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Clinical Significance of the Expression profiles of P2X7R, NLRP3 and CXCL16 in Patients with Gouty Arthritis-Induced Kidney Injury

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ABSTRACT The researchers aimed to study the clinical significance of the expression profiles of purinergic ligand-gated ion channel 7 receptor (P2X7R), nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) and C-X-C motif chemokine receptor 16 (CXCL16) in patients with gouty arthritis (GA)-induced kidney injury (KI). A total of 120 GA patients admitted between January 2020 and January 2022 were enrolled. The P2X7R, NLRP3 mRNA and CXCL16 levels of the KI group had negative correlations with eGFR and positive correlations with Scr and CysC ($P<0.05$). The areas under the receiver operator characteristic curves of P2X7R, NLRP3 mRNA, and CXCL16 indicated that the three indices had high sensitivity and specificity for the diagnosis of GA-induced KI, and the combined detection was most effective. GA-induced KI patients have elevated levels of P2X7R, NLRP3 mRNA and CXCL16. Hence, these indices are valuable for the early diagnosis of GA-induced KI.

INTRODUCTION

Gouty arthritis (GA) is triggered by the deposition of urate crystals in joints and other tissues (Cabãu et al. 2020). Its main clinical manifestations include gouty nephropathy, subcutaneous tophus, and urinary calculi. In severe cases, GA may be manifested as joint deformity and even dysfunction, serious influencing the life quality as well as physical and mental health of patients (Macaya et al. 2015; Marin et al. 2018). GA is one of the most common inflammatory arthritis in clinical practice and mostly occurs in middle-aged males (Evavold et al. 2019), with a prevalence of 1.1 percent in China (Liu et al. 2015). Reflecting the onset of GA to a certain extent, GA-induced kidney injury (KI) aggravates the disease and makes the treatment more difficult (Zhang et al. 2022). Hence, it is necessary to find indices suitable for the early diagnosis of GA-induced KI.

As an ion channel protein distributed in many types of tissues and organs, purinergic ligand-gated ion channel 7 receptor (P2X7R) can participate in the inflammatory response of GA (Shi et al. 2017). Besides, nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) is a molecular target for the treatment of many diseases, which can regulate the inflammatory response of the human body by interacting with caspase-1 to form the inflammasome (Swanson et al. 2019). C-X-C motif chemokine receptor 16 (CXCL16), as a key member of the chemokine family, plays a mediating role in the accumulation of mononuclear cells, with high expression level in the synovial tissue of patients with rheumatoid arthritis (Aglietti et al. 2017). At present, the expressions of P2X7R, NLRP3, and CXCL16 in GA-induced KI patients and their values for diagnosis and treatment have rarely been reported.

Objectives

The aim of this study was to provide a basis for the clinical diagnosis and treatment of GA-induced KI by detecting the expressions of P2X7R, NLRP3 and CXCL16.

MATERIAL AND METHODS

General Data

A total of 120 GA patients admitted to our hospital from January 2020 to January 2022 were enrolled according to the following inclusion criteria: (1) all patients met the diagnosis criteria

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in "*Guidelines for the Diagnosis and Treatment of Primary Gout*" (Chinese Rheumatology Association 2011), (2) they signed the informed consent after being informed of the research methods and purpose, and (3) the study was approved by the ethics committee of our hospital. The exclusion criteria were as follows: (1) patients with chronic nephritis, complicated renal failure, history of renal surgery or urinary system diseases, (2) those who had to take long-term medication due to illness which affected their renal function and metabolism, or (3) those complicated with severe trauma, infection, hypertension, diabetes mellitus or malignant tumors.

According to the Kidney Disease Improving Global Outcomes criteria (Stevens et al. 2013), the enrolled patients were divided into a KI group (n=52) and a non-KI (NKI) group (n=68). Meanwhile, 60 healthy individuals admitted to the hospital for physical examination in the same period were included as a control group. The KI group consisted of 45 males and 7 females [34-75 years old, (46.85±4.62) years old on average], with 20 at the acute exacerbation stage of GA and 32 at the complete remission stage. The NKI group was composed of 60 males and 8 females [36-77 years old, (47.12±4.54) years old on average], with 30 at the acute exacerbation stage of GA and 38 at the complete remission stage. Moreover, the control group consisted of 48 males and 12 females [35-78 years old, (45.96±4.70) years old on average]. The three groups had comparable general data (P>0.05).

