Introduction

Osteoarthritis (OA) is a chronic, debilitating joint disease characterized by the degeneration of articular cartilage, sclerosis of the subchondral bone, and osteophyte formation. This work aimed at estimating the level of urinary C-terminal telopeptide of type II collagen (CTX-II) as a biomarker of cartilage turnover and to determine its relation with radiological and functional assessment of knee OA.

Patients and methods

The current study included 40 postmenopausal women with symptomatic knee OA fulfilling the American Rheumatism Association clinical diagnostic criteria for knee OA. A total of 20 healthy volunteers were enrolled as a control group. Patients were assessed radiologically using the Kellgren–Lawrence grading system and functionally using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). Urinary CTX-II was measured for the patient and control groups.

Results

There was no statistically significant difference as regards age and BMI between patients and controls. Disease duration affects both function assessed using the WOMAC and cartilage degradation assessed using urinary CTX-II. There was a statistically significant correlation between the WOMAC and urinary CTX-II, whereas there was no statistically significant correlation between the Kellgren–Lawrence scale and both urinary CTX-II and the WOMAC.

Conclusion

This study further confirms that urinary CTX-II is an index of early cartilage degradation in knee OA even before radiological changes occur. The functional assessment using the WOMAC is an easy inexpensive method in reflecting cartilage degradation. Moreover, this work supports the lack of association between the functional status of knee OA patients assessed using the WOMAC and their radiological severity measured using the Kellgren–Lawrence grading scale.

Keywords:

knee osteoarthritis, urinary C-terminal telopeptide of type II collagen, Western Ontario and McMaster Universities Osteoarthritis Index

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The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) is generally recommended as the most sensitive, condition-specific instrument [13–16]. It is a disease specific self-report questionnaire for measurement of the symptoms of OA of the hips and knees. The WOMAC has a reliability, validity, and sensitivity to changes in the health status of patients with knee OA [17,18].

The aim of the present work was to estimate the biomarker of cartilage turnover CTX-II in urine and to determine its relation with both radiological severity and the functional assessment of knee OA.

Patients and methods

The current study included 40 postmenopausal women with symptomatic knee OA fulfilling the American Rheumatism Association clinical diagnostic criteria for knee OA [19]. Patients were randomly selected from those attending the Outpatient Clinic of Physical Medicine, Rheumatology, and Rehabilitation Department at University of Alexandria. Patients having any of the following conditions were excluded from the study: inflammatory knee disorders, other arthropathies, metabolic bone disease, serious systemic diseases, neoplasms, history of knee trauma or surgery and previous intra-articular injections. The control group consisted of 20 postmenopausal healthy female volunteers without clinical or radiological evidence of knee OA. Their ages and BMI were matched to that of the patient group.

Height and weight were measured with the individual barefoot and lightly dressed. The BMI was calculated as BMI = weight (kg)/height (m²).

The most symptomatic knee was assessed:

(a) Using a weight-bearing anteroposterior radiograph of the knee and,
(b) By means of Functional Assessment using the WOMAC [13,15] 3.1 Likert version.

The former was recorded with the patient standing with toes pointed straight ahead, knees fully extended, and weight equally distributed on both feet. Radiographs were scored using the Kellgren–Lawrence (K–L) grading system. A K–L grade of 0 indicates that no radiographic features of OA are present, a K–L grade of 1 is defined as doubtful joint space narrowing (JSN) and possible osteophytic lipping, and a K–L grade of 2 denotes the presence of definite osteophytes and possible JSN on anteroposterior weight-bearing radiograph. Higher disease progression is graded as K–L 3, presented with multiple osteophytes, definite JSN, sclerosis, and possible bony deformity, and, lastly, grade 4 is defined by large osteophytes, marked JSN, severe sclerosis, and definitely bony deformity [20]. The WOMAC 3.1 Likert version had five response levels for each item. It represents different degrees of intensity (none, mild, moderate, severe, or extreme) that were scored from 0 to 4. The final score for the WOMAC was determined by adding the aggregate scores for pain, stiffness, and function. Scores ranged from 0 to 96 for the total WOMAC, where 0 represents the best health status and 96 the worst possible status. The higher the score, the poorer is the function.

