The Association of Elevated Serum Lipocalin-2 Levels With Diabetic Peripheral Neuropathy in Type 2 Diabetes

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Abstract

Background

A variety of studies have demonstrated the role of lipocalin-2 (LCN2) in both diabetes and neurological disorders. Nevertheless, the relationship between LCN2 and diabetic peripheral neuropathy (DPN) needs to be elucidated in humans. Therefore, this study was aimed to investigate the association of LCN2 with DPN in type 2 diabetes (T2D).

Methods

A total 207 participants with T2D were included in this study. All participants were classified into DNP group and non-DNP (NDPN) group based on the Toronto Clinical Neuropathy Scoring (TCNS). Demographic and biochemical parameters were measured. Serum LCN2 levels were determined using an enzyme-linked immunosorbent assay method.

Results

Serum LCN2 levels in DNP group were higher than those in NDPN group ($p = 0.001$). Stratification analysis according to the tertiles of serum LCN2 levels showed that with the LCN2 level elevated, the number of participants with DPN increased, whereas the number of participants with NDPN decreased (trend $p = 0.003$). Moreover, serum LCN2 levels positively correlated to TCNS scores, which reflects neuropathy severity ($r = 0.438$, $p = 0.000$). Multivariate stepwise regression analysis showed that BMI, triglycerides and diastolic pressure were independently associated with serum LCN2 in DPN. Additionally, logistic regression analysis demonstrated that LCN2 ($OR = 1.009$) and diabetes duration ($OR = 1.058$) were independently associated with the occurrence of DPN in T2D.

Conclusions

Our report reveals the association of serum LCN2 with DPN in T2D. LCN2 might be used to evaluate DPN severity and serve a role in the pathogenesis of DPN.

Background

Type 2 diabetes (T2D), the most common form of diabetes, has fallen into the leading causes of disability and death worldwide. The harms of T2D are arisen from its complications such as neuropathy, nephropathy, retinopathy, and cardiovascular disease. Diabetic peripheral neuropathy (DPN), the most prevalent and troublesome complication of T2D, affects up to 50% individuals [1, 2]. DPN commonly with symptoms of pain, paresthesia or numbness leads to lower quality of life, increased morbidity and huge economic burdens. A variety of factors related to the pathophysiological processes of T2D including hyperglycemia, the formation of intracellular advanced glycation end products, oxidative stress, mitochondrial dysfunction, inflammatory cascades have been demonstrated to be implicated in the

development and progression of DPN [3]. However, the precise mechanisms underlying the development and progression of DPN remain elusive.

Lipocalin-2 (LCN2), also known as 24p3 or neutrophil gelatinase-associated lipocalin, is a secreted glycoprotein in response to numerous physiological and pathological stimuli [4]. A variety of studies have demonstrated that LCN2 broadly expresses in many tissues such as adipose tissue, liver, kidneys, lungs, and the brain, and serve roles in both metabolic and neurological disorders. Our previous study and other colleagues' studies indicate that serum LCN2 levels are significantly higher in diabetes and diabetic complications such as diabetic retinopathy, diabetic nephropathy and cardiovascular diseases [5-9]. For neurological diseases, the role of LCN2 has been implicated in diabetic encephalopathy, and some other neurological diseases in animal models with metabolic disturbance [10, 11]. Most recently, a report by Bhusal et al. indicated that glial-derived LCN2 played an important role in the pathogenesis of DPN via PDK2-lactic acid axis in the dorsal root ganglion (DRG) in mice model [12]. Nevertheless, the relationship between LCN2 and DPN in humans remains unclear. Therefore, we conducted a study to investigate the association between serum LCN2 and DPN in individuals with T2DM.

Materials And Methods

Participants

A total of 207 participants with T2D from the Department of Endocrinology, the First Affiliated Hospital of University of Science and Technology of China were included in this study from September 2018 to February 2021. T2D was diagnosed according to the 1999 World Health Organization criteria. All participants with T2D were classified into DNP group and non-DNP (NDPN) group. The diagnosis of DPN was based on the Toronto Clinical Neuropathy Scoring (TCNS) [13]. 107 participants with TCNS scores ≥ 6 were assigned to DNP group, and 100 participants with scores < 6 were assigned to NDPN group. The clinical parameters including sex, age and body mass index (BMI) were matched in the two groups. The exclusion criteria were as follows: (a) type 1 diabetes, gestational diabetes, specific types of diabetes or acute complications of diabetes; (b) neuropathy caused by other diseases or drugs; (c) severe arteriovenous vascular disease (e.g. venous embolism, lymphangitis); (d) neurotoxicity caused by drugs, especially chemotherapeutic drugs, and nerve damage caused by metabolic poisons caused by renal insufficiency; (e) chronic kidney disease $\geq$ stage 3b; (f) any amputation other than involving the toes or fingers.

