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Association of peripheral nesfatin-1 with early stage diabetic nephropathy

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ABSTRACT

Background: Nesfatin-1 is a newly found anorectic neuropeptide with potent metabolic regulatory effects that its circulating levels are shown to be elevated in diabetes. We compared serum nesfatin-1 in patients with type 2 diabetes and microalbuminuria (30 mg/day ≤ urinary albumin excretion (UAE) <300 mg/day) with their control patients with type 2 diabetes and normoalbuminuria (UAE <30 mg/day).
Patients and methods: In a cross sectional setting, 44 adult patients with type 2 diabetes and microalbuminuria and 44 control patients with type 2 diabetes and normoalbuminuria were evaluated. Serum levels of nesfatin-1 along with demographic, clinical and biochemical factors associated with diabetes was measured.
Results: Mean peripheral concentrations of nesfatin-1 were significantly higher in patients with diabetes who had microalbuminuria compared to normoalbuminuric control patients (175.27 ± 25.96 pg/ml vs. 134.66 ± 23.18 pg/ml, respectively; p value < 0.001). Significant positive correlations were found between circulating nesfatin-1 levels and the following case-mix variables: duration of diabetes, glycated hemoglobin, plasma creatinine, UAE and serum uric acid. In the multivariate logistic regression and after adjustment for a constellation of potentially confounding variables associated with diabetic kidney disease (DKD), circulating nesfatin-1 was the only variable significantly associated with microalbuminuria (odds ratio [95% confidence interval] = 1.224 [1.007–1.487], p value = 0.042).
Conclusion: In patients with type 2 diabetes, circulating nesfatin-1 appears to be associated with microalbuminuria independent of other established risk factors of DKD. The underlying pathophysiological mechanisms and the prognostic significance of this association remain to be elucidated.

What is already known about this subject?

- Previous studies found significantly higher levels of circulating nesfatin-1 in diabetes and associated metabolic derangements.
- There are scant data available on the alterations of peripheral nesfatin-1 in chronic microvascular complications of diabetes.

What are the new findings?

- We found significantly higher serum nesfatin-1 in the presence of microalbuminuria.

How might these results change the focus of research or clinical practice?

- Nesfatin-1 is a potential early biomarker of diabetic kidney disease (DKD), suggesting a prognostic correlation between peripheral nesfatin-1 and the severity and poorer control of diabetes.

1. Introduction

Diabetic kidney disease (DKD) is a chronic microvascular complication of diabetes which may lead to end-stage renal disease [1–3]. It is suggested that in people with diabetes, hyperglycemia,

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by inducing the production of advanced glycation end-products, leads to structural alterations in proteins, and these processes are presumed to be the key roots in the pathogenesis of diabetic nephropathy [4].

Nesfatin-1 is a newly found neuropeptide consisting of 82 amino acids with predominantly anorectic effects that participates in the regulation of hunger and fat storage and is also characterized as a potent regulator of metabolism. Nesfatin-1 is expressed in several tissues including the pancreatic islet cells and the central nervous system (CNS) [5,6]. Being mostly generated in the hypothalamus nuclei, nesfatin-1 has the ability to cross the blood–brain barrier without molecular saturation. Through further observations it was shown that nesfatin-1 suppresses food intake after being intracerebroventricularly injected, the injection decreases food intake in a dose-dependent manner while the injection of a nesfatin-1 neutralizing antibody stimulates appetite [5–7]. Nesfatin-1 has been reported to possess an anti-hyperglycemic effect which is peripheral and time-, dose- and insulin-dependent [8]. Recent experimental studies have also linked nesfatin-1 to enhanced peripheral and hepatic insulin sensitivity, through promoting peripheral glucose uptake and decreasing gluconeogenesis via different mechanistic pathways [9,10].

Nesfatin-1 has been studied in several metabolic dysregulations such as the diabetes, epilepsy, and inflammation [11–14]. Studies have shown that plasma levels of nesfatin-1 are elevated in patients with type 2 diabetes [15]. Raised nesfatin-1 levels were significantly associated with impaired glucose tolerance, body mass index (BMI), glycated hemoglobin (HbA_{1c}), fasting plasma glucose (FPG), and 2-h postprandial plasma glucose [9,15]. Studies have investigated nesfatin-1 as a potential cause of feeding disturbance in patients with chronic kidney disease (CKD), suggesting that nesfatin-1 may have a negative correlation with total protein intake in these patients [11,16,17].

However, no previous study to the best of our knowledge has described possible alterations of circulating nesfatin-1 levels in patients with DKD. Based on recent reports of elevated nesfatin-1 levels in patients with diabetes, nesfatin-1, being an anti-hyperglycemic and insulin sensitizer neuropeptide, is biologically plausible to be related to the pathogenesis of DKD. The purpose of this study was to comparatively determine serum nesfatin-1 levels in a group of patients with type 2 diabetes and microalbuminuria and their diabetic controls with normoalbuminuria.

