# CTX-II and YKL-40 in early diagnosis and treatment evaluation of osteoarthritis

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**Abstract.** This study investigated the value of C-terminal telopeptides of collagen type II (CTX-II) and YKL-40 in early diagnosis and treatment evaluation of osteoarthritis (OA). A total of 90 patients with OA diagnosed and treated in The First Affiliated Hospital, Guangzhou Medical University from March 2015 to January 2018 were selected as the study group. At the same time, 50 healthy elderly were included as the control group. The study group was divided into three subgroups including group A (29 cases, 500 mg glucosamine sulfate), group B (29 cases, 50 mg diacerein) and group C (32 cases, 500 mg glucosamine sulfate and 50 mg diacerein). Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was used to assess the severity and treatment of arthritis. Enzyme-linked immunosorbent assay was used to measure the concentration of CTX-II and YKL-40 in serum. WOMAC scores in the study A, B and C groups were significantly higher than those in the control group (P<0.001). Serum CTX-II and YKL-40 concentrations were higher in the study group than in the control group (P<0.001). Sensitivity of serum CTX-II combined with YKL-40 in the diagnosis of OA was 90% and the specificity was 78%. CTX-II and YKL-40 levels in different Kellgren Lawrence (K-L) grades were significantly different (P<0.001), and increased with the increase of K-L grade. Concentrations of serum CTX-II and YKL-40 before treatment in the study group was positively correlated with WOMAC score (P<0.001). At 3, 6 and 9 weeks after the beginning of treatment, serum concentrations of CTX-II and YKL-40 decreased significantly (P<0.001). At 3 weeks of treatment, CTX-II was positively correlated with YKL-40 concentration and WOMAC score (r=0.406, P<0.001; r=0.430, P<0.001); CTX-II was positively correlated with YKL-40 concentration and WOMAC score at 6 weeks of treatment (r=0.350, P<0.001; r=0.358, P<0.001); CTX-II was positively correlated with YKL-40 concentration and WOMAC score at 9 weeks after treatment (r=0.370, P<0.001; r=0.394, P<0.001). Combined detection of serum CTX-II and YKL-40 can improve the sensitivity of early OA diagnosis, and it has an important diagnostic value for early OA patients. Therefore, it can be used as a biological indicator for early OA diagnosis, severity assessment, and evaluation of treatment effects.

## Introduction

Osteoarthritis (OA) is a degenerative joint disease mainly affecting the elderly (1). With the growth of aging population, incidence of OA shows an increasing trend. Most elderly people show symptoms of systemic multi-articular OA, and approximately 60% of OA elderly patients need to be treated (2). At present, the pathogenesis of OA has not yet been elucidated, and clinically there is no effective means for diagnosing early OA. Magnetic resonance imaging (MRI) and X-ray are common methods for diagnosing OA, but it has certain limitations. Joints are often affected even when the X-ray results are normal. MRI has a higher resolution in the diagnosis of early OA, however, the application of MRI is limited by the high cost (3,4). Although there are many clinical methods for treating OA, the treatment cycle is long and the effect is often not ideal. With the development of OA, joints are gradually destroyed, and internal structure of the joint undergoes pathological changes and disorders. In clinical practice, it often manifests as joint pain, swelling, morning stiffness, and poor joint stability. In severe cases, joint deformity and function may also occur (5,6). Therefore, early diagnosis and treatment of OA is particularly important. With the development of molecular biology, the application of biological markers in the diagnosis of OA have attracted increasing attention. C-terminal telopeptides of collagen type II (CTX-II) is a biomarker that can reflect the pathological changes and metabolism of joint tissue. It can be detected in urine, blood, and synovial fluid (7). YKL-40 is a member of the 18 glycosyl hydrolase family and is widely distributed in synoviocytes and chondrocytes (8). Studies have shown that CTX-II and YKL-40 are closely related to the pathological changes of articular cartilage and can

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reflect the degree of inflammation of OA (9). Glucosamine is an important component of cartilage tissue. Glucosamine supplementation can reduce the destruction of cartilage tissue and cells. Diacerein can induce cartilage production and has anti-inflammatory, analgesic and antipyretic effects. Both glucosamine and diacerein supplementation can relieve joint pain and improve joint activity, thereby delaying the course of OA (10). Clinically, WOMAC is a scoring system specially designed for hip and knee arthritis, which can assess the severity of arthritis and its therapeutic effect according to the related symptoms and signs of patients (11). Previous studies on CTX-II and YKL-40 mainly focused on the development of OA articular cartilage tissue. There are few studies on the diagnostic value of serum CTX-II and YKL-40 in patients with OA. This study examined the expression of CTX-II and YKL-40 in the serum of patients with early OA and explored the role of CTX-II and YKL-40 in the diagnosis of early OA, assessment of disease status and evaluation of therapeutic effect.