Collection and Treatment of Specimens

First, 3 mL of fasting peripheral venous blood was drawn from each subject in the morning. After 10 minutes of centrifugation (3,000 r/min), the supernatant was collected and stored at -80°C prior to use. Strictly following the kit's instructions (R&D Systems, USA), the enzyme-linked immunosorbent assay was performed to determine the levels of serum P2X7R and CXCL16.

Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

First, 3 mL of venous blood was extracted from each subject, placed in an anticoagulant tube containing ethylene diamine tetraacetic acid, and then added an equal volume of lymphocyte separation medium. After being uniformly mixed, the solution was centrifuged to obtain PBMCs.

Detection of Relative Expression Level of NLRP3 mRNA in PBMCs

The test tube containing PBMCs was added 1 mL of TRIzol lysis buffer, followed by high-speed centrifugation to extract total RNA and to determine its content. RNA reverse transcription was conducted strictly following the instructions of corresponding kit (Thermo Fisher Scientific, USA). The primer sequences are listed in Table 1. The relative expression level of NLRP3 mRNA was calculated by ΔΔCT, and GAPDH gene was used as the internal reference.

Table 1: Primer sequences

Gene	*Primer sequence*
NLRP3	Forward: 5'-CATGAGTGCTGCTTCGACAT-3'
	Reverse: 5'-GCTTCAGTCCCACACAGA-3'
GAPDH	Forward: 5'-AGACAGCCGCATCTTCTTGT-3'
	Reverse: 5'-CTTGCCGTGGGTAGAGTCAT-3

Detection of Renal Function

AU5400 automatic biochemical analyzer (Olympus, Japan) was used to detect the levels of serum creatinine (Scr) and cystatin C (CysC).

Observation Indices

Multi-group comparison was performed for the levels of P2X7R, NLRP3 mRNA, CXCL16 and indices related to renal function. Pearson's correlation analysis was conducted to explore the correlations of the levels of P2X7R, NLRP3 mRNA and CXCL16 with indices related to the renal function of the KI group. Receiver operating characteristic (ROC) curves were plotted to predict the values of P2X7R, NLRP3 mRNA and CXCL16 for the diagnosis of GA-induced KI. Based on the ROC curves, the specificity, sensitivity and Youden index were calculated.

Statistical Analysis

Statistical analysis was conducted using SPSS 20.0 software. The expression levels of

P2X7R, NLRP3 mRNA, and CXCL16 and other measurement data were expressed as ($\chi \pm s$) and subjected to the *t* test, and the count data were subjected to the chi-square (χ^2) test. The correlations between factors were examined with Pearson's correlation analysis. Analysis of variance and LSD-t test were applied to multigroup and pairwise comparisons, respectively. ROC curves were plotted to predict the values of P2X7R, NLRP3 mRNA and CXCL16 for the diagnosis of GA-induced KI. The statistically significant difference between variables was confirmed when P<0.05.

RESULTS

P2X7R, NLRP3 mRNA and CXCL16 Levels of Three Groups

The KI group had significantly higher levels of P2X7R, NLRP3 mRNA and CXCL16 than those of NKI and control groups (P<0.01). The levels of the three indices in the NKI group were significantly higher than those of the control group (P<0.01) (Table 2).

Renal Function-related Indices of Three Groups

Compared with NKI and control groups, the KI group had a significantly lower eGFR level and higher levels of Scr and CysC (P<0.01). NKI and control groups had similar levels of eGFR, Scr and CysC (P>0.05) (Table 3).

Correlations of P2X7R, NLRP3 mRNA and CXCL16 with Renal Function-related Indices of the KI Group

P2X7R, NLRP3 mRNA and CXCL16 had negative correlations with eGFR (P<0.05), and positive correlations with Scr and CysC (P<0.05) in the KI group (Table 4).

