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Attenuate Synovial Fluid Uncarboxylated Matrix Gla-Protein (ucMGP) Concentrations Are Linked with Radiographic Progression in Knee Osteoarthritis

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. Decreased serum and synovial fluid (SF) uncarboxylated matrix Gla-protein (ucMGP) levels have been detected in OA patients. This study was carried out to investigate the association of serum and synovial fluid (SF) ucMGP levels with radiographic severity in knee OA patients.

Objectives. This study was carried out to investigate the association of serum and synovial fluid (SF) ucMGP levels with radiographic severity in knee OA patients.

Material and Methods. One hundred sewenty eight knee OA patients and 160 healthy controls among the outpatients in our hospital were enrolled in the study. Anteroposterior knee X-ray plains were taken to evaluate the radiographic severity of the affected knee. The radiographic assessment of knee OA was performed according to the Kellgren-Lawrence criteria. ucMGP levels in the serum and synovial fluid were examined utilizing enzyme-linked immunosorbent assay method (ELISA).

Results. The mean serum ucMGP levels of the knee OA patients was significantly lower than that of healthy controls [2603 (1919~3222) nmol/LVS 2811 (1926~3619) nmol/L, $p = 0.045$]. Synovial fluid ($r = –0.479$, $p = 0.05$) was negatively correlated with radiographic severity.

Conclusions. In conclusion, this study revealed a significant decrease in the SF ucMGP levels of OA patients and illustrated a negative correlation of SF ucMGP levels with the extent of radiographic severity in patients with knee OA. The present findings indicate that ucMGP in SF might serve as a novel biomarker for assessing OA progression (**Adv Clin Exp Med 2015, 24, 6, 1013–1017**).

Key words: osteoarthritis, radiographic severity, matrix Gla-protein.

Osteoarthritis (OA) is the most prevalent disease of articular joints characterized by joint space narrowing on X-ray, progressive cartilage degradation, secondary synovial inflammation and osteophyte formation [1]. The knee is the most commonly affected joint, and knee osteoarthritis has developed into a major concern among elderly individuals [2]. Since OA progression is a continuing pathological process, biomarkers for accurately and sensitively predicting the increased risk of OA progression have been widely used in recent years [3–4]. The osteophyte associated with osteoarthritis (OA) is a bony outgrowth formed at the margins of the affected joint through endochondral ossification-like processes. However, the mechanism of osteophyte formation and its pathogenesis are unclear. Matrix Gla protein (MGP), a mineralization inhibitor, present manly in bone, cartilage, and vascular smooth muscle, have been associated clinically with conditions of abnormal calcification [5]. Recent studies showed that MGP could be mainly quantified by its inactive form of uncarboxylated matrix Gla-protein in synovial fluid or serum [6, 7]. Mineralization of the extracellular matrix is a key process required for normal development and function of skeletal tissues [7]. Studies have shown that constant expression of MGP blocks endochondral and intramembranous ossification in the limb [8]. In addition, MGP could inhibit soft connective tissues

calcification [8]. It has been reported that in OA patients, levels of ucMGP were significantly lower than controls and other inflammatory diseases [7]. Based on the data, we hypothesized that ucMGP in serum and synovial fluid may play crucial roles in the pathophysiology of OA progression. However, there have been no studies illustrating the relationship between ucMGP levels and disease severity. Therefore, the present study was aimed to illustrate the correlation between serum and SF levels of ucMGP and radiographic severity in knee OA patients.

Material and Methods

Study Subjects

One hundred and seventy-eight patients with OA undergoing diagnostic or therapeutic knee hyaluronic acid injection in General Hospital of People's Liberation Army from December 2012 and March 2014 were enrolled in the study. Both clinical symptomatic and radiographic criteria diagnosis of OA were established referred to the American College of Rheumatology [9]. One hundred and sixty sex- and age-matched subjects with normal knee radiographs were recruited as controls. Patients were excluded if they have posttraumatic arthritis, previous or current joint infection, deposition arthritis (e.g. gout or CPPD), systemic inflammatory or autoimmune disorders. Radiographic severity was assessed on the basis of the Kellgren and Lawrence (KL) grading system [10]. KL grade ≥ 2 in at least one knee only be selected in the current study. Participants who had no signs of radiographic changes of the knee were regarded as healthy controls. The higher grade of the two knees was used for analysis. None of the patients received any treatment for knee OA before the study.

