Comparative study of CTX-II, Zn\textsuperscript{2+}, and Ca\textsuperscript{2+} from the urine for knee osteoarthritis patients and healthy individuals

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Abstract
The aim of the study was to explore the relationship between the concentration of CTX-II, Zn\textsuperscript{2+}, and Ca\textsuperscript{2+} in urine and knee osteoarthritis (KOA).

Eighty-two patients with KOA and 20 healthy volunteers were enrolled. Anteroposterior and lateral position x-rays of knee joints were collected. The images were classified according to Kellgren-Lawrence radiographic grading criterion. The patients were divided into group grade I, group grade II, group grade III, and grade IV. The concentration of CTX-II in the urine was detected by enzyme-linked immunosorbent assay. The concentration of Zn\textsuperscript{2+} and Ca\textsuperscript{2+} in urine was detected by inductively coupled plasma atomic emission spectrometry.

Compared with the healthy individuals, the concentration of CTX-II was significantly higher in KOA patients. The concentration of CTX-II in KOA patients from high to low was as follows: group IV, group III, group II, and group I. There was no significant difference between group I and healthy individuals. The concentration of Zn\textsuperscript{2+} and Ca\textsuperscript{2+} in urine of KOA patients was higher than that in healthy individuals. There was no difference in each KOA group.

The concentration of CTX-II is instrumental to diagnose the progress of KOA. The concentration of Zn\textsuperscript{2+} and Ca\textsuperscript{2+} in urine is helpful for early diagnosis of KOA.

Abbreviations: ELISA = enzyme-linked immunosorbent assay, ICP-AES = inductively coupled plasma atomic emission spectrometry, KOA = knee osteoarthritis, MRI = magnetic resonance imaging, sCOMP = serum cartilage oligometric matrix protein.

Keywords: Ca\textsuperscript{2+}, collagen type II, knee osteoarthritis, urine, Zn\textsuperscript{2+}

1. Introduction
Knee osteoarthritis (KOA) is a common disease for middle-aged and old people. The chronic and painful symptoms are seriously harmful to human health. Epidemiological investigation showed that over one-third of people aged over 60 years have KOA in United States.[1] In China, over 50% of people aged over 50 have KOA. Also, 80% female and 70% male aged over 65 suffered from the disease.[2] With the aging society, the population of KOA is still increasing. It is estimated that 25 million in United States and 8 million in Japan suffered from it.[3,4]

Traditionally, clinical symptoms and radiography were employed to diagnose KOA. Magnetic resonance imaging (MRI) was also used to diagnose the structural change of bone.[5]

However, lack of early diagnostic markers and makers for progression lead to serious results. Some researchers identified several markers, such as C-telopeptide fragments of type II collagen (CTX-II) in urine, uric acid in joint fluid, serum cartilage oligometric matrix protein (sCOMP), and serum adipokines.[6–10] Proteomics also revealed some novel markers, such as hemopexin, custerin, alpha-1acid glycoprotein-2, macrophage stimulating protein, Fib3 (Fibulin 3 peptides)-1, and Fib3-2.[11,12] However, the predictive value should be further explored.

Some elements, such as Ca\textsuperscript{2+}, play important role in keeping osmotic pressure of joint fluid and supplying nutrients. The unbalance of these elements occurs in different diseases. The test of these elements in joint fluid is helpful to diagnose the related diseases. Periodical examination of these elements is helpful to monitor the progress of the disease and effect of treatment.[13,14] Reagan et al.[15] cultured chondrocyte with different elements, observed the effect, and found that elements have a certain role in repairing the cartilage cell regeneration. Krachler and Domers,[13] Krachler et al.[14] and Yazar et al.[16] found that microelements have a certain role in diagnosing osteoarthritis. However, the potential of Zn\textsuperscript{2+} and Ca\textsuperscript{2+} in KOA is still unclear.

In this study, we will explore the predictive value of CTX-II, Zn\textsuperscript{2+}, and Ca\textsuperscript{2+} in KOA, especially different progression.

2. Study population and methods

2.1. Study population
In all, 82 KOA patients and 20 healthy individuals were enrolled in this study. The study was approved by Review Board of Guilin Medical College. The basic information of subjects is shown in Table 1. The following patients were excluded: patients with liver...
and kidney dysfunction; patients with bone and joint disease in hip and ankle, cartilage metabolic diseases, such as ankylosing spondylitis, and rheumatoid arthritis; nonmenopause\textsuperscript{[17]}; patients taking medicine of hormone and cartilage metabolism.

2.2. Grouping

Kellgren-Lawrence radiographic grading criterion was employed to assess KOA patients. Three professional doctors analyzed the radiography. Group I: suspicious narrow in joint space and suspicious osteophyte; group II: osteophyte and normal or suspicious narrow in joint space; group III: medium osteophyte, obvious narrow in joint space, subchondral bone sclerosis, and possible deformity; group IV: large osteophyte, obvious narrow in joint space, serious subchondral bone sclerosis, and obvious deformity.

