ORIGINAL RESEARCH

Correlation Between Plasma NLRP3, IL-1β, and IL-18 and Diabetic Nephropathy in Patients With Type 2 Diabetes

Yangyang Yuan, MMed; Liqin Li, MD; Xiaoyan Wang, MMed; Pu Zhang, MMed; Jun Wang, MMed; Yanxin Xiao, MD

ABSTRACT

Context • Diabetic nephropathy (DN) is a common microvascular complication in diabetic patients. The pathogenesis of DN is complex. Inflammatory response may play a key role as a common downstream pathway.

Objective • The study intended to explore the relationship between the levels of plasma nucleotide-binding oligomeric domain-like receptor protein 3 (NLRP3 inflammasome), interleukin-1β (IL-1β), and IL-18 and the progression of type 2 diabetic nephropathy to clarify their relationship with type 2 diabetes mellitus (T2DM) and to provide evidence for clinical treatment.

Design • The research team performed a controlled observational study.

Setting • The study took place at Baoding No. 1 Central Hospital in Baoding, Hebei, China.

Participants • Participants were 153 patients with T2DM who received treatment at the hospital between October 2020 and October 2021. The research team allocated 30 participants without evidence of DN to the control group. Based on the DN stage, the team assigned the 123 remaining participants to one of five observation groups: (1) 32 participants with stage 1 DN to the DN1 group, (2) 31 participants with stage 2 DN to the DN2 group, (3) 30 participants with stage 3 DN to the DN3 group, (4) 30 participants with stage 4 DN to the DN4 group, and (5) 29 participants with stage 5 DN to the DN5 group.

Outcome Measures • The research team measured participants' levels of "nucleotide binding oligomeric domain-like receptor protein 3" (NLRP3), interleukin-1 beta (IL-1β), and IL-18 and used the Spearman rank correlation analysis to determine the correlation between those levels and the DN stages.

Results • The levels of NLRP3 , IL-1β and IL-18 in all the five observation groups were significantly higher than those in the control group (all $P < .01$). The levels were also significantly higher: (1) in the DN2, DN3, DN4, and DN5 groups than those in the DN1 group (all $P < .01$); (2) in the DN3, DN4, and DN5 groups than those in the DN2 group (all $P < .01$); (3) in the DN4 and DN5 groups than those in the DN3 group (all $P < .01$); and (4) in the DN5 groups than those in the DN4 group (all $P < .01$). The Spearman rank correlation analysis showed that the NLRP3, IL-1β, and IL-18 levels were significantly positively correlated with the DN stage ($P = .01$).

Conclusions • NLRP3, IL-1β and IL-18 played an important role in the progression of T2DM, and their levels increased with the aggravation of DN. Therefore, the plasma levels of NLRP3, IL-1β and IL-18 can be useful as indicators of the occurrence and development of DN and can provide clinical guidance for the early diagnosis of DN and for the determination and adjustment of treatment plans. (*Altern Ther Health Med.* 2023;29(4):52-56).

Yangyang Yuan, MMed, Physician; **Liqin Li, MD**, Physician; **Xiaoyan Wang, MMed**, Physician; **Pu Zhang; MMed**, Physician; **Jun Wang, MMed**, Physician; **Yanxin Xiao, MD**, Physician; Department of Endocrinology, Baoding No. 1 Central Hospital, Baoding, Hebei, China.