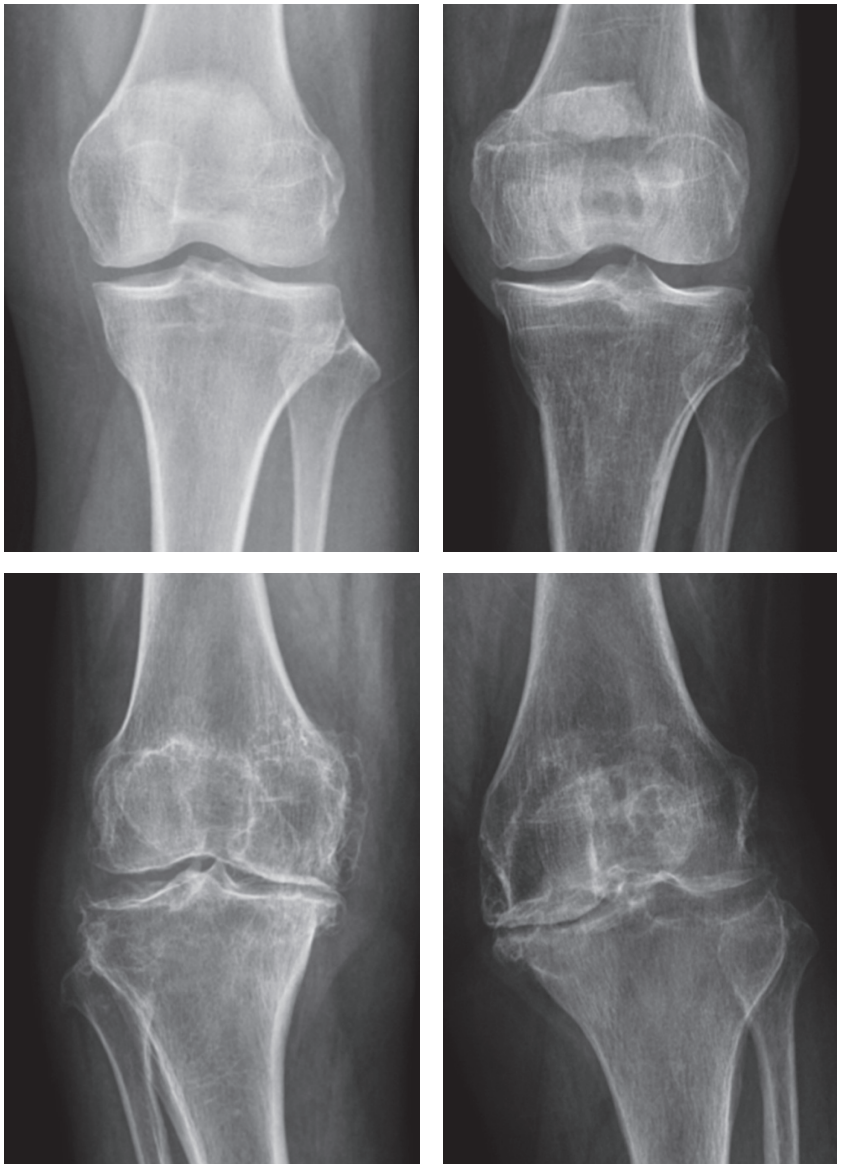

Fig. 1. Osteoarthritis Kellgren-Lawrence grading system
A) K-L grading I;
B) K-L grading II;
C) K-L grading III;
D) K-L grading IV

The ethics committee of Orthopedic Hospital of General Hospital of PLA agreed the research protocol. All the subjects were informed of the whole process and signed the consent form.

