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S Honsawek

J Poonphakdi

B Deepaisarnsakul

A Tanavalee

P Yuktanandana

See next page for additional authors

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S Honsawek, J Poonphakdi, B Deepaisarnsakul, A Tanavalee, P Yuktanandana, S Ngarmukos, and S. Tantavisut

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Expression of matrix metalloproteinase-1 in knee osteoarthritis: Association with disease severity

Sittisak Honsawek*
Jariya Poonphakdi* Benjamad Deepaisarnsakul**
Aree Tanavalee*** Pongsak Yuktanandana***
Srihatach Ngarmukos*** Saran Tantavisut***

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Background : *Osteoarthritis (OA) is characterized by the progressive loss of articular cartilage and osteophyte formation resulting in pain, stiffness, reduced motion, swelling, crepitus, and disability. The aim of this study is to investigate plasma and synovial fluid matrix metalloproteinase-1 (MMP-1) levels of patients with primary knee OA and to examine their relationship with disease severity.*

Methods : *Thirty-two patients aged 53 - 83 years old with knee OA and 10 healthy individuals were recruited into this study. Disease severity was determined using weight-bearing anteroposterior radiographs of the affected knee. The radiological grading of knee OA was performed according to Kellgren-Lawrence grading system. MMP-1 levels in both plasma and synovial fluid were evaluated using enzyme-linked immunosorbent assay.*

* Department of Biochemistry, Faculty of Medicine, Chulalongkorn University
** Department of Central Laboratory, Taksin Hospital
***Department of Orthopaedics, Faculty of Medicine, Chulalongkorn University

Results : *The mean plasma MMP-1 levels were higher in OA patients compared to controls; however, the difference was not statistically significant (198.0 ± 63.5 vs. 94.2 ± 2.4 pg/ml, P = 0.1). MMP-1 levels in synovial fluid of OA patients (2632.8 ± 525.2 pg/ml) were 13-fold higher than in corresponding blood samples (P <0.001), and were 26-fold higher than in the plasma of healthy controls (P <0.001). Subsequent analysis revealed that synovial fluid MMP-1 levels of knee OA patients were positively correlated with OA grading (r = 0.873, P< 0.001).*

Conclusions : *MMP-1 levels in synovial fluid are positively associated with the severity of joint damage in knee OA. Synovial fluid MMP-1 may serve as a biomarker for determining disease severity and could play a possible role in the pathogenesis of osteoarthritis.*

Keywords : *MMP-1, knee osteoarthritis, severity, synovial fluid.*

Reprint request: Honsawek S. Department of Biochemistry, Faculty of Medicine Chulalongkorn University and King Chulalongkorn Memorial Hospital Rama IV road, Patumwan, Bangkok 10330, Thailand. Email: Sittisak.H@chula.ac.th

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บทนำ : *โรคข้อเสื่อม (osteoarthritis) เป็นโรคข้อที่พบได้บ่อย ซึ่งทำให้เกิดอาการเจ็บปวด บวมบริเวณข้อ ข้อตึงฝืด เคลื่อนไหวได้จำกัด คลำได้ความรู้สึกกรอบแกรบบริเวณข้อ และทำให้ข้อพิการได้ วัตถุประสงค์ของการศึกษานี้เพื่อตรวจวิเคราะห์ระดับโปรตีน MMP-1 ในพลาสมาและในน้ำไขข้อของผู้ป่วยโรคข้อเข่าเสื่อมปฐมภูมิและศึกษาความสัมพันธ์กับระดับความรุนแรงของโรค*

วิธีการศึกษา : *การศึกษานี้ประกอบด้วยผู้ป่วยโรคข้อเข่าเสื่อมจำนวน 32 ราย ตั้งแต่อายุ 53 - 83 ปี และคนปกติที่มีสุขภาพดีที่มีอายุใกล้เคียงกัน 10 ราย การจัดระดับความรุนแรงของโรคอาศัยเกณฑ์ของ Kellgren - Lawrence บนภาพถ่ายรังสีข้อเข่าในท่ายืนที่มีพยาธิสภาพในท่าหน้า – หลัง และทำการตรวจระดับ MMP-1 ในพลาสมาและในน้ำไขข้อด้วยวิธี enzyme–linked immunosorbent assay*

