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Plasma and synovial fluid autotaxin correlate with severity in knee osteoarthritis

Thomas Mabey, Pimpisa Taleongpong, Wanvisa Udomsinprasert, Napaphat Jirathanathornnukul, Sittisak Honsawek *

Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok 10330, Thailand

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ABSTRACT

Background: This study aimed to investigate the relationships between plasma and synovial autotaxin and the severity in knee osteoarthritis (OA) patients.
Methods: A total of 90 participants (70 knee OA patients and 20 controls) were recruited. Autotaxin and high-sensitivity C-reactive protein (hs-CRP) levels were determined. The symptomatic and radiographic severity of OA was assessed using the Western Ontario McMaster University Osteoarthritis Index (WOMAC) scores and the Kellgren–Lawrence grades.
Results: OA patients had significantly higher circulating autotaxin and hs-CRP than controls. Plasma autotaxin was directly correlated with synovial fluid autotaxin ($r = 0.639$, $P < 0.001$). Additionally, plasma and synovial fluid autotaxin were associated with radiographic severity ($P < 0.001$). Furthermore, plasma and synovial fluid autotaxin levels were positively correlated with WOMAC scores ($r = 0.558$, $P < 0.001$ and $r = 0.371$, $P = 0.002$, respectively).
Conclusion: Plasma and synovial fluid autotaxin levels were positively correlated with the severity of OA. Thus, autotaxin has potential as a biomarker reflecting the severity of knee OA.

1. Introduction

Osteoarthritis (OA) is a degenerative joint disorder that involves progressive changes in all joint structures initiated by a combination of mechanical, genetic and age-associated factors [1–3]. Other risk factors associated with OA include gender, obesity, poor nutrition, and muscle weakness [3]. Despite the etiology of OA being unclear, inflammation has been strongly associated with the destruction of extracellular matrix resulting in the softening and degradation of cartilage tissue, remodeling of subchondral bone, and increased bone vascularization, accompanied by the clinical symptoms of the disease including pain, joint swelling, early morning stiffness, and disability [4,5]. Radiographic examination can reveal key pathological features including joint space narrowing, as the result of the loss of articular cartilage, and abnormalities in bone structure for example osteophyte formation, subchondral sclerosis, and subchondral bone cyst [6].

Autotaxin (ATX) or ecto-nucleotide pyrophosphatase/phosphodiesterase (ENPP)-2 is a 125 kDa glycoprotein that belongs to the ENPP family [7]. It is secreted as lysophospholipase D (lysoPLD), an active enzyme with a distinct catalytic domain able to hydrolyze lysophosphatidylcholine (LPC) into lysophosphatidic acid (LPA) and choline [8]. Abundant concentrations of LPA can be detected in biological fluids including serum, synovial fluid, and plasma. Serum is the best characterized source of LPA [9] and studies have shown that the majority of circulating LPA is produced by the lysoPLD activity of autotaxin [10–13]. LPA initiates and mediates a variety of cellular signaling pathways by binding to six cell surface-expressed guanine-nucleotide-binding protein (G-protein)-coupled receptors named LPA_{1-6} [14].

Previous studies have highlighted that local LPA production plays a critical role in stimulating cell proliferation, migration, and cytokine and matrix metalloproteinase (MMP) production in chronic inflammatory diseases including rheumatoid arthritis (RA) [9]. Increased expression of autotaxin mRNA detected in synovial fibroblasts (SFs) of arthritic mouse models can lead to local LPA production and subsequent LPA-dependent SF stimulation [15]. This leads to increased cellular adhesion, migration, and enhanced production of pro-inflammatory cytokines and MMPs [15]. SFs isolated from OA patients were shown to express significant amounts of autotaxin mRNA [15]. Furthermore, expression of LPA_{1-3} has been observed in human OA SFs [16], suggesting a potential role of autotaxin and LPA signaling in the pathogenesis of OA.

* Corresponding author at: Department of Biochemistry, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital, 1873 Rama IV road, Patumwan, Bangkok 10330, Thailand. Tel./fax: +66 2 256 4482.
E-mail address: Sittisak.H@chula.ac.th (S. Honsawek).

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Table 1
Baseline clinical characteristics of knee OA patients and controls.

	OA patients	Controls	*P*
Number	70	20	
Age (y)	68.9 ± 0.9	68.1 ± 0.7	NS
Gender (female/male)	56/14	15/5	NS

OA, osteoarthritis.

2. Methods

2.1. Study population

The present study was conducted in accordance with the guidelines of the Declaration of Helsinki, and a written informed consent was obtained from all 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.