Laboratory Methods

One to two milliliters of synovial fluid was obtained from the affected knee before hyaluronic acid injection for the first time. Then, all the specimens were handled to centrifugation to remove joint debris and cells, and placed to cold storage immediately at −80°C until ELISA performing. Blood samples were obtained from all the subjects in the fasting state, then centrifuged and stored at −80°C until examination after clotting. Levels of ucMGP in SF and serum were conducted utilizing commercial ELISA according to the manufacturer's operation steps. In brief, anti-ucMGP (IBL, Minneapolis, America) was added to a micro-well plate by polyclonal rabbitanti-mouse IgG (Santa Cruz Biotech, Texas, America). Serum samples and standards were mixed with b-ucMGP35––54(a tracer biotinylated peptide with residues 35–54 in human MGP), and then transferred to the already washed micro-well plate and incubated overnight. Then streptavidine-peroxidase incubation and tetramethylbenzidine staining were conducted after washing. H_2SO_4 was added to cease the reaction and the plate was read at 450 nm.

Statistical Analysis

SPSS statistical software (SPSS for Windows 15.0, Inc., Chicago, IL, USA) was used for all statistical calculations. The data is presented as means ± SD or median (interquartile range). The Kolmogorov-Smirnov test was performed to analyze the data normality. Unpaired *t*-test, Mann--Whitney *U* test, were used to evaluate significance in basic clinical parameters between knee OA patients and healthy controls. ucMGP levels in serum and SF were compared between knee OA patients with different KL grades using Kruskal-Wallis test. Statistical significance of the correlation of ucMGP levels in serum and SF with disease severity was determined using Spearman coefficient.

Results

Baseline Clinical Parameters

The baseline clinical parameters between patients with knee OA and the healthy controls are depicted in Table 1. No significant differences exist in age, sex, and BMI between the two groups. Significant lower levels of serum ucMGP were found in knee OA patients compared with healthy controls [2603 (1919~3222) nmol/LVS 2811 (1926~3619) nmol/L, p = 0.045] (Table 1).

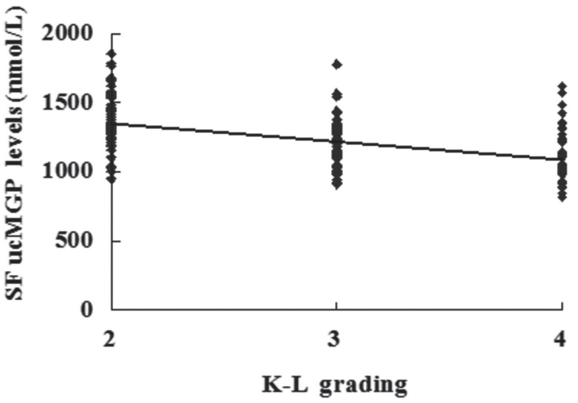

Fig. 2. Relationship between SF ucMGP levels and K-L grading system

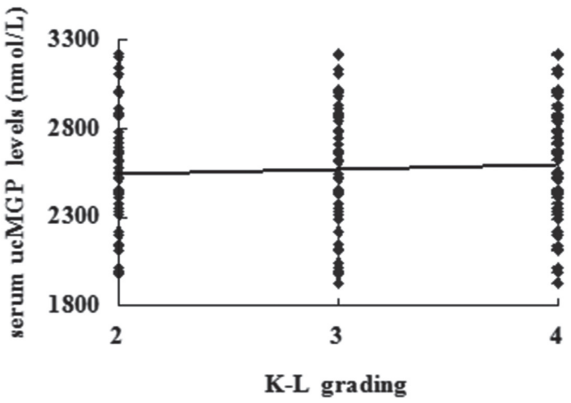

Fig. 3 Relationship between serum ucMGP levels and K-L grading system

Table 2. The ucMGP levels of serum and SF in knee OA patients with different KL grades (nmol/L)

	K-L grading II	K-L grading III	K-L grading IV	P	r
No.	65	63	50		
SF ucMGP levels	1379 (947~1853)	1277 (909~1777)[&]	1192 (814~1633)[&§]	< 0.001	–0.479
Serum ucMGP levels	2591 (1983~3222)	2614 (1929~3201)	2619 (1919~3213)	> 0.05	0.026

All values are expressed as median (interquartile range); [&] – p < 0.001 vs. KL grade II; [§] – p < 0.001 vs. KL grade III.

