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Original Article

IL-17 expression in synovial fluid and synovial membrane in patients with knee osteoarthritis

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Abstract: Objective: In the present study, we aimed to detect the levels of IL-17 in the serum, synovial fluid, and synovial membrane of patients with knee osteoarthritis (KOA) and investigate their association with the severity of articular cartilage damage and synovitis. Methods: According to the American College of Rheumatology criteria for osteoarthritis (OA) diagnosis, 46 cases diagnosed with knee OA (KOA group) were selected from patients that underwent arthroscopic surgery at our hospital from June 2012 to June 2014. Additional 46 cases with other knee joint diseases (non-KOA group) were included as controls. The IL-17 levels in synovial fluid and serum were assayed by ELISA, and those in the synovial membrane were assayed by immunohistochemistry. The severity of knee articular cartilage damage and synovitis-related pathological changes was evaluated by arthroscopy using the Outerbridge and Ayral scores, respectively. Results: IL-17 levels in the synovial fluid and synovial membranes of the KOA group were significantly higher than in the non-KOA group ($P < 0.05$). The levels of IL-17 in the synovial fluid and synovial membranes were positively correlated with the serum level of high-sensitivity C-reactive protein (HS-CRP), the Outerbridge score, and the Ayral score in the KOA group. Conclusion: IL-17 levels in the synovial fluid and synovial membrane are increased in KOA patients and are positively correlated with KOA severity.

Keywords: Osteoarthritis, IL-17, synovial fluid, synovial membrane

Introduction

Osteoarthritis (OA) is a chronic joint disease characterized by degeneration, destruction, hyperplasia, and synovitis of the articular cartilage and subchondral bone [1]. Obesity, trauma, age, gender (more common in women), and biochemical factors are the main risk factors for OA [2]. However, the pathogenesis of OA has not been explicitly documented [3].

Magnetic resonance imaging (MRI) and arthroscopy are important methods to evaluate the severity of knee osteoarthritis (KOA). However, these methods have a limited role in KOA diagnosis. Therefore, it is necessary to explore quantitative and sensitive methods for the examination of KOA. In recent years, research has shown that biomarker testing is a potential method for the early diagnosis of KOA. The radiographic grade of KOA is associated with a number of biomarkers in knee synovial membranes, such as P-selectin, cartilage oligomeric matrix protein, proteoglycans, and high-sensitivity C-reactive protein (HS-CRP) [4]. It has been demonstrated that IL-17 might play a crucial role in KOA pathogenesis and is closely related to pain [5]. The accurate and effective regulation of IL-17 signaling can prevent inflammation. In the present study, the IL-17 levels in serum, synovial fluid, and synovial membrane from KOA patients were tested to analyze their association with the severity of knee articular cartilage damage and synovitis-related pathological changes.

Materials and methods

Patients

According to the American College of Rheumatology (ACR) criteria for the diagnosis of KOA, 46 patients diagnosed with OA (KOA group) were selected from patients who underwent arthroscopic surgery at Weifang No. 2 People's Hospital from June 2012 to June 2014. Addi-

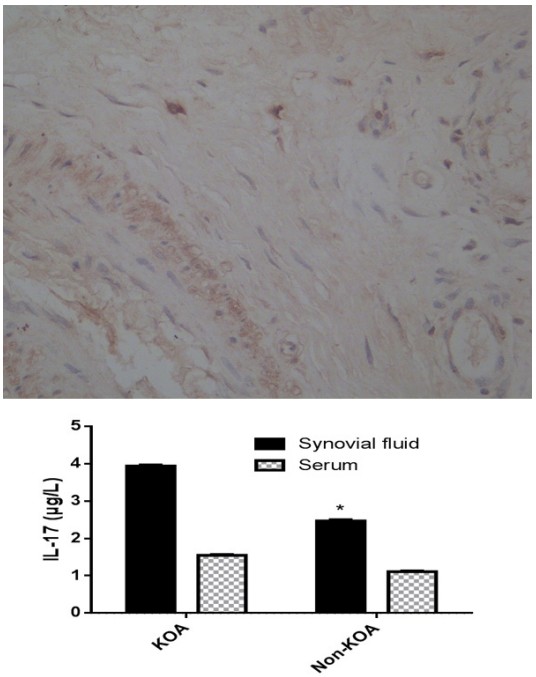

Figure 1. Expression of IL-17 in the synovial membrane. Synovial membrane sections were prepared, and IL-17 expression was examined by immunohistochemistry. The sections were observed under the microscope. Five fields of view at high magnification (400 ×) were chosen randomly from each stained section.

