Relationship between Osteoarthritis and Leptin Concentrations in Synovial Fluid

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Purpose: Leptin may play an important role in the pathophysiology of osteoarthritis. This study investigated whether leptin concentration in synovial fluid is related to the radiographic severity of osteoarthritis.

Materials and Methods: Synovial fluids were obtained from 29 osteoarthritis patients who underwent knee surgery and 10 who had no abnormality on articular cartilage during arthroscopic examination. The progression of osteoarthritis was classified by Kellgren Lawrence grading scale. The concentrations of leptin was measured with commercial enzyme-linked immnosorbent assay kits.

Results: A significant increase in synovial fluid concentrations was observed in osteoarthritis patients (6.7 ± 4.1 ng/ml) compared to the control (2.4 ± 1.3 ng/ml). Leptin levels were increased with advancing osteoarthritis stage, resulting in the highest level in stage IV patients (10.7 ± 4.9 ng/ml; range 4.7-15.8) compared to that of stage I patients (4.0 ± 2.0 ng/ml; range 1.2-7.3). In osteoarthritis patients, age showed a significant correlation with leptin concentrations.

Conclusion: This study shows that synovial fluid leptin concentrations were closely related to the radiographic severity of osteoarthritis, and suggests that the age of patient may influence synovial fluid leptin concentrations during osteoarthritis progression.

Key Words: Osteoarthritis, Synovial fluid, Biochemical markers, Leptin

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— 92 —
Introduction

Osteoarthritis (OA) is a disease in which great metabolic changes of chondrocytes are observed. It represents one of the most frequent and disabling diseases encountered in elderly individuals.

Objective and measurable standards for OA are a prerequisite for an appropriate treatment of OA. To date, radiographic grading is the most useful method for evaluating disease progression. Much more precise evaluation has been possible thanks to the 3-dimensional, multisectional images provided by magnetic resonance imaging and direct arthroscopic examination. However, these methods have their limitations in early detection of the disease and there is not yet such clear standards.

There is a great potential in the use of biochemical markers of arthritis for a quantitative, reliable and sensitive detection of OA. It has been proposed that biochemical markers may be used to provide diagnostic tests, to stage disease, to predict later disease progression, and to predict and monitor therapeutic response. Recently, Takahashi et al. showed a relationship between radiographic grading of OA and the biochemical markers for arthritis. However, few reasonable biomarkers are currently used in clinical applications. If such markers were to become available, effective drug treatment would be possible and the timing and choice of surgery could be improved. Therefore, it is very important to make progress in the study of biochemical markers available.

In response to cartilage damage, various growth factors and cytokines have been observed in synovial fluid (SF) and synovial tissues from patients with joint diseases. Leptin was initially thought to be an adipocyte-derived small protein (16 kd), but currently it may be considered a pleiotropic hormone involved in the control of various physiological processes, such as lipid homeostasis, reproductive function, angiogenesis, or bone growth. Figenchaa et al. reported the effect of leptin on chondrocytes and the expression of leptin receptor in normal human cartilage. Recently, leptin was detected in SF obtained from patients with OA and human OA chondrocytes were shown to produce both leptin and growth factors.

Previously, Marks et al. showed a relationship between an increased risk of OA and high body mass index (BMI), which was well known to have a positive correlation with adipocyte-derived leptin. Taken together, these results indicate a potential role of leptin in the pathophysiology of OA. However, the effect of leptin on the progression of OA remains controversial due to two opposing mechanisms of leptin. Furthermore, studies on changes in leptin levels related to the severity of OA were limited. Therefore, this study was aimed to investigate whether leptin concentrations in SF is related to the radiographic severity of OA.

Materials and Methods

Synovial fluids (SF) were obtained from 29 OA patients who underwent knee surgeries (5 men and 24 women; mean age, 62.7 years; range 48-87 years). Knee OA was diagnosed from clinical and radiologic evaluation, based on the American College
of Rheumatology criteria\textsuperscript{10}. The surgeries included 7 total knee replacement arthroplasties, 14 microfractures, and 8 cartilage debridement with meniscectomies. The progression of OA was classified according to Kellgren Lawrence grading scale\textsuperscript{13} by our musculoskeletal radiologist; 8 patients with stage I, 4 with stage II, 12 with stage III, and 5 with stage IV. As a control study, we obtained SF from 10 individuals (7 men and 3 women; mean age, 36.3 years; range, 16~48 years) who had no abnormalities on articular cartilage during arthroscopic examination and were radiologically evaluated as normal. The study was conducted in conformity with the declaration of Helsinki principles, and written informed consent was obtained from all participants.