## Materials and methods

*General information*. Diagnostic experiment of 90 patients with OA diagnosed and treated in The First Affiliated Hospital, Guangzhou Medical University (Guangzhou, China) from March 2015 to January 2018 were selected as the study group. The study group included 38 males and 52 females, and age ranged from 49 to 78 years, with an average age of 58.8±6.7 years. Kellgren Lawrence (K-L) (12) classification: 30 cases of grade I, 23 cases of grade II, 19 cases of grade III and 18 cases of grade IV. Inclusion criteria: i) Patients met OA diagnostic criteria established by the American College of Rheumatology (ACR; Atlanta, GA, USA) (11); ii) K-L (13) grade <1, imaging shows no osteophyte hyperplasia and joint space is normal; and iii) knee pain and soreness last for at least 4 months. Exclusion criteria: i) Patients who have previously received knee joint treatment; ii) patients with fever or skin lesions at the site of the disease; iii) patients with severe hepatorenal and hematopoietic disorders; iv) patients with other bony diseases such as gout and bone cancer; v) individuals with a history of mental illness or having a family history of mental illness; and vi) age ≤39 years or age ≥85 years. At the same time, 50 healthy elderly individuals were selected as the control group. The control group included 23 males and 27 females, and age ranged from 43 to 75 years, with a mean age of 59.2±5.1 years. The study was approved by the Ethics Committee of The First Affiliated Hospital, Guangzhou Medical University, and all participants signed an informed consent.

*Grouping and treatment*. The study group was divided into three subgroups including group A [29 cases, oral intake of 500 mg glucosamine sulfate (14), batch no. H20090305; Hubei Aipu Bio-Engineering Co., Ltd., Wuhan, China], group B [29 cases, oral intake of 50 mg diacerein (15), batch no. J20100150; Kunming Jida Pharmaceutical Co., Ltd., Kunming, China] and group C (32 cases, oral intake of 500 mg glucosamine sulfate and 50 mg diacerein). Treatment was performed twice a day. Patients' adverse reactions and toxic side effects during the treatment were recorded.

*Observation of curative effect*. Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was used to assess the severity of arthritis and its therapeutic effect. WOMAC is the best self-assessment scale for OA. It includes joint pain, joint stiffness and daily activities. The total score was 20 points for joint pain, 8 points for stiff and 68 points for daily activities. Higher scores indicate more serious conditions. WOMAC scores were evaluated before and at 3, 6 and 9 weeks after the beginning of treatment to assess symptom improvement and functional recovery.

*Sample collection and detection*. Fasting venous blood was extracted from each participant at 1 week gap, before drug treatment, 3, 6 and 9 weeks of treatment. The serum was separated by centrifugation at 3,000 x g (Hunan Pingfan Technology Co., Ltd., Changsha, China) and was stored at -20°C. The concentrations of CTX-II and YKL-40 in serum were detected by enzyme-linked immunosorbent assay (ELISA) using human CTX-II ELISA kit (Shanghai Guye Biotechnology Co., Ltd., Shanghai, China) and human YKL-40 ELISA kit (Qingdao Jieshikang Biotech Co., Ltd., Qingdao, China) according to the instructions of the kit. The kit was kept at room temperature for 30 min before use, and test sample, standard and blank wells were set. Enzyme-labeled reagents and samples were not added into the black wells. The remaining wells were added with 100 $\mu$l of the test samples or standard samples. After mixing, the microtiter plates were covered with membranes and incubated at 37°C for 2 h. After that, the liquid was discarded. After air drying, 100 $\mu$l of working solution A was added into each well. The wells were covered and incubated for 1 h at 37°C. After that, the liquid was discarded. After spin drying, the plate was washed three times with automatic plate washer (Nanjing Detie Laboratory Equipment Co., Ltd., Nanjing, China). Then, 100 $\mu$l of working solution B was added into each well and the wells were covered and incubated for 1 h at 37°C. After that, the liquid was discarded. After spin dry, the plate was washed 3 times and 90 $\mu$l of substrate solution was added into each well. The wells were covered with membrane, followed by incubation in the dark at room temperature for 20 min. Then, 50 $\mu$l of stop solution was added into each well, and the OD value of each well was immediately detected at 450 nm using an enzyme-labeled analyzer (Shanghai Xinzhuang Instrument Co., Ltd., Shanghai, China) to calculate the concentrations of CTX-II and YKL-40.

*Statistical analysis*. SPSS v.20.0 (Beijing Netscape Technology Co., Ltd., Beijing, China) was used for statistical analysis. Measured data were expressed as mean ± standard deviation. t-test was used for comparison of the measurement data between two groups. Chi-square test was used to compare enumeration data between the groups. One-way analysis of variance was used for comparisons among multiple groups. Comparison of data at multiple time-points was performed using the repeated measures analysis of variance and the post hoc test was LSD. Intragroup comparisons were compared twice by LSD t-test. Diagnostic performance of serum CTX-II and YKL-40 concentrations for OA was evaluated using receiver operating characteristic (ROC) curves. Correlation analysis was performed using Pearson's correlation coefficient. P<0.05 was considered to indicate a statistically significant difference.