Values of P2X7R, NLRP3 mRNA and CXCL16 for Diagnosis of GA-induced KI

As revealed by ROC curves, P2X7R, NLRP3 mRNA and CXCL16 all had high diagnostic sensitivity and specificity for GA-induced KI, and the combined detection using these indices was

Table 2: P2X7R, NLRP3 mRNA and CXCL16 levels of three groups (χ±s)

Group	*n*	*P2X7R (pg/mL)*	*NLRP3 mRNA*	*CXCL16 (ìg/L)*
KI group	52	668.45± 302.47	2.59± 0.42	2.96± 0.45
NKI group	68	429.68± 125.64	1.92± 0.38	1.98± 0.10
Control group	60	360.57± 82.59	0.98± 0.22	1.12± 0.08
F		41.480	303.306	733.781
P		<0.001	<0.001	<0.001

Table 3: Renal function-related indices of three groups (χ±s)

Group	*n*	*P2X7R (pg/mL)*	*NLRP3 mRNA*	*CXCL16 (ìg/L)*
KI group	52	71.63±11.96	91.68± 25.47	2.95± 0.89
NKI group	68	97.85± 6.89	44.96± 5.20	0.96± 0.30
Control group	60	100.72± 6.75	43.28± 4.91	0.88± 0.25
F		202.485	203.584	268.797
P		<0.001	<0.001	<0.001

Table 4: Correlations of P2X7R, NLRP3 mRNA and CXCL16 with renal function-related indices among patients in KI group

Index	*P2X7R*		*NLRP3 mRNA*		*CXCL16*	
	r	*P*	*r*	*P*	*r*	*P*
eGFR	-0.534	0.000	-0.562	0.000	-0.633	0.000
Scr	0.429	0.022	0.602	0.000	0.467	0.014
CysC	0.417	0.025	0.563	0.000	0.451	0.016

more valuable for diagnosis than the detection using any single index (Table 5 and Fig. 1).

DISCUSSION

GA is an acute/chronic metabolic disease induced by various factors, such as heredity, lifestyle, and the environment, mostly by purine metabolism disorders, abnormal increase of serum uric acid, and urate deposition (Veerappan et al. 2017; Zheng et al. 2018). The disease is manifested as acute or chronic arthritis, as well as pain and even deformity in joints. Without timely treatment, it may damage the kidneys, thus triggering chronic interstitial nephritis, acute and chronic renal failure and even uremia (Steinbach et al. 2018). The pathogenesis of GA-induced KI is related with the deposition of urate in renal tissue due to the decreased urate clearance rate in renal tubules (Charmoy et al. 2016). At the early stage of GA, the urine of patients does not change evidently, thus easily causing missed diagnosis, misdiagnosis and even delay in treatment (Lin et al. 2020). To improve GA diagnosis and treatment, it is now crucial to find reliable early diagnostic indices.

Table 5: Value of P2X7R, NLRP3 mRNA and CXCL16 for diagnosis of GA-induced KI

Variable	*AUC*	*P*	*Specificity*	*Sensitivity*	*Youden index*
P2X7R	0.787	<0.001	0.734	0.770	0.504
NLRP3 mRNA	0.878	<0.001	0.853	0.866	0.719
CXCL16	0.823	<0.001	0.792	0.816	0.608
Combined detection	0.964	<0.001	0.918	0.942	0.860