Urinary CTX-II was measured for both groups using ELISA, a sandwich enzyme immunoassay for the in-vitro quantitative measurement of CTX-II in urine. Second-void morning urine samples were obtained for the assessment of urinary CTX-II [21–25]. Thereby, the circadian changes in biomarker levels over the day and changes due to food intake were neglected [25,26].

All participants were informed about the aims of the study and the study protocol, and informed consent was obtained before the study.

Data were analyzed using the statistical package for the social sciences (SPSS, version 20; SPSS Inc., an IBM Company, Chicago, Illinois, USA) software. Differences were considered as significant if P value was 0.05 or greater.

Results

The present study included 40 postmenopausal women clinically diagnosed as having knee OA. Their mean age was 56.97 ± 3.573 years. The control group consisted of 20 postmenopausal healthy female volunteers with matched age and BMI. Their mean age was 54.9 ± 3.507 years. The disease duration of OA patients ranged from 3 to 12 years (mean: 6.61 ± 2.5 years). The WOMAC score, including its items pain, stiffness, and physical function, ranged from 30 to 73, with a mean of 57.025 ± 11.145.

On radiographic assessment using the K–L grading scale, six patients were of grade 1, 16 patients were of grade 2, and 18 patients were of grade 3.

There was no statistically significant difference as regards age and BMI between patients and controls, whereas urinary CTX-II was statistically higher in patients than in controls (Table 1).

There were statistically significant positive correlations between the disease duration and both WOMAC and
urinary CTX-II. No correlation could be detected between the disease duration and the K–L scale. BMI had no significant effect on WOMAC, K–L scale, and urinary CTX-II (Table 2).

A statistically significant positive correlation was found between urinary CTX-II and WOMAC. No correlation could be found between urinary CTX-II and the K–L scale (Table 3), as well as between WOMAC and the K–L scale (Table 4).

**Discussion**

In this study, we assessed urinary CTX-II as a biochemical marker of cartilage degradation and investigated if there was relation between it and both radiological severity and the functional assessment of knee OA among postmenopausal women.

The effect of sex, age, and BMI on the urinary CTX-II level is well established in the literature [24,25]; the selection of patients was made to justify these variables as much as possible. The selected patients were all postmenopausal women. Evidence indicates that OA and cartilage degradation could be related to sex hormones, as decreased estrogen levels in both animal models and women after menopause are associated with increased cartilage degradation [24,26]. Moreover, a previous study on monkeys showed that ovariectomy induced OA lesions of articular cartilage [27]. The increased urinary CTX-II concentration with increased age was explained previously to reflect the increased prevalence of radiographic OA with increasing age [25]. The selection of the studied patient’s age was made randomly and it ranged from 50 to 60 years. The mean range of our patients was 56.9 years. This age is younger than the mean age in the studies conducted among other populations [28,29], but is in accordance with previous Egyptian studies on OA patients [30,31]. This may be attributed to heavy physical activity among the studied patients. Rossignol et al. [32] found that early onset of OA was seen among heavy workers, with almost 40% of patients reporting their first symptoms before the age of 50. In order to avoid the influence of BMI on urinary CTX-II concentration, the selection of the control group was made to be matched with the patient group. Moreover, the circadian rhythm could influence the urinary CTX-II concentration and thus second-void morning urine samples were obtained to exclude diurnal variations [22,23].