Table 1. IL-17 levels in the serum and synovial fluid of patients with KOA and non-KOA (μg/L)

Group	Cases	Synovial fluid	Serum
KOA	46	3.94 ± 0.030	1.55 ± 0.02
Non-KOA	46	2.47 ± 0.03	1.11 ± 0.02
T	-	2.83	0.026
P	-	0.042	0.928

tionally, 46 cases with other knee joint diseases (non-KOA group) were included as controls. The KOA group included 22 males and 24 females, ranging in ages from 45-81 years and an average age of 60 years; the mean body mass index was 18.1 to 26.9, and the mean course of disease lasted from 8 to 87 months. The non-KOA group included 25 males and 21 females, ranging in ages from 43-80 years and an average age of 58 years; there were 28 cases of meniscus injury and 18 cases of anterior cruciate ligament injury. The study was approved by the ethics committee of our hospital. A signed informed consent form was obtained from all the patients.

The diagnostic criteria for KOA according to the ACR criteria for the diagnosis of KOA include: (1) knee pain for most of the time for a month, (2) snapping joint activities, (3) morning stiffness ≤ 30 min, (4) age ≥ 40 years, (5) knee joint swelling with snapping, and (6) knee joint swelling without snapping. OA was diagnosed upon the observation of at least the combinations (1), (2), (3) and (4), or (1), (2), (3) and (5), or (1) and (6).

The exclusion criteria included other associated inflammatory arthritis or autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus or gout; a history of steroid injections or non-steroidal drug use over the past three months, a history of severe trauma in the knee joint, or associated severe liver and kidney dysfunction and cardiovascular disease.

The inclusion criteria included (1) arthroscopic anterior cruciate ligament reconstruction, and (2) meniscus angioplasty or arthroscopy.

Arthroscopic evaluation of articular cartilage damage and synovitis-related pathological changes

The severity of articular cartilage damage and synovitis was evaluated in accordance with the findings of knee arthroscopy using the Outerbridge [6] and Ayral scores [7], respectively.

Collection and preservation of serum and synovial fluid specimens

For the serum specimens, the patients were required to fast for drink eight hours before the operation. Four-milliliter fasting blood specimens were collected through the ulnar vein in the morning. The specimens were placed in sterile tubes without any anticoagulant and centrifuged at 800 g for 15 min. The serum specimens were retained and dispensed into micro-centrifuge tubes immediately. Each tube was labeled and frozen at -80°C.

Synovial fluid specimens: After anesthesia, 4-5 mL synovial fluid specimens were extracted by puncturing the knee joint cavity through the lateral supra-patellar approach. The specimens were collected in serum tubes and centrifuged at 3,000 rpm for 15 min within 2 hours of sur-

Table 2. IL-17 levels in the synovial membrane of patients with KOA and non-KOA (μg/L)

Group	Cases	Synovial membrane
KOA	46	43.60 ± 6.28
Non-KOA	46	32.22 ± 5.73
T	-	3.39
P	-	0.02

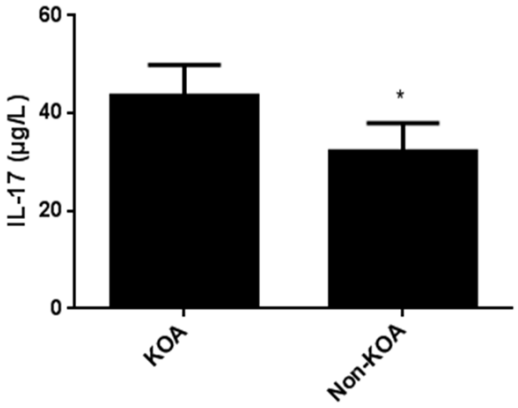

Figure 2. IL-17 levels in the serum and synovial fluid of patients with KOA and non-KOA. IL-17 protein expression was examined by ELISA.

gery. The supernatants were then collected into micro-centrifuge tubes and stored at -80°C.

