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RESEARCH ARTICLE

Association of chemerin levels in synovial fluid with the severity of knee osteoarthritis

Ke Huang, Gang Du, Lin Li, Hongsuo Liang, and Bo Zhang

Department of Orthopaedic Surgery, The Second People's Hospital of Nanning, Guangxi, P.R China

Abstract
Context: Chemerin has been implicated to be correlated with inflammation.

Objective: This study aims to determine the association of chemerin levels in serum and synovial fluid (SF) with the disease severity of patients with knee Osteoarthritis (OA).

Methods: 124 patients with knee OA and 76 healthy controls were enrolled in this study.

Results: Chemerin levels in serum were significant higher with regard to paired SF. Chemerin levles in SF of knee OA patients were correlated with disease severity evaluated by KL grading criteria.

Conclusion: Chemerin levels may be involved in the pathophysiology of the development and progression of OA.

Keywords: Chemerin, synovial fluid, severity, osteoarthritis

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Introduction

Osteoarthritis (OA) is a chronic degenerative joint disease characterized by the loss of articular cartilage, which is caused by the imbalance between cartilage matrix synthesis and degradation (Clements et al. 2006). Risk factors, such as obesity, trauma, age, and female gender are considered to play important roles in the development and progression of OA (Blagojevic et al. 2010). However, the underlying causes of OA are still unknown.

Currently, magnetic resonance imaging and direct arthroscopic examination are still the main methods for evaluating the OA progression. However, these methods are unsatisfactory due to their limitations in the early detection, the debate of critical standards, as well as traumatic defects. Thus, it is essential to identify more quantitative, reliable, and sensitive detection methods of OA. Biomarkers may serve as potential predictors for the presence, progression, and severity of OA. Recent evidence revealed a significant association between the radiographic grading of OA and some biochemical markers in the synovial fluid (SF), including P selectin (Cheng et al. 2010), cartilage oligomeric matrix protein, aggrecan (El-Arman et al. 2010), nuclear factor kappa B ligand (Ellabban et al. 2011), as well as CXCL8, and CCL5 (Pierzchala et al. 2011). Furthermore, urinary type II collagen C-telopeptide (CTX-II) was shown to be associated with radiographic signs of joint damage (Garnero et al. 2005).

Recent studies showed that pro-inflammatory mediators are involved in the pathophysiology of OA (Li et al. 2009). Inflammatory cytokines in the synovial membrane are generally considered to promote the pathological changes of OA by increasing cartilage degradation and inducing hyperalgesia (Orita et al. 2011). The increased systematic inflammation marker in the plasma and SF of OA patients has been associated with the radiographic severity of patients with hand, knee, or hip OA (Wolfe 1997; Conrozier et al. 2000). Chemerin, a newly discovered adipokine, has been shown to be associated with inflammatory markers including high sensitivity C reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α; Lehrke et al. 2009; Weigert et al. 2010). In addition, human chondrocytes were demonstrated to express both the

Address for Correspondence: Ke Huang, M.D., Department of Orthopaedic Surgery, The Second People's Hospital of Nanning, 13 Dancun Road, 530031, Nanning, Guangxi, P.R China. Tel/Fax: 86-0771-4835822. E-mail: gxhuangke@126.com

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receptor ChemR23 and the ligand chemerin. Chemerin stimulation further promotes inflammatory signaling in chondrocytes, which is characterized by an enhanced secretion of cytokines and metalloproteases (Berg et al. 2010). Consequently, chemerin is hypothesized to be involved in the mechanism of OA, and chemerin levels in the serum and SF may be correlated with the severity of knee OA.

The current study aims to determine the correlation between serum and SF chemerin concentrations and the radiographic disease severity in patients with knee OA in order to assess its role in the pathophysiology of OA.

