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Elevated Circulating and Synovial Fluid Endoglin Are Associated with Primary Knee Osteoarthritis Severity

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Background and Aims. Osteoarthritis (OA) is a chronic degenerative joint disorder of the synovial joint characterized by loss of articular cartilage, osteophyte formation, and alterations of subchondral bone. The aim of this study was to evaluate endoglin levels in both plasma and synovial fluid of patients with primary knee OA and to determine their relationship with disease severity.

Methods. Thirty nine patients with primary knee OA and 15 healthy controls were recruited in this study. The radiographic grading of OA in the knee was performed using the Kellgren and Lawrence classification. Endoglin concentrations in both plasma and synovial fluid were analyzed using a sandwich enzyme-linked immunosorbent assay.

Results. The average value of plasma endoglin in patients with knee OA was significantly higher compared with that of healthy controls (5.16 ± 0.22 vs. 4.43 ± 0.3 ng/mL, $p = 0.03$). Although endoglin levels in synovial fluid were higher with respect to paired plasma samples, the difference was not significant (5.41 ± 0.32 vs. 5.16 ± 0.22 ng/mL, $p = 0.3$). Additionally, plasma endoglin levels exhibited a positive correlation with synovial fluid endoglin levels ($r = 0.52$, $p = 0.001$). Further analysis revealed that plasma endoglin levels significantly correlated with disease severity ($r = 0.38$, $p = 0.02$). Furthermore, the synovial fluid levels of endoglin also positively correlated with disease severity ($r = 0.55$, $p = 0.001$).

Conclusions. These findings indicate that endoglin in plasma and synovial fluid is correlated with progressive joint damage in knee OA. Endoglin is likely to be useful as a biomarker for determining disease severity and may play a possible role in the pathogenesis of osteoarthritis.

Key Words: Biomarker, Disease severity, Endoglin, Osteoarthritis, Plasma, Synovial fluid.

Introduction

Osteoarthritis (OA) is the most common form of degenerative joint disease that progressively results in loss of joint function and is the leading cause of physical disability and impaired quality of life. The principal features of OA include subchondral bone sclerosis, articular cartilage breakdown and alterations of the synovium on both morphological and biochemical levels leading to episodic synovitis (1). These three joint compartments can be sources of production of growth factors and cytokines, as well as mediators conventionally associated with inflammation, which promote progressive joint destruction in OA (2). Diagnosis of OA relies upon history and physical examination, but x-rays demonstrating osteophyte formation, joint space narrowing, and subchondral bone cysts are diagnostic. The precise etiology of OA remains entirely obscure. Various biochemical and biomechanical factors plausibly play a substantial role in the development of OA.

Endoglin, also known as CD105, is a 180-kDa homodimeric transmembrane co-receptor for members of the transforming growth factor-β (TGF-β) superfamily and shares 71%

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sequence similarity with the transmembrane and cytoplasmic domains of β-glycan, another TGF-β superfamily co-receptor (3). This large type I integral membrane glycoprotein is a cell-surface accessory receptor for the fibrogenic key cytokines TGF-β1 and TGF-β3 and modulates the TGF-β signaling pathway by interacting with the TGF-β receptor complexes (4). There is growing evidence that overexpression of endoglin may be implicated in angiogenesis, inflammation, and wound healing (5). Endoglin is constitutively expressed on vascular endothelial cells and chondrocytes and may be essential for the regulation of epithelial/mesenchymal transition, which has a critical role in embryonic development, tissue repair, and fibrogenesis by binding TGF-β1 (6,7). It has been previously reported that endoglin was expressed on human chondrocytes and formed a heteromeric complex with the TGF-β signaling receptors and β-glycan (8). In addition, endoglin expression was upregulated in synovial tissues of OA implicating that it may play a possible role in the perpetuation of inflammation in OA (9).

Recently, it has been suggested that several growth factors and cytokines are present in the circulation and synovial fluid of patients with OA and play important roles in the pathogenesis of OA (10−14). To our knowledge, however, there are no reports on the association of circulating and synovial fluid levels of endoglin with disease severity in primary knee OA. Thus, we postulated that increased endoglin levels in plasma and synovial fluid may be related to the severity of clinical outcomes in knee OA patients. Accordingly, the current study was conducted to measure both plasma and synovial fluid levels of endoglin in patients with knee OA and investigate the relationships between plasma and synovial fluid endoglin with the disease severity of primary knee OA.

Materials and Methods

Subjects

Ethical approval of this study was obtained from the Institutional Review Board on Human Research of the Faculty of Medicine, Chulalongkorn University. The current study was conducted in compliance with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from patients and healthy volunteers prior to their participation in the study.

