Association of CCL13 Levels in Serum and Synovial Fluid With the Radiographic Severity of Knee Osteoarthritis

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Objective: CCL13, a recently identified CC chemokine, plays an important role in the process of joint destruction, which is considered a common cause for osteoarthritis (OA). This study aims to examine the relation of CCL13 levels in serum and synovial fluid (SF) with the radiographic severity of OA.

Methods: CCL13 levels in serum and SF were evaluated using enzyme-linked immunosorbent assay method in 240 patients with knee OA and 134 control subjects. The progression of OA was classified using the Kellgren-Lawrence (KL) system by evaluating x-ray changes observed in anteroposterior knee radiography.

Results: Knee OA patients had higher levels of serum CCL13 compared with control subjects. Knee OA patients with KL grade 4 showed significantly elevated CCL13 levels in serum and SF compared with those with KL grades 2 and 3. Knee OA patients with KL grade 3 had significantly higher SF levels of CCL13 compared with those with KL grade 2. CCL13 levels in serum and SF of knee OA patients were significantly correlated with disease severity evaluated by KL grading criteria.

Conclusions: CCL13 levels in serum and SF were correlated with the radiographic severity of OA. CCL13 levels in serum and SF may serve as a biomarker for the progression of OA.

Key Words: CCL13, serum, synovial fluid, severity, osteoarthritis

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Osteoarthritis (OA) is a chronic degenerative joint disease leading to pain, stiffness, reduced motion, swelling, crepitus, and disability.[1] It is characterized by articular cartilage degradation, combined with bony outgrowth at joint margin, and chronic nonspecific inflammation of synovium.[2] The knee is the most clinically significant site of primary OA involvement.[3] It is likely that numerous etiologic factors, such as aging, obesity, being female, smoking, genetics, joint injury, inflammation, and some mechanical and metabolic factors, participate in the pathogenesis and are well accepted risk factors for OA.[4]

Chemokines, some small chemoattractant cytokines, are classified into 4 families on the basis of the location of cysteine residues. The 4 chemokine groups are CC, C, CXC, and CX3C, where C is a cysteine and X is any amino-acid residue, and their receptors are consequently named as CCR, CR, CXCR, and CX3CR.[5] Chemokines and their receptors are involved in a variety of inflammatory diseases including OA by recruiting leukocytes to the inflammatory site.[6] CCL13, also called monocyte chemoattractant protein 4, is a family member of CC chemokine. CCL13 are considered to be implicated in joint destruction via the recruitment and retention of monocytes and T lymphocytes into the joints.[7] Recent study showed that CCL13 is elevated in serum from patients with OA compared with healthy individuals.[8] These results suggest that CCL13 may be involved in the mechanism of OA.

This study aims to determine whether CCL13 levels in serum and synovial fluid (SF) of patients with knee OA are correlated with the disease severity.

MATERIALS AND METHODS

Patients

This study consisted of 182 patients diagnosed with OA of at least 1 knee according to clinical symptomatic criteria (American College of Rheumatology) and radiographic criteria. Patients with additional inflammatory arthritis or autoimmune 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. Furthermore, SF of each subjects were examined using polarized microscopy by 2 experienced rheumatologists. Those with calcium pyrophosphate deposition disease crystals were also excluded from this study. One hundred thirty-four age- and sex-matched subjects with no clinical and radiological evidence of OA served as the control group. All participants provided written informed consent, and the Committee on Medical Ethics of our hospital, in accordance with the Declaration of Helsinki of the World Medical Association, approved the study protocol.

Radiographic Assessment of OA

Disease severity was graded by 2 graders who were blinded to the results according to the system of Kellgren and Lawrence (KL).[9] The subjects who had radiographic OA of KL grade 2 or greater in at least 1 knee were defined as knee OA patients. Control subjects were defined as not having radiographic knee OA as indicated by KL grade of 0 for both knees. The grading scale used for analysis was the one found higher upon comparison between both knees.

Laboratory Methods

Venous blood was obtained at 7:00 AM after overnight fasting. Before any treatment on OA, SF was obtained from OA patients who received the treatment of hyaluronic acid injection for the first time. The CCL13 levels in serum and SF were quantified using enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN).

Statistical Analysis

The data are presented as means ± SD or median (interquartile range). Data normality were analyzed using Kolmogorov-Smirnov test. Comparison of the characteristics between patients with knee OA and control subjects was performed

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TABLE 1. Characteristics Between Patients With Knee OA and Control Subjects

Characteristics	Patients With Knee OA (n = 182)	Control Subjects (n = 134)	*P*
Age, y	61.23 ± 10.90	61.56 ± 7.66	0.761
Sex (male/female), n	76/106	54/80	0.794
CCL13 in serum, pg/mL	69.70 (56.92–80.12)	50.93 (39.77–62.26)	<0.001
CCL13 in SF, pg/mL	46.31 (39.11–55.35)		

using unpaired *t* test, Mann-Whitney *U* test, or χ^2 test. Kruskal-Wallis test was utilized to compare the difference of CCL13 levels in serum and SF among knee patients with different KL grades. The correlation of CCL13 levels in serum and SF with disease severity was determined by Spearman correlation analysis and a multinomial logistic regression analysis. All statistical analysis was performed using SPSS (SPSS Inc, Chicago, IL) for Windows 13.0. Differences were considered significant at $P < 0.05$.

