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ORIGINAL ARTICLE

Correlation and Prediction of IL-10Ra and DKK-4 Plasma Levels in Patients with Calcium Oxalate Urolithiasis

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SUMMARY

Background: **Calcium oxalate urolithiasis, commonly referred to as kidney stones, is a prevalent condition marked by the development of solid crystals within the urinary system. Currently, there is no established treatment to cure calcium oxalate urolithiasis. The underlying processes of calcium oxalate urolithiasis remain unclear, and the aim of this study was to clarify the correlation and prediction of IL-10Ra and DKK-4 plasma levels in patients with calcium oxalate urolithiasis.**

Methods: **In this study, we explored the role of body mass index, eating habits score, levels of IL-10Ra and DKK-4, C-reactive protein, white blood cell count, blood urea nitrogen, and blood uric acid in HCC. In total, 85 patients with COU, attending our hospitals between January 2022 and June 2023, were enrolled in this study as experimental group (n = 85), and 85 healthy individuals, who underwent physical examinations during the same period, were collected as control group (n = 85). The obtained blood samples were collected for further testing. Numerous assays, including ELISA assay, western blot, and qRT-PCR, were employed to investigate the role of IL-10Ra and DKK-4 in calcium oxalate urolithiasis.**

Results: **The results indicate that the upregulation of IL-10Ra and DKK-4 may accelerate the progression of calcium oxalate urolithiasis. The logistic regression analysis indicates that the levels of IL-10Ra and DKK-4 positively correlated with calcium oxalate urolithiasis.**

Conclusions: **In summary, the expression levels of IL-10Ra and DKK-4 positively correlated with the progression of calcium oxalate urolithiasis, suggesting that IL-10Ra and DKK-4 could serve as potential predictive factors and risk factors for calcium oxalate urolithiasis.**

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KEYWORDS

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INTRODUCTION

Calcium oxalate urolithiasis (COU), commonly referred to as kidney stones, is a condition marked by the accumulation of solid crystalline deposits, predominantly composed of calcium oxalate, in the kidneys or urinary system [1,2]. These concretions may also incorporate materials such as calcium phosphate, uric acid, and struvite. Individuals with COU often experience acute pain in the flank or side, which may radiate to the lower abdomen and groin, accompanied by nausea, vomiting,

and hematuria [3,4]. Current treatments for COU are largely aimed at alleviating symptoms; however, they do not address the underlying condition, allowing for the potential progression of the disease despite ongoing management. Previous studies [5] have provided evidence suggesting a potential relationship between the IL-10Ra, DKK-4, and COU.

Interleukin-10 receptor alpha (IL-10Ra) is a pivotal protein in the immune system, functioning as a receptor for interleukin-10 (IL-10), an essential anti-inflammatory cytokine that modulates immune responses and sustains the immune equilibrium [6,7]. Within the realm of calcium oxalate urolithiasis (COU), inflammation and immune reactions are recognized contributors to the pathogenesis of renal calculi. Oxalate crystal deposition in the renal tissue can incite inflammation, leading to cellular damage and the mobilization of immune cells [8]. It is conceivable that aberrant regulation of IL-10Ra may influence the immune dynamics in COU. Nonetheless, the intricate mechanisms by which IL-10Ra operates in COU remain obscure [9]. Comprehensive research is imperative to delineate IL-10Ra's specific function in COU and to determine how its modulation could affect the disease's onset and progression.

Dickkopf-related protein 4 (DKK-4) belongs to the Dickkopf family, known for modulating the Wnt signaling pathway. Studies have linked DKK-4 to a range of physiological and pathological processes, such as embryonic development, tissue regeneration, and oncogenesis [10,11]. In calcium oxalate urolithiasis (COU), aberrant proliferation and differentiation in renal tubules or the urinary tract may lead to kidney stone formation. DKK-4 could influence these cellular activities by altering Wnt-mediated signaling pathways that govern cell growth and differentiation. Nonetheless, DKK-4's precise role in COU remains elusive, and further research is needed to elucidate the specific role of DKK-4 in COU. Here, we conducted a clinical study to investigate the precise role of IL-10Ra and DKK-4 plasma levels in patients with COU.