Data collection and demographic measurement

All participants were questioned and physical examined by trained doctors and nurses to obtain the information on age, sex, weight, height, blood pressure, illness and medical therapy history. The BMI was calculated as the weight/height². Blood pressure (BP) was tested in triplicate after at least 30 min of rest, and the average of three recordings was recorded.

Laboratory measurements

Venous blood samples were collected from all participants after an overnight fast of 10-12 hours. Fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TG), creatinine (Cr) (in urine or serum) were assayed by an automatic biochemistry analyzer (7600-020 Chemical Analyzer, Hitachi, Japan). Hemoglobin A1c (HbA1c) was measured by affinity chromatography with an HbA1c radiometer (Bio-Rad Laboratory Inc., Hercules, CA, USA). Urinary albumin was assayed using immune turbidimetry kits purchased from Northern Biotechnology Research Institute (Beijing, China). Urine albumin creatinine ratio (UACR) was calculated as the urine albumin / urine creatinine. Fasting C-peptide (FCP) was assayed by electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany). The estimated glomerular filtration rate was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

Serum LCN2 levels were assayed using an enzyme-linked immunosorbent assay (ELISA) kit (Shanghai Xitang Biotechnology Co. Ltd, Shanghai, China). Procedures were according to the manual instructions by kit provider.

Assessment of DPN

DPN was diagnosed using TCNS based on neuropathic symptoms, signs and the presence of abnormal nerve conduction [13]. All participants underwent electromyogram tests to evaluate the median nerve motor and sensory branches, the motor and sensory branches of the ulnar nerve, the motor and sensory branches of the radial nerve, the tibial nerve and the peroneal nerve. The TCNS, a validated and reliable scale, has been used to grade DPN severity [14]. The clinical neuropathy score ranges from a minimum of 0 to a maximum of 19 points. Six points are derived from symptoms, eight from lower-limb reflexes, and five from sensory examination distally at the toes. A higher score indicates more severe disability.

Statistical Analysis

Statistical analyses were conducted using SPSS software (version 20.0, SPSS Inc., Chicago, IL, USA). Continuous variables with normal distribution were expressed as means $\pm$ standard deviation (SD), skewed variables as medians with inter-quartile ranges, and categorical variables as frequencies. All variables were tested for normality using *Kolmogorov-Smirnov* test. Skewed distributed variables including TG and UACR were logarithm transformed to normality for further analyses. To compare the differences in two groups, independent *t*-test was performed for the normally distributed variables, and χ^2 -test for categorical variables. To compare the differences in the three groups stratified according to LCN2 tertiles χ^2 -test was used followed by Bonferroni method. The correlation between LCN2 and TCNS score was performed by Spearman's correlation analysis. The Pearson's correlation analysis was used to examine the correlation between serum LCN2 and clinical parameters in DPN. Multivariate stepwise regression analysis was further performed to assess the association between serum LCN2 and clinical parameters after adjusting for potential confounders. Multiple logistic regression analysis was performed using the occurrence of DPN as a dependent variable. The confounders included the variables that had

been reported to be associated with DPN. The p value less than 0.05 was considered to be statistically significant.

Results

Characteristics of the participants

As shown in Table 1, there were no statistically significant differences in sex, age, and BMI between both DPN group and NDPN group (all $p > 0.05$). When compared with the NDPN group, patients in DPN group had longer diabetes durations ($p = 0.008$). For the other variables that might affect serum LCN2 levels including HbA1c, FBG, FCP, TC, TG, UACR, eGFR and blood pressure, no statistically significant differences were observed between the two groups (all $p > 0.05$).

Serum LCN2 levels and DPN

The serum LCN2 levels in DPN group were significant higher than those in NDPN group ($p = 0.001$; Fig.1). Stratification analysis according to the tertiles of serum LCN2 levels showed that with the LCN2 levels elevated, the number of patients with DPN increased, whereas the number of patients with NDPN decreased (trend $p = 0.003$; Fig.2). Participants with DPN in T3 or T2 group had higher serum LCN2 levels than those in T1 group (T2 vs T1, $p = 0.004$; T3 vs T1, $p = 0.002$).

