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Original Article

The relationship between the serum and synovial fluid osteopontin (OPN), cartilage oligomeric matrix protein (COMP), and the severity of knee osteoarthritis (KOA) assessed by radiology

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Abstract: Objective: To investigate the relationship between the serum and synovial fluid osteopontin (OPN), cartilage oligomeric matrix protein (COMP), and the severity of knee osteoarthritis (KOA) assessed by radiology. Methods: 108 patients with KOA in our hospital were enrolled in a study group (SG). 108 patients in the same period were included in a control group (CG). The patients in the CG needed knee surgery due to a meniscus injury or other trauma. An enzyme linked immunosorbent assay (ELISA) was used to determine the expression levels of OPN and COMP. The Kellgren-Lawrence (KL) system was adopted to grade the development of KOA. The correlation between the OPN and COMP expression levels and the KL grades was analyzed using a Spearman correlation coefficient. The Pearson correlation coefficient was introduced to analyze the correlation between the OPN and COMP expressions. Results: The serum and synovial fluid COMP and OPN expression levels in the SG were higher than those in the CG ($P < 0.05$). The expression trend of COMP and OPN in patients with different K-L grades was as follows: Grade IV expression > grade III expression > grade II expression > grade I expression ($P < 0.05$). Both the serum COMP and OPN expression levels were positively correlated with the K-L grades ($r=0.723$, $P < 0.05$; $r=0.799$, $P < 0.05$). Both the synovial fluid COMP and OPN had a positive correlation with the K-L grades ($r=0.701$, $P < 0.05$; $r=0.784$, $P < 0.05$). There was a positive correlation between the COMP and OPN expression levels in the SG ($r=0.340$, $P < 0.05$; $r=0.612$, $P < 0.05$). Conclusions: COMP and OPN are highly expressed in the serum and synovial fluid of patients with KOA. The expression levels are positively correlated with the disease severity as assessed using radiology, which may be a biological measure to evaluate the conditions of patients with KOA.

Keywords: Knee osteoarthritis, osteopontin, cartilage oligomeric matrix protein, radiology

Introduction

Arthritis is a chronic joint inflammation. It is characterized by a progressive degeneration of articular cartilage and subchondral sclerosis. The knee joint is the most common pathogenic site in osteoarthritis [1, 2]. One study [3] suggested that obesity, smoking, joint injury, and other mechanical or metabolic factors are the risk factors for knee osteoarthritis (KOA). However, the pathogenesis of KOA is not known. The diagnosis mainly depends on imaging. For example, the X-ray Kellgren-Lawrence grading diagnostic criteria are always used for the assessment of the severity of KOA. However, the disease has developed to a very serious stage when obvious lesions are observed under X-rays. But this indicates that the value of X-rays in the early diagnosis of patients is not high [4, 5]. Therefore, one study [6] proposed that a more accurate and sensitive method should be found to evaluate and diagnose KOA. In recent years, some scholars [7] have said that changes in the biomarkers in the body fluid of patients with KOA can reflect the metabolism of articular cartilage and the progression of KOA.

Both cartilage oligomeric matrix protein (COMP) and osteopontin (OPN) are the metabolic mark-

ers of KOA. As a macromolecular substance in the extracellular matrix of articular cartilage, COMP is secreted in the cartilage, blood vessels, and synovium. As an inflammation-related multifunctional protein, OPN is abundantly expressed in bones. Meanwhile, OPN also plays an important role in the differentiation of cartilage into the bone during fracture repair [8, 9]. A previous study [10] showed that the further degradation of articular cartilage may lead to the up-regulation of COMP expression in the pathogenesis of KOA. Another study [11] suggested that the degeneration of articular cartilage promotes the release of OPN into the synovial fluid.

In this study, it was suspected that the serum and synovial fluid COMP and OPN can be tested to determine the conditions of patients with KOA. Therefore, the expression levels of COMP and OPN were tested. The relationship with the X-ray KL grades was analyzed. Thus, a more accurate measure was provided for the evaluation of the conditions of patients with KOA.

