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Expressions and clinical significance of interleukin-1β and cyclooxygenase-2 in serum and synovia of patients with gouty arthritis

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Abstract

***Background**: To explore the expressions and clinical significance of interleukin-1β (IL-1β) and cyclooxygenase-2 (COX-2) in serum and synovia of patients with gouty arthritis (GA). **Materials and Methods**: A total of 110 GA patients hospitalized from January 2016 to September 2019 were selected as observation group, with 55 at remission stage (observation group I) and 55 at acute attack stage (observation group II). Another 55 healthy volunteers were selected as control group. The correlations of IL-1β and COX-2 with uric acid (UA) were explored by Spearman's analysis. IL-1β and COX-2 expression levels were compared at different time points after treatment. **Results**: IL-1β, COX-2 and UA levels of patients were significantly higher in observation group I and II than those in the control group, and higher in the observation group II than those in observation group I. IL-1β and COX-2 were positively correlated with UA ($P<0.05$). ROC curve analysis showed that the optimal cut-off values of IL-1β and COX-2 for predicting GA were 18.23 and 9.12 pg/mL, and AUC values were 0.702 and 0.714 ($P<0.001$), respectively. On admission and after treatment for 3, 7, and 10 days, IL-1β and COX-2 levels of observation group II significantly exceeded those of observation group I ($P<0.05$). Compared with the efficacy group, after treatment for 14 days, IL-1β and COX-2 levels in serum and synovia significantly increased in the non-efficacy group ($P<0.05$). **Conclusion**: IL-1β and COX-2 levels in serum and synovia of GA patients are evidently higher than those in the control group, which rise with the aggravation of disease.*

***Keywords**: Interleukin-1β, Cyclooxygenase-2, Gouty arthritis*

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Introduction

Gouty arthritis (GA) is a kind of metabolic rheumatism caused by urate depositing in joints and surrounding connective tissues due to purine metabolic disorder (1). Arthralgia is the most common initial symptom of GA, with lancinating and gnawing pains, affecting the life quality of patients (2). The complex pathogenesis of GA remains unclear, although there are several hypotheses regarding the main causes of GA, namely genetic factor, environment, immunization, endocrine causes, and diet (3). In recent years, the role of immunoreactions in GA onset receives more and more attention. Inflammatory factor is the important regulating factor of immune response. Inflammatory factor level is closely associated with severity of lesions in acute GA patients (4). Interleukin-1β (IL-1β) is a member of IL-1 family, playing an important regulating role in mechanism of immune inflammation. Besides, IL-1β can induce the degradation of extracellular matrix of chondrocytes, and plays an important role in the damage of cartilage in arthritis disease (5). Cyclooxygenase-2 (COX-2) is a kind of bifunctional enzyme, the key enzyme for synthesis of prostaglandin E2, while prostaglandin E2 is an important factor to participate in the inflammation of tissues around the joint, synovium, and other lesions, playing an important role in the occurrence and development of arthritis (6). This study aims to explore expressions of IL-1β and COX-2 levels in serum and synovium in GA patients and their guidance value for clinical intervention.

Materials and Methods

Clinical data

A total of 110 GA patients hospitalized from January 2016 to September 2019 were selected as observation group, of which, there were 82 male patients, 28 female patients, aged (56.31 ± 5.13) years old on average. Among them, there were 55 cases of remission stage (observation group I), and 55 cases of acute attack stage (observation group II).

Inclusion criteria:

1. Patients were diagnosed with GA in accordance with the *Gouty Arthritis Diagnosis Standard* formulated by American College of Rheumatology in 2015 (7), and
2. Patients and their family members signed the Informed Consent Form, and approvals were received from the Ethics Committee of our hospital.

Exclusion criteria:

1. GA patients with other arthritis diseases including osteoarthritis and rheumatoid arthritis,
2. those with severe hepatic and renal dysfunction or gastrointestinal disease,
3. those with malignant tumor, hematologic disease or autoimmune disease,
4. patients in pregnancy or lactation, or
5. GA patients with incomplete medical data.

Another 55 healthy volunteers who received physical examinations at the Physical Examinations Center of our hospital during the same period were enrolled as control group, and their age and gender were matched with those of GA patients.

Data collection

The basic data of all the subjects, including their age, gender, course of diseases, body mass index (BMI), and history of diabetes, hypertension, and coronary heart disease were collected.