ผลการศึกษา : *ระดับค่าเฉลี่ย MMP-1 ในพลาสมาของผู้ป่วยโรคข้อเข่าเสื่อมสูงกว่าคนปกติ แต่ไม่มีความแตกต่างทางสถิติ (198.0 ± 63.5 vs 94.2 ± 2.4 pg/ml, P = 0.1) ระดับของโปรตีน MMP-1 ในน้ำไขข้อของผู้ป่วยโรคข้อเข่าเสื่อม (2632.8 ± 525.2 pg/ml) มีค่าสูงกว่าในพลาสมาอย่างมีนัยสำคัญ (P <0.001) โดยมีระดับโปรตีน MMP-1 ในน้ำไขข้อสูงมากกว่าในพลาสมาถึง 13 เท่า และมีระดับสูงมากกว่าในพลาสมาของคนปกติถึง 26 เท่า นอกจากนี้ระดับโปรตีน MMP-1 ในน้ำไขข้อมีสหสัมพันธ์โดยตรงกับระดับความรุนแรงของโรคบนภาพถ่ายรังสี (r = 0.873, P< 0.001)*

บทสรุป : *ผลจากการศึกษาสรุปได้ว่าระดับโปรตีน MMP-1 ในน้ำไขข้อมีสหสัมพันธ์โดยตรงกับระดับความรุนแรงของโรคข้อเข่าเสื่อม โปรตีน MMP-1 ในน้ำไขข้อนี้อาจนำมาใช้เป็นดัชนีบ่งชี้ทางชีวเคมีของความรุนแรงในโรคข้อเสื่อมและมีบทบาทสำคัญต่อการเกิดกระบวนการเสื่อมสภาพของโรคข้อเสื่อม*

คำสำคัญ : *MMP-1; โรคข้อเข่าเสื่อม; ความรุนแรงของโรค; น้ำไขข้อ.*

Osteoarthritis (OA) is characterized by the progressive destruction of articular cartilage with joint-space narrowing, osteophyte formation, subchondral sclerosis, and synovitis. [1] It is a degenerative joint disease leading to pain, stiffness, reduced motion, swelling, crepitus, and disability. The pathology of OA involves in the whole joint including the menisci, ligaments, periarticular muscles, joint capsule, and the infrapatellar fat. One of the present methods to examine the affected joint is radiological assessment which reflects disease severity by grading the joint degeneration. Kellgren-Lawrence grading scale representing disease severity has been the most widely used system. [2] The etiology and pathogenesis of OA remain unclear, but they have been related to several physiological factors such as obesity and aging. [3] Nonetheless, biochemical factors have been recognized as playing an potential role in OA development.

The matrix metalloproteinases (MMPs) have been considered the main enzymes responsible for degradation of aggrecan and collagen in cartilage. [4, 5] Proinflammatory cytokines and MMPs have been shown to be present in the synovial fluid and synovial tissue of OA patients. [6, 7] MMPs are a group of Zn^{2+} dependent extracellular enzymes that play a key role in normal and pathological tissue remodeling and have the combined ability to degrade all components of the extracellular matrix. [8, 9] Based on domain structure and substrate specificity, MMPs can be divided into subclasses (collagenases, gelatinases, stromelysins, and membrane type MMPs).[10] Several studies have shown that expression of several MMPs is elevated in cartilage and synovial tissues of patients with rheumatoid arthritis (RA) and OA or of animal OA model.[11-13] MMP-1 belongs to the collagenase subgroup of the MMP family. It is able to cleave the triple helical chains of type II collagen in articular cartilage and play an important role in abnormal collagen turnover in OA. [14, 15]

Although synovial fluid levels of several cytokines have been investigated in patients with knee OA, there have not been documented the association of synovial fluid levels of MMP-1 with disease severity in primary knee OA. [16-18] We have postulated that MMP-1 in synovial fluid might be related to the radiographic severity in knee OA patients. The objective of this study is to investigate plasma and synovial fluid levels of MMP-1 in primary knee OA patients, and to evaluate the association between synovial fluid MMP-1 levels and the radiographic grading of knee osteoarthritis.

