Case Control Study

Relationship between serum Dickkopf-1 and albuminuria in patients with type 2 diabetes

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Abstract

BACKGROUND
Diabetic kidney disease is a microvascular complication of diabetes with complex pathogenesis. Wingless signaling-mediated renal fibrosis is associated with diabetic kidney disease. Dickkopf-1, a negative regulator of Wingless, has been proven to participate in renal fibrosis, glucose metabolism, and inflammation. However, whether serum Dickkopf-1 levels are associated with diabetic kidney disease remains unclear.

AIM
To assess the relationship between serum Dickkopf-1 levels and albuminuria in individuals with type 2 diabetes.

METHODS
Seventy-three type 2 diabetes patients and 24 healthy individuals were enrolled in this case-control study. Diabetic individuals were separated into normal albuminuria, microalbuminuria, and macroalbuminuria groups based on their urinary albumin/creatinine ratios (UACRs). Clinical characteristics and metabolic indices were recorded. Serum Dickkopf-1 levels were determined by enzyme-linked immunosorbent assay.

RESULTS
No significant difference in serum Dickkopf-1 levels was found between healthy individuals and the normal albuminuria group. However, the levels in the microalbuminuria group were significantly lower than those in the normal albuminuria group (P = 0.017), and those in the macroalbuminuria group were the
lowest. Bivariate analysis revealed that serum Dickkopf-1 levels were positively correlated with hemoglobin A1c level ($r = 0.368$, $P < 0.01$) and estimated glomerular filtration rate ($r = 0.339$, $P < 0.01$), but negatively correlated with diabetes duration ($r = -0.231$, $P = 0.050$), systolic blood pressure ($r = -0.369$, $P = 0.001$), serum creatinine level ($r = -0.325$, $P < 0.01$), and UACR ($r = -0.459$, $P < 0.01$). Multiple and logistic regression showed that serum Dickkopf-1 levels were independently associated with UACR (odds ratio $= 0.627$, $P = 0.021$).

**CONCLUSION**

Serum Dickkopf-1 levels are negatively associated with UACR. Lower serum Dickkopf-1 levels could be a critical risk factor for albuminuria in diabetes.

**Key Words:** Dickkopf-1; Albuminuria; Diabetic kidney disease; Type 2 diabetic mellitus; Wingless; Microalbuminuria

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**INTRODUCTION**

Diabetic kidney disease (DKD), the key cause of end-stage kidney disease, is a severe microvascular complication of diabetes[9]. Because of the multiple factors underlying induction of DKD, its pathophysiology remains not fully elucidated[8-9]. Previous studies indicated that hemodynamic changes and metabolic disorders initiate a sequence of events that accelerate the progression of DKD. Activation of the renin-angiotensin-aldosterone system, secretion of proinflammatory factors and cytokines, and dysregulation of multiple intracellular pathways are involved in DKD progression[10].

The Wingless (Wnt) signaling pathway is essential in cell proliferation, cell migration, stem cell maintenance, tissue repair, and embryonic development[6,7]. In recent years, the Wnt signaling pathway has received considerable attention due to its roles in kidney disease, cancer, bone disease, diabetes, rheumatoid arthritis, and Alzheimer’s disease[4]. Dysregulation of Wnt signaling has been shown to contribute to abnormal kidney function (e.g., renal fibrosis, ischemic injury, and acute renal failure), leading to podocyte injury, mesangial cell dysfunction, and extracellular matrix deposition[5].

Dickkopf-1, together with dickkopf-2, dickkopf-3, and dickkopf-4, belonging to the dickkopf family, is an antagonist of Wnt signaling. It is a secreted glycoprotein and is widely expressed in various tissues, including the skin, osteocytes, endothelial tissue, and placenta. Dickkopf-1 can bind to lipoprotein-receptor-related protein 5/6 and interrupt the formation of lipoprotein-receptor-related protein and Wnt protein complex to inhibit the canonical Wnt signaling. Thus, it is characterized as a comprehensive regulator of Wnt signaling involved in inflammation, atherosclerosis, and regulation of glucose metabolism[11]. As such, Dickkopf-1 has been proposed to influence disease in individuals with diabetes[12]. However, the relationship between serum Dickkopf-1 levels and DKD has not been established thus far. Considering that most individuals with diabetes are type 2 diabetes with complicated patho-
physiological mechanisms, we aimed to assess the serum Dickkopf-1 levels in type 2 diabetic individuals with different albuminuria stages and further explore the potential relationship between them.

