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Original Article

Effects of arthroscopic knee surgery on IL-1β, CXCL13 and TNF-α in the knee joint fluid of knee osteoarthritis patients and their correlation with clinical outcomes

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Abstract: Aims: To investigate IL-1β, CXCL13, and TNF-α expression in knee joint fluid before and after knee arthroscopic debridement for knee osteoarthritis. Methods: A total of 57 patients who underwent knee arthroscopic debridement were recruited. Patients' visual analog scale (VAS) scores and Lysholm scores were evaluated. The knee joint synovial fluid was extracted during surgery. All patients were divided into five groups according to Outerbridge cartilage damage score. The synovial fluids were extracted during intra-articular injection of sodium hyaluronate. Enzyme-linked immunosorbent assay (ELISA) was applied to detect interleukin-1β (IL-1β), chemokine C-X-C motif ligand 13 (CXCL13), and tumor necrosis factor-α (TNF-α) expression in knee joint fluid. Results: IL-1β, CXCL13, and TNF-α expression in knee joint fluid were significantly lower after the procedure compared to those before the procedure ($P<0.05$). The postoperative VAS scores were significant lower than preoperative ones, while postoperative Lysholm scores were significant higher than preoperative ones (*P* values <0.05). In addition, IL-1β, CXCL13, and TNF-α expression were significant higher in patients with more severe cartilage damages compared to those in patients with milder damages. Conclusion: Arthroscopic debridement can reduce IL-1β, CXCL13, and TNF-α expression in knee joint synovial fluid in patients with knee osteoarthritis.

Keywords: Arthroscopic debridement, osteoarthritis, interleukin-1β, chemokine C-X-C motif ligand 13, tumor necrosis factor-α

Introduction

Knee Osteoarthritis (KOA), also known as knee degenerative osteoarthritis is the most common form of arthritis in the knee. In recent years, KOA incidence was increasing gradually worldwide. Currently epidemiological survey has found that 66% of KOA prevalence in people 50 years of age and older [1]. Among them, the prevalence of men was higher than that in women [2]. KOA treatment includes surgery and non-surgical approaches. In 1960, the arthroscopic technique was used in clinical practice for the first time. Arthroscopic debridement has become an important method for KOA diagnosis and treatment because of mild trauma, quick recovery, and low cost [3]. In addition, the etiology and pathogenesis of KOA has become emphasis of research. The pathogenesis of KOA has not been completely elicited yet. However the role of immune system dysfunction in the occurrence and development of KOA has drawn more and more attention [4, 5].

As an important inflammatory cytokines, interleukin-1 (IL-1) including IL-1α and IL-1β plays a role in the pathogenesis of KOA. Normal cartilage cells produce only small amounts of IL-1β. However, KOA patients have higher levels of IL-1β found in the synovium and cartilage. In addition, recent studies have found high expression of IL-1α and IL-1β in cartilage cells and matrix. Yang L [6] compared IL-1β content in joint synovial fluid between KOA patients and healthy subjects. They found significantly higher IL-1β content in KOA patients than that of healthy subjects, which can be used as indicator to diagnose and evaluate the severity of KOA.

CXCL13 plays an important role in genetic susceptibility to exogenous infection of individuals,

which is one of the direct causes T cell subsets and B cell ratio imbalance *in vivo* [7]. Follicular T helper cell (Tfh) is a recently discovered T helper cell. CXCR5 and interleukin-21 (IL-12) are expressed and secreted on the surface of Tfh. CXCR5 interacts with B cell to produce chemokine-1 (BCA-1, also known as CXCL13) in order to attract Tfh to the germinal center (GC) formed by the secondary lymphoid follicle formation (germinal center, GC). In turn, Tfh activated B cells to produce antibodies.

Tumor necrosis factor-α (TNF-α) is an important mediator in natural and acquired immunity mediating immune response and acute inflammation, such as activating inflammatory chemokines in occurrence and development of inflammation. Previous studies have shown that TNF-α and its receptor demonstrated positive immunostaining in interstitial cells of articular cartilage in KOA animal models, which is highly correlated with the severity of KOA.

The present study was designed to investigate the IL-1β, CXCL13, and TNF-α expression in the synovial fluid before and after knee arthroscopic debridement in patients with KOA, and its relationship with clinical outcomes. We aim to present a theoretical basis to providing arthroscopic debridement to KOA patients.

