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Original Article

Are synovial levels of WISP-1 associated with knee osteoarthritis severity and cartilage loss in elderly population?

Yubao Gong, Chen Yang, Jianguo Liu, Xin Qi

Department of Bone and Joint Surgery, The First Hospital of Jilin University, Changchun, Jilin Province, China

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Abstract: Objectives: WNT1 inducible signaling pathway protein-1 (WISP-1) is highly expressed in the hypertrophic zone of cartilage growth plate and has been shown to be able to induce articular cartilage damage in animal models of osteoarthritis, but its associations with Synovitis in patient with osteoarthritis has not been studied. This study aims to determine the associations between synovial levels of WISP-1 with symptoms and signs, demographic data, knee radiographic score, Synovitis degree and cartilage loss in osteoarthritis (OA) patients. Methods: A total of 89 randomly selected subjects (mean 63 years old, ranging 52-78 years old, 73% female) were studied. Demographic data were documented. Synovial level of WISP-1 was assessed by enzyme-linked immunosorbent assay (ELISA) kits. The grading of OA was determined by Kellgren/Lawrence score. Synovitis severity was assessed using MRI. The loss of cartilage was assessed by whole-organ MRI scoring (WORMS). Pearson's correlation coefficient (r) was used to determine correlations among WISP-1 level with symptoms and signs of OA, demographic data, knee radiographic score, synovitis severity and cartilage loss. Results: The synovial fluid WISP-1 level in knees of OA patients was significantly higher in OA patient group with synovitis than in group without synovitis ($P<0.001$). Moreover, there was no difference in WOMAC score or WORMS score between these two groups. The synovial fluid WISP-1 level was not correlated with severity of OA damage shown by radiography ($r=0.004$, $P=0.981$). Conclusions: These findings indicate that the synovial fluid WISP-1 level is correlated with synovitis in patient with osteoarthritis and may be used as a clinical indicator to facilitate the diagnosis, evaluation and treatment of synovitis in patient with osteoarthritis.

Keywords: Osteoarthritis, synovitis, knee, WISP-1, radiographic, MRI, older adults

Introduction

Osteoarthritis (OA) is one of the most common degenerative diseases, which results in the destruction of cartilage and bone, and ultimately leading to joint dysfunction. The cause of the disease is still unknown, although obesity, genetic factors, and injury have all been associated with increased risk of OA [1, 2]. Although in most cases the initial event leading to OA occurs within the cartilage or subchondral bone [3, 4], the synovial tissue of many OA patients also shows morphological changes, with marked inflammatory responses [5, 6].

Wnt1 inducible signaling pathway protein-1 (WISP-1) belongs to the CCN-family of growth factors and has been shown to induce bone formation and fibrosis [7, 8]. WISP-1 was shown to be able to regulate chondrocyte and macrophage MMP and aggrecanase expression and is capable of inducing articular cartilage damage in models of OA [9]. Recent study shows that WISP-1 was involved in the pathologic mechanism in experimental and human OA [10].

This study was aimed to explore the associations between synovial WISP-1 level with demographic data, knee radiographic score, synovitis severity, cartilage loss in OA patients (WORMS score), WOMAC and HSS score.

Materials and methods

Subjects

Eighty-nine patients were randomly selected from the out-patient orthopaedic clinic of our

