JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
ORIGINAL ARTICLE

Elevated expression levels of serum insulin-like growth factor-1, tumor necrosis factor-α and vascular endothelial growth factor 165 might exacerbate type 2 diabetic nephropathy

Xiang Li[1†], Ting-Ting Wu[2†], Juan Chen[3*], Wen Qiu[1]

[1]Department of Clinical Laboratory, Huai'an Hospital Affiliated of Xuzhou Medical University, [2]Department of Clinical Laboratory, The Fourth People's Hospital of Huai'an, and [3]Department of Endocrinology, Huai'an First People's Hospital, Nanjing Medical University, Huai'an, China

Keywords
Insulin-like growth factor-1, Type 2 diabetic nephropathy, Vascular endothelial growth factor 165

***Correspondence**
Juan Chen
Tel.: +86-0517-8492-7745
Fax: +86-0517-8492-7745
E-mail address: chenjuan20160418@163.com

J Diabetes Investig 2017; 8: 108–114

doi: 10.1111/jdi.12542

ABSTRACT

Aims/Introduction: The present study aimed to determine the associations between expressions of insulin-like growth factor-1 (IGF-1), tumor necrosis factor-α (TNF-α) and vascular endothelial growth factor 165 ($VEGF_{165}$) in serum, and occurrence and development of type 2 diabetic nephropathy (DN).

Materials and Methods: A total of 108 patients diagnosed as DN were randomly selected, including 50 patients in the microalbuminuria group, 44 patients in the macroalbuminuria group and 14 patients in the renal insufficiency group. Meanwhile, 97 healthy people were collected as a normal control group. Urinary albumin (UALB) and urine creatinine (Cr) of all participants were measured for 24 h, with their ratio (UALB/Cr) being calculated. Enzyme-linked immunosorbent assay was used to detect the serum concentrations of IGF-1, TNF-α and $VEGF_{165}$.

Results: The expressions of serum IGF-1, TNF-α and $VEGF_{165}$ in the type 2 DN patients were significantly higher than those in the control group (all $P < 0.05$). The expressions of serum IGF-1, TNF-α and $VEGF_{165}$ in the type 2 DN patients were positively correlated with UALB/Cr (all $P < 0.05$). As type 2 DN worsened, the expressions of serum IGF-1, TNF-α and $VEGF_{165}$ increased, and type 2 DN severity had positive correlations with serum IGF-1, TNF-α and $VEGF_{165}$ concentrations (all $P < 0.05$). There was a positive association between IGF-1 and TNF-α, IGF-1 and $VEGF_{165}$, and TNF-α and $VEGF_{165}$ (all $P < 0.05$). Logistic regression analysis showed that IGF-1 and $VEGF_{165}$ were associated with the progression of DN (both $P < 0.05$).

Conclusions: Elevated expression levels of serum IGF-1, TNF-α and $VEGF_{165}$ might exacerbate type 2 DN.

INTRODUCTION

As type 2 diabetes progresses, various complications will emerge in patients with this disease, including nephropathy, atherosclerosis, peripheral neuropathy and retinopathy[1]. Among these complications, diabetic nephropathy (DN) is a common microvascular complication, which is one of the leading causes of diabetes-related death[2]. The main pathological changes of DN include glomerular hypertrophy, proteinuria, thickening of glomerular basement membrane, as well as expansion and proliferation of the mesangial matrix, eventually leading to fibrosis and sclerosis of the glomerulus[3]. Proteinuria is the main form of DN, and 39% of diabetes patients worldwide suffer from microalbuminuria[4]. It is believed that the pathogenesis of DN is mainly related to glycometabolic disorder, abnormal hemodynamics, oxidative stress, cytokine and genetic factors[5]. Type 2 diabetes is a type of low-grade chronic inflammation, accompanied by increased concentration of inflammatory factors[6]. With mutual effects and constraints, cytokines constitute a complex cytokine network, which is closely related with the occurrence of DN[7].