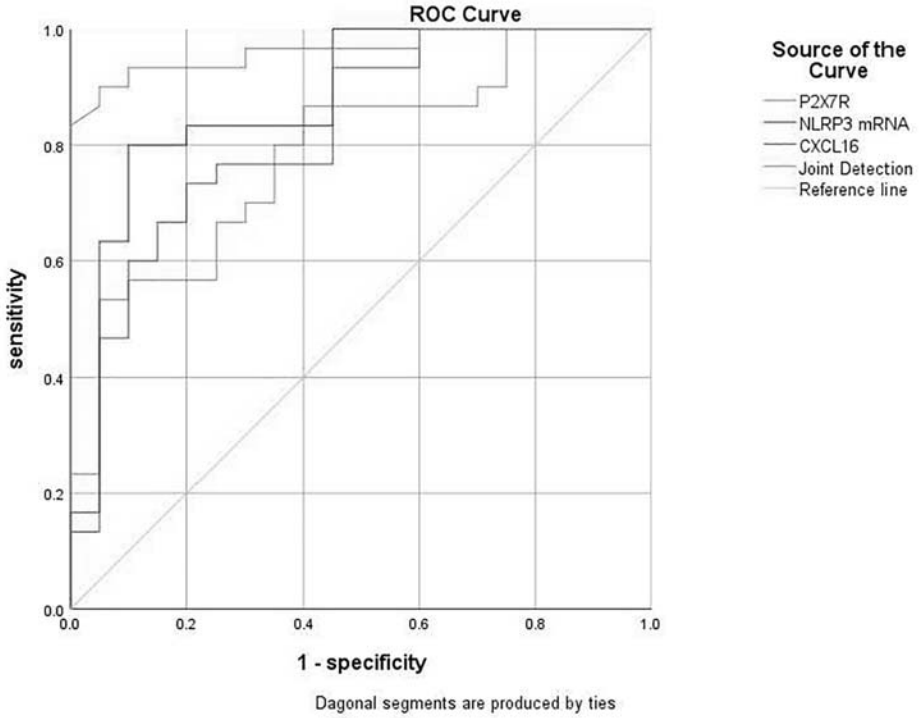

Fig. 1. ROC curves of P2X7R, NLRP3 mRNA, and CXCL16 for diagnosis of GA-induced KI

P2X7R participates in the expressions of inflammatory response mediators in the synovial tissue of joints (Li et al. 2023). The expression level of P2X7R in GA patients has been correlated with multiple inflammatory factors and the erythrocyte sedimentation rate (Pirzada et al. 2020). Under the stimuli of ATP, P2X7R binds panx-1 to induce the formation of non-selective pores (Kiçik et al. 2019; Marín-Aguilar et al. 2020). P2X7R, as an upstream molecule of NLRP3, is only expressed in a variety of cells including neutrophils, dendritic cells, and microglia. It also regulates the expression of NLRP3 at the protein and mRNA levels (Smola et al. 2017). Nonetheless, whether NLRP3 exerts a direct effect on NLRP3 inflammasome remains unclear.

NLRP3 inflammasome is a macromolecular multi-protein complex composed of NLRP3, caspase-1 (effector protein), and apoptosis-associated speck-like protein containing a CARD (adaptor protein). It plays a crucial role in the treatment of diseases such as cardiovascular disease, lung injury, GA, and Parkinson's syndrome (Han et al. 2016; Xing et al. 2018). The possible mechanism of NLRP3 participating in the inflammatory response of GA is as follows. The inhibition of NLRP3 is relieved under the stimulation of ATP and monosodium urate to induce oligomerization, completing inflammasome assembly through recruiting adaptor protein and pro-Caspase-1 containing CARD to provide a platform for the generation and secretion of various inflammatory response mediators, and also participating in the prevention and treatment of GA inflammatory responses by impeding the assembly or activation of inflammatory complexes (Lee et al. 2016).

Expressed on the surface of various cells including macrophages, fibroblasts, and dendritic cells, CXCL16 exists as a soluble molecule or binds the membrane of the cell surface, which plays an essential role in GA onset and progression (Le et al. 2019). In this study, the levels of P2X7R, NLRP3 mRNA and CXCL16 in the KI group were significantly higher than those of NKI and control groups, and the NKI group had significantly higher levels of these indices than those of the control group. Thus, the detection using P2X7R, NLRP3 mRNA, and CXCL16 levels in GA patients had high values for diagnosing KI and evaluating the renal function. In addition, the KI group had a significantly lower level of eGFR and higher levels of Scr and CysC than those of NKI and control groups, which indicated the existence of severe KI in GA patients, being consistent with a previous literature (Xiao et al. 2019).

