Int J Clin Exp Med 2020;13(2):726-733
www.ijcem.com /ISSN:1940-5901/IJCEM0099623

Original Article

The expression levels of interleukin-6 (IL-6), IL-12, and matrix metalloproteinase-13 (MMP-13) in the synovial fluid and synovium in hip osteoarthritis and their relationship with disease progression

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Received July 14, 2019; Accepted September 10, 2019; Epub February 15, 2020; Published February 28, 2020

Abstract: Objective: To investigate the expressions of interleukin-6 (IL-6), interleukin-12 (IL-12), and matrix metalloproteinase-13 (MMP-13) in the synovial fluid and synovium in hip osteoarthritis and their relationship with disease progression. Methods: Seventy-two patients with hip osteoarthritis (study group, age 50-80 years) and 43 patients with total hip replacement caused by trauma (control group, age 40-78 years) who were admitted to our hospital from March 2012 to March 2014 were included in our survey. The levels of IL-6, IL-12, and MMP-13 were determined by ELISA; the correlations between IL-6, IL-12, MMP-13 and the Kellgren-Lawrence (K-L) classification were tested using a Spearman analysis; the correlations between IL-6, IL-12, MMP-13 were tested using a Pearson analysis; the critical levels and application values of IL-6, IL-12, and MMP-13 in the K-L classification were also determined using an ROC curve. Results: The levels of IL-6, IL-12, and MMP-13 in the synovial fluid of the study group were higher than those of the control group (all $P<0.05$). The results of the analysis among the patients with different K-L grades showed that there were significant differences in the levels of IL-6, IL-12, and MMP-13 in the study group. With the increase in the K-L grades, the levels of IL-6, IL-12, and MMP-13 increased (all $P<0.05$). A Spearman correlation analysis showed that IL-6 ($r=0.771$, $P<0.001$), IL-12 ($r=0.463$, $P<0.001$), and MMP-13 ($r=0.625$, $P<0.001$) were positively correlated the with K-L classification. The Pearson correlation analysis showed that IL-6 was positively correlated with IL-12 ($r=0.346$, $P=0.003$), MMP-13 ($r=0.518$, $P<0.001$), and IL-12 was positively correlated with MMP-13 ($r=0.536$, $P<0.001$). The critical values of IL-6, IL-12 and MMP-13 for the differentiating K-L classification I from II were 144.00 pg/mL, 143.00 pg/mL, 226.80 ng/mL, and the area under the curve (AUC) values were 0.995, 0.645, and 0.785 respectively; for the K-L classifications II to III, the values were 184.70 pg/mL, 193.40 pg/mL and 264.60 ng/mL, and the AUC values were 0.555, 0.760, and 0.740, respectively. Conclusion: The expression levels of IL-6, IL-12, and MMP-13 in the synovial fluid of patients with hip osteoarthritis increased with the severity of the osteoarthritis. In addition, IL-6, IL-12, and MMP-13 had a good correlation with the K-L classification and had a good application value in evaluating the severity of osteoarthritis.

Keywords: Hip osteoarthritis, IL-6, IL-12, MMP-13

Introduction

Osteoarthritis is a chronic degenerative disease characterized by the degeneration of articular cartilage, subchondral sclerosis, and a narrowing of joint space. It is caused by aging, obesity, strain, trauma, and other factors and mostly occurs in the hip and knee joints [1, 2]. According to epidemiological data, osteoarthritis affects nearly 4% of the world's population [3]. In 2003, osteoarthritis was the sixth most common cause of major disability in the world, and it is expected to rise to become the fourth most common major disability cause by 2020, having a serious impact on society and families [4, 5].