MATERIALS AND METHODS

Participants
This case-control study involved 73 type 2 diabetic individuals who had an age of onset > 18 years and 24 healthy volunteers. Type 2 diabetes was diagnosed based on the 1999 World Health Organization criteria. The exclusion criteria were as follows: (1) Presence of acute complications of diabetes, such as diabetic ketosis, lactic acidosis, hyperglycemia, or hyperosmolarity; (2) Presence of comorbid thyroid disease, adrenal disease, or other endocrine diseases; (3) Diagnosis with osteoporosis; (4) Presence of serious heart, liver, hematological system, autoimmune, neoplastic, or acute cardiovascular diseases; (5) Presence of operation or acute infection; and (6) Presence of other systemic diseases that can induce proteinuria. The study was approved by the Medical Ethics Committee of the Affiliated Hospital of Weifang Medical University. In addition, informed consent was obtained from all individuals. The individuals with diabetes were categorized into normal albuminuria group (urine albumin creatinine ratios (UACR) < 30 mg/g), microalbuminuria group (UACR, 30–300 mg/g), and macroalbuminuria group (UACR, > 300 mg/g). Twenty-four healthy volunteers were included in a control group.

Measurements
Medical history and clinical characteristics (sex, age, height, weight, and blood pressure) were recorded in the morning during patients’ clinic visits. Blood samples for analysis of metabolic indices were also collected. Urine samples were obtained for urinary albumin and creatinine testing. Body mass index (BMI), UACR, and estimated glomerular filtration rate (eGFR) were calculated. HOMA-IR [fasting glucose × fasting insulin (μU/mL)/22.5] was used to calculate insulin resistance.

Renal function and lipids were assessed using an autoanalyzer (Cobas 8000, Roche, Basel, Switzerland). Hemoglobin A1c (HbA1c) was detected by using a high-performance liquid chromatography system (Bio-Rad, United States). Fasting insulin and C-peptide levels were tested by chemiluminescence (e601, Roche). Serum Dickkopf-1 concentrations were assayed by ELISA employing a human Dickkopf-1-specific antibody, with a range of 10 to 1000 pg/mL (R&D systems Catalog DKK100, United States). Urinary albumin was measured by immune turbidimetry and creatinine was measured with a chemistry analyzer (AU2700, Olympus, Tokyo, Japan).

Statistical analysis
Parametric variables are presented as the mean (SE), and nonparametric variables (HOMA IR and triglycerides) are expressed as medians (IQR). Logarithmic transformations were applied to the nonparametric variables prior to analysis. One-way analysis of variance (ANOVA) was performed for multiple comparisons, followed by Tukey post hoc comparison. Pearson’s correlation was used to examine relationships between variables. Multivariate linear regression models were used to estimate the determinants of Dickkopf-1. Logistic regression analyses indicated the risk factors in diabetic patients with proteinuria. The sample size was calculated using G. Power 3.1 (Germany) with the accepted minimum level of α = 0.05 and β = 0.2 (power = 0.8). IBM SPSS Statistics, version 20.0, was used to perform data analyses. A value of $P < 0.05$ was accepted as statistically significant.

RESULTS

Cohort and clinical characteristics of individuals with diabetes
Normal healthy individuals were matched for age and sex with diabetic individuals. No significant differences were observed regarding sex, age, and BMI among diabetic patients with different stages of albuminuria. Higher blood pressure was found in patients in the macroalbuminuria group ($P < 0.01$). As expected, the fasting plasma glucose and HbA1c levels of diabetic individuals were significantly higher than those of normal controls ($P < 0.0001$ for both). However, with respect to C-peptide or insulin levels, no significant differences among groups were observed. Lipid profile tests
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revealed that triglyceride levels were elevated and high-density lipoprotein cholesterol levels were lower in patients with diabetes ($P < 0.05$). Additionally, no significant difference in low-density lipoprotein cholesterol was found among the four groups. Compared with healthy individuals, the microalbuminuria and macroalbuminuria groups exhibited significantly elevated serum urea nitrogen, creatinine, and uric acid levels ($P < 0.05$ for all); they also exhibited lower eGFR ($P < 0.05$).

