Association between the Severity of Knee Osteoarthritis and Serum Cartilage Biomarker Levels

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Background: *Osteoarthritis (OA) is the most common form of arthritis. However, there has been no cost-effective tool for the investigation of the severity and progression of the disease because using OA standard diagnostic methods causes cartilage damage.*
Objective: *To evaluate the relationship between serum chondroitinsulphate WF6 (CS- WF6) and hyaluronate (HA) and the severity of knee OA according to Kellgren-Lawrence (K/L) grades of radiographic severity and minimal joint space width (JSW).*
Material and Method: *One-hundred and twenty-six patients with OA (knee) according to K/L grades were classified into four groups. The JSW of the tibiofemoral joint were measured from standing PA radiographs. Serum CS-WF6 and HA were analyzed by the ELISA based technique. One-way analysis of variance, Bonferroni's method and Kendall's tau coefficient relation test were performed to evaluate the association of K/L grades and JSW with levels of CS-WF6 and HA, respectively.*
Results: *Serum CS-WF6 levels in grade 4 were significantly increased when compared with the other grades ($p<0.05$). The serum HA level did not show any significant difference among the grades of severity. The serum CS-WF6 level showed a significant negative correlation with the JSW and its levels rose rapidly to the level beyond 300 ng/ml. There was no correlation found between the levels of serum HA and JSW.*
Conclusion: *WF6 levels may be useful in identifying patients at risk of rapid progression reflected by a point of an abruptly high WF6 level. The determination of WF6 in the serum showed increasing levels in more severe grades, so it could be useful in monitoring the effectiveness of treatment. There were some limitations because of broad distribution and overlap with the normal range. Thus, it may not be suitable as a diagnostic tool.*

Keywords: *Biomarkers, Cartilage, Osteoarthritis, Chondroitin sulphate WF6*

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Osteoarthritis (OA) is the most common form of arthritis of the joints often causing permanent disability[1]. From the economic point of view, the cost of treatment of OA is 1% of the Gross Domestic Product[2]. OA is considered a slowly progressive disease altering the bone, cartilage and synovial tissue metabolism due to an imbalance between synthetic and catabolic functions; however, the catabolic function is predominant. A plain radiograph is the gold standard to estimate the extent of the disease but its wide margin of error in precision and poor sensitivity lead to late detection of joint degradation and poor treatment monitoring. Magnetic Resonance Imaging (MRI) gives a more accurate and sensitive image, but it is used less often due to cost and limited availability. The most established method for assessing joint damage in OA from the plain radiograph is measurement of the minimal joint space width (JSW)[3,4], which currently is the gold standard for evaluating the use of disease modifying OA drugs (DMOADS); whereas, the use of Kellgren-Lawrence grades (K/L grades) of radiographic severity[5] is accepted worldwide as the most accurate severity grading system. However, when radiological diagnosis is made, articular damage has occurred and it has a high variation in inter- and intra-observer reliability even between experienced orthopedic surgeons[6]. Chondroitin and glucosamine are considered as DMOADS for their anti-catabolic and anti-inflammatory effects. They are especially noted for their cartilage preservation effect in animal studies[7], so these drugs should be prescribed in early OA. As OA is often detected late using the plain

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radiograph, is it suitable for evaluating the uses of DMOADS?

In OA, the articular cartilage matrix molecules are released into the blood and urine and then can be detected. In previous studies, several biomarkers, described in OA, show change more rapidly than radiographic assessment and clinical presentation[2,8-10]. Therefore, they may be useful for identifying patients at risk of progression, early detection, monitoring therapeutic responses and evaluating the use of DMOADS.

Chondroitin sulfate binds with molecules of proteoglycan, a molecule of hyaluronate (HA) and collagen to form cartilage tissue. When proteoglycan, which is synthesized to repair the damaged cartilage, is destroyed in the metabolism process, it can be detected by using an antibody, which is specific to newly-synthesized chondroitin sulfate (neopeptide). A newly developed WF6 monoclonal antibody, which is specific to chondroitin-6-sulfate and chondroitin 2; 6-sulfate is called chondroitin WF6 epitope (CS-WF6). CS-WF6 is found at higher levels in patients who are older than fifty-five years of age, an age group which has a significantly high prevalence of OA when compared to other age groups[11]. Thus, this biological marker may represent the change of the joint in OA.

