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Plasma and synovial fluid sclerostin are inversely associated with radiographic severity of knee osteoarthritis

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ABSTRACT

Objective: The purpose of this study was to analyze sclerostin in plasma and synovial fluid of knee osteoarthritis (OA) patients and to investigate the association between sclerostin levels and radiographic severity.

Design and methods: A total of 190 subjects (95 knee OA patients and 95 healthy controls) were recruited in the present study. Sclerostin levels in plasma and synovial fluid were assessed using an enzyme-linked immunosorbent assay. OA grading was performed using the Kellgren–Lawrence classification.

Results: Plasma sclerostin levels were significantly lower in OA patients than in healthy controls ($P = 0.004$). Additionally, sclerostin levels in plasma were significantly higher with respect to paired synovial fluid ($P < 0.001$). Moreover, sclerostin levels in plasma and synovial fluid demonstrated a significant inverse correlation with the radiographic severity of knee OA ($r = -0.464$, $P < 0.001$ and $r = -0.592$, $P < 0.001$, respectively). Subsequent analysis revealed that there was a positive correlation between plasma and synovial sclerostin levels ($r = 0.657$, $P < 0.001$).

Conclusions: Sclerostin was significantly lower in OA plasma samples when compared with healthy controls. Plasma and synovial fluid sclerostin levels were inversely associated with the radiographic severity of knee OA. Therefore, sclerostin may be utilized as a biochemical marker for reflecting disease severity in primary knee OA.

Introduction

Osteoarthritis (OA) is a progressive degenerative joint disease which particularly affects weight bearing joints, predominantly the hips and knees, with which pain, joint swelling, reduced motion, and stiffness are commonly associated. The pathophysiology of OA is contributed to by three main joint tissue types; the synovium, cartilage, and subchondral bone. Articular cartilage destruction with joint space narrowing, osteophyte formation, subchondral bone sclerosis, and synovitis are characteristics of osteoarthritis [1]. The exact aetiology of OA remains obscure but there are a number of known associated risk factors including age, obesity, alterations in joint mechanical stability, genetic predisposition, and previous joint trauma [2]. Nevertheless, loss of articular cartilage, subchondral sclerosis, and bone remodelling have been known to play important roles in OA development.

Wnt/β-catenin signalling has a substantial role in bone and cartilage homeostasis in the adult skeleton, and has been implicated in the process of cartilage degradation in osteoarthritis [3]. Sclerostin, encoded by the *SOST* gene, is an exclusively osteocyte-derived protein that contains a signal peptide for secretion and a cysteine-knot motif [4]. The amino acid sequence of sclerostin has a 20–24% similarity with a specific subfamily of cysteine knot-containing proteins termed the DAN (differential screening-selected gene aberrative in neuroblastoma) family of secreted proteoglycans [5]. Sclerostin is secreted as a monomer in contrast to many other cysteine-knot proteins which form disulfide-linked homodimers [6]. Inactivating mutations in the *SOST* gene can cause sclerosteosis and van Buchem disease which are bone dysplasia disorders characterized by progressive skeletal overgrowth [7–9]. Sclerostin is expressed by terminally differentiated cells embedded in mineralized matrix including osteocytes and hypertrophic chondrocytes [10,11]. Its downregulation in osteocytes by physical loading of bone contributes to the mechanical sensor function of osteocytes and the subsequent increase in bone growth [12].

The negative regulatory actions of sclerostin occur through the canonical Wnt/β-catenin signalling pathway by binding specifically to low density lipoprotein-related protein 5 and 6 (LRP5/6) and inhibiting their association with Frizzled receptors [13,14]. This binding inhibits the pathway which would normally lead to, among other outcomes, bone formation and can subsequently impact on osteogenesis. Sclerostin has also been shown to be produced in interleukin (IL)-1α stimulated

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chondrocytes within joint cartilage. This was found to be potentially beneficial in protecting against cartilage degradation [15]. In addition, decreased sclerostin expression of osteocytes is associated with increased cortical bone density in hip OA, and sclerostin has previously been shown to be expressed by chondrocytes in mineralized cartilage, indicating a potential role for sclerostin in OA pathogenesis [3].

To our knowledge, no previous study has examined sclerostin with respect to its relationship with radiographic severity in primary knee osteoarthritis. Thus, the objectives of this study were to compare sclerostin levels in plasma and synovial fluid from OA patients and healthy controls, and additionally to investigate the association between plasma and synovial fluid sclerostin levels and radiographic severity in primary knee OA.

