Original article

Synovial joint fluid cytokine levels in hip disease

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Abstract

Objective. The purpose of this study was to evaluate cytokine level characteristics in the hip joint fluid, including rapidly destructive coxopathy (RDC), OA, osteonecrosis (ON) of the femoral head and RA.

Methods. Thirty-three hips with RDC, 57 with OA, 36 with ON and 10 with RA were included in the study. OA hips were divided into two groups: 20 hips with early OA without joint space narrowing and 37 hips with terminal OA. ON hips were divided into three groups: 13 hips with $< 3$ mm collapse, 14 hips with $> 3$ mm collapse and 9 hips with terminal ON. Joint fluid was collected during surgery. Cytokine levels including IL-1β, IL-6, IL-8 and TNF-α were measured using homogeneous time-resolved fluorescence.

Results. All measured cytokine levels in RDC were significantly higher than those in OA ($P < 0.05$). Terminal OA showed higher cytokine levels than those in early OA ($P < 0.05$). IL-6 and TNF-α levels in the ON group with $> 3$ mm collapse were higher than those found in the ON group with $< 3$ mm collapse. In comparing cytokine levels between RDC, terminal OA, RA and terminal ON, RDC showed significantly higher IL-8 levels than terminal OA and RA ($P < 0.05$).

Conclusion. IL-8 levels in RDC were higher than in the other hip diseases. The IL-8 level may reflect the aggressiveness of joint destruction in RDC, and IL-6 and TNF-α levels may also reflect ongoing destruction in OA and ON.

Key words: synovial fluid, cytokine, hip disease, rapidly destructive coxopathy, osteoarthritis.

Introduction

The inflammatory cytokines play an important role in joint destruction by stimulating osteoclast cells [1, 2]. Osteoclast cells are activated by cytokines such as IL-1β, IL-6 and TNF-α [1, 2], causing joint destruction in RA patients. Therefore biologic drugs including IL-6 inhibitors [3] and TNF-α inhibitors [4, 5] have been developed to prevent joint destruction and have achieved improved clinical results. Several studies have reported that knee joint synovial fluid cytokine levels with RA were significantly higher than those found in cases of OA [6, 7].

Osteoclast cells and inflammatory cytokines play a role in hip diseases, including rapidly destructive coxopathy (RDC). RDC produces destruction of the femoral head and/or acetabulum within 6–12 months, mostly in elderly females, and causes severe hip pain and disability [8]. Radiographically, joint space narrowing occurs first and destruction of the femoral head and acetabulum follow [9]. Histologically, RDC creates severe cartilage and bone destruction along with invasion of nonspecific granulation tissue composed of macrophages and fibroblastic cells [10]. Mature and activated osteoclasts occur in the synovial membrane and granulomatous bone marrow lesions occur in the focal destructive portion of the femoral heads [11]. Femoral head bone resorption is brought about through the production of IL-6 in RDC hips [12]. Osteoclast cells also occur at the reparative zone of the collapsed femoral head in hips with osteonecrosis (ON) of the femoral head [13]. The effect of inflammatory cytokines on the osteoclast cells in ON hips has not been documented; however,
cytokine gene polymorphisms have a predictive role for the development of ON [14].

Thus inflammatory cytokines have been proposed to play a role in joint destruction in hip disease. Cytokines have been reported in the synovial fluid of diseased knees [6, 7]; however, there have been few studies concerning the synovial joint fluid cytokine levels of diseased hips [15, 16]. No study has evaluated the relationship between the severity of hip joint destruction and hip joint synovial fluid cytokine levels.

The purpose of this study is (i) to determine the characteristics of the cytokine levels in hip diseases including RDC, OA, RA and ON and (ii) to clarify the relationship between the severity of joint destruction and cytokine levels using synovial joint fluid collected during hip surgery. We hypothesized that RDC would show higher levels of cytokines in the hip joint fluid and that the severity of joint destruction is influenced by the cytokine level in hip joint fluid.