[†]These authors are regarded as co-first author.
Received 2 March 2016; revised 11 May 2016; accepted 22 May 2016

Insulin-like growth factor-1 (IGF-1) is a kind of multifunctional cell proliferation regulatory factor that plays a pivotal role in the growth and development of organisms, glycometabolism, and lipid metabolism[8]. Levin-Iaina *et al.*[9] found that non-obese diabetic rats suffered from early kidney hypertrophy, with IGF-1 accumulation in the kidneys, which suggested that IGF-1 was acting on the development of DN. Tumor necrosis factor-α (TNF-α) is a polypeptide hormone with double biological effects. It can promote synthesis and release of inflammatory cytokines, and provide an immune protective effect on the one hand and participate in immunopathological injuries of organisms on the other hand[10]. Vascular endothelial growth factor (VEGF), also called vascular permeability factor, can promote vascularization and vascular permeability, and sustain the normal operation of microcirculatory system functions[11]. VEGF is expressed as several alternately spliced isoforms, such as $VEGF_{165}$ and $VEGF_{121}$. VEGF165 is predominant, with optimal bioavailability, and plays an instrumental role in VEGF biological potency, whereas $VEGF_{121}$ is less potent but freely diffusible[12]. A recent study found that, under diabetic state, the expression of VEGF might be upregulated by an increase of cytokines and glycation end-products, activation of protein kinase C, and high tension in the glomerulus[13]. At present, the action mechanism of different cytokines to DN has not yet been definitely concluded[5]. The present study carried out correlation analyses between the expression levels of serum IGF-1, TNF-α and $VEGF_{165}$, and type 2 DN to provide a foundation for the diagnosis and treatment of type 2 DN.

MATERIALS AND METHODS

Participants

A total of 108 patients diagnosed as DN at the Huai'an First People's Hospital, Nanjing Medical University, Huai'an, China, between September 2013 and September 2015 were randomly selected as a case group, and were allocated into the microalbuminuria group (50 patients; 30 mg/g ≤ urine protein/urine creatinine ≤ 300 mg/g), macroalbuminuria group (44 patients, 300 mg/g < urine protein/urine creatinine) and the kidney failure group (14 patients) according to the DN staging method of Mogensen[14]. Meanwhile, 97 healthy examinees whose age and sex corresponded to the case group were collected as a healthy control group. The pathological diagnosis of DN conforms to diagnostic criteria in a study reported by Tervaert *et al.*[15] Kidney injury was considered to be caused by diabetes mellitus if the majority of diabetes mellitus patients met any of the following criteria: (i) more than 3 months of persistent macroalbuminuria; and (ii) diabetic retinopathy accompanied by microalbuminuria, over 3 months of persistent glomerular filtration rate <60 mL/(min·1.73 m^2) or chronic kidney disease. The exclusion criteria of DN groups were as follows: (i) patients who had tumors, tuberculosis, cardiovascular or cerebrovascular diseases, other endocrine metabolic diseases, or kidney diseases including mesangial proliferative nephropathy, membranous nephropathy, nephrotic syndrome or hypertensive renal disease; (ii) pregnant women or breast-feeding women; and (iii) patients suffering from stressful situations, such as surgery and trauma. The study was carried out with the approval of the ethics committee of the Huai'an First People's Hospital, Nanjing Medical University. Informed consent was obtained from all participants.

Experimental method

All participants were fasted overnight, and approximately 3 mL of elbow venous blood was collected from each participant in the morning and stored in ice. Urinary albumin (UALB) and urine creatinine (Cr) of all participants were measured for 24 h, with their ratio (UALB/Cr) being calculated. The elbow venous blood was centrifuged for 10 min at 1,200 *g* at 4°C. Supernatant was separated and packed, and then immediately stored at −80°C for further detection. Enzyme-linked immunosorbent assay (Wuhan Boster Biological Engineering Co., Ltd., Wuhan, China) was used to detect the concentrations of IGF-1, TNF-α and $VEGF_{165}$ in serum. Absorbance (A) of each well [optical density (OD)] value was measured by full wavelength spectrophotometer, with a detection wavelength of 450 nm and calibration wavelength of 570 nm. A standard curve was drawn using the A (OD) value as the ordinate, and standard substance concentration as abscissa to calculate concentrations of IGF-1, TNF-α and $VEGF_{165}$ in serum.

Clinical observation indexes

Patients' basic information was recorded at admission, including sex, age and duration of diabetes. All patients received examinations of total cholesterol, triglyceride, blood urea nitrogen (BUN), serum creatinine, blood β2-microglobulin (MG), urine β2-MG, UALB/Cr, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein-cholesterol (VLDL-C) by an automatic biochemical analyzer.

Statistical analysis

Data were analyzed by applying SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Categorical data were presented as percentage or rate, with comparisons carried out by χ^2-test. Continuous data that conform to a normal distribution were shown as mean ± standard deviation, with least significant difference *t*-test to analyze the differences between two groups and one-way analysis of variance (ANOVA) among multiple groups. Pearson's correlation analysis was used for the correlation between the levels of IGF-1, TNF-α and $VEGF_{165}$, and UALB/Cr, urine protein/urine creatinine and cytokines. Spearman's correlation analysis was used for the correlation between DN severity and IGF-1, TNF-α and $VEGF_{165}$. $P < 0.05$ shows significant difference.