MATERIALS AND METHODS

Patients

In total, 85 patients with COU, attending our hospitals between January 2022 and June 2023, were enrolled in this study as experimental group (n = 85), and 85 healthy individuals, who underwent physical examinations during the same period, were collected as control group (n = 85). All the participants of the study were informed about the experiment, and they provided written consent, as sanctioned by the Ethics Committee of our hospital. Criteria for inclusion into the experimental group: COU was confirmed through non-enhanced CT scan (NCCT) or CT enhancement with three-dimensional reconstruction (CTU), followed by either inpatient surgery or extracorporeal shock wave lithotripsy. The experimental group was diagnosed with calcium oxalate urolithiasis by using infrared spectroscopy. Exclusion criteria: patients with neurological disorders, cardiovascular diseases, renal insufficiency, congenital urinary anomalies, urinary tumors, and other types of secretory urolithiasis were excluded.

Collection of the clinical material from the patients

Upon admission to the hospital, patients in the experimental and in the control group were assessed for age, gender, body mass index (BMI), eating habits score, levels of IL-10Ra and DKK-4, C-reactive protein (CRP), white blood cell count (WBC), blood urea nitrogen (BUN), and blood uric acid (BUA). Both the control and the experimental group underwent routine blood testing. Subsequently, blood samples were collected from both groups for further experimental analysis.

ELISA assay

To assess the concentrations of IL-10Ra and DKK-4 in blood specimens from both experimental and control group, we utilized the ELISA assay technique. Sample preparation entailed cell disruption by using a lysing solution, followed by centrifugation at 4°C and 10,000 rpm for 10 minutes. We then meticulously collected the supernatant, the clear fluid above the pellet, for subsequent IL-10Ra and DKK-4 protein assay. To determine the levels of IL-10Ra and DKK-4 in the collected blood samples, we employed an ELISA kit, according to the manufacturer's protocol.

Western blotting

Blood specimen proteomic profiling entailed fractionation through 10% SDS-PAGE. Proteins isolated from these samples were subsequently transferred onto a PVDF membrane. To prevent non-specific binding, the membrane underwent a TBST wash. Primary antibodies targeting the protein of interest and a housekeeping protein (GAPDH), procured from Bioworld Technology, Inc., in China, were applied to the membrane, which was then incubated at 4°C overnight. Post-incubation, another TBST rinse was carried out to remove unattached primary antibodies. The membrane was later introduced to secondary antibodies, also from Bioworld Technology, Inc., China, and allowed to interact for two hours at room temperature. Excess secondary antibodies were then washed off with TBST. For protein visualization, the membrane was treated with ECL chemiluminescent substrate, and the resultant protein bands were analyzed.

qRT-PCR analysis

Total RNA extraction was performed by using TRIzol reagent (Beyotime, Shanghai) in accordance with the manufacturer's protocol. mRNA and cDNA synthesis were conducted with the mRNA reverse transcription kit (Beyotime, Shanghai). Quantitative analysis was executed by employing SYBR Green PCR Mix (Vazyme Biotech, Shanghai) on a real-time PCR system. We cal-

culated the relative mRNA expression levels by utilizing the $2^{-\Delta\Delta Ct}$ method, normalizing them against GAPDH. Each experiment was conducted in triplicate. The primer sequences utilized are listed below:
IL-10Ra forward:
5'- ATGAGAAGTGCCTGGTCTCC -3',
reverse:
5'- TCA GGGAGCCAAGGTAGGAA -3';
DKK-4 forward:
5'- ATGGAGAGGTGACTC AGACAG-3',
reverse:
5'- TCAGGCATCCAGTTTTGACTC-3';
β-actin forward:
5'-CGGTCAGGTCATCACTATC-3',
reverse:
5'-CAGGGCAGTAATCTCCTTC-3'.

Statistical analysis
Statistical analyses were performed by utilizing SPSS software version 20.0. To summarize categorical variables, frequencies and percentages were employed, while continuous variables were characterized by their mean and standard deviation (M ± SD). The chi-squared test assessed differences in categorical data, and the *t*-test was used to examine variations in the continuous variables between the two groups. A p-value of less than 0.05 was considered statistically significant. Logistic regression analysis was conducted to investigate the association between IL-10Ra, DKK-4, and calcium oxalate urolithiasis.

