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ORIGINAL ARTICLE

Clinical significance of serum interleukin-8 and soluble tumor necrosis factor-like weak inducer of apoptosis levels in patients with diabetic nephropathy

Shu-yan Liu, Jie Chen, Yong-feng Li*

Department of Endocrinology, The First Affiliated Hospital of Henan Polytechnic University (Jiaozuo Second People's Hospital), Jiaozuo, China

Keywords
Diabetic nephropathy, Interleukin-8, Soluble tumor necrosis factor-like weak inducer of apoptosis

***Correspondence**
Yong-feng Li
Tel.: +86-138-3917-1285
Fax: +86-391-262-5785
E-mail address:
liyongfeng017@126.com

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ABSTRACT

Aims/Introduction: Recent studies suggest that chronic inflammatory responses are important in the development of diabetic nephropathy (DN). Various inflammatory and angiogenesis molecules affect the pathogenesis and progression of DN. Inflammation damages the microcirculation and causes kidney damage. In the present study, we studied changes in interleukin-8 (IL-8) and soluble tumor necrosis factor-like weak inducer of apoptosis (sTWEAK) levels in patients with DN, and investigated the clinical significance of these two inflammatory factors.

Materials and Methods: Participants were categorized into healthy controls (n = 30) and patients with type 2 diabetes mellitus (n = 124). The type 2 diabetes mellitus group was further subdivided into the normoalbuminuria (n = 34), microalbuminuria (MAU; n = 46,) and proteinuria (MaAU; n = 44,) groups. Patients with DN were included in the MAU and MaAU groups. Total cholesterol, triglyceride, low-density lipoprotein cholesterol, glycosylated hemoglobin, fasting blood glucose, 2-h postprandial blood glucose, blood urea nitrogen, serum creatinine, 24-h urine microalbumin, IL-8 and sTWEAK levels were measured. Logistic regression was used to analyze the factors associated with proteinuria.

Results: In the healthy controls, normoalbuminuria, MAU and MaAU groups, we found that IL-8 levels increased, whereas sTWEAK levels decreased ($P < 0.05$). IL-8 might be an independent risk factor and serum sTWEAK a protective factor for MAU and MaAU. Serum levels of sTWEAK, IL-8 and microalbumin were significantly correlated in the MAU and MaAU groups.

Conclusions: Serum IL-8 and sTWEAK levels might be markers that can be used for an early diagnosis of DN.

INTRODUCTION

Diabetic nephropathy (DN) is a severe microvascular complication of diabetes, which has become a common cause of chronic renal failure and a major cause of death due to diabetes. Its pathogenesis has not been completely explained, but is known to be related to multiple factors, including hemodynamic changes, heredity factors, inflammatory mediators, impaired glucose metabolism and cytokines[1].

Recently, the role of the inflammatory response in the pathogenesis of DN has been emphasized. The interaction of numerous inflammatory cells, adhesion factors, chemokines and growth regulatory factors promote an inflammatory cascade that ultimately leads to the development of DN. Interleukin-8 (IL-8) is a chemokine with extensive sources and diversified biological functions[2], which can induce leukocyte (e.g., neutrophil) chemotaxis and activation[3]. IL-8 is also involved in the development of complications, such as diabetic retinopathy, DN, cardiovascular disease (CVD) and infections. Serum IL-8 levels were found to change before a significant decline in renal function among patients with kidney injuries[4]. Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is a glycoprotein of the tumor necrosis factor

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superfamily that circulates in plasma in the soluble form (sTWEAK)[5,6]. TWEAK shows different degrees of expression during organ (including the heart, skeletal muscles and the kidney) damage[4,7]. It regulates cell proliferation, death, differentiation and inflammatory responses. Recently, sTWEAK has been found to promote the proliferation of renal parenchymal cells in patients with DN. Furthermore, it causes the fibrosis of renal tubules and increases the exudation of inflammatory cells in the kidneys[8], which can gradually decrease sTWEAK levels with a decline in renal function[9].

