

Original Article

Elevated chemerin levels in synovial fluid and synovial membrane from patients with knee osteoarthritis

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Abstract: To test the serum, synovial fluid and synovial membrane levels of the adipokine chemerin in patients with knee osteoarthritis (KOA) and investigate their relationships with the severity of articular cartilage damage and synovitis. According to the American College of Rheumatology criteria for diagnosis of osteoarthritis (OA), 30 cases with OA diagnoses (OA group) were selected from patients who underwent arthroscopic surgery in our hospital from June 2013 to February 2014. Another 30 cases with other knee joint diseases (non-OA group) were included as controls. The synovial fluid and serum levels of chemerin were assayed by ELISA, and the synovial membrane level of chemerin was assayed by the immunohistochemical method. The severity of the knee articular cartilage damage and synovitis-related pathological changes were evaluated by arthroscopy using the Outerbridge and Ayrar scores, respectively. The synovial fluid and synovial membrane levels of chemerin in the OA group were higher than those in the non-OA group. Statistically significant differences were found between the two groups in the synovial fluid and synovial membrane levels of chemerin ($P < 0.05$). The synovial fluid and synovial membrane levels of chemerin were positively correlated with the serum level of high-sensitivity C-reactive protein (HS-CRP), Outerbridge score and Ayrar score in the OA group. The synovial fluid and synovial membrane levels of chemerin are increased in KOA patients and are positively correlated with the severity of KOA.

Keywords: Osteoarthritis, chemerin, synovial fluid, synovial membrane

Introduction

Osteoarthritis (OA) is a chronic joint disease characterized by the 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 risk factors for OA. The pathogenesis of OA has not been explicitly documented [2].

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 the diagnosis of KOA. 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 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 oligo-

meric matrix protein, proteoglycan and high-sensitivity C-reactive protein (HS-CRP) [3]. Chemerin is a novel adipokine that was identified in 2007 and plays a critical role in the development of coronary atherosclerosis, metabolic syndrome and other diseases [4]. In the present study, the serum, synovial fluid and synovial membrane levels of chemerin in KOA patients were tested to analyze their relationships with the severity of knee articular cartilage damage and synovitis-related pathological changes.

Materials and methods

Clinical data

According to the American College of Rheumatology (ACR) criteria for diagnosis of OA, 30 cases with OA diagnoses (OA group) were selected from patients who underwent arthroscopic surgery in our hospital from June 2013 to February 2014. Another 30 cases with other

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knee joint diseases (non-OA group) were included as controls. The OA group included 6 males and 24 females, aged 43-80 years old, with an average age of 61 years; the mean body mass index was 18.2-26.8, and the mean course of disease lasted 6-90 months. The non-OA group included 15 males and 15 females, aged 41-80 years old, with an average age of 39 years; there were 18 cases of meniscus injury and 12 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 of the patients.

Diagnostic criteria for OA: According to the ACR criteria for diagnosis of KOA: (1) knee pain most of the time for a month; (2) snapping joint activities; (3) morning stiffness \leq 30 min; (4) age \geq 40 years; (5) knee joint swelling with snapping; and (6) knee joint swelling without snapping. OA was diagnosed upon the observation of at least (1), (2), (3) and (4), or (1), (2), (3) and (5), or (1) and (6).

Exclusion criteria: Other associated inflammatory arthritis or autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus or gout; a history of steroid injection 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.

Inclusion criteria: (1) arthroscopic anterior cruciate ligament reconstruction; and (2) meniscus angioplasty or arthroscopy.

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

The severities of the articular cartilage damage and synovitis were evaluated in accordance with the findings of knee arthroscopy using the Outerbridge [5] and Ayrar scores [6], respectively.

Collection and preservation of serum and synovial fluid specimens

Serum specimens: Patients were fasted 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 non-anticoagulant tubes and centrifuged at 3,000 rpm for 15 min. Serum specimens were retained and dispensed into Eppendorf 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 a puncture in the knee joint cavity through the lateral suprapatellar approach. The specimens were kept in serum tubes and centrifuged at 3,000 rpm for 15 min within 2 hours after surgery. The supernatants were collected into Eppendorf tubes and kept at -80°C .

ELISA assay of chemerin in synovial fluid and serum

Synovial fluid and serum levels of chemerin were assayed by ELISA using a commercial kit (R&D, USA). 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 then, 50 μL of 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 liquid. 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 on a shaker for 5 s to thoroughly mix the substrate and termination solutions. The absorbance of the reaction mixture at 450 nm wavelength (A value) was measured. The sample concentrations of chemerin were calculated from the standard curve.