An important feature of osteoarthritis is the overproduction of inflammatory mediators. Interleukin can enhance the production of

matrix metalloproteinase (MMP) to change the synthesis and metabolism of chondrocytes [6, 7]. For example, an elevated level of interleukin-6 (IL-6) is closely related to the occurrence and prognosis of osteoarthritis. IL-6 is negatively correlated with the Kellgren-Lawrence (K-L) classification, and for each elevated logarithmic level, the risk of the poor prognosis of osteoarthritis will increase by 1.34 times. Blocking IL-6 may be a strategy for the treatment of osteoarthritis [8]. MMP inhibitors can improve the joint injury of osteoarthritic rats, and inhibit cartilage degradation and osteophyte formation, which are also potential targets for the treatment of osteoarthritis [9]. IL-12 can inhibit the proliferation of chondrocytes induced by transforming growth factor-beta (TGF-β), promote the production of nitric oxide, stimulate normal human articular chondrocyte to express a variety of genes, including inducible nitric oxide synthase, inducible cyclooxygenase, IL-6 and interstitial acid, regulate chondrocyte response and promote cartilage degradation [10, 11]. These studies have proved the important roles of interleukin and MMP in osteoarthritis. However, there are few studies on IL-6, IL-12, and MMP-13 in hip osteoarthritis, especially IL-12 and MMP-13. The relationship between IL-12 and the progression of hip osteoarthritis is not clear.

Therefore, this study examines the expression of IL-6, IL-12, and MMP-13 in hip osteoarthritis, so as to explore the relationship between IL-6, IL-12, MMP-13 and the progression of hip osteoarthritis, and to provide guidance for clinical treatment.

Materials and methods

Research object

Seventy-two patients with hip osteoarthritis (study group) aged 50-80 years were selected from March 2012 to March 2014 in the Zhejiang Provincial Hospital of Traditional Chinese Medicine. Forty-three patients (the control group) aged 40-78 years who underwent total hip replacement due to trauma in the same period were also selected. Inclusion criteria: All patients in the study group met the 2007 guidelines for the diagnosis of osteoarthritis formulated by the Scientific Association of the Chinese Medical Association [12]. All patients in the study group were diagnosed using X-ray radiology and graded using K-L radiography [8]. The patients in the control group had no history of joint disease and were all patients with femoral head ischemia caused by trauma who needed total hip replacements. Exclusion criteria: K-L grade IV for the study group, other systemic inflammatory syndrome, serious endocrine dysfunction, pregnant women, cardiovascular, liver, and kidney disease, neurological and psychiatric history, chronic pain syndrome, language communication difficulties, joint bone tumors, various cancer bone metastases, recent acute stroke, autoimmune diseases for both two groups. The study was approved by the Ethics Committee of Zhejiang Provincial Hospital of Traditional Chinese Medicine, and informed consents were signed by the patients or their families.

Observation indicators

The levels of IL-6, IL-12 and MMP-13 were determined by ELISA. The correlations between IL-6, IL-12, MMP-13, and K-L grading, as well as the correlation between IL-6, IL-12, and MMP-13 expression levels were analyzed. The critical levels and application values of IL-6, IL-12, and MMP-13 in K-L grading were determined.

Detection method

The patients in the study group were tested by the imaging staff who used an XR-500-1 medical X-ray machine produced by Dongguan Momentum Positron Technology Co., Ltd. One chief physician and two physicians who have worked for more than 6 years were assigned to determine the K-L classifications in the study group. The synovial fluid was collected by clinicians during the operations. The supernatant was collected using centrifugation and the expression levels of IL-6, IL-12, and MMP-13 were detected by ELISA. The detection kits were purchased from Shanghai Yubo Biotechnology Co., Ltd. and the product numbers were IC-IL6-p, IC-IL18-p, and IC-MMP 13-p, respectively, and we strictly followed the manufacturer's instructions. A Berthold Microporous Plate Multifunctional Analyser LB 942 was purchased from Shanghai Flash Spectrum Biotechnology Co., Ltd. the product number SuPerMax 3000AL.