Moreover, in all subjects, a Spearman's analysis showed that serum LCN2 level positively correlated to TCNS score, which reflects neuropathy severity ($r = 0.438$, $p = 0.000$; Fig 3).

The association of serum LCN2 with clinical parameters in DPN

To examine the association of clinical parameters with serum LCN2, Pearson's correlation analysis was performed in DPN group. Serum LCN2 was shown to be positively correlated to BMI ($r = 0.289$, $p = 0.003$), and TG ($r = 0.217$, $p = 0.024$), respectively, and negatively correlated to DBP ($r = -0.204$, $p = 0.035$; Table 2). No significant correlations were observed between serum LCN2 and age, diabetes duration, HbA1c, FBG, TC, UACR, and SBP (all $p > 0.05$).

To further determine independent clinical parameters affecting serum LCN2, multivariate stepwise regression analysis was performed using sex, age, BMI, HbA_{1c}, UACR, eGFR, TG and DBP as the independent variables. As shown in Table 3, sex ($\beta = 21.933$, $p = 0.022$), BMI ($\beta = 4.666$, $p = 0.002$), TG ($\beta = 37.390$, $p = 0.022$) and DBP ($\beta = -1.835$, $p = 0.000$) were shown to be independently associated with serum LCN2 levels in DPN.

Multiple logistic regression analysis

Multiple logistic regression analysis was performed using the occurrence of DPN as a dependent variable in all subjects. As shown in Table 4, the independent factors for DPN were LCN2 ($OR = 1.009$) and

diabetes duration ($OR = 1.058$), respectively. Other variables were excluded in this model including sex, age, BMI, HbA1c and UACR.

Discussion

The mechanisms underlying the pathogenesis of DPN have been attributed to impaired glucose metabolism and dyslipidemia [15]. The dysfunctions of metabolic pathways characterized by hyperglycemia and dyslipidemia cause an imbalance of the mitochondrial redox state and inflammatory processes, thereby leading to neuronal and glial cell injuries, which are accepted as crucial mechanisms of the pathogenesis of DPN [3]. Increasing evidence has demonstrated the roles of LCN2 in modulating the activities of glial cells, recruiting immune cells and amplifying neuroinflammation, consequently resulting in neuronal demyelination and apoptosis [4]. Besides, as an iron-binding protein, LCN2-mediated oxidative stress promotes neuronal injury [10, 16]. The LCN2 levels are known to be elevated in circulation in diabetes [5-9, 17]. Consistently, increased LCN2 expressions have also been described in the brain regions in both ob/ob mice and mice fed high-fat diets, the two classical models with metabolic disorders characterized by obesity, hyperglycemia, dyslipidemia, systemic inflammation, and neuroinflammation [10, 18, 19]. LCN2-related reactive oxygen species genes, which contribute to neurodegeneration, has been shown to be differentially expressed in the hippocampus of wild-type and ob/ob mice [10]. Noteworthy, the involvement of LCN2 has recently been implied in the neurological disorders from the studies in diabetic rodent models [10, 11]. Bhusal et al. found that the expression of LCN2 in the hippocampus was increased in STZ-induced diabetic mice models [11, 20]. Deletion of *Lcn2* gene ameliorated diabetes-induced reactive gliosis and expression of pro-inflammatory cytokines in the hippocampus, subsequently decreasing neuronal loss in the hippocampus. Moreover, diabetes-associated cognitive deficits were improved in LCN2 KO mice compared to wild-type mice in diabetic conditions.

Preclinical studies strongly suggest the presumable role of LCN2 in the pathogenesis of DPN in humans. This study herein firstly reported the association of LCN2 with DPN in humans. In this study, serum LCN2 levels were shown to be elevated in individuals with DPN (Fig 1), and the ratio of individuals with DPN to all diabetic individuals increased with the increase in the serum LCN2 levels (Fig 2). Furthermore, multivariable regression analysis showed that serum LCN2 level was independently correlated with the occurrence of DPN in individuals with T2D. Additionally, we found that with the increase in serum LCN2 levels, the TCNS scores for DPN were increased (Fig 3). Application of TCNS in clinical studies has confirmed its role in documenting and monitoring DPN [14, 21, 22]. A higher score indicates more severe disability. Given that LCN2 has been recently shown to be stable in the circulation, this study suggests LCN2 as a biomarker in the evaluation of DPN severity [23].