Table I. Baseline data of the study and control groups [n(%)]/(mean ± SD).

| Indexes | Study group (n=90) | Control group (n=50) | t/$\chi^2$ | P-value |
|---|---|---|---|---|
| Sex | | | 0.187 | 0.723 |
| Male | 38 (42.22) | 23 (46.00) | | |
| Female | 52 (57.78) | 27 (54.00) | | |
| Age, years | 58.8±6.7 | 59.2±5.1 | 0.367 | 0.714 |
| Smoking | | | 0.083 | 0.855 |
| Yes | 32 (35.56) | 19 (38.00) | | |
| No | 58 (64.44) | 31 (62.00) | | |
| BMI, kg/m$^2$ | 24.13±4.83 | 25.13±3.16 | 1.315 | 0.190 |
| Cre, mmol/l | 10.16±1.08 | 10.26±0.76 | 0.579 | 0.563 |
| UA, $\mu$mol/l | 193.23±22.14 | 186.14±23.47 | 1.777 | 0.077 |
| ALT, U/l | 19.41±8.46 | 21.62±8.04 | 1.507 | 0.134 |
| AST, U/l | 18.63±7.26 | 19.74±8.16 | 0.828 | 0.408 |
| Glu, mmol/l | 6.01±0.98 | 5.87±1.06 | 0.786 | 0.432 |
| r-GT, U/l | 43.53±17.63 | 45.15±16.78 | 0.529 | 0.597 |
| WOMAC score | 47.38±10.41 | 5.16±2.12 | 28.310 | <0.001 |

SD, standard deviation; BMI, body mass index; Cre, creatinine; UA, uric acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Glu, blood glucose; r-GT, r-glutamyl transferase; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.
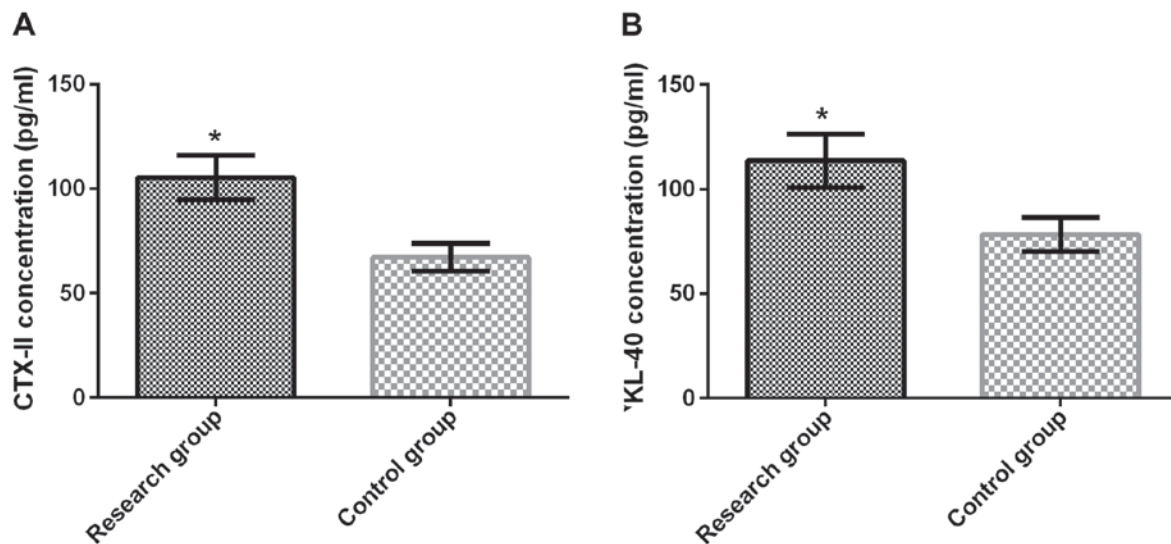


Figure 1. Comparison of serum CTX-II and YKL-40 concentrations in the study and control groups. ELISA results showed that (A) serum CTX-II concentration in the study group was significantly higher than that of the control group (t=23.010, P<0.001); (B) serum YKL-40 concentration in the study group was significantly higher than that of the control group (t=17.50, P<0.001). *P<0.01, compared with the control group. CTX-II, C-terminal telopeptides of collagen type II.

## Results

*General information.* There were no significant differences in sex, age, smoking habit, body mass index (BMI), creatinine (Cre), uric acid (UA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood glucose (Glu), r-glutamyl transferase (r-GT) among the study and control groups (P>0.05). WOMAC scores of the study groups A-C were significantly higher than those of the control group (t=28.310, P<0.001; Table I).

*Serum CTX-II and YKL-40 concentrations in study and control groups.* Concentrations of serum CTX-II and YKL-40 in the study group were 105.41±10.63 pg/ml and 113.58±12.87 pg/ml, respectively. Concentrations of serum CTX-II and YKL-40 in the control group were 67.12±6.74 pg/ml and 78.26±8.12 pg/ml, respectively. Serum CTX-II concentrations in the study group were significantly higher than those in the control group (P<0.001). Serum YKL-40 concentrations in the study group were also significantly higher than those in the control group (P<0.001; Fig. 1A and B).