Statistical analysis

SPSS 22.0 software (Asia Analytics Formerly SPSS, China) was used for the statistical analy-

Table 1. Baseline characteristics ($\bar{x}$±sd)

	Control group (n=43)	Study group (n=72)	χ^2/t	P
Gender (n, %)			0.047	0.828
Male	26 (60.47)	45 (62.50)		
Female	17 (39.53)	27 (37.50)		
Age (years)	58.60±12.20	58.40±13.60	0.091	0.728
BMI (kg/m^2)	22.17±2.95	23.47±3.68	1.968	0.052
Height (cm)	162.34±16.72	158.75±16.25	1.134	0.259
Weight (kg)	56.33±6.27	58.39±6.84	1.611	0.110
K-L classification (n, %)				
Grade I		27		
Grade II		32		
Grade III		13		
Femoral BMD (g/cm^2)	0.78±0.15	0.82±0.19	1.178	0.241
RBC count (10^9/L)	4.24±1.33	4.22±1.26	0.081	0.936
WBC count (10^9/L)	7.15±2.14	7.23±2.08	0.197	0.844
Hemoglobin (g/L)	120.83±20.69	119.41±19.67	0.367	0.714
Creatinine (μmol/L)	53.17±18.64	55.32±19.14	0.589	0.557
Uric acid (μmol/L)	296.37±42.58	285.72±41.14	1.326	0.188
ALT (U/L)	17.22±11.35	18.15±10.17	0.454	0.651
AST (U/L)	25.15±14.68	24.83±14.48	0.114	0.909

Note: K-L: Kellgren-Lawrence; BMI: body mass index; BMD: bone mineral density; RBC: red blood cell; WBC: white blood cell; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

sis. Measuring data were expressed as the mean ± deviation ($\bar{x}$±sd). Counting data were expressed as n (%). Counting data were compared using an χ^2 test. A variance analysis was used for the comparison of measurement data among groups. An LSD test was used as a post-test. An independent sample *t* test was used for the comparison of measurement data between two groups. We used a Spearman analysis to determine the correlations between IL-6, IL-12, MMP-13, and the K-L classification. We used a Pearson analysis to determine the correlations of IL-6, IL-12, and MMP-13. The critical levels and application values of IL-6, IL-12, and MMP-13 in the K-L classification were determined using an ROC curve. P<0.05 was considered statistically significant.

Results

Baseline characteristics

There were 43 patients in the control group, including 26 males (60.47%) and 17 females (39.53%) aged 58.6±12.2 years. There were 72 patients in the study group, including 45 males (62.50%) and 27 females (37.50%) aged 58.4±13.6. There were no significant differences in the sex ratios or ages between the two groups (P>0.05). There were no significant differences in the other data of the two groups, such as body mass index (BMI), height, weight, femoral bone density, or some indexes of liver and kidney function (P>0.05) (**Table 1**).

Quantification results of IL-6, IL-12 and MMP-13

The expression levels of IL-6, IL-12, and MMP-13 in the synovial fluid of the two groups were significantly different, and the expression levels of IL-6, IL-12, and MMP-13 in the study group were higher than the levels in the control group (P<0.05). The analysis results of the patients with different K-L grades in the study group showed that there were significant differences in the expression levels of IL-6, IL-12, and MMP-13 among the patients with different K-L grades. With an increase of K-L grades, the expression levels of IL-6, IL-12, and MMP-13 increased (P<0.05) (**Tables 2**, **3**).

Analysis of the correlations among IL-6, IL-12, MMP-13, and the K-L classification

A Spearman correlation analysis showed that IL-6 (r=0.771, P<0.001), IL-12 (r=0.463, P<0.001), and MMP-13 (r=0.625, P<0.001) were

Table 2. The expression levels of IL-6, IL-12, and MMP-13 in the two groups ($\bar{x}$±sd)

	Control group (n=43)	Study group (n=72)	t	P
IL-6 (pg/mL)	78.46±14.74	163.56±16.85	27.429	<0.001
IL-12 (pg/mL)	17.37±6.15	157.71±29.35	30.901	<0.001
MMP-13 (ng/mL)	36.19±11.14	231.48±58.25	21.712	<0.001

Table 3. The expression levels of IL-6, IL-12, and MMP-13 among different K-L grades in the study group ($\bar{x}$±sd)

	Grade I (n=27)	Grade II (n=32)	Grade III (n=13)	F	P
IL-6 (pg/mL)	125.31±12.18	173.75±13.42*	186.62±18.58*,#	123.115	<0.001
IL-12 (pg/mL)	142.48±22.96	159.22±25.54*	171.43±28.65*,#	18.195	<0.001
MMP-13 (ng/mL)	182.57±44.65	225.97±56.18*	285.90±58.44*,#	17.270	<0.001

Note: Compared with grade I, *P<0.05; compared with grade II, #P<0.05.

