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Serum and Synovial Fluid Levels of CCL18 are Correlated with Radiographic Grading of Knee Osteoarthritis

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

AG **Yun Zhou**
BCE **Juwu Chen**
CDF **Guohui Yang**

Department of Emergency Center, First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, P.R. China

Corresponding Author: Yun Zhou, e-mail: zzufzy@126.com
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Background: Chemokines are involved in the pathogenesis of osteoarthritis (OA). CCL18, a member of the chemokines family, is observed in synovial fluid (SF) of OA patients. The aim of this study was to determine the association between CCL18 levels in serum and SF with radiographic knee OA.





Material/Methods: This study was conducted in a population of 308 patients with knee OA. The radiological knee OA was graded by the Kellgren-Lawrence grading system.

Results: Serum levels of CCL18 in knee OA patients were markedly higher than those in healthy controls. Serum and SF levels of CCL18 increased with the severity of KL grades and were correlated with disease severity.

Conclusions: The CCL18 levels in serum and SF are correlated with the severity of OA.

MeSH Keywords: **Chemokines • Osteoarthritis, Knee • Synovial Fluid • Thymic Factor, Circulating • Trauma Severity Indices**

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Background

Osteoarthritis (OA) is the most prevalent joint disease. Articular cartilage destruction, bony outgrowth, and synovitis are the characteristic abnormalities in this disease [1]. The etiology and pathogenesis of OA remains poorly understood. Recently, inflammation has been reported to be a key mediator in the development of OA [2]. A series of inflammatory markers, including interleukin-6 (IL-6) [3], tumor necrosis factor- α (TNF- α) [3], C-reactive protein [4], and YKL-40 [5], were found to be correlated with the severity of knee or hip OA.

Chemokines are a large family of soluble proteins involved in leukocyte activation and traffic during inflammatory responses [6]. All chemokines share certain primary structural similarities, including a conserved 4-cystein motif. Four chemokine subfamilies have been described based on the positions of certain cysteine residues (CXC, CC, C, and CX3C) [7]. CCL18, also termed pulmonary and activation-regulated chemokine (PARC), has been described. CCL18 is either constitutively expressed or induced in monocytes/macrophages and dendritic cells, and chemoattracts T and B lymphocytes [8]. Recently, CCL18 was reported to be highly expressed in articular cartilage and synovial tissue of patients with OA compared with healthy controls [9], indicating the possible role of CCL18 in the development and pathogenesis of OA.

Although there have been studies investigating differences in CCL18 levels between OA patients and healthy controls, no investigation has focused on the association between CCL18 levels and radiographic knee OA. The aim of the present study was to determine the association between CCL18 levels in serum and SF with radiographic knee OA.

Material and Methods

Patients

A total of 308 patients diagnosed with knee OA by the American College of Rheumatology criteria were included in the present study. These patients (129 males and 179 females) were aged 43 to 81 years with mean age of 61.68 ± 8.17 years. The exclusion criteria were inflammatory arthritis, knee damage, aseptic osteonecrosis, and taking corticosteroid or nonsteroidal anti-inflammatory drugs (NSAIDs) during the past 3 months. We also enrolled 150 age- and sex-matched subjects with no clinical and radiological evidence of OA, arthritis, or other joint diseases as controls. The control group subjects (59 males and 91 females) were aged 46 to 79 years with mean age of 62.18 ± 7.35 years.

All participants provided written informed consent and our Hospital Ethics Committee approved the study.

Radiographic assessment of OA

Knee radiography was performed with participants standing on both legs with fully extended knee and the X-ray beam was centered at the level of the joint. The Kellgren and Lawrence (KL) grading system was utilized to assess radiographic severity. The definition of OA was having KL grade ≥ 2 in at least 1 knee. Controls were defined as those with KL grades of 0 in the knees.

Laboratory methods

Venous blood was collected after overnight fasting. SF was taken from the knee that had worse OA using sterile knee puncture before the first hyaluronic acid treatment. Then SF was immediately separated by centrifugation to remove cells and joint debris after SF sample collection and stored at -80°C until use. A commercially available enzyme-linked immunosorbent assay (R&D Systems Inc., Minneapolis, MN, USA) was utilized to examine serum and SF levels of CCL18.

Statistical analysis

The data of the current study were analyzed by SPSS version 13.0 software program (SPSS Inc., Chicago, IL). The results are expressed as means \pm SD or median (25th percentile, 75th percentile), as appropriate. Kolmogorov-Smirnov test was used to assess normality of distribution. The variables among OA patients and controls were compared using chi-square test, unpaired t-test, or Mann-Whitney U test, as appropriate. Kruskal-Wallis test was utilized to compare the difference in C-reactive protein (CRP) levels and CCL18 levels in serum and SF between knees of patients with different KL grades. The correlation of CRP levels and CCL18 levels in serum and SF with disease severity was assessed by Spearman correlation analysis and multinomial logistic regression analysis. Spearman correlation analysis was utilized to examine the correlation of CRP levels with CCL18 levels in serum and SF. *P*-value < 0.05 was set as the level of statistical significance.