This adjustment of age, sex, and BMI between the patient and control groups was made to isolate the effect of cartilage degradation on urinary CTX-II level in the patient group.

| Variables | Patients (n = 40) (mean ± SD) | Controls (n = 20) (mean ± SD) | t | P |
|-----------|-------------------------------|--------------------------------|---|---|
| Age       | 56.97 ± 3.57                  | 54.9 ± 3.50                    | 2.16 | 0.05 |
| BMI       | 32.19 ± 1.35                  | 31.54 ± 0.75                   | 2.00 | 0.06 |
| Urinary CTX-II | 435.45 ± 64.3 | 234.95 ± 9.91                   | 13.8 | 0.0001* |

**Table 2** The correlation between urinary C-terminal telopeptide of type II collagen and the Western Ontario and McMaster Universities Osteoarthritis Index, Kellgren–Lawrence score, and disease duration and body mass index

| Variables          | WOMAC            | Kellgren–Lawrence scale | Urinary CTX-II |
|--------------------|------------------|-------------------------|---------------|
| R                  | P                | R                       | P             |
| Disease duration   | 0.609*           | 0.000                   | 0.293         |
| BMI                | 0.032            | 0.844                   | 0.058         |
| Urinary CTX-II     | 0.628*           | 0.000                   | 0.723         | 0.008 | 0.962 |

WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; *Statistically significant at P ≤ 0.05.

| Variables | Urinary CTX-II (mean ± SD) | Controls (mean ± SD) | t | P |
|-----------|-----------------------------|----------------------|---|---|
| WOMAC     | 0.950*                      | 0.000                |
| Kellgren–Lawrence scale | 0.088 | 0.590 |

| Variables | WOMAC (mean ± SD) | Controls (mean ± SD) | t | P |
|-----------|-------------------|----------------------|---|---|
| Kellgren–Lawrence score | 0.088 | 0.590 |

WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; *Statistically significant at P ≤ 0.05.

The WOMAC among the studied patients ranged from 30 to 73, with more than 60% of them above 50. None of them had WOMAC above 73 as the individuals in this study had to be mobile enough to visit the rehabilitation center three times per week.

According to the K–L scale, the majority of the studied patients were of grade 2, which means small osteophytes, possible narrowing of the joint. None of them were of grade 4, because this study included mobile and relatively healthier individuals. A limitation of using
plain radiographs for detecting cartilage degradation is that significant cartilage degradation must have occurred in order to be visible on a radiograph [33].

The high level of urinary CTX-II in the patient group as compared with the control group was found in this study. Indeed urinary CTX-II is a specific marker for cartilage degradation [25].

In the current study, there were significant positive correlations between duration of joint disease and both urinary CTX-II ($r = 0.628$, $P = 0.000$) and WOMAC score ($r = 0.609$, $P = 0.000$). This expected finding is attributed to the fact that, the longer the disease duration, the longer the duration of cartilage destruction, leading to more release of biomarkers and more clinical findings. However, no significant correlation could be found between the disease duration and the K–L grading scale. This is in accordance with the findings of Garnero et al. [3], who reported weak associations of urinary CTX-II with prevalent radiographic knee OA, and contradictory to the findings of Cubukcu et al. [34], who demonstrated that age and disease duration were found to be positively associated with the K–L grading scale.

In our study, a significant positive correlation was found between WOMAC and urinary CTX-II. The greater the cartilage degeneration, the greater the release of biomarkers and presence of pain and disability that occurs even before radiological changes. In contrast, no significant correlation could be found between urinary CTX-II and the K–L grading scale. Many studies have been conducted to investigate the relationship between radiographic severity and disability in knee OA. There is a widespread belief that there is a high discordance between clinical and radiographic knee OA. In a study of over 6000 patient, Hannan et al. [35] found that around half of those patients with radiographic K–L score of 2–4 reported knee pain. Falaffi et al. [36] displayed that the level of disability experienced by patients with knee OA has been shown to correlate more accurately with their age and psychological involvement than with their radiographic scores.