*Diagnostic value of serum CTX-II and YKL-40 concentrations for OA*. ROC curve of serum CTX-II and YKL-40 concentrations in diagnosis of OA was plotted. Area under the curve (AUC) of serum CTX-II in the diagnosis of OA was 0.886 [95% confidence interval (CI): 0.930 to 0.942], optimal cut-off value for diagnosis of OA was 0.70, diagnostic sensitivity was 84% and specificity was 86%. AUC of serum YKL-40 in the diagnosis of OA was 0.880 (95% CI: 0.822 to 0.939), optimal cut-off value for diagnosis of OA was 0.62, diagnostic sensitivity was 82%, and specificity was 80%. ROC curve for the diagnosis of OA using combination of serum CTX-II and YKL-40 was plotted. AUC for the diagnosis of OA by serum CTX-II combined with YKL-40 was 0.880 (95% CI: 0.820 to 0.939), optimal cutoff value for diagnosis of OA was 0.78, diagnostic sensitivity was 90%, and specificity was 78% (Fig. 2).

*Serum CTX-II and YKL-40 levels of different K-L grades*. CTX-II and YKL-40 concentrations were significantly higher in grades II-IV patients than in grade I (P<0.001). CTX-II and YKL-40 concentrations were significantly higher in patients with grade III and IV than in patients with K-L grade II (P<0.001). The concentrations of CTX-II and YKL-40 in patients with grade IV were significantly higher (P<0.001) than those with grade III. Concentrations of CTX-II and YKL-40 increased with the increase of K-L classification (Fig. 3A and B).

*Correlation between serum CTX-II and YKL-40 concentrations and WOMAC score before treatment in the study group*. Serum CTX-II concentrations in the study group was positively correlated with WOMAC score (r=0.357, P<0.001). Serum YKL-40 concentrations was positively correlated with WOMAC score (r=0.327, P=0.001; Fig. 4A and B).

*WOMAC scores before and after treatment in groups A-C*. There was no significant difference in WOMAC scores before treatment and at 3, 6 and 9 weeks after the beginning of treatment among groups A-C (P>0.05). Compared with pre-treatment scores, WOMAC scores decreased significantly at 3, 6, and 9 weeks in groups A-C (P<0.001). Comparison of scores at 3 weeks after the beginning of treatment, WOMAC scores of groups A-C decreased significantly at 6 and 9 weeks (P<0.001) and the scores at 6 weeks after the beginning of treatment, WOMAC scores of groups A-C decreased significantly at 9 weeks (P<0.001; Table II).

*Changes of serum CTX-II concentrations before and after treatment in groups A-C*. There was no significant difference in serum CTX-II concentrations before treatment and at 3, 6 and 9 weeks after the beginning of treatment among groups A-C (P>0.05). Compared with pre-treatment scores, serum CTX-II concentrations decreased significantly at 3, 6 and 9 weeks in groups A-C (P<0.001). Comparison of scores at 3 weeks after the beginning of treatment, serum CTX-II concentrations of groups A-C decreased significantly at 6 and 9 weeks (P<0.001). Comparison of scores at 6 weeks after the beginning of treatment, serum CTX-II concentrations of groups A-C decreased significantly at 9 weeks (P<0.001; Table III).
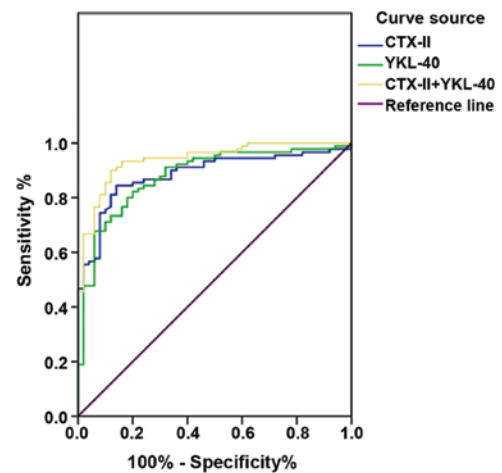


Figure 2. Diagnostic value of serum CTX-II and YKL-40 concentrations for OA. ROC curve showed that AUC of serum CTX-II in the diagnosis of OA was 0.886 (95% CI: 0.930 to 0.942), optimal cut-off value for diagnosis of OA was 0.70, diagnostic sensitivity was 84% and specificity was 86%. AUC of serum YKL-40 in the diagnosis of OA was 0.880 (95% CI: 0.822 to 0.939), optimal cut-off value for diagnosis of OA was 0.62, diagnostic sensitivity was 82%, and specificity was 80%. ROC curve for the diagnosis of OA using combination of serum CTX-II and YKL-40 was plotted. AUC for the diagnosis of OA by serum CTX-II combined with YKL-40 was 0.880 (95% CI: 0.820 to 0.939), optimal cut-off value for diagnosis of OA was 0.78, diagnostic sensitivity was 90%, and specificity was 78%. CTX-II, C-terminal telopeptides of collagen type II; OA, osteoarthritis; ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval.