Results

Clinical parameters of OA patients and healthy control

There were no marked differences in age and sex between these 2 groups (Table 1).

The serum and SF CCL18 levels

CCL18 levels in knee OA patients and healthy controls are displayed in Table 1. There were relatively higher serum CCL18 and CRP levels in knee OA subjects than in healthy controls ($P < 0.001$ and $P < 0.001$, respectively).

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

Characteristics	Knee OA patients (n=308)	Healthy controls (n=150)	P value
Age (years)	61.68±8.17	62.18±7.35	0.522
Gender (male/female)	129/179	59/91	0.603
CRP (mg/L)	2.37 (1.46–3.40)	1.55 (0.87–1.94)	<0.001
CCL18 in serum (ng/mL)	54.13 (41.91–69.72)	38.97 (31.29–48.12)	<0.001
CCL18 in SF (ng/mL)	31.24 (23.62–39.06)		

Table 2. CRP and CCL18 levels of serum and SF in knee OA patients with different KL grades.

CCL18 (ng/mL)	Grade 2 (n=96)	Grade 3 (n=122)	Grade 4 (n=90)	P value
CRP (mg/L)	1.82 (1.23–2.45)	2.53 (1.53–3.93)*	3.10 (1.84–4.66)*,**	<0.001
CCL18 in serum (ng/mL)	45.85 (37.00–54.10)	49.56 (39.25–61.36)*	72.26 (60.78–83.81)*,**	<0.001
CCL18 in SF (ng/mL)	27.00 (20.91–31.24)**	28.49 (22.88–35.61)*	45.43 (33.55–53.00)*,**	<0.001

P*<0.01 vs. KL grade 2; *P*<0.01 vs. KL grade 3.

CCL18 levels in knee OA patients with different KL grades

As shown in Table 2, serum and SF levels of CCL18, as well as serum CRP levels, increased with the increase of KL grades.

Correlation of KL grades with other variables

Spearman correlation analysis indicated the significant associations of CCL18 levels in both serum and SF with KL grades (*r*=0.560, *P*<0.001 and *r*=0.525, *P*<0.001, Figures 1 and 2, respectively). Furthermore, CRP levels were also correlated with KL grades (*r*=0.406, *P*<0.001) (Figure 3). Multinomial logistic regression analysis also showed a positive correlation of serum and SF CCL18 levels, as well as CRP levels with KL grades (*P*<0.001, *P*<0.001, and *P*<0.001, respectively).

Correlation of serum and SF CCL18 levels with CRP

Spearman correlation analysis indicated serum and SF CCL18 levels were both associated with CRP in knee OA patients (*r*=0.194, *P*=0.001 and *r*=0.230, *P*<0.001, respectively)

Discussion

Radiological examination using magnetic resonance imaging and direct arthroscopic examination are the main methods used to evaluate OA disease progression. However, use of these methods is limited due to high cost, lack of clear critical

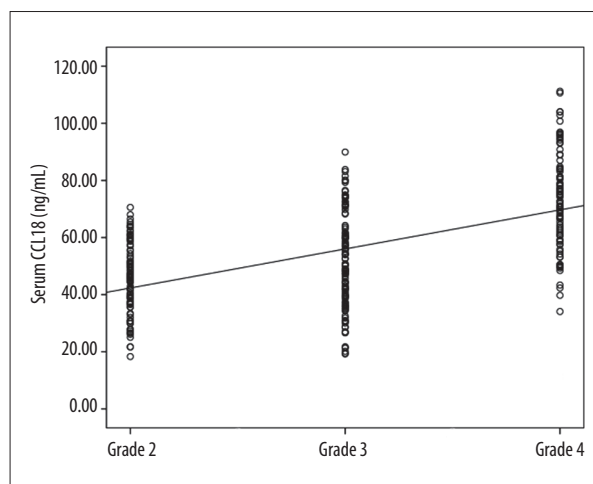


Figure 1. Correlation of serum CCL18 levels with different KL grades.

standards, and traumatic defects [10]. Nowadays, biomarkers are utilized in early disease diagnosis, disease progression assessment, and therapeutic effects [11]. The current results revealed a close correlation between radiographic severity of OA and CCL18 levels in serum and SF. This indicates the possible role of CCL18 levels in predicting the severity of OA. Furthermore, other chemokines, including macrophage inflammatory protein-1 α (CCL3) [12], interferon- γ -inducible protein-10 (CXCL10) [13], and stromal cell-derived factor-1 (CXCL12) [14], were also found to be associated with the severity of OA.