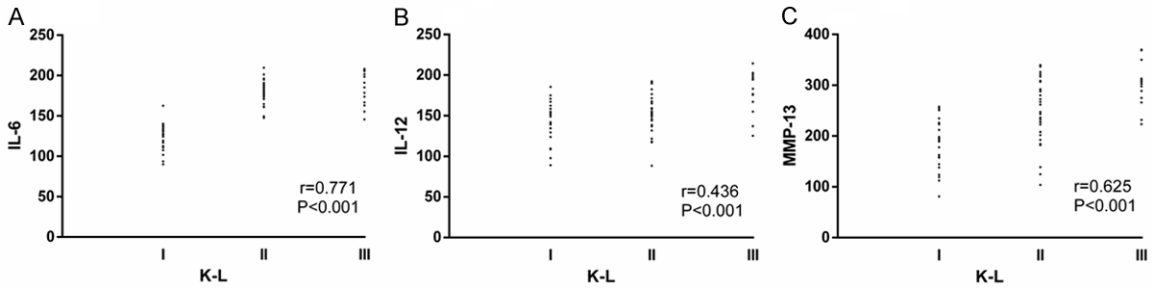

Figure 1. Spearman correlation analysis of IL-6, IL-12, MMP-13, and K-L classification. A: Correlation analysis of IL-6 and K-L classification. B: Correlation analysis of IL-12 and K-L classification. C: Correlation analysis of MMP-13 and K-L classification.

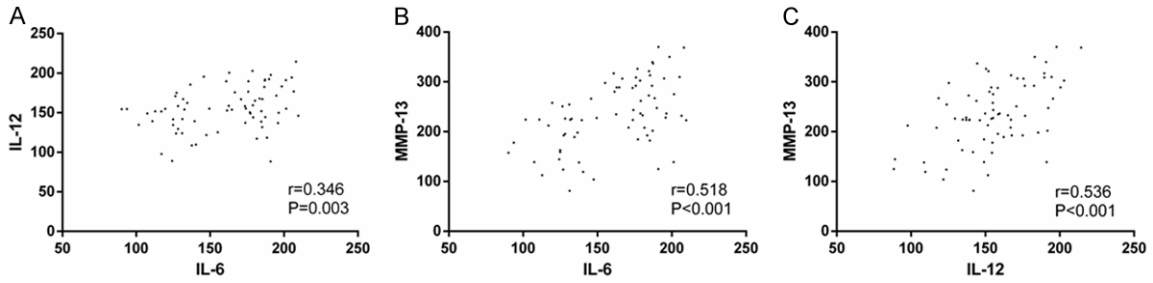

Figure 2. Pearson correlation analysis of IL-6, IL-12 and MMP-13. A: Correlation analysis of IL-6 and IL-12. B: Correlation analysis of IL-6 and MMP-13. C: Correlation analysis of IL-12 and MMP-13.

positively correlated with the K-L classification (**Figure 1**).

Correlation analysis of IL-6, IL-12, and MMP-13

A Pearson correlation analysis showed that IL-6 was positively correlated with IL-12 (r=0.346, P=0.003), MMP-13 (r=0.518, P<0.001), and IL-12 was positively correlated with MMP-13 (r=0.536, P<0.001) (**Figure 2**).