In many published studies, it has been shown that the K–L score was not related to WOMAC score but it was important to follow-up the progress of the disease [37,38]. The Framingham Osteoarthritis Study found that 10% of people aged 63 years and over had symptomatic knee OA in the presence of radiographic changes [39]. Individuals with radiographic evidence of OA may be asymptomatic at any time. In the relevant literature, results are contradictory as some studies [35,36] reported no association between pain scores and radiographic features and others [40–42] found that radiographic features of OA were significantly associated with knee pain. In this study, radiological findings did not correlate with the severity of pain as assessed with WOMAC. These results may be due to our patients’ characteristics as they were mostly categorized as mild-to-moderate for radiographic features. In contrast, conventional radiography, which is the most commonly used imaging modality, may not identify bony changes related to pain in early knee OA. Radiographs demonstrate structural changes rather than disease severity. Conventional radiography permits only limited assessment of the three knee compartments, provides only an approximation of articular cartilage change with measurement of JSN, and poorly characterizes other soft tissues [43].

### Conclusion

This study further confirms that urinary CTX-II is an index of early cartilage degradation in knee OA even before radiological changes occurs. The functional assessment using WOMAC is an easy inexpensive method in reflecting cartilage degradation. Moreover, this work supports the lack of association between the functional status of knee OA patients assessed with WOMAC and their radiological severity measured using the K–L grading scale.

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### Conflicts of interest

There are no conflicts of interest.

### References

1. Alkan BM, Fidan F, Tosun A, Ardiçöğlu Ö. Quality of life and self-reported disability in patients with knee osteoarthritis. Mod Rheumatol 2014; 24:166–171.
2. Duddy J, Kirwan JR, Széchenyi B, Clarke S, Granell R, Volkov S. A comparison of the semiflexed (MTP) view with the standing extended view (SEV) in the radiographic assessment of knee osteoarthritis in a busy routine X-ray department. J Rheumatol 2005; 44:349–351.
3. Garnero P, Piperno M, Ginneys E, Christgau S, Delmas PD, Vignon E. Cross sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee osteoarthritis: relations with disease activity and joint damage. Ann Rheum Dis 2001; 60:619–626.
4. Garnero P, Ayal X, Rousseau JC, Christgau S, Sandell LJ, Dougados M, et al. Uncoupling of type II collagen synthesis and degradation predicts progression of joint damage in patients with knee osteoarthritis. Arthritis Rheum 2002; 46:2613–2624.
5 Christgau S, Garnero P, Fledelius C, Moniz C, Ensig M, Gineys E, et al. Collagen type II C-telopeptide fragments as an index of cartilage degradation. Bone 2001; 29:209–215.

6 Vignon E, Garnero P, Delmas P, Avouac B, Bettica P, Boers M, et al. Recommendations for the registration of drugs used in the treatment of osteoarthritis: an update on biochemical markers. Osteoarthritis Cartilage 2001; 9:289–293.

7 Poole AR. Biochemical/immunochemical biomarkers of osteoarthritis: utility for prediction of incident or progressive osteoarthritis. Rheum Dis Clin North Am 2003; 29:803–818.

8 Rafterd JH, Reinhold FP Beckstrom KJ, Risberg M, Årøen A. Relationship between CTX-II and patient characteristics, patient-reported outcome, muscle strength, and rehabilitation in patients with a focal cartilage lesion of the knee: a prospective exploratory cohort study of 48 patients. BMC Musculoskeletal Disord 2014; 15:99.

9 Dahliert B, Billinghurst C, Manner P, Ionescu M, Reiner A, Tanzer M, et al. Collagenase-mediated cleavage of type II collagen is selectively enhanced in osteoarthritis cartilage and can be arrested with a synthetic inhibitor which spares collagenase-1 (MMP-1). Arthritis Rheum 2000; 43:673–682.

10 Squires G, Ionescu M, Okouneff S, Poole AR. The pathobiology of focal lesions development in aging human articular cartilage and molecular matrix changes characteristic of osteoarthritis. Arthritis Rheum 2003; 48:1261–1270.