Thirty nine patients aged 50−78 years diagnosed with primary knee OA (34 females and 5 males; mean age 67.8 ± 1.1 years) according to the criteria of the American College of Rheumatology and 15 normal healthy individuals (12 females and 3 males; mean age 65.5 ± 0.6 years) were recruited into the study. All patients underwent total knee arthroplasty at our institute between January 2008 and August 2008. Participants were excluded on the basis of having arthropathy due to gout, pseudogout, rheumatoid arthritis, systemic lupus erythematosus, psoriasis, hemochromatosis, previous knee injury, or previous joint infection. Patients with any systemic inflammatory or autoimmune disorders or any types of malignant or chronic illnesses were not included in this study.

Disease severity was evaluated using weight-bearing anteroposterior x-rays of the affected knee. Knee radiographs were determined according to the Kellgren and Lawrence classification (15): grade 1, questionable 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 in volumes of 1−2 mL using sterile knee puncture just prior to 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 1 day before surgery, centrifuged to remove cells and debris, and stored at −80 °C until used. Double-blind quantitative detection of endoglin in plasma and synovial fluid was used with a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) (Quantikine, R&D Systems, Minneapolis, MN) according to manufacturer's instructions. Briefly, standards of recombinant human endoglin, plasma, and synovial fluid samples were added to 96-well microtiter plates precoated with mouse monoclonal antibody against human endoglin and incubated for 2 h at room temperature. The wells were then washed four times with washing buffer and incubated for 2 h at room temperature with a horseradish peroxidase-conjugated monoclonal antibody against endoglin. After four 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 absorbance was measured at 450 nm using automated microplate reader. Recombinant human endoglin was used to generate a linear standard calibration curve (range 0−10 ng/mL). The manufacturer-reported precision was 2.8−3.2% (intra-assay) and 6.3−6.7% (inter-assay).

Statistical Analysis

Statistical analysis was performed using the statistical package for social sciences (SPSS) software, v.16.0 for Windows. All values are expressed as mean ± SEM. Comparisons between two groups of individuals were done using Student's independent *t*-test for unpaired data. The significance of differences in the measured values between

groups (Kellgren and Lawrence classification) was performed using one-way analysis of variance (ANOVA). Correlation among the concentration of endoglin in the plasma and synovial fluid and disease severity was analyzed by Pearson's correlation coefficient (r). A p value <0.05 was considered to indicate statistical significance.

Results

Thirty nine plasma and synovial fluid samples from patients with primary knee OA and 15 plasma samples from healthy controls were obtained for assessment of endoglin concentrations. As illustrated in Figure 1, OA patients had higher elevated plasma endoglin levels compared to healthy controls (5.16 ± 0.22 vs. 4.43 ± 0.3 ng/mL, $p = 0.03$). Notwithstanding that endoglin levels in synovial fluid were higher with respect to paired plasma samples, the difference was not significant (5.41 ± 0.32 vs. 5.16 ± 0.22 ng/mL, $p = 0.3$).

In accordance with the Kellgren and Lawrence (KL) classification, 11 patients were KL grade 2, whereas 13 patients were KL grade 3, and 15 patients were KL grade 4 osteoarthritis. Plasma and synovial fluid levels of endoglin were evaluated and compared in relation to radiological KL grading of OA. Plasma endoglin levels from KL grade 2 were 4.46 ± 0.23 ng/mL, those from KL grade 3 were 5.09 ± 0.35 ng/mL, and those from KL grade 4 were 5.74 ± 0.43 ng/mL (Figure 2). The results showed that plasma endoglin levels in KL grade 4 were significantly greater than those of KL grade 2 ($p = 0.01$). Even though the mean plasma levels of endoglin in KL grade 3 were higher than those in KL grade 2, the difference was not significant ($p = 0.08$). Furthermore, the synovial fluid levels of endoglin from KL grade 2 were 3.93 ± 0.23 ng/mL, those from KL grade 3 were 5.25 ± 0.33 ng/mL, and those from KL grade 4 were 6.64 ± 0.64 ng/mL (Figure 3). Results showed that synovial fluid endoglin levels in KL grade 4 were significantly elevated compared with those of KL grade 2 ($p = 0.001$) and 3 ($p = 0.04$). Moreover,