HA, an important component of the cartilage component of cartilage and synovium, is widely studied and a correlation between its level, severity of inflammation and number of joints involved in rheumatoid arthritis is found[12]. In OA, HA is consistently associated with radiographic progression in both hip and knee and displays more rapid change in its levels than changes seen in the radiographs[13,14].

This paper describes the investigation into the value of these two biological markers for predicting the severity of knee osteoarthritis, when compared to assessment by plain radiograph using minimal joint space width (JSW) and K/L grades.

Material and Method

Patients

This cross-sectional study consisted of 126 patients (102 women) with primary knee OA diagnosed using the American College of Rheumatology criteria. The exclusion criteria were history of active rheumatic diseases and previous musculoskeletal injury or surgery that could be responsible for secondary osteoarthritis. Patients had no known cancer, liver or kidney disease. Patients who had had intra- articular steroid injections within 30 days or more than three injections in the previous six months and HA in the previous 3 months were also excluded. No patients had ever used oral glucosamine or chondroitin. Patients who had carried out vigorous activities in the two days before serum collection were also excluded. Most of the patients periodically used analgesics and non-steroidal anti-inflammatory drugs but not routinely. Age, gender, height and weight were recorded, and then the body mass index was calculated individually. All patients were informed about the procedures in detail and their consent was obtained under the approval of the Research Ethics committee, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

Radiographic assessment

All patients had a standardized standing posterior-anterior radiograph taken and were classified into four grades according to K/L grades as follows: grade 0, no radiographic features of OA present; grade 1 doubtful joint space narrowing and possible osteophytic lipping; grade 3, multiple osteophytes, definite joint space narrowing, sclerosis, possible bony deformity; grade 4, large osteophytes, marked joint space narrowing and definitely bony deformity[5]. The inter-bone distance at the narrowest point (JSW) was then measured by three orthopedists using a computer program (CMU-PAC system).

Biochemical marker measurements

A fasting morning blood sample was taken at 9.00 am from the most visible vein around the elbow. Samples were centrifuged at 6,500 g for ten minutes for serum preparation within 1 hour after the collection and stored at -20°c until assayed.

Serum hyaluronate

Serum HA was measured by the ELISA-based assay for HA using biotinylated HA-binding proteins. Serum or standard HA (Healon® Advanced Medical Optics Uppsala Ab, Uppsala, Sweden) at 19-10,000 ng/mL in 6% w/v BSA in phosphate buffered saline pH 7.4 was added to 1.5 mL plastic tubes containing biotinylated HA-binding proteins (1:200 in 0.05 M Tris-hydrochloride buffer, pH 8.6). After the tubes were incubated at room temperature for 1 hour, the samples were added to the microplate wells, which were pre-coated with 100 mg/mL umbilical cord HA and blocked with 1% BSA (150 µL/well). The microplate was then incubated at room temperature for 1 hour. After incubation, the wells were washed and 100 µL of peroxidase-conjugated antibiotin antibody

was added to each well. The microplate was then incubated at room temperature for another hour. Ortho-phenylenediamine substrate was used to detect the conjugated antibody.

Serum WF6

Serum WF6 was measured using competitive ELISA with a mAb-WF6 standard (i.e. shark aggrecan at a concentration of 19-10,000 ng/mL). The five-fold serum was diluted in 6% w/v bovine serum albumin (BSA) in Tris-EDTA buffer (0.1 M Tris-hydrochloride, pH 7.4, containing 0.15 M sodium chloride, 0.1% Tween 20 and 0.1% BSA)[15,16], and an equal volume of WF6 (cell culture supernatant 1:200 dilution) was then added into a 1.5 mL plastic tube. These tubes were incubated at 37°C for 1 hour before the samples were added to the microplate wells, which were pre-coated with the A1 fraction of the shark aggrecan. The plates were incubated at 37°C for 1 hour, after which the wells were washed and 100 µL of ortho-phenylenediamine was added. The reaction was stopped after 10 minutes using 50 µL of 4 M sulfuric acid per well, and absorbance was determined at 492/690 nm using a microplate reader.