Detection of laboratory indices

Fasting peripheral venous blood (5 mL) was collected from patients at acute attack stage in observation groups in the morning of admission and the 3rd, 7th, 10th, and 14th day after the treatment, and synovia was collected by puncture of articular cavity of the injured limb. Then, the supernatant of both samples was separately collected by centrifugation, whereas 5 mL peripheral venous blood was collected from patients at remission stage in observation groups

and patients in control group on the morning of the examination day, without puncture, and no synovial sample was collected.

Fasting plasma glucose (FPG), total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were obtained by conventional laboratory test, IL-1β and COX-2 levels in serum and synovia were tested by enzyme linked immunosorbent assay, and uric acid (UA) level was tested using uricase method.

Treatment methods

The same basic therapy and colchicine medication intervention were given to all the patients. 1) Basic therapy: best rest, keeping warm, low purine diet, alcohol prohibition, drinking water 3000 mL/d. Sodium Bicarbonate Tablets was administered (os, after meal, 1.0 g/time, 3 times/day). 2) Colchicine intervention: Colchicine was given at 0.5 mg/time, 3 times per day, and then the frequency was reduced to 2 times per day for 7 consecutive days and then 1 time per day for 4 consecutive days, followed by drug withdrawal. At the same time, etoricoxib was taken orally at 120 mg/time/day for three days and then 60 mg/time/day for seven days, and then withdrawn.

Evaluation of therapeutic effects

According to previous literature (8), the efficacy on acute GA patients (observation group II) was evaluated. Cured: clinical symptoms disappeared completely, joint function integral <5 points, and physicochemical indices were normal. Obviously effective: clinical symptoms were improved obviously, and 5 points ≤ joint function integral ≤ 7 points. Effective: clinical symptoms were somewhat improved, 7 points ≤ joint function integral ≤ 10 points. Ineffective: clinical symptoms were not improved, joint function integral >10 points. Cured, obviously effective and effective patients were divided as obviously effective group, while ineffective patients were divided as non-efficacy group. There were 90 patients in the obviously effective group, and 20 patients in non-obviously effective group.

Statistical analysis

SPSS 19.0 software was employed for data analysis. Quantitative data were expressed as mean ± standard deviation (SD), *t*-test was used for comparison between two groups, and *F*-test was adopted for comparison among multiple groups. Numerical data were expressed as frequency or ratio, and chi-square (χ^2) test was utilized for comparison between the two groups. The predictive values of IL-1β and COX-2 for GA were explored by plotting ROC curves. Spearman's correlation analysis was employed to study the correlations of IL-1β and COX-2 with UA. Two-sided P<0.05 suggested that the diffidence was statistically significant.

Results

Baseline clinical data

The baseline data of patients were compared among groups, and the results (**Table 1**) showed that there were no statistically significant differences in age, gender, course of disease and BMI among the groups (P>0.05). All patients in observation groups had history of diabetes, hypertension and coronary heart disease, while those in control group had no history of the above diseases.

Laboratory indices

Laboratory test indices were compared among all the groups (**Table 2**). The levels of FPG, LDL-C, IL-1β, COX-2 and UA in observation group I and II were higher than those in the control group (P<0.05), while HDL-C was lower than that in the control group (P<0.05). There were no statistically significant differences in FPG, LDL-C, and HDL-C between observation groups I and II (P>0.05). IL-1β, COX-2, and UA levels were significantly higher in observa-

Table 1. Baseline clinical data [`x ± s, n (%)]

Index	Observation group I (n=55)	Observation group II (n=55)	Control group (n=55)	F/χ^2	P
Age (years old)	56.28±5.32	56.39±5.13	56.24±5.14	0.843	0.278
Gender [male/female (n)]	37/18	38/17	39/16	0.170	0.918
Course of Disease (year)	8.04±0.45	8.03±0.34	-	0.031	0.896
BMI	22.01±1.13	21.96±1.24	21.89±1.21	1.362	0.457
History of diabetes	6(10.91)	5(9.09)	-	0.101	0.751
History of hypertension	6(10.91)	9(16.36)	-	0.695	0.406
History of coronary heart disease	4(7.27)	3(5.45)	-	0.153	0.696

Table 2. Laboratory test indices (`x ± s)