RESULTS

Comparison of observation indexes

There was no statistical significance in sex, age, and course of disease of patients between the case and

Table 1 | Clinical data comparison between the control group and the case group

Groups	Case group (n = 108)	Control group (n = 97)	P
Sex			
Female	57	42	0.208
Male	51	55	
Age (years)	54.35 ± 7.96	53.21 ± 7.45	0.293
Course of disease (years)	5.87 ± 2.25	–	–
TC (mmol/L)	4.73 ± 1.31	4.32 ± 1.12	0.018
TG (mmol/L)	1.81 ± 0.37	1.59 ± 0.39	<0.001
HDL-C (mmol/L)	1.54 ± 0.29	1.88 ± 0.19	<0.001
LDL-C (mmol/L)	2.67 ± 0.88	2.23 ± 0.61	<0.001
VLDL-C (mmol/L)	0.95 ± 0.40	0.74 ± 0.31	<0.001
BUN (mmol/L)	6.83 ± 0.89	6.31 ± 0.71	<0.001
Scr (μmol/L)	98.84 ± 17.19	90.65 ± 15.09	<0.001
Blood β_2-MG (μg/L)	2,402.54 ± 509.95	2,179.97 ± 529.11	0.003
Urine β_2-MG (μg/L)	398.15 ± 95.12	349.75 ± 91.24	<0.001
UALB/Cr (mg/g)	81.68 ± 34.92	17.22 ± 8.72	<0.001

Blood β2-MG, β2-microglobulin of serum; BUN, blood urea nitrogen; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; Scr, serum creatinine; TC, total cholesterol; TG, triglyceride; UALB/Cr, urinary albumin/urine creatinine; urine β2-MG, β2-microglobulin of urine; VDL-C: very low-density lipoprotein-cholesterol.

control group (all $P > 0.05$). The case group had higher total cholesterol, triglyceride, LDL-C, VLDL-C, BUN, serum creatinine (Scr), blood β2-MG, urine β2-MG and UALB/Cr, and lower HDL-C compared with the control group (all $P < 0.05$; Table 1).

Expression levels of serum IGF-1, TNF-α and $VEGF_{165}$

The expressions of serum IGF-1, TNF-α and $VEGF_{165}$ in the case group were 254.53 ± 76.01 ng/mL, 17.38 ± 5.21 pg/mL and 55.41 ± 18.96 pg/mL, respectively, which were all higher than those in the control group (192.15 ± 33.14 ng/mL, 7.05 ± 1.57 pg/mL and 25.89 ± 6.42 pg/mL, all $P < 0.05$; Figure 1).

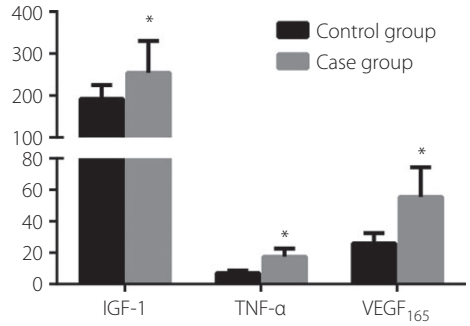

Figure 1 | Comparison of serum insulin-like growth factor-1 (IGF-1), tumor necrosis factor-α (TNF-α) and vascular endothelial growth factor 165 ($VEGF_{165}$) expression levels between the control group and case group (*$P < 0.05$ when compared with the control group).

Correlation analyses between serum levels of three proteins and DN

Pearson's correlation analyses showed that UALB/Cr was correlated with serum IGF-1 ($r = 0.721$), TNF-α ($r = 0.696$) and $VEGF_{165}$ ($r = 0.650$; all $P < 0.01$) in patients with type 2 DN (Figure 2).

Serum IGF-1, TNF-α and $VEGF_{165}$ levels, and severity of DN

It was found that DN worsened, and expression levels of IGF-1, TNF-α and $VEGF_{165}$ in serum also elevated as proteinuria of DN patients increased (Table 2). There were remarkable differences among groups (all $P < 0.05$). IGF-1 showed the largest increment from 193.11 ± 37.61 ng/mL to 355.68 ± 32.10 ng/mL. Spearman's correlation analysis confirmed that IGF-1, TNF-α and $VEGF_{165}$ were positively correlated with the severity of DN ($r = 0.801$, $r = 0.477$, $r = 0.723$; all $P < 0.05$).

