Correlation of plasma and synovial fluid osteopontin with disease severity in knee osteoarthritis

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Abstract

Objectives: The purposes of this study were to examine osteopontin levels in both plasma and synovial fluid of patients with primary knee osteoarthritis (OA) and to investigate their relationship with severity of the disease.

Design and methods: Thirty-two patients aged 53–83 years with knee OA and 15 healthy controls were enrolled in this study. Anteroposterior knee radiographs were taken to determine the disease severity of the affected knee. The radiographic grading of OA in the knee was performed by using the Kellgren–Lawrence criteria. Osteopontin levels in the plasma and synovial fluid were measured using enzyme-linked immunosorbent assay.

Results: The mean plasma osteopontin concentration of the knee OA patients was significantly higher compared with that of healthy controls (168.8±15.6 vs 67.2±7.7 ng/mL, P<0.0001). Osteopontin levels in synovial fluid were significantly higher with respect to paired plasma samples (272.1±15.0 vs 168.8±15.6 ng/mL, P<0.001). In addition, plasma osteopontin levels showed a positive correlation with synovial fluid osteopontin levels (r=0.373, P=0.035). Subsequent analysis showed that plasma osteopontin levels significantly correlated with severity of disease (r=0.592, P<0.001). Furthermore, the synovial fluid levels of osteopontin also correlated with disease severity (r=0.451, P=0.01).

Conclusion: The data suggest that osteopontin in plasma and synovial fluid is related to progressive joint damage in knee OA. Osteopontin may serve as a biochemical marker for determining disease severity and could be predictive of prognosis with respect to the progression of knee OA.

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Keywords: Osteopontin; Osteoarthritis; Plasma; Synovial fluid

Introduction

Osteoarthritis (OA) is a chronic degenerative joint disease that is characterized by the progressive destruction of articular cartilage with varying degrees of severity within a given joint [1]. The clinical features of OA include pain, stiffness, reduced motion, swelling, crepitus, and deformity. Pathologically, the disease manifests itself by cartilage loss, subchondral bone, sclerosis and cyst, and marginal osteophytes. Diagnosis is relied upon symptoms and signs in conjunction with radiography. Although clinical research on OA has been extensively investigated, the etiology of this disease remains poorly elucidated. Several biochemical and biomechanical factors are considered for the pathogenesis.

Osteopontin is a highly phosphorylated and sulfated glycoprotein with a molecular weight in the range of 44–66 kDa [2]. Osteopontin is one of the major noncollagenous bone matrix proteins produced by various cell types, including activated T cells, macrophages, osteoblasts and chondrocytes [3]. Osteopontin expression during chondrocyte maturation is one of the important events involved in cartilage-to-bone
transitions in fracture repair [4]. There is evidence suggesting that osteopontin functions as a proinflammatory cytokine and plays a critical role particularly in the regulation of tissue repair and remodeling [5]. Osteopontin may be involved in the molecular pathogenesis of osteoarthritis, contributing to progressive degeneration of articular cartilage [6]. Expression of osteopontin mRNA isolated from human OA cartilage was enhanced as compared with normal cartilage. Indeed, osteopontin was shown to be upregulated in human OA chondrocytes [7]. Moreover, osteopontin was found to be expressed in bone forming cells and hypertrophic chondrocytes of the embryonic epiphyseal growth plates [8].

Although circulating and/or synovial fluid levels of several cytokines have been investigated in patients with knee OA, there have been no detailed studies on plasma and synovial fluid levels of osteopontin in various clinical stages of primary knee OA [9–13]. Therefore, we hypothesized that osteopontin in plasma and synovial fluid may be associated with the severity of clinical outcomes in knee OA patients. To prove this hypothesis we examine the plasma and synovial fluid levels of osteopontin in knee OA patients and healthy controls. The purposes of the present study were to investigate the concentrations of osteopontin in both plasma and synovial fluid of patients with primary knee osteoarthritis, and to evaluate the possible correlations with the radiographic grading of knee OA.

Patients and methods

Patients

The present study was approved by the Ethical Committee on Human Research of the Faculty of Medicine, Chulalongkorn University and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from the patients and healthy volunteers prior to their participation in this study.

Thirty-two patients aged 53–83 years with primary knee osteoarthritis (28 females and 4 males; mean age 70.5±1.3 years) according to the criteria of the American College of Rheumatology and 15 normal healthy individuals (10 female and 5 male; mean age 65.5±0.6 years) were enrolled in the study. The severity of the disease was determined using weight-bearing anteroposterior radiographs of the affected knee. Knee radiographs were evaluated according to the Kellgren and Lawrence classification [14]: grade 1, doubtful narrowing of joint space and possible osteophytic lipping; grade 2, definite osteophytes and possible narrowing of joint space; grade 3, moderate multiple osteophytes, definite narrowing of joints space, some sclerosis and possible deformity of bone contour; grade 4, large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour. The grading scale used for analysis was the higher of the two knees.