*Changes of serum YKL-40 concentrations before and after treatment in groups A-C*. There was no significant difference in serum YKL-40 concentrations before treatment and at 3, 6 and 9 weeks after the beginning of treatment among groups A-C (P>0.05). Compared with pre-treatment scores, serum YKL-40 concentrations decreased significantly at 3, 6 and 9 weeks in groups A-C (P<0.001). Comparison of scores at 3 weeks after the beginning of treatment, serum YKL-40 concentrations of groups A-C decreased significantly at 6 and 9 weeks (P<0.001). Comparison of scores at 6 weeks after the beginning of treatment, serum YKL-40 concentrations of groups A-C decreased significantly at 9 weeks (P<0.001; Table IV).

*Correlation between serum CTX-II and YKL-40 concentration and WOMAC score at 3, 6 and 9 weeks of treatment in OA patients*. At 3 weeks of treatment, CTX-II was positively correlated with YKL-40 concentration and WOMAC score (r=0.406, P<0.001; r=0.430, P<0.001); CTX-II was positively correlated with YKL-40 concentration and WOMAC score at 6 weeks of treatment (r=0.350, P<0.001; r=0.358, P<0.001); At 9 weeks of treatment, serum CTX-II was positively correlated with YKL-40 concentration and WOMAC score (r=0.370, P<0.001; r=0.394, P<0.394; Fig. 5A-F).

*Safety analysis*. None of the patients experienced any discomfort or toxicity during the treatment of this study.

## Discussion

OA is the most common joint disease in middle-aged and elderly people. Pathological basis of OA mainly include degenerative

Table II. WOMAC scores before and after treatment in groups A-C (mean ± SD).

| Time-points | Group A (n=29) | Group B (n=29) | Group C (n=32) | F | P-value |
|---|---|---|---|---|---|
| Before treatment | 45.63±9.58 | 46.17±10.22 | 50.26±11.25 | 1.830 | 0.166 |
| 3 weeks | 38.83±8.70[a] | 39.41±7.85[a] | 35.41±7.85[a] | 2.182 | 0.118 |
| 6 weeks | 30.16±6.45[a,b] | 32.10±5.72[a,b] | 29.74±5.74[a,b] | 1.324 | 0.271 |
| 9 weeks | 23.26±4.08[a-c] | 21.42±5.01[a-c] | 20.52±4.17[a-c] | 2.990 | 0.055 |
| F | 49.300 | 58.170 | 83.400 | | |
| P-value | <0.001 | <0.001 | <0.001 | | |

[a]Compared with pre-treatment level, $P<0.01$; [b]compared with 3 weeks of treatment, $P<0.01$; [c]compared with 6 weeks of treatment, $P<0.01$. WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; SD, standard deviation.
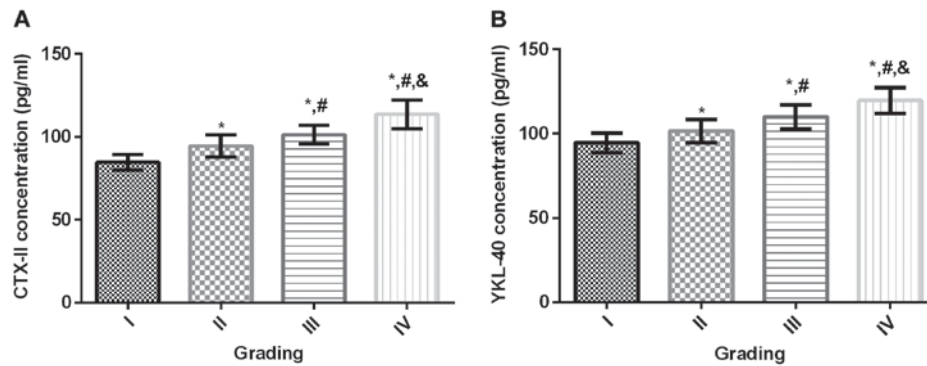