Correlation analyses between DN severity and IGF-1, TNF-α and $VEGF_{165}$

Pearson's correlation analyses showed that DN severity was positively correlated with the concentrations of serum IGF-1 ($r = 0.726$), TNF-α ($r = 0.628$) and $VEGF_{165}$ ($r = 0.632$; all $P < 0.05$; Figure 3).

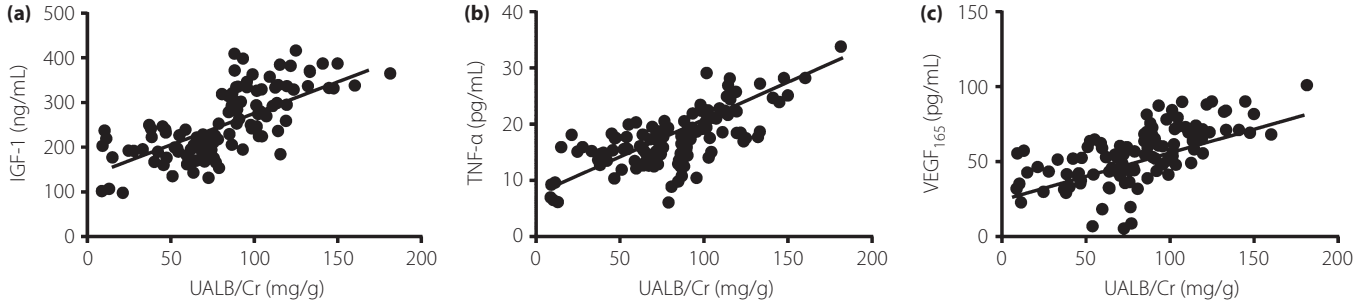

Figure 2 | Correlation analyses between urinary albumin/urine creatinine (UALB/Cr), and concentrations of serum insulin-like growth factor-1 (IGF-1), tumor necrosis factor-α (TNF-α) and vascular endothelial growth factor 165 ($VEGF_{165}$) in type 2 diabetic nephropathy (DN) patients.

Table 2 | Comparisons of serum insulin-like growth factor-1, tumor necrosis factor-α and vascular endothelial growth factor 165 levels among patients with different severity of diabetic nephropathy

Groups	IGF-1 (ng/mL)	TNF-α (pg/mL)	$VEGF_{165}$ (pg/mL)
Little proteinuria group (n = 50)	193.11 ± 37.47*,**	15.01 ± 3.49*,**	41.98 ± 14.39*,**
Large-amount proteinuria group (n = 44)	292.14 ± 57.45**	18.57 ± 5.17**	63.39 ± 13.02**
Kidney failure group (n = 14)	355.68 ± 32.10	22.10 ± 6.19	78.27 ± 12.06

*P < 0.05 when compared with the large-amount proteinuria group; **P < 0.05 when compared with the kidney failure group. DN, diabetic nephropathy; IGF-1, insulin-like growth factor-1; TNF-α, tumor necrosis factor-α; $VEGF_{165}$, vascular endothelial growth factor165.

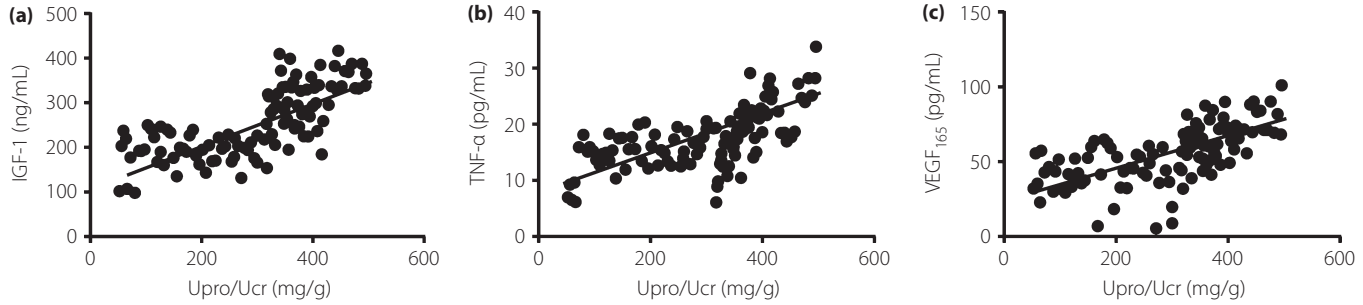

Figure 3 | Correlation analyses between urine protein/urine creatinine (Upro/Ucr), and concentrations of serum insulin-like growth factor-1 (IGF-1), tumor necrosis factor-α (TNF-α) and vascular endothelial growth factor 165 ($VEGF_{165}$) of the type 2 diabetic nephropathy (DN) patients.