Corresponding author: Yangyang Yuan, MMed
E-mail: m15612199379@163.com

Diabetic nephropathy (DN) is a common microvascular complication in diabetic patients.[1] When the disease becomes hard to control, it's the second leading cause of chronic renal failure and an important cause of death in diabetic patients.[2]

As one of the most common and serious complications in diabetic patients, DN involves many factors and links in its occurrence and development process, and it can eventually lead to irreversible, progressive kidney damage; renal failure; and an increase in disability and fatality for diabetic patients. Its diagnosis and treatment have received extensive clinical attention.[3]

In recent years, clinicians have commonly used the urinary aldosterone excretion rate (UAE), 24-hour urinary-protein quantification, and levels of serum creatinine to assess the degree of renal injury in DN. However, the early stage of DN lacks clinical manifestations, with the disease being hidden, and the above indexes can still be in the normal range at that point. The concentration of DN increases significantly only in the case of severe renal injury, which often leads to clinicians missing the best treatment opportunity. An effective means for early diagnosis and treatment of DN in clinical practice is still lacking.[4] It's very important to find simple, rapid, and sensitive indicators and methods for the detection of early DN that are clinically feasible

The pathogenesis of type 2 diabetes mellitus (T2DM) and the pathogenesis of DN are complex. Wang et al found that the occurrence and development of T2DM and its complications involved a variety of inflammatory cytokines.[5] Donate-Correa et al found that the pathogenesis of DN may be related to various factors, such as abnormal glucose metabolism, renal hemodynamic changes, oxidative stress, cytokines, inflammatory responses, and genetic factors, and that inflammatory response may play a key role as a common downstream pathway.[6]

Inflammatory Factors

Nucleotide binding oligomeric domain-like receptor protein 3 (NLRP3) is an important regulatory protein involved in inflammation and apoptosis. After activation, NLRP3 is assembled into NLRP3 inflammasome, which can further promote the secretion and release of interleukin-1 beta (IL-1β), IL-18, and other inflammatory factors as well as various effector molecules, causing inflammation.[7]

Wada and Makino found that NLRP3 is involved in DN's occurrence and development.[8] At present, NLRP3 inflammasome dominates the inflammatory mechanism, and clinicians have universally recognized its important role in promoting the release of downstream inflammatory factors and mediating the occurrence and development of DN.[9]

NLRP3 is the core protein of the inflammasome in a protein complex consisting of NLRP3, apoptosis-related speck-like protein (ASC), and aspartic acid-specific cysteine proteinase-1 (caspase-1). Wu et al and Yaribeygi et al reported in animal experiments that the pathological changes of DN could significantly decrease after loss or drug inhibition of NLRP3 expression.[10,11] Wu et al also found significantly increased NLRP3 expression in the renal tissues of patients with T2DM and significantly increased serum IL-1β and IL-18 content in patients with positive NLRP3.[12]

NLRP3 activated by high blood sugar can activate Caspase-1 through ASC and promote release of IL-1β and IL-18, to participate in the oxidative-stress and inflammatory-reaction processes; promote renal, tubule interstitial fibrosis; reduce the glomerular filtration rate; and ultimately damage the kidneys.[13,14] At the same time, two studies have shown that activated NLRP3 can directly induce apoptosis of renal, tubular epithelial cells and participate in the process of apoptosis in DN.[15,16]

Current Study

The current study intended to explore the relationship between the levels of plasma NLRP3, IL-1β, and IL-18 and the progression of type 2 diabetic nephropathy to clarify their relationship with T2DM and to provide evidence for clinical treatment.

METHODS

Participants

The research team performed an observational study, which took place at Baoding No. 1 Central Hospital in Baoding, Hebei, China. Potential participants for the five observation groups were patients with T2DM that was complicated with different stages of diabetic nephropathy (DN), who received treatment at the hospital between October 2020 and October 2021. Potential participants for a control group were patients with T2DM without evidence of DN who received treatment in the same period.

The study included potential participants in one of the groups if they met the diagnostic criteria for T2DM in the *Chinese Guidelines for the Prevention and Treatment of Type 2 Diabetes* (*2016 Edition*)[17]. The study included potential participants in one of the DN groups if they met the diagnostic criteria for DN that the Nephropathy Branch of the Chinese Association of Traditional Chinese Medicine formulated in 2007.[18]

The study excluded potential participants from any of the groups if they: (1) had glomerulonephritis, hypertensive kidney damage, or other renal diseases, (2) had recently used nephrotoxic drugs and performed vigorous exercise; (3) had diabetic ketosis or infection; or (4) had heart failure, a blood-system disease, an immune-system disease, tumors, or other systemic disease.