RESULTS

Baseline Clinical Parameters

The baseline clinical parameters of patients with knee OA and control subjects are presented in Table 1. There were no clinically meaningful differences in age and sex between the 2 groups.

The CCL13 Levels in Serum

Patients with knee OA had significantly higher serum CCL13 levels compared with control subjects ($P < 0.001$) (Table 1).

CCL13 Levels in Knee OA Patients With Different KL Grades

The CCL13 levels in serum and SF of knee OA patients with different KL grades are shown in Table 2. Knee OA patients with KL grade 4 had significantly elevated CCL13 levels in serum and SF than did those with KL grades 2 and 3. Furthermore, the CCL13 levels in SF of knee OA patients with KL grade 3 were significantly higher compared with those with KL grade 2 ($P = 0.006$). However, no significant differences in the CCL13 levels of serum were observed between patients with KL grades 2 and 3 ($P = 0.067$).

Association of Clinical Parameters With KL Grades

Spearman correlation analysis showed that the CCL13 levels in serum and SF were correlated to KL grades ($r = 0.304$ [$P = 0.002$] and $r = 0.390$ [$P < 0.001$], respectively). Multinomial logistic regression analysis indicated that CCL13 levels in serum and SF were both positively associated with KL grades ($P < 0.001$ and $P < 0.001$, respectively).

DISCUSSION

This study provides the first report of the association of CCL13 levels in serum and SF with radiographic severity of knee OA. The results indicated that serum levels of CCL13 were significantly elevated in knee OA patients compared with control subjects. In addition, Spearman correlation analysis showed that CCL13 levels in serum and SF were correlated with KL grades.

Iwamoto et al.[10] reported that CCL13 enhanced fibroblast-like synoviocyte (FLS) proliferation by activating the extracellular signal–regulated kinase mitogen-activated protein kinase cascade. It has been accepted that FLS proliferation is involved in the pathogenesis of OA. Fibroblast-like synoviocyte proliferation in the synovial tissue contributes to chronic inflammation and the destruction of articular cartilage through promoting the production of a variety of cytokines, chemokines, and matrix metalloproteinases (MMPs).[11] This indicates that CCL13 may contribute to the articular cartilage degradation by promoting the proliferation of FLS and eventually lead to the development of OA. In addition, extracellular signal–regulated kinases play an important role in the up-regulation of expression of MMP, such as MMP-1, MMP-2, and MMP-9.[12] Osteoarthritis is characterized by the degradation of extracellular matrix components.[13] Matrix metalloproteinase is a family of structurally related calcium- and zinc-dependent proteolytic enzymes that is involved in the degradation of many different components of extracellular matrix.[14] Therefore, it is hypothesized that CCL13 may be involved in the pathogenesis of OA indirectly by promoting different MMPs.

Chemokines are small chemoattractant cytokines that play key roles in the accumulation of inflammatory cells at the site of inflammation.[15] Inflammatory factors have been shown to be associated with pathogenesis of OA.[16] CCL13 may contribute to the development and progression of OA indirectly by enhancing inflammation response. On the other hand, interferon γ significantly stimulated CCL13 production in human chondrocytes, and this effect was enhanced in combination with interleukin 1β or tumor necrosis factor α.[10] Hence, CCL13 is hypothesized to interact with inflammatory cytokines and then amply the inflammatory response in chondrocytes and at last lead to the damage of cartilage.

Recently, chemokines have been shown to be involved in the mechanism of OA. Chemokines such as interferon c–inducible protein 10 (CXCL10),[17] stromal cell-derived factor 1 (CXCL12),[18] and macrophage inflammatory protein 1α (CCL3)[19] were all indicated to be associated with the severity of OA. Therefore, we hypothesized that CCL13 may be also involved in the pathogenesis of OA and evaluated the correlation of CCL13 levels in serum and SF with the disease severity of OA. Our results indicated that knee OA patients had significantly elevated serum levels of CCL13 compared with control subjects. This is consistent with another study that also found that the expressions of CCL13 in cartilage

TABLE 2. CCL13 Levels of Serum and SF in Patients With Knee OA With Different KL Grades

CCL13, pg/mL	Grade 2 (n = 59)	Grade 3 (n = 73)	Grade 4 (n = 50)	*P*
Serum	62.91 (50.76–75.52)	69.12 (58.59–78.54)	76.36 (65.04–83.52)*†	<0.001
SF	42.50 (34.45–50.50)†	47.97 (39.43–55.10)*	54.66 (42.04–60.74)*†	<0.001

*$P < 0.01$ versus KL grade 2.

†$P < 0.01$ versus KL grade 3.

and the concentration of CCL13 protein in serum were both significantly higher in OA patients compared with control subjects.[8] In addition, the present study indicated that CCL13 levels in serum and SF were correlated with KL grades. These results point to the role of CCL13 in the pathophysiology of OA. CCL13 levels in serum and SF may be a biomarker to assess the progression of OA.

This study has several potential limitations. First, the sample size was not large enough to reach definitive conclusions. Further studies with great numbers are warranted. Second, our study is of cross-sectional design, and the causative relation must be confirmed by future longitudinal studies. Last, we did not assess the differences of CCL13 levels in SF levels between knee OA patients and control subjects because of ethical concerns.

In conclusion, CCL13 levels in serum and SF were positively correlated with the severity of knee OA. CCL13 levels in serum and SF may serve as a new biomarker in addition of the traditional methods for assessing the risk and severity of knee OA.

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