ROC analysis results

The critical values of IL-6, IL-12 and MMP-13 to classify grade I and grade II were 144.00 pg/mL, 143.00 pg/mL, and 226.80 ng/mL, and the AUC values were 0.995, 0.645, and 0.785 respectively. To classify grade II and grade III, the values were 184.70 pg/mL, 193.40 pg/mL, and 264.60 ng/mL, and the AUC values were

Table 4. ROC analysis results

		IL-6 (pg/mL)	IL-12 (pg/mL)	MMP-13 (ng/mL)
K-L-I-II	AUC	0.995	0.645	0.785
	95%CI	0.985-1.006	0.504-0.785	0.668-0.901
	Critical values	144.00	143.00	226.80
	Specificity	100%	78.13%	68.75%
	Sensitivity	96.3%	48.15%	85.19%
K-L-II-III	AUC	0.555	0.760	0.740
	95%CI	0.334-0.777	0.586-0.933	0.588-0.892
	Critical values	184.70	193.40	264.60
	Specificity	43.75%	46.15%	43.75%
	Sensitivity	100.00%	100.00%	100.00%

Note: AUC: Area under the curve; CI: Confidence interval.

0.555, 0.760 and 0.740, respectively (**Table 4**, **Figure 3**).

Discussion

The etiology of hip osteoarthritis is very complex. It is a kind of nonspecific arthritis mainly caused by the loss of hyaline cartilage and the remodeling of subchondral bone. The patient's joint space is narrowed, and then osteophytes and loose bodies form around the joints, resulting in an impairment or even loss of joint function [13, 14]. The K-L classification is the most widely used method for the clinical diagnosis of osteoarthritis, which has an important reference value that osteoarthritis surgeons use to formulate strategies for osteoarthritis treatment. Patients above grade II often need surgical treatment, but the K-L classification needs to be evaluated according to the results of radiological diagnosis. The evaluation results are influenced by clinical factors, which also increases the cost of treatment [15]. In recent years, many cytokines have been found to play an important role in the study of osteoarthritis, such as interleukin and MMPs [6, 7]. Therefore, this study analyzed the relationship between IL-6, IL-12, MMP-13, and the K-L classification and determined the critical diagnostic level in order to provide guidance for the clinical evaluation of the severity of osteoarthritis.

We first analyzed the differences in IL-6, IL-12, and MMP-13 expressions in synovial fluid between osteoarthritis patients and non-osteoarthritis patients. The levels of IL-6, IL-12, and MMP-13 in the synovial fluid of osteoarthritis patients were significantly higher than those of the non-osteoarthritis patients. This result has also been verified in previous studies [16-18]. Many scholars believe that inflammation is one of the mechanisms of osteoarthritis. In some histological, magnetic resonance imaging and ultrasonic imaging diagnostic studies of osteoarthritis, many patients have synovial inflammation changes [19, 20]. IL-6 is one of the most representative cytokines in the inflammatory reaction. IL-6 can activate the STAT-3 signaling pathway to induce chondrocyte catabolism, which is closely related to the degree of articular cartilage destruction [16]. After the expression of IL-6 is inhibited, the severity of osteoarthritis in model mice is significantly reduced [21]. IL-12 is produced by activated macrophages, lymphocytes, and other cells. Like IL-6, IL-12 can promote the catabolism of chondrocytes [22]. IL-12 can also induce an increase of inducible nitric oxide synthase and cyclooxygenase II to produce nitric oxide and prostaglandin E2. Nitric oxide can inhibit the migration of chondrocyte and the synthesis of proteoglycan. The ability to repair cartilage tissue in patients with osteoarthritis is extremely low, which further affects the repair of cartilage tissue in patients with osteoarthritis [23, 24]. Prostaglandin E2 is a metabolite of arachidonic acid. An elevated level of prostaglandin E2 is an important cause of pain in patients with osteoarthritis. It can increase blood circulation and vascular permeability, which play a key role in the progress of cartilage destruction in osteoarthritis, and it also participates in the remodeling of cartilage and bone in osteoarthritis patients [25]. MPP-13 is a collagenase that degrades the extracellular matrix of cartilage. It belongs to the MPP family. It can decompose collagen type I, collagen type II, and collagen type III. Among them, collagen type II is an important component of cartilage tissue. It forms a network structure with proteoglycan to maintain the integrity of cartilage tissue [26, 27].