All patients agreed to participate in the study and signed informed consent forms. The hospital's Ethics Committee (Approval No: 2020-025) approved the study's protocols.

Procedures

Groups. The research team assigned participants to the five observation groups according to their urinary microalbumin and renal function, based on the Mogensen clinical staging method for nephropathy[19]: (1) stage 1, the DN1 group; (2) stage 2, the DN2 group; (3) stage 3, the DN3 group; (4) stage 4, the DN4 group; and (5) stage 5 the DN5 group. The research team assigned participants to the control group if they didn't have DN.

Data collection. All patients fasted for at least 12 h, and the research team drew 5 ml of participants' venous blood in the early morning. The team divided the blood samples into two parts: (1) used one part for evaluation of participants' renal function using an automatic biochemical analyzer (Hitachi 7600, Tokyo, Japan) and (2) centrifuged the other part at 3000 r/min for 15 min, stored it in a refrigerator at -80°C, and then used the samples to measure the NLRP3, IL-1β and IL-18 in the plasma, using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA)

Table 1. Comparison of Demographic Characteristics of the Six Groups

Characteristic	Control Group n = 30 n (%) Mean ± SD	DN1 Group n = 32 n (%) Mean ± SD	DN2 Group n = 31 n (%) Mean ± SD	DN3 Group n = 30 n (%) Mean ± SD	DN4 Group n = 30 n (%) Mean ± SD	DN5 Group n = 29 n (%) Mean ± SD
Gender						
Male	16 (53.33)	16 (50.00)	17 (54.84)	16 (53.33)	16 (53.33)	16 (55.17)
Female	14 (46.67)	16 (50.00)	14 (45.16)	14 (46.67)	14 (46.67)	13 (44.83)
Age	55.70 ± 9.32	54.03 ± 9.77	55.03 ± 9.23	55.83 ± 8.67	56.03 ± 9.21	56.69 ± 7.85
BMI	27.45 ± 2.18	27.39 ± 2.02	27.65 ± 2.29	27.58 ± 2.13	27.54 ± 2.16	27.50 ± 2.25

Abbreviations: BMI, body mass index; DN, diabetic nephropathy; DN1, stage 1; DN2, stage 2; DN3, stage 3; DN4, stage 4; DN5, stage 5.

Table 2. Comparison of the Levels of NLRP3, IL-1β, and IL-18 Between the Diabetic Nephropathy (DN) and Control Groups

Factors	Control Group n = 30 Mean ± SD	DN1 Group n = 32 Mean ± SD	DN2 Group n = 31 Mean ± SD	DN3 Group n = 30 Mean ± SD	DN4 Group n = 30 Mean ± SD	DN5 Group n = 29 Mean ± SD
NLRP3	100.75 ± 2.54	130.57 ± 2.25	150.69 ± 2.30	200.63 ± 2.38	260.63 ± 2.28	380.63 ± 2.33
IL-1β	4.52 ± 1.48	13.46 ± 1.37	17.59 ± 1.41	23.62 ± 1.59	37.58 ± 1.44	53.56 ± 1.50
IL-18	7.62 ± 2.34	15.60 ± 2.24	22.60 ± 2.28	35.61 ± 2.33	45.62 ± 2.35	56.61 ± 2.34
		Comparison: Control and D-1 Groups	**Comparison: Control and D-2 Groups**	**Comparison: Control and D-3 Groups**	**Comparison: Control and D-4 Groups**	**Comparison: Control and D-5 Groups**
NLRP3	*P* value	<.01[a]	<.01[a]	<.01[a]	<.01[a]	<.01[a]
IL-1β	*P* value	<.01[a]	<.01[a]	<.01[a]	<.01[a]	<.01[a]
IL-18	*P* value	<.01[a]	<.01[a]	<.01[a]	<.01[a]	<.01[a]
		Comparison: D-1 and D-2 Groups	**Comparison: D-1 and D-3 Groups**	**Comparison: D-1 and D-4 Groups**	**Comparison: D-1 and D-5 Groups**	**Comparison: D-2 and D-3 Groups**
NLRP3	*P* value	<.01[b]	<.01[b]	<.01[b]	<.01[b]	<.01[c]
IL-1β	*P* value	<.01[b]	<.01[b]	<.01[b]	<.01[b]	<.01[c]
IL-18	*P* value	<.01[b]	<.01[b]	<.01[b]	<.01[b]	<.01[c]
		Comparison: D-2 and D-4 Groups	**Comparison: D-2 and D-5 Groups**	**Comparison: D-3 and D-4 Groups**	**Comparison: D-3 and D-5 Groups**	**Comparison: D-4 and D-5 Groups**
NLRP3	*P* value	<.01[c]	<.01[c]	<.01[d]	<.01[d]	<.01[e]
IL-1β	*P* value	<.01[c]	<.01[c]	<.01[d]	<.01[d]	<.01[e]
IL-18	*P* value	<.01[c]	<.01[c]	<.01[d]	<.01[d]	<.01[e]