Index	Observation group I (n=55)	Observation group II (n=55)	Control group (n=55)
FPG (mmol/L)	6.72±2.08*	6.69±1.95*	6.35±1.42
TC (mmol/L)	4.31±1.03	4.37±1.02	4.28±1.02
TG (mmol/L)	1.43±0.74	1.44±0.73	1.29±0.62
LDL-C (mmol/L)	2.58±0.31*	2.69±0.32*	2.35±0.22
HDL-C (mmol/L)	1.17±0.42*	1.03±0.43*	1.44±0.42
IL-1β (pg/mL)	19.45±5.12*	34.13±5.69*#	15.28±4.12
COX-2 (pg/mL)	9.58±2.54*	18.34±2.29*#	8.35±2.12
UA (μmol/L)	367.24±91.45*	558.32±92.74*#	218.51±68.61

* P<0.05 *vs.* control group, # P<0.05 *vs.* observation group I.

tion group II than those in observation group I (P<0.05). The TC and TG levels displayed no statistically significant differences among all the groups (P>0.05). The results revealed that the FPG, LDL-C, HDL-C, IL-1β, COX-2 and UA levels in observation groups had differences with those in the control group, and with exacerbation of the disease in patients, IL-1β, COX-2, and UA showed an upward trend.

ROC curve analysis results of predictive values of IL-1β and COX-2 for GA

ROC curve analysis showed that the optimal cut-off values of serum IL-1β and COX-2 for predicting GA were 18.23 pg/mL and 9.12 pg/mL, and AUC values were 0.702 (95%CI: 0.687-0.812, P<0.001) and 0.714 (95%CI: 0.694-0.85, P<0.001), respectively (**Figure 1**). Thus, both their predictive values were both high.

Correlations of IL-1β and COX-2 with UA in serum

Correlations of IL-1β and COX-2 with UA were further explored using Spearman correlation analysis (**Figure 2**). The results uncovered that IL-1β and COX-2 had significantly positive correlations with UA. It showed that IL-1β and COX-2 may increase with the increase in UA expression level.

IL-1β and COX-2 levels in serum and synovia at different times after treatment

Changes in expressions of IL-1β and COX-2 in serum and synovia were compared at different times after treatment in observation groups I and II (**Table 3**). It was discovered that in observation groups I and II, the levels of IL-1β and COX-2 in serum and synovia on the 7th, 10th, and 14th day after the treatment were significantly lower than those on admission (P<0.05). On admission and

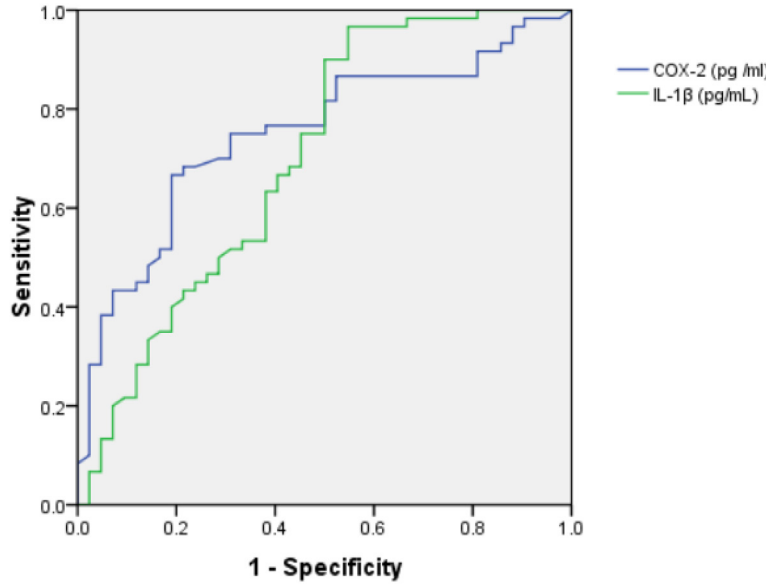

Fig. 1. ROC curve analysis results of predictive values of IL-1β and COX-2 for GA.

the 3rd, 7th, and 10th day after the treatment, the IL-1β and COX-2 levels in serum and synovia in observation group II were significantly higher than those in observation group I (P<0.05).

