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Correlations between CXCL13, IL-24 genes and wrist arthritis

G. ZHAO[1,2], J.-Y. MI[2], X.-Y. PAN[2], X. ZHANG[2], Y.-J. RUI[1,2]

[1]Department of Medicine, Soochow University, Suzhou, China
[2]Department of Hand Surgery, Wuxi 9th People's Hospital, Wuxi, China

Abstract. – OBJECTIVE: To investigate the relationship between B lymphocyte chemokine 1 (CXCL13) and interleukin-24 (IL-24) gene and wrist arthritis.

PATIENTS AND METHODS: A total of 122 cases of patients with wrist arthritis treated in our hospital from May 2013 to April 2016 were randomly selected as wrist arthritis group, while 120 normal subjects were selected as normal control group. Venous blood was collected from all patients in normal control group and wrist arthritis group, respectively. Rheumatoid factor (RF), human C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) in venous blood were measured. The visual analogue scale (VAS) score was used to statistically analyze the pain of subjects in normal control and wrist arthritis groups; the wrist flexion and extension activities of subjects in normal control group and wrist arthritis group were measured. The expressions of CXCL13 and IL-24 mRNA in synovial tissue of normal control group and wrist arthritis group were detected by reverse transcription-polymerase chain reaction (RT-PCR). Western blotting was used to detect the expressions of CXCL13 and IL-24 in normal control group and wrist arthritis group.

RESULTS: The levels of CRP, RF, and ESR in the normal control group were within the normal range, but the levels of CRP, RF, and ESR in the wrist arthritis group were significantly higher than those in the normal control group. VAS scores and joint flexion extension activities in the normal control group were at normal levels. The VAS score of wrist arthritis group was significantly higher than that of normal control group, and the joint flexion extension activities were significantly lower than that in the normal control group. The results of RT-PCR showed that the expression of CXCL13 mRNA in synovial tissue of wrist arthritis was significantly higher than that in the normal control group, while the expression of IL-24 mRNA in synovial tissue of wrist arthritis was significantly lower than that in the normal control tissues. Western blotting showed that the expression of CXCL13 in synovial tissue of wrist arthritis was significantly higher than that in the normal control group, while the expression of IL-24 in synovial tissue of wrist arthritis was significantly lower than that in normal control groups. Analysis of variance showed that the expressions of CXCL13 and IL-24 in normal control group and wrist arthritis group had statistically significant differences ($p<0.01$).

CONCLUSIONS: The abnormal expressions of CXCL13 and IL-24 are closely related to the occurrence and development of wrist arthritis. This study shows that CXCL13 and IL-24 have important research values in wrist arthritis. CXCL13 and IL-24 expressions can be used as new indicators of the diagnosis and treatment of wrist arthritis.

Key Words:
CXCL13, IL-24, Wrist arthritis.

Introduction

Wrist arthritis refers to a variety of joint inflammations, including the rheumatic, rheumatoid, traumatic, osteoarthritis and purulent arthritis[1]. Early lesions of the wrist arthritis can damage the synovium, and in later stage may even lead to cartilage and bone damage[2]. Wrist arthritis is seriously endangering people's lives and health. The most common treatment is physiotherapy, but the effect is not satisfied. Many patients do not pay enough attention to the treatment of wrist arthritis, and will not receive treatment until the later stage[3]. Therefore, it is important to find a practical and reliable method in the diagnosis and treatment of wrist arthritis. Some studies have clarified that B lymphocyte chemokine 1 (CXCL13) and interleukin-24 (IL-24) genes are the genes related to inflammation. CXCL13 can induce inflammation and IL-24 has a good anti-inflammatory effect[4,5]. The primary purpose of this study was to investigate a new effective method to diagnose and treat wrist arthritis, based on the role of CXCL13, IL-24 genes in the treatment of wrist arthritis.

Corresponding Author: Yongjun Rui, MD; e-mail: wxswkryj@163.com

Patients and Methods

Patients

Form May 2013 to April 2016, 122 patients diagnosed as wrist arthritis (52 males and 70 females aged 28 to 55 years old) were chosen from our hospital. Additionally, 120 patients without a history of wrist arthritis were selected as normal control group, including 48 males and 72 females aged 30 to 56 years old. There were no significant differences in sample size, gender and age between the two groups. This study was approved by the Ethics Committee of Wuxi 9th People's Hospital. Signed written informed consents were obtained from all participants before the study.