SF were aspirated from the suprapatellar pouch before the operations, using an 18-gauge needle. SF samples were collected in sterile plastic tubes and centrifuged at 10,000 g for 15 minutes at 4°C to remove cells and joint debris. The supernatants were stored at -80°C until biochemical assay was performed. Leptin was measured by a sandwich commercial ELISA kits (R&D system, Abingdon, UK) according to the manufacturer’s instruction. In brief, 100 µl of SF samples and standard were added to microplate wells. 100 µl biotinylated anti-human leptin (Biotin Conjugate) solution was added into each well and the mixture was incubated for 2 hours at room temperature. After thorough wash, 100 µl stabilized chromogen was added to each well and was incubated for 30 minutes at room temperature. After the stop solution was added to each well. the absorbance was read at 450nm and leptin concentration was calculated with standard cover based on the standard concentrations. The minimum detectable dose of human leptin of this kit was 3.5 pg/ml.

All data were expressed as means and standard deviation. Leptin concentrations between OA patients and the controls were compared and analyzed with Student’s t-test and one-way ANOVA. P≤ 0.05 was considered significant.

Results

Table 1 shows the demographic characteristics of OA patients and the control. There were significant differences between OA patients and the control with respect to age and gender distribution and SF leptin concentrations (P<0.05). Age of OA patients (mean 62.7 years; range 48~87 years) was older than that (mean 36.4 years; range 16~48) of the control. The proportion of females was higher in the OA group whereas the proportion of males was higher in the control. Mean leptin concentrations were 6.7 ng/ml (range 0.5~15.8) in OA patients and 2.4 ng/ml (range 1.0~4.6) in the control.

<table>
<thead>
<tr>
<th></th>
<th>OA patients (n=29)</th>
<th>Control (n=10)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)*</td>
<td>62.7±10.7 (48~87)</td>
<td>36.4±11.4 (16~48)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male/Female</td>
<td>5/24</td>
<td>7/3</td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml)*</td>
<td>6.7±4.1 (0.5~15.8)</td>
<td>2.4±1.3 (1.0~4.6)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*: Values are presented as mean ± SD.
In order to investigate whether these differences in age and sex between both groups affected leptin concentrations, we compared the correlation of SF leptin concentrations with age and sex. In OA patients, age showed a significant positive correlation with leptin concentrations (P<0.01). However, in the control, no correlation was found between the two variables (Fig. 1). In OA patients, mean SF leptin concentrations were higher in women (6.33 ng/ml) than in men (2.78 ng/ml), although a significant difference was not observed. However, in the controls, mean SF leptin concentrations were similar between men (2.83 ng/ml) and women (1.53 ng/ml) (Table 2).

SF leptin concentrations were analyzed according to the severity of OA. There was no significant difference in age among patients in each stage. In all OA stages, the proportion of females was greatly higher compared to that of males and the ratio of males to females was not different with each OA stage. However, it was observed that leptin levels were increased with advancing OA stage, resulting in the highest level in stage IV patients, whose mean level was 10.7 ng/ml.

Table 2. Comparison of synovial fluid concentrations of leptin in according to sex

<table>
<thead>
<tr>
<th></th>
<th>Leptin (ng/ml)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OA</td>
<td></td>
<td></td>
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<tr>
<td>Men (n=5)</td>
<td>2.78±2.01</td>
<td>p=0.07</td>
</tr>
<tr>
<td>Women (n=24)</td>
<td>6.33±4.19</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men (n=7)</td>
<td>2.83±1.39</td>
<td>p=0.16</td>
</tr>
<tr>
<td>Women (n=3)</td>
<td>1.53±0.35</td>
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</tbody>
</table>

Note: Values are presented as mean ± SD.

Table 3. Comparison of synovial fluid concentrations of leptin according to the severity of OA

<table>
<thead>
<tr>
<th></th>
<th>stage I (n=8)</th>
<th>stage II (n=4)</th>
<th>stage III (n=12)</th>
<th>stage IV (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)*</td>
<td>61.3±14.0 (48~87)</td>
<td>53.8±4.9 (48~60)</td>
<td>63.6±9.5 (50~83)</td>
<td>70.0±7.9 (60~80)</td>
</tr>
<tr>
<td>Male / Female</td>
<td>1/7</td>
<td>2 / 2</td>
<td>2 / 10</td>
<td>0 / 5</td>
</tr>
<tr>
<td>Leptin (ng/ml)*</td>
<td>4.0±2.0 (1.2~7.3)</td>
<td>6.8±4.2 (0.5~9.7)</td>
<td>5.4±3.4 (2.0~12.4)</td>
<td>10.7±4.9* (4.7~15.8)</td>
</tr>
</tbody>
</table>

*: Values are presented as mean ± SD. * P<0.05.