Patients and methods

The Institutional Review Board of the Osaka University Graduate School of Medicine approved the study (ID: 071023) and written informed consent to use case information was obtained from all patients prior to enrolment, in accordance with the Declaration of Helsinki. One hundred and thirty-six hips in 127 patients who underwent hip surgery, including total hip arthroplasty (THA), rotational acetabular osteotomy (RAO) and hip arthroscopy, between 2004 and 2012 at our institution and affiliated hospitals were included in the present study. The mean patient age at the time of surgery was 59 years (range 16–85 years). Subjects consisted of 28 males and 99 females. There were 33 hips with RDC [5 males, 28 females; mean age (± S.D.) 75 (6) years], 57 hips with OA [6 males, 51 females; mean age (± S.D.) 56 (19) years], 10 hips with RA [10 females; mean age (± S.D.) 68 (9) years] and 36 hips with ON [18 males, 18 females; mean age (± S.D.) 51 (17) years]. OA hips were divided into two groups: 20 early OA hips, including those with acetabular dysplasia or labral tear without joint space narrowing with a Kellgren and Lawrence (KL) grade of 0–1, and 37 hips with terminal OA with a KL grade of 3–4 [17]. ON hips were divided into three groups based on the Japanese Ministry of Health, Labour and Welfare stage classification. There were 14 hips with <3 mm osteonecrotic collapse (stage 3A ON), 13 hips with >3 mm osteonecrotic collapse (stage 3B ON) and 9 hips with terminal ON (stage 4 ON) [18]. The RDC diagnosis was made with plain hip radiographs or MRI. RDC findings included a diffuse low-signal intensity area in the femoral head and neck on T1-weighted images and a high-signal intensity area on T2-weighted images with or without rapidly destructive findings [9]. All hips with RDC, terminal OA, RA and stage 4 ON showed widespread joint space obliteration. The joint space was preserved in all hips with early OA, stage 3A ON and stage 3B ON.

The hip joint synovial fluid was collected by puncture during the hip surgery, including RAO and hip arthroscopy for early OA and THA for other hip diseases before the capsule was released. The samples were centrifuged at 715 g for 10 min to remove the debris and blood cells, and the supernatant was immediately stored at −80°C. We attempted to collect synovial fluid from all consecutive surgical hips, however, we were unable to obtain samples in all hips. We were able to collect synovial fluid in 100% of RDC and RA hips, 82% of early OA hips, 78% of ON hips and 33% of terminal OA hips.

The cytokine levels, including IL-1β, IL-6, IL-8 and TNF-α, were measured using homogeneous time-resolved fluorescence (HTRF; Cisbio International, Bagnols, France) [19, 20], which reduced the cost and experimental time while increasing accuracy. Cytokine levels were compared with ELISA throughout [21]. The cytokines were detected with anti-cytokine monoclonal antibodies labelled with XL665 and cryptate. These mouse monoclonal antibodies recognize distinct epitopes of human cytokines and they do not cross-react with cytokines from other species. The specific signal released after binding both monoclonal antibodies to each cytokine is proportional to the cytokine concentration in the sample or standard. An 80-μl fluid sample was needed to measure the cytokine concentrations for each of the four cytokines. First, cryptate and XL665 was added to the reconstruction buffer for each of the cytokines and gently mixed. The second step involved gently mixing 20 μl of joint fluid and 20 μl of mixed reagent. Next, we placed the samples on a specialized plate and incubated them for 2 or 3 h. Cytokine concentrations were examined using the Artemis TR-FRET microplate reader for HTRF (Cosmo Bio Co., Tokyo, Japan) following incubation. Concentrations were determined by comparing the samples with standard samples whose concentrations were determined preliminarily.

We compared the cytokine levels between samples from patients with RDC, OA, RA and ON. We also compared the cytokine levels between ON stages and between early OA and terminal OA to clarify the relationship between the severity of joint destruction and the cytokine level. To remove the influence of the severity of joint destruction, we compared RDC with terminal OA, RA and stage 4 ON.

Statistical analysis

Statistical analysis was performed with a t-test for two-group comparison, including a comparison between early OA and terminal OA. For multiple comparisons, the Steel–Dwass test was used in comparing RDC, OA and ON, and between stage 3A ON, stage 3B ON and stage 4 ON, while the Steel test was used in comparing RDC and terminal OA, RA and stage 4 ON. Statistical software (SPSS version 20.0J for Windows; IBM, Armonk, NY) was used for all statistical analyses. Significance was established at \( P < 0.05 \).

