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**ORIGINAL ARTICLE**

# Evaluation of the Level of Type II Collagen C-Terminal Telopeptide in Urine in Patients with Early Knee Osteoarthritis.

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## ABSTRACT

**Background:** Knee osteoarthritis is a multifactorial disorder that has been identified as a major source of disability. Greater urinary C-telopeptide pieces of type II collagen (UCTXII) amounts were associated with greater activity of the disease scores; thus, the purpose of the present research was to compare urinary CTX-II levels between patients with early KOA and control subjects, as well as to find out the relationship between urinary CTX-II levels, radiographic detection of OA, and outcome reported by patients.

**Methods:** This study involved 90 people, 45 with osteoarthritis (cases) and 45 healthy people (control group). All patients received a diagnosis with KOA using the 2016 ACR clinical and radiological categorization criteria. Everyone who participated in the study signed a written consent form. We included individuals with Kellgren-Lawrence (KL) grade 0 or 1 knee OA in our study.

**Results:** There was a statistically significant beneficial relationship among UCTXII and the WOMAC, the visual analogue scale score, C-reactive protein, and the rate of erythrocyte sedimentation at the Western Ontario and McMaster Colleges. There was, however, no substantial link among UCTXII and age or BMI. The optimum Urinary CTXII threshold value for distinguishing osteoarthritis patients from controls was > 85.25. This point demonstrated a high level of sensitivity and specificity, as well as a statistically substantial result.

**Conclusion:** According to our findings, urine C-telopeptide fragments of type II collagen (UCTXII) constitute an efficient, harmless, and viable method for detecting early knee OA.

**Keywords:** Western Ontario and McMaster Universities Osteoarthritis Index, Urinary C-Terminal Telopeptide, Type II Collagen.

## INTRODUCTION

Osteoarthritis (OA) is the most common joint disease, causing significant disability in most adults over the age of 55 [1]. The knee is the most affected joint, manifested with pain, stiffness, and considerable functional disability especially in women [2]. OA patients can be treated earlier if the disease is detected in its early stages. As a result, looking for a morning OA scanning tool is a critical step in the identification and therapy of OA [3].

Age, gender, trauma, misuse, heredity, and weight may all contribute to the occurrence of damage in various joint compartments These hazards may serve as catalysts for cartilage biologic reactions that are aberrant, affecting bone, cartilage, and synovial membrane, resulting in the OA-specific characteristics over time [4] and, in advanced stages, joint contractures, muscle atrophy, and limb deformity [5]. Many biomarkers have been studied to better understand the prognosis and pathogenesis of osteoarthritis [6]. C-telopeptide fragments of type II collagen (CTX-II), which are produced during articular cartilage breakdown and excreted in urine, are one such biomarker. Urinary CTX-II levels have been found to be associated with the presence and severity of knee OA [7].

Collagen type II degradation in urine can be evaluated by looking for segments of hexagonal (Helix-II) or C-telopeptide (CTX-II) areas detected largely in cartilage made from hyaline. CTX-II has received increasing attention., and it is higher in osteoarthritic individuals than in normal, and it

predicts the severity and progression of knee OA [6]. CTX-II may be a specific marker of cartilage degradation that can be used for clinical monitoring of OA [8]. Patients with high CTX-II levels have an 8-fold higher chance of developing OA. The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) is widely regarded as the most sensitive, condition specific tool [9].

WOMAC is a disease-specific self-report questionnaire used to assess hip and knee OA symptoms. The WOMAC has been shown to be reliable, valid, and sensitive to changes in the health status of patients with knee OA [10]. This study aimed to examine urine CTX-II levels in individuals who have early osteoarthritis of the knee and control subjects, as well as to look for a link between urinary CTX-II levels, radiographic extent of OA, and outcomes reported by patients.