RESULTS

Comparison of the clinical data
Table 1 delineates the clinical and demographic data of the experimental and the control group. This data encompasses body mass index (BMI), eating habits score, levels of IL-10Ra and DKK-4, C-reactive protein (CRP), white blood cell count (WBC), blood urea nitrogen (BUN), and blood uric acid (BUA). Analysis of these parameters indicates that there are marked disparities in BMI, IL-10Ra and DKK-4 levels, CRP, WBC, and BUN as well as differences in the pharmaceutical treatments when comparing the experimental and the control group. Conversely, no notable differences were observed in terms of gender and age when contrasting patients with COU and healthy persons.

Effect of IL-10Ra and DKK-4 protein in patients with COU
Figure 1 illustrates the use of Western blotting in evaluating the protein expression levels of IL-10Ra and DKK-4 protein in individuals without calcium oxalate urolithiasis and those with calcium oxalate urolithiasis. The findings reveal that the levels of IL-10Ra and DKK-4 proteins were significantly elevated in patients with calcium oxalate urolithiasis (experimental group) compared to the health control group, with statistical significance indicated by a p-value of less than 0.05. This suggests a link between the increased levels of IL-10Ra and DKK-4 proteins and the development of type 2 diabetes.

Effect of IL-10Ra and DKK-4 mRNA in patients with COU
In the diagram labeled as Figure 2, the technique of Western blotting is depicted as a method for assessing the concentration of specific proteins, namely IL-10Ra and DKK-4, within two distinct groups: those unaffected by calcium oxalate urolithiasis disease and those suffering from it. The comparative analysis presented in the results indicates a pronounced increase in the levels of both IL-10Ra and DKK-4 proteins in the subjects diagnosed with calcium oxalate urolithiasis, when set against the healthy control subjects. This variation in the protein expression is statistically significant, as denoted by a p-value lower than 0.05. Therefore, the data suggest a potential association between the upregulation of these proteins and the susceptibility to developing calcium oxalate urolithiasis in individuals with a history of calcium oxalate urolithiasis.

Analysis of the correlation between IL-10Ra and DKK-4 levels in patients with calcium oxalate urolithiasis
To clarify the relationship between IL-10Ra and DKK-4 levels in individuals with calcium oxalate urolithiasis, a binary logistic regression analysis was performed. The results from the logistic regression analysis showed that IL-10Ra levels (OR = 1.132, 95% CI 0.998 - 1.141, $p < 0.05$) positively correlated with calcium oxalate urolithiasis, and IL-10Ra served as a predictive factor for the development of calcium oxalate stones. Similarly, the logistic regression analysis indicated that levels of DKK-4 (OR = 1.165, 95% CI 0.997 - 1.134, $p < 0.05$) positively correlated with calcium oxalate urolithiasis, and DKK-4 served as a predictive factor for the development of calcium oxalate stones.

DISCUSSION

Calcium oxalate urolithiasis is a common condition, characterized by the formation of kidney stones that are primarily composed of calcium oxalate crystals [12]. Management involves a combination of symptomatic relief, stone passage facilitation, and preventive measures to reduce the risk of recurrence. Prevention strategies for calcium oxalate urolithiasis aim to reduce the risk of developing kidney stones that are primarily composed of calcium oxalate crystals. By identifying these risk factors and predictors, individuals at higher risk for calcium oxalate urolithiasis can take preventive measures to reduce the likelihood of developing kidney stones [13,14]. This study focuses on the potential correlation between IL-10Ra and DKK-4 levels in patients with calcium oxalate urolithiasis. The findings of our

Table 1. Demographic and participants general data.

Variable	Experimental group	Control group	t/χ^2	p
Age (years)	45.52 ± 13.92	47.46 ± 16.62	-1.78	0.065
Gender, female (%)	60 (70.6%)	55 (64.7%)	1.64	0.146
BMI (kg/m^2)	26.34 ± 4.56	25.12 ± 3.96	2.84	0.004
IL-10Ra (pg/mL)	463.83 ± 79.48	223.86 ± 41.91	11.94	0.000
DKK-4 (ng/mL)	917.34 ± 234.80	332.85 ± 70.06	10.67	0.000
CRP (mg/L)	4.16 ± 5.52	2.24 ± 2.36	3.64	0.002
WBC (10^9/L)	8.32 ± 3.21	5.91 ± 1.52	10.72	0.001
BUN (mmol/L)	5.74 ± 4.68	5.14 ± 1.25	2.43	0.017

Table 2. Logistic regression analysis.