Materials and methods

General information

108 patients with KOA in our hospital were categorized as the study group (SG), including 60 males and 48 females. The patients were aged (47.57±5.29) on average. 108 patients who needed joint surgery due to a meniscus injury or some other trauma and who agreed to have synovial fluid extracted in the same period were included in the control group (CG). The inclusion and exclusion criteria were as follows: Patients meeting the diagnostic criteria for osteoarthritis of the knee were included [12]. Patients with severe immune system disease, other malignancies, joint infections, severe liver and kidney dysfunction, cognitive and communication disorders, and/or who did not cooperate with the study were excluded. All the patients or their family members agreed to participate in the study and signed the informed consent form. This study was approved by the Ethics Committee of Weifang Second People's Hospital.

Severity grading of KOA

All the patients in the SG were given an X-ray and graded from I to IV according to the Kellgren-Lawrence (K-L) grading criteria [13].

Sample collection

3 ml of fasting venous blood was collected from each patient. The blood was centrifuged at a speed of 3000 r/min. The serum was separated and stored at -80°C for follow-up testing. All the joint synovial fluid samples were collected during each knee joint surgery. After centrifugation at the speed of 3000 r/min for 10 min, the supernate was separated for the test. The ELISA method (COMP ELISA kit was purchased from Cloud-Clone Corp. Wuhan; OPN ELISA kit was purchased from Jonln Biotechnology Co., Ltd.) was used to test the expression levels of COMP and OPN in the serum and synovial fluid.

Test methods

All test procedures were performed strictly in accordance with the kit instructions. The details were as follows: First, the COMP and OPN antibodies were respectively coated using a 96-well plate. 100 μL of the COMP standard and synovial fluid was injected into each well for the COMP test. Similarly, 100 μL of the OPN standard and synovial fluid was injected into each well for the OPN test. Then, incubation was performed at 37°C. Washing was repeated 7 times after the incubation. 100 μL of a biotin labelled antibody was added to each well. The same volume of saline was added to the blank wells as a control, and then incubated at 4°C for 30 min. After the incubation, washing was continuously performed 9 times. After drying the washing solution, 50 μl of substrate was added to each well. Incubation was continued for 30 min at room temperature in a dark place. Finally, 50 μL of stop solution was added to each well. The absorbance value was tested using ELIASA at 450 mm.

Outcome measures

(1) The expression levels of the serum and synovial fluid COMP and OPN were compared between the SG and the CG. (2) The patients with RA were divided into grades I, II, III, and IV using K-L grading. The expression levels of COMP and OPN in the patients with different K-L grades were compared. (3) The correlation of the serum and synovial fluid COMP and OPN and the K-L grades in SG was analyzed. (4) A correlation analysis of the COMP and OPN expression levels in the serum and synovial fluid in the SG was performed.

Table 1. The general data of the two groups of patients

Factor	Study group n=108	Control group n=108	X^2/t	P
Gender			0.019	0.891
Male	60 (55.56)	61 (56.48)		
Female	48 (44.44)	47 (43.52)		
Age			0.019	0.892
≥ 47	56 (51.85)	57 (52.78)		
< 47	52 (48.15)	51 (47.22)		
BMI (kg/m^2)			0.019	0.890
≤ 22	62 (45.00)	63 (43.33)		
> 22	46 (55.00)	45 (56.67)		
Is there a history of smoking?			0.020	0.888
Yes	68 (62.96)	69 (63.89)		
No	40 (37.04)	39 (36.11)		
Is there a history of drinking?			0.019	0.892
Yes	55 (50.93)	56 (51.85)		
No	53 (49.07)	52 (48.15)		
History of surgery			0.023	0.880
Yes	31 (28.70)	30 (27.78)		
No	77 (71.30)	78 (72.22)		
Coagulation function				
APTT s	27.98±1.26	28.03±1.23	0.295	0.768
PT s	11.25±1.05	11.21±1.07	0.277	0.782
FIB g/l	3.02±0.24	3.05±0.21	0.978	0.329
Renal function index (μmol/L)				
Creatinine	54.65±4.27	55.27±4.33	1.060	0.291
Urea	5.36±0.34	5.37±0.41	0.195	0.846
Uric acid	290.26±10.26	291.38±11.09	0.770	0.442

Table 2. A comparison of the serum COMP and OPN expression levels between the two groups

Factor	Study group n=108	Control group n=108	t	P
COMP (ng/L)	236.71±25.27	76.83±17.96	53.59	< 0.001
OPN (ng/L)	776.14±98.91	145.62±75.33	52.70	< 0.001