We then analyzed the correlation between IL-6, IL-12, MMP-13, and the K-L classification. A Spearman correlation analysis showed that IL-6, IL-12, MMP-13, and the K-L classification were positively correlated, which provided a possibility for IL-6, IL-12, MMP-13 to replace the

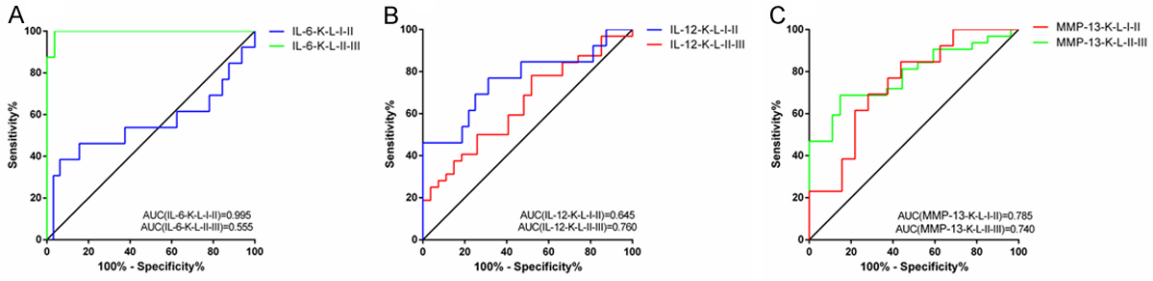

Figure 3. ROC analysis of IL-6, IL-12 and MMP-13. A: Value of IL-6 in differentiating K-L grades I, II, and III. B: Value of IL-12 in differentiating K-L grades I, II, and III. C: Value of MMP-13 in differentiating K-L grades I, II, and III.

K-L classification. However, in previous studies, IL-6 levels in the synovia of patients with knee osteoarthritis were negatively correlated with K-L grade [15], which was completely contrary to our results. In another study, there was no significant difference in the serum IL-6 levels between early (K-L grade I-II) and late (K-L grade III-IV) [28]. There are also studies showing that the level of IL-6 in peripheral blood increases with the severity of knee osteoarthritis [29]. These studies have not discussed this contradiction. We speculate that this may be related to the original source of the specimens and the location of osteoarthritis, but there are few studies on IL-6 in hip osteoarthritis. This assumption will be verified in future studies. The levels of IL-12 in the plasma, synovial fluid, and articular cartilages of patients with primary knee osteoarthritis were significantly increased, and they were positively correlated with the severity of imaging [30]. This is similar to our results, but there are no reports on MMP-13 and the severity of hip osteoarthritis. Although some of our results are controversial or not validated, the role of IL-6, IL-12 and MMP-13 in the development of osteoarthritis is clear, which supports our results but suggests that we need a more rigorous experimental design.

Finally, we determined the critical levels of IL-6, IL-12, and MMP-13 among the K-L grades. The critical values of IL-6, IL-12, and MMP-13 between K-L grade I and II were 144.00 pg/mL, 143.00 pg/mL and 226.80 ng/mL, respectively. The critical values for distinguishing K-L grades II from III were 184.70 pg/mL, 193.40 pg/mL and 264.60 ng/mL, respectively. The AUC values all exceeded 0.5, which further increased the possibility of IL-6, IL-12, and MMP-13 replacing the K-L grade. It was found that IL-6 was more valuable than IL-12 and MMP-13 in evaluating K-L grade I and II patients, but IL-12 and MMP-13 were more advantageous in evaluating K-L grade II and III patients. However, this study did not analyze the expression levels of IL-6, IL-12 and MMP-13 in the peripheral blood, so it is impossible to judge whether IL-6, IL-12, and MMP-13 in the serum have the same value in this experiment, but this will be covered in our future research.

In summary, the expression levels of IL-6, IL-12, and MMP-13 in the synovial fluid of patients with hip osteoarthritis increased with the severity of osteoarthritis. In addition, IL-6, IL-12, MMP-13, and the K-L classification have a good correlation, which has a good application value in evaluating the severity of osteoarthritis.

Disclosure of conflict of interest

None.

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References

[1] Nelson AE. Osteoarthritis year in review 2017: clinical. Osteoarthritis Cartilage 2018; 26: 319-325.