Fig. 1. Relationship between synovial fluid concentrations of leptin and age in OA patients group (left panel) and the controls (right panel). In OA patients, a significant correlation between age and SF leptin levels was observed (P=0.0003).
ng/ml (range 4.7–15.8) whereas that of stage I patients was 4.0 ng/ml (range 1.2–7.3) (Table 3).

**Discussions**

Little information has been provided about the changes in leptin in the different stages of OA. The present study revealed that SF leptin concentrations were increased in OA patients and that they were closely related to radiographic severity of OA. Especially, marked increase in SF leptin concentrations were found in patients with the most advanced OA stage. This result is consistent with that of Dumond et al.\(^6\), who showed that the pattern and level of leptin expression were related to the grade of cartilage destruction. These results suggest that the measurement of leptin in synovial fluid may provide information that will be useful in the early detection of OA changes and in the assessment of the progression of OA.

The reasons for increased leptin levels in SF with advancing OA stage are not clear. However, it seems that leptin may contribute to the repair of damaged articular cartilage during OA progression by stimulating the synthesis of proteoglycan. IGF-1, TGF-β1 in cartilage\(^6,29\). Proteoglycan is main component of extracellular matrix. IGF-1 and TGF-β1 stimulate chondrocytes to repair damaged extracellular matrix by forming cell clusters and increasing their anabolic activity\(^3,21,22\).

On the other hand, there is a possibility that increased leptin levels of SF in OA patients may stimulate the degeneration of OA. Leptin also has a pro-inflammatory effect on cartilage\(^30\). The increased expression of leptin in markedly damaged cartilage, together with elevated leptin levels in synovial fluid suggest that leptin may trigger cartilage destruction by increasing matrix metalloprotease (MMP)-9 and MMP-13 in a dose-dependent manner\(^24\). MMPs are thought to be major mediators of cartilage destruction and association of MMPs with proinflammatory cytokine (IL-1, TNF-α) is essential process in degenerative changes of chondrocyte\(^7,27\).

In addition, leptin activate type 2 nitric oxide synthase (NOS2) while increasing the IL-1 activation of IFN-γ\(^19\) and NOS2\(^20\). Nitric oxide has well documented pro-inflammatory effects on joint cartilage, triggering the loss of chondrocyte phenotype, inducing chondrocyte apoptosis and the activation of MMPs.

One of the limitations of our present study is the difference of age and sex distribution in OA patients group versus the normal group. Indeed, leptin levels are higher in women than in men even when adjusted for body mass index, which may be relevant to the influence of sex on the development or frequency of certain diseases, such as osteoarthritis\(^2,26\). Previously, Hickey et al.\(^11\) reported a higher level of SF and serum leptin in women than in men. Our present study also showed a remarkable increased SF leptin concentrations in women compared to men although there was no significant difference. Therefore, further study is needed for leptin levels considering age and sex factors to exclude a possibility that these factors can influence leptin levels in OA progression.

Degenerative changes in articular cartilage may proceed more rapidly in women...
during the normal aging process. The susceptibility of women to cartilage damage is reflected in the difference in the ratio of women to men between the control and OA groups\(^2\). In this respect, in the present study, higher ratio of women and men in OA patients group compared the controls appears to be the cause for increased SF leptin concentration in OA patients group.

However, the present study showed that SF leptin concentration had a tendency to increase with advancing OA stage although there was no significant difference in the ratio of women and men according to the OA severity. This result implies that the increased SF leptin concentrations in OA patients group may be influenced more by the occurrence of OA disease than by the different distribution of sex.

Uesaka et al.\(^{28}\) reported that chondroitin sulfate isomers in SF showed a decreasing tendency with increasing age. As such, age can also influence the biochemical levels in SF. They showed that the biochemical variables had a poor correlation with age, but a relatively strong inverse correlation with the severity of OA, suggesting that the changes in chondroitin sulfate isomers concentrations in OA patients are influenced more by the severity of OA than by age. However, in the present study, age showed a strong positive correlation with SF leptin levels in OA patients. Therefore, further study is needed to clearly define whether the increased SF leptin concentrations in OA patients is attributed to age.

**Conclusion**

In conclusion, this study shows that synovial fluid leptin concentrations were closely related to the radiographic severity of osteoarthritis, and suggests that age of patient may influence synovial fluid leptin concentrations during osteoarthritis progression.

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