Materials and methods

Patients

A total of 124 patients diagnosed with knee OA according to the criteria of the American College of Rheumatology were enrolled in the present study. Patients with additional inflammatory arthritis or auto-immune diseases, including rheumatoid arthritis, systemic lupus erythematosus, and gout, who had received corticosteroid injection, or nonsteroidal anti-inflammatory drugs within the past 3 months; or who had experienced trauma were excluded. Control participants were recruited from healthy subjects with medical check-up in our hospital and matched to the cases by age, gender, and body mass index (BMI). They had no clinical and radiological evidence of OA, arthritis or other joint diseases. All subjects in control and case groups were Han population from South China.

This study was approved by the ethics committee of our hospital, and informed consent was obtained from all participants.

Clinical and laboratory data collection

Clinical data including age, height, and weight were indentified from the medical records. BMI was calculated as weight in kilograms divided by height squared in meters (kg m^{-2}). Before any treatment on OA, SF and serum samples were obtained from OA patients who received the treatment of hyaluronic acid injection for the first time. Serum samples were also collected from the healthy subjects as the controls. Then chemerin levels in the serum and SF were measured by an enzyme-linked immunosorbent assay (Phoenix Pharmaceuticals, Inc, Burlingame, CA, USA) (Coefficient of variations (CVs) for intra assay: 5–7%; CVs for inter assay: 12–15%; detection limit range: 0–100 μg/L). CRP was tested using an auto biochemistry instrument (Hitachi 7170; Tokyo, Japan).

Assessment of radiographic outcomes

Disease severity was graded by two graders who were blinded to the results according to the system of Kellgren and Lawrence (1957). The subjects who had radiographic knee OA of KL grade ≥2 in at least one knee were defined as knee OA patients. Healthy controls were defined as having neither radiographic knee OA as indicated by KL grades of 0 for both knees. When the patients were affected in both knees, the grading of the worst affected knee was used for analysis.

Statistical analysis

The data are presented as means ± SD. Statistical analyses were performed using SPSS version 16.0 software. Data normality was analyzed using the Kolmogorov-Smirnov test. The values were analyzed by the Student t-test to compare the differences between patients with knee OA and healthy controls. Categorical variables were analyzed by the χ^2 tests. The association of chemerin levels with other characteristics was analyzed using Spearmen correlation analysis. Characteristics were compared between knee patients with different KL grades using one-way ANOVA or χ^2 tests. The Spearmen correlation coefficient was calculated to determine the relationship between serum and SF chemerin levels and the severity of OA. A multinomial logistic regression analysis was performed to determine the association of various factors with the severity of OA. As the chemerin and CRP levels were not normally distributed, logarithmic (log) transformed values were used for multiple linear regression analysis. A P value of less than 0.05 was considered statistically significant.

Results

Baseline clinical parameters

The baseline clinical parameters of patients with knee OA and healthy controls are presented in Table 1. Knee OA patients showed higher levels of serum CRP compared with healthy controls. No significant differences in age, gender, and BMI were observed between the two groups.

The chemerin levels in serum and SF

The levels of serum chemerin in knee OA patients were higher than those in healthy controls, however, the difference was not statistically significant ($P > 0.05$). Chemerin levels in the serum of knee OA patients were significantly higher compared with those in paired SF ($P < 0.001$). Chemerin levels in serum and SF of knee OA patients and serum levels of chemerin in healthy controls are shown in Figure 1.

Table 1. The characteristics between patients with knee OA and healthy controls.

Characteristics	Knee OA patients	Healthy controls	P value
Age (years)	58.94 ± 8.15	59.55 ± 7.55	0.599
Gender (male/female)	44/80	26/50	0.855
BMI (kg/m^2)	24.99 ± 2.84	24.63 ± 2.36	0.366
CRP (mg/L)	1.49 ± 0.52	2.92 ± 0.96	<0.001

BMI, body mass index; CRP, C reactive protein; OA, osteoarthritis.

