatory cells develop in DKD because of the persistent chronic inflammation through proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-18, and tumour necrosis factor-α (TNF-α).[37] It has been reported that nesfatin-1 producing neurons at the level of hypothalamus and brainstem are highly sensitive to peripheral inflammatory stimulation by these cytokines.[38] Therefore, the activation of defence mechanisms against metabolic disturbance and inflammation may lead to a remarkable elevation in nesfatin-1 concentrations in some DKD persons.

This is the first report to suggest a clinically relevant relation between renal tissue damage and serum nesfatin-1 concentrations. Our results that statistically more severe tubulointerstitial lesions were observed in persons with lower serum nesfatin-1 concentrations suggest a possible clinical utility of serum nesfatin-1 concentrations as a biomarker for diabetic renal tubular damage. Thus, nesfatin-1 may reduce renal damage by reducing the oxidative stress especially in renal tubules.

A previous study using an ischemia-reperfusion model rat showed that nesfatin-1 exhibited antioxidant effects by enhancing superoxide dismutase (SOD) and catalase (CAT) activity, thereby inhibiting elevated malondialdehyde (MDA) levels. Furthermore, nesfatin-1 reduced tubular mitochondrial stress and decreased tubular apoptosis, indicating its potential as a therapeutic agent for renal disorders.[39] *Tezcan et al*.[40] proved that nesfatin-1 suppressed the expression of α-smooth muscle actin (α-SMA) and improved renal fibrosis by using an unilateral ureteral obstruction (UUO) model rat. It may be possible that similar phenomenon occurred in human renal tissue observed in our study, and nesfatin-1 reduced renal damage by decreasing the oxidative stress especially renal tubules.

Limitations

This study has some limitations. First, the sample size of this study was small. Second, there was no healthy control group. Third, the impact of prescribed medicines has not been investigated. Some diabetic drugs, such as thiazolidinedione derivatives and DPP-4 inhibitors,[41, 42] have been reported to affect serum nesfatin-1 concentrations. Therefore, it is difficult to generalise the results of this study. Further studies are required to validate the present findings on the protective effect of serum nesfatin-1 on renal tubules, including studies involving persons with earlier-stages of DKD.

Conclusion

In conclusion, we found that the more severe the tubulointerstitial damage, the lower the serum nesfatin-1 concentrations in persons with renal biopsy-proven DKD. This indicates that serum nesfatin-1 concentrations may be a potential biomarker for tubulointerstitial disorders and may lead to better results in the diagnosis and treatment of patients in the future.

Compliance with Ethical Standards

Conflict of interest

SA received scholarship donation from Kyowa Kirin, Daiichi-Sankyo, Tanabe-Mitsubishi. The remaining authors declare no conflict of interest.

Ethical approval

All procedures involving human participants were in accordance with ethical standards of the institution at which the studies were conducted (approval number in Ethics Committee of the Kindai University Hospital: R02-092), and with standards of the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in this study.

