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

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**Keywords**
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**ABSTRACT**

**Aims/Introduction:** The present study aimed to determine the associations between expressions of insulin-like growth factor-1 (IGF-1), tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) 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-\(\alpha\) and VEGF\(_{165}\).

**Results:** The expressions of serum IGF-1, TNF-\(\alpha\) 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-\(\alpha\) 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-\(\alpha\) and VEGF\(_{165}\) increased, and type 2 DN severity had positive correlations with serum IGF-1, TNF-\(\alpha\) and VEGF\(_{165}\) concentrations (all \(P < 0.05\)). There was a positive association between IGF-1 and TNF-\(\alpha\), IGF-1 and VEGF\(_{165}\), and TNF-\(\alpha\) 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-\(\alpha\) 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\(^7\).
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. Levine-laina et al. 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. 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. VEGF is expressed as several alternately spliced isoforms, such as VEGF165 and VEGF121. VEGF165 is predominant, with optimal bioavailability, and plays an instrumental role in VEGF biological potency, whereas VEGF121 is less potent but freely fusible. 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. At present, the action mechanism of different cytokines to DN has not yet been definitely concluded. The present study carried out correlation analyses between the expression levels of serum IGF-1, TNF-α and VEGF165, 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 Hua’an First People’s Hospital, Nanjing Medical University, Hua’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. 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. 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 m²) 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 Hua’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 VEGF165 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 abcissa to calculate concentrations of IGF-1, TNF-α and VEGF165 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 χ²-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 VEGF165 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 VEGF165. 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 comparison between the control group and the case group**

| Groups         | Case group | Control group | 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 ± 15.99 | <0.001 |
| Blood β2-MG (μg/L) | 2,402.54 ± 509.95 | 2,179.97 ± 529.11 | 0.003 |
| Urine β2-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.

The case group had higher total cholesterol, triglyceride, LDL-C, VLDL-C, and lower HDL-C compared with the control group (all P < 0.05; Table 1).

**Expression levels of serum IGF-1, TNF-α and VEGF165**

The expressions of serum IGF-1, TNF-α and VEGF165 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).

**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 VEGF165 (r = 0.650; all P < 0.01) in patients with type 2 DN (Figure 2).

**Serum IGF-1, TNF-α and VEGF165 levels, and severity of DN**

It was found that DN worsened, and expression levels of IGF-1, TNF-α and VEGF165 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 VEGF165 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 VEGF165**

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 VEGF165 (r = 0.632; all P < 0.05; Figure 3).
Correlation among IGF-1, TNF-α and VEGF165

As shown in Figure 4, there was a positive association between IGF-1 and TNF-α ($r = 0.458$), IGF-1 and VEGF165 ($r = 0.588$), and TNF-α and VEGF165 ($r = 0.428$; all $P < 0.05$).

Logistic regression analysis

With DN progression as a dependent variable, IGF-1, TNF-α, VEGF165, 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 VEGF165 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. In recent years, many researchers have paid attention to the effect of cytokines on the occurrence and development of DN. In the present study, we found that expression levels of IGF-1, TNF-α and VEGF165 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. found that elevated total cholesterol...
and triglyceride might play a contributing role in the progression of DPN. Zheng et al.\textsuperscript{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.\textsuperscript{20} and Papanas et al.\textsuperscript{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-\( \alpha \) and VEGF\textsubscript{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\textsuperscript{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\textsuperscript{23}. Gu et al.\textsuperscript{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.\textsuperscript{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\textsuperscript{26}. However, Akturt et al.\textsuperscript{27} showed that mean serum IGF-I levels in diabetic patients were lower than the non-diabetic controls. Interestingly, Kim et al.\textsuperscript{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.\textsuperscript{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-\( \alpha \) is a potential factor that causes fibrosis sclerosis, and plays a significant role in the acute phase of inflammation and sclerosis process\textsuperscript{30}. However, a constant large amount of release of TNF-\( \alpha \), 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-\( \alpha \) can induce proliferation of intrarenal cells, stimulation of expression adhesion molecules, excessive extracellular matrix and other inflammation mediators, which leads to direct damage to mesangial cells\textsuperscript{31}. Taslimipar et al.\textsuperscript{32} found that TNF-\( \alpha \) 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-\( \alpha \) level was positively correlated with UALB and urinary Cr. Consistent with our study, Navarro et al.\textsuperscript{33} showed that TNF-\( \alpha \) 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\textsuperscript{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\textsuperscript{35}. It was shown in the study of Veron et al.\textsuperscript{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, mesentry activation, TGF-P generation and glomerular sclerosis, thus exacerbating the progress of DN. By adding VEGF inhibitors, Wang et al.\textsuperscript{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.\textsuperscript{38} supported that VEGF\textsubscript{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\textsubscript{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-\( \alpha \) and VEGF\textsubscript{165} in type 2 DN patients during different periods increased in various degrees and showed correlation, which

### Table 3 | Logistic regression analysis

| Index    | B   | SE  | Wald | Sig. | Exp(B) | 95% Cl    |
|----------|-----|-----|------|------|--------|-----------|
| IGF-1    | 0.07| 0.02| 8.46 | 0.004| 1.07   | 1.02-1.13 |
| TNF-\( \alpha \) | -0.10| 0.21| 0.22 | 0.638| 0.91   | 0.60-1.36 |
| VEGF\textsubscript{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 \( \beta\text{-MG} \) | 0.00| 0.00| 0.42 | 0.516| 1.00   | 1.00-1.00  |
| Urine \( \beta\text{-MG} \) | 0.00| 0.01| 0.12 | 0.725| 1.00   | 0.98-1.01  |

Blood \( \beta\text{-MG} \), \( \beta\text{-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-\( \alpha \), tumor necrosis factor-\( \alpha \); urine \( \beta\text{-MG} \), \( \beta\text{-microglobulin of urine; VEGF\textsubscript{165}, vascular endothelial growth factor 165; VLDL-C, very low density lipoprotein-cholesterol.} \)
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.

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DISCLOSURE
The authors declare no conflict of interest.

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