Methods

Study participants

This study has been approved by the Institutional Review Board (IRB) on Human Research of the Faculty of Medicine, Chulalongkorn University and has been conducted in agreement 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 old with primary knee osteoarthritis (28 females and 4 males; mean age 70.5 $\pm$ 1.3 years) according to the criteria of the American College of Rheumatology [19] were recruited 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 Kellgren and Lawrence

(KL) classification. [2] 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 joint 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 one found higher upon comparison between both knees. We also recruited 10 gender and age matched subjects (mean age 65.5 ± 0.6 years) with normal knee radiographs as controls. None of the participants had underlying diseases such as diabetes, histories of corticosteroid medication, other forms of arthritis, cancer, or other chronic inflammatory diseases.

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^{O}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^{O}C until used. Double-blind quantitative detection of MMP-1 in plasma and synovial fluid was performed using commercial enzyme-linked immunosorbent assay (ELISA) (Quantikine, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. Briefly, standards of recombinant human MMP-1, plasma, and synovial fluid samples were added to 96-well microtiter plates precoated with mouse monoclonal antibody against human MMP-1 and incubated for 2 hours at room temperature. The wells were then washed four times with washing buffer and incubated for 2 hours at room temperature with a horseradish peroxidase-conjugated monoclonal antibody against MMP-1. After four washes, substrate solution was added to each well, and the plate was incubated for 30 minutes 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 microplate reader. Recombinant human MMP-1 was used to generate a standard curve.

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 one-way analysis of variance (ANOVA). Pearson's correlation coefficient was employed to determine the correlation among the concentration of MMP-1 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

Ten plasma and 32 synovial fluid samples from knee OA patients and 10 plasma samples from healthy controls were acquired for measurement of MMP-1 concentrations. Plasma and synovial fluid MMP-1 levels of knee OA patients and plasma of controls are shown in Figure 1. OA patients had higher plasma MMP-1 concentrations compared to healthy controls (198.0 ± 63.5 *vs.* 94.2 ± 2.4 pg/ml, P = 0.1). Although the mean plasma MMP-1 levels were higher in OA patients compared to controls, the difference was not statistically significant. MMP-1 levels in

synovial fluid of OA patients (2632.8 ± 525.2 pg/ml) were 13-fold higher than in corresponding blood samples (P <0.001), and were 26-fold higher than in the plasma of healthy controls (P <0.001).

With regard to the radiological KL classification, patients were categorized into 3 groups in relation to OA grading. Eleven patients were classified as grade 2, eleven as grade 3, and ten as grade 4. As demonstrated in Figure 2, MMP-1 concentrations in synovial fluid were elevated significantly as the disease severity increased. Subsequent analysis revealed that synovial fluid MMP-1 levels of knee OA patients positively correlated with OA grading (r = 0.873, P< 0.001).

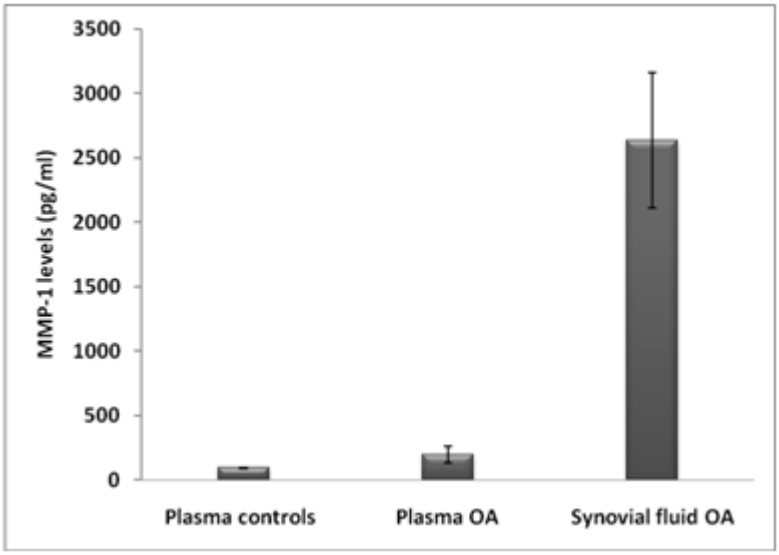

Figure 1. MMP-1 levels in plasma and synovial fluid of patients with OA and controls.