ELISA for IL-17 in synovial fluid and serum

The levels of IL-17 in synovial fluid and serum were assayed by ELISA using a commercial kit (R&D, USA). The blank control, standard, and sample wells were set according to the manufacturer's instructions. The test samples were thawed to room temperature, and 100-μL aliquots of each sample were added into monoclonal antibody-coated 96-well plates. The plates were shaken well, and 50 μL of the affinity substance was added per well, followed by shaking. The reaction solution was incubated at room temperature for 30 min and rinsed repeatedly with a washing solution. Next, 200 μL of TMB color-developing reagent was added to each well and incubated for 15 min. Finally, a termination solution was added at 50 μL per well. The plates were shaken for 5 s to thoroughly mix the substrate and termination solutions. The absorbance of the reaction mixture was measured at 450 nm (A value). The sample concentrations of IL-17 were calculated from the standard curve.

Serum HS-CRP analysis

Serum samples were removed from -70°C storage and were completely thawed to room temperature. The HS-CRP concentration in each sample of the KOA group was assayed by a rate nephelometric immunoassay.

Immunohistochemical assay for IL-17

Tissue specimens of synovial membrane were collected during arthroscopic surgery. The specimens were washed twice with normal saline within one hour of surgery and then cut into approximately 0.5 cm × 0.5 cm × 0.5 cm blocks. The tissue blocks were fixed with 10% paraformaldehyde, embedded in paraffin, and sectioned into 4-5 μm-thick slices. The pathology sections were prepared after surgery, and IL-17 expression was assayed by immunohistochemistry. The sections were examined under the microscope. Five fields of view at high magnification (400 ×) were chosen randomly from each stained section. One brownish-yellow particle representing a positive result was marked within one of the fields of view and was then used as a standard for automatic detection of all positive results in the field of view.

Statistical analysis

Statistical analysis was performed with SPSS 18.0. Continuous data were expressed as the mean ± standard deviation (X ± SD). Comparison of means was performed between groups by the *t*-test. Pearson correlation analysis was conducted on the average integral optical density of various indices. A *P* value less than 0.05 was considered statistically significant.

Results

IL-17 expression in the synovial membrane of KOA patients

Tissue specimens of the synovial membrane were prepared after surgery, and IL-17 expression was assayed by immunohistochemistry. The specimen sections were examined by microscopy. One brownish-yellow particle representing a positive result was marked within one of the fields of view and was then used as

Table 3. Correlations between IL-17 levels in the serum, synovial fluid, and synovial membrane and serum HS-CRP concentration in OA patients

Group	Serum	Synovial fluid	Synovial membrane
IL-17 (μg/L)	1.55 ± 0.03	3.94 ± 0.03	43.6 ± 6.28
HS-CRP (mg/L)	1.91 ± 0.30	1.90 ± 0.30	1.90 ± 0.30
R	0.56	0.87	0.43
P	< 0.05	< 0.001	< 0.001

Table 4. Correlations between IL-17 levels in the synovial fluid and synovial membrane and the Outerbridge score of articular cartilage damage in patients with osteoarthritis

	Synovial membrane	Synovial fluid
IL-17 (μg/L)	43.6 ± 6.28	1.55 ± 0.03
Outerbridge score	18.71 ± 1.3	18.71 ± 1.3
R	0.68	0.13
P	< 0.05	0.007

Table 5. Correlations between IL-17 levels in the synovial fluid and synovial membrane and the Ayral score of synovitis in patients with osteoarthritis

	Synovial membrane	Synovial fluid
IL-17 (μg/L)	43.6 ± 6.280	1.55 ± 0.03
Ayral score	29.38 ± 0.40	29.38 ± 0.40
R	0.30	0.17
P	< 0.05	< 0.05

a standard for automatic detection of all the positive results in the field of view (**Figure 1**).

IL-17 levels were elevated in the synovial fluid and synovial membrane of KOA patients

IL-17 levels in the synovial fluid were elevated in patients with KOA compared with the non-KOA group (3.94 ± 0.03 *vs*. 2.47 ± 0.03 μg/L, P = 0.042). However, in the KOA patients, plasma IL-17 levels were not statistically different from those in the non-KOA patients (P > 0.05) (**Table 1**; **Figure 1**). As seen from **Table 2** and **Figure 2**, IL-17 levels in the synovial membrane of patients with KOA were significantly increased compared with those in non-KOA patients (43.60 ± 6.28 *vs*. 32.22 ± 5.73 μg/L, P = 0.02).