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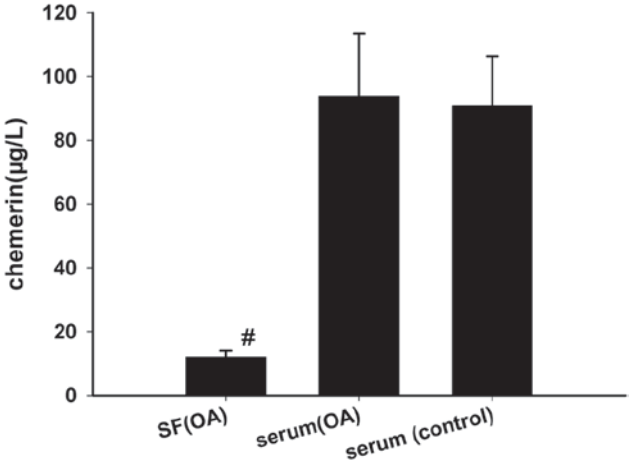

Figure 1. Chemerin levels in serum and SF. There were no significant differences in the serum chemerin levels between patients with knee OA and healthy controls ($P > 0.05$). Chemerin levels in SF were lower than in paired serum samples in knee OA patients ($P < 0.001$). OA, osteoarthritis; SF, synovial fluid.

Association of chemerin levels with other characteristics

In the healthy control group, the serum chemerin levels were significantly correlated with BMI (r = 0.373, $P = 0.001$) and CRP (r = 0.394, $P < 0.001$). However, no significant association was found between serum chemerin levels with age (r = 0.068, $P = 0.557$) and gender (r = 0.111, $P = 0.341$). In the knee OA patients group, the chemerin levels in serum and SF were both correlated with BMI (r = 0.387, $P < 0.001$ and r = 0.305, $P = 0.001$, respectively) and CRP (r = 0.394, $P < 0.001$ and r = 0.412, $P < 0.001$, respectively). There were no significant associations of chemerin levels in serum and SF with age (r = 0.061, $P = 0.502$ and r = 0.078, $P = 0.390$, respectively) and gender (r = 0.125, $P = 0.167$ and r = 0.109, $P = 0.230$, respectively).

Chemerin levels in knee OA patients with different KL grades

The chemerin levels of serum and SF in knee OA patients with different KL grades are displayed in Table 2. Knee OA patients with KL grade 4 showed significantly elevated chemerin levels in SF compared with those with KL grade 2 and 3. However, no significant differences in the chemerin levels of SF were found between patients with KL grade 2 and 3. Furthermore, there were no significant differences in the serum chemerin levels between knee OA patients with different KL grades.

Higher levels of serum CRP were found in knee OA patients with KL grade 4 compared with those with KL grade 2 and 3. There were no significant differences in age, gender, and BMI between knee OA patients with different KL grades.

Association of clinical parameters with KL grades

Spearmen correlation analysis showed that the chemerin levels in SF were positively correlated with KL grades (r = 0.367, $P < 0.001$). However, no significant association was found between serum levels of chemerin and KL grades (r = 0.129, $P = 0.152$). Multinomial logistic regression analysis was used to examine the association of various clinical factors with KL grades. The results indicated that chemerin levels of SF were still positively associated with KL grades after adjusting for age, gender, BMI, and CRP ($P < 0.001$). There was no significant association between serum levels of chemerin and KL grades after multinomial logistic regression analysis ($P = 0.981$).

Table 2. The characteristics in knee OA patients with different KL grades.

Chemerin (μg/L)	Grade 2 (n = 37)	Grade 3 (n = 55)	Grade 4 (n = 32)	*P* value
Age (years)	59.46 ± 8.99	58.73 ± 7.87	58.72 ± 7.82	0.901
Gender (male/female)	13/24	21/34	10/22	0.808
BMI (kg/m^2)	24.69 ± 2.70	24.76 ± 2.90	25.72 ± 2.85	0.239
CRP(mg/L)	2.57 ± 0.65	2.78 ± 0.72	3.56 ± 0.97	0.034
Chemerin levels in serum (μg/L)	90.88 ± 16.13	92.45 ± 18.98	97.52 ± 15.97	0.261
Chemerin levels in SF (μg/L)	11.24 ± 1.78	11.61 ± 1.98	13.37 ± 2.01	<0.001

BMI, body mass index; CRP, C reactive protein; OA, osteoarthritis.