[2] Rousseau JC, Sornay-Rendu E, Bertholon C, Garnero P and Chapurlat R. Serum periostin is associated with prevalent knee osteoarthritis and disease incidence/progression in women: the OFELY study. Osteoarthritis Cartilage 2015; 23: 1736-1742.

[3] da Costa BR, Reichenbach S, Keller N, Nartey L, Wandel S, Juni P and Trelle S. Effectiveness of non-steroidal anti-inflammatory drugs for

the treatment of pain in knee and hip osteoarthritis: a network meta-analysis. Lancet 2017; 390: e21-e33.
[4] Machado GC, Maher CG, Ferreira PH, Pinheiro MB, Lin CW, Day RO, McLachlan AJ and Ferreira ML. Efficacy and safety of paracetamol for spinal pain and osteoarthritis: systematic review and meta-analysis of randomised placebo controlled trials. BMJ 2015; 350: h1225.
[5] Qin J, Barbour KE, Murphy LB, Nelson AE, Schwartz TA, Helmick CG, Allen KD, Renner JB, Baker NA and Jordan JM. Lifetime risk of symptomatic hand osteoarthritis: the johnston county osteoarthritis project. Arthritis Rheumatol 2017; 69: 1204-1212.
[6] Nasi S, Ea HK, So A and Busso N. Revisiting the role of interleukin-1 pathway in osteoarthritis: interleukin-1alpha and -1beta, and NLRP3 inflammasome are not involved in the pathological features of the murine menisectomy model of osteoarthritis. Front Pharmacol 2017; 8: 282.
[7] Akhtar N, Khan NM, Ashruf OS and Haqqi TM. Inhibition of cartilage degradation and suppression of PGE2 and MMPs expression by pomegranate fruit extract in a model of post-traumatic osteoarthritis. Nutrition 2017; 33: 1-13.
[8] Livshits G, Zhai G, Hart DJ, Kato BS, Wang H, Williams FM and Spector TD. Interleukin-6 is a significant predictor of radiographic knee osteoarthritis: the Chingford study. Arthritis Rheum 2009; 60: 2037-2045.
[9] Janusz MJ, Bendele AM, Brown KK, Taiwo YO, Hsieh L and Heitmeyer SA. Induction of osteoarthritis in the rat by surgical tear of the meniscus: Inhibition of joint damage by a matrix metalloproteinase inhibitor. Osteoarthritis Cartilage 2002; 10: 785-791.
[10] Futani H, Okayama A, Matsui K, Kashiwamura S, Sasaki T, Hada T, Nakanishi K, Tateishi H, Maruo S and Okamura H. Relation between interleukin-18 and PGE2 in synovial fluid of osteoarthritis: a potential therapeutic target of cartilage degradation. J Immunother 2002; 25 Suppl 1: S61-64.
[11] Petrovic-Rackov L and Pejnovic N. Clinical significance of IL-18, IL-15, IL-12 and TNF-alpha measurement in rheumatoid arthritis. Clin Rheumatol 2006; 25: 448-452.
[12] Cutolo M, Berenbaum F, Hochberg M, Punzi L and Reginster JY. Commentary on recent therapeutic guidelines for osteoarthritis. Semin Arthritis Rheum 2015; 44: 611-617.
[13] Yang M, Jiang L, Wang Q, Chen H and Xu G. Traditional Chinese medicine for knee osteoarthritis: an overview of systematic review. PLoS One 2017; 12: e0189884.
[14] Bai Z, Guo XH, Tang C, Yue ST, Shi L and Qiang B. Effects of artesunate on the expressions of insulin-like growth factor-1, osteopontin and C-Telopeptides of type II collagen in a rat model of osteoarthritis. Pharmacology 2018; 101: 1-8.
[15] Zhao TJ, Zhang HM, Chen WH, Jing L, Sun G, Gu LJ, Zhang C and Yin T. Grading treatment of hip osteoarthritis. Chin J Orthopa Trauma 2010; 23: 665-667.
[16] Stannus O, Jones G, Cicuttini F, Parameswaran V, Quinn S, Burgess J and Ding C. Circulating levels of IL-6 and TNF-alpha are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. Osteoarthritis Cartilage 2010; 18: 1441-1447.
[17] Jiang L, Bao J, Zhou X, Xiong Y and Wu L. Increased serum levels and chondrocyte expression of nesfatin-1 in patients with osteoarthritis and its relation with BMI, hsCRP, and IL-18. Mediators Inflamm 2013; 2013: 631251.
[18] Han S, Han MS and Park HR. Transglutaminase-2 in osteoarthritis: MMP-13 production through enhanced FOXO3A nuclear translocation. Osteoarthritis Cartilage 2018: 26: S110.
[19] Oehler S, Neureiter D, Meyer-Scholten C and Aigner T. Subtyping of osteoarthritic synoviopathy. Clin Exp Rheumatol 2002; 20: 633-640.
[20] Rhodes LA, Conaghan PG, Radjenovic A, Grainger AJ, Emery P and McGonagle D. Further evidence that a cartilage-pannus junction synovitis predilection is not a specific feature of rheumatoid arthritis. Ann Rheum Dis 2005; 64: 1347-1349.
[21] Latourte A, Cherifi C, Maillet J, Ea HK, Bouaziz W, Funck-Brentano T, Cohen-Solal M, Hay E and Richette P. Systemic inhibition of IL-6/Stat3 signalling protects against experimental osteoarthritis. Ann Rheum Dis 2017; 76: 748-755.
[22] Ansari MY, Khan MN and Ahmad I. OP0105 Zcchc6 deletion downregulates il-6 and reduces the severity of experimental osteoarthritis in mice. Ann Rheum Dis 2018; 77: 103.
[23] Alaaeddine N, Antoniou J, Moussa M, Hilal G, Kreichaty G, Ghanem I, Abouchedid W, Saghbini E and Di Battista JA. The chemokine CCL20 induces proinflammatory and matrix degradative responses in cartilage. Inflamm Res 2015; 64: 721-731.
[24] Patra S, Muthuraman MS, Meenu M, Priya P and Pemaiah B. Anti-inflammatory effects of royal poinciana through inhibition of toll-like receptor 4 signaling pathway. Int Immunopharmacol 2016; 34: 199-211.
[25] Aikawa J, Uchida K, Takano S, Inoue G, Iwase D, Miyagi M, Mukai M, Shoji S, Sekiguchi H and Takaso M. Regulation of calcitonin gene-related peptide expression through the COX-2/mPGES-1/PGE2 pathway in the infrapatellar fat pad in knee osteoarthritis. Lipids Health Dis 2018; 17: 215.

