Association of plasma and synovial fluid interferon-γ inducible protein-10 with radiographic severity in knee osteoarthritis

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A B S T R A C T

Objectives: The objective of this study was to investigate interferon-γ inducible protein-10 (IP-10) concentrations in plasma and synovial fluid of patients with knee osteoarthritis (OA) and to analyze their relationship with disease severity.

Design and methods: Forty OA patients and 15 healthy controls were enrolled in this study. OA grading was performed according to the Kellgren–Lawrence criteria. IP-10 levels in plasma and synovial fluid were assessed using enzyme-linked immunosorbent assay.

Results: Plasma IP-10 levels in the knee OA patients were significantly lower than those of controls (P=0.006). IP-10 levels in plasma were markedly higher with regard to paired synovial fluid (P=0.001). Furthermore, IP-10 concentrations in plasma and synovial fluid displayed significant inverse correlation with radiographic severity (r=−0.713, P<0.001 and r=−0.561, P=0.001, respectively). Subsequent analysis revealed that plasma IP-10 levels were positively correlated with synovial fluid IP-10 levels (r=0.424, P=0.006).

Conclusions: IP-10 levels in both plasma and synovial fluid were inversely associated with the severity of knee OA. Accordingly, IP-10 could serve as a biomarker for determining disease severity and might play a possible role in the pathophysiology of osteoarthritis.

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Introduction

Osteoarthritis (OA) is a chronic, age-related, degenerative joint disease resulting in pain, stiffness, reduced motion, swelling, crepitus, and disability. It is characterized by articular cartilage degradation, combined with bony outgrowth at joint margin, and chronic nonspecific inflammation of synovium [1]. The diagnosis of OA is generally based on clinical and radiographic changes, which reflect disease severity by grading the joint destruction. In addition, there are two main categories of biochemical markers presently studied as a promising indicator for OA. One includes components of extracellular matrix of the involved joint; the other includes cytokines that metabolize the molecules of the joint tissues [2]. A number of studies have provided detailed insights into the pathophysiological and biochemical aspects of OA; however, the mechanisms underlying the progressive loss of articular cartilage remain largely unknown.

Chemokines are small chemoattractant cytokines produced by tissue cells and leukocytes and play a major aspect in the accumulation of inflammatory cells and immune response at the site of inflammation [3]. Chemokines are classified into four subfamilies based on the position of the two N-terminal cysteine residues. The four chemokine groups are CXC, CC, C and CX3C, where C is a cysteine and X is any amino acid residue [4–6]. The CXC chemokines and CC chemokines are the most extensively investigated subfamilies [7,8]. The CXC family can be further subdivided into CXC chemokines containing the ELR (glutamic-leucine-arginine) motif, which plays a major role in neutrophil chemotaxis, and the non-ELR CXC chemokines, such as interferon-γ inducible protein-10 (IP-10), a monokine induced by interferon-γ, interferon-inducible T cell alpha chemoattractants [9]. These chemokines have been demonstrated to be essential in selective accumulation of mononuclear cells into synovium, resulting in initiation and progression of synovitis, especially in OA [10–13].

Previous studies have demonstrated that numerous cytokines are associated with clinical parameters of disease severity in knee OA and may play possible roles in the pathogenesis of OA [14–16]. Therefore, we have postulated that IP-10 in plasma and synovial fluid might be associated with the disease severity in knee OA patients. The purpose
of this study was to investigate the association between plasma and synovial IP-10 concentrations and the radiographic severity in patients with knee osteoarthritis in order to determine its role in OA pathophysiology.

**Materials and methods**

Subjects

Forty patients (35 females and 5 males) diagnosed with knee osteoarthritis according to the criteria of the American College of Rheumatology, and 15 healthy volunteers with no clinical and radiological evidence of OA (12 females and 3 males) were enrolled in the present study. The present study was conducted in agreement with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from the patients and healthy volunteers prior to their participation in the study. This study was approved by the Institutional Review Board on Human Research of the Faculty of Medicine, Chulalongkorn University.