The present study compared the serum levels of IL-8 and sTWEAK in healthy individuals and type 2 diabetes mellitus patients with normoalbuminuria (NMAU), microalbuminuria (MAU) and proteinuria (MaAU; of these, patients with MAU and MaAU were considered as having DN), and probed the clinical significance of IL-8 and sTWEAK levels for diagnosing and assessing the progression of DN.

METHODS

Study participants

The present study was approved by our hospital's ethics committee (institutional review board number: 20150013), and was carried out in accordance with the Declaration of Helsinki in 1995 (as revised in Fortaleza, Brazil, October 2013). All patients granted written informed consent to participate in the study.

Healthy adults (n = 30) who underwent a physical examination at the outpatient department of The First Affiliated Hospital of Henan Polytechnic University (Jiaozuo Second People's Hospital), Jiaozuo, China, from October 2015 to May 2016 were enrolled as the NC group. Patients with type 2 diabetes mellitus were further subdivided into those with NMAU (n = 34), MAU (n = 46) and MaAU (n = 44). Patients with NMAU, MAU and MaAU were defined as having a 24-h urine microalbumin (MAL) level of <30 mg/24 h, 30–300 mg/24 h and >300 mg/24 h, respectively. We excluded patients with infectious diseases, acute infections, heart failure, hyperthyroidism, tumors, immune system diseases, hematological system disorders, and moderate-to-severe hepatic and renal insufficiency. The following data were recorded for all participants: sex, age, disease course, disease history, ankle brachial index (ABI) and risk factors for DN (hypertension, hyperlipidemia and obesity).

Data collection

Fasting venous blood samples (4 mL) were collected from the arms of all patients on the morning of the second day after admission. Enzyme-linked immunosorbent assays were utilized to determine the serum levels of IL-8 (for optimal IL-8 ELISA® kit; Dakota Biotechnology Co., Ltd., Wuhan, China) and sTWEAK (soluble tumor necrosis factor micro apoptosis inducers enzyme-linked immunosorbent assay kit; Huamei Biological Engineering Co., Ltd., Wuhan, China). We also measured total cholesterol, triglyceride (TG), low-density lipoprotein cholesterol, glycosylated hemoglobin (HbA1c), fasting blood glucose, fasting insulin, 2-h postprandial blood glucose, blood urea nitrogen, serum creatinine (Scr) and MAL levels. The insulin resistance index (homeostasis model assessment for insulin resistance = FPG × fasting insulin/22.5) was assessed using the homeostasis model assessment steady-state model. Other relevant auxiliary examinations, such as electrocardiography and liver/kidney color Doppler ultrasonography, were also carried out.

Statistical analysis

SPSS 19.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. The χ^2-test was utilized to compare quantitative data between groups. Different statistical methods were used in accordance with the data distribution patterns. For data with normal distribution, the indicators in each group were expressed as mean ± standard deviation. For data with non-normal distribution, the indicators in each group were expressed as the median (minimum and maximum values). The Kruskal–Wallis test and rank-sum test were utilized to compare data among the four groups, the Mann–Whitney U-test was applied for intragroup pairwise comparisons in the case of significant intergroup differences, and Spearman's correlation test was used to analyze the correlations among all indicators. In patients with type 2 diabetes mellitus, a binary logistic regression model was used to investigate the factors associated with MAU and MaAU. $P < 0.05$ was considered statistically significant.