Materials and methods

Study subjects

The present study was conducted in agreement with the guidelines of the Declaration of Helsinki, and 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. The sample size was designed according to the standardized effect size of 0.7, using Student's *t*-test to compare means of continuous variables, a statistical power of 0.8, and a *P*-value of 0.05. Therefore, at least 35 subjects were required in each group.

Ninety-five patients (79 females and 16 males) were diagnosed with primary knee osteoarthritis according to the criteria of the American College of Rheumatology, and 95 healthy volunteers with no clinical and radiological evidence of OA (77 females and 18 males) were enrolled in the present study. All patients were randomly selected and scheduled to undergo diagnostic or therapeutic arthroscopy or total knee arthroplasty in our hospital between January 2009 and August 2011. Clinical data were carefully reviewed to exclude any forms of secondary OA and inflammatory joint diseases. 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.

Knee radiography was taken when each participant was standing on both legs with fully extended knees and the X-ray beam was centred at the level of the joint. Assessment of radiographic severity was performed using the Kellgren and Lawrence (KL) grading system [16]. 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 grading scale used for analysis was the one found higher upon comparison between both knees.

Laboratory methods

Following a 12-h overnight fast, venous blood samples were collected into ethylenediamine tetraacetic acid (EDTA) tubes, centrifuged, and stored immediately at −80 °C until analysis. Synovial fluid was aspirated from the affected knee of OA patients using sterile knee puncture just prior to surgery when the arthroscopy or total knee arthroplasty was performed. The specimen was then centrifuged to remove cells and joint debris and then stored at −80 °C for further measurement.

Plasma and synovial fluid sclerostin levels were measured using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) development kit (R&D Systems, Minneapolis, MN, USA). According to the manufacturer's instructions, recombinant human sclerostin standards, plasma, and synovial fluid samples were pipetted into each well, which was pre-coated with mouse monoclonal antibody specific for sclerostin. After incubating for 2 h at room temperature, every well was washed thoroughly four times with washing buffer. Then, a horseradish peroxidase-conjugated polyclonal antibody specific for sclerostin was added to each well and incubated for a further 2 h at room temperature. After four 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 colour intensity was measured with an automated microplate reader at 450 nm. The amount of colour generated is directly proportional to the amount of sclerostin in the sample. Sclerostin concentration was determined by a standard optical density–concentration curve. Twofold serial dilutions of recombinant human sclerostin with a concentration of 31.3–2000 pg/mL were used as standards. The intra- and inter-assay coefficients of variation (CVs) were 1.8–2.1% and 8.2–10.8%, respectively. The sensitivity of this assay was 3.8 pg/mL.

Statistical analysis

Statistical analysis was carried out using the statistical package for social sciences (SPSS) software, version 16.0 for Windows. 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 Tukey post hoc test if ANOVA showed significance. Pearson's correlation coefficient (r) was employed to determine correlations between plasma and synovial fluid sclerostin and clinical characteristics. Data were expressed as a mean ± standard error of the mean. *P*-values < 0.05 were considered to be statistically significant for differences and correlations.

Results

Ninety-five OA patients, aged 49–84 years, and 95 controls, aged 50–80 years were enrolled in the present study. The baseline clinical characteristics of the subjects are displayed in Table 1. There were no statistically significant differences in the ages or gender ratios (female/male) between OA patients and healthy controls. As shown in Fig. 1, plasma sclerostin levels were significantly lower in OA patients than in healthy controls (920.7 ± 62.6 pg/mL vs. 1177.8 ± 72.3 pg/mL, $P = 0.004$). Sclerostin concentrations in synovial fluid of OA patients were nearly twofold lower than in paired plasma samples (526.8 ± 51.3 pg/mL vs. 920.7 ± 62.6 pg/mL, $P < 0.001$). Plasma sclerostin levels exhibited a positive correlation with synovial fluid sclerostin levels ($r = 0.657$, $P < 0.001$) (Fig. 2).

In order to reduce the confounding effect of age, using the mean age of 70 years, the study population was divided into a middle-aged (those <70 years of age, n = 51) and an elderly (those ≥70 years of age, n =

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

	OA patients	Controls	*P*
Number	95	95	
Age (years)	69.5 ± 0.8	68.2 ± 0.7	0.2
Gender (female/male)	79/16	77/18	0.5

OA = Osteoarthritis.