Variable	B	SE	Wald	p	Exp (B)	95% CI
IL-10Ra (pg/mL)	0.124	43.039	8.32	0.00	1.132	0.998 - 1.141
DKK-4 (ng/mL)	0.063	18.872	6.56	0.00	1.165	0.997 - 1.134

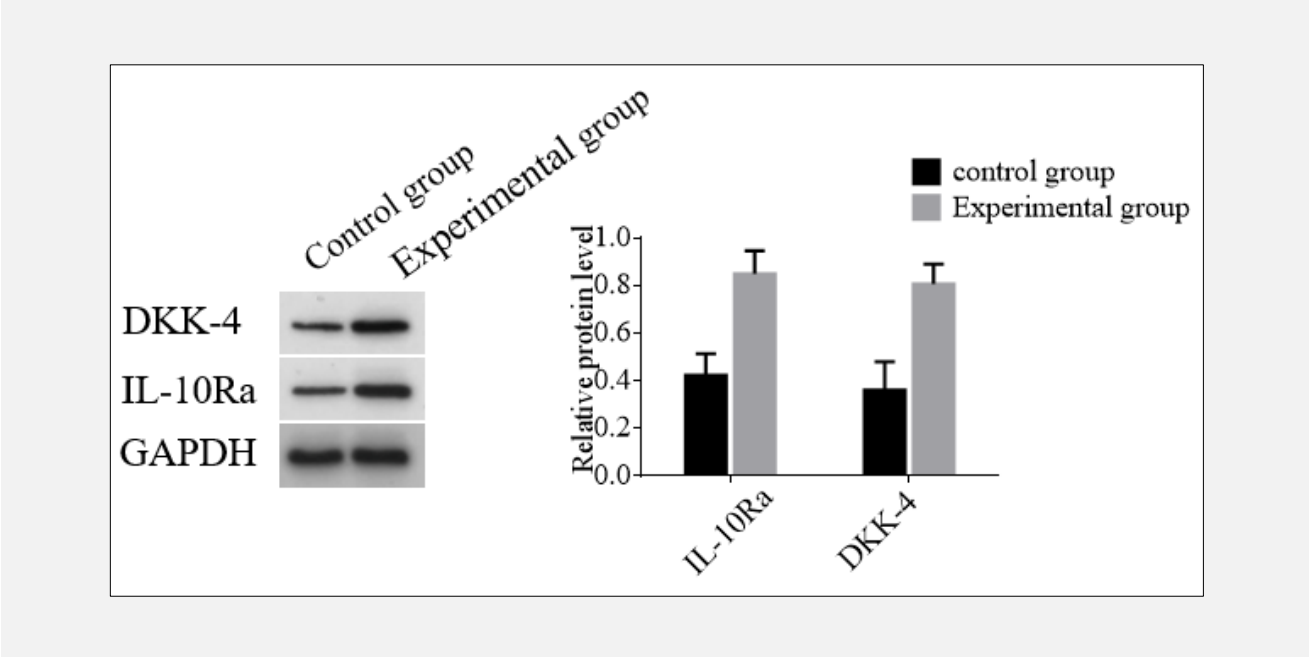

Figure 1. The relative expression levels of IL-10Ra and DKK-4 proteins in the blood specimens from individuals without calcium oxalate urolithiasis and from those with calcium oxalate urolithiasis.

The relative protein expression levels were measured by Western blot (WB).

study reveal that the expression levels of IL-10Ra and DKK-4 were significantly elevated in patients with calcium oxalate urolithiasis (experimental group), compared to the health control group. Furthermore, IL-10Ra and DKK-4 positively correlated with calcium oxalate urolithiasis and serve as predictive factors for its development.

IL-10Ra, also known as Interleukin-10 receptor alpha, is a protein that plays a crucial role in the immune system [15]. It serves as a receptor for the cytokine inter-