## METHODS

A total of 45 male and female patients with early KOA were invited to participate in this study. They were selected from the Physical Medicine, Rheumatology and Rehabilitation Department of Mansoura University Hospital's outpatient clinic. They were all diagnosed with knee OA according on the 2016 ACR clinical and radiological diagnostic criteria [11]. Each participant in the study signed a written consent form. The institution of research board of faculty of medicine, Mansoura University, code: MS/21.05.1500, approved this study. The control group consisted of forty-five (45) apparently healthy volunteers who were matched for age and gender. This study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki).

This study involved adult males and females aged 40 and up who were diagnosed with knee OA based on the Kellgren-Lawrence (KL) score (at least one knee joint was classified K-L 1). Patients who did not have an intra-articular lesion that required surgery or were likely to require surgery during the research period. We excluded from this study any subjects under the age of 40, with a KL records greater than grade 1, a history of arthroscopy within the previous 6 months, present or prior acute inflammatory joint disease or any other rheumatic illness, a history of consumption of any osteoarthritis instructed medication or the use of hyaluronic acid injections within the six-month period before inclusion, acute significant trauma, and a history of chronic kidney disease (glomerular filtration rate (GFR)).

All patients in the current investigation were subjected to a history taking that included their personal information (name, date of birth, profession, where they live, marital relationship, and special behaviors such as cigarette smoking) and present history, which included evaluation of pain regarding the site, beginning, duration, course, nature, what exacerbate pain and what decrease it , symptoms related to other rheumatic conditions such as fever, oral ulcer, sensitivity to light, slipping hair, rashes of the skin, irritation of mouth, difficulty swallowing, Raynaud's phenomenon, as well as other symptoms indicating different system affections such as cerebral, cardiovascular, pulmonary, gastrointestinal, genitourinary, vascular, and cutaneous manifestations. We inquired about previous knee injuries, whether direct or indirect, previous surgery on the knee, previous intra-articular injection, and previous hospitalization, therapeutic history, and family history of comparable condition or rheumatic disease.

In this study, physical examination included general examination in the form of general circumstance, movement patterns, and skin color, vital signs (pulse, blood pressure, respiratory rate, and body temperature), body measurements (weight, height, and BMI), skin for subcutaneous swelling (nodules, tophi), or other skin lesions suggesting collagen diseases, and lymph node examination, while systemic examination included cardiovascular examination, chest examination, abdominal examination, neurological examination, and lymph node assessment.

All patients had bilateral weight bearing anteroposterior x-rays of the knee and were rated according to the Kellgren-Lawrence score (0-4) [12]. Anteroposterior and lateral views of the spine was performed to rule out OA or spine spondylosis.

Second-void morning urine samples were collected for the evaluation of urinary CTX-II, complete blood count, ESR, CRP, serum creatinine, serum uric acid, synovial fluid aspiration and evaluation in patients with effusion, and urinary CTX-II.

For assay of Urinary CTX-II, a urine sample was collected in a sterile container and centrifuged for 20 minutes at a speed of 2000-3000 R.P.M (rotation per minute), centrifuged again if precipitation appears after removing the supernatant. A double-antibody sandwich ELISA was used in the package to ascertain the status of human beings CTX-II inside tests. Addition of CTX-II to a monoclonal antibody-containing enzyme microplate that has already been applied using human CTX-II monoclonal immunoglobulin exposure, then addition of biotin-labeled CTX-II antibodies coupled with Streptavidin-HRP to form immunological complex, then exposure and rinsing repeated to eliminate the separated enzyme. When Chromogen Reagent A, B, is added the color of the

fluid turns blue, and when acid is added, the color transforms to yellow. The color chroma and the amount of human material CTX-II levels in the specimen were found to be directly associated.