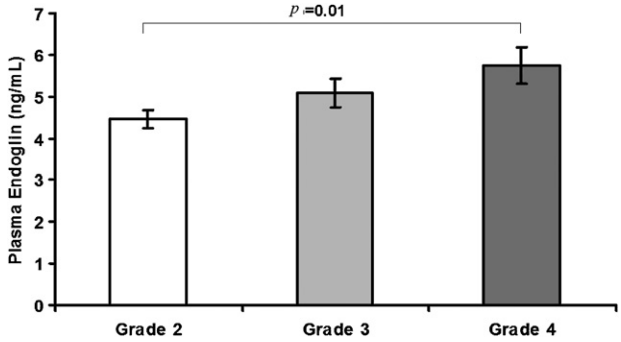

Figure 2. Comparison of plasma endoglin levels in patients with OA classified according to Kellgren and Lawrence grading scale.

the mean synovial fluid levels of endoglin in KL grade 3 were higher than those in KL grade 2 ($p = 0.002$). We subsequently investigated the relationship between plasma and synovial fluid levels of endoglin and the severity of OA. Plasma endoglin levels significantly correlated with disease severity ($r = 0.38$, $p = 0.02$). Synovial fluid levels of endoglin also positively correlated with disease severity ($r = 0.55$, $p = 0.001$). In addition, plasma endoglin levels exhibited a positive correlation with synovial fluid endoglin levels ($r = 0.52$, $p = 0.001$) (Figure 4).

Discussion

Endoglin, an accessory component for TGF-β receptor system, functions as a modulator of cellular TGF-β responses (16). This auxiliary receptor binds TGF-β1, TGF-β3, activin, BMP-2, and BMP-7 in the presence of the signaling receptor type I and II and regulates the effect of TGF-β on extracellular matrix synthesis (6,17,18). Therefore, the presence of endoglin as a part of the TGF-β receptor complex could be pivotal in determining the extent of extracellular matrix protein accumulation following the binding of TGF-β1. Endoglin contains the RGD (Arg-Gly-Asp) sequence in an exposed region of the extracellular domain (19). This is an essential recognition motif for

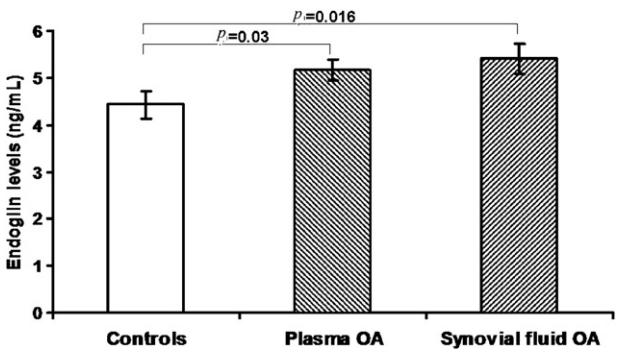

Figure 1. Endoglin levels in plasma and synovial fluid of patients with osteoarthritis (OA) and healthy controls.

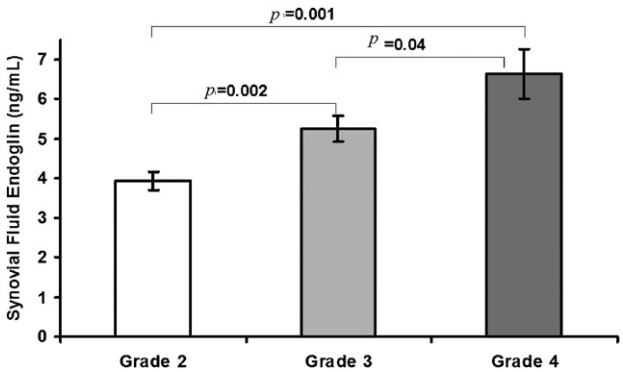

Figure 3. Comparison of synovial fluid endoglin levels in patients with OA classified according to Kellgren and Lawrence grading scale.

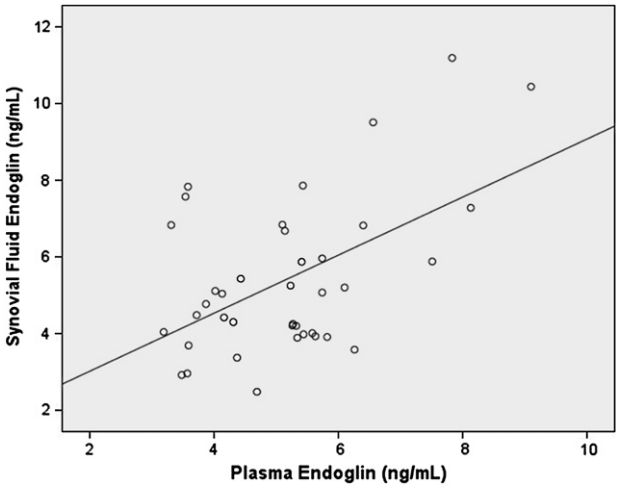

Figure 4. Correlation between plasma endoglin level and synovial fluid endoglin level in patients with OA ($r = 0.52$, $p = 0.001$).

a number of extracellular matrix proteins such as fibronectin, collagen, laminin and vitronectin for their specific integrin receptors. It is unclear whether TGF-β1 binding to endoglin in chondrocytes and in the cartilage would lead to an increase or decrease in TGF-β signaling.