Table 3. A comparison of the COMP and OPN expression levels in the synovial fluid of the two groups of patients

Factor	Study group n=108	Control group n=108	t	P
COMP (ng/L)	762.31±35.69	426.72±33.37	71.38	< 0.001
OPN (ng/L)	1219.86±143.24	621.83±65.22	39.49	< 0.001

Statistical methods

The data in this study were statistically analyzed using SPSS 18.0 (Bizinsight [Beijing] Information Technology Co., Ltd.). The enumeration data were analyzed using a chi-squared test. The measurement data were expressed as the means ± standard deviations. A single factor analysis of variance was used for the comparisons among the groups. The LSD test method was adopted for the pairwise comparisons. The Pearson correlation coefficient was used to carry out the correlation analysis of the COMP and OPN expression levels with the K-L grades. The figures were plotted using GraphPad Prism 6. $P < 0.05$ implied a significant difference.

Results

General information

There were no significant differences in terms of gender, age, BMI, history of smoking, history of drinking, or history of surgery between the two groups (P > 0.05). The patients were comparable (**Table 1**).

Comparison of the COMP and OPN expression levels between the two groups

The expression levels of COMP and OPN in the SG were higher than those in the CG (P < 0.05) (**Tables 2**, **3**).

The expression levels of COMP and OPN in the serum and synovial fluid of patients with different K-L grades

According to the X-ray K-L grading criteria, the patients were divided into grade I (n=29), II (n=27), III (n=27), and IV (n=26). The expression

Table 4. The COMP expression levels in the serum and synovial fluid of the patients with different K-L grades (ng/L)

Factor	I grade n=29	II grade n=26	III grade n=27	IV grade n=26	F	P
Serum	193.55±16.78	226.43±19.84*	257.18±22.33**	298.69±26.73***	117.2	< 0.001
Joint synovial fluid	612.16±25.47	748.13*±26.86	835.23**±31.65	966.72±35.63***	674.4	< 0.001

Note: *, **, ***compared with the I group, P < 0.05; *compared with **, ***, P <0.05; **compared with ***, P < 0.05.

Table 5. The OPN expression levels in the serum and synovial fluid of the patients with different K-L grades (ng/L)

Factor	I grade n=29	II grade n=26	III grade n=27	IV grade n=26	F	P
Serum	711.36±74.31	769.63±81.55*	833.91±86.25**	905.23±99.47***	25.94	< 0.001
Joint synovial fluid	921.54±103.72	1134.62±112.45*	1374.82±126.93**	1523.54±142.05***	130.6	< 0.001

Note: *, **, ***compared with the I group, P < 0.05; *compared with **, ***, P < 0.05; **compared with ***, P < 0.05.

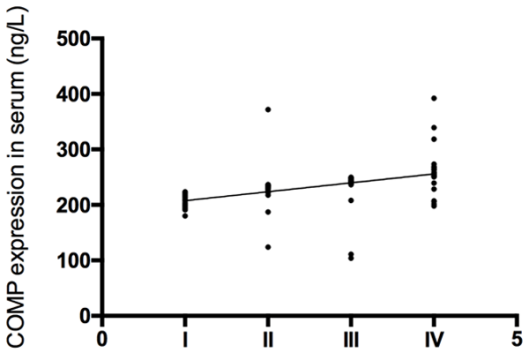

Figure 1. The correlation between COMP and K-L grading in the serum of the study group patients. The correlation of serum COMP with K-L grades in SG. The serum COMP was positively correlated with the K-L grades (r=0.723, P < 0.05).

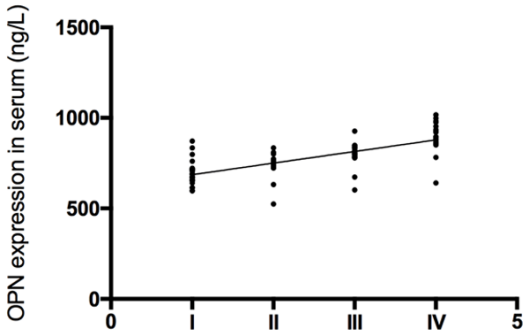

Figure 2. The correlation between OPN and K-L grading in the serum of the study group patients. The correlation of serum OPN with the K-L grades in SG. The serum OPN was positively correlated with the K-L grades (r=0.799, P < 0.05).