Assessment of pain and function were based upon *Visual Analogue Scale (VAS)*, which is a 0-10 scale for determining the severity of knee discomfort. The level of pain varies from 0 (no pain) to 10 (worst pain), and upon *WOMAC (Western Ontario and McMaster Universities Osteoarthritis Index)* [13], which is a three-part questionnaire, each section evaluates a separate item, such as discomfort, stiffness, and functional impairment in ADL. The first portion includes 5 inquiries regarding discomfort, the second portion includes 2 inquiries concerning morning and immobility stiffness, and the last portion includes 17 inquiries about challenge in doing everyday activities. All these questions are rated on a scale of 0 to 4. The overall score runs from 0 to 96. The pain scale spans from 0 to 20. The stiffness score ranges from 0 to 8. The functional limitation score varies between 0 and 68 (Fig 1).

**Statistical analysis:**The SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA) was used for programming, manufacturing, and evaluating the obtained data. The data distribution was checked for normalcy using the Shapiro Walk test. To represent qualitative data, percentages and proportions in relation were used. The chi square test (2) and Fisher exact were used to calculate the difference between the qualitative variables, as illustrated. Standard deviation (SD) was used to express quantitative data as mean. The Mann Whitney U test was used for non-normally distributed data (non-parametric data), while the t-test for independent samples was employed for contrasting two distinct sets of regularly dispersed parameters. The Youden index J was used for establishing the ideal cutoff value, which is the ROC curve point furthest away from the vertical line of parity [maximum (sensitivity + specificity)].

## RESULTS

The current study involved 90 people, 45 with osteoarthritis (cases) and 45 healthy people (control group). Table (1) shows that the mean age in the cases group was 44.93 ± 3.94 years and the mean age in the control group was 46.42 ± 7.74 years, where there is not a statistically significant distinction between the two subgroups (p= 0.253).

The cases group had a total of eight men (17.8%) with 37 females (82.2%), whereas the control group had twelve men (26.7%) and 33 females (73.3%), with no statistically significant distinction between the two classes (p=0.310). The mean body mass index in the cases group was 36.01 ± 6.36 Kg/m2 and the mean BMI in the control group was 32.06 ± 4.95 Kg/m2 with statistically significant difference between the two groups (p= 0.253). The prevalence of DM was 15.6% and 22.2% in the cases and the control group respectively, with no statistically significant difference between the two groups (p= 0.419). The prevalence of hypertension was 11.1% and 26.7% in the cases and the control group respectively, with no statistically significant difference between the two groups (p= 0.059). The prevalence of positive family history was 64.4% in the cases group that was statistically significantly higher compared to the control group (33.3%) (p= 0.003).

Table (2) demonstrates the mean WOMAC score in the cases group was 16.96 ± 9.08 and the median score was 16 with range between 4 and 40. The mean pain at rest score in the cases group was 0.67 ± 1.11 and the median score was 0 with range between 0 and 4. The mean pain at activity score in the cases group was 3.67 ± 1.83 and the median score was 3 with range between 1 and 3.The KL score was 1 in all the cases. All the cases had Joint crepitus on palpation. There were 41 cases (91.1%) with knee tenderness, 30 cases (66.7%) with Knee pain induced by movement and there were 3 cases (6.7%) with limited range of movement.

Table (3) shows that the median ESR in the cases group was 15 mm/h (range: 4-30) and in the group in charge was 13 mm/h (range: 4-30), with no statistically significant distinction between the two classes (p= 0.621). The mean CRP in the cases group was 2.5 mg/dl (range: 1.2-4.6) and in the group in charge was 2.3 mg/dl (range: 1.2-5.8), with no significant statistical distinction among the two classes (p= 0.381). The median CTXII level in the cases group was 224.3 (range: 59.4-362.8) and in the group in charge was 55.4 (range: 0-141.5). The level of CTXII was statistically considerably greater in the group of instances (p < 0.001).

Table (4) shows that, there was a significant positive relationship with Urinary CTXII and WOMAC, Pain at activity score, CRP, and ESR in the cases cohort. Other variables didn't show a statistically significant correlation.

Table (5) shows the best cutoff point of Urinary CTXII to identify osteoarthritis cases from the control was > 85.25. This point showed high degree of sensitivity and specificity, with high statistically significant value (p < 0.001).