Results

RDC hip synovial fluid showed significantly higher cytokine levels in IL-1β \( (P < 0.0001, \text{Steel–Dwass test}) \). IL-6
Table 1: The differences in synovial fluid cytokine levels between hip disease categories.

<table>
<thead>
<tr>
<th>Comparisons between each hip disease (P values)</th>
<th>RDC OA</th>
<th>RDC RA</th>
<th>RDC ON</th>
<th>OA RA</th>
<th>OA ON</th>
<th>RA ON</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of hips</td>
<td>33</td>
<td>10</td>
<td>36</td>
<td>57</td>
<td>36</td>
<td>10</td>
</tr>
<tr>
<td>IL-1β, median (range), pg/ml</td>
<td>261 (33–6223)</td>
<td>73 (0–5675)</td>
<td>147 (40–1413)</td>
<td>544 (45–5221)</td>
<td>***</td>
<td>**</td>
</tr>
<tr>
<td>IL-6, median (range), pg/ml</td>
<td>783 (25–6282)</td>
<td>347 (0–3427)</td>
<td>523 (137–3433)</td>
<td>142 (25–3931)</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>IL-8, median (range), pg/ml</td>
<td>3305 (330–9534)</td>
<td>316 (42–5020)</td>
<td>873 (307–4008)</td>
<td>771 (178–9606)</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>TNF-α, median (range), pg/ml</td>
<td>348 (32–5980)</td>
<td>193 (97–7715)</td>
<td>245 (0–9580)</td>
<td>1435 (327–3972)</td>
<td>P = 0.02</td>
<td>978 (0–1070)</td>
</tr>
</tbody>
</table>

Statistical analysis was performed by using Steel-Dwass test. Blank: P > 0.1, P < 0.05, ***P < 0.001.

Discussion

Some authors have examined the cytokine levels in knee joint synovial fluid. The levels of IL-1β, IL-8 and TNF-α in the RA knee joint fluid were significantly higher than those in the OA knee [6, 7]. High-grade destruction of the OA knee showed significantly higher levels of IL-1β than did OA knees with low-grade destruction [22]. However, there were few reports concerning cytokine levels in the hip joint. Although hip joint fluid IL-1β levels with acetabular dysplasia have been measured, the samples were collected after injection of 10 ml of saline inside the joint capsule [15]. Therefore they did not have an accurate cytokine level. In the present study, joint fluid was obtained from 33 hips with RDC than in 37 hips with terminal OA. No previous study evaluated other inflammatory cytokine levels, including IL-6, IL-8 and TNF-α, in other hip
**TABLE 2** Statistical comparison of cytokine levels in synovial fluid between OA stages

<table>
<thead>
<tr>
<th>Number of hips</th>
<th>Early OA</th>
<th>Terminal OA</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β, median (range), pg/ml</td>
<td>50 (0–413)</td>
<td>86 (8–5675)</td>
<td>0.004</td>
</tr>
<tr>
<td>IL-6, median (range), pg/ml</td>
<td>44 (0–1127)</td>
<td>542 (48–3427)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IL-8, median (range), pg/ml</td>
<td>163 (42–737)</td>
<td>190 (42–5020)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TNF-α, median (range), pg/ml</td>
<td>90 (3–500)</td>
<td>207 (63–5290)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

a=t-test.

**Fig. 1** Comparison of the cytokine levels in the OA hip synovial joint fluid between early stage and terminal stage.

Terminal OA hips showed significantly higher levels of IL-1β, IL-6, IL-8 and TNF-α than early OA hips.