RESULTS

We found no statistically significant differences for sex, age, body mass index, low-density lipoprotein cholesterol and blood urea nitrogen levels between the NC, NMAU, MAU and MaAU groups ($P > 0.05$; Table 1). In contrast, significant differences between the NC and the other three groups were found for the disease course, hypertension, non-alcoholic fatty liver disease, ABI, homeostasis model assessment for insulin resistance, HbA1c, fasting blood glucose, postprandial blood glucose, total cholesterol, TG, Scr, MAL, IL-8 and sTWEAK levels ($P < 0.05$). When comparing the NMAU, MAU and MaAU groups, we detected no statistically significant differences in hypertension, non-alcoholic fatty liver disease, homeostasis model assessment for insulin resistance, fasting blood glucose, postprandial blood glucose, total cholesterol and TG ($P > 0.05$). From healthy individuals, to those with diabetes and DN, a decrease in the ABI was noted ($P < 0.05$). Similarly, the concentrations of IL-8 and MAL were increased ($P < 0.05$), whereas the concentration of sTWEAK was decreased ($P < 0.05$; Figure 1).

The factors associated with MAU in patients with type 2 diabetes mellitus were investigated using logistic regression (Table 2). Age, disease course, HbA1c, TG and Scr levels were not significantly associated ($P > 0.05$), whereas ABI, IL-8 and sTWEAK were significantly associated ($P < 0.05$). ABI and sTWEAK were protective factors (relative risk [RR] <1), and IL-8 was a risk factor (RR > 1).

Table 1 | Comparison between the four groups

Group	NC (n = 30)	NMAU (n = 34)	MAU (n = 46)	MaAU (n = 44)	t-test/χ^2	P
Sex (male/female)	15/15	15/19	23/23	18/26	6.996	0.072
Age (years)	51.0 ± 11.0	52.0 ± 8.0	55.0 ± 10.0	57.0 ± 12.0	2.591	0.055
Disease course (years)	-	6.00 ± 5.40†	8.00 ± 5.50†,‡	9.0 ± 5.20†,‡,§	6.767	0.016
Hypertension, n (%)	0 (0.0)	16 (47.1)†	25 (54.3)†	26 (59.1)†	29.83	<0.001
NAFLD, n (%)	0 (0.0)	8 (23.5)†	9 (19.6)†	10 (22.7)†	8.18	0.043
BMI (kg/m^2)	23.35 ± 3.88	25.05 ± 3.84	24.10 ± 3.16	23.15 ± 3.37	2.166	0.094
ABI	1.22 ± 0.67	1.17 ± 0.47	0.86 ± 0.39†,‡	0.85 ± 0.47†,‡	5.898	<0.001
HbA1c (%)	5.20 (4.70, 6.10)	10.3 (6.60, 15.0)†	9.30 (7.00, 15.7)†,‡	10.2 (8.00, 13.2)†,§	79.676	<0.001
HOMA-IR	1.61 ± 0.65	2.05 ± 0.71†	2.13 ± 0.67†	2.22 ± 0.59†	5.735	<0.001
FBG (mmol/L)	4.60 (4.20, 5.40)	9.00 (3.80, 18.9)†	9.40 (4.20, 20.5)†	9.61 (6.10, 18.3)†	59.477	<0.001
PBG (mmol/L)	6.20 (5.30, 7.20)	19.1 (10.6, 30.4)†	17.0 (12.8, 26.3)†	18.8 (12.3, 27.8)†	76.438	<0.001
CHOL (mmol/L)	4.20 (3.63, 5.63)	5.08 (3.00, 6.48)†	4.75 (2.70, 6.10)†	5.05 (3.34, 6.29)	8.829	0.032
TG (mmol/L)	1.20 (0.73, 1.62)	1.74 (0.38, 3.52)†	1.76 (0.62, 6.54)	1.77 (0.41, 3.29)†	23.573	<0.001
LDL (mmol/L)	2.47 (2.02, 3.56)	2.46 (1.35, 3.19)	2.55 (1.33, 3.77)	2.84 (1.14, 3.78)	2.856	0.414
BUN (mmol/L)	4.67 ± 0.41	4.63 ± 1.25	4.49 ± 1.25	4.45 ± 1.57	0.399	0.754
Scr (μmol/L)	55.37 ± 6.57	65.59 ± 12.89†	62.57 ± 11.44†	72.36 ± 20.08†,‡,§	9.068	<0.001
MAL (mg/24 h)	9.00 ± 4.33	25.29 ± 5.62†	147.02 ± 53.53†,‡	855.34 ± 367.07†,‡,§	166.681	<0.001
sTWEAK (ng/mL)	241.79 ± 4.43	182.86 ± 12.85†	165.41 ± 10.76†,‡	112.31 ± 15.53†,‡,§	711.933	<0.001
IL-8 (ng/mL)	16.99 ± 0.55	26.16 ± 1.63†	33.96 ± 4.09†,‡	62.94 ± 17.74†,‡,§	160.038	<0.001