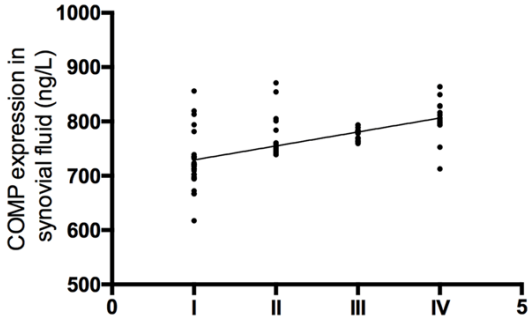

Figure 3. The correlation between COMP and K-L grades in the synovial fluid of the patients in the study group. The correlation of the synovial fluid COMP and K-L grades in the SG. The synovial fluid COMP had a positive correlation with the K-L grades (r=0.701, P < 0.05).

trends of COMP and OPN are shown below: Grade IV expression > grade III expression > grade II expression > grade I expression (P < 0.05) (**Tables 4**, **5**).

Correlation of the serum and synovial fluid COMP and OPN expressions with K-L grades in SG

The COMP and OPN expression levels in the serum were positively correlated with the K-L grades (r=0.723, P < 0.05; r=0.799, P < 0.05). The COMP and OPN expression levels in the synovial fluid had a positive correlation with the K-L grades (r=0.701, P < 0.05; r=0.784, P < 0.05) (**Figures 1**-**4**).

Correlation analysis of the COMP and OPN expressions

The serum and synovial fluid COMP expression was positively correlated with the OPN expres-

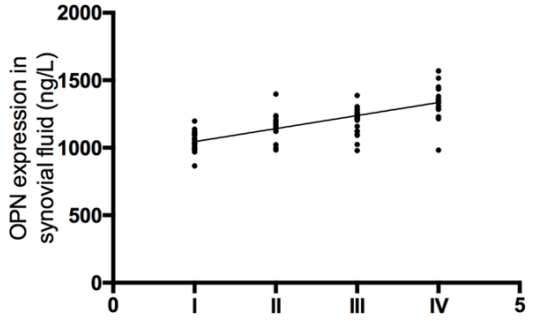

Figure 4. The correlation between OPN and the K-L grades in the synovial fluid of the patients in the study group. The correlation of synovial fluid OPN and the K-L grades in the SG. The synovial fluid OPN had a positive correlation with the K-L grades (r=0.784, P < 0.05).

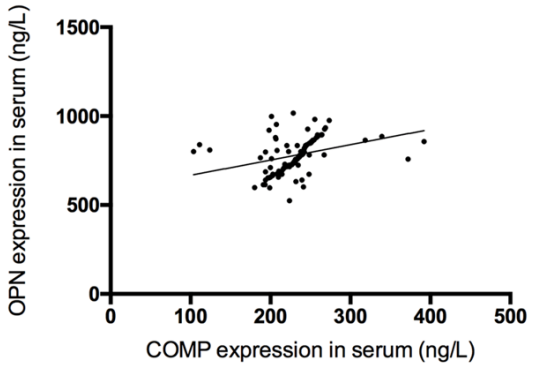

Figure 5. A correlation analysis of the COMP and OPN expressions in the serum of the study group patients. A correlation analysis of the COMP and OPN expression in the serum of the study group patients. The serum COMP expression was positively correlated with the serum OPN (r=0.340, P < 0.05).

sion (r=0.340, P < 0.05; r=0.612, P < 0.05) (**Figures 5**, **6**).

Discussion

Knee osteoarthritis is a chronic osteoarticular disease. It is characterized by a progressive degeneration and injury of the knee articular cartilage [14]. A previous study [15] showed that the biochemical index changes of the knee articular cartilage occur earlier than the morphological changes. Therefore, some [16] consider that the biological factors in patients' body fluid can be used for an early assessment of the severity of KOA.