Figure 3. Comparison of CTX-II and YKL-40 concentrations in different K-L grades. ELISA results showed that (A) CTX-II and (B) YKL-40 concentrations were significantly higher in patients with grades II-IV than in patients with K-L grade I (*$P<0.001$). CTX-II and YKL-40 concentrations were significantly higher in patients with grades III and IV than in patients with K-L grade II (#$P<0.001$). The concentrations of CTX-II and YKL-40 in patients with grade IV were significantly higher (&$P<0.001$) than those with grade III. CTX-II, C-terminal telopeptides of collagen type II; K-L, Kellgren Lawrence.
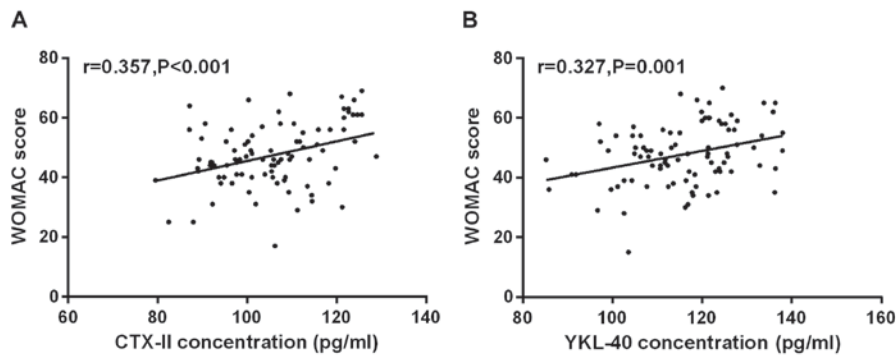


Figure 4. Correlation of serum CTX-II and YKL-40 concentrations with WOMAC scores in OA patients. (A) Pearson's test results showed that serum CTX-II concentration in OA patients was positively correlated with WOMAC score (r=0.357, $P<0.001$). (B) Serum YKL-40 concentration was also positively correlated with WOMAC score (r=0.327, $P=0.001$). CTX-II, C-terminal telopeptides of collagen type II; OA, osteoarthritis; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

changes of articular cartilage and the hyperosteogeny. Incidence of this disease increases with aging (16). OA causes irreversible damage to a certain extent. OA not only brings inconvenience to the daily life of patients, but also causes a heavy burden on their families. Therefore, early diagnosis of OA has attracted increasing attention (17). Compared with expensive MRI and traumatic arthroscopy, molecular biology markers have the advantages of affordable price and early detection. In recent years, biomarkers have been increasingly used in the diagnosis of OA.

Pathological changes of OA are manifested as the loss or abnormal synthesis of glycoprotein in cartilage matrix, which makes the base of the joint thinner and surface cartilage softer, resulting in pathological hyperplasia and formation of osteophytes (18). Collagen type II is an important component of articular cartilage and is involved in the reconstruction and repair of articular cartilage. When OA occurs, the process of reconstruction and repair of articular cartilage is accelerated, and the concentration of CTX-II in body fluids also increases (19). CTX-II is produced by the cleavage of

Table III. Serum CTX-II concentrations before and after treatment in groups A-C (pg/ml)/(mean ± SD).

| Time-points | Group A (n=29) | Group B (n=29) | Group C (n=32) | F | P-value |
|---|---|---|---|---|---|
| Before treatment | 101.11±11.62 | 104.26±10.89 | 105.43±12.17 | 1.113 | 0.333 |
| 3 weeks | 91.13±8.63[a] | 88.41±9.01[a] | 92.13±8.45[a] | 1.470 | 0.235 |
| 6 weeks | 83.56±7.15[a,b] | 82.18±6.71[a,b] | 86.12±7.27[a,b] | 2.465 | 0.090 |
| 9 weeks | 72.87±7.01[a-c] | 73.52±6.97[a-c] | 70.13±7.53[a-c] | 1.935 | 0.150 |
| F | 53.370 | 6.530 | 80.170 | | |
| P-value | <0.001 | <0.001 | <0.001 | | |

[a]Compared with pre-treatment level, P<0.01; [b]compared with 3 weeks of treatment, P<0.01; [c]compared with 6 weeks of treatment, P<0.01. CTX-II, C-terminal telopeptides of collagen type II; SD, standard deviation.

Table IV. Serum YKL-40 concentrations before and after treatment in groups A-C (pg/ml)/(mean ± SD).

| Time-points | Group A (n=29) | Group B (n=29) | Group C (n=32) | F | P-value |
|---|---|---|---|---|---|
| Before treatment | 114.56±12.65 | 116.14±13.28 | 112.63±10.26 | 0.647 | 0.525 |
| 3 weeks | 103.14±11.63[a] | 105.41±9.78[a] | 101.74±10.03[a] | 0.940 | 0.394 |
| 6 weeks | 92.41±9.86[a,b] | 93.45±10.26[a,b] | 89.93±8.63[a,b] | 1.097 | 0.338 |
| 9 weeks | 83.74±8.41[a-c] | 84.11±8.45[a-c] | 80.17±9.12[a-c] | 1.942 | 0.149 |
| F | 44.610 | 50.410 | 66.360 | | |
| P-value | <0.001 | <0.001 | <0.001 | | |

[a]Compared with pre-treatment level, P<0.01; [b]compared with 3 weeks of treatment, P<0.01; [c]compared with 6 weeks of treatment, P<0.01. SD, standard deviation.