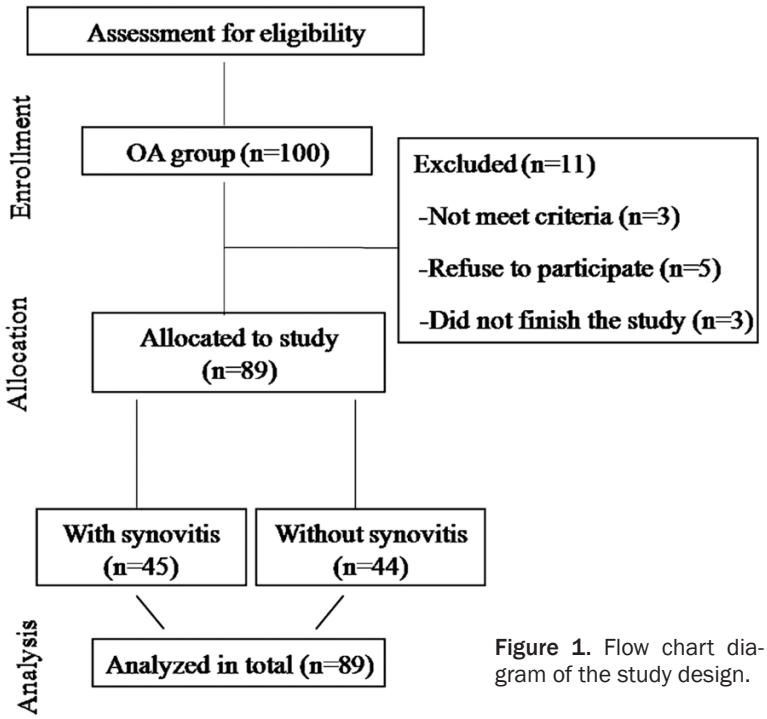

Figure 1. Flow chart diagram of the study design.

hospital from Aug 2011. All patients have primary OA of the knee with intermittent or constant pain. The inclusion criteria were: 1). Age older than 40 years, 2). Primary knee OA diagnosed according to the clinical and radiological criteria of the American College of Rheumatology (ACR) [11], and 3). Disease severity grade of ≥2 based on the Kellgrene/Lawrence radiographic system [12].

Exclusion criteria were: 1). Presence of other rheumatic diseases, 2). Traumatic knee lesions, 3). Other intra-articular or systemic corticosteroid use within the 3 months before enrollment, 4). Visco supplement injection, arthroscopy, and aspiration or lavage of the studied knee within 6 months.

Synovitis is a common complication of Osteoarthris. Synovitis is the inflammation of a synovial membrane, usually painful, particularly on motion, and characterized by swelling, due to effusion in a synovial sac. The signs of synovitis were first examined upon physical examination by the presence of knee warmth and/or effusion, and then confirmed by imaging studies. This study was approved by the hospital ethics committee, and written informed consent was obtained from all participants.

Demographic data

Age and gender were documented. Height was measured to the nearest 0.1 cm using a stadiometer. Weight was measured to the nearest 0.1 kg using a single pair of electronic scales. Body mass index [BMI; weight (kg)/height^2 (m^2)] was also calculated. Self-report of smoking status and diseases including asthma, cardiovascular disease, and diabetes were recorded.

WISP-1 protein concentration measurement

Synovial fluid was withdrawn from consented patients following the induction of anesthesia and before other procedure, such as viscosupplement injection, arthroscopy, and aspiration or lavage of the affected knee.

Synovial fluid was isolated in a serum separator tube and samples were allowed to clot for 30 minutes before centrifugation for 10 minutes at approximately 3000× g. Serum was removed and studied immediately or aliquoted and stored samples at -80°C. WISP-1 concentration was measured by human WISP-1 (Wnt-induced secreted protein1; also CNN_4) ELISA kit (Rapidbio, USA) following the manufacturer's instruction.

Knee X-ray and knee pain assessment

The degree of arthritis in the affected knee was evaluated with a weight-bearing posterior-anterior (PA) radiograph by an orthopedic surgeon, using the Kellgren-Lawrence (KL) classification (**Table 1**) [12]: grade 0, no radiological changes; 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.

Table 1. Synovial fluid WISP-1 level in OA patients

Characteristic categorical variables	Total	Synovitis	Non-synovitis	*P* value
Numbers of patients	89	45	44	
Age (years)	58.1±9.8	57.3±11.9	59.4±10.8	0.876
Female (%)	73.0	66.7	79.5	
BMI (kg/m^2)	27.5 (4.3)	27.3 (5.8)	27.6 (4.9)	0.256
K/L grading				
Grade 2	32	15	7	
Grade 3	40	19	21	
Grade 4	17	3	14	
WOMAC score	28.8±10.3	27.3±8.6	29.5±11.5	0.059
Synovitis severity	5.3	7.3 (5-12)	1.2 (0-4)	<0.001
WORMS score	43.3±13.4	45.7±7.6	41.5±9.7	0.347

Data are expressed as mean plus/minus standard deviation (SD). *P* values are for differences between groups with and without synovitis.