Knee radiography was taken when each participant was standing on both legs with fully extended knee and the X-ray beam was centered at the level of the joint. Assessment of radiographic severity was performed using the Kellgren and Lawrence (KL) grading system [17]. Depending on changes observed in conventional weight-bearing anteroposterior radiographs of the affected knee in extension, osteoarthritis was divided into 5 grades (0 to 4); grade 0 (normal findings), no X-ray changes; grade 1 (questionable), doubtful narrowing of joint space and possible osteophyte tipping; grade 2 (mild), definite osteophytes and possible joint space narrowing; grade 3 (moderate), multiple moderate osteophytes, definite narrowing of joint space, bone sclerosis and possible deformity of bone contour; grade 4 (severe), large osteophytes, marked joint space narrowing, severe sclerosis, and deformity of bone contour. OA patients were defined as having radiographic knee OA of KL grade ≥ 2 in at least 1 knee. Controls were defined as having neither radiographic hip OA nor knee OA, as indicated by KL grades of 0 for both hips and both knees. The grading scale used for analysis was the one found higher upon comparison between both knees.

**Laboratory methods**

Venous blood samples collected from all participants were centrifuged and stored immediately at −80 °C until analysis. Synovial fluid was taken from the most affected knee during a total knee arthroplasty. The specimen was then centrifuged to remove cells and stored −80 °C until analysis. Synovial fluid, two synovial fluid samples were selected and analyzed for human IP-10 at serial two fold dilutions. The recovery ranged between 90.8% and 108.7% with an overall recovery of 100.3%.

The sensitivity of this assay was 1.0 pg/mL. For the technical validity of measurements in synovial fluid, two synovial fluid samples were selected and analyzed for human IP-10 at serial two fold dilutions. The sensitivity of this assay was 1.0 pg/mL. For the technical validity of measurements in synovial fluid, two synovial fluid samples were selected and analyzed for human IP-10 at serial two fold dilutions. The sensitivity of this assay was 1.0 pg/mL. For the technical validity of measurements in synovial fluid, two synovial fluid samples were selected and analyzed for human IP-10 at serial two fold dilutions. The recovery ranged between 90.8% and 108.7% with an overall recovery of 100.3%.

**Statistical analysis**

Statistical analysis was carried out using the statistical package for social sciences (SPSS) software, version 16.0 for Windows. Demographic data between patients and controls were compared by chi-square tests and unpaired Student’s t, tests, where appropriate. Comparisons between the groups were performed using one-way analysis of variance (ANOVA) with Tukey post hoc test if ANOVA showed significance. Correlations between plasma and synovial fluid IP-10 and disease severity were assessed using Pearson’s correlation coefficient (r). Data were expressed as a mean ± standard error of the mean. P-values < 0.05 were considered to be statistically significant for differences and correlations.

**Results**

Forty knee OA patients at age of 53–91 years and 15 healthy individuals at age of 59–83 years were recruited in the present study. No clinically meaningful difference in age was observed between the OA patients and healthy controls (69.7 ± 1.2 vs. 72.1 ± 1.9 years, P = 0.5). Plasma and synovial fluid IP-10 concentrations of knee OA patients and plasma levels of healthy controls are demonstrated in Fig. 1. Plasma IP-10 levels were lower in OA patients than in healthy participants (378.5 ± 21.6 pg/mL vs. 498.3 ± 38.0 pg/mL, P = 0.006). IP-10 levels in synovial fluid of OA patients (154.7 ± 25.6 pg/mL) were 2.5-fold lower than in corresponding blood samples (P = 0.001), and were 3-fold lower than in the plasma of healthy controls (P < 0.001). There was a positive correlation between plasma and synovial IP-10 concentration (r = 0.424, P = 0.006) (Fig. 2).

According to the radiographic KL classification, patients were categorized into 3 groups in relation to OA grading. Fourteen patients were classified as grade 2, thirteen as grade 3, and thirteen as grade 4. As shown in Table 1, knee OA patients with higher radiographic OA severity (≥ KL grade 2) were 3.5-fold lower than in the plasma of healthy controls (P = 0.001). In addition, the associations between plasma and synovial fluid levels of IP-10 and the disease severity of osteoarthritis were statistically evaluated. The plasma concentrations of IP-10 were inversely correlated with knee OA severity (r = −0.713, P < 0.001) (Fig. 3). Further analysis

![Fig. 1. IP-10 levels in plasma and synovial fluid of patients with OA and healthy controls.](image-url)
revealed that synovial fluid IP-10 levels of knee OA patients were also negatively correlated with OA grading ($r = -0.561, P < 0.001$) (Fig. 4).