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References

1. International Diabetes Federation. IDF Diabetes Atlas, 10th

edn. Brussels, Belgium: 2021. https://www.diabetesatlas.org Accessed 7 May 2022.
2. Thomas, MC, et al. (2015) Diabetic kidney disease. Nat Rev Dis Primers, 1, 15018.
3. Afkarian M, et al. (2016) Clinical manifestations of kidney disease among US adults with diabetes, 1988-2014. JAMA, 316(6), 602–610.
4. Iwai T, et al. (2018) Diabetes mellitus as a cause or comorbidity of chronic kidney disease and its outcomes: the gonryo study. Clinical and experimental nephrology, 22(2), 328–336.
5. Wada J, Makino H. (2013) Inflammation and the pathogenesis of diabetic nephropathy. Clinical science (London, England: 1979), 124(3), 139–152.
6. Han DC, et al. (2001) Leptin stimulates type I collagen production in db/db mesangial cells: glucose uptake and TGF-beta type II receptor expression. Kidney international, 59(4), 1315–1323.
7. Rüster C, Wolf G. (2013) Adipokines promote chronic kidney disease. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association, 28 Suppl 4, iv8–iv14.
8. Oh-I S, et al. (2006) Identification of nesfatin-1 as a satiety molecule in the hypothalamus. Nature, 443(7112), 709–712.
9. Schalla MA, Stengel A. (2018) Current Understanding of the Role of Nesfatin-1. Journal of the Endocrine Society, 2(10), 1188–1206.
10. Xu Y, Chen F. (2020) Antioxidant, Anti-Inflammatory and Anti-Apoptotic Activities of Nesfatin-1: A Review. Journal of inflammation research, 13, 607–617.
11. Tanida M, et al. (2015) Hypothalamic Nesfatin-1 Stimulates Sympathetic Nerve Activity via Hypothalamic ERK Signaling. Diabetes, 64(11), 3725–3736.
12. Ishida E, et al. (2012) Nesfatin-1 induces the phosphorylation levels of cAMP response element-binding protein for intracellular signaling in a neural cell line. PloS one, 7(12), e50918.
13. Brailoiu GC, et al. (2007) Nesfatin-1: distribution and interaction with a G protein-coupled receptor in the rat brain. Endocrinology, 148(10), 5088–5094.
14. Prinz P, et al. (2016) Peripheral and central localization of the nesfatin-1 receptor using autoradiography in rats. Biochemical and biophysical research communications, 470(3), 521–527.
15. Irannejad A, et al. (2017) Association of peripheral nesfatin-1 with early stage diabetic nephropathy. Pathophysiology: the official journal of the International Society for Pathophysiology, 24(1), 17–22.
16. Korani MA, Sonbol A. (2018) The relation between plasma levels of nesfatin-1 and different grades of diabetic kidney disease in patients with type 2 diabetes. Egypt J Intern Med 30, 68–71.
17. Li QC, Wang HY, Chen X, Guan HZ, Jiang ZY. (2010) Fasting plasma levels of nesfatin-1 in patients with type 1 and type 2 diabetes mellitus and the nutrient-related fluctuation of nesfatin-1 level in normal humans. Regulatory peptides, 159(1-3), 72–77.
18. Araki E, et al. (2020) Japanese Clinical Practice Guideline for Diabetes 2019. Diabetology international, 11(3), 165–223.
19. Aydin S, et al. (2009) Nesfatin-1 and ghrelin levels in serum and saliva of epileptic patients: hormonal changes can have a major effect on seizure disorders. Molecular and cellular biochemistry, 328(1-2), 49–56.
20. Tervaert TW, et al. (2010) Pathologic classification of diabetic nephropathy. Journal of the American Society of Nephrology: JASN, 21(4), 556–563.
21. Zhai T, et al. (2017) Circulating Nesfatin-1 Levels and Type 2 Diabetes: A Systematic Review and Meta-Analysis. Journal of diabetes research, 2017, 7687098.
22. Schenk M, et al. (2020) Intra-individual reproducibility of galectin-1, haptoglobin, and nesfatin-1 as promising new biomarkers of immunometabolism. Metabolism open, 6, 100034.
23. Nakata M, Manaka K, Yamamoto S, Mori M, Yada T. (2011) Nesfatin-1 enhances glucose-induced insulin secretion by promoting Ca (2+) influx through L-type channels in mouse islet β-cells. Endocrine journal, 58(4), 305–313.
24. Gonzalez R, et al. (2011) Nesfatin-1 exerts a direct, glucose-dependent insulinotropic action on mouse islet β- and MIN6 cells. The Journal of endocrinology, 208(3), R9–R16.
25. Wu D, et al. (2014) Hypothalamic nesfatin-1/NUCB2 knockdown augments hepatic gluconeogenesis that is correlated with inhibition of mTOR-STAT3 signaling pathway in rats. Diabetes, 63(4), 1234–1247.
26. Akour A, et al. (2017) Levels of metabolic markers in drug-naive prediabetic and type 2 diabetic patients. Acta diabetologica, 54(2), 163–170.
27. Zhang Z, et al. (2012) Increased plasma levels of nesfatin-1 in patients with newly diagnosed type 2 diabetes mellitus. Experimental and clinical endocrinology & diabetes: official journal, German Society of Endocrinology [and] German Diabetes Association, 120(2), 91–95.
28. Mirakhor Samani S, Ghasemi H, Rezaei Bookani K, Shokouhi B. (2019) SERUM NESFATIN-1 LEVEL IN HEALTHY SUBJECTS WITH WEIGHT-RELATED ABNORMALITIES AND NEWLY DIAGNOSED PATIENTS WITH TYPE 2 DIABETES MELLITUS: A CASE-CONTROL STUDY. Acta endocrinologica (Bucharest, Romania: 2005), -5(1), 69–73.
29. Fouque D, et al. (2008) A proposed nomenclature and diagnostic criteria for protein-energy wasting in acute and chronic kidney disease. Kidney international, 73(4), 391–398.
30. Carrero JJ, et al. (2018) Global Prevalence of Protein-Energy Wasting in Kidney Disease: A Meta-analysis of Contemporary Observational Studies From the International Society of Renal Nutrition and Metabolism. Journal of renal nutrition: the official journal of the Council on Renal Nutrition of the National Kidney Foundation, 28(6), 380–392.
31. Vettoretti S, et al. P. (2020) Frailty phenotype and multi-domain impairments in older patients with chronic kidney disease. BMC geriatrics, 20(1), 371.
32. Ramanjaneya M, et al. (2010) Identification of nesfatin-1 in human and murine adipose tissue: a novel depot-specific adipokine with increased levels in obesity. Endocrinology, 151(7), 3169–3180.
33. Anwar GM, et al. (2014) Nesfatin-1 in childhood and adolescent obesity and its association with food intake, body composition and insulin resistance. Regulatory peptides,