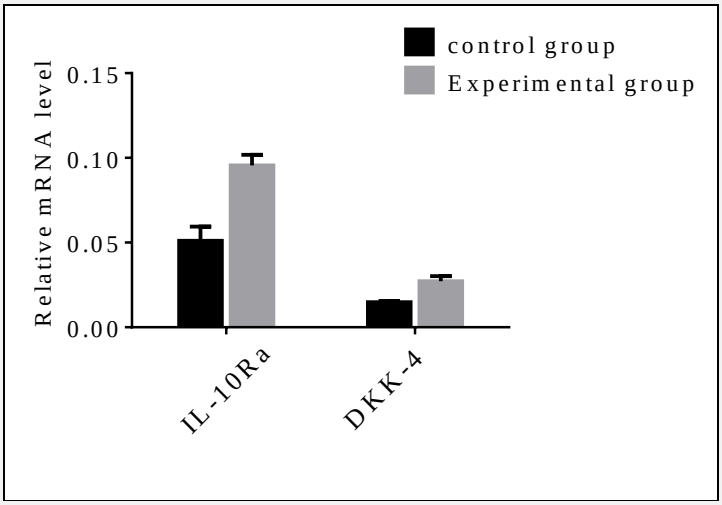

Figure 2. The relative expression levels of IL-10Ra and DKK-4 mRNA in the blood specimens from the control and the experimental group.

The relative mRNA expression levels were measured by qRT-PCR.

leukin-10 (IL-10), an anti-inflammatory cytokine that plays a crucial role in regulating immune responses and sustaining an immune tolerance [16]. IL-10Ra is present on the surface of several immune cells, such as macrophages, dendritic cells, and B cells. Upon binding with IL-10, IL-10Ra triggers a signaling pathway that suppresses pro-inflammatory reactions and enhances anti-inflammatory mechanisms. Research on the role of IL-10Ra in calcium oxalate urolithiasis is an emerging area of interest [17]. The specific involvement of IL-10Ra in this condition is still being elucidated. Kumar et al. [5] has investigated the role of IL-10Ra in calcium oxalate urolithiasis; the findings showed that IL-10Ra participates in the calcium oxalate urolithiasis process by regulating oxalate impaired metabolism, immune response, and the production of mitochondrial ROS in both monocytes and macrophages. Our findings indicated that patients with calcium oxalate urolithiasis exhibited significantly elevated levels of IL-10Ra expression, compared to the healthy control group. Furthermore, the expression level of IL-10Ra positively correlated with the development of calcium oxalate urolithiasis, indicating that IL-10Ra may serve as a potential predictive factor and risk factor for calcium oxalate urolithiasis.

DKK-4, also known as Dickkopf-4, is a protein in the Dickkopf family, which regulates the Wnt signaling pathway. This pathway plays a critical role in a variety of cellular functions, such as growth, cell division, specialization, and maintaining the tissue balance [18]. The pathogenesis of calcium oxalate urolithiasis involves complex interactions among several factors, such as urinary supersaturation with calcium and oxalate, urinary pH, and the presence of crystal formation inhibitors and promoters. The Wnt signaling pathway, regulated by proteins like DKK-4, has been implicated in the development and progression of kidney stones. As a modulator of Wnt signaling, DKK-4 may play a potential role in calcium oxalate urolithiasis. However, the precise role of DKK-4 in calcium oxalate urolithiasis is still elusive. Further research is needed to elucidate the specific role of DKK-4 in calcium oxalate urolithiasis and to explore its potential as a therapeutic and predicting target for the prevention or treatment of calcium oxalate urolithiasis [19]. Yang Y et al. [20] has investigated the Wnt pathway in calcium oxalate urolithiasis; the findings showed that DKK-4 participates in the calcium oxalate urolithiasis development by controlling the Wnt pathway. Our study results indicated that the expression levels of DKK-4 were significantly elevated in patients with calcium oxalate urolithiasis, compared to the healthy control group. Moreover, the expression level of DKK-4 positively correlated with the progression of calcium oxalate urolithiasis, suggesting that DKK-4 could serve as a potential predictive factor and risk factor for this condition.

A limitation of the present study is that DKK-4 controlling the Wnt pathway is complex, and the study may oversimplify the interactions within this pathway or fail to account for other relevant pathways that could influence the cell function in patients with COU, which is why further study needs to be managed. The current study has clarified the correlation and prediction of IL-

10Ra and DKK-4 plasma levels in patients with calcium oxalate urolithiasis, indicating that IL-10Ra and DKK-4 could serve as potential predictive factors and risk factors for calcium oxalate urolithiasis.

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Availability of Data and Materials:
The datasets used and/or analyzed during this study are available from the corresponding author upon reasonable request.

Consent for Publication:
All of the authors consented to the publication of this research.

Ethical Approval and Consent to Participate:
Ethical approval was given by the Jinshan Hospital, Fudan University, and written informed consent was obtained from all patients.

Declaration of Interest:
All authors declare that there are no conflicts of interest in regard to this study.

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