**Comparison of serum Dickkopf-1 levels among the four groups**

No significant difference in serum Dickkopf-1 levels between healthy individuals and all diabetic individuals was found ($6.63 \pm 0.29$ ng/mL vs $6.13 \pm 0.23$ ng/mL; $P = 0.2598$). However, among patients with diabetes, the serum Dickkopf-1 level was the lowest in patients with macroalbuminuria ($4.73 \pm 0.13$ ng/mL). Patients with microalbuminuria had a lower mean serum Dickkopf-1 level, compared with patients with normal albuminuria ($6.14 \pm 0.36$ ng/mL vs $7.52 \pm 0.43$ ng/mL; $P = 0.017$) (Table 1).

**Univariate correlations with serum Dickkopf-1 levels**

Correlation analysis revealed that serum Dickkopf-1 levels were positively correlated with HbA1c ($r = 0.368$, $P = 0.001$) and eGFR ($r = 0.339$, $P = 0.003$), whereas it had negative correlations with diabetes duration ($r = -0.231$, $P = 0.050$), systolic blood pressure ($r = -0.369$, $P = 0.001$), serum creatinine levels ($r = -0.325$, $P = 0.005$), uric acid levels ($r = -0.375$, $P < 0.01$), and UACR ($r = -0.459$, $P < 0.01$). Notably, Dickkopf-1 remained negatively correlated with UACR ($r = -0.268$, $P = 0.029$) in patients with diabetes after being adjusted for sex, age, diabetes duration, HbA1c, eGFR, and uric acid levels. No statistical correlations were found between serum Dickkopf-1 levels and lipids, including triglyceride, total cholesterol, low-density lipoprotein and high-density lipoprotein (Table 2).

**Multivariate correlations with serum Dickkopf-1 levels**

Predictors of Dickkopf-1 levels were determined among variables that showed significant univariate associations with Dickkopf-1, by means of multivariate linear regression analysis. After adjustments for age, sex, diabetes duration, and HbA1c levels, the analysis revealed that Dickkopf-1 levels were independently associated with UACR (beta coefficient $= -0.280$; $R^2 = 0.395$, $P = 0.025$), but not with systolic blood pressure, serum creatinine level, or uric acid level. Logistic regression analyses indicated that Dickkopf-1 levels were strongly associated with UACR in diabetic individuals (odds ratio $= 0.627$, $P = 0.021$) (Tables 3 and 4).

**DISCUSSION**

The present study revealed that serum Dickkopf-1 levels declined as the degree of albuminuria increased in diabetic individuals. Notably, Dickkopf-1 levels were independently and negatively related with UACR. These findings indicate that Dickkopf-1 is independently associated with the occurrence of proteinuria in diabetes patients.

Dickkopf-1 has been suggested to play roles in diabetes and DKD; however, there have been few investigations of these relationships. Although serum Dickkopf-1 levels were similar between healthy individuals and all diabetic individuals in our study, Dickkopf-1 levels were positively associated with HbA1c levels in the further correlation analysis. This is consistent with the conclusion by Franceschi et al[13], who showed similar serum Dickkopf-1 levels between children with type 1 diabetes and healthy children[13]. However, Lattanzio et al[13] observed elevated serum Dickkopf-1 levels in type 2 diabetic patients; it decreased upon treatment with acarbose or rosiglitazone. Our study suggested that Dickkopf-1 levels were positively correlated with HbA1c levels; thus, we speculate that Dickkopf-1 may participate in diabetes through modulation of glucose metabolism. However, the specific mechanism merits further exploration in a future study.