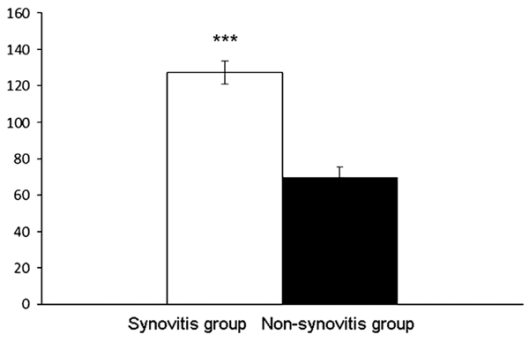

Figure 2. Concentration of WISP-1 in synovial fluid in patient with or without synovitis. Data are representative of experiments repeated at least three times. ***: P<0.001.

Knee pain under various situations (on flat surface, going up/down stairs, at night, sitting/lying and standing upright) was assessed by a self-administered questionnaire using the Western Ontario McMaster Osteoarthritis Index (WOMAC) with a 10-point scale from 0 (no pain, stiffness or function problems) to 9 (most severe pain, stiffness or severe function problems) [13]. Each component of joint pain was summed to create a total pain (0-45) score. Prevailing knee pain was defined as a total score of ≥1.

Synovitis severity assessment using MRI

A non-invasive method was used to assess the severity of synovitis [14]. Imaging was performed without contrast agent, using a 1.5 T and a knee coil. To examine the synovial membrane, two axial sequences were included in MRI: a T2-weighted (synovial fluid) and a gradient echo (GRE) (synovial membrane). Synovial membrane thickness was measured on four regions of interest (ROI): medial and lateral recesses, and medial and lateral suprapatellar bursa, with each graded/scored from 0 to 3, for a maximum of 12.

Knee cartilage volume measurements

The loss of cartilage was assessed by whole-organ MRI scoring (WORMS) [15]. MRI images of the knee were acquired with a 1.5 Tesla scanner using a circumferential knee coil. Cartilage signal and morphology were scored in each of the 14 articular-surface regions(excluding region S) using the fat-suppressed T2-weighted FSE images and the FS-3D SPGR images with an eight-point scale: 0=normal thickness and signal; 1=normal thickness but increased signal on T2-weighted images; 2.0=partial-thickness with focal defect <1 cm in greatest width; 2.5=full-thickness with focal defect <1 cm in greatest width; 3=multiple areas of partial-thickness (Grade 2.0) with defects intermixed with areas of normal thickness, or a Grade 2.0 with defect wider than 1 cm but <75% of the region; 4=diffuse (≥75% of the region) partial-thickness loss; 5=multiple areas of full-thickness loss (grade 2.5) or a grade 2.5 lesion wider than 1 cm but <75% of the region; 6=diffuse (≥75% of the region) full-thickness loss. The maximum cartilage scores for the entire knee were 84.

Statistical analysis

Statistical analysis was performed using the statistical package for social sciences (SPSS) software, version 16.0 for Windows. WISP-1 protein concentration between patients with and without synovitis was compared by chi-square tests and unpaired Student's *t* test where appropriate.

Pearson's correlation coefficient (r) was used to determine correlation among concentrations of

Table 2. Synovial fluid WISP-1 concentration in osteoarthritis patients

	Total	KL Grade 2	KL grade 3	KL grade 4	r value	*P* value
Numbers	89	32	40	17		
WISP-1 (pg/ml)	100.3±48.0	106.4±60.9	94.5±23.0	95.4±37.8	0.004	0.981

Data were expressed as mean plus/minus standard error of the mean (SEM). *P* value is for difference between Kellgren and Lawrence (KL) subgroups.

WISP-1 in synovial fluid with demographic data, knee x ray, WOMAC score, synovitis severity, WORMS score. Data were expressed as mean ± standard error of the mean (SEM). *P* values <0.05 were considered statistically significant.

Results

The study design of this project was shown as in **Figure 1**. Eighty-nine synovial fluid samples from knees of OA patients were used for WISP-1 concentration measurement. The patients were divided into two groups: with synovitis or without synovitis. Characteristics of the study population are shown in **Table 1**.

There was no statistically significant difference in age, BMI, WOMAC score, synovitis severity, and WORMS score between groups with and without synovitis. As shown in **Figure 2**, OA patients with synovitis had higher synovial fluid WISP-1 concentrations compared with that of non-synovitis group (127.2±6.4 vs. 69.9±5.4 pg/ml, P<0.001).

According to Kellgrene/Lawrence radiographic system classification, OA patients were classified into three groups. 32 patients were KL grade 2, 40 were KL grade 3 and 17 were KL grade 4. As shown in **Table 2**, radiographic severity of the osteoarthritis was not correlated with WISP-1 levels in synovial fluid.