**TABLE 3** Cytokine levels in synovial fluid in each ON stage

<table>
<thead>
<tr>
<th>Number of hips</th>
<th>Stage 3A ON</th>
<th>Stage 3B ON</th>
<th>Stage 4 ON</th>
<th>Comparison between each ON stage (P-values)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13</td>
<td>14</td>
<td>9</td>
<td>3A–3B</td>
</tr>
<tr>
<td>IL-1β, median (range), pg/ml</td>
<td>102 (25–620)</td>
<td>193 (79–3913)</td>
<td>285 (46–3931)</td>
<td>*</td>
</tr>
<tr>
<td>IL-6, median (range), pg/ml</td>
<td>428 (45–1436)</td>
<td>1021 (324–5221)</td>
<td>567 (123–2473)</td>
<td>†</td>
</tr>
<tr>
<td>IL-8, median (range), pg/ml</td>
<td>413 (178–2932)</td>
<td>1328 (311–9606)</td>
<td>875 (192–8551)</td>
<td>*</td>
</tr>
<tr>
<td>TNF-α, median (range), pg/ml</td>
<td>126 (31–468)</td>
<td>283 (28–5672)</td>
<td>394 (138–3368)</td>
<td>*</td>
</tr>
</tbody>
</table>

Statistical analysis was performed by using Steel-Dwass test. Blank: P ≥ 0.1, †P < 0.1, *P < 0.05, **P < 0.01, ***P < 0.001.
diseases or the relationship between the severity of joint destruction and cytokine levels. We measured joint fluid cytokine levels for the four hip disease categories in the present study. We showed higher IL-1β and TNF-α levels in the hip joint fluid with RDC than that with terminal OA, and showed higher IL-8 levels than not only terminal OA, but also RA. In addition, we demonstrated the relationship between the severity of the joint destruction and cytokine levels.

The present study has some limitations. First, the number of subjects in the RA and each of the ON stages was small. Further studies are needed to fully evaluate differences in cytokine levels between these diseases categories. Second, synovial fluid was not collected from all hips and this occurred most often in the hips with OA. We could obtain synovial fluid in only one-third of the terminal OA hips, although joint fluid could be obtained from almost all hips with RDC, RA and ON. The joint fluid was ordinarily evident in joints with higher degrees of inflammation. Therefore OA hips with collected synovial fluid might show greater inflammation and higher cytokine concentrations than OA hips with less synovial fluid, which is difficult to aspirate. We might therefore have overvalued the cytokine levels by evaluating only the joint fluid, which could be aspirated. Third, the patients' background data, including age and gender, significantly differed between hip disease categories. Patients with RDC showed older age, and patients with ON included more male patients than the other groups. In addition, patients with early OA showed younger age than other hip categories because the indication of RAO for early OA was young age. Although we should adjust the age and gender between hip disease categories to evaluate the cytokine level differences, it is impossible because the differences in the patients' background data are too large for adjustment in early OA and ON. Therefore we compared RDC with terminal OA in patients adjusted for age and gender, and RDC also showed higher levels of IL-1β, IL-8 and TNF-α.

