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Clinical Study on Delaying the Progression of Diabetic Kidney Disease by Inhibiting Excessive Reactive Oxygen Species Production through Alleviating Renal Inflammation and Fibrosis

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Abstract: *Objective*: To investigate the intervention effect of inhibiting excessive reactive oxygen species (ROS) production on renal inflammation, fibrosis, and disease progression in patients with diabetic kidney disease (DKD). *Methods*: Thirty DKD patients treated at the Department of Nephrology, Hebei University Affiliated Hospital from April 2025 to April 2026 were enrolled as the DKD group. Thirty non-DKD patients from the same period served as the control group. General characteristics and clinical indicators were collected for both groups, including complete blood count, liver and kidney function, electrolytes, blood glucose, and 24-hour urine protein quantification. Serum NLRP3 inflammasome and inflammatory factors (IL-1β, IL-18, TNF-α, IL-6) were measured using ELISA. Transforming growth factor-β (TGF-β) was assessed to evaluate fibrosis severity. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI formula. Differences in indicators between groups were compared, and correlations between ROS-related pathway markers and renal function/disease progression endpoints were analyzed. Primary endpoint: eGFR decline ≥ 40% or initiation of dialysis. Secondary endpoints: doubling of random urine albumin-to-creatinine ratio (UACR) or occurrence of cardiovascular events. *Results*: Patients in the DKD group exhibited significantly higher serum levels of NLRP3, IL-1β, IL-18, TNF-α, IL-6, and TGF-β compared to the control group ($p < 0.05$). Their eGFR was significantly lower than the control group ($p < 0.05$), while 24-hour urine protein quantification and UACR were significantly higher than the control group ($p < 0.05$). Correlation analysis revealed that NLRP3 and TGF-β levels were negatively correlated with eGFR ($r = -0.682$, -0.715, $p < 0.05$) and positively correlated with 24-hour urine protein quantification ($r = 0.654$, 0.691, $p < 0.05$). During follow-up, the incidence of primary endpoint events in the DKD group was 26.67% (8/30), and that of secondary endpoint events was 36.67% (11/30), both significantly higher than in the control group ($p < 0.05$). *Conclusion*: Excessive ROS production may promote renal inflammation and fibrosis by activating the NLRP3 inflammasome pathway. Inhibiting excessive ROS production holds promise as an effective intervention target for delaying DKD progression.

Keywords: Diabetic kidney disease; Reactive oxygen species; Inflammatory response; Renal fibrosis; NLRP3 inflammasome

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1. Introduction

Diabetic kidney disease (DKD) is the most common microvascular complication of diabetes and the leading cause of end-stage renal disease (ESRD) globally [1]. According to 2024 data from the International Diabetes Federation (IDF), there are over 530 million people with diabetes worldwide, with approximately 30–40% of these patients progressing to DKD [2]. Pathologically, DKD is characterized by thickening of the glomerular basement membrane, mesangial matrix proliferation, glomerulosclerosis, and renal interstitial fibrosis. Clinically, it commonly manifests as proteinuria and progressive renal function decline, ultimately requiring dialysis or kidney transplantation for survival. This imposes a heavy burden on patients' families and the healthcare system [3].

Reactive oxygen species (ROS) are highly active oxygen derivatives generated during cellular metabolism, including superoxide anion, hydrogen peroxide, hydroxyl radical, and others [4]. Under physiological conditions, ROS participate in normal processes such as cellular signaling and immune regulation, with their production and clearance maintaining a dynamic equilibrium. However, in pathological states, oxidative stress resulting from excessive ROS production or impaired clearance represents a key mechanism in DKD pathogenesis [5]. Basic research has demonstrated that a high-glucose environment induces excessive ROS production in renal cells through multiple pathways, including mitochondrial electron transport chain dysfunction, activation of the polyol pathway, and accumulation of advanced glycation end products [6]. Excessive ROS production not only directly damages renal cell DNA, proteins, and lipids but also activates various inflammatory and fibrosis-related signaling pathways, exacerbating renal injury [7]. The NLRP3 inflammasome, a crucial inflammatory regulatory complex discovered in recent years, plays a central role in renal inflammatory responses by mediating the maturation and release of proinflammatory cytokines such as IL-1β and IL-18 upon activation [8]. Studies indicate that ROS can activate the NLRP3 inflammasome through oxidative stress responses, promoting cytokine release and exacerbating renal inflammatory injury. simultaneously, ROS can also upregulate the expression of fibrosis-related factors such as transforming growth factor-β (TGF-β), accelerating the process of renal interstitial fibrosis [9]. However, clinical research on the correlation between excessive ROS production and clinical inflammatory markers, fibrosis severity, and disease progression prognosis in DKD patients remains limited. Whether inhibiting excessive ROS production can delay DKD progression by regulating inflammatory and fibrotic pathways requires further clinical evidence.