Subjects and methods

The subjects

A total of 57 patients with KOA who underwent knee arthroscopic debridement in our hospital from December 2014 to March 2015 were recruited in this study. All patients KOA diagnosis are consistent with 2007 Chinese Medical Association Orthopaedic Surgery Branch revised diagnostic criteria. 57 patients includes 28 males and 29 females aged 57-88 years, average (61.5 ± 14.2) years. The KOA duration is from 0.1 to 14 years, average (18 ± 5.7) months. See Supplementary Table 1.

Reagents and instruments

The enzyme-linked immunosorbent assay (ELISA) kits for IL-1β, CXCL13, and TNF-α were purchased from R & D Systems Inc. (Minneapolis, MN, USA). Bio-Rad 680 Microplate Reader was purchased from Bio-Rad Laboratories, Inc. (Hercules, CA, USA). The arthroscopy system Dyonics was purchased from Smith & Nephew (Memphis, TN, USA).

Method

VAS and Lysholm scores before and after surgery: The VAS and Lysholm scores of 57 KOA patients were evaluated before the surgery and at the six-month follow-up by the same orthopedic physician. VAS scores range from 0 to 10 points [8]. 0: painless; 1-3: a slight pain which can be tolerated; 4-6: pain affects sleep but still can be endured; 7-10: intense pain affecting and sleep, which cannot be tolerated any longer. Lysholm score is an internationally applied rating scale for knee function general evaluation, which includes limp, support, locking, instability, pain, swelling, stair-climbing, and squatting. >90 is defined as excellent; 84-90 is defined as good; 65-83 is defined as fair; <65 is defined as poor.

Specimen collection: The knee joint synovial fluid was extracted before the surgery and at the six-month follow-up by the same orthopedic physician. A total of 2 mL synovial fluid was extracted as specimens. The supernatant was separated after centrifugation, which was kept in the -80°C freezer for future ELISA testing.

Arthroscopic debridement: A total of 57 KOA patients underwent knee arthroscopic debridement conducted by the same orthopedic physician. Its brief procedure was as follows. All parts of the knee joint were explored. All free tissues, synovial hyperplasia, and wrinkled shade were removed. In addition, meniscal injuries were sutured and capsular contractures were released.

Outerbridge cartilage injury score: Based on arthroscopic findings during the procedure, the Outerbridge scores were rated as follows. Normal was rated 0; Mild articular cartilage surface blisters were rated 1 point; Shallow ulcers on the articular cartilage surface with diameter <1.25 cm were rated 2 points; Deep ulcers on articular cartilage surface with diameter ≥1.25 cm were rated 3 points; Full-thickness articular cartilage damages were rated 4 points.

Cytokines measurement: The concentration of IL-1β, CXCL13, and TNF-α in joint synovial fluid was determined by ELISA using the commercially available enzyme-linked immunoabsor-

Table 1. The Outerbridge scores based on cartilage damages among patients with KOA

Outerbridge scores	N	Male (n)	Female (n)	Age (year, x ± s)
0	10	5	5	58.5 ± 1.5
1	11	5	6	57.7 ± 2.1
2	14	7	7	63.5 ± 1.7
3	15	7	8	65.1 ± 1.0
4	7	4	3	61.5 ± 1.5

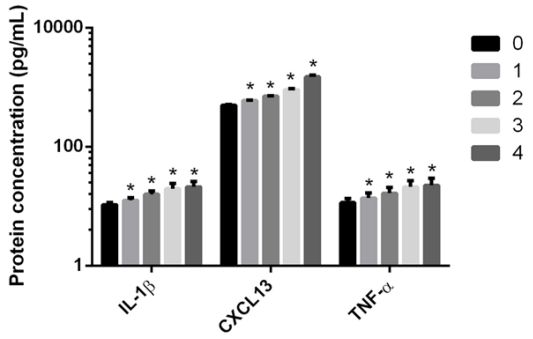

Figure 1. IL-1β, CXCL13, and TNF-α expression levels in patients with different Outerbridge groups (*P<0.05 compared with the group with 0 point).

bent assays according to the manufacturer's instructions (Quantikine, R & D Systems, Minneapolis, MN, USA). Optical densities were determined using a microtiter plate reader (Multiscan RC Type 351; Labsystems, Helsinki, Finland) at 405 nm, with a correction wavelength set at 540 or 570 nm. The blank was subtracted from the duplicate readings for each standard and sample without any knowledge of survival or other clinical data. Concentrations are expressed as pg/mL for IL-1β, CXCL13, and TNF-α. All of the analyses and calibrations were carried out at least in duplicate. The mean values were used for statistical analyses.