Coincident with our observations, a most recent study on DPN in mice model has revealed that LCN2 expressions in both DRG and sciatic nerve increase significantly in DPN mice [12]. Under the conditions of diabetes, LCN2 from satellite glial cells mediates macrophage infiltration into DRG, stimulates the release of inflammatory cytokines such as tumor necrosis factor- α , and enhances neuronal inflammatory response. PDK2, the key regulator of mitochondrial function, is typically up-regulated in diabetic

conditions and promotes glycolytic metabolism, along with increased DRG lactic acid production, consequently leading to neurotoxicity. LCN2 contributes to the pathogenesis of DPN via PDK2-lactic axis in DRG of diabetic mice. These findings above may provide mechanistic interpretations for our clinical observations.

A variety of factors including renal function, proteinuria, blood glucose, lipid and blood pressure have been extensively described to influence serum LCN2 levels in individuals with T2D [5, 17, 24, 25]. To minimize the potential differences in the comparison of serum LCN2 levels between DPN and NDPN groups, we carefully characterized and matched participants according to sex, age and BMI. Notably, no statistically significant differences were indicated in other parameters including HbA1c, FBG, FCP, TC, TG, UACR, eGFR and blood pressure. The only statistically significant difference in the two groups was longer diabetes duration for individuals in the DPN group. This could be explained by the fact that diabetes duration is the risk factor for DPN in T2D [26].

In this study, both BMI and TG were shown to be independent factors associated with serum LCN2 levels, consistently with previous clinical studies [24, 25, 27]. Dysregulation of LCN2 has been tied to obesity, metabolic syndrome and cardiovascular diseases, mainly through its ability to bind to lipids like fatty acids [28]. For example, LCN2 could bind to the fatty acid retinoic acid to mediate thermogenesis and lipid metabolism in adipose tissue [29]. Additionally, down-regulation of LCN2 was shown to attenuate the metabolism of arachidonic acid, impairing energy homeostasis in mice study [30]. This study indicated a negative association of LCN2 with DBP, whereas such association was shown to be positive in previous studies [25, 31]. This discrepancy may be attributed to the heterogeneity of the studied population across the distinct studies.

A recent study has indicated that a LCN2 monoclonal antibody significantly reduces cerebral infarction and neurological deficits after stroke, suggesting targeting LCN2 as a promising intervention for the therapy of neurological diseases. Another study showed that treatment with an anti-LCN2 antibody prevented LCN2-related neuroinflammation and neuronal death in vitro [20]. Therefore, the association of LCN2 with DPN described in this study suggests a presumable strategy for the treatment of DPN.

Limitations should be noted in this study. This was a cross-sectional study, which could not provide the causal relationship between increased serum LCN2 levels and the development and progression of DPN. Moreover, the sample size of this study was limited. Prospective studies with larger sample size are required to unravel the role of LCN2 in the pathogenesis of DPN.

Conclusions

This report is the first study on the association of serum LCN2 with DPN in T2D. LCN2 might be used to evaluate DPN severity. Moreover, LCN2 might serve a role in the pathogenesis of DPN. Novel strategies for the intervention of DPN would be beneficial from further studies on the relationship between LCN2 and DPN.

Abbreviations

BMI: Body mass index; BP: Blood pressure; Cr: Creatine; DPN: Diabetic peripheral neuropathy; DRG: Dorsal root ganglion; DBP: Diastolic blood pressure; eGFR: estimated glomerular filtration rate; FCP: Fasting C-peptide; FBG: Fasting blood glucose; HbA1c: Hemoglobin A1c; LCN2: Lipocalin-2; NDPN: Non-diabetic peripheral neuropathy; SBP: Systolic blood pressure; SD: Standard deviation; T2D: Type 2 diabetes; TCNS: Toronto Clinical Neuropathy Scoring; TC: Total cholesterol; TG: Triglycerides; UACR: Urine albumin creatinine ratio.

Declarations

Ethics approval and consent to participate

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author (W.W.) upon reasonable request.

Competing interests

The authors declare no competing interests.