Correlation among IGF-1, TNF-α and $VEGF_{165}$

As shown in Figure 4, there was a positive association between IGF-1 and TNF-α (r = 0.458), IGF-1 and $VEGF_{165}$ (r = 0.588), and TNF-α and $VEGF_{165}$ (r = 0.428; all P < 0.05).

Logistic regression analysis

With DN progression as a dependent variable, IGF-1, TNF-α, $VEGF_{165}$, total cholesterol, triglyceride, LDL-C, VLDL-C, BUN, serum creatinine, blood β2-MG, urine β2-MG as independent variables were included into logistic regression analysis. The results showed that IGF-1 and $VEGF_{165}$ were associated with the progression of DN (both P < 0.05), whereas other indexes failed to have an association with the progression of DN (all P > 0.05; Table 3).

DISCUSSION

Cytokine is one of the major mediators composing the immune system, and change of immunological function is one of the leading causes of DN occurrence[16]. In recent years, many researchers have paid attention to the effect of cytokines on the occurrence and development of DN[17]. In the present study, we found that expression levels of IGF-1, TNF-α and $VEGF_{165}$ in serum increased as DN worsened, and were positively related to UALB/Cr.

The present study initially showed that total cholesterol, triglyceride, LDL-C, VLDL-C, BUN, Scr, blood β2-MG, urine β2-MG and UALB/Cr were higher, and HDL-C was lower in the type 2 DN patients compared with the healthy individuals. Similarly, Wiggin *et al.*[18] found that elevated total cholesterol

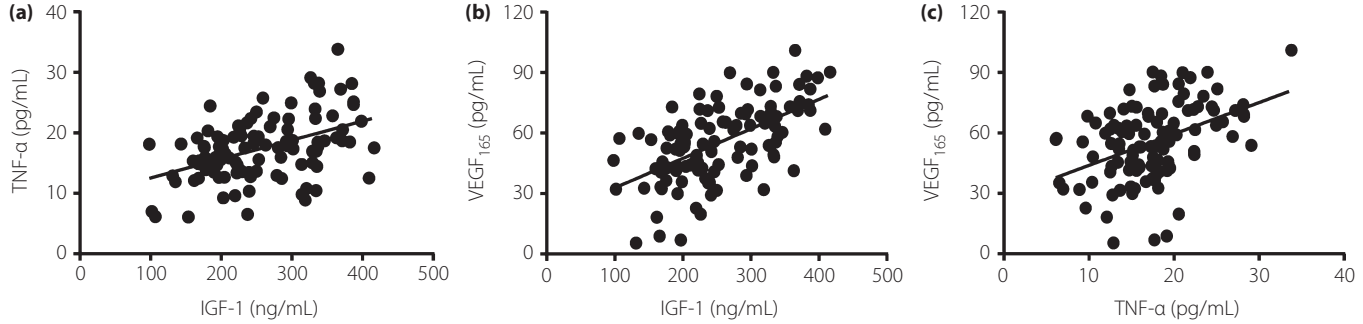

Figure 4 | Correlation analyses among concentrations of serum insulin-like growth factor-1 (IGF-1), tumor necrosis factor-α (TNF-α) and vascular endothelial growth factor 165 ($VEGF_{165}$) in the type 2 diabetic nephropathy (DN) patients.

Table 3 | Logistic regression analysis

Index	B	SE	Wald	Sig.	Exp(B)	95% CI	
IGF-1	0.07	0.02	8.46	0.004	1.07	1.02	1.13
TNF-α	−0.10	0.21	0.22	0.638	0.91	0.60	1.36
$VEGF_{165}$	0.31	0.12	6.69	0.010	1.37	1.08	1.73
Total cholesterol	0.18	0.81	0.05	0.825	1.20	0.24	5.85
Triglyceride	−2.30	1.99	1.33	0.249	0.10	0.00	4.99
HDL-C	0.14	3.41	0.00	0.968	1.14	0.00	918.06
LDL-C	0.91	0.96	0.91	0.341	2.49	0.38	16.36
VLDL-C	1.90	2.00	0.90	0.342	6.69	0.13	336.97
BUN	0.58	0.83	0.49	0.486	1.78	0.35	8.97
Serum creatinine	−0.03	0.05	0.33	0.563	0.97	0.89	1.07
Blood β2-MG	0.00	0.00	0.42	0.516	1.00	1.00	1.00
Urine β2-MG	0.00	0.01	0.12	0.725	1.00	0.98	1.01