For data with non-normal distribution, the indicators in each group are expressed as the median (minimum and maximum values). For data with normal distribution, the indicators in each group are expressed as mean ± standard deviation. †Compared with the control group (NC) $P < 0.05$. ‡Compared with the normoalbuminuria group (NMAU) $P < 0.05$. §Compared with the microalbuminuria group (MAU) $P < 0.05$. ABI, ankle brachial index; BMI, body mass index; BUN, blood urea nitrogen; CHOL, total cholesterol; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment for insulin resistance; IL-8, interleukin 8; LDL, low-density lipoprotein cholesterol; MaAU, proteinuria group; MAL, 24-h urine microalbumin; NAFLD, non-alcoholic fatty liver disease; NC, control group; PBG, postprandial blood glucose; Scr, serum creatinine; sTWEAK, soluble tumor necrosis factor-like weak inducer of apoptosis; TG, triglyceride.

We then investigated the factors associated with MaAU (MAL >300 mg/24 h) in patients with type 2 diabetes mellitus (Table 3). HbA1c, TG and ABI were not significantly associated ($P > 0.05$). Age, disease course, Scr, IL-8 and sTWEAK levels were all significantly associated with MaAU ($P < 0.05$); sTWEAK was a protective factor (RR <1), whereas age, disease course, IL-8 and Scr were risk factors (RR > 1).

Correlation analysis showed that in the MAU group, sTWEAK demonstrated a strong negative linear correlation with IL-8 ($r = -0.760$) and MAL ($r = -0.945$); IL-8 showed a strong positive linear correlation with MAL ($r = 0.806$). In the MaAU group, TWEAK showed a significant negative linear correlation with IL-8 ($r = -0.651$); sTWEAK showed a strong negative linear correlation with MAL ($r = -0.820$) and IL-8 showed a strong positive linear correlation with MAL ($r = 0.822$).

DISCUSSION

Diabetic nephropathy is a common and severe microangiopathy that can occur in patients with diabetes[10]; its major pathological changes include glomerular hypertrophy, mesangial matrix expansion and obvious thickening of the basilar membrane, resulting in nodular or diffuse glomerulosclerosis[11]. Most cases of DN show no obvious clinical symptoms early in the disease. Although renal puncture is an important criterion for the diagnosis of DN, patients are reluctant to undergo this procedure; therefore, it cannot be routinely used for patients with type 2 diabetes mellitus. The clinical test with the highest sensitivity and the best predictive value for an early diagnosis of DN is the measurement of albuminuria in urinary tests[12]; measuring MAL is considered the 'gold standard'[13]. Although, clinically, MAL is used for an early diagnosis of DN, it is affected by a variety of factors, such as systemic or urinary tract infections, bleeding, vigorous exercise, or kidney damage related to drugs[14]. Therefore, MAL alone cannot provide a timely and accurately diagnosis DN. This necessitates the development of more accurate and specific diagnostic markers for DN.