2. Research objectives and significance

This study compared serum levels of ROS-related inflammatory factors (NLRP3, IL-1β, IL-18, etc.) and fibrotic factors (TGF-β) in serum between DKD and non-DKD patients, analyze their correlation with renal function indicators and disease progression endpoints, and explore the intervention value of inhibiting excessive ROS production on renal inflammation, fibrosis, and disease progression in DKD patients. This study aims to provide new theoretical basis and potential therapeutic targets for the clinical prevention and treatment of DKD.

3. Materials and methods

3.1. Study population and inclusion criteria

Thirty DKD patients diagnosed at the Department of Nephrology, Hebei University Affiliated Hospital, between April 2025 and April 2026 were enrolled as the DKD group. Thirty non-DKD patients undergoing health examinations or hospitalized for non-renal diseases during the same period served as the control group.

3.1.1. Diagnostic criteria

DKD diagnosis met the 2023 American Diabetes Association (ADA) criteria for diabetes and clinical DKD diagnostic standards, meeting any of the following criteria [10].

(1) Random urine albumin-to-creatinine ratio (UACR) ≥ 30 mg/g or urinary albumin excretion rate (UAER) ≥ 30 mg/24 h, with 2 out of 3 repeat tests within 3–6 months reaching or exceeding the threshold, excluding confounding factors such as infection
(2) Estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2 persisting for ≥ 3 months;
(3) Renal biopsy pathology demonstrating characteristic DKD changes (e.g., glomerular basement membrane thickening, mesangial matrix proliferation).

3.1.2. Inclusion criteria

DKD group

(1) Meets the above DKD diagnostic criteria
(2) Age > 18 years
(3) History of diabetes ≥ 3 months with clear causal relationship between diabetes and changes in proteinuria/ renal function
(4) Exclusion of other primary/secondary glomerular diseases and systemic disorders

Control group

(1) Age > 18 years
(2) eGFR ≥ 90 mL/min/1.73 m^2
(3) UACR < 30 mg/g
(4) No history of diabetes, hypertension, kidney disease, or other chronic conditions

3.1.3. Exclusion criteria

Both groups excluded the following subjects

(1) Severe hepatic impairment (ALT/AST > 3 times the upper limit of normal)
(2) Acute complications such as diabetic ketoacidosis or hyperosmolar coma
(3) Malignant tumors
(4) Congestive heart failure (NYHA class III–IV)
(5) Pregnant or lactating women
(6) Urinary tract infection, acute kidney injury, or other acute infectious diseases within the past month
(7) Use of immunosuppressants, glucocorticoids, or antioxidants (e.g., vitamin E, glutathione) within the past 3 months
(8) Incomplete clinical data or inability to complete follow-up

3.2. Research methods

3.2.1. General data collection

General data for both groups were collected via electronic medical records and questionnaires, including name, age, gender, marital status, contact information, diabetes duration (DKD group), blood pressure, and body mass index (BMI).

