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 occurs. 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
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 ± 3.50.7 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.

### Table 1 Age, body mass index, and urinary C-terminal telopeptide of type II collagen of the patient and the control group

<table>
<thead>
<tr>
<th>Variables</th>
<th>Patients (n = 40) (mean ± SD)</th>
<th>Controls (n = 20) (mean ± SD)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>56.97 ± 3.57</td>
<td>54.9 ± 3.50</td>
<td>2.16</td>
<td>0.05</td>
</tr>
<tr>
<td>BMI</td>
<td>32.19 ± 1.35</td>
<td>31.54 ± 0.75</td>
<td>2.00</td>
<td>0.06</td>
</tr>
<tr>
<td>Urinary CTX-II</td>
<td>435.45 ± 64.3</td>
<td>234.95 ± 9.91</td>
<td>13.8</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

CTX-II, C-terminal telopeptide of type II collagen; *Statistically significant at \( P \leq 0.05 \).

### Table 2 The correlation between urinary C-terminal telopeptide of type II collagen with the Western Ontario and McMaster Universities Osteoarthritis Index and the Kellgren–Lawrence scale

<table>
<thead>
<tr>
<th>Variables</th>
<th>WOMAC</th>
<th>Kellgren–Lawrence scale</th>
<th>Urinary CTX-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>P</td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>Disease duration</td>
<td>0.690*</td>
<td>0.000</td>
<td>0.170</td>
</tr>
<tr>
<td>BMI</td>
<td>0.032</td>
<td>0.844</td>
<td>0.058</td>
</tr>
</tbody>
</table>

CTX-II, C-terminal telopeptide of type II collagen; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; *Statistically significant at \( P \leq 0.05 \).

### Table 3 The correlation between the Western Ontario and McMaster Universities Osteoarthritis Index, Kellgren–Lawrence score, and urinary C-terminal telopeptide of type II collagen with disease duration and body mass index

<table>
<thead>
<tr>
<th>Variables</th>
<th>WOMAC</th>
<th>Kellgren–Lawrence score</th>
<th>Urinary CTX-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>P</td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>WOMAC</td>
<td>0.950*</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Kellgren–Lawrence score</td>
<td>0.088</td>
<td>0.590</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4 The correlation between the Western Ontario and McMaster Universities Osteoarthritis Index and the Kellgren–Lawrence scale

<table>
<thead>
<tr>
<th>Variables</th>
<th>WOMAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>0.154</td>
</tr>
<tr>
<td>P</td>
<td>0.342</td>
</tr>
</tbody>
</table>

WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

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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Dr. Hayam Mostafa and Dr. Sarah Sayed El Tawab performed the research, analyzed the data and wrote the paper. Dr. Ahmed Mohamed Moghazy performed the laboratory evaluation of the participants and wrote the paper.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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