Pickup *et al.*[15] first reported in 1997 that inflammatory cytokines and oxidative stress played important roles in the pathogenesis and progression of DN. DN is associated with high glucose levels and hemodynamic disturbance, which can lead to intrinsic renal cell injury, secretion of pre-inflammatory mediators, migration of white blood cells to the injured site, secretion of additional chemokines and stimulation of renal mesangial cells to secrete collagen fiber, laminin and fibronectin, thus leading to glomerulosclerosis. Renal tubular epithelial cells secrete immune mediators and cytokines, which allow aggregation of the renal interstitial mononuclear cells/macrophages, leading to renal interstitial inflammatory fibrosis[16]. Macrophages, adipocytes and endothelial cell-derived

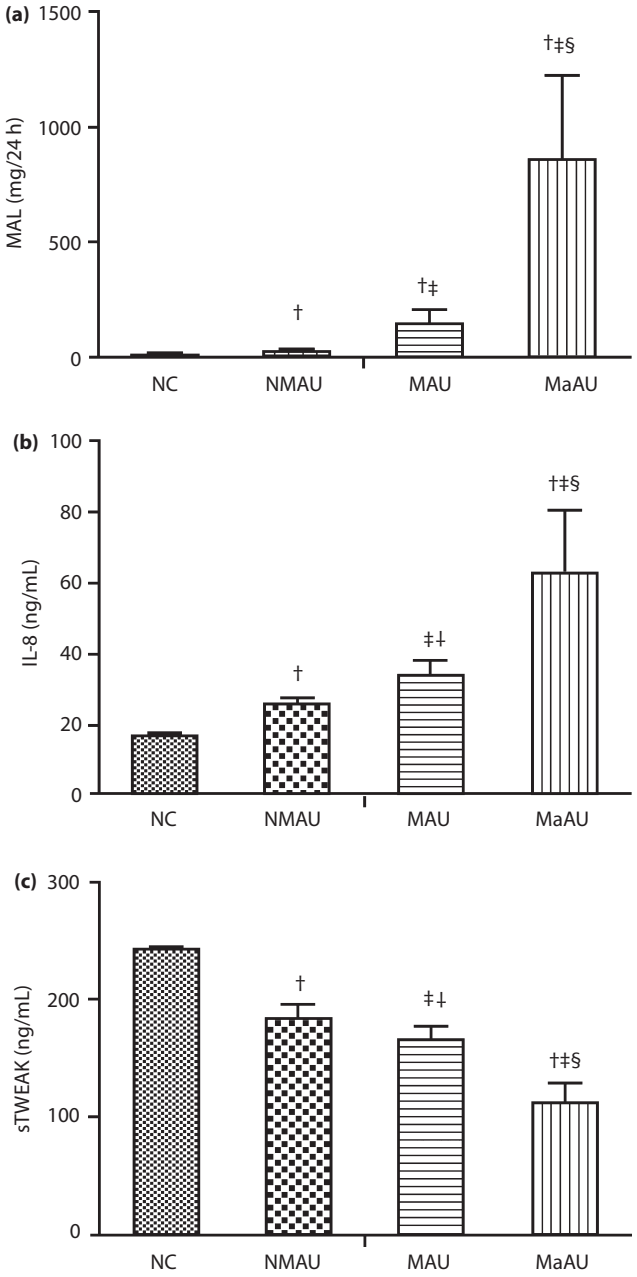

Figure 1 | Comparison of 24-h urine microalbumin (MAL), serum interleukin 8 (IL-8) and soluble tumor necrosis factor-like weak inducer of apoptosis (sTWEAK) levels between the control (NC), normoalbuminuria (NMAU), microalbuminuria (MAU) and proteinuria (MaAU) groups. (a) Comparison of MAL levels between the NC, NMAU, MAU and MaAU groups. (b) Comparison of serum IL-8 levels between the NC, NMAU, MAU and MaAU groups. (c) Comparison of serum sTWEAK levels between the NC, NMAU, MAU and MaAU groups. †Compared with NC: $P < 0.05$. ‡Compared with NMAU: $P < 0.05$. §Compared with MAU: $P < 0.05$.

inflammatory cytokines can all participate in the pathogenesis and progression of DN[17]. Some researchers have hypothesized that a long-term micro-inflammatory state and immune factors might related to the pathogenesis of DN[16], whereas others have proposed that inflammation is the key factor in the development of DN[18].