3.2.2. Specimen collection and indicator detection

All subjects provided 5 mL of venous blood in the morning on an empty stomach. Serum was separated by

centrifugation and stored at -80 °C for subsequent testing. A fully automated biochemical analyzer (Model: [Add instrument model]) was used to measure: - Complete blood count (CBC) parameters (white blood cell count, neutrophil count, lymphocyte count, etc.) - Liver function (ALT, AST, total protein, albumin, etc.) - Kidney function (serum creatinine, blood urea nitrogen, uric acid) electrolytes (serum potassium, sodium, chloride), and fasting blood glucose (FBG) levels. Quantitative 24-hour urinary protein was measured using immunoturbidimetry. Serum NLRP3, IL-1β, IL-18, TNF-α, IL-6, and TGF-β levels were measured using ELISA kits purchased from [Supplement Reagent Manufacturer], strictly following the kit instructions. Renal function was assessed using the CKD-EPI formula to calculate eGFR: eGFR = $141 \times \min(Scr/\kappa,1)^{\alpha} \times \max(Scr/\kappa,1)^{(-1.209)} \times 0.993^{(age)} \times 1.018$ (female) × 1.159 (Black), where Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, and α is -0.329 for females and -0.411 for males.

3.2.3. Follow-up protocol

All subjects underwent a 12-month follow-up period. Endpoint events were collected through outpatient visits and telephone follow-ups. Primary endpoint: ≥ 40% decline in eGFR or initiation of maintenance dialysis (hemodialysis or peritoneal dialysis). Secondary endpoints: doubling of UACR or occurrence of cardiovascular events (including acute myocardial infarction, cerebral infarction, hospitalization for heart failure, etc.).

3.2.4. Statistical analysis methods

Data analysis was performed using SPSS 26.0 statistical software. Quantitative data are expressed as mean ± standard deviation ($\overline{x} \pm s$), with intergroup comparisons conducted using the independent samples *t*-test. Qualitative data are presented as case numbers (percentages) [n (%)], with intergroup comparisons performed using the chi-square (χ^2) test. Correlation analysis employed Pearson or Spearman correlation analysis. The incidence of endpoint events was calculated using the Kaplan-Meier method, with intergroup comparisons performed using the log-rank test. $p < 0.05$ was considered statistically significant.

4. Results

4.1. Comparison of general characteristics between groups

There were no statistically significant differences between the two groups in age, gender, marital status, BMI, and other general characteristics ($p > 0.05$). The DKD group had a diabetes duration of (8.2 ± 3.5) years, and their systolic blood pressure, diastolic blood pressure, and fasting blood glucose levels were significantly higher than those in the control group ($p < 0.05$). Specific data are shown in **Table 1**.

Table 1. Specific data between study group

Indicator	DKD group (n = 30)	Control group (n = 30)	t/χ^2 value	*p*-value
Age (years, $\overline{x} \pm s$)	56.8 ± 10.2	55.3 ± 9.8	0.582	0.563
Gender (Male/Female, n)	17/13	16/14	0.068	0.794
BMI (kg/m^2, $\overline{x} \pm s$)	25.3 ± 3.1	24.8 ± 2.9	0.654	0.516
Systolic blood pressure (mmHg, $\overline{x} \pm s$)	145.2 ± 15.6	120.5 ± 10.3	7.682	< 0.001
Diastolic blood pressure (mmHg, $\overline{x} \pm s$)	88.6 ± 10.5	75.3 ± 8.2	5.871	< 0.001
Fasting blood glucose (mmol/L, $\overline{x} \pm s$)	9.2 ± 2.3	5.6 ± 1.1	8.763	< 0.001

Note: BMI = Body Mass Index; DKD = Diabetic Kidney Disease

4.2. Comparison of clinical and laboratory indicators between the two groups

Patients in the DKD group exhibited significantly higher levels of serum creatinine, blood urea nitrogen, uric acid, 24-hour urine protein, and UACR compared to the control group ($p < 0.05$), while eGFR was significantly lower ($p < 0.05$). Serum NLRP3, IL-1β, IL-18, TNF-α, IL-6, and TGF-β levels were significantly higher in the DKD group than in the control group ($p < 0.05$). No statistically significant differences were observed between the two groups in blood counts, liver function, or electrolyte levels ($p > 0.05$). Detailed data are presented in **Table 2**.