Main Reagents

Bicinchoninic acid (BCA) Protein Assay Kit (Beyotime, Shanghai, China); TRIzol Total RNA Extraction Kit (Tiangen, Beijing, China); reverse transcriptase-polymerase chain reaction (RT-PCR) Reverse Transcription Kit (Tiangen, Beijing, China); anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH), anti-human CXCL13 and anti-IL-24 monoclonal antibody and secondary antibody (Cell Signaling Technology, Danvers, MA, USA).

Methods

Index Determination

Inflammation Index Determination

The venous blood from normal control group and wrist arthritis group was collected. After treatment, rheumatoid factor (RF), human C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were detected.

Wrist Related Index Determination

Visual analogue scale (VAS) score was used to analyze the pain of subjects in normal control group and wrist arthritis group, and wrist flexion and extension activities of subjects in normal control group and wrist arthritis group were also measured.

Real-Time PCR Analysis

The synovial tissues from normal control group and wrist arthritis group were rapidly transferred to 1 mL TRIzol reagent and fully ground into homogenate. After being allowed to stand at room temperature for 5 min, the sample was totally lysed, centrifuged at 12000 g at 4°C for 5 min. The supernatant was carefully removed. Chloroform was added to the supernatant and mixed well. After standing at room temperature for 5 min, the supernatant was carefully removed via centrifugation at 12000 g for 15 min at 4°C. Then, the same volume of isopropanol was added, followed by standing at room temperature for 10 min. After centrifugation at 12000 g, at 4°C for 10 min, the precipitate was taken, 75% ethanol was added, and mixed well to wash RNA precipitation. Finally, RN as-free water was added to completely dissolve the precipitate. Then, the ratio of optical density (OD) 260/OD280 and the RNA concentration were measured. According to the instructions and the primer sequence template shown in Table I, the PCR products were amplified by RT-PCR.

Western Blotting

The synovial tissues collected from normal control group and wrist arthritis group were washed with ice saline. According to the whole protein extraction kit instructions, the IP lysate (containing PMSF and protease inhibitor) was added and the tissues were ground thoroughly on ice. Then, the tissue homogenate was centrifuged at 12000 g at 4°C for 10 min. The supernatant was taken and centrifuged at 12000 g for 20 min at 4°C, and the supernatant was still taken. Next, according to the instructions of the protein kit, the protein was quantified and the same amount of total protein was added into the sample solution. The sample was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under constant voltage of 220 V until the bromophenol blue reached the bottom of the gel. The gel was isolated and the protein was transferred to the polyvinylidene fluoride (PVDF) membrane based on the molecular weight of the target protein. Then, protein-coated PVDF membranes were blocked in 5% skim milk powder for 3 h at room temperature on a shaker and incubated in the corresponding primary antibody (1:1000) overnight at 4°C. On the second day, TBS-0.1% Tween-20 (TTBS) was used to wash the membrane fully for 3 times (10 min); then, the secondary antibody (1:2000) was incubated at room temperature for 1 h, and it was washed with TTBS (10 min/times). At last, enhanced chemiluminescence (ECL) was added for color development.

Statistical Analysis

The experimental data were expressed as mean ± standard deviation and the experimental results were analyzed by Statistical Product and Service Solutions (SPSS) 17.0 statistical software (SPSS Inc., Chicago, IL, USA). The means between the two groups were compared by *t*-test. One-way