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Authors' Contributions

WW conceived and designed this study, wrote this manuscript and participated in the data analysis. ZX, XRS and WZ contributed to data collection and analysis. YX and MYX contributed to laboratory test and helped in editing the manuscript. YSD contributed to the conception of this study, and edited the manuscript. SWX, JPW and ZZ contributed to the study design and critical revision of the manuscript for important intellectual content. All authors read and approved the final version of the manuscript.

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Tables

Table 1 General characteristics of the participants

	NDPN group (<i>n</i> = 100)	DPN group (<i>n</i> = 107)	<i>p</i>
Age(years)	54.45±11.89	57.18±13.04	0.118
Male, <i>n</i> (%)	61(61.00)	71(66.36)	0.381
Diabetes duration (years)	7.49±6.77	10.14±7.29	0.008**
BMI (kg/m ²)	24.34±3.67	24.17±3.16	0.713
FBG (mmol/L)	8.40±3.33	8.86±3.05	0.303
HbA _{1c} (%)	8.76±2.17	9.04±2.16	0.344
FCP (nmol/L)	0.35±0.22	0.32±0.19	0.426
TC (mmol/L)	4.20±0.84	4.31±0.95	0.366
TG ^Δ (mmol/L)	1.47(1.00-2.16)	1.53(1.00-2.43)	0.506
SBP(mmHg)	129.45±16.03	130.26±17.79	0.731
DBP(mmHg)	82.99±8.91	81.15±9.64	0.156
UACR ^Δ (mg/g)	14.85(9.80-26.25)	17.00(10.54-33.13)	0.285
eGFR (mL/min/1.73 m ²)	109.87±18.95	108.13±17.20	0.490

Continues variables are expressed as mean ± SD, median (25th–75th percentile) or as *n* (%). BMI, body mass index; DBP, diastolic blood pressure; DPN, diabetic peripheral neuropathy; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; FCP, Fasting C-peptide; HbA_{1c}, hemoglobin A1c; NDPN, non-diabetic peripheral neuropathy; SBP, systolic blood pressure; TG, triglyceride; TC, total cholesterol; UACR, urine albumin creatinine ratio. ^Δ Logarithm transformations were carried out before analysis. ** *p* < 0.01.

Table 2 Pearson's correlation analysis of variables with serum LCN2 in DPN

Variable	<i>r</i>	<i>p</i>
Age	-0.042	0.669
Diabetes duration	0.044	0.655
BMI	0.289	0.003
HbA _{1c}	-0.091	0.350
FBG	-0.023	0.812
SBP	-0.146	0.133
DBP	-0.204	0.035
TG	0.217	0.024
TC	0.013	0.896
UACR	0.013	0.893

BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; HbA_{1c}, glycosylated hemoglobin A1c; SBP, systolic blood pressure; TG, triglyceride; TC, total cholesterol; UACR, urine albumin creatinine ratio. Significant differences indicated in bold.

Table 3 Multivariate stepwise regression analysis of serum LCN2 in DPN

Independent variables	Dependent variable: serum LCN2			
	<i>β</i>	<i>SE</i>	<i>Standard β</i>	<i>p</i>
Sex	21.933	9.425	0.202	0.022
BMI	4.666	1.505	0.286	0.002
TG	37.390	16.116	0.215	0.022
DBP	-1.835	0.485	-0.344	0.000

BMI, body mass index; DBP, diastolic blood pressure; TG, triglyceride.

Table 4 Logistic regression analysis

Independent variables	Dependent variable: occurrence of DPN				
	β	SE	p	OR	95%CI
LCN2	0.009	0.003	0.002	1.009	1.003-1.015
Diabetes duration	0.057	0.026	0.030	1.058	1.006-1.116

LCN2, lipocalin-2.

Figures

Figure 1

Comparison of serum LCN2 levels between NDPN and DPN groups. DPN, diabetic peripheral neuropathy; NDPN, non-diabetic peripheral neuropathy; LCN2, lipocalin-2.

Figure 2

Prevalence of DPN and NDPN participants stratified according to the tertiles of serum LCN2 levels T1(< 108.93ng/ml), T2(109.13ng/ml-145.97ng/ml), T3(> 145.97ng/ml). DPN, diabetic peripheral neuropathy; NDPN, non-diabetic peripheral neuropathy. ** p < 0.01.

Figure 3

Positive correlation between TCNS score and serum LCN2 levels in DPN. DPN, diabetic peripheral neuropathy; LCN2, lipocalin-2; NDPN, non-diabetic peripheral neuropathy; TCNS, Toronto Clinical Neuropathy Scoring