Data analysis

Demographic characteristic data of K/L grades and the relation of HA/ WF6 and K/L grades were analyzed using one-way analysis of variance and Bonferroni's method. Kendall's tau correlation coefficient was used to analyze the relation of HA/WF6 and JSW. All statistical analyses were performed using STATA software (version 10.0). The results were considered statistically significant at p-value <0.05.

Results

One hundred and twenty-six patients (102 women) with primary knee OA were recruited into the present study. The mean age in K/L grade 1 was significantly lower than other grades and JSW showed a significant difference between K/L grades. Shared similar demographic characteristics including sex and BMI were shown in each group according to K/L grades (Table 1).

Serum HA were not significantly different between K/L grades (Table 2, Fig. 1A) and there was no correlation observed between HA and JSW (Fig. 1B). On the other hand, WF6 was significantly higher when grade 4 was compared with the others (Table 2, Fig. 2A). WF6 showed a negative correlation compared to JSW and its values rose rapidly after 300 ng/ml (Fig. 2B).

Discussion

The standard diagnosis criteria of OA is based on clinical and plain radiographic findings, but when clinical and radiographic findings are established the articular damage has already occurred, so biochemical markers are now being identified and have proved useful in determining diagnosis, prognosis

Table 1. Baseline demographic and characteristics of patients

Characteristic	Total n = 126	K/L grade 1 n = 32	K/L grade 2 n = 30	K/L grade 3 n = 34	K/L grade 4 n = 30
Age (years)	64.25±8.80	57.81±4.30*	64.33±7.67	67.20±7.59	67.57±11.12
Sex (male/female)	24/102	2/30	2/25	9/25	4/26
BMI (kg/m^2)	24.34±3.99	24.02±3.90	24.15±2.95	23.53±2.95	25.76±4.53
JSW (mm)*	2.70±1.50	4.50±0.44	3.31±0.45	2.22±0.82	0.72±0.36

Data presented as mean ± SD or n

K/L = Kellgren and Lawrence grading system; BMI = body mass index; JSW = minimal joint space width

* Data are statistically significantly different between groups (p<0.05; one-way analysis of variance and Bonferroni's method)

Table 2. Comparison of serum biomarkers according to Kellgren-Lawrence grades of radiographic severity

Serum biomarker	Total n = 126	K/L grade 1 n = 32	K/L grade 2 n = 30	K/L grade 3 n = 34	K/L grade 4 n = 30
HA (ng/ml)	241.22±351.16	222.90±384.66	160.93±89.53	219.45±138.15	375.92±562.78
WF6 (ng/ml)	694.63±976.97	180.40±302.76	271.80±259.04	660.23±687.56	1,725.98±1,353.77*

K/L = Kellgren and Lawrence grading system; HA = hyaluronate

* Data are statistically significantly different between groups (p<0.05; one-way analysis of variance and Bonferroni's method)

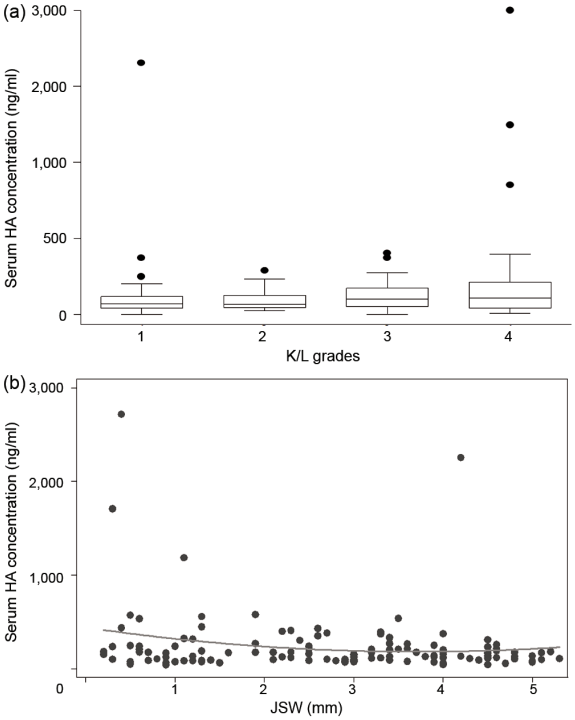

Fig. 1 Comparison of serum HA and severity of OA. (a) Simple box plot represented correlation between Serum HA and K/L grades. (b) Correlation between serum HA and JSW.