DKD is a severe microvascular complication of diabetes with characteristic pathological changes comprising glomerular sclerosis, as well as glomerular basement membrane thickening, mesangial cell expansion, and tubular apoptosis. Increasing degrees of albuminuria/proteinuria have been regarded as indicators of DKD progression. The Wnt pathway has been verified to participate in renal fibrosis and play a dichotomous role in DKD pathogenesis. Modulation of Wnt over-activation has been shown to improve albuminuria; downregulation of the Wnt pathway could induce renal injury and fibrosis[13,14,15]. Exogenous administration of nitric oxide donors...
As an endogenous inhibitor of the Wnt pathway, Dickkopf-1 has also been reported to contribute to microvascular complications of diabetes. Qiu et al. first reported that reduced serum Dickkopf-1 levels led to retinal Wnt pathway activation; thus, Dickkopf-1 could serve as an indicator of diabetic retinopathy. Li et al. found that Dickkopf-1 reduced podocyte apoptosis, which was associated with calcium influx and oxidative stress induced by Wnt signaling in the context of high glucose levels. Wang et al. showed that Dickkopf-1 suppressed podocyte injury by inhibiting Wnt pathway signaling that had been activated by high glucose-induced expression of β-arrestin1/2. Activation of the ubiquitin C-terminal hydrolase L1 enzyme, triggered by the Wnt pathway, can also be attenuated by Dickkopf-1, thereby reducing podocyte injury. In this study, lower Dickkopf-1 levels were observed in the microalbuminuria and macroalbuminuria groups, compared with healthy individuals. Moreover, serum 

### Table 1 Clinical and metabolic characteristics of the four groups

|                          | Normal healthy group | Normal albuminuria group | Microalbuminuria group | Macroalbuminuria group |
|--------------------------|----------------------|--------------------------|------------------------|------------------------|
| n                        | 24                   | 24                       | 25                     | 24                     |
| Sex (M/F)                | 7/17                 | 11/13                    | 11/14                  | 15/9                   |
| Age (years)              | 51 ± 3               | 54 ± 1                   | 57 ± 2                 | 59 ± 2                 |
| Diabetes duration (yr)   | -                    | 5.19 ± 1.33             | 9.23 ± 1.38            | 12.83 ± 1.39          |
| BMI (kg/m²)              | -                    | 25.21 ± 0.67            | 25.52 ± 0.91           | 26.10 ± 0.72         |
| SBP (mmHg)               | 111.90 ± 3.12        | 134.5 ± 4.26            | 143.5 ± 4.86           | 156.1 ± 2.64         |
| DBP (mmHg)               | 70.92 ± 1.83         | 85.92 ± 2.11            | 84.80 ± 3.05           | 87.50 ± 2.52         |
| FPG (mmol/L)             | 4.83 ± 0.09          | 9.98 ± 0.56             | 10.10 ± 0.72           | 9.69 ± 0.86          |
| HDL (mmol/L)             | 5.68 ± 0.05          | 8.90 ± 0.38             | 8.66 ± 0.36            | 8.46 ± 0.34          |
| FCP (ng/mL)              | 1.68 ± 0.14          | 1.84 ± 0.17             | 1.88 ± 0.22            | 1.99 ± 0.28          |
| FINS (uIU/mL)            | 7.84 ± 0.88          | 9.67 ± 1.71             | 8.21 ± 1.34            | 15.93 ± 5.48         |
| HOMA-IR                  | 1.40 (0.95, 2.38)    | 2.91 (1.87, 5.88)       | 3.84 (2.12, 5.12)      | 2.47 (0.66, 8.01)    |
| TG (mmol/L)              | 0.73 (0.42, 1.33)    | 1.69 (0.87, 2.67)       | 1.85 (1.07, 2.29)      | 2.10 (1.31, 5.07)    |
| TC (mmol/L)              | 4.67 ± 0.18          | 5.03 ± 0.21             | 4.62 ± 0.30            | 6.18 ± 0.40         |
| LDL (mmol/L)             | 2.73 ± 0.16          | 2.85 ± 0.18             | 2.73 ± 0.19            | 3.43 ± 0.28          |
| HDL (mmol/L)             | 1.47 ± 0.06          | 1.31 ± 0.09             | 1.10 ± 0.07            | 1.12 ± 0.09          |
| SCr (μmol/L)             | 58.43 ± 1.71         | 58.79 ± 2.40            | 76.56 ± 5.93           | 106.3 ± 8.53        |
| BUN (mmol/L)             | 4.45 ± 1.71          | 4.87 ± 0.25             | 6.46 ± 0.55            | 8.28 ± 0.57         |
| UA (mmol/L)              | 257.0 ± 14.30        | 270.1 ± 16.08           | 337.8 ± 21.93          | 367.7 ± 19.00       |
| eGFR (mL/min/1.73 m²)    | 126.2 ± 5.34         | 128.2 ± 5.77            | 105.2 ± 7.17           | 75.14 ± 7.50        |
| UACR (mg/g)              | 6.55 ± 0.59          | 9.47 ± 1.63             | 103.7 ± 13.03          | 581.3 ± 46.75       |
| Dickkopf-1 (ng/mL)       | 6.63 ± 0.29          | 7.52 ± 0.43             | 6.14 ± 0.36            | 4.73 ± 0.13         |