The statistical analysis

IBM SPSS 17.0 statistical software was used to analyze data. Continuous data were expressed as mean ± standard deviation (SD). Categorical data were expressed as percentages. Intergroup comparisons were performed using Chi-square test (or Fisher's exact test) or analysis of variance or rank sum test. All the tests were performed using a two-sided test of difference, where the inspection level α of 0.05 and a difference with P<0.05 were considered statistically significant.

Results

Outerbridge scores based on cartilage damage in patients with KOA

Based on Outerbridge scores, among 57 cases of KOA patients, there were 10 patients who were rated 0 point, 11 patients who were rated 1 point, 14 patients who were rated 2 points, 15 patients who were rated 3 points, and 7 patients who were rated 4 points. In addition, there were 5 males and 5 females with average age (58.5 ± 1.5) years among 10 patients who were rated 0 point; there were 5 males and 6 females with average age (57.7 ± 2.1) years among 11 patients who were rated 1 point; there were 7 males and 7 females with average age (63.5 ± 1.7) years among 14 patients who were rated 2 points; there were 7 males and 8 females with average age (65.1 ± 1.0) years among 15 patients who were rated 3 points; there were 4 males and 3 females with average (61.5 ± 1.5) years among 7 patients who were rated 4 points. There was no significant difference in terms of average age and gender composition between groups (P>0.05) (**Table 1**).

IL-1β, CXCL13, and TNF-α expression levels in patients with different Outerbridge groups

According to the results of the ELISA test, IL-1β, CXCL13, and TNF-α expression in the group with Outerbridge 0 point was 10.6 ± 1.0 pg/mL, 501.1 ± 12.3 pg/mL, and 11.5 ± 1.9 pg/mL respectively; it was 12.5 ± 1.4 pg/mL, 600.5 ± 10.5 pg/mL, and 13.7 ± 3.1 pg/mL in the group with Outerbridge 1 point. The expression was significant increased as Outerbridge scores were increased (**Figure 1**).

IL-1β expression levels among outerbridge cartilage injury groups before and after procedures

According to the results of the ELISA test, preoperative IL-1β expression levels were significantly higher than postoperative ones among all groups with different Outerbridge scores (*p* values <0.05) (**Figure 2**).

CXCL13 expression levels among outerbridge cartilage injury groups before and after procedures

According to the results of the ELISA test, preoperative CXCL13 expression levels were

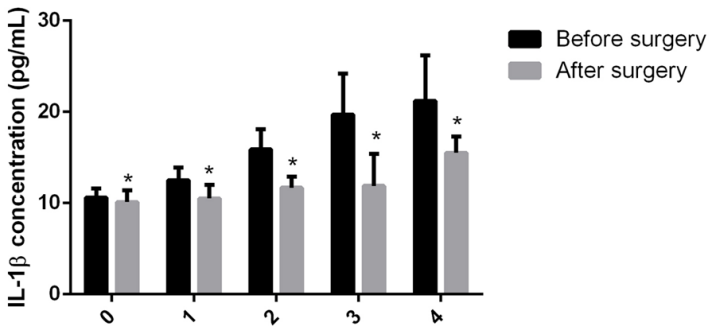

Figure 2. IL-1β expression levels among Outerbridge cartilage injury groups before and after surgeries (pg/mL, x ± SD).

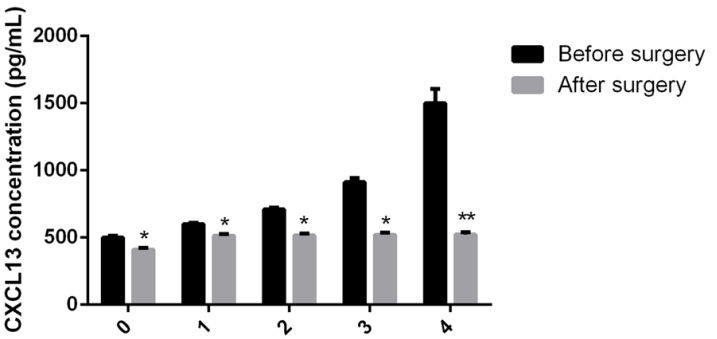

Figure 3. CXCL13 expression levels among Outerbridge cartilage injury groups before and after surgeries (pg/mL, x ± SD).