Blood β2-MG, β2-microglobulin of serum; BUN, blood urea nitrogen; CI, confidence interval; Exp(B), exponentiation of the B coefficient; IGF-1, insulin-like growth factor-1; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; SE, standard error; Sig., significance; TNF-α, tumor necrosis factor-α; urine β2-MG, β2-microglobulin of urine; $VEGF_{165}$, vascular endothelial growth factor 165; VLDL-C, very low density lipoprotein-cholesterol.

and triglyceride might play a contributing role in the progression of DPN. Zheng *et al.*[19] reported a significant elevation in urinary albumin excretion, serum creatinine and BUN in the DN group compared with normal controls. Also, Ji *et al.*[20] and Papanas *et al.*[21] also reported increased levels of LDL, but decreased levels of HDL in DM patients with DNP.

Also, the present study showed that IGF-1, TNF-α and $VEGF_{165}$ levels in the type 2 DN patients were higher than that in the healthy individuals, and they increased as the disease progressed. IGF-1 is responsible for the growth and regeneration of an organism, boosting cell proliferation and differentiation by acting on autoreceptors[22]. A possible reason why IGF-1 could promote DN occurrence is that IGF-1 could lead to matrix accumulation and proliferation of glomeruli mesangial cells[23]. Gu *et al.*[24] found that IGF-1 could promote transport of glucose in cells, thereby increasing glucose uptake by mesangial cells under glucose condition, and boosting proliferation of mesangial cells. Zhang *et al.*[25] found that IGF-1 would result in lipid deposition in mesangial cells, which then formed foam cells, and thereby damaging the functions of mesangial cells. It was also reported that IGF-1 could inhibit the activity of metalloproteinase-2 to promote collagen degradation in part of the kidney, which resulted in glomerular sclerosis and eventually led to the death of end-stage DN patients[26]. However, Akturk *et al.*[27] showed that mean serum IGF-I levels in diabetic patients were lower than the non-diabetic controls. Interestingly, Kim *et al.*[28] showed although a significant reduction of serum IGF-I levels was observed in patients with type 1 diabetes mellitus, remarkably increased serum levels of IGF-I and IGFBP-3 levels were detected in individuals with glucose intolerance including type 2 diabetes. In addition, the present study also found that serum IGF-1 level was positively correlated with urinary albumin and urine creatinine. Unfortunately, a study reported by Fujiwara *et al.*[29] found that urinary IGF-I, but not serum IGF-I, showed a significant negative correlation with creatinine clearance. The differences in sample size, diagnostic criteria of DN or inclusion and exclusion criteria of participants might explain different outcomes. TNF-α is a potential factor that causes fibrosis sclerosis, and plays a significant role in the acute phase of inflammation and sclerosis process[30]. However, a constant large amount of release of TNF-α, or the imbalance between the factor and other immunomodulatory factors will give rise to pyrexia and lesion in organisms. At present, it is acknowledged that TNF-α can induce proliferation of intrinsic renal cells, stimulation of expression adhesion molecules, excessive extracellular matrix and other inflammation mediators, which leads to direct damage to mesangial cells[31]. Taslipinar *et al.*[32] found that TNF-α could stimulate mesangial cells to produce oxygen free radicals and increase metabolites of lipid peroxidase, which gave rise to injuries of intracellular membranes and basement membranes, eventually promoting proteinuria. In addition, the present study also found that serum TNF-α level was positively correlated with UALB and urinary Cr. Consistent with our study, Navarro *et al.*[33] showed that TNF-α level in patients with type 2 diabetes at an early stage of nephropathy was independently linked with urinary albumin excretion. VEGF is a mitogen specifically for endothelial cells[34]. In normal physiological conditions, a small amount of VEGF functions to sustain endothelial fenestration, and moderate VEGF can protect and repair glomerular endothelial cells[35]. It was shown in the study of Veron *et al.*[36] that a large quantity of VEGF could promote cell division in glomerular capillaries, increase vasoactive substances such as plasmin and colloid enzyme, dissolve the microblood basement membrane, decrease the number of anions in the glomerular basement membrane, influence the mechanical and charge barrier of the filtration membrane, and enhance vascular permeability, thereby resulting in proteinuria; furthermore, VEGF could also cause an increased activity of mononuclear giant cells in the glomerulus, mesentery activation, TGF-P generation and glomerular sclerosis, thus exacerbating the progress of DN. By adding VEGF inhibitors, Wang *et al.*[37] improved the albumin excretion rate and histological changes of DN rats, and repaired the expression of specific genes of podocytes, suggesting that VEGF inhibitors might become an important assistant means in the treatment of DN. Largely consistent with the present study, Lenz *et al.*[38] supported that $VEGF_{165}$ might contribute to the development of albuminuria in diabetic nephropathy as a result of type 2 diabetes instead of type 1 diabetes. Also, the present study discovered that serum $VEGF_{165}$ level was positively correlated with UALB and urinary Cr. The possible reason that can explain this association should be further explored.