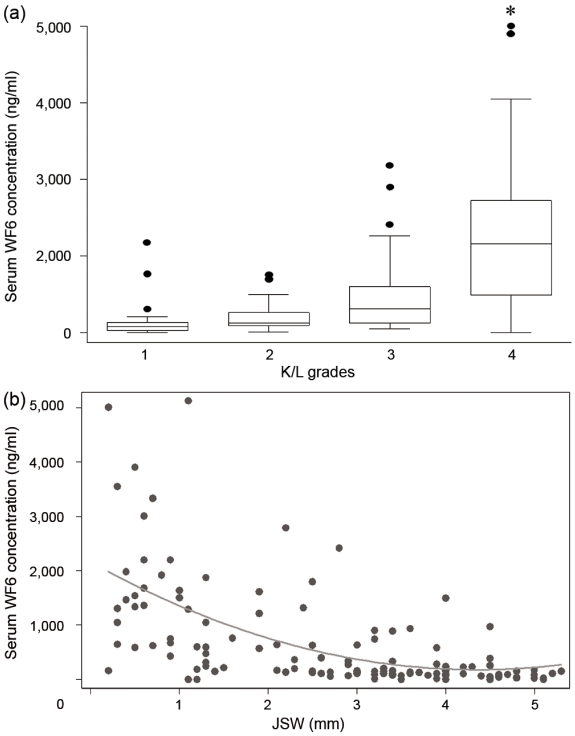

Fig. 2 Comparison of serum WF6 and severity of OA. (a) Simple box plot represented correlation between Serum HA and K/L grades. (b) Correlation between serum WF6 and JSW.
* Data are statistically significant between group difference.

efficacy of the drugs and interventions in OA before articular damage is so extreme. Glucosamine has anti-catabolic and anti-inflammatory effects by inhibiting prostaglandins E_2, nitric oxide and matrix metalloproteinases in vitro[17]. Moreover, inhibition of osteoclasts and the induction of osteoblast differentiation effects of glucosamine are shown resulting in the limitation of bone resorption and enhancing bone deposition in mice cells[18]. In human chondrocyte and synovial cells, glucosamine has been shown to induce HA synthesis, an important component of cartilage and synovium[19]. A cartilage preservation effect is shown in animal models in the anterior cruciate ligament transection using glucosamine hydrochloride[7]. Chondroitin's anti-apoptotic effects on the chondrocytes and inhibition of NF-8B in the mouse model may be an important mechanism in the anti-inflammatory and chondroprotective effect in OA[20,21]. These drugs may not have benefit in terms of chondroprotection when cartilage eburnation occurs in advanced OA but using standard criteria, articular damage occurs so several biological markers are identified which show rising of their levels before radiological features of OA are established. Serum cartilage oligomeric protein (sCOMP), a non-collagenous protein, is widely studied and its association is observed with hip related symptoms and clinical signs of hip OA before radiographic findings[22]. For detection of disease progression, increased C-terminal cross-linked telopeptide type II collagen (CTX-II) levels and HA are associated with rapid progression of both hip and knee OA[23-25].

HA, a high molecular-weight-proteoglycan, an important component of articular cartilage, has been studied as a biomarker for OA reflecting synovial inflammation and cartilage degradation. In the study reported by Pothacharoen, HA levels detected by newly developed assays have shown a few high serum levels in OA patients and its value has no difference from that of the normal subjects[12]. In the present study, the authors also found no correlation between serum HA and severity of OA determined by K/L grades and JSW.

WF6 levels were increased in the more severe K/L grades, but a significant correlation was found

only when grade 4 was compared with the others, whereas the levels of WF6 show a significant negative correlation with the JSW, which indirectly represents the cartilage volume. WF6 is one of the catabolic cartilage markers in OA when its levels rise rapidly at 300 ng/ml. At this level, the WF6 value may represent the starting point of severe cartilage catabolism, which may be used as a cut point for determining the use of DMOADS because beyond this point, the anti-catabolic effect of DMOADS, may not inhibit the process of the disease. In aspects of diagnosis potential, WF6 have been increased in many OA patients and its average is significantly higher than that seen in healthy subjects[12]. However, like most biomarkers, WF6 had significantly increased levels in some OA patients but some had normal levels[26,27]. Thus, threshold values could not be determined.