The relation of the cytokine levels with the histology of the femoral head specimens was not evaluated. However, the diagnosis and disease staging was evaluated by radiographs, mainly early OA, terminal OA and stage 3A/3B/4 ON in the present study. We think that the degenerative histological grading of the femoral head specimen in each disease category is comparable to the radiological staging.
IL-1\( \beta \) and TNF-\( \alpha \) levels were significantly higher in RDC than terminal OA, and the IL-8 levels were significantly higher in RDC hips than in both terminal OA and RA hips, even when removing the influences of joint destruction. Inflammatory cytokines, including IL-1\( \beta \), IL-6 and TNF-\( \alpha \), have been implicated as the joint destruction factor activating the osteoclast cells in patients with RA \cite{1, 2}. IL-8 has been reported to play an important role in not only RA \cite{23, 25}, but also aseptic loosening of hip prostheses \cite{26, 27} and tumour bone metastasis from lung and breast cancer \cite{28-30}. Synovial fluid IL-8 levels have been reported to correlate with the severity of osteolysis around loosened hip prostheses, whose relation with osteoclast cells has been reported \cite{27, 31}. IL-8 has further been reported to stimulate both human osteoclast formation and bone resorption in tumour metastasis \cite{28}. The IL-8 mechanism of action does not require RANK ligand (RANKL) pathway activation, but involves the expression and activation of the specific IL-8 receptor on the osteoclast cell surfaces and their precursors \cite{28}. Osteoclast cells have been activated in the synovial membrane and the femoral head in RDC hips \cite{11}. IL-8 and other cytokines might have promoted RDC hip joint destruction via osteoclast cell activation. IL-8 also induces a massive accumulation of neutrophils, which produce neutrophil elastase, leading to cartilage destruction \cite{32}. Clinical correlation of IL-8 levels with tumour aggressiveness \cite{33, 34} and with the severity of osteolysis in hip prosthesis loosening \cite{27} have also been reported. These facts are in agreement with the aggressiveness of the joint destruction in hips with RDC, as joint space narrowing, which means cartilage destruction, occurred first, with subsequent collapse of the femoral head or acetabulum \cite{9}. Any treatments in the early stage of RDC, such as bisphosphonates or inhibitors of IL-8, for the prevention of joint destruction may be applied. It has been reported that injection of IL-8 in the knee joints of rabbits causes cartilage destruction, and it has also been shown that an inhibitor of neutrophil elastase prevented the destruction of cartilage \cite{32}. In addition, RDC radiographically resembles OA, ON, RA, neuropathic osteoarthropathy and septic arthritis \cite{35-38}. IL-8 levels in the hip synovial joint fluid may be useful in diagnosing RDC and in helping to distinguish RDC from other hip diseases.
There was a relationship between the severity of joint destruction and synovial fluid cytokine levels. It has been reported that more severely involved joints showed higher IL-1β levels for OA knees [22]. Terminal OA showed higher levels of IL-1β, IL-6, IL-8 and TNF-α than early OA hips in the present study, and stage 3B hips showed significantly higher IL-6 and TNF-α levels than stage 3A hips. Stage 3B hips also had a tendency towards higher IL-6 levels than stage 4 hips in the ON group, although there was no statistical significance (Fig. 2). IL-6 and TNF-α were the common factors between OA and ON. These findings suggested that early stage disease processes show lower cytokine levels and more advanced stages show higher cytokine levels commensurate with a greater inflammatory status. In addition, the terminal stage disease process shows comparatively low cytokine levels as a type of burn-out status. RDC hips might show higher cytokine levels before joint destruction. On the other hand, there were no differences in cytokine levels between terminal OA, RA and stage 4 ON. The inflammatory reaction, including cytokine levels, did not differ in the terminal stages among the chronic osteoarthritic diseases. We could not clearly prove that elevated cytokine levels were the cause of RDC or the result of joint destruction. This is because all hips with RDC showed widespread joint space obliteration (stage 3) [9]. It is difficult to acquire joint fluid samples before joint destruction because most patients with RDC had come to the hospital due to severe hip pain after joint destruction. The severity of joint destruction influenced the IL-6 and TNF-α levels in the ON group. Progression of joint destruction in ON hips occurred due to mechanical stress, including collapse of the subchondral necrotic area. However, RDC hips initially showed joint space narrowing without subchondral destruction [9]. The underlying pathological condition between these diseases differed, and changes in the cytokine levels might differ in each disease.

Hip joint puncture to investigate synovial fluid cytokine levels is an invasive procedure and requires exposure to radiation. Serum IL-8 levels have been correlated with synovial fluid IL-8 levels of the hip joints with implant loosening [27]. We will need to investigate further the use of serum cytokine levels as a diagnostic tool.

In conclusion, synovial fluid IL-8 levels were significantly higher in RDC hips than in both terminal OA and RA hips. High joint fluid IL-8 levels might indicate high osteoclast cell activity in RDC hips. IL-6 and TNF-α levels in the joint fluid may also reflect ongoing destruction in OA and ON hips. Further study, including measurement of the cytokine levels in the joint fluid before destruction of the hip joint, will be needed to elucidate the underlying pathological mechanisms involved in producing RDC.

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**Disclosure statement:** The authors have declared no conflicts of interest.

### References


### Rheumatology key messages

- Hip joint fluid IL-8 levels were significantly higher in RDC than in OA and RA.
- The severity of joint destruction influenced IL-6 and TNF-α levels in the hip joint fluid.


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