In conclusion, the expression levels of serum IGF-1, TNF-α and $VEGF_{165}$ in type 2 DN patients during different periods increased in various degrees and showed correlation, which

suggested that IGF-1, TNF-α and $VEGF_{165}$ all participated in the occurrence and development of DN, and might act as an effective index to monitor DN condition changes. However, because of the complexity and diversity of DN mechanisms, the relationship of cytokines, including IGF-1, TNF-α and $VEGF_{165}$, and pathogenesis require further investigation and clinical verification.

ACKNOWLEDGMENT

We would like to acknowledge the reviewers for their helpful comments on this paper.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Akinsegun A, Akinola Olusola D, Sarah JO, *et al*. Mean platelet volume and platelet counts in type 2 diabetes: mellitus on treatment and non-diabetic mellitus controls in lagos, nigeria. *Pan Afr Med J* 2014; 18: 42.
2. Korish AA, Abdel Gader AG, Korashy HM, *et al*. Camel milk attenuates the biochemical and morphological features of diabetic nephropathy: inhibition of Smad1 and collagen type IV synthesis. *Chem Biol Interact* 2015; 229: 100–108.
3. Li J, Wu HM, Zhang L, *et al*. Heparin and related substances for preventing diabetic kidney disease. *Cochrane Database Syst Rev* 2010. doi: 10.1002/14651858.CD005631.pub2.
4. Rossing P. Diabetic nephropathy: worldwide epidemic and effects of current treatment on natural history. *Curr Diab Rep* 2006; 6: 479–483.
5. Raptis AE, Viberti G. Pathogenesis of diabetic nephropathy. *Exp Clin Endocrinol Diabetes* 2001; 109(Suppl 2): S424–S437.
6. Devaraj S, Dasu MR, Jialal I. Diabetes is a proinflammatory state: a translational perspective. *Expert Rev Endocrinol Metab* 2010; 5: 19–28.
7. Shao Y, Cheng Z, Li X, *et al*. Immunosuppressive/anti-inflammatory cytokines directly and indirectly inhibit endothelial dysfunction–a novel mechanism for maintaining vascular function. *J Hematol Oncol* 2014; 7: 80.
8. Ding J, Sullivan DA. The effects of insulin-like growth factor 1 and growth hormone on human meibomian gland epithelial cells. *JAMA Ophthalmol* 2014; 132: 593–599.
9. Levin-laina N, Iaina A, Raz I. The emerging role of NO and IGF-1 in early renal hypertrophy in STZ-induced diabetic rats. *Diabetes Metab Res Rev* 2011; 27: 235–243.
10. Fritzenwanger M, Meusel K, Jung C, *et al*. Cardiotrophin-1 induces tumor necrosis factor alpha synthesis in human peripheral blood mononuclear cells. *Mediators Inflamm* 2009; 2009: 489802.
11. Anderson SM, Siegman SN, Segura T. The effect of vascular endothelial growth factor (VEGF) presentation within fibrin matrices on endothelial cell branching. *Biomaterials* 2011; 32: 7432–7443.
12. Zhang S, Zhau HE, Osunkoya AO, *et al*. Vascular endothelial growth factor regulates myeloid cell leukemia-1 expression through neuropilin-1-dependent activation of c-met signaling in human prostate cancer cells. *Mol Cancer* 2010; 9: 9.
13. Veron D, Bertuccio CA, Marlier A, *et al*. Podocyte vascular endothelial growth factor (vegf(1)(6)(4)) overexpression causes severe nodular glomerulosclerosis in a mouse model of type 1 diabetes. *Diabetologia* 2011; 54: 1227–1241.
14. Chawla V, Roshan B. Non-proteinuric diabetic nephropathy. *Curr Diab Rep* 2014; 14: 529.
15. Tervaert TW, Mooyaart AL, Amann K, *et al*. Pathologic classification of diabetic nephropathy. *J Am Soc Nephrol* 2010; 21: 556–563.
16. Wu CC, Sytwu HK, Lin YF. Cytokines in diabetic nephropathy. *Adv Clin Chem* 2012; 56: 55–74.
17. Navarro JF, Mora C. Diabetes, inflammation, proinflammatory cytokines, and diabetic nephropathy. *Sci World J* 2006; 6: 908–917.
18. Wiggin TD, Sullivan KA, Pop-Busui R, *et al*. Elevated triglycerides correlate with progression of diabetic neuropathy. *Diabetes* 2009; 58: 1634–1640.
19. Zheng M, Lv LL, Cao YH, *et al*. A pilot trial assessing urinary gene expression profiling with an mrna array for diabetic nephropathy. *PLoS One* 2012; 7: e34824.
20. Ji ZY, Li HF, Lei Y, *et al*. Association of adiponectin gene polymorphisms with an elevated risk of diabetic peripheral neuropathy in type 2 diabetes patients. *J Diabetes Complications* 2015; 29: 887–892.
21. Papanas N, Katsiki N, Papatheodorou K, *et al*. Peripheral neuropathy is associated with increased serum levels of uric acid in type 2 diabetes mellitus. *Angiology* 2011; 62: 291–295.
22. Huat TJ, Khan AA, Pati S, *et al*. Igf-1 enhances cell proliferation and survival during early differentiation of mesenchymal stem cells to neural progenitor-like cells. *BMC Neurosci* 2014; 15: 91.
23. Kamenicky P, Mazziotti G, Lombes M, *et al*. Growth hormone, insulin-like growth factor-1, and the kidney: pathophysiological and clinical implications. *Endocr Rev* 2014; 35: 234–281.
24. Gu T, Falhammar H, Gu HF, *et al*. Epigenetic analyses of the insulin-like growth factor binding protein 1 gene in type 1 diabetes and diabetic nephropathy. *Clin Epigenetics* 2014; 6: 10.
25. Zhang YJ, Chen YN, Zheng SS, *et al*. [HMGB1/SREBP-1 mediated IFN-gamma-induced lipid deposition in mouse mesangial cells]. *Zhongguo Ying Yong Sheng Li Xue Za Zhi* 2013; 29: 6–10.
26. Kumar PA, Brosius FC 3rd, Menon RK. The glomerular podocyte as a target of growth hormone action: implications for the pathogenesis of diabetic nephropathy. *Curr Diabetes Rev* 2011; 7: 50–55.