2. Patients and methods

2.1. Detailed protocol and study participants

In a cross-sectional setting, 44 adult patients with type 2 diabetes and microalbuminuria (30 mg/day ≤ urinary albumin excretion [UAE] <300 mg/day) and 44 control patients with type 2 diabetes and normoalbuminuria (UAE <30 mg/day) were evaluated from 1 August 2014 to 31 February 2015. All patients were referred for routine follow-up in the outpatient diabetes clinic of a university-affiliated general hospital (Vali-Asr Hospital, Tehran University of Medical Sciences). Participants were receiving oral antihyperglycemic agents and/or insulin, antihypertensive individuals were taking either angiotensin converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) and statins were used in patients with dyslipidemia. Diagnosis of type 2 diabetes was made according to the criteria established by the American Diabetes Association [18]. An experienced ophthalmologist, blinded to the biochemical and clinical assessments, examined diabetic retinopathy according to the American Academy of Ophthalmology definitions. For this purpose, Volkor ocular lens (+90 or +78) indirect ophthalmoscopy was employed using a slit lamp biomicroscopy (Topcon, Tokyo, Japan) and fluorescein angiography [19,20]. Extensive details on diagnosis of diabetic neuropathy is available elsewhere [21]. Exclusion criteria were serum creatinine >2 mg/dL, history of renal disease prior to the onset of diabetes, presence of any sign or symptom of inflammatory renal disease and non-compliance to treatment (e.g. due to side effects of drugs or irregular consumption of medications). Patients suspected of having renal disease unrelated to diabetic nephropathy were excluded from the present study and were referred to the nephrology clinic for further evaluations. Other exclusions were cigarette/tobacco smoking, alcohol consumption, obesity (BMI ≥ 30 mg/kg^2), evidence of renal damage and dysfunction (estimated glomerular filtration rate [eGFR] < 60 ml/min/1.73 m^2 or presence of macroalbuminuria [UAE >300 mg/day]), current or recent urinary tract infection, active viral/bacterial infection, evidence of fatty liver degenerative disease (nonalcoholic fatty liver disease and nonalcoholic steatohepatitis) [22], use of drugs known to affect serum nesfatin-1 such as anti-epileptic drugs [12] and previous history of chronic macrovascular complications due to diabetes [23]. All patients provided written informed consent prior to enrollment and the protocol was approved by the local ethics committee of Tehran University of Medical Sciences.

2.2. Clinical and biochemical assessments

Recruited patients underwent through physical examination and laboratory measurements and baseline demographics (age, sex, duration of diabetes mellitus [DDM], drug use), and clinical and biochemical measures were determined. Waist circumference was taken at the end of normal expiration in a horizontal plane, midway between the inferior margin of the ribs and superior border of the iliac crest, and was rounded to the nearest 0.1 cm. BMI was calculated according to the Quetelet equation (i.e., weight in kg/height in m^2) Systolic blood pressure (Sbp) and diastolic blood pressure (Dbp) were measured after at least 5 min of rest in the sitting position, by using a standard mercury sphygmomanometer. The average of two measurements made at least 5 min apart was used for the analysis. Venous blood samples were collected following an overnight 12-h fast at 7–8 AM for all participants, with 500 Kallikrein inhibitor Unit (KiU) of the protease inhibitor aprotinin being added during the blood withdrawal. Serum FPG, HbA_{1c}, total cholesterol, triglyceride, low-density lipoproteins cholesterol (LDL-C), high-density lipoproteins cholesterol (HDL-C), blood urea nitrogen (BUN), uric acid, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT)and alkaline phosphatase (aP) were measured in a certified standard laboratory. The FPG measurements (intra- and interassay coefficients of variation being 2.1% and 2.6%, respectively) were carried out using the glucose oxidase method and HbA_{1c} (%) was measured using the high-performance liquid chromatography (Skylight Biotech, Akita, Japan). Serum triglyceride, total cholesterol, LDL-C and HDL-C were measured using the enzymatic methods (Parsazmun, Karaj, Iran). Patients were informed to collect 24-h urine samples on three occasions within few days after the visit. Gold standard method of 24-h urine collections helps avoid the false positive results associated with the use of albumin to creatinine ratio in a spot sample as the detection method. Completeness of the collected sample was attested by measuring urinary creatinine excretion. A repeat measurement was requested if creatinine excretion levels were lower than 20 mg/kg per 24 h and 15 mg/kg per 24 h for men and women, respectively. UAE was determined by calorimetric methods using the commercial kits (ZiestChem Diagnostics, Tehran, Iran), with the average value obtained from three consecutive collections used for analysis. The diagnosis of early stage DKD was defined by the presence of microalbuminuria (30 mg/day ≤ UAE <300 mg/day) on at least two out of three consecutive measurements. The