Discussion

Osteoarthritis is a heterogeneous and multifactorial process of chronic degenerative joint disorder with major features of articular cartilage destruction along with bone, muscle, ligament, and synovial involvement [1]. It is characterized pathologically by the loss of cartilage, subchondral bone sclerosis or cyst, and osteophyte formation. The development of preventive or therapeutic strategies for OA seemingly depends on identification of pathophysiological mechanisms and biochemical markers that underlie progressive joint deterioration. Until now, etiopathogenesis of OA is still unknown. Nevertheless, many potential factors have apparently demonstrated the abilities to increase the risk of OA.

Interferons are a family of proteins first identified by their ability to induce cellular resistance to infection by many viruses. Interferon γ-inducible protein-10 (IP-10), an important non-ELR-CXC chemokine, was found to be a gene induced by IFN-γ in a human myeloid cell line, human mononuclear cells, fibroblasts, and endothelial cells [19]. The importance of IP-10 expression during synovial inflammation in OA has recently been emphasized [20,21]. It has been previously demonstrated that circulating IP-10 is present in rheumatoid arthritis (RA), psoriatic arthritis, and osteoarthritis [13,22–24]. However, the association between circulating and synovial fluid levels of IP-10 and radiographic severity has never been investigated in knee OA patients. To our knowledge, data on the relationship between IP-10 levels in plasma and synovial fluid and severity of knee OA have as yet not been documented in the literature. This study has been the first to illustrate that IP-10 was measured in both plasma and synovial fluid obtained from primary knee OA patients, and that IP-10 levels were inversely associated with radiographic grading of knee OA.

The most interesting finding in this study has been that IP-10 levels were decreased in plasma of patients with primary knee OA compared to the controls. Our results revealed that plasma IP-10 levels were positively correlated with synovial fluid IP-10 levels; however, IP-10 concentrations in plasma were remarkably higher than those observed in paired synovial fluid samples. These findings implied that IP-10 was released from extra-articular tissues into a systemic circulation. The source of IP-10 could be originated from the local tissues (inflamed synovium, cartilage, and subchondral bone) and extra-articular tissues. Previous studies have demonstrated that IP-10 was expressed in synovial cells, articular cartilage chondrocytes and subchondral bone osteoblasts in rheumatoid arthritis and osteoarthritis [20,21,25,26]. IP-10 levels in plasma and synovial fluid were measured in a well-defined knee OA population at every stage of disease, and were significantly lower in end-stage knee OA patients compared with early OA patients. This observation suggests a significant decrease in the systemic and local production of IP-10 in patient with advanced knee OA. The mechanisms

Table 1

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<th>KL Grade 4</th>
<th>P</th>
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<td>14</td>
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<td>Synovial fluid IP-10 (pg/mL)</td>
<td>154.7±25.6</td>
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<td>121.2±18.9</td>
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<td>Plasma IP-10 (pg/mL)</td>
<td>375.5±21.6</td>
<td>510.9±18.8</td>
<td>335.7±36.4</td>
<td>278.6±18.6</td>
<td>0.001</td>
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IP-10= interferon-γ inducible protein-10.
KL= Kellgren and Lawrence.

Fig. 2. Scattergram showing the positive correlation between plasma and synovial fluid IP-10 concentrations in OA patients ($r = 0.424, P = 0.006$).

Fig. 3. Scattergram showing the negative correlation between plasma IP-10 levels in OA patients and disease severity classified according to Kellgren and Lawrence grading scale ($r = -0.713, P < 0.001$).

Fig. 4. Scattergram showing the negative correlation between synovial fluid IP-10 levels in OA patients and disease severity classified according to Kellgren and Lawrence grading scale ($r = -0.561, P < 0.001$).
of IP-10 reduction in the circulation and synovial fluid of OA patients remain to be investigated further.

In contrast with our findings, Patel and colleagues have demonstrated that IP-10 levels in the circulation were significantly lower than those observed in paired synovial fluid samples in RA [27]. This finding accorded with that of de Jager et al., which showed a lower circulating IP-10 concentration compared to synovial fluid IP-10 in Juvenile idiopathic arthritis [28]. Additionally, the serum levels of IL-8, IP-10, and RANTES were significantly elevated in active RA [29]. The serum RANTES level may be a useful marker for disease activity and the serum IP-10 level can be used as an index of prognosis in RA [29]. The explanation for these conflicting results remains unclear but may be attributed to differences in disease advancement, populations or assays applied, and/or to incomplete control for confounding variables.