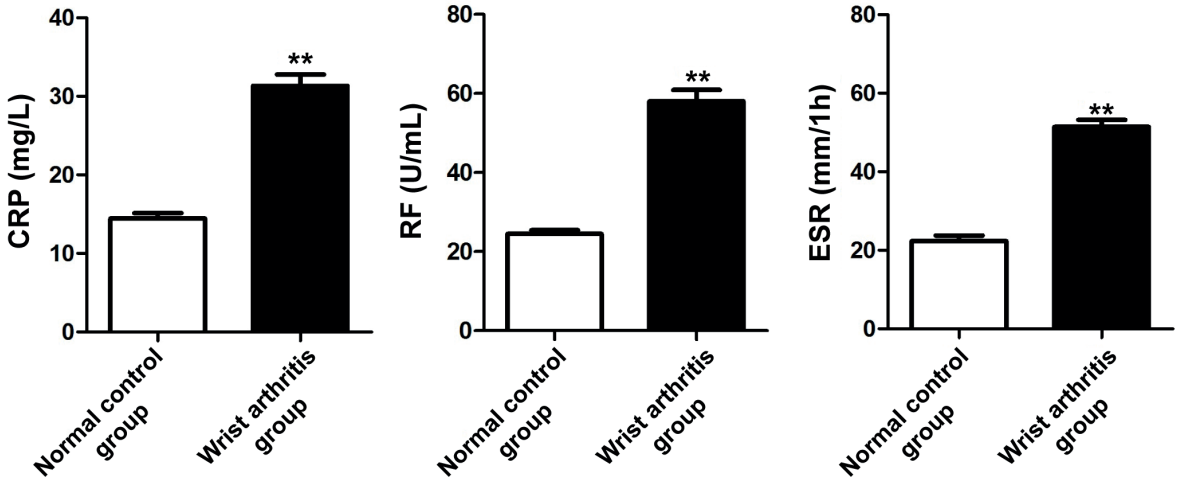

Figure 1. Measurement results of CRP, RF and ESR in normal control group and wrist arthritis group. The expressions of CRP, RF and ESR in wrist arthritis group are significantly higher than those in normal control group; $^{**}p<0.01$.

ANOVA was used to compare the means among groups. *p*-test was used for the pairwise comparison. $p<0.05$ suggested that the difference was statistically significant.

Results

Inflammation Index Measurement Results

As can be seen from Figure 1, the CRP, RF and ESR levels in normal control group were within the normal range, while the levels of CRP, RF and ESR in wrist arthritis group were significantly higher than those in normal control group. Our results suggested that the CRP, RF and ESR expressions are abnormal in patients with wrist arthritis, and a large number of inflammatory factors are aggregated in the body.

Wrist Related Index Measurement Results

As shown in Figure 2, VAS score and joint flexion extension activities of normal control group were all at normal levels, while VAS score of wrist arthritis group was significantly higher than that of normal control group, and joint flexion extension activities were significantly lower than those of normal control group. Patients with wrist arthritis had significant pain accompanied by dyskinesia of joint flexion and extension.

RT-PCR Results of CXCL13 and IL-24 mRNA Expressions in Normal Control Group and wrist Arthritis group

The total RNA extracted from the synovial tissue samples of normal control group and wrist arthritis group was detected by RT-PCR. The expression of CXCL13 mRNA in the synovial tissue of wrist arthritis was significantly higher than that in normal control group, but the expression of IL-24 mRNA was significantly lower than that in normal control group. The results are shown in Figure 3. It can be seen that the expressions of inflammatory factors in wrist arthritis are increased, while the expressions of anti-inflammatory factors are decreased. In addition, the diagnostic ROC curve values of CX-

Table I. RT-PCR primer sequences for CXCL13 and IL-24 mRNA.

Gene name	Primer sequence
CXCL13	5'-3' CTCTGCTTCTCATGCTGCTG
	3'-5' TGAGGGTCCACACACACAAT
IL-24	5'-3' CTGCATCCAGGTCAGAAGAATT
	3'-5' GAGCAGATAAGAATTTCACACAGG
β-actin	5'-3' GAGCCGGGAAATCGTGCGT
	3'-5' GGAAGGAAGGCTGGAAGATG

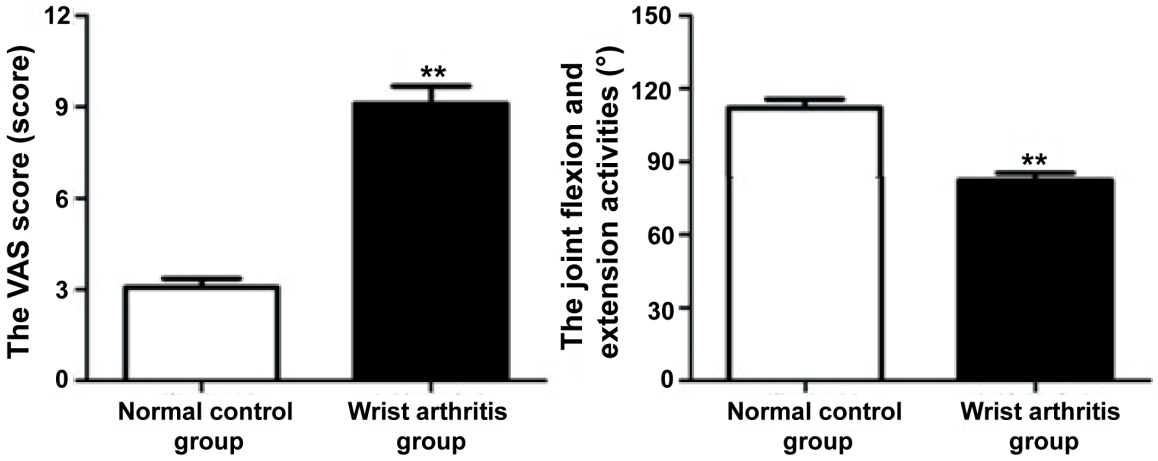

Figure 2. VAS score and joint flexion and extension activity in normal control group and wrist arthritis group. The VAS score of wrist arthritis group is significantly higher than that of normal control group, while the joint flexion and extension activities is significantly lower than that of normal control group. Compared with normal control group, $^{**}p<0.01$.