Discussion

WISP-1 is a member of the CCN family of secreted, extracellular matrix (ECM)-associated signaling proteins (CCN intercellular signaling protein). WISP-1 has been shown to be able to promote mesenchymal cell proliferation and osteoblastic differentiation, as well as repress chondrocyte differentiation [7, 8]. WISP-1 binds to bone morphogenetic protein 2 (BMP2) and enhances BMP2's function in osteogenesis [16]. WISP-1 expression was significantly increased in the synovium and cartilage of mice with OA, but not with arthritis. WISP-1 expression was also shown to be elevated in humans with OA. WISP-1 regulates chondrocyte and macrophage MMP and aggrecanase expression and is capable of inducing articular cartilage damage in models of OA [10]. WISP-1 produced either in synovium or chondrocytes after stimulation by canonical Wnt could inhibit Smad2 and drive the chondrocyte towards hypertrophy and terminal differentiation [17]. WISP-1 was also shown to be able to inhibit the differentiation of precursor cells into chondrocytes and may also affect chondrocyte phenotype [7].

The expression of WISP-1 was shown to be increased in the synovium and cartilage of articular joint tissue in mice with experimental OA and human with OA [10]. In a whole-genome oligonucleotide array study of the RNA extracted from damaged and intact cartilage areas within the same joint of the knee in patients with OA, WISP-1 was found to be one of the six genes upregulated [18]. In the current study, we further examined the expression of WISP-1 in synovial fluid in human with OA. Our result indicated that the expression of WISP-1 was increased when synovitis was present in OA patients. This confirms that synovium is involved in the pathogenesis of OA. The WISP-1 in synovial fluid was mainly from the synovium. However, the synovial levels of WISP-1 in OA patient were not correlated with knee radiographic score (K/L degree), cartilage loss in OA patients (WORMS score), WOMAC, or HSS score. This means that WISP-1 participated in cartilage damage process during synovitis, but not directly correlated to the damage result. However, the synovial fluid WISP-1 level in knees was significantly higher in osteoarthritis patients with synovitis than in those without synovitis which indicates that the synovial fluid WISP-1 level is correlated with synovitis in patient with osteoarthritis and may be used as a clinical indicator to facilitate the diagnosis, evaluation and treatment of synovitis in patient with osteoarthritis.

There are many studies about the target point in OA pathogenesis, the cytokine IL-1 has been studies extensively, however, anti-IL-1 treatment has not been proved to be effective [19]. WISP-1 is a downstream factor in the Wnt pathway. Identifying downstream Wnt signaling effector molecules, such as WISP-1 will enable us to find more specific therapeutic approaches which will be safer and more efficient for diseases such as OA. For example, the antibody against WISP-1 will target the Wnt pathway in the synovium, since it cannot pass the cartilage matrix and not able to affect the pathway inside cartilage cell, so this could be an ideal treatment method [20]. So the WISP-1 may be a promising alternative therapeutic target in the future. Furthermore, the WISP-1 may work as an indicator for synovitis severity and therapeutic effect.

Our current study can be improved in the following ways. First, there is no comparison with healthy people in our study. An obvious question is, is WISP-1 elevated only among patients with OA or also elevated in healthy people? Second, all cases in our study with synovitis are OA patients, is WISP-1 increased in synovitis of non-OA patients? Third, is the expression of WISP-1 in blood also elevated? Answers to these questions will be part of our future studies.

In summary, we explored the relationship of WISP-1 expression in the synovial fluid with clinical manifestation and found that the synovial fluid WISP-1 level in knees of OA patients was significantly higher in group with synovitis than in group without synovitis.

Conclusion

WISP-1 level in synovial fluid is higher in OA patients with synovitis, indicating that the synovial fluid WISP-1 level is correlated with synovitis in patient with osteoarthritis. Our study is the first study exploring the relationship of WISP-1 level with Synovitis severity and cartilage loss in osteoarthritis patient.

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Disclosure of conflict of interest

None.

Address correspondence to: Xin Qi, Department of Bone and Joint Surgery, The First Hospital of Jilin University, 71 Xinmin Street, Changchun 130021, Jilin Province, China. Tel: 86-0431-81875599; E-mail: dr.qixin@hotmail.com

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