Possibly, the increase of P2X7R activates mononuclear macrophages, drives the differentiation and maturation of inflammatory cells, and participates in the synthesis and release of various inflammatory factors playing crucial roles in the occurrence of GA-induced KI (Liu et al. 2022). Moreover, the elevation of CXCL16 level in patients with GA-induced KI may be a stress response mostly originating from metabolic changes (Kim et al. 2023). Given the role of NLRP3 inflammasome in regulating inflammatory response, its relative expression level can reflect the response intensity of GA patients (Yang et al. 2020). The rising expression level of NLRP3 mRNA further activates the downstream inflammatory pathway, contributes to the apoptosis or pyroptosis of renal tubular cells, leads to the imbalance between protease and anti-protease, and degrades the extracellular matrix (Wen et al. 2021). In this study, P2X7R, NLRP3 mRNA, and CXCL16 all had high diagnostic sensitivity and specificity for GA-induced KI, and the combined detection had a higher diagnostic value than that of the detection using any single index. Hence, these indices can be used as biomarkers for the early diagnosis of GA-induced KI.

CONCLUSION

In conclusion, the increasing P2X7R, NLRP3 mRNA and CXCL16 levels of patients with GA-induced KI have close correlations with renal function. These indices have high values for the diagnosis of GA-induced KI and can be used as early diagnostic indices.

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The first two authors contributed equally to this study.

RECOMMENDATIONS

Further multicenter studies with larger sample sizes are in need to verify the roles of P2X7R, NLRP3 mRNA and CXCL16 in the early diagnosis of GA-induced KI.

ABBREVIATIONS

CysC: Cystatin C; CXCL16: C-X-C motif chemokine receptor 16; GA: gouty arthritis; KI: kidney injury; NKI: non-KI; P2X7R: purinergic ligand-gated ion channel 7 receptor; PBMCs: peripheral blood mononuclear cells; ROC: receiver operating characteristic; Scr: serum creatinine.

REFERENCES

Aglietti RA, Dueber EC 2017. Recent insights into the molecular mechanisms underlying pyroptosis and gasdermin family functions. *Trends Immunol,* 38: 261-271.

Cabãu G, Crisan TO, Klück V et al. 2020. Urate induced immune programming: Consequences for gouty arthritis and hyperuricemia. *Immunol Rev,* 294: 92-105.

Charmoy M, Hurrell BP, Romano A et al. 2016. The Nlrp3 inflammasome, IL-1β, and neutrophil recruitment are required for susceptibility to a nonhealing strain of Leishmania major in C57BL/6 mice. *Eur J Immunol,* 46: 897-911.

Chinese Rheumatology Association 2011. Guidelines for the diagnosis and treatment of primary gout. *Chin J Rheumat,* 15: 410-413.

Evavold CL, Kagan JC 2019. Defying Death: The (W)hole truth about the fate of GSDMD pores. *Immunity,* 50: 15-17.

Han Q, Bing W, Di Y et al. 2016. Kinsenoside screening with a microfluidic chip attenuates gouty arthritis through inactivating NF-κB signaling in macrophages and protecting endothelial cells. *Cell Death Dis,* 7: e2350.

Kiçik A, Tüzün E, Erdoðdu E et al. 2019. Neuroinflammation mediators are reduced in sera of Parkinson's disease patients with mild cognitive impairment. *Noro Psikiyatr Ars,* 57: 15-17.

Kim SK, Choe JY, Park KY 2023. CXCL12 and CXCR4 as novel biomarkers in uric acid-induced inflammation and patients with gouty arthritis. *Biomedicines,* 11: 649.

Le Daré B, Victoni T, Bodin A et al. 2019. Ethanol upregulates the P2X7 purinergic receptor in human macrophages. *Fundam Clin Pharmacol,* 33: 63-74.

Lee SW, Lee SS, Oh DH et al. 2016. Genetic association for P2X7R rs3751142 and CARD8 rs2043211 polymorphisms for susceptibility of gout in Korean men: Multi-center Study. *J Korean Med Sci,* 31: 1566-1570.