IL-1β and COX-2 levels in serum and synovia of patients with different therapeutic effects

The changes of IL-1β and COX-2 levels in serum and synovia of patients were compared between obviously effective group and non-obviously effective group on admission and the 14th day after the treatment. The results (**Table 4**) showed that there were no statistical differences in the IL-1β and COX-2 levels in serum and synovia between the two groups on admission (P>0.05). Compared with data on admission, IL-

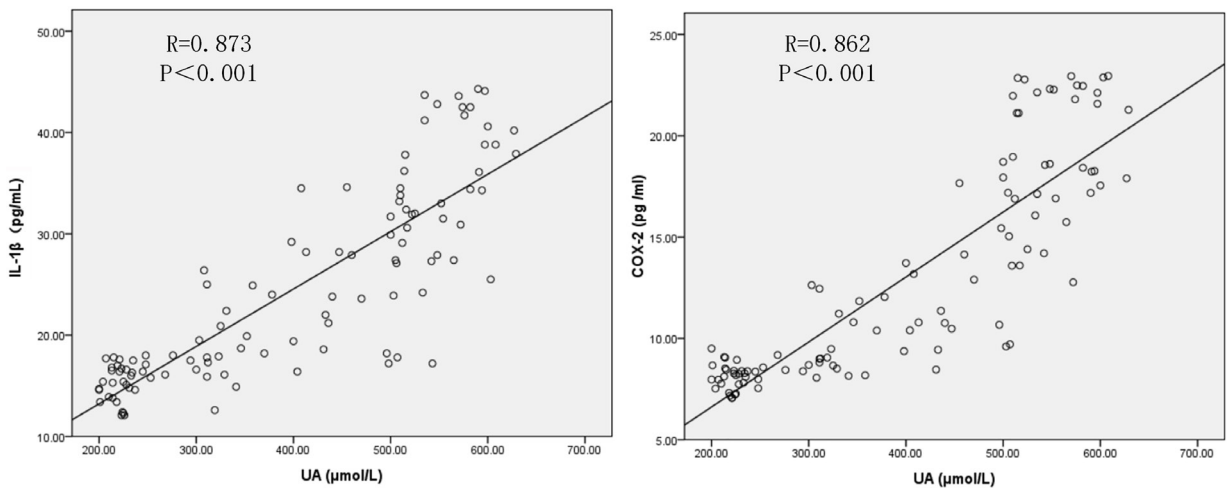

Fig. 2. Correlations of IL-1β and COX-2 with UA in serum.

Table 3. IL-1β and COX-2 levels in serum and synovia at different times after treatment (`x ± s)

Index	On admission	3 d after treatment	7 d after treatment	10 d after treatment	14 d after treatment
IL-1β in serum					
Observation Group I (n=55)	19.45±5.12	17.81±2.13	16.78±2.19*	16.53±3.24*	15.61±2.12*
Observation Group II (n=55)	34.13±5.69#	31.51±3.28#	25.09±3.56*#	19.14±3.23*#	18.45±2.01*
IL-1β in synovia					
Observation Group I (n=55)	35.11±3.36	33.32±3.16	27.54±3.11*	23.67±3.29*	18.43±2.51*
Observation Group II (n=55)	45.56±3.12#	41.12±3.23#	34.56±3.31*#	24.89±3.07*	19.47±3.11*
COX-2 in serum					
Observation Group I (n=55)	9.58±2.54	9.11±1.54*	8.74±1.22*	8.54±1.25*	8.34±1.24*
Observation Group II (n=55)	18.34±2.29#	17.98±1.98#	16.49±2.03*#	12.54±2.11*#	9.43±1.43*
COX-2 in synovia					
Observation Group I (n=55)	21.41±4.32	19.63±5.56	14.29±5.02*	11.20±5.08*	10.47±1.32*
Observation Group II (n=55)	32.54±4.23#	24.00±6.87#	19.48±6.21*#	14.45±5.67*#	11.52±1.05*

*P<0.05 *vs.* on admission, #P<0.05 *vs.* observation group I.

Table 4. IL-1β and COX-2 levels in serum and synovia of patients with different therapeutic effects (`x ± s)

Index	Obviously effective group (n=90)		Non-obviously effective group (n=20)	
	On admission	14 d after treatment	On admission	14 d after treatment
IL-1β in serum	28.11±5.43	16.07±2.35*	29.31±5.46	19.76±2.03*#
IL-1β in synovia	41.26±3.56	18.78±2.56*	42.01±3.47	20.45±3.16*#
COX-2 in serum	16.25±3.46	8.58±1.32*	16.45±3.52	9.89±1.43*#
COX-2 in synovia	27.35±3.29	10.32±1.05*	27.58±3.34	11.89±1.07*#

*P<0.05 *vs.* on admission, #P<0.05 *vs.* obviously effective group.