*P < 0.05 vs normal healthy group. 
*P < 0.05 vs normal albuminuria group.

**P < 0.05 vs microalbuminuria group.** 
BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FPG: Fasting plasma glucose; HbA1c: Glycosylated hemoglobin A1c; FCP: Fasting C peptide; FINS: Fasting insulin; TC: Total cholesterol; TG: Triglyceride; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; Scr: Serum creatinine; BUN: Blood urea nitrogen; UA: Uric acid; eGFR: Estimated glomerular filtration rate; UACR: Urinary albumin/creatinine ratio.

Dickkopf-1 reportedly alleviated mesangial cell apoptosis and proteinuria in diabetic rats through the restoration of Wnt signaling. Spironolactone has been proposed to prevent mesangial cell apoptosis in DKD by upregulating Wnt protein expression. Conversely, over-activation of the Wnt pathway has been found to worsen albuminuria by contributing to podocyte injury. Modulation of Wnt pathway over-activation can improve albuminuria, mesangial cell dysfunction, and extracellular matrix deposition.

As an endogenous inhibitor of the Wnt pathway, Dickkopf-1 has also been reported to contribute to microvascular complications of diabetes. Qiu et al. first reported that reduced serum Dickkopf-1 levels led to retinal Wnt pathway activation; thus, Dickkopf-1 could serve as an indicator of diabetic retinopathy. Li et al. found that Dickkopf-1 reduced podocyte apoptosis, which was associated with calcium influx and oxidative stress induced by Wnt signaling in the context of high glucose levels. Wang et al. showed that Dickkopf-1 suppressed podocyte injury by inhibiting Wnt pathway signaling that had been activated by high glucose-induced expression of β-arrestin1/2. Activation of the ubiquitin C-terminal hydrolase L1 enzyme, triggered by the Wnt pathway, can also be attenuated by Dickkopf-1, thereby reducing podocyte injury. In this study, lower Dickkopf-1 levels were observed in the microalbuminuria and macroalbuminuria groups, compared with healthy individuals. Moreover, serum
Dickkopf-1 levels successively decreased as UACR increased. It was speculated that decreased Dickkopf-1 level induced abnormal Wnt signaling pathway activation, thus leading to aggravated renal cell damage and increased albuminuria production. Taken together, these results indicated that lower Dickkopf-1 is a risk factor for proteinuria.

Besides, the analysis showed that Dickkopf-1 was positively correlated with HbA1c level and eGFR, but negatively correlated with disease course, systolic blood pressure, serum creatinine, and UACR. Consistent with the findings by Qiu et al\(^2\), we speculate...
that various factors (e.g., hyperglycemia) may lead to abnormal secretion of Dickkopf-1; diminished Dickkopf-1 levels may over-activate the Wnt signaling pathway and upregulate angiogenic factors (e.g., vascular endothelial growth factor), thereby promoting renal damage, neovascularization, and proteinuria\(^\text{[19]}\). Additionally, Dickkopf-1 has been found to inhibit cell fibrosis, suggesting that Dickkopf-1 may participate in the modulation of fibrosis during DKD progression\(^\text{[20]}\). Further analyses are required to elucidate the underlying mechanisms in the pathophysiology of DKD.