Li X, Wan A, Liu Y et al. 2023. P2X7R Mediates the synergistic effect of ATP and MSU crystals to induce acute gouty arthritis. *Oxid Med Cell Longev,* 2023: 3317307.

Lin X, Shao T, Huang L et al. 2020. Simiao decoction alleviates gouty arthritis by modulating proinflammatory cytokines and the gut ecosystem. *Front Pharmacol,* 11: 955.

Liu L, Wang D, Liu M et al. 2022. The development from hyperuricemia to gout: Key mechanisms and natural products for treatment. *Acupunct Herb Med,* 2: 25-32.

Liu R, Han C, Wu D et al. 2015. Prevalence of hyperuricemia and gout in mainland China from 2000 to 2014: A systematic review and meta-analysis. *BioMed Res Int,* 2015: 762820.

Macaya F, Adrover A, Díaz I 2015. Periarticular nodules, arthritis and chronic nephropathy. *Reumatol Clin,* 11: 401-402.

Marin M, Maalouf NM 2018. Effects of pharmacological reversal of hyperuricemia on features of the metabolic syndrome in patients with gouty arthritis. *J Investig Med,* 66: 1031-1036.

Marín-Aguilar F, Lechuga-Vieco AV, Alcocer-Gómez E et al. 2020. NLRP3 inflammasome suppression improves longevity and prevents cardiac aging in male mice. *Aging Cell,* 19: e13050.

Pirzada RH, Javaid N, Choi S 2020. The roles of the NLRP3 inflammasome in neurodegenerative and metabolic diseases and in relevant advanced therapeutic interventions. *Genes,* 11: 131.

Shi J, Gao W, Shao F 2017. Pyroptosis: Gasdermin-mediated programmed necrotic cell death. *Trends Biochem Sci,* 42: 245-254.

Smola S, Trimble C, Stern PL 2017. Human papillomavirus-driven immune deviation: Challenge and novel opportunity for immunotherapy. *Ther Adv Vaccines,* 5: 69-82.

Steinbach A, Riemer AB 2018. Immune evasion mechanisms of human papillomavirus: An update. *Int J Cancer,* 142: 224-229.

Stevens PE, Levin A 2013. Evaluation and management of chronic kidney disease: Synopsis of the kidney disease: Improving global outcomes 2012 clinical practice guideline. *Ann Intern Med,* 158: 825-830.

Swanson KV, Deng M, Ting JP 2019. The NLRP3 inflammasome: Molecular activation and regulation to therapeutics. *Nat Rev Immunol,* 19: 477-489.

Veerappan Ganesan AP, Eisenlohr LC 2017. The elucidation of non-classical MHC class II antigen processing through the study of viral antigens. *Curr Opin Virol,* 22: 71-76.

Wen L, Yang H, Ma L 2021. The roles of NLRP3 inflammasome-mediated signaling pathways in hyperuricemic nephropathy. *Mol Cell Biochem,* 476: 1377-1386.

Xiao Q, Zhu X, Yang S et al. 2019. LPS induces CXCL16 expression in HUVECs through the miR-146a-mediated TLR4 pathway. *Int Immunopharmacol,* 69: 143-149.

Xing J, Liu Y, Chen T 2018. Correlations of chemokine CXCL16 and TNF-α with coronary atherosclerotic heart disease. *Exp Ther Med,* 15: 773-776.

Yang G, Lee HE, Moon SJ et al. 2020. Direct binding to NLRP3 pyrin domain as a novel strategy to prevent NLRP3 driven inflammation and Gouty Arthritis. *Arthrit Rheumatol,* 72: 1192-1202.

Zhang J, Yang M, Cheng X et al. 2022. Acute kidney injury in a patient with gout and polyarticular septic arthritis: A case report. *Int Urol Nephrol,* 54: 2755-2756.

Zheng R, Tao L, Jian H et al. 2018. NLRP3 inflammasome activation and lung fibrosis caused by airborne fine particulate matter. *Ecotoxicol Environ Saf,* 163: 612-619.

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