It has been previously shown that endoglin mRNA expression was present in peripheral blood mononuclear cells and synovial tissues of patients with rheumatoid arthritis (RA) and OA (20,21). Recent studies have also reported that endoglin protein was expressed on human chondrocytes at high concentrations and was detectable in synovial tissue of patients with RA and with OA (8,9). However, the relationship between endoglin levels in plasma and synovial fluid and disease severity has never been investigated in patients with knee OA. To our knowledge, no published data regarding concentrations of endoglin in plasma and synovial fluid and their relationship with severity of knee OA have been previously documented in the literature. This study is the first to demonstrate that endoglin was identified in both plasma and synovial fluid obtained from patients with primary knee OA, and that endoglin positively correlated with radiographic grading of knee OA.

The most interesting and novel finding in this report is that concentrations of endoglin were increased in both plasma and synovial fluid of patients with knee OA compared with the control plasma levels. Endoglin levels in plasma and synovial fluid were detected in a well-defined knee OA population at any disease stage and were significantly higher in end-stage knee OA patients compared with early OA patients. This observation indicates a significant increase in the systemic and local production of endoglin in primary knee OA.

Several aspects of the causes of high plasma and synovial fluid endoglin in knee OA patients, especially in those with end-stage knee OA, merit discussion. Increased circulating endoglin in OA patients may be attributed to production of endoglin secreted from the injured joints. The source of endoglin in synovial fluid is likely to be synovial lining cells, endothelial cells in the synovial tissues and chondrocytes. Previous studies have shown that endoglin was present in endothelial cells in synovial tissue, and its expression was also increased in OA compared to normal synovial tissue lining cells (9). Moreover, endoglin was expressed in human chondrocytes at levels comparable with endothelial cells (8). These findings suggest that endogenous endoglin may be involved in the pathogenesis of OA.

It is conceivable that cell lysis, leakage, death, or inflammation may be associated with the release of endoglin (22). Synovitis and degenerative alterations of articular cartilage may be precipitating factors in the release of endoglin into the synovial fluid of knee joints in OA patients. Further investigations will be needed to determine whether the increased levels of endoglin in synovial fluid and plasma related to higher levels of endoglin in the joint tissues or an increase in the cellular and tissue damage in the joints as the disease progresses. The elevated concentration of endoglin in the synovial fluid of knee OA suggests that local tissue (synovial and cartilage tissue) has the potential to release endoglin into the joints. The mechanisms of endoglin elevation in the circulation and synovial fluid of patients with OA remain to be elucidated.

It should be noted that there are some inherent limitations to this study. First, our study was based on a relatively small number of enrolled patients. A further study with a random sample of a larger population will be required to make a more definite conclusion. Second, only endoglin production has been examined in both plasma and synovial fluid. Additional histological studies for endoglin expression may reveal more valuable information on the pathogenic role of endoglin in OA. Third, the study was limited to those patients who attended our hospital. Consequently, the results may not be directly applicable to subjects from other ethnic groups. Moreover, the results obtained from our study cannot necessarily be extrapolated in men because of female predominance of subjects with knee OA. Finally, this study was cross-sectional in its design and, therefore, no conclusions regarding cause and effect relationships can be drawn. However, prospective longitudinal studies can now be designed to document disease progression and define the precise role of endoglin in knee OA.

To summarize, we demonstrated a significant increase in the systemic and local expression of endoglin and identified a strong association with the degree of radiographic progression in patients with primary knee OA. The results of the present study support the finding that endoglin may be a useful prognostic indicator to reflect the disease severity of knee OA. Further, endoglin concentration in plasma positively correlated with that in synovial fluid. This is the first study to identify such a correlation and should be followed-up with a prospective study in order to substantiate these findings. Although underlining mechanisms of

this association and their cause-and-effect relationships are not entirely clear in the current study, these findings may contribute to the understanding of the role of endoglin in the pathogenesis of chronic degenerative joint disease.

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