IL-8, which can be expressed in vascular endothelial cells, fibroblasts, monocytes and epithelial cells, can mediate a series of cascade reactions stimulating the migration of white blood cells, the formation of neutrophil peroxide, as well as lysosome release, activation and chemotaxis. Thus, IL-8 is thought to be involved in the pathogenesis and progression of complications, such as diabetic retinopathy, DN, CVD and infections. IL-8 was found to stimulate neutrophils[19], leading to a significant promotion of human renal mesangial cell proliferation. Furthermore, IL-8 can induce oxidative stress[20], changes in vascular permeability, increased endothelial coagulation ability and reduced diastolic function, resulting in abnormal blood flow regulation that contributes to the development of DN.

Tumor necrosis factor-like weak inducer of apoptosis is a tumor necrosis factor superfamily cytokine, consisting of 249 amino acids, that has a molecular weight of 27 kD. As for most tumor necrosis factor members, the TWEAK protein is present as a membrane-bound (mTWEAK) and soluble (sTWEAK) form after furin protease proteolytic cleavage. Both forms are biologically active and can bind to their only signal transduction receptor, fibroblast growth factor-induced 14 (Fn14)[21]. *In vitro* studies have shown that mTWEAK, which can act as a collocation signal molecule, and sTWEAK through Fn14[22,23], lead to qualitatively different activity states. A combination of the two factors is involved in cell proliferation, apoptosis, cytokine production, angiogenesis and mediated immune injury during organ damage during the pathophysiology of immune system-mediated tissue damage and disease[24].

Some authors have observed a low level of sTWEAK in patients with atherosclerosis, and a negative correlation between sTWEAK levels and endometrial/medial thickness in asymptomatic patients; furthermore, sTWEAK levels were identified as a CVD indicator[25]. Therefore, sTWEAK can be considered as a potential biomarker of CVD[26]. Urbonaviciene *et al.*[27] found that, in patients with severe ischemic intermittent claudication, sTWEAK levels were significantly reduced. However, they also found that sTWEAK levels and atherosclerosis in the lower extremities showed a positive correlation. According to a recent prospective case–control study[28], low serum sTWEAK levels can be used to predict type 2 diabetes mellitus. In that study, a lower sTWEAK serum level was found in the type 2 diabetes mellitus group when compared with the NC group, suggesting a link between sTWEAK concentration and type 2 diabetes mellitus.[28] Although low sTWEAK levels were shown to have a protective effect on vascular diseases, the mechanism of this association has not yet been fully elucidated, and several hypotheses have been proposed. One hypothesis for the decrease in sTWEAK levels is the uptake of sTWEAK by Fn14 receptors[29]. Fn14 is expressed in renal innate cells, including mesangial cells, podocytes and endothelial cells. Fn14 expression is significantly increased during acute and chronic

Table 2 | Binary logistic regression analysis of the factors associated with microalbumin in patients with type 2 diabetes mellitus

Variable	Regression coefficient (B)	Significance level (P)	Relative risk (RR)	95% CI of the OR	
				Lower limit	Upper limit
Age (years)	–0.008	0.663	0.992	0.959	1.027
Disease course (years)	0.075	0.082	1.078	0.990	1.172
HbA1c (%)	–0.187	0.052	0.830	0.689	0.999
TG (mmol/L)	0.138	0.453	1.148	0.801	1.645
ABI	–0.607	0.019	1.267	1.102	1.533
Scr (μmol/L)	0.007	0.579	1.007	0.982	1.033
IL-8 (pg/mL)	0.721	0.002	2.056	1.310	3.225
sTWEAK (pg/mL)	–0.167	<0.001	0.846	0.783	0.914

Dependent variable, the presence/absence of microalbumin. ABI, ankle brachial index; CI, confidence interval; HbA1c, glycosylated hemoglobin; IL-8, interleukin 8; OR, odds ratio; Scr, serum creatinine; sTWEAK, soluble tumor necrosis factor-like weak inducer of apoptosis.