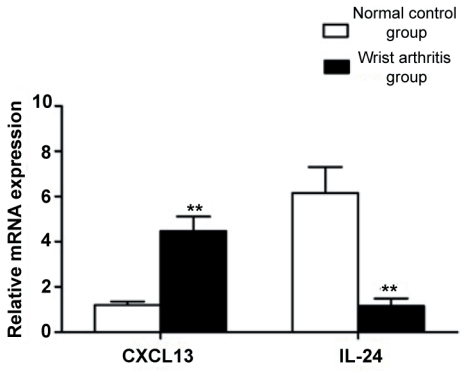

Figure 3. RT-PCR results of CXCL13 and IL-24 mRNA in normal control group and wrist arthritis group. The expression of CXCL13 mRNA in the synovial tissue of wrist arthritis is significantly higher than that in normal control group, whereas the expression of IL-24 mRNA is significantly lower than that in normal control group. Compared with normal control group, $^{**}p<0.01$.

CLB and IL-24 in wrist arthritis are 0.826 and 0.854 (Figure 4A and 4B).

Western Blotting Results of CXCL13 and IL-24 in Normal Control Group and wrist Arthritis Group

As shown in Figure 5 Western blotting of the synovial tissue samples from normal control group and wrist arthritis group revealed that the expression of CXCL13 protein in the synovial tissue of wrist arthritis was significantly higher than that in normal control group, while the expression of IL-24 protein was significantly lower than that in normal control group.

Discussion

Arthritis is a common chronic disease. At present, there are more than 100 million arthritis patients in China. Nowadays, arthritis often occurs in both elderly people and young people[6-8]. Arthritis can lead to limitation of joint activity and permanent loss of joint function in several severe cases[9]. Wrist joints play a key role in people's daily life, so wrist arthritis should be diagnosed and treated correctly and effectively.

CXCL13, as an important member in the chemokine family, is primarily associated with the body's immune system[10,11]. CXCL13, also as the first CXC family gene detected in the liver and lymph nodes, induces the production of inflammation that affects many diseases[12-15]. IL-24, a suppressor of inflammatory interleukin discovered this year, has the properties of inhibiting tumors, inhibiting inflammation and repairing damage[16,17]. Although CXCL13 and IL-24 have been reported in many diseases, such as a variety of cancers, immune diseases and inflammatory diseases, there are not many reports on the research of CXCL13 and IL-24 in arthritis[18-20]. In summary, this work aimed to investigate the roles of CXCL13 and IL-24 in wrist arthritis by studying the expression of CXCL13 and IL-24 in normal control group and wrist arthritis group.

In this study, 122 cases of patients with chronic arthritis and 120 normal subjects were selected. The venous blood samples of both groups were collected for the detection of RF, CRP and ESR, respectively. Our results showed that the levels of

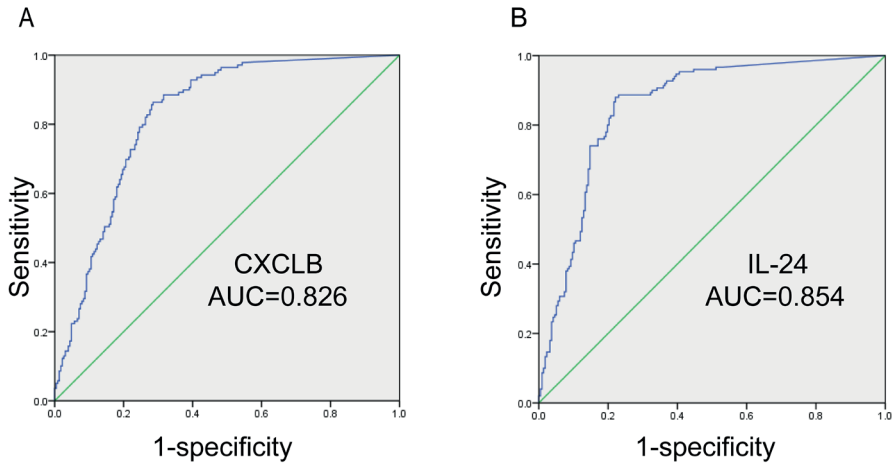

Figure 4. The ROC curve of the diagnostic value of CXCLB and IL-24 in wrist arthritis. ***A,*** The ROC curve of the diagnostic value of CXCLB in wrist arthritis is 0.826. ***B,*** The ROC curve of the diagnostic value of IL-24 in wrist arthritis is 0.854.