2.3. ELISA

Enzyme-linked immunosorbent assay (ELISA) was employed to detect the CTX-II level in urine. Morning urine (5–10mL) was collected and centrifuged at 2500rpm/min for 20 minutes. The supernate was transferred to centrifuge tube and kept at \( -80^\circ C \). After dissolution, the samples were diluted 5 times. The diluted samples and standard sample (100\( \mu \)L) were added into coated wells and incubated at 37\( ^\circ C \) for 1 hour. Except for blank well, 50\( \mu \)L color-developing agents A and B were added into each well for 15 minutes in dark place. Stop solution was used to stop the reaction. The optical density (OD) value at 450nm was recorded.

2.4. Measurement of Zn\textsuperscript{2+} and Ca\textsuperscript{2+}

HNO\textsubscript{3} (8mL) was added to 2mL urine and mixed well. Full spectrum direct reading plasma spectrometer was used to examine the Zn\textsuperscript{2+} and Ca\textsuperscript{2+} in urine. The working parameters of instrument were as follows: incident power, 1.1kW; argon flow, 0.5L/min.

2.5. Statistical analysis

SPSS 18.0 was used to analyze the data. Independent-samples \( t \) test was used for statistical comparisons between 2 groups. One-way analysis of variance (ANOVA) followed by a least-significant difference test was used for statistical comparisons among multiple groups. The significance was assured when the \( P \) value was less than .05.

3. Results

3.1. The CTX-II concentration in different groups

Compared with the control group (218.341 \( \pm \) 22.270) pg/mL, the CTX-II concentration in KOA (261.235 \( \pm \) 39.944) pg/mL was higher (\( F=43.722, P<.001 \)). The concentration of CTX-II in KOA patients from high to low was as follows: group IV, group III, group II, and group I (\( F=334.402, P<.001 \)). There was no significant difference between group I and healthy individuals (\( P>.05 \)) (Fig. 1).

3.2. The concentration of Zn\textsuperscript{2+} and Ca\textsuperscript{2+} in different groups

The concentration of Zn\textsuperscript{2+} in KOA (0.7000 \( \pm \) 0.1736 mg/L) was higher than that in the control group (0.3764 \( \pm \) 0.2163 mg/L) (\( F=53.401, P<.001 \)). The concentration of Ca\textsuperscript{2+} in KOA (204.3536 \( \pm \) 71.6828 mg/L) was also higher than that in the control group (80.4635 \( \pm \) 39.3493 mg/L) (\( F=55.379, P<.0001 \)). There was no significant difference among group I, group II, group III, and group IV (\( P>.05 \)) (Figs. 2 and 3).

4. Discussion

Healthy cartilage matrix mainly contains collagen type II. Type II collagen in cartilage was degraded continuously through matrix

| Table 1  | The general information of subjects (\( x \pm s \)). |
|----------|--------------------------------------------------|
| Group    | Case number | Age, y | Male | Female |
| Control  | 20          | 57.6 ± 6.1 | 8    | 12     |
| Group I  | 19          | 56.7 ± 6.9 | 9    | 10     |
| Group II | 21          | 59.1 ± 5.9 | 10   | 11     |
| Group III| 25          | 59.9 ± 3.9 | 11   | 14     |
| Group IV | 17          | 60.6 ± 4.8 | 8    | 9      |

\( ^\circ C \)
metalloproteinase (MMPs), synthesized by chondrocyte, osteoclast and synoviocytes. Osteoarthritis is a disease characterized by continuous joint damage, including the wear of cartilage and change of synovial tissues. Studies showed that the increased expression of MMP-1, MMP-2, and MMP-9 proteins might be associated with the pathogenesis of osteoarthritis (OA). MMPs are Zn2+ and Ca2+-dependent proteinases, and the activity of MMPs are regulated by Ca2+ concentration. Type II collagen was degraded by MMP-1 into 3/4 or 1/4 fragments. These fragments can also be degraded by other MMPs into CTX-II. These fragments can also be degraded by other MMPs into CTX-II.

In this study, we found that the concentration of Zn2+ and Ca2+ was higher in KOA patients than healthy individuals, and Zn2+ and Ca2+ in urine from KOA patients and found that CTX-II was positively related to the KOA radiographic grading. The concentration of Zn2+ and Ca2+ was higher in KOA patients than healthy individuals, but not related to KOA radiographic grading. The concentration of Zn2+ and Ca2+ in urine is also helpful for early diagnosis for KOA.

5. Conclusions

In summary, we examined the change of CTX-II, Zn2+, and Ca2+ in urine from KOA patients and found that CTX-II was positively related to the KOA radiographic grading. The change of Zn2+ and Ca2+ was higher in KOA patients than healthy individuals, but not related to KOA radiographic grading. The concentration of CTX-II is instrumental to diagnose the progress of KOA. The concentration of Zn2+ and Ca2+ in urine is also helpful for early diagnosis for KOA.

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Figure 3. Comparison of the concentration of Zn2+ in urine between control group and each KOA group (* means P<.05 vs control group). KOA=knee osteoarthritis.
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