Serum HS-CRP analysis

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

Immunohistochemical assay of chemerin

Tissue specimens of synovial membrane were taken during arthroscopic surgery. The specimens were washed twice with normal saline within one hour after surgery and cut into approximately 0.5 cm \times 0.5 cm \times 0.5 cm blocks. The tissue blocks were fixed with 10% paraformaldehyde, embedded in paraffin and sectioned into 4-5 μm -thick slices. Pathological sections were prepared after surgery, and chemerin expression was assayed by immunohistochemistry. The section specimens were

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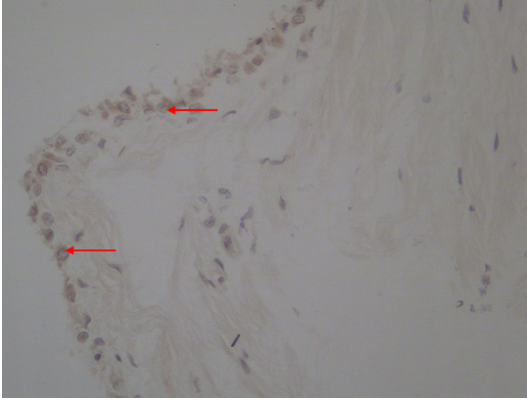


Figure 1. Microphotograph of chemerin (red arrows) in synovium membrane. Synovial membrane sections were prepared and chemerin expression was examined by immunohistochemistry. The section specimens were observed under the microscope. Five fields of view at high magnification (400 ×) were chosen randomly from each stained section.

Table 1. Serum and synovial fluid levels of chemerin in patients with OA and non-OA (ng/L)

Group	Cases	Synovial fluid	Serum
OA	30	3943.26 ± 29.78	1552.16 ± 28.93
Non-OA	30	2467.54 ± 30.66	1113.62 ± 32.15
<i>T</i>	-	2.83	0.026
<i>P</i>	-	0.042	0.928

Table 2. Synovial membrane level of chemerin in patients with OA and non-OA (µg/L)

Group	Cases	Synovial membrane
OA	30	43.60 ± 6.28
Non-OA	30	32.22 ± 5.73
<i>T</i>	-	3.39
<i>P</i>	-	0.02

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 then used as a standard for automatic detection of all the positive results in the field of view.

Statistical analysis

Statistical analysis was performed using SPSS 18.0. Continuous data were expressed as the mean ± standard deviation ($X \pm SD$). Comparison of the 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

Chemerin expression in synovial membrane of KOA patients

Tissue specimens of synovial membrane were prepared after surgery and chemerin expression was assayed by immunohistochemistry. The section specimens were examined under the microscope. One brownish-yellow particle representing a positive result was marked within one of the fields of view and then used as a standard for automatic detection of all the positive results in the field of view (**Figure 1**).

Synovial fluid and synovial membrane levels of chemerin in the OA patients were elevated

Chemerin levels in synovial fluid were elevated in patients with OA compared with non-OA group (3943.26 ± 29.78 vs. 2467.54 ± 30.66 ng/L, *P* = 0.042). However, in OA patients, plasma chemerin levels were not statistically different from non-OA patients (*P* > 0.05) (**Table 1**). As seen from **Table 2**, synovial membrane levels of chemerin in patients with OA were significantly increased compared with non-OA 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 plasma, synovial fluid and synovial membrane levels of chemerin were all significantly correlated with serum HS-CRP concentration (**Table 3**).

Synovial fluid and synovial membrane levels of chemerin are positively correlated with the severity of KOA

Next, we evaluated the severity of the knee articular cartilage damage and synovitis-related pathological changes by arthroscopy using the Outerbridge and Ayril scores. We found that the synovial fluid (1552.16 ± 28.93 ng/L) and synovial membrane (43600 ± 6280 ng/L) levels of chemerin were positively correlated with both Outerbridge (**Table 4**) and Ayril scores (**Table 5**). These results indicated that as the concentration of chemerin increased in the synovial tissue, the severity of synovitis was correspondingly increased.