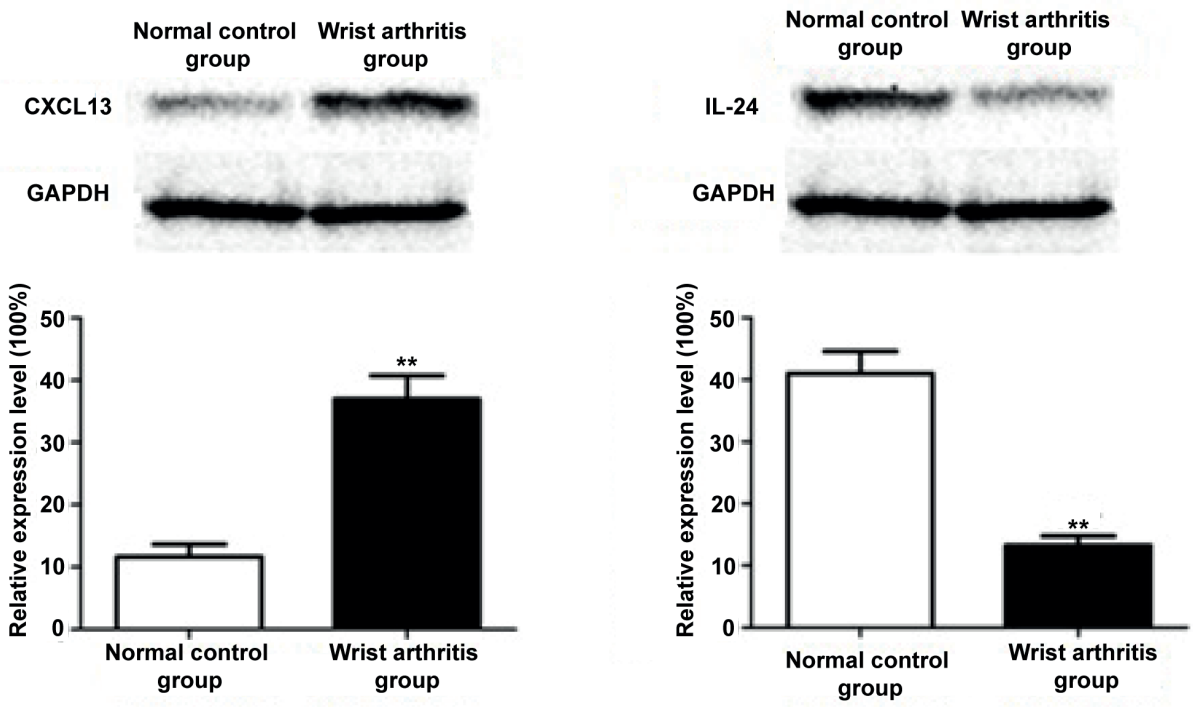

Figure 5. Western blotting results of CXCL13 and IL-24 proteins in normal control group and wrist arthritis group. The expression of CXCL13 protein in the synovial tissue of wrist arthritis is significantly higher than that in normal control group, while the expression of IL-24 protein is significantly lower than that in normal control group. Compared with normal control group, $^{**}p<0.01$.

CRP, RF and ESR in normal control group were within the normal ranges, while the levels of CRP, RF and ESR in wrist arthritis group were significantly higher than those in normal control group. VAS scores and joint flexion and extension activities of normal control group were all at normal levels, but the VAS score of wrist arthritis group was significantly higher than that of normal control group; meanwhile, the joint flexion and extension activities were significantly lower than those of normal control group. The results of RT-PCR showed that the expression of CXCL13 mRNA in

synovial tissue of wrist arthritis was significantly higher than that in normal control group, while the expression of IL-24 mRNA in synovial tissue of wrist arthritis was significantly lower than that in normal control group. Western blotting showed that the expression of CXCL13 in synovial tissue of wrist arthritis was significantly higher than that in normal control group, while the expression of IL-24 in synovial tissue of wrist arthritis was significantly lower than that of normal control group.

Conclusions

Our study confirmed that the abnormal expressions of CXCL13 and IL-24 are closely related to the occurrence and development of wrist arthritis. This can provide a new theoretical basis for exploring the mechanism of wrist arthritis, and a new direction for its diagnosis and treatment.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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