Seventy patients (56 females and 14 males) diagnosed with knee osteoarthritis according to the criteria of the American College of Rheumatology [17], and 20 healthy volunteers with no clinical or radiological evidence of OA (15 females and 5 males) were enrolled in the present study. No participant had underlying diseases such as diabetes, advanced liver or renal diseases, histories of medication interfering with bone metabolism (such as corticosteroids or bisphosphonates), other forms of arthritis, cancer or other chronic inflammatory diseases.

2.2. Radiographic definitions

Knee radiography was taken when each participant was standing on both legs with fully extended knees and the X-ray beam was centered at the concentration of the joint. Assessment of radiographic severity was performed using the Kellgren and Lawrence (KL) classification [18]. 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 lipping; 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; and 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 grade used for analysis was the one found higher upon comparison between both knees.

2.3. Symptomatic definitions

The symptomatic severity of the disease was determined according to the Western Ontario McMaster University Osteoarthritis Index (WOMAC) [19]. The index consists of three main categories including pain, stiffness, and physical function. Total WOMAC scores range from 0 to 100. A higher score represents worse outcome.

2.4. Laboratory methods

Following a 12-h overnight fast, venous blood samples were collected from all participants, centrifuged, and stored immediately at −80 °C until the day of analysis. High-sensitivity C-reactive protein (hs-CRP) was analyzed by Immunoturbidimetric method using a Cobas 6000 automated analyzer (Roche Diagnostics). Synovial fluid was taken from the most affected knee during diagnostic or therapeutic arthroscopy or total knee replacement. The specimen was then centrifuged to remove cells and joint debris and then stored at −80 °C for further measurement.

Plasma and synovial fluid autotaxin concentrations were measured using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) development kit (R&D Systems). According to the manufacturer's protocol, recombinant human autotaxin standards, plasma, and synovial fluid samples were added into each well of a microplate, which was pre-coated with a monoclonal antibody against autotaxin. After incubating for 2 h at room temperature, every well was washed thoroughly 4 times with wash buffer. Then, a horseradish peroxidase-conjugated polyclonal antibody specific for autotaxin was pipetted into each well and incubated for a further 2 h at room temperature. After 4 washes, substrate solution was pipetted into the wells and then the microplate was incubated for 30 min at room temperature with protection from light. Finally, the reaction was stopped by the stop solution and the optical density was measured with an automated microplate reader at 450 nm. The amount of color generated is directly proportional to the amount of autotaxin in the sample. Autotaxin concentration was determined by a standard optical density–concentration curve. Twofold serial dilutions of recombinant human autotaxin with a concentration of 0.781–50 ng/ml were used as standards. The intra- and inter-assay CVs were 2.6–3.7% and 2.9–4.7%, respectively. The sensitivity of this assay was 0.157 ng/ml.

2.5. Statistical analysis

Statistical analysis was performed using the statistical package for social sciences (SPSS) software, ver 16.0Tests of normality and test of homogeneity of variances was employed to determine the subject's age, WOMAC, hs-CRP, and autotaxin values in the plasma and synovial fluid. Analysis of covariance (ANCOVA) indicated that age and gender were not potentially confounding factors in the study. 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 a Tukey post hoc test if ANOVA showed significance. Comparisons between groups were made using Mann–Whitney *U* test (for 2 groups) or Kruskal–Wallis test (>2 groups) when the variances were not equal among the groups. Correlations between plasma and synovial fluid autotaxin, hs-CRP, and disease severity were assessed using Pearson's correlation coefficient (*r*). Data were expressed as a mean ± standard error of the mean. A $P < 0.05$ was considered to be statistically significant for differences and correlations.

3. Results

3.1. Autotaxin concentrations in plasma and synovial fluid of knee OA patients compared to healthy controls

The baseline clinical characteristics of the subjects are displayed in Table 1. There were no statistically significant differences in the ages

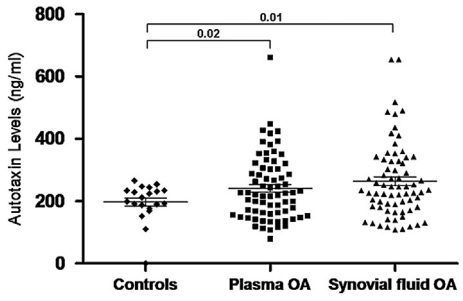

Fig. 1. Autotaxin levels in plasma and synovial fluid of OA patients and healthy controls.

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