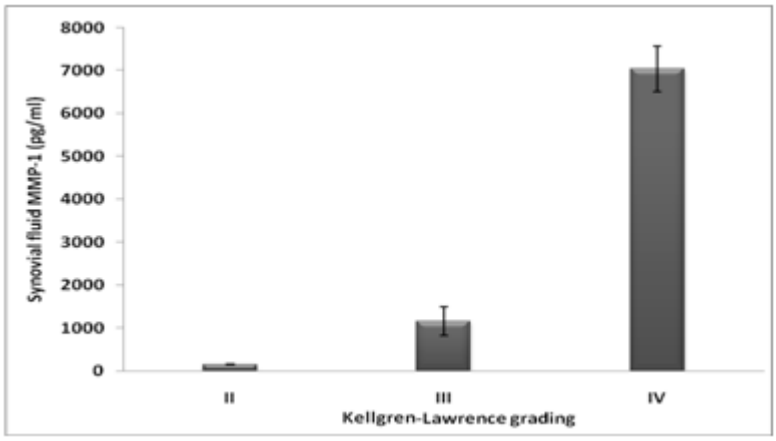

Figure 2. Comparison of synovial fluid MMP-1 levels in patients with OA classified according to Kellgren and Lawrence grading scale.

Discussion

It has been documented that MMP-1 was detected in synovial fluid of patients with RA and with OA. [9] However, the investigation of MMP-1 levels in the plasma and synovial fluid in relation to disease severity has never been specifically determined in knee OA patients. To the best of our knowledge, no study dealing with correlation of plasma and synovial fluid levels of MMP-1 and severity of knee OA has been previously documented in the literature. This study is the first to show that MMP-1 was detected in both plasma and synovial fluid derived from patients with primary knee OA, and that synovial fluid MMP-1 positively correlated with the severity of OA.

The present study shows a marked increase of synovial fluid MMP-1 levels compared to the plasma levels of knee OA patients and controls. Our results suggest enhanced local and systemic production of MMP-1 in the primary knee osteoarthritis. There are two possible reasons to explain why MMP-1 levels in synovial fluid were elevated. First, high MMP-1 levels in synovial fluid are possibly caused by either the release of MMP-1 residing in extracellular matrix, or the increase in its production, or both processes. Second, synoviocytes and chondrocytes in the local tissues including the synovial membrane and articular cartilage could express endogenous MMP-1 in an autocrine/paracrine manner to increase endogenous MMP-1 in synovial fluid.

In the present study, the data show that plasma and synovial fluid levels of MMP-1 may play a plausible role in the pathogenesis of OA. Measurements of synovial levels of MMP-1 could likely be used as a biochemical marker for determining disease severity and might be predictive of prognosis with respect to the progression of knee osteoarthritis. Further researches may provide additional information regarding the value of MMP-1 as a potential marker to monitor the disease progression.

It should be noted; however, that a limitation of this study is that the sample size was small to make definite conclusion. Our available data should be confirmed in a large number of subjects. In addition, incomplete assessment of potential confounders and the effect of joint sites other than knee need to be taken into consideration. It is possible that the elevated plasma and synovial MMP-1 levels found in primary knee OA patients play a role in the progression of cartilage degeneration. Lastly, the female predominance of patients with OA may lead to a potential bias in comparing the levels of MMP-1 since the controlled data obtained from a large sample size are not available at present.

To sum up, patients with primary knee OA had elevated levels of plasma MMP-1 compared with healthy controls. We performed this study with the goal of relating synovial fluid MMP-1 levels to the radiological progression of knee OA. We showed that synovial fluid MMP-1 levels were significantly correlated with the magnitude of OA radiographic progression. MMP-1 measurement may not only serve as a biochemical marker of disease progression but also has the potentiality to contribute to the fundamental processes underlying the pathogenesis of primary knee OA. Additional longitudinal studies are required to elucidate the influence of MMP-1 on disease outcome. Future investigations will be needed to shed light on the possible role of MMP-1 involved in the pathogenesis of chronic degenerative joint disorder, with the aim of developing effective pharmacological agents to delay the progression to osteoarthritis.

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