[a]*P* < .01, indicating that the levels of NLRP3, IL-1β, and IL-18 were significantly higher in the DN1, DN2, DN3, DN4, and DN5 groups than those of the control group

[b]*P* < .01, indicating that the levels of NLRP3, IL-1β, and IL-18 were significantly higher in the DN2, DN3, DN4, and DN5 groups than those of the DN1 group

[c]*P* < .01, indicating that the levels of NLRP3, IL-1β, and IL-18 were significantly higher in the DN3, DN4, and DN5 groups than those of the DN2 group

[d]*P* < .01, indicating that the levels of NLRP3, IL-1β, and IL-18 were significantly higher in the DN4 and DN5 groups than those of the DN3 group

[e]*P* < .01, indicating that the levels of NLRP3, IL-1β, and IL-18 were significantly higher in the DN5 group than those of the DN4 group

Abbreviations: DN1, stage 1; DN2, stage 2; DN3, stage 3; DN4, stage 4; DN5, stage 5; IL-1β, interleukin-1 beta; NLRP3, nucleotide binding oligomeric domain-like receptor protein 3

Table 3. Correlation of NLRP3, IL-1β and IL-18 With the Stage of T2DM Nephropathy

	NLRP3		IL-1β		IL-18	
Correlating Item	**R Value**	***P* value**	**R Value**	***P* value**	**R Value**	***P* value**
T2DM Nephropathy Stage	0.952	.01[a]	0.968	.01[a]	0.987	.01[a]

[a]*P* < .01, indicating that NLRP3, IL-1β, and IL-18 were significantly correlated with the T2DM nephropathy stage

Abbreviations: IL-1β, interleukin-1 beta; NLRP3, nucleotide binding oligomeric domain-like receptor protein 3; T2DM, type 2 diabetes mellitus.

with a kit purchased from Shanghai Enzyme-linked Biotechnology (Shanghai, China), following the kit's instructions strictly. In addition, taking the midstream urine of the experimental and control participants and measured the microalbumin using a BA400 automatic special protein instrument (Biosystems, Barcelona, Spain).

Outcome measures. The research team measured participants' levels of NLRP3, IL-1β, and IL-18 and performed a correlation analysis to determine the correlation between the NLRP3, IL-1β, and IL-18 levels and the stage of diabetic nephropathy.

Outcome Measures

The levels of NLRP3, IL-1β and IL-18 were compared between the observation group and the control group at different stages. Spearman grade correlation analysis was used to observe NLRP3, IL-1β, IL-18 levels and their correlation with diabetic nephropathy stage.