Laboratory methods

Synovial fluid was aspirated from the affected knee during surgery, when a total knee arthroplasty was performed, centrifuged to remove cells and joint debris and stored immediately at −80 °C until the day of measurement. Blood samples were collected from the same patients one day before surgery, centrifuged to remove cells and debris, and stored at −80 °C until used. Double-blind quantitative detection of osteopontin in plasma and synovial fluid was performed using commercial enzyme-linked immunosorbent assay (ELISA) (Immuno-Biological Laboratories Co., Gunma, Japan) according to the manufacturers’ instructions. Briefly, standards of recombinant human osteopontin, plasma, and synovial fluid samples were added to 96-well microtiter plates precoated with rabbit polyclonal antibody against osteopontin and incubated for 1 h at room temperature. The wells were then washed seven times with washing buffer and incubated for 30 min at 4 °C with a horseradish peroxidase-labeled mouse monoclonal antibody to human osteopontin. After nine washes, substrate solution was added to each well, and the plate was incubated for 30 min at room temperature in the dark. Finally, the reaction was stopped with the stop solution, and then absorbance was measured at 450 nm using automated microtiter plate reader. The osteopontin concentration was calculated by the standard curve. Twofold serial dilutions of recombinant human osteopontin with a concentration of 5–320 ng/mL were used as standards. The sensitivity of this assay was 3.3 ng/mL.

Statistical analysis

Statistical analysis was carried out with the statistical package for social sciences (SPSS) software, version 16.0 for Windows. Comparisons between the groups were performed using Student’s t-test for unpaired data. Nonparametric test was used to analyze the difference of age between controls and OA patients. Pearson’s correlation coefficient was employed to determine the correlation among the concentration of osteopontin in the plasma and synovial fluid and the disease severity. Data were expressed as a mean±SEM. P values <0.05 were considered statistically significant.

Results

 Plasma and synovial fluid osteopontin of knee OA patients and plasma osteopontin of controls are demonstrated in Fig. 1. There was no difference of age between healthy controls and the OA patients (65.5±0.6 vs 70.5±1.3 years, P=0.6). OA patients had higher plasma osteopontin concentrations compared to healthy controls (168.8±15.6 vs 67.2±7.7 ng/mL, P<0.0001). Osteopontin levels in synovial fluid were significantly higher with respect to paired plasma samples (272.1±15.0 vs 168.8±15.6 ng/mL, P<0.001). In order to compare synovial fluid levels of osteopontin as a function of age among OA patients, they were categorized into 3 age groups as follows: less than 65 years of age (n=9), 65 to 75 years of age (n=12), and more than 75 years of age (n=11). As illustrated in Fig. 2, there was no statistically significant difference in synovial fluid osteopontin levels among patients with OA (P=0.5). Interestingly, plasma osteopontin levels showed a positive correlation with synovial fluid osteopontin levels (r=0.373, P=0.035).
According to the Kellgren and Lawrence (KL) grading scale, 8 patients were KL grade 2, whereas 12 patients were KL grade 3 and 12 patients were KL grade 4 osteoarthritis. The plasma and synovial fluid levels of osteopontin were analyzed and compared in relation to radiological KL grading of OA. The plasma osteopontin levels from KL grade 2 were 69.8 ± 23.1 ng/mL; those from KL grade 3 were 193.8 ± 17.9 ng/mL; and those from KL grade 4 were 209.8 ± 21.8 ng/mL. These results showed that plasma osteopontin levels in KL grade 3 and 4 were significantly higher than those of KL grade 2 (P < 0.001). Although the mean plasma levels of osteopontin in KL grade 4 were greater than those in KL grade 3, the difference was not statistically significant (P = 0.835). In addition, the synovial fluid levels of osteopontin from KL grade 2 were 191.9 ± 36.7 ng/mL; those from KL grade 3 were 300.5 ± 10.2 ng/mL; and those from KL grade 4 were 297.1 ± 22.3 ng/mL. The data revealed that synovial fluid osteopontin levels in KL grade 3 and 4 were significantly elevated compared with those of KL grade 2 (P < 0.01). We further analyzed the correlation between the plasma and synovial fluid levels of osteopontin and the severity of osteoarthritis. The plasma osteopontin levels significantly correlated with severity of disease (r = 0.592, P < 0.001) (Fig. 3). The synovial fluid levels of osteopontin also correlated with disease severity (r = 0.451, P = 0.01) (Fig. 4).

Discussion

Osteopontin, a member of the SIBLING (small integrin-binding ligand N-linked glycosylated protein) family, is present in extracellular fluids, at sites of inflammation, and in the extracellular matrix of mineralized tissues [3,15]. This protein also known as early T cell activation gene-1 (Eta-1) is abundant in bone, where it mediates important cell–matrix and cell–cell interactions [16]. Osteopontin facilitates the attachment of osteoclasts to the bone matrix via an interaction with cell surface αvβ3 integrin and CD44, the hyaluronic acid receptor [17]. It contains the arginine–glycine–aspartate (RGD) integrin-binding motif for the adherence of many cell types [18]. An essential function of osteopontin in osteogenesis is the
inhibition of bone mineralization [19]. Osteopontin is a strong chemoattractive and proinflammatory molecule and may be involved in a number of physiologic and pathologic events including angiogenesis, apoptosis, inflammation, wound healing and tumor metastasis [20].