mature type II collagen and passes through joint blood, synovial fluid and urine in the form of nano-collagen (20). YKL-40 is a type of chitinase-like 3-protein that can reflect the state of endothelial cell damage such as cell migration, adhesion and reorganization (21). Concentration of YKL-40 in normal individuals is low, but it is widely present in articular chondrocytes and is mainly found in the surface and middle layers of cartilage (22). Results of this study showed that concentrations of serum CTX-II and YKL-40 in the study group were significantly higher than those of the control group, and concentrations of CTX-II and YKL-40 increased with the increase of k-l classification. Concentrations of serum CTX-II and YKL-40 before treatment were positively correlated with the WOMAC score, indicating that CTX-II and YKL-40 have important diagnostic value for early OA. Concentrations of CTX-II and YKL-40 can be used as an objective reference for the severity of OA. Meulenbelt *et al* (23) measured the concentration of CTX-II in urine of patients with OA and found that the level of CTX-II in OA patients was significantly increased, and there was a significant correlation with the JOA score of hip, hand, articular surface and knee joints, suggesting that CTX-II may be a sensitive marker of OA activity. Väänänen *et al* (24) showed that expression of YKL-40 in the synovial fluid of OA patients was significantly upregulated, suggesting a significant correlation between YKL-40 and the degree of inflammation in OA patients. Previous studies on CTX-II and YKL-40 mainly focused on advanced OA. Our study further confirmed the diagnostic value of CTX-II and

YKL-40 for early OA. We further found that the sensitivity of CTX-II combined with YKL-40 in the diagnosis of OA was 90% and the specificity was 78%, while the sensitivity of CTX-II alone in the diagnosis of OA was 84%, and the specificity was 86%. For YKL-40 alone, sensitivity was 82% and specificity was 80%. The combination of the two may improve the sensitivity of early OA diagnosis.

Glucosamine and diacerein are commonly used supplements in clinical treatment of OA patients. Diacerein can suppress the vicious circle of joint inflammation by reducing the production of inflammatory mediators, stabilize the articular cartilage environment, thereby delaying the progression of OA and improving the clinical symptoms of OA patients (25). Glucosamine is one of the components of articular cartilage. It participates in glycosylation of lipids and proteins in articular cartilage cells and metabolism of articular chondrocytes. It promotes the production of bone marrow mesenchymal stem cells and inhibits malignant cycle of joint inflammation (26). The study of Wen *et al* (27) showed that oral glucosamine can delay the development of OA, relieve pain and regulate the metabolism of chondrocytes in OA rats. Wilkens *et al* (28) reported that glucosamine has the properties of restoring cartilage and anti-inflammation, and can reduce the pain related disability in patients with degenerative lumbar OA. Pelletier *et al* (29) confirmed that diacetaminophen had good clinical efficacy for patients with OA, and believed that the optimal dose of diacetaminophen was 100 mg/day. Therefore, it was shown that glucosamine and diacetone have good clinical effects on OA.
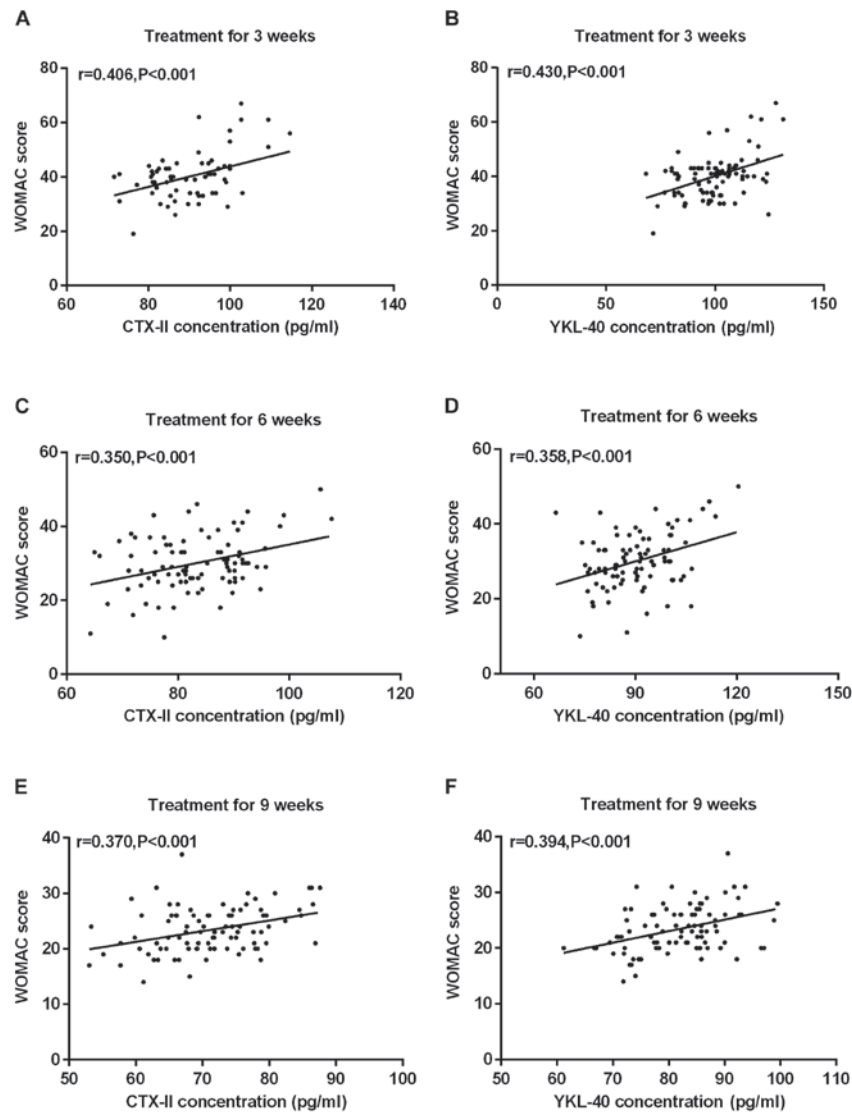
Figure 5. Correlation between serum CTX-II and YKL-40 concentration and WOMAC score in OA patients at 3, 6 and 9 weeks. (A) Pearson test results showed that the serum CTX-II concentration of patients with OA was positively correlated with the WOMAC score at 3 weeks of treatment (r=0.406, P<0.001). (B) At 3 weeks of treatment, serum YKL-40 concentration in OA patients was positively correlated with WOMAC score (r=0.430, P<0.001). (C) The serum CTX-II concentration of patients with OA was positively correlated with the WOMAC score at 6 weeks of treatment (r=0.350, P<0.001). (D) At 6 weeks of treatment, serum YKL-40 concentration of OA patients was positively correlated with WOMAC score (r=0.358, P<0.001). (E) At 9 weeks of treatment, serum CTX-II concentration of OA patients was positively correlated with WOMAC score (r=0.370, P<0.001). (F) At 9 weeks of treatment, serum YKL-40 concentration in OA patients was positively correlated with WOMAC score (r=0.394, P<0.001). CTX-II, C-terminal telopeptides of collagen type II; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; OA, osteoarthritis.