Table 1. Baseline characteristics

	OA patients (n = 178)	Healthy controls (n = 160)	p-value
Age (y)	62.8 ± 7.4	63.2 ± 8.0	0.357
Sex (f/m)	114/64	94/66	0.218
BMI (kg/m^2)	23.6 ± 2.6	22.9 ± 2.9	0.489
Serum ucMGP levels (nmol/L)	2603 (1919~3222)	2811 (1926~3619)	0.045
SF ucMGP levels (nmol/L)	1230 (814~1853)		

Basic data are expressed as the mean ± SD. Serum/SF ucMGP levels were given as median (interquartile range).

The ucMGP Levels in the Serum and SF in OA Patients

According to the Kellgren and Lawrence (KL) grading system, 65 were regarded as KL grade II, whereas 63 patients were KL grade III, and 50 patients were KL grade IV knee OA. The serum and SF levels of ucMGP were analyzed and compared in relation to radiological KL grading of OA. The serum ucMGP levels from KL grade II were 2591 (1983~3222) nmol/L, those from KL grade III were 2614 (1929~3201) nmol/L, and those from KL grade IV were 2619 (1919~3213) nmol/L (Table 2). The results showed that differences of serum ucMGP levels between KL grade II and III, IV did not reach any significance ($p > 0.05$).

The SF ucMGP levels from KL grade II were 1379(947~1853) nmol/L, those from KL grade III were 1277 (909~1777) nmol/L, and those from KL grade IV were 1192(814~1633) nmol/L (Table 2). The results showed that SF ucMGP levels in KL grade IV were lower higher than those of KL grade II and III ($p < 0.001$). And also SF ucMGP levels in KL grade III were significantly lower than those of KL II ($p < 0.001$) (Table 2).

Association of Clinical Parameters with KL Grades

The ucMGP in SF were both associated with KL grades ($r = –0.479$, $p < 0.001$) by Spearman correlation analysis. Differences of serum ucMGP levels between KL grade II and III, IV did not reach any significance ($p > 0.05$).

Discussion

To the best of our knowledge, this is the first clinical study showing the close relationship between serum and synovial fluid ucMGP levels and radiographic severity in knee OA patients. We have demonstrated for the first time that synovial fluid instead of serum ucMGP concentrations were negatively correlated with radiographic progression of knee OA. Our results indicated that ucMGP may serve as a novel and reliable biomarker for reflecting radiographic severity in knee OA patients.

The most widely used method to identify OA disease progression is radiological assessment through MRI which reflects OA progression by grading degeneration of the joint. However, this method is limited because of its high financial cost and controversial critical standards [11]. In recent years, biomarkers have been proven to provide valuable information for the early disease diagnosis, prognosis, and pharmacological targets in many diseases [12].

Osteophyte formation in OA occurs through a series of highly coordinated biological processes that include MSC proliferation and differentiation, deposition and remodeling of the cartilage matrix, vascular invasion, and bone marrow formation [13]. Osteophyte is also a significant mark in evaluate the radiographic progression in OA [14]. Matrix Gla protein (MGP), a mineralization inhibitor, have been associated clinically with conditions of abnormal calcification [15]. Studies have shown that MGP polymorphism, is associated with radiographic hand osteoarthritis [16] and levels of ucMGP were significantly lower than controls and other inflammatory diseases [7]. In this study, we examined the role of ucMGP in OA progression. Our findings point to a possible role of MGP in the progression of knee OA. There is an even stronger suggestion that the inactive form of MGP-ucMGP levels in SF may serve as a novel biomarker to assess the progression of OA. However, further study should be carried out to identify the findings and more works should be done to illustrate the mechanism.

There are several limitations to be taken into account in this study. First, this is a cross-sectional study performed in a relatively small sample among Chinese people. Therefore, our findings

should be validated by further longitudinal studies in a larger population sample. Second, we did not examine ucMGP levels in SF from healthy controls due to ethical concerns. Last, we did not assess whether the hyaluronic acid injection had an effect on the serum and SF levels of ucMGP in knee OA patients.

In conclusion, this study revealed a significant decrease in the SF ucMGP levels of OA patients and illustrated a negative correlation of SF ucMGP levels with the extent of radiographic severity in patients with knee OA. The findings confirm that ucMGP in the SF may be useful prognostic biomarker for the disease severity of knee OA.

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