188, 21–24.
34. Ding S, et al. (2015) Serum nesfatin-1 is reduced in type 2 diabetes mellitus patients with peripheral arterial disease. Medical science monitor: international medical journal of experimental and clinical research, 21, 987–991.
35. Johansen KL, Lee C. (2015) Body composition in chronic kidney disease. Current opinion in nephrology and hypertension, 24(3), 268–275.
36. Dong J, et al. (2013) Nesfatin-1 stimulates fatty-acid oxidation by activating AMP-activated protein kinase in STZ-induced type 2 diabetic mice. PloS one, 8(12), e83397.
37. Lontchi-Yimagou E, Sobngwi E, Matsha TE, Kengne AP. (2013) Diabetes mellitus and inflammation. Current diabetes reports, 13(3), 435–444.
38. Bonnet MS, et al. (2009) Central nesfatin-1-expressing neurons are sensitive to peripheral inflammatory stimulus. Journal of neuroinflammation, 6, 27.
39. Jiang G, et al. (2015) The protective effect of nesfatin-1 against renal ischemia-reperfusion injury in rats. Renal failure, 37(5), 882–889.
40. Tezcan N, et al. (2022) Nesfatin-1 treatment preserves antioxidant status and attenuates renal fibrosis in rats with unilateral ureteral obstruction. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association, 37(7), 1238–1248.
41. Yamada M, et al. (2010) Troglitazone, a ligand of peroxisome proliferator-activated receptor-{gamma}, stabilizes NUCB2 (Nesfatin) mRNA by activating the ERK1/2 pathway: isolation and characterization of the human NUCB2 gene. Endocrinology, 151(6), 2494–2503.
42. Chen K, Zhuo T, Wang J, Mei Q. (2018) Saxagliptin Upregulates Nesfatin-1 Secretion and Ameliorates Insulin Resistance and Metabolic Profiles in Type 2 Diabetes Mellitus. Metabolic syndrome and related disorders, 16(7), 336–341.