In this study, the relationship between the serum and synovial fluid COMP and OPN

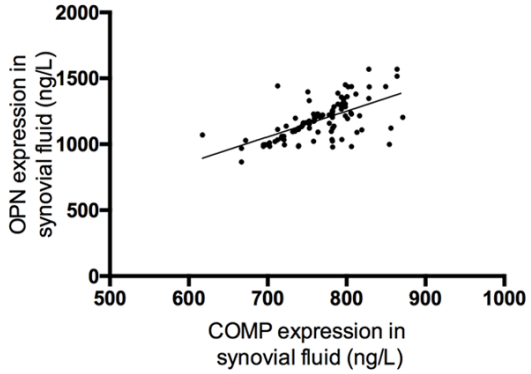

Figure 6. A correlation analysis of the COMP and OPN expression in the synovial fluid of the patients in the study group. A correlation analysis of the COMP and OPN expressions in the synovial fluid of the patients in the study group. The synovial fluid COMP expression was positively correlated with the synovial fluid OPN expression (r=0.612, P < 0.05).

expressions and the K-L grades was investigated. As a homologous pentamer extracellular matrix glycoprotein (ECM) secreted mainly by chondrocytes and synovial cells, COMP has obvious tissue specificity [17]. When articular cartilage is damaged, the degradation of the cartilage matrix and the repair of chondrocytes can cause an increase in the synthesis of COMP [18]. The study results showed that the expression of the serum and synovial fluid COMP in the SG was remarkably higher than it was in the CG. A previous study [19] showed that the COMP expression in the synovial fluid of rabbits in the CG was significantly lower than it was in the knee joint group. The result is consistent with the conclusion in this study. As a functional glycoprotein, OPN is known as a T-cell early activating factor. It can mediate the occurrence of the inflammatory response and promote the further destruction of the joint [20]. In this study, the serum and synovial fluid OPN expressions in the SG were significantly higher than they were in the CG. A previous study [21] showed that OPN can interact with CD44, a hyaluronic acid receptor on the cell surface. As a result, osteoclasts are more likely to attach to the surface of the bone matrix. Thus, the cartilage is damaged. The study has also suggested that the OPN expression in patients with KOA was remarkably up-regulated. The result is consistent with the conclusion in this study.

The expression trend of COMP and OPN in the serum and synovial fluid of patients with KOA

was determined. Then, the patients' conditions were graded according to the K-L criteria. The correlation of the COMP and OPN expressions with the severity of KOA was analyzed. The results showed that the expression trend of COMP and OPN was as follows: Grade IV expression > grade III expression > grade II expression > grade I expression. It indicated that the expressions of COMP and OPN increase with the progression of the disease. Namely, the higher the expressions of COMPA and OPN, the more serious the articular cartilage injury is. A previous study [22] showed that collagen and non-collagen protein are the main macromolecular constitutive substances in human articular cartilage. COMA plays an important role in the interaction between cells and the cell matrix. It is also the main component of the non-collagen protein of cartilage. Therefore, as one study [23] suggested, the determination of the synovial fluid COMP content can reflect the degree of articular cartilage injury. Another study [24] showed that the COMP markers produced in the cartilage are released into the blood after a joint injury. The degree of articular cartilage injury can be evaluated by testing the blood COMP level. A study [25] also showed that the expression levels of COMP can reflect the synovial lesions of the patients. All the results proved the conclusion in the current study. Another study [26] also suggested that the more serious the degeneration of the articular cartilage, the higher the expression level of OPN. All the studies mentioned above and the results in this study indicated that COMP and OPN can be used as important biological markers for judging the conditions of patients with KOA. Finally, the correlation between COMP and OPN in the serum and synovial fluid was analyzed. The results showed that the COMP expression was positively correlated with the OPN expression both in the serum and in the synovial fluid. A previous study [27] showed that OPN is a downstream factor of the Wnt/β-catenin singling pathway. After the Wnt/β-catenin signaling pathway is activated, the expression of OPN will be up-regulated. As a result, relevant cartilage lesions are further caused. However, no study on the relationship between COMP and OPN has been released. As important biomolecules for the assessment of the severity of KOA, the relationship between COMP and OPN will be further investigated in future studies.

In summary, both COMP and OPN show high expressions in the serum and synovial fluid of patients with KOA. The expression levels have a positive correlation with the disease severity as assessed using radiology. They can be used as a biological indicator for the assessment of the conditions of patients with KOA. However, there are still some limitations with regard to this study. For example, the mechanism of COMP and OPN in KOA was not investigated in vitro. The specific relationship between COMP and OPN was not analyzed. It will be further investigated in future studies.

Disclosure of conflict of interest

None.

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