Inflammatory pathways are known to play a key role in the development of OA. Inflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α are detectable in OA and are capable of activating matrix metalloproteinases (MMPs) [30]. Among them, MMP-3 (stromelysin) is a major regulator in cartilage degeneration and joint destruction. Plasma MMP-3 levels were shown to be predictive of knee joint space narrowing in patients with progressive radiographic knee OA [31]. Additionally, circulating levels of TNF-α and IL-6 were associated with radiographic knee osteoarthritis and knee cartilage loss, suggesting that low level inflammation may play a role in the pathogenesis of knee OA [32]. In addition to inflammatory mediators, breakdown of articular cartilage matrix during OA leads to the generation of constituent fragments that may serve as biochemical markers of OA [2]. Cartilage oligomeric matrix protein (COMP), a member of the thrombospondin family of glycoproteins, has been proposed as a molecular marker of cartilage degradation in OA [33]. Recent investigations have shown that COMP predicts disease progression and cartilage loss in radiographic knee OA and appears a useful biomarker of early OA [34–36]. Furthermore, serum hyaluronic acid (HA) level positively correlated with the severity of radiographic knee OA and could function as a predictor of disease progression in osteoarthritis of the knee, indicating that serum HA has the potential to be valuable for the diagnosis of the presence and severity of knee OA [37,38]. Recently, high levels of COMP and HA were associated with incident radiographic knee OA outcomes, implying prognostic utility of COMP and HA for incident knee OA [39]. In our study, circulating and synovial fluid IP-10 were inversely related to radiographic grading of knee OA. Therefore, IP-10 may be useful as a biochemical marker to reflect disease severity in knee OA patients.

There were several limitations in the present study. First, this study included a small number of patients because we carefully selected primary knee OA patients undergoing total knee replacement without any disease complications. Nonetheless, plasma and synovial fluid IP-10 levels were significantly correlated with radiographic severity of OA, implying that our findings shed light on the role of IP-10 as a possible biomarker in the knee OA patients. A further investigation conducted on a random sample of a larger population will be essential to draw more definite conclusion. Secondly, we did not assess functional impairment and pain in these patients. Additional studies are required to determine whether IP-10 is correlated with functional impairment (WOMAC or Lequesne score) and/or pain (visual analogue scale). Thirdly, we cannot exclude the possibility that the low plasma IP-10 levels in the patients with knee OA in this study were an epiphenomenon reflecting some unknown mechanisms. More researches will be required to demonstrate disease progression and define the precise role of IP-10 in knee OA. Furthermore, we mainly analyzed IP-10 levels in the plasma and synovial fluid, but not its expression in the intra-articular tissues. Therefore, additional investigations of tissue IP-10 expression are needed to elucidate the pathogenic mechanisms of IP-10 in OA. Finally, we did not measure biomarkers of cartilage destruction including COMP and MMPs. Future studies on biochemical markers of cartilage damage and their correlation with IP-10 may help identify more pieces of the degenerative jigsaw of OA; nevertheless, the challenge remains to piece them together to originate a rational solid hypothesis pertaining to their exact role.

In summary, the present study showed that plasma IP-10 levels in knee OA patients were significantly lower than those in healthy controls. Synovial fluid IP-10 levels were markedly reduced with respect to paired plasma IP-10 samples. Whether low IP-10 levels in knee OA patients are the consequence or a possible contributing factor of the disease requires further studies. Moreover, plasma and synovial fluid IP-10 levels were inversely correlated with radiographic severity of knee OA patients, suggesting that IP-10 levels might be used as a biomarker for determining disease severity in knee OA. These findings supported the hypothesis which portrayed IP-10 as a protective factor in osteoarthritis. Prospective longitudinal studies are warranted to gain insight into the potential utility of IP-10 as a biochemical marker of disease progression and prognosis. In addition, IP-10 levels in plasma were directly correlated with those in synovial fluid. This study has been the first to demonstrate such a correlation and additional studies will be necessary to define the mechanisms underlying this association.

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References