Statistical Analysis

The research team used SPSS 19.0 software (IBM Corporation, New York, USA) for analysis of the data. The team: (1) expressed measurement data as means ± standard deviations (SDs) and used the t test to compare data between the groups; (2) expressed counting data as numbers (N) and percentages (%) and used the chi squared test (χ^2) to compare data between the groups, and (3) used the Spearman rank correlation analysis determine the correlation. $P < .05$ was considered to be statistically significant.

RESULTS

Participants

No significant differences existed in gender, age, or body mass index (BMI) between the six groups ($P > .05$), indicating comparability (Table 1).

NLRP3, IL-1β, and IL-18 Levels

Table 2 shows that the levels of NLRP3, IL-1β and IL-18 in all the five observation groups were significantly higher than those in the control group (all $P < .01$).The higher the DN stage was, the higher the levels of NLRP3, IL-1β and IL-18 were. The levels of NLRP3, IL-1β, and IL-18 were significantly higher: (1) in the DN2, DN3, DN4, and DN5 groups than those of the DN1 group (all $P < .01$); (2) in the DN3, DN4, and DN5 groups than those of the DN2 group (all $P < .01$); (3) in the DN4 and DN5 groups than those of the DN3 group (all $P < .01$); and (4) in the DN5 group than those of the DN4 group (all $P < .01$).

Correlation Analysis

Table 3 shows that the NLRP3, IL-1β, and IL-18 levels were significantly positively correlated with the stages of DN (all $P = .01$).

DISCUSSION

The current study showed that serum the NLRP3, IL-1β, and IL-18 levels in the five DN groups were significantly higher than those in the T2DM group without DN, the control group. Therefore, those inflammatory factors have significance in the diagnosis of DN patients. In participants in the early stages of DN, the NLRP3, IL-1β and IL-18 levels were significantly higher than those of the in the control group, suggesting early diabetic kidney injury, which is significant in early diagnosis. These results also consisted with the Kim et al report.[20] At the same time, the NLRP3, IL-1β and IL-18 levels showed a significant increasing trend with the progression of DN stages, indicating that the above indexes could be increasing the severity of the DN, which may serve as a reference for the DN stages.[21]

The Spearman correlation analysis found a correlation between the NLRP3, IL-1β and IL-18 levels and DN, and those indicator levels were positively correlated with the stages of DN, As said in the review,the inflammatory response has an important role in the pathophysiology of diabetic nephropathy.[22] Some former studies also found the occurrence of inflammatory reaction can promote the aggravation of DN-induced kidney injury,and activation of the NOD-like receptor thermal protein domain associated protein 3 inflammasome can not only lead to the occurrence of inflammatory response, but also induce pyroptosis[23],which indicating that NLRP3 played an important role in the development of DN in patients.

IL-18/IL-1β is expressed in renal tissue and is upregulated by several stimuli including hyperglycemia. The expression/urinary level of IL-18/IL-1β is positively correlated with the progression of diabetic nephropathy and the urinary albumin excretion rate.[23] Deng et al found the serum IL-1β and IL-18 content of NLRP3 positive patients also increased significantly.[24] All results indicated these factors may become targets for early diagnosis and treatment of diabetic nephropathy patients.

Although this study confirmed the association of NLRP3, IL-1β, and IL-18 with type 2 diabetic nephropathy, there were some limitations. Firstly, the sample size was limited, which just met the minimum requirements. Secondly, the literature was limited to make proper comparisons.From another perspective, it highlighted the importance of pioneering research. Efforts shall be made to conduct studies with larger sample sizes in the future.

CONCLUSIONS

NLRP3, IL-1β and IL-18 played an important role in the progression of T2DM, and their levels increased with the aggravation of DN. Therefore, the plasma levels of NLRP3, IL -1β, and IL-18 can be useful as indicators of the occurrence and development of DN in clinical practice and can provide clinical guidance for the early diagnosis of DN and for the determination and adjustment of treatment plans.

AUTHORS' DISCLOSURE STATEMENT
The authors all declared none of the conflict of interest.

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