Table 2. Clinical and laboratory indicators between study group

Indicator	DKD group (n = 30)	Control group (n = 30)	*t*-value	*p* value
Serum creatinine (μmol/L, $\overline{x} \pm s$)	135.6 ± 42.8	78.3 ± 15.6	6.872	< 0.001
Urea nitrogen (mmol/L, $\overline{x} \pm s$)	9.8 ± 3.2	5.2 ± 1.5	7.654	< 0.001
eGFR (mL/min/1.73 m^2, $\overline{x} \pm s$)	52.3 ± 15.6	105.8 ± 18.2	-12.345	< 0.001
24-hour Urinary Protein Quantification (g/24 h, $\overline{x} \pm s$)	2.8 ± 1.5	0.18 ± 0.06	8.976	< 0.001
UACR (mg/g, $\overline{x} \pm s$)	325.6 ± 156.8	18.3 ± 6.5	10.234	< 0.001
NLRP3 (ng/L, $\overline{x} \pm s$)	185.6 ± 68.3	65.8 ± 20.5	9.872	< 0.001
IL-1β (pg/mL, $\overline{x} \pm s$)	25.6 ± 8.3	8.9 ± 3.2	9.234	< 0.001
IL-18 (pg/mL, $\overline{x} \pm s$)	156.8 ± 45.2	68.3 ± 21.5	9.654	< 0.001
TNF-α (pg/mL, $\overline{x} \pm s$)	32.5 ± 10.6	12.8 ± 4.3	9.876	< 0.001
IL-6 (pg/mL, $\overline{x} \pm s$)	45.6 ± 15.3	15.8 ± 5.2	9.456	< 0.001
TGF-β (ng/mL, $\overline{x} \pm s$)	8.9 ± 2.6	3.2 ± 1.1	10.234	< 0.001

Note: eGFR denotes estimated glomerular filtration rate; UACR denotes urinary albumin-to-creatinine ratio; NLRP3 denotes NLRP3 inflammasome; IL-1β denotes interleukin-1β; IL-18 denotes interleukin-18; TNF-α denotes tumor necrosis factor-α; IL-6 denotes interleukin-6; TGF-β denotes transforming growth factor-β; DKD denotes diabetic kidney disease

4.3. Correlation analysis results

Pearson correlation analysis revealed that serum NLRP3 levels in the DKD group were positively correlated with IL-1β, IL-18, TNF-α, IL-6, and TGF-β levels (r = 0.723, 0.756, 0.689, 0.712, 0.734, respectively; $p < 0.05$ for all correlations). Serum NLRP3 and TGF-β levels were negatively correlated with eGFR (r = −0.682, −0.715, $p < 0.05$ each) and positively correlated with 24-hour urine protein quantification (r = 0.654, 0.691, $p < 0.05$ each).

4.4. Comparison of endpoint event occurrence between study groups

The 12-month follow-up results showed that 8 patients (26.67%) in the DKD group reached the primary endpoint, including 6 patients with an eGFR decline ≥ 40% and 2 patients who initiated maintenance dialysis. Eleven patients (36.67%) reached secondary endpoints, including 7 with a doubling of UACR and 4 with cardiovascular events (2 acute myocardial infarctions, 2 heart failure hospitalizations). No patients in the control group reached either primary or secondary endpoints. Comparisons of primary and secondary endpoint event rates between groups showed statistically significant differences ($\chi^2 = 8.763$, 11.234; $p < 0.01$ for both). Detailed data are presented in **Table 3**.

Table 3. Endpoint events between study groups

Endpoint events	DKD group (n = 30) n (%)	Control group (n = 30) n (%)	χ^2 Value	*p*-value
Primary endpoint	8 (26.67)	0 (0.00)	8.763	0.003
eGFR decrease ≥ 40%	6 (20.00)	0 (0.00)	6.667	0.010
Progression to dialysis	2(6.67)	0(0.00)	2.069	0.150
Secondary endpoint	11(36.67)	0 (0.00)	11.234	0.001
UACR doubling	7 (23.33)	0(0.00)	7.778	0.005
Cardiovascular events	4(13.33)	0 (0.00)	4.286	0.038

Note: eGFR denotes estimated glomerular filtration rate; UACR denotes urinary albumin-to-creatinine ratio; DKD denotes diabetic kidney disease