Discussion

Previous studies revealed no significant differences in the levels of serum adiponectin between OA patients and healthy controls (Honsawek et al. 2010; Choe et al. 2011). Similar results were found in the present study. Our results indicated that no remarkable differences in the serum chemerin concentrations were observed between knee OA patients and healthy subjects. However, the serum levels of other adipokines including resistin (Choe et al. 2011), visfatin (Chen et al. 2010), and apelin (Hu & Feng 2011) were significantly elevated in the OA patients compared with the healthy controls. This inconsistency may be mainly due to the different systemic effects caused by different adipokines in OA patients.

CRP has been demonstrated to be correlated with the severity of OA (Wolfe 1997; Stürmer et al. 2004). The current results also indicated that knee OA patients with KL grade 4 had higher levels of serum CRP compared with those with KL grade 2 and 3. In addition, our results revealed that the chemerin levels in serum and SF were correlated with the serum CRP levels. Similar results were found in other studies. Serum chemerin levels were positively correlated with serum CRP levels in patients with metabolic syndrome (Dong et al. 2011), type 2 diabetes (Weigert et al. 2010; Hu et al. 2011), and incident dialysis (Yamamoto et al. 2010). This indicates that chemerin is correlated with inflammation. Chemerin may play a role in the mechanism of OA as an inflammatory factor.

The present study indicated that the chemerin levels in SF were significantly elevated in knee OA patients with KL grade 4 compared with those with KL grade

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2 and 3. The chemerin levels in the SF were positively correlated with the severity of knee OA. This is consistent with the findings on other adipokines. Adipokines, including adiponectin and leptin, were demonstrated to be associated with the severity of OA (Ku et al. 2009; Honsawek et al. 2010). Currently, radiological investigation is the traditional method to examine the affected joint and assess the disease progression of OA. Our findings suggest that chemerin levels in SF may serve as a new biomarker for predicting or assessing the risk and severity of knee OA.

Chemerin has been suggested to be involved in the pathogenesis of OA. Chemerin is a chemotactic peptide that binds to the G protein-coupled receptor ChemR23 (Meder et al. 2003). Chemerin and ChemR23 were expressed in cultured human articular chondrocytes from OA patients. The expression levels of pro-inflammatory cytokines, including IL-6, IL-8, IL-1β, and TNF-α, as well as the matrix metalloproteases, such as MMP-1, MMP-2, MMP-3, MMP-8, and MMP-13, were significantly elevated in the cultured articular chondrocytes after stimulation with recombinant chemerin (Berg et al. 2010). These inflammatory cytokines were demonstrated to promote pathological OA (Kapoor et al. 2011). MMPs are considered to be key factors in the remodeling process of the cartilage matrix. Increased MMPs are suggested to be correlated with the induction of cartilage destruction in OA patients (Iannone & Lapadula 2003). These findings indicate that chemerin could contribute to the deterioration of cartilage tissue by promoting an inflammatory signal pathway and the secretion of enzymes that digest the extracellular matrix. In another study, chemerin expression was found to be increased after IL-1β stimulation in chondrocytes (Conde et al. 2011). This suggests that chemerin may interact with the inflammatory factors and then amplify the inflammatory effects in chondrocytes, ultimately causing damage to the cartilage.

The limitation of the present study should be considered. First, this is a cross-sectional study performed on a relatively small sample. Therefore, our findings should be validated by further studies with a larger sample size. Second, chemerin levels in SF from healthy controls were not assessed because of ethical concerns. Whether there are significant differences in the chemerin levels of SF between knee OA patients and healthy controls is unclear.

In conclusion, the chemerin levels in SF may be positively correlated with the severity of knee OA. Aside from the traditional methods, the chemerin levels in SF may serve as a new biomarker for assessing the risk and severity of knee OA.

Declaration of interest

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