1β and COX-2 levels in serum and synovia were significantly reduced in the two groups on the 14th day after the treatment (P<0.05). The IL-1β and COX-2 levels in serum and synovia in non-obviously effective group were significantly higher than those in obviously effective group on the 14th day after the treatment (P<0.05).

Discussion

In recent years, the incidence rate of GA has been rising obviously. Its initial manifestations are mainly acute pain of joints and movement disorder affecting the daily life of patients. Its pathogenesis is unclear with hypothesis of certain genetic factor and bad diet structure. In addition, GA patients usually suffer from diabetes, obesity, cardio-cerebrovascular disease, or other diseases (2,3). This study found that patients in all the observation groups had history of varying degrees of diabetes, hypertension or coronary heart disease, with FPG and LDL-C rising and HDL-C reducing obviously, in line with the view above.

The important role of inflammatory response in GA has been gradually recognized in recent years. GA is an immune response to the secretion of inflammatory factors due to the phagocytizing of urate microcrystals after the formation of urate microcrystals, activation of synovial endothelial cells and exudation of mononuclear macrophages caused by the changes in urate concentration in joint fluid (9). IL-1β is one of the major inflammatory factors excreted by mononuclear macrophages, it participates in the occurrence and development of multiple inflammatory diseases, and plays an important role in the inflam-

mation of tissues with deposited monosodium urate crystals in GA patients (10). COX-2 is also called Prostaglandin oxidase reductase, and it is the key enzyme of synthesis and initiation of prostaglandin E2 that is the main media participating in the pathological process of gout (11). In addition, the expression of COX-2 in inflammatory response is soared to increase the pathological synthesis of prostaglandin E2, thus intensifying the development of inflammation and joint injury (12). Moreover, IL-1β can induce the high expression of COX-2 in endothelial cells (13). This study found that the IL-1β and COX-2 levels in serum in observation groups were significantly higher than those in the healthy control group. With the worsened conditions of patients, IL-1β and COX-2 levels showed a rising trend. It is suggested that IL-1β and COX-2 are closely associated with the occurrence and development of the GA in patients.

Rising of UA level will increase UA crystals in joints, thus stimulating mononuclear macrophages to excrete inflammatory factors (14). This study also found that the UA level in the serum in observation groups was significantly higher than that in the healthy control group and increasingly higher with worsening condition of patients. Therefore, further analysis was performed on the correlations of IL-1β and COX-2 with UA expression level, and the results showed that IL-1β and COX-2 were positively correlated with UA. ROC curve analysis exhibited that the optimal cut-off values of serum IL-1β and COX-2 for predicting GA were 18.23 pg/mL and 9.12 pg/mL, and AUC values were 0.702 and 0.714 ($P<0.001$), respectively, suggesting high predictive values.

Increase in UA may promote mononuclear macrophages to excrete IL-1β, induce expression of COX-2, stimulate generation of prostaglandin E2, damage cartilage extracellular matrix, and enhance permeability of vascular endothelial cells, further stimulating the release of inflammatory factors including IL-1β, and generating a vicious circle.

In this study, the changes of expressions of IL-1β and COX-2 in serum and synovia during clinical treatment were further observed, and it was found that the IL-1β and COX-2 levels in serum and synovia were significantly reduced from the 7th day after the treatment, suggesting that such levels are reduced with better condition of patients and that more than seven days of anti-inflammatory treatment can obviously improve the inflammatory response, thus effectively relieving the pain of patients. For IL-1β and COX-2 levels in serum and synovia in patients with different efficacies, the results of this study showed that the IL-1β and COX-2 levels on the 14th day after treatment were lower than those on admission in the two groups, while IL-1β and COX-2 levels in obviously effective group were lower than those in non-obviously effective group. It revealed that monitoring of IL-1β and COX-2 levels in serum and synovia has certain reference value for guiding clinical treatment and evaluating clinical efficacy. However, with of the limited sample size, the above results may have deviation, as such, more samples are still needed for further verification.

In conclusion, IL-1β and COX-2 levels in serum and synovia in GA patients are clearly higher than those in healthy patients in the control group, and they rise with the worsening condition of patients. Monitoring of IL-1β and COX-2 levels in serum and synovia is helpful for evaluation of clinical efficacy, with high predictive values.

Conflict of interest

The author declares no competing interest.

Authors' contribution

GC conceived and designed the study.
CX reviewed and edited the manuscript.

LZ performed the experiments.
LX provided the mutants.
ZC and YL wrote the paper.
All authors read and approved the manuscript.

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