Table 3 | Binary logistic regression analysis of the factors associated with proteinuria in patients with type 2 diabetes mellitus

Variable	Regression coefficient (B)	Significance level (P)	Relative risk (RR)	95% CI of the OR	
				Lower limit	Upper limit
Age (years)	0.069	0.003	1.071	1.023	1.122
Disease course (years)	0.197	<0.001	1.218	1.100	1.349
HbA1c (%)	0.213	0.064	1.238	0.988	1.552
TG (mmol/L)	0.148	0.383	1.160	0.831	1.618
ABI	–0.983	0.216	1.025	0.895	1.079
Scr (μmol/L)	0.038	0.008	1.039	1.010	1.069
IL-8 (pg/mL)	6.486	0.036	1.410	1.244	1.782
sTWEAK (pg/mL)	5.757	0.043	0.883	0.676	0.972

Proteinuria, microalbumin >300 mg/24 h. Dependent variable, microalbumin >300 mg/24 h = 1 vs microalbumin ≤300 mg/24 h = 0. ABI, ankle brachial index; CI, confidence interval; HbA1c, glycosylated hemoglobin; IL-8, interleukin 8; OR, odds ratio; Scr, serum creatinine; sTWEAK, soluble tumor necrosis factor-like weak inducer of apoptosis.

inflammation kidney damage; an increase in the sTWEAK ligand can lead to a decrease in peripheral serum sTWEAK levels[30]. Recently, sTWEAK has been found to promote the proliferation of renal parenchymal cells, the fibrosis of renal tubules and an increase in the exudation of inflammatory cells in patients with DN[8]; sTWEAK levels might gradually decrease with a decline in renal function[9]. These findings show the value of sTWEAK in diagnosing DN.

The results of the present study found that, with DN progression, serum IL-8 levels gradually increased and sTWEAK levels gradually decreased. Logistic regression analysis showed that serum IL-8 levels were an independent risk factor for type 2 diabetes mellitus patients with MAU and MaAU. Serum IL-8 levels were an independent risk factor for MAU and MaAU, whereas serum sTWEAK levels were an independent protective factor for MAU and MaAU in patients with type 2 diabetes mellitus. The concentration of IL-8 increased with an increase in renal damage. Furthermore, serum IL-8 concentration was highly correlated with MAL in the MAU and MaAU groups, and sTWEAK concentration showed a negative correlation with IL-8 and MAL in patients with DN. These findings suggest that serum IL-8 and sTWEAK have important roles in the development of DN. We suggest that IL-8 and sTWEAK levels could be used for an early assessment of DN damage and as indicators of type 2 diabetes mellitus progression.

We also found that ABI was a protective factor for DN; however, we detected no difference in ABI between the NC and the NMAU groups or between the MAU and MaAU groups. Our data only showed that the ABI level in the MAU and MaAU groups were lower than those in the NC and MAU groups. This relationship will require further analysis in future studies.

In conclusion, serum IL-8 and sTWEAK levels might be sensitive markers for diagnosing DN, as they play important roles in the occurrence and progression of DN. The limitations of the present study include its small sample size and that all participants were residents of Henan Province, China. As this was a cross-sectional study, no causal relationship between IL-8/sTWEAK levels and DN could be established. However, as we found an RR < 1 for sTWEAK and >1 for IL-8, we hypothesize that sTWEAK might be a protective factor for DN, whereas IL-8 might be a risk factor for DN. Further prospective cohort studies with larger sample sizes are required to explore the causal relationship between these two indicators and DN.

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DISCLOSURE

The authors declare no conflict of interest.

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