11 Poole AR. Cartilage in health and disease. In: Koopman W, editor Arthritis and allied conditions: a textbook of rheumatology. 14th ed Philadelphia: Lippincott, Williams & Wilkins; 2001. 226–284.

12 Atley LM, Sharma L, Clemens JD, Shaffer K, Pietka TA, Riggins JA, et al. The collagen II CTX degradation marker is generated by collagenase 3 and in urine reflects disease burden in knee OA patients. Trans Orthop Res Soc 2000; 25:168.

13 Guermazi M, Poiradeau S, Yahia M, Mezganni M, Fernandez J, Habib Elleuch M, et al. Translation; adaptation, and validation of Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) for an Arab population: the Slax modified WOMAC. Osteoarthritis Cartil 2004; 12:259–268.

14 Escobar A, Quintana JM, Bilbao A, Aróstegui I, Lafuente I, Vidaurreta I. Responsiveness and clinically important differences for the WOMAC and SF-36 after total knee replacement. Osteoarth Cartil 2007; 15:273–280.

15 Bellamy N. WOMAC osteoarthritis index: a user's guide. London: University of Western Ontario; 1995.

16 Ehrich EW, Davies GM, Watson DJ, Bolognese JA, Seidenberg BC, Bellamy N. Minimal perceptible clinical improvement with the Western Ontario and McMaster Universities osteoarthritis index questionnaire and global assessments in patients with osteoarthritis. J Rheumato 2000; 27:2635–2641.

17 Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW. Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy with patients of osteoarthritis of the hip or knee. J Rheumatol 1988; 15:1833–1840.

18 Hmamouchi I, F Al, Tahiri L, Khazzani H, EL Mansouri L, Sanea A, et al. Clinically important improvement in the WOMAC and predictor factors for response to non-specific non-steroidal anti-inflammatory drugs in osteoarthritis patients: a prospective study. BMC Research Notes 2012; 5:58.

19 Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. Arthritis Rheum 1986; 29:1039–1049.

20 Kellgren JH, Lawrence JS. Radiological assessment of osteo-arthritis. Ann Rheum Dis 1957; 16:494–502.

21 Jansen NW, Roosendaal G, Lundin B, Heijnen L, Mauser-Bunschoten E, Bijlsma JW, et al. The combination of the biomarkers urinary C-terminal telopeptide of type II collagen, serum cartilage oligomeric matrix protein, and serum chondroitin sulfate 446 reflects cartilage damage in hemophilic arthropathy. Arthritis Rheum 2009; 60:290–298.

22 Andersson ML, Peterson IF, Karlsson KE, Jonsson EN, Mansson B, Heinigard D, et al. Diurnal variation in serum levels of cartilage oligomeric matrix protein in patients with knee osteoarthritis or rheumatoid arthritis. Ann Rheum Dis 2006; 65:1490–1494.

23 Kong SY, Stabler TV, Criscione LG, Elliott AL, Jordan JM, Kraus VB, et al. Diurnal variation of serum and urine biomarkers in patients with radiographic knee osteoarthritis. Arthritis Rheum 2006; 54:2496–2504.

24 Dam EB, Bjorjalsen I, Karstad MA, Qvist P, Christiansen C. Increased urinary excretion of C-telopeptides of type II collagen (CTX-II) predict cartilage loss over 21 months by MRI. Osteoarthritis Cartilage 2009; 17:384e389.

25 Reijman M, Hazes JM, Biema-Weintra SM, Koes BW, Christgu S, Uttenfliet AG, et al. A new marker for osteoarthritis-cross sectional and longitudinal approach. Arthritis Rheum Dis 2004; 59:2471–2478.