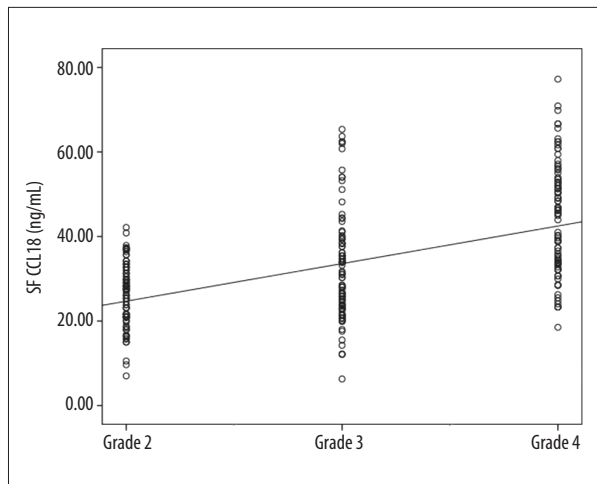


Figure 2. Correlation of SF CCL18 levels with different KL grades.

CCL18 mRNA expression levels were found to be significantly higher in the cartilage samples from OA patients than those in controls [9]. In addition, serum levels of CCL18 were also markedly elevated in OA patients compared to controls [9]. Similar results were found in the current study. Our results also indicated the relative higher CCL18 levels in serum of knee OA patients. These findings suggest that CCL18 serves as a key mediator in the development of OA.

Matrix metalloproteinases (MMPs) are considered to be the key factor in the remodeling process of cartilage matrix. The production of MMPs is suggested to be correlated with cartilage destruction in OA process [15]. Takayasu reported that MMP-3 was significantly enhanced by stimulation of fibroblast-like synoviocytes (FLS) with CCL18 [16]. Furthermore, overexpression of CCL18 in mouse lungs enhanced the release of MMP-2, and MMP-9 [17]. This indicates that the excessive production of different MMPs stimulated by CCL18 may be detrimental in OA cartilage, thereby contributing to their characteristic degradation.

Chemokines are chemotactic cytokines that regulate leukocyte migration during inflammatory responses, as well as homeostatic trafficking of lymphocytes and dendritic cells. CCL18 is either constitutively expressed or induced in monocytes/macrophages and dendritic cells, and chemoattracts T and B lymphocytes. CCL18 was shown to promote the expression of

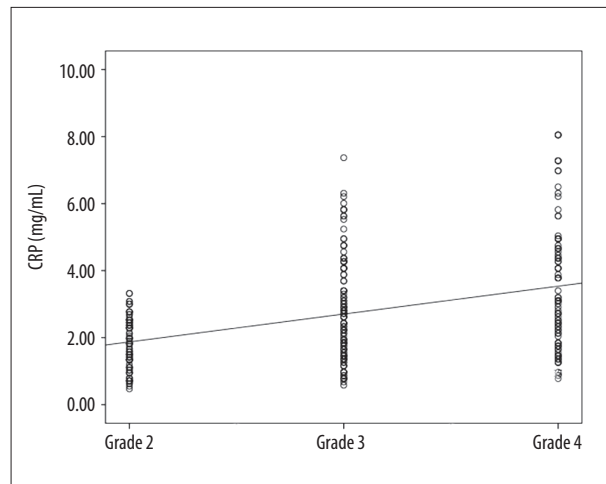


Figure 3. Correlation of serum CRP levels with different KL grades.

interleukin 6 (IL-6) in cultured FLS established from synovial tissues of OA patients [16]. This indicates that CCL18 could contribute to deterioration of cartilage tissue by promoting the inflammatory signal pathway. In addition, inflammatory factors, such as tumor necrosis factor- α , IL-4, and IL-13, were found to induce the secretion of CCL18 in blood polymorphonuclear neutrophils or synovial tissue from rheumatoid arthritis patients [18,19]. This suggests that CCL18 may contribute to the progression of cartilage damage and OA indirectly through promoting inflammatory response.

The limitations of the present study should be considered. First, it was designed as a cross-sectional study. Further prospective investigations should be performed. Second, we did not evaluate CCL18 levels in SF from healthy controls due to ethical concerns. Third, the sample size was not large enough and further studies with larger sample sizes are warranted.

Conclusions

CCL18 levels in serum and SF were closely correlated with the radiographic grading of knee OA. The CCL18-mediated inflammatory pathway may be involved in the mechanism of OA. This needs to be confirmed by further study.

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