27. Akturk M, Arslan M, Altinova A, *et al*. Association of serum levels of IGF-I and IGFBP-1 with renal function in patients with type 2 diabetes mellitus. *Growth Horm IGF Res* 2007; 17: 186–193.
28. Kim MS, Lee DY. Insulin-like growth factor (IGF)-I and IGF binding proteins axis in diabetes mellitus. *Ann Pediatr Endocrinol Metab* 2015; 20: 69–73.
29. Fujihara M, Uemasu J, Kawasaki H. Serum and urinary levels of insulin-like growth factor i in patients with chronic renal disease and diabetes mellitus: its clinical implication. *Clin Nephrol* 1996; 45: 372–378.
30. Pehlivan Y, Onat AM, Ceylan N, *et al*. Serum leptin, resistin and TNF-α levels in patients with systemic sclerosis: the role of adipokines in scleroderma. *Int J Rheum Dis* 2012; 15: 374–379.
31. Zelova H, Hosek J. Tnf-alpha signalling and inflammation: interactions between old acquaintances. *Inflamm Res* 2013; 62: 641–651.
32. Taslipinar A, Yaman H, Yilmaz MI, *et al*. The relationship between inflammation, endothelial dysfunction and proteinuria in patients with diabetic nephropathy. *Scand J Clin Lab Invest* 2011; 71: 606–612.
33. Navarro JF, Mora C, Maca M, *et al*. Inflammatory parameters are independently associated with urinary albumin in type 2 diabetes mellitus. *Am J Kidney Dis* 2003; 42: 53–61.
34. Gao YF, Shu Y, Yang L, *et al*. A graphic method for identification of novel glioma related genes. *Biomed Res Int* 2014; 2014: 891945.
35. Morita S, Furube E, Mannari T, *et al*. Vascular endothelial growth factor-dependent angiogenesis and dynamic vascular plasticity in the sensory circumventricular organs of adult mouse brain. *Cell Tissue Res* 2015; 359: 865–884.
36. Veron D, Reidy KJ, Bertuccio C, *et al*. Overexpression of vegf-a in podocytes of adult mice causes glomerular disease. *Kidney Int* 2010; 77: 989–999.
37. Wang S, Li Y, Huang YJ. [The effects of VEGF-R inhibitor on podocytopathy of rats with type I diabetic nephropathy]. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 2011; 27: 1003–1006.
38. Lenz T, Haak T, Malek J, *et al*. Vascular endothelial growth factor in diabetic nephropathy. *Kidney Blood Press Res* 2003; 26: 338–343.