26 Karstad MA, Bjorjalsen AC, Bay Jensen AC, Henriksen K, Rils BJ, Christiansen C. Biochemical markers identify influences on bone and cartilage degradation in osteoarthritis — the effect of sex, Kellgren–Lawrence score (KL) score, body mass index (BMI), oral salmon calcitonin (SCT) treatment and diurnal variation. BMC Musculoskeletal Disorders 2010; 11:125.

27 Ham KD, Looser RF, Lindgren BR, Carlson SC. Effects of long term estrogen replacement therapy on osteoarthritis severity in cynomolgous monkeys. Arthritis Rheum 2002; 46:1956–1964.

28 Cimen OB, Incel NA, Yapiy C, Apaydin D, Erdogan C. Obesity related measurements and joint space width in patients with knee osteoarthritis. J Med Sci 2004; 109:159–164.

29 Miller GD, Nicklas BJ, Looser RF. Inflammatory biomarkers and physical function in older, obese adults with knee pain and self-reported osteoarthritis after intensive weight-loss therapy. J Am Geriatr Soc 2008; 56:644–651.

30 Darwish AF, Abdel-Ghany HS, El-Sherbini YM. Diagnostic and prognostic value of some biochemical markers in early knee osteoarthritis. Egypt Rheumatol 2012; 34:1-8.

31 Shereif W, Hassanin AA. Comparison between uses of therapeutic exercise and heat application on relieve pain and improvement of physical function for patients with knee osteoarthritis. Life Sci J 2011; 8:388–396.

32 Rossignol M, Leclerc A, Allaret FA, Rozenberg S, Valat JP, Vouac BA, et al. Primary osteoarthritis of hip, knee, and hand in relation to occupational exposure. Occup Environ Med 2005; 62:772–777.

33 Cibere J, Zhang H, Garnero P, Poole AR, Lobanok T, Saane T, et al. Association of biomarkers with pre-radiographically defined and radiographically defined knee osteoarthritis in a population based study. Arthritis Rheum 2009; 60:1372–1380.

34 Cubukcu D, Sarsan A, Alkan H. Relationship between pain, function and radiographic findings in osteoarthritis of the knee: a cross-sectional study. Arthritis Rheum 2012; 1–5.

35 Hannan MT, Felson DT, Pincus T. Analysis of the discordance between radiographic changes and knee pain in osteoarthritis of the knee. J Rheumatol 2000; 27:1513–1517.

36 Falaffi F, Cavaliere F, Noli M, Ferracelli G. Analysis of disability in knee osteoarthritis. Relationship with age and psychological variables but not with radiographic score. J Rheumatol 1991; 18:1581–1586.

37 Küçü G, Yank B, Atalar H, Gütlen G. Associated factors with pain and disability in patients with knee osteoarthritis. Turk J Rheumatol 2010; 25:77–81.

38 Rupperten TN, Ozcipka F, Lüring C, Pennekamp PH, Grifka J. Is there a correlation between the clinical, radiological and intra-surgical findings of osteoarthritis of the knee? A prospective study on 103 patients. Z Orthop Unfall 2007; 145:430–435.

39 Felson TD, Naimark A, Anderson J. The prevalence of knee osteoarthritis in the elderly. The Framingham Osteoarthritis Study. Arthritis Rheum 1987; 30:914–918.

40 McAlindon TE, Cooper C, Kirwan JR, Dieppe PA. Determinants of disability in osteoarthritis of the knee. Ann Rheum Dis 1993; 52:258–262.

41 Lethbridge-Cejku M, Scott WJ, Reichle R, Ettinger WH, Zonderman A, Costa A. Association of radiographic features of osteoarthritis of the knee with pain: data from the Baltimore Longitudinal Study of Aging. Arthritis Care Res 1995; 8:182–188.

42 Cicuttini FM, Baker J, Hart DJ, Spector TD. Association of pain with radiological changes in different compartments and views of the knee joint. Osteoarth Cartilage 1996; 4:143–147.

43 Hodler J, Resnick D. Current status of imaging of articular cartilage. Skeletal Radiol 1996; 25:703–709.