HS-CRP is a sensitive indicator for evaluating KOA. Our results showed that the IL-17 levels in plasma, synovial fluid, and synovial membrane were all significantly correlated with serum HS-CRP concentrations (**Table 3**).

IL-17 levels in the synovial fluid and synovial membrane are positively correlated with KOA severity

Next, we evaluated the severity of knee articular cartilage damage and synovitis-related pathological changes by arthroscopy using the Outerbridge and Ayral scores. We found that the IL-17 levels in synovial fluid (1.55 ± 0.03 μg/L) and synovial membrane (43.6 ± 6.28 μg/L) were positively correlated with both the Outerbridge (**Table 4**) and the Ayral scores (**Table 5**). These results indicated that as the concentration of IL-17 increased in the synovial tissue, the severity of synovitis correspondingly increased.

Discussion

OA is a chronic joint disease characterized by the degeneration, destruction, hyperplasia, and synovitis of the articular cartilage and subchondral bone [8]. Advanced KOA patients often struggle with limited joint mobility, joint deformity, severe pain, and even disability. Ultimately, these patients have to undergo surgical treatment such as joint replacement. KOA patients already undergo significant degeneration of the knee joint prior to the observation of the typical X-ray changes and clinical manifestations [8]. Hence, early diagnosis of KOA is of particular importance. With the rapid development of molecular biology techniques in recent years, scholars have proposed that testing for the associated biomarkers in synovial fluid may be useful for the early diagnosis of KOA [9].

IL-17 might play a crucial role in the pathogenesis of rheumatoid arthritis (RA) [10-13], psoriatic arthritis (PsA) [14], or OA, and is closely related to pain [5]. Previous studies have examined synovial IL-17 levels only in the synovial fluid of OA patients and the control group. The same results have been obtained in our study [5]. However, we showed that the IL-17 levels in the synovial membranes of the KOA group were higher than those in the non-KOA group. Statistically significant differences were found between these two groups with respect to the IL-17 levels in the synovial fluid and synovial membrane.

HS-CRP is secreted by hepatocyte nuclei and adipocytes and is regulated by pro-inflammatory factors. According to existing research, the association between serum HS-CRP and KOA includes the following: (1) HS-CRP is a sensitive indicator for evaluating KOA, (2) HS-CRP is associated with the symptoms of OA patients, including pain and joint function, and (3) HS-CRP has no significant statistical difference with the Kellgren-Lawrence score or the degree of joint space narrowing [15, 16]. The results of the present study showed that the serum HS-CRP level was significantly correlated with the IL-17 levels in the serum, synovial fluid, and the synovial membrane. Previous research has indicated that CRP is closely associated with KOA severity. A recent study also showed that serum CRP levels in KOA are higher in KL4 patients than in KL2 and KL3 patients. Moreover, our results showed that the IL-17 levels in the serum and synovial membrane were correlated with the CRP level. Similar results have also been reported in literature.

In this study, IL-17 levels in the synovial fluid and synovial membrane were positively correlated with the Ayral score of synovitis. This correlation was higher between the synovial membrane level of IL-17 and the Ayral score, i.e. as the IL-17 concentration in the synovial membrane increased; the severity of synovitis was correspondingly increased, as was the Ayral score. This result indicates that the level of IL-17 reflects the severity of synovitis. The results from the present study also showed that IL-17 levels in both the synovial fluid and the synovial membrane were positively correlated with the Outerbridge score of cartilage damage. This correlation was higher between the IL-17 concentration in the synovial membrane and the score of the articular cartilage damage, i.e., as IL-17 concentration increased, the severity of the articular cartilage damage increased correspondingly, as did the Outerbridge score. This indicates that IL-17 concentration reflects the severity of articular cartilage damage.

KOA is pathologically manifested as synovitis and differing degrees of articular cartilage damage. Arthroscopic evaluation by the Ayral and Outerbridge scores could objectively reflect the severity of KOA. In the present study, IL-17 levels in the serum, synovial fluid, and synovial membrane were determined in KOA patients, and were found to be positively correlated with the Ayral and Outerbridge scores. Therefore, we conclude that the IL-17 levels in the synovial fluid or membrane reflect the severity of synovitis in KOA to some extent, and infer that IL-17 might be another valuable biomarker to reflect KOA severity.

Disclosure of conflict of interest

None.

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