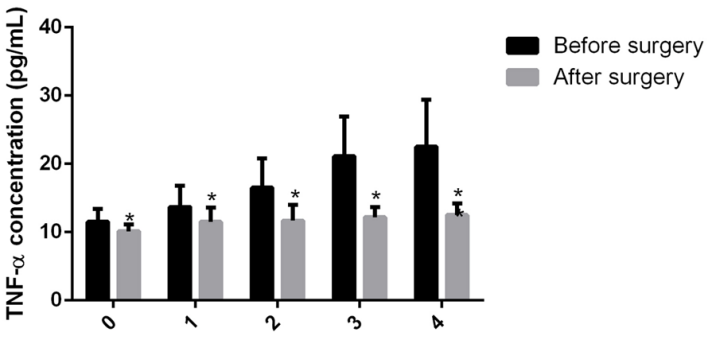

Figure 4. TNF-α expression levels among Outerbridge cartilage injury groups before and after surgeries (pg/mL, x ± SD).

significantly higher than postoperative ones among all groups with different Outerbridge scores (*p* values <0.05) (**Figure 3**).

TNF-α expression levels among outerbridge cartilage injury groups before and after procedures

According to the results of the ELISA test, TNF-α preoperative expression levels were significantly higher than postoperative ones among all groups with different Outerbridge scores (*p* values <0.05) (**Figure 4**).

The comparison of VAS scores before and after surgeries

According to VAS scores, all patients had varying degrees of pain before surgery and 33 patients were painless after surgery. The difference was statistically significant (P<0.01). There was 18 and 17 patients with mild pain before and after surgeries respectively without statistically significant difference (P>0.05). A total of 27 patients had moderate patients before surgery and 7 patients after surgery with statistically significant difference (P<0.05). A total of 12 patients had severe patients before surgery and no patients after surgery with statistically significant difference (P<0.01) (**Table 2**).

The comparison of lysholm scores before and after surgeries

According to Lysholm scores, a total of 18 patients were rated poor before surgeries with no patient after surgeries. The difference was statistically significant (P<0.01). There were a total of 29 and 2 patients who were rated fair before and after surgeries respectively with statistically significant difference (P<0.05). A total of 10 and 13 patients were rated well before and after surgeries respectively without statistically significant difference (P>0.05). There was no patient who was rated excellent before surgeries. A total of 42 patients were rated excellent after surgeries. The difference was statistically significant (P<0.01) (**Table 3**).

Discussion

KOA is characterized by degenerative changes in articular cartilage and hyperplasia of subchondral bone, which is one of the most com-

Table 2. The comparison of different groups based on VAS scores before and after surgeries

Groups	VAS	Preoperative (n)	Postoperative (n)	P
Painless	0	0	33	<0.01
Mild pain	1-3	18	17	>0.05
Moderate pain	4-6	27	7	<0.05
Severe pain	7-10	12	0	<0.01

Table 3. The comparison of different groups based on Lysholm scores before and after surgeries

Groups	Lysholm	Preoperative (n)	Postoperative (n)	P
Poor	<65	18	0	<0.01
Fair	65-83	29	2	<0.05
Good	84-90	10	13	>0.05
Excellent	>90	0	42	<0.01

mon joint diseases worldwide. According to the latest WHO survey, the incidence of all kinds of osteoarthritis was about 60%-70% in the population aged over 50 years. Currently there are approximately 190 million patients with osteoarthritis worldwide [8]. As China has entered the aging society, about 60% of population aged 60 years has KOA according to statistics. Therefore, exploring an effective minimally invasive surgical treatment for KOA has great clinical benefits.

Cytokines in joint synovial fluid has the regulatory function to mediate communication between cells and plays an important role in the inflammatory response. These cytokines are produced in the synovial membrane, and then was spread through the synovial fluid to activate chondrocytes secrete inflammatory cytokines. Previous studies have demonstrated that cytokines play an important role in the development of KOA. TNF-α, CXCL13, and IL-1β are the three important inflammatory cytokines contributing to the development of KOA. Lipopolysaccharides (LPS) stimulate synovial tissue to secrete IL-1, which stimulates I and III collagen synthesis, inhibits II and IX collagen synthesis that are critical for synthesis of proteoglycan, and regulates synthesis of proteolytic enzymes such as cartilage metalloproteinases (MMP). In addition, IL-1 can cause osteoarthritis by stimulating osteoblasts altering the normal load-bearing structures in joints. In the joint synovial fluid of patients with KOA, there are a large number of inflammatory cytokines including IL-1β and TNF-α, which may be one of the causes resulting in synovitis and pain in patients [9, 10].