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Table 3. Correlations between serum, synovial fluid and synovial membrane levels of chemerin and serum HS-CRP concentration in OA patients

Group	Serum	Synovial fluid	Synovial membrane
Chemerin (ng/L)	1552.16 ± 28.93	3943.26 ± 29.78	43600 ± 6280
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 synovial fluid and synovial membrane levels of chemerin and the Outerbridge score of articular cartilage damage in patients with osteoarthritis

	Synovial membrane	Synovial fluid
Chemerin (ng/L)	43600 ± 6280	1552.16 ± 28.93
Outerbridge score	18.71 ± 1.3	18.71 ± 1.3
R	0.68	0.13
P	< 0.05	0.007

Table 5. Correlations between synovial fluid and synovial membrane levels of chemerin and the Ayril score of synovitis in patients with osteoarthritis

	Synovial membrane	Synovial fluid
Chemerin (ng/L)	43600 ± 6280	1552.16 ± 28.93
Ayril score	29.38 ± 0.40	29.38 ± 0.40
R	0.30	0.17
P	< 0.05	< 0.05

Discussion

KOA is a chronic joint disease characterized by the degeneration, destruction, hyperplasia and synovitis of the articular cartilage and subchondral bone. Advanced KOA patients often struggle with limited joint mobility, joint deformity, severe pain and even disability. Ultimately, these patients will have to undergo surgical treatment such as joint replacement. Prior to the observation of typical X-ray changes and clinical manifestations, KOA patients have undergone significant degeneration of the knee joint. 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 associated biomarkers in synovial fluid may be useful in the early diagnosis of KOA.

Adipokines have an important role in the development and progression of OA. It is possible that research on adipokines may yield a breakthrough in the early diagnosis of KOA. Chemerin is a novel adipokine identified in 2007, and it is a small protein consisting of 163 amino acid residues.

Chemerin is secreted in the form of pre-protein and exhibits biological activity after being hydrolyzed by cysteine and serine proteases [7]. Previous research has indicated that there is no significant difference in the serum level of adiponectin between OA patients and the control group. The same results have been obtained in a few recent studies. Our results showed that the serum level of chemerin had no significant difference between the OA and normal control groups. However, the serum levels of other adipokines, such as resistin, visfatin and endogenous ligands, all displayed significant differences in OA patients from those of normal individuals. These differences may be related to different systemic reactions caused by various adipokines in OA patients [8, 9].

HS-CRP is secreted by hepatocyte nuclei and adipocytes and is regulated by pro-inflammatory factors. According to the 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 [10, 11]. In the present study, the results showed that the serum HS-CRP level was significantly correlated with the serum, synovial fluid and synovial membrane levels of chemerin. Previous research has indicated that CRP is closely associated with the severity of KOA. A recent study also showed that the serum CRP level in KOA is higher in KL4 patients than in KL2 and KL3 patients. Moreover, our results showed that the serum and synovial membrane levels of chemerin were correlated with the CRP level. Similar results have been reported in the literature. It is documented that the serum level of chemerin is positively correlated with the CRP level in

patients with metabolic syndrome and type 2 diabetes [12, 13].

In this study, the synovial fluid and synovial membrane levels of chemerin were positively correlated with the Ayril score of synovitis. This correlation was higher between the synovial membrane level of chemerin and the Ayril score. That is, as the concentration of chemerin increased in the synovial membrane, the severity of synovitis was correspondingly increased, as was the Ayril score. This result indicates that the level of adipokine concentration reflects the severity of synovitis. The latest research overseas has shown that chemerin and its receptor, CMKLR1, are also expressed in synovial fibroblasts. Chemerin has a role in chemotactic insulin reaction and inflammatory protein release in the metabolism of synovial fibroblasts, while the presence of synovitis is a critical mediator of the damage and chronic persistence of OA. Therefore, we conclude that the synovial fluid or membrane levels of chemerin to some extent reflect the severity of synovitis in KOA.

Iannone et al. [14] reported that chemerin factor and ChemR23 are expressed in articular chondrocytes (AC). The results from the present study also showed that both the synovial fluid and synovial membrane levels of chemerin were positively correlated with the Outerbridge score of cartilage damage. This correlation was higher between the chemerin concentration in the synovial membrane and the score of the articular cartilage damage; that is, as the chemerin concentration increased, the severity of the articular cartilage damage was correspondingly increased, as was the Outerbridge score. This result indicates that the level of adipokine concentration reflects the severity of the articular cartilage damage.

KOA is pathologically manifested as synovitis and different degrees of articular cartilage damage. Arthroscopic evaluation by Ayril and Outerbridge scores could objectively reflect the severity of KOA. In the present study, the chemerin in the serum, synovial fluid and synovial membrane was detected in KOA patients, and the chemerin levels were found to be positively correlated with the Ayril and Outerbridge scores. Therefore, we infer that chemerin may be another valuable biomarker to reflect the severity of KOA.

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Disclosure of conflict of interest

None.

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