It has been reported that osteopontin was present in plasma and synovial fluid of patients with rheumatoid arthritis and with OA [21]. However, the relationship between osteopontin levels in the plasma and synovial fluid and disease severity has never been specifically determined. To the best of our knowledge, no study dealing with correlation of osteopontin levels in plasma and synovial fluid and severity of knee OA has been previously reported in the literature. This study is the first to demonstrate that osteopontin was detected in both plasma and synovial fluid obtained from patients with primary knee OA, and that osteopontin levels positively correlated with the severity of OA.

The present study revealed a marked increase of osteopontin levels in both plasma and synovial fluid of patients with knee osteoarthritis compared to the control plasma levels. Our findings suggest enhanced local and systemic production of osteopontin in the primary knee osteoarthritis. It is of interest to note that osteopontin levels of synovial fluid were significantly higher than those seen in paired plasma samples. Elevated levels of osteopontin in synovial fluid are possibly caused by either the release of osteopontin residing in extracellular matrix, or the increase in its production, or both processes. The source of the osteopontin in the synovial fluid is presumably to be the local tissues (such as the synovial membrane and articular cartilage). Previous studies have demonstrated the immunohistochemical expression of osteopontin in the synovial lining cells, fibroblasts in the synovial tissues, and articular chondrocytes [6,7,21]. It is suggested that cell adhesion, migration or inflammation could be involved in the release of osteopontin [20]. Synovitis and degenerative changes of articular cartilage is likely to be facilitating factors in the release of osteopontin into the synovial fluid.

In accordance with our observations, recent studies have showed that osteopontin protein deposition and mRNA expression increased with morphological signs and the severity of matrix degradation in human osteoarthritic cartilage [7]. These findings indicate that osteopontin expression in osteoarthritic cartilage strongly correlates with the severity of OA disease. In addition, Attur et al. have revealed the increased expression of osteopontin in human OA cartilage; the expression of osteopontin mRNA was highly upregulated as compared with normal cartilage [6]. They also found that addition of recombinant osteopontin to human OA-affected cartilage under ex vivo conditions suppressed spontaneous production of nitric oxide and prostaglandin E2. These findings suggest that osteopontin is overexpressed in OA cartilage and functions as an endogenous inhibitor of production of inflammatory mediators in cartilage. Furthermore, Yaki et al. showed that the advanced OA cartilage had significantly higher osteopontin mRNA expression than the minimal OA cartilage [22]. The patient matched comparison of minimal and advanced OA cartilage displayed alterations in gene expression that may be involved in OA progression.

These results indicate that plasma and synovial fluid levels of osteopontin may play a significant role in the pathogenesis of OA. Measurements of plasma and/or synovial levels of osteopontin could possibly serve as a biochemical parameter for determining disease severity and may be predictive of prognosis with respect to the progression of osteoarthritic disease process. Longitudinal studies may provide further information regarding the value of osteopontin as a potential marker to monitor the course of OA. Additional investigation will be needed to evaluate the knee radiographs among the control group because they presumably have no knee pain, but might have radiographic evidence of OA.

This study has had several limitations. First, the sample size was not large enough to arrive at definitive conclusions. Secondly, we investigated only those patients who attended Chulalongkorn Memorial Hospital, a tertiary care center, for evaluation or treatment of knee OA. Thirdly, the cross-sectional design of our study precluded addressing whether the analyzed level of osteopontin predicted alteration in severity in patients with knee OA.

The determination of an accurate cut-off value for circulating osteopontin level is one critical issue in its clinical application. The documented normal circulating osteopontin levels in previous reports are highly variable, with a range from 31 ng/mL to 200 ng/mL [23–25]. The exact reason is unclear but it could be attributed to the different assay systems and conditions of sample collection utilized in those studies. Another critical issue that limits the applicability of osteopontin as a specific marker for osteoarthritis is that the osteopontin level is also increased in a variety of cancers. Elevation of circulating osteopontin levels has been recently reported in melanoma, ovarian cancer, pancreatic cancer, colon cancer, breast cancer, lung cancer, and hepatocellular carcinoma [26–30]. Further studies with standardized assay system in a large population are warranted to gain insight into the potential utility of osteopontin in OA patients.

In conclusion, patients with primary knee OA had higher levels of plasma osteopontin compared with healthy controls. Osteopontin concentrations in plasma and synovial fluid significantly correlated with the severity of disease. Further investigations are in progress to elucidate the contribution of osteopontin to the pathogenesis of the degenerative process of osteoarthritis.

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