As KOA becomes more severe, IL-1β and TNF-α expression levels are increased accordingly [11, 12]. In the present study, with the Outerbridge scores was increasing from 0 to 4 points, IL-1β, CXCL13, and TNF-α expression levels were gradually increasing accordingly. The above results have demonstrated that the extent of the cartilage damage in patients with KOA can be determined by measuring the expression of inflammatory cytokines in synovial fluid, particularly the expression of IL-1β, CXCL13 and TNF-α.

Since the 1960s, the arthroscopy technique has been applied in clinical treatment for the first time. It has become an important minimally invasive method of diagnosis and treatment for KOA because of its rapid recovery, fewer complications, and low cost characteristics [13-15]. During arthroscopic procedure, the joint cavity can be rinsed in order to eliminate pain and swelling caused by inflammatory mediators, swelling. The ruptured cartilage, synovial tissue, and loose debris can be removed. And meniscal and ligament damage can be repaired. It can restore joint stability, prevent further deterioration, relieve symptoms, and improve joint function.

Jackson et al. [16] used the arthroscope to clear loose debris and remove damaged meniscus for a total of 200 patients with knee pain. Their results demonstrated that clinical symptom was significantly improved after the procedure. Sprague et al. [17] used arthroscopic debridement techniques for 69 cases of KOA patients, including surgical synovectomy, osteophyte removal, and damaged meniscus repairment. The statistics showed the effective rate was 74%. Dervin et al. [18] carried out a long-term follow-up up to 24 months for the 126 cases of patients who underwent arthroscopic debridement. The SF-36 Health Survey and WOMAC score rating scale were used as criteria to evaluate clinical efficacy. The results showed that 56 cases reached excellent postoperative joint function (44%, 56/126), 38 cases reached good postoperative joint function (30%, 38/126), and 32 cases had fair

postoperative joint function (26%, 32/126). Finally, a total of 27 patients (21%, 27/126) underwent knee replacement, three cases underwent elective tibial osteotomy, and two cases had arthroscopic debridement again. Ambrozaitis et al. [19] have shown that the clinical effective rate of arthroscopic debridement was between 50% and 70% after a long period of follow-up. Krystallis et al. [20] evaluated and analyzed the clinical indications for arthroscopic debridement. They found that arthroscopic debridement had a good clinical effect if KOA onset was emergent, joint cavities had stenosis, and preoperative joint function was good.

The present study showed that the expression levels of IL-1β, CXCL13 and TNF-α in knee joint synovial fluids were significantly reduced after surgeries compared with those before surgeries. The differences were statistically significant (*P* values <0.05). In addition, the postoperative VAS scores were decreased significantly compared with preoperative ones; and the postoperative Lysholm scores were significantly increased compared with preoperative ones. The differences were statistically significant (*P* values <0.05). The above results suggest that arthroscopic debridement can achieve the desired clinical effect for KOA patients. Although arthroscopic debridement cannot change the pathological process KOA, it can restore joint structure, relieve joint pain, and improve joint function in the short term. Therefore, patients with KOA can postpone arthroplasty and arthrodesis. It is proved to be a minimally invasive surgical method with great clinical values.

Conclusion

Arthroscopic debridement for KOA can reduce IL-1β, CXCL13 and TNF-α expression in knee joint synovial fluid. The reduction of IL-1β, CXCL13 and TNF-α expression is in accordance with the improvement of clinical symptoms in patients with KOA. Therefore, they can be used as predictive indicators of long-term prognosis.

Disclosure of conflict of interest

None.

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Supplementary Table 1. Characteristics of the study population

Variables	All patients	Never smokers	Ever smokers	*P*-value
Patients, n (%)	57 (100%)	25 (44%)	32 (56%)	
Age (years), mean ± SD	61.5 ± 11.4	62.0 ± 11.8	60.0 ± 10.1	0.5389
Age, n (%)				
<65 years	26 (46%)	14 (56%)	12 (38%)	0.5106
≥65 years	31 (54%)	11 (44%)	20 (62%)	
Sex, n (%)				
Female	29 (51%)	23 (79%)	6 (23%)	0.0001
Male	28 (49%)	2 (7%)	26 (93%)	
KOA Duration (months), mean ± SD	18.0 ± 5.7	19.5 ± 1.7	17.2 ± 9.1	0.5451