In summary, WF6 levels may be useful in identifying patients at risk of rapid progression reflected by an abrupt high level. The determination of WF6 in serum shows an increase in more severe grades of OA, so it could be useful in monitoring the effectiveness of treatment, although it has some limitations because of broad distribution and overlap with the normal range. Therefore, it may not be suitable as a diagnostic tool.

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What is already known on this topic?

WF6 was identified by Faculty of Medicine, Chiang Mai University. In the past, this biomarker showed statistically significant in people who older than 55 years, an age group which has a significantly high prevalence of OA and WF6 average levels in OA patients were significantly above in healthy control. However, the difference among severity of OA has never been determined.

What this study adds?

This study showed correlation between the severity of OA and WF6 levels especially determined by the JSW which indirectly represents the cartilage volume but WF6 levels have broad distribution and some overlapping with the normal range so WF6 may be useful in monitoring the effectiveness of treatment but may not suitable for diagnostic tool.

Potential conflicts of interest

None.

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ความสัมพันธ์ระหว่างความรุนแรงของข้อเข่าเสื่อมและสารบ่งชี้ทางชีวภาพในเลือด

อภิรักษ์ แสงสิน, ดำเนินสันต์ พฤกษากร, ศิริชัย ลือวิฑูรเวชกิจ, พีรพรรณ โปธาเจริญ, ปรัชญา คงทวีเลิศ

ภูมิหลัง: *โรคข้อเสื่อมเป็นโรคข้อที่พบบ่อยที่สุดซึ่งทำให้เกิดภาวะทุพพลภาพ ปัจจุบันยังไม่มีเครื่องมือตรวจวินิจฉัยที่มีประสิทธิภาพ เพราะการใช้เกณฑ์มาตรฐานการวินิจฉัยในปัจจุบันกว่าจะทำการวินิจฉัยก็เกิดความเสียหายต่อกระดูกอ่อนผิวข้อแล้ว*

วัตถุประสงค์: *เพื่อหาความสัมพันธ์ระหว่างสารบ่งชี้ทางชีวภาพคอนดรอยตินซัลเฟต WF6 (CS-WF6) และไฮยาลูโลแนน (HA) ในกระแสเลือดผู้ป่วยโรคข้อเสื่อมกับความรุนแรงของโรคข้อเข่าเสื่อม*

วัสดุและวิธีการ: *ผู้ป่วยข้อเข่าเสื่อม 126 ราย ถูกแบ่งระดับความรุนแรงของโรคเป็นสี่กลุ่มตาม Kellgren-Lawrence (K/L grades) และความกว้างของข้อเข่าที่แคบที่สุดถูกวัดจากภาพถ่ายรังสีในท่ายืน สารบ่งชี้ทางชีวภาพคอนดรอยตินซัลเฟต WF6 (CS-WF6) และไฮยาลูโลแนน (HA) จากตัวอย่างเลือด ถูกวัดด้วยวิธี ELISA ความรุนแรงของโรคข้อเข่าเสื่อมและสารบ่งชี้ทางชีวภาพ ถูกนำมาหาความสัมพันธ์ทางสถิติ*

ผลการศึกษา: *เมื่อนำระดับของคอนดรอยตินซัลเฟต WF6 (CS-WF6) ของผู้ป่วยข้อเข่าเสื่อมระดับที่ 4 ตาม K/L grades เปรียบเทียบกับระดับอื่น ๆ พบว่ามีค่าเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ และพบมีความสัมพันธ์ในเชิงลบอย่างมีนัยสำคัญทางสถิติ กับความกว้างของข้อเข่าที่แคบที่สุด ส่วนระดับไฮยาลูโลแนน (HA) นั้นไม่พบความสัมพันธ์กับความรุนแรงของโรค*

สรุป: *สารบ่งชี้ทางชีวภาพคอนดรอยตินซัลเฟต WF6 (CS-WF6) ในเลือดอาจมีประโยชน์ในการบ่งบอกผู้ป่วยที่มีความเสี่ยง ในการเกิดการลุกลามของโรคอย่างรวดเร็ว และพบว่าคอนดรอยตินซัลเฟต WF6 (CS-WF6) นี้มีค่าสูงขึ้นตามความรุนแรงของ โรคข้อเข่าเสื่อมจึงอาจจะใช้ในการติดตามการรักษา*