Our study also found that long diabetes duration, higher blood pressure, and lower eGFR were strongly correlated with UACR. This is due to the well-known pathophysiology that long-term/chronic hyperglycemia causes hemodynamic changes, including glomerular hyperfiltration, high perfusion, and excess pressure; these changes lead to upregulation of the renin-angiotensin-aldosterone system, overproduction of cytokines, and dysregulation of the redox homeostasis and multiple intracellular signaling pathways, which worsen DKD\(^\text{[21]}\). Thus, the positive correlation between Dickkopf-1 and eGFR, together with the negative correlation between Dickkopf-1 and diabetes duration, systolic blood pressure, serum creatinine level, and UACR in our study convinced a relationship of Dickkopf-1 and DKD.

Our study has certain limitations. As a cross-sectional study without follow-up, the significance of changes in serum Dickkopf-1 levels and DKD development remains unknown. Although decreased Dickkopf-1 was found in patients with DKD patients, further studies are required to investigate how Dickkopf-1 is involved in this shedding process. Finally, a small number of participants and selection bias also affect the limitation of the conclusion.

**CONCLUSION**

In summary, this study revealed that circulating Dickkopf-1 concentrations are associated with UACR and successively decrease with the progression of albuminuria in type 2 diabetic individuals. The results imply that Dickkopf-1 participates in the development of DKD. However, large-scale follow-up studies are warranted to confirm the findings and elucidate the underlying mechanism.

**ARTICLE HIGHLIGHTS**

**Research background**

Diabetic kidney disease (DKD) is a microvascular complication of diabetes with complex pathogenesis. Wingless signaling-mediated renal fibrosis is associated with DKD. Dickkopf-1, a negative regulator of Wingless, has been proven to be participating in renal fibrosis, glucose metabolism, and inflammation. However, whether serum Dickkopf-1 levels are associated with diabetic kidney disease remains unclear.

**Research motivation**

Are there any correlations between serum Dickkopf-1 levels and glucose levels or albuminuria in type 2 diabetic individuals? Answering this question will provide significant insight into understanding the roles of Dickkopf-1 in DKD.

**Research objectives**

In this study, we assessed the relationship between serum Dickkopf-1 levels and albuminuria in individuals with type 2 diabetes. This will be helpful for the exploration of the mechanism of Dickkopf-1 in DKD.

**Research methods**

Seventy-three type 2 diabetes and 24 healthy individuals were enrolled in this case-control study. Diabetic individuals were separated into normal albuminuria, microalbuminuria, and macroalbuminuria groups based on their urinary albumin/creatinine ratios (UACR). Clinical characteristics and metabolic indices were recorded. Serum Dickkopf-1 levels were determined by enzyme-linked immunosorbent assay.
Research results
No significant difference in serum Dickkopf-1 levels was found between healthy individuals and the normal albuminuria group. However, the levels in the microalbuminuria group were significantly lower than those in the normal albuminuria group, and those in the macroalbuminuria group were the lowest. Bivariate analysis revealed that serum Dickkopf-1 levels were positively correlated with hemoglobin A1c levels and estimated glomerular filtration rate, but negatively correlated with diabetes duration, systolic blood pressure, serum creatinine level, and UACR. Multiple and logistic regression showed that serum Dickkopf-1 levels were independently associated with UACR.

Research conclusions
We have identified that serum Dickkopf-1 levels are negatively associated with UACR. Lower serum Dickkopf-1 levels could be a critical risk factor for albuminuria in diabetes.

Research perspectives
Dickkopf-1, as an endogenous inhibitor of the Wnt pathway, mediates various effects on the microvascular complications of diabetes, including DKD. The value of the study allows scientists to better understand the mechanisms of DKD for treatment in the future.

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