[26] Zhang W, Zheng J, Chen J and Huang L. The influence of connective tissue growth factor on rabbit ligament injury repair. Saudi Pharm J 2017; 25: 498-503.
[27] Mehdizadeh A, Gardiner BS, Lavagnino M and Smith DW. Predicting tenocyte expression profiles and average molecular concentrations in achilles tendon ECM from tissue strain and fiber damage. Biomech Model Mechanobiol 2017; 16: 1329-1348.
[28] Shimura Y, Kurosawa H, Sugawara Y, Tsuchiya M, Sawa M, Kaneko H, Futami I, Liu L, Sadatsuki R, Hada S, Iwase Y, Kaneko K and Ishijima M. The factors associated with pain severity in patients with knee osteoarthritis vary according to the radiographic disease severity: a cross-sectional study. Osteoarthritis Cartilage 2013; 21: 1179-1184.
[29] Ansari MY, Khan NM and Haqqi TM. ZCCHC6 (TUT7) deletion inhibit the expression of IL-6 and MMP-13 and reduces the severity of post-traumatic osteoarthritis in a murine model. Osteoarthritis Cartilage 2018; 26: S97-S98.
[30] Wang Y, Xu D, Long L, Deng X, Tao R and Huang G. Correlation between plasma, synovial fluid and articular cartilage Interleukin-18 with radiographic severity in 33 patients with osteoarthritis of the knee. Clin Exp Med 2014; 14: 297-304.