In this study, glucosamine, diacerein and their combination were used to treat patients with early OA. Results showed that there were no significant differences in WOMAC score, serum CTX-II and YKL-40 concentrations before and at 3, 6 and 9 weeks after treatment among groups A, B and C. At 3, 6 and 9 weeks after the beginning of treatment, WOMAC score and serum concentrations of CTX-II and YKL-40 decreased significantly (P<0.001). CTX-II was positively correlated with YKL-40 concentration and WOMAC score at 3, 6 and 9 weeks of treatment. Glucosamine alone, diacerein alone and the combination showed similar therapeutic effects, which may be explained by the short treatment cycle.

Serum CTX-II and YKL-40 concentrations showed a decreasing trend during the course of treatment. The degree of decline was significantly correlated, so CTX-II and YKL-40 may become biological indicators for the treatment effect

evaluation of OA patients. Manicourt et al (30) showed that oral administration of salmon calcitonin in patients with knee OA can reduce the expression of CTX-II, MMP-1 and MMP-3, and it is believed that the expression level of these biomarkers can predict the change of knee joint space. Väänänen et al (22) found that plasma YKL-40 levels are associated with disease activity of rheumatoid arthritis during treatment. Plasma YKL-40 is a biomarker for predicting RA disease activity and can be used to guide RA remission therapy. Previous studies mainly focused on CTX-II and YKL-40 in advanced OA, while the use of CTX-II and YKL-40 for assessing treatment efficacy of early OA patients is rare. In this study early OA patients were included. Therefore, we confirmed that concentrations of serum CTX-II and YKL-40 can be used as biological indicators for evaluating the therapeutic effects of treatment of early OA patients.

This study was conducted in strict accordance with the inclusion and exclusion criteria. There was no difference in sex, age, smoking habit, BMI, Cre, UA, ALT, AST, Glu, and r-GT among the study subgroups A-C and the control group. Results confirmed the potential of CTX-II and YKL-40 in the diagnosis of the early stages of OA, determination of disease activity, and treatment assessment. However, the regulatory mechanism of CTX-II and YKL-40 in the development of OA has not yet been elucidated. The treatment time is short and the sample size is small. In future studies, we will expand the sample size, extend treatment time, and conduct an in-depth investigation on the mechanisms of actions of CTX-II and YKL-40 in OA.

In conclusion, combined detection of serum CTX-II and YKL-40 can improve the sensitivity of OA diagnosis, and it has an important diagnostic value for early OA patients. It can be used as a biological indicator for OA diagnosis, severity assessment, as well as evaluation of treatment effects.

## Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

## Authors' contributions

PW drafted the manuscript. PW and JS were mainly devoted to collecting and interpreting the general data. PW, JS and DQ performed ELISA. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

The study was approved by the Ethics Committee of The First Affiliated Hospital, Guangzhou Medical University (Guangzhou, China). Signed informed consents were obtained from the patients or guardians.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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