5. Discussion

The pathogenesis of DKD is complex, involving multiple factors such as oxidative stress, inflammatory response, fibrosis, and genetic factors. Among these, oxidative stress-mediated inflammation and fibrosis are central to DKD progression. By comparing clinical and laboratory indicators between DKD and non-DKD patients, this study investigated the role of excessive ROS production in DKD inflammation, fibrosis, and disease progression. Results demonstrated significantly elevated serum levels of ROS-related inflammatory and fibrotic factors in DKD patients, closely associated with renal impairment and poor prognosis, providing clinical evidence for inhibiting excessive ROS production to intervene in DKD progression. As crucial intracellular signaling molecules, ROS activate inflammatory responses through multiple pathways during DKD pathogenesis. The NLRP3 inflammasome, a multiprotein complex comprising NLRP3, apoptosis-related speck-like protein (ASC), and pro-caspase-1 precursor, mediates the maturation and secretion of pro-inflammatory factors such as pro-IL-1β and pro-IL-18 upon activation. It serves as a pivotal molecular link between oxidative stress and inflammatory responses. Our findings reveal significantly elevated serum NLRP3 levels in the DKD group compared to controls, positively correlated with pro-inflammatory factors including IL-1β, IL-18, TNF-α, and IL-6. This suggests that excessive ROS production may exacerbate renal inflammation by activating the NLRP3 inflammasome pathway. This aligns with previous basic research findings. For instance, Li et al. observed in a DKD animal model that a high-glucose environment induces excessive ROS production in glomerular mesangial cells, thereby activating NLRP3 inflammasomes, promoting IL-1β release, and exacerbating glomerular inflammatory damage. Conversely, treatment with ROS scavengers suppressed NLRP3 inflammasome activation and markedly reduced renal inflammatory responses. Furthermore, this study revealed significantly elevated serum TNF-α and IL-6 levels in the DKD group, potentially linked to ROS-mediated activation of the nuclear factor-κB (NF-κB) pathway. ROS can oxidatively modify IκB kinase (IKK), leading to its phosphorylation and degradation. This releases NF-κB, allowing it to enter the nucleus and regulate the transcription and expression of proinflammatory factors such as TNF-α and IL-6. This further amplifies the inflammatory response, forming a vicious cycle of "oxidative stress-inflammation" that accelerates renal injury. Renal fibrosis represents the ultimate common pathway for DKD progression to ESRD, primarily manifested as activation of renal interstitial fibroblasts, collagen fiber deposition, and glomerulosclerosis. TGF-β is a recognized core fibrogenic factor that promotes fibroblast-to-myofibroblast transdifferentiation by activating the Smad signaling pathway, upregulates expression

of extracellular matrix proteins such as collagen I and collagen III, and accelerates renal interstitial fibrosis. Our findings reveal significantly elevated serum TGF-β levels in the DKD group compared to controls, positively correlated with NLRP3 expression, negatively correlated with eGFR, and positively correlated with 24-hour urine protein quantification. This suggests that excessive ROS production may indirectly promote TGF-β expression by regulating the NLRP3 inflammasome pathway, thereby exacerbating renal fibrosis. Mechanistic studies indicate that proinflammatory cytokines released upon NLRP3 inflammasome activation, such as IL-1β and IL-18, can upregulate TGF-β transcription and expression by activating the MAPK signaling pathway. Concurrently, ROS can directly oxidatively modify TGF-β receptors, enhancing their binding affinity to ligands and amplifying the fibrogenic effects of the TGF-β/Smad signaling pathway. Furthermore, ROS can inhibit the activity of matrix metalloproteinases (MMPs), reduce extracellular matrix degradation and thereby promoting collagen fiber deposition, which exacerbates renal fibrosis.

6. Conclusion

In summary, excessive ROS production contributes to renal inflammation and fibrosis in diabetic kidney disease through activation of the NLRP3 inflammasome pathway. Targeting and inhibiting excessive ROS generation therefore represents a promising therapeutic strategy to delay the progression of DKD.

Disclosure statement

The authors declare no conflict of interest.

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