**Table 1:** Demographic data analysis and clinical history in the two groups being studied.

| | | Cases team (N=45) | | The observer team (N=45) | | The value assessment |
|---|---|---|---|---|---|---|
| **Age (in years)** | | 44.93 ± 3.94 | | 46.42 ± 7.74 | | t= -1.150<br>P = 0.253 |
| **Gender** | **Male** | 8 | 17.8% | 12 | 26.7% | $\chi 2$= 1.029<br>P = 0.310 |
| | **Female** | 37 | 82.2% | 33 | 73.3% | |
| **BMI (Kg/m$^2$)** | | 36.01 ± 6.36 | | 32.06 ± 4.95 | | t= 1.322<br>P = 0.284 |
| **Diabetes mellitus** | | 7 | 15.6% | 10 | 22.2% | $\chi 2$= 0.653<br>P = 0.419 |
| **Hypertension** | | 12 | 26.7% | 5 | 11.1% | $\chi 2$= 3.554<br>P = 0.059 |
| **Positive family history of osteoarthritis** | | 29 | 64.4% | 15 | 33.3% | $\chi 2$= 8.715<br>P = 0.003* |

Continuous information reported as mean standard deviation.
Numbers are used to represent information that is categorical (percentage)
t = t-test for samples that were independent
$\chi 2$= Analysis of chi-squares
*: High numerically (P less than 0.05)
BMI: Body mass index

**Table 2:** Assessment of function, pain, radiological findings and Clinical manifestations in the case group.

| | | Cases group (N=45) |
|---|---|---|
| **WOMAC score** | **Mean ± SD** | 16.96 ± 9.08 |
| | **Median (range)** | 16 (4 -40) |
| **Pain at rest score** | **Mean ± SD** | 0.67 ± 1.11 |
| | **Median (range)** | 0 (0 -4) |
| **Pain at activity score** | **Mean ± SD** | 3.67 ± 1.83 |
| | **Median (range)** | 3 (1 -8) |
| **KL score** | | 1 |
| **Joint crepitus on palpation** | | 45(100%) |
| **Knee tenderness** | | 41 (91.1%) |
| **Knee pain induced by movement** | | 30 (66.7%) |
| **Limited range of movement** | | 3 (6.7%) |
| **Quadriceps wasting** | | 0 (0%) |
| **Knee joint swelling** | | 0 (0%) |

Continuous information reported as mean standard deviation ± SD & median (Variety)
KL: Kellgren and Lawrence
SD: Deviation from the mean

**Table 3:** Analysis of the laboratory data and urinary CTXII level in the two study groups.

| | Cases group (N=45) | Control group (N=45) | Test of Significance | P value |
|---|---|---|---|---|
| ESR (mm/h) | 15 (4-30) | 13 (4-30) | z = -0.494 | 0.621 |
| CRP (mg/dl) | 2.5 (1.2-4.6) | 2.3 (1.2-5.8) | z = -0.876 | 0.381 |
| **Urinary CTX II level** | | | | |
| Median (Range) (pg/ml) | 224.3 (59.4-362.8) | 55.4 (0-141.5) | | |

Continuous information reported as mean standard deviation ± SD & median (Variety)
t= independent samples t-test z= U-test. The use of Mann
*: High numerically (P less than 0.05)
CRP: stands for C-reactive protein.
ESR: stands for erythrocyte sedimentation rate.

**Table 4:** Correlation of urinary CTXII level with clinical and laboratory data in the cases group

| | | **Urinary CTXII** |
|---|---|---|
| Age | $r_s$ | -0.251 |
| | P | 0.096 |
| WOMAC | $r_s$ | 0.515 |
| | P | **< 0.001*** |
| Pain at rest | $r_s$ | 0.125 |
| | P | 0.411 |
| Pain at activity | $r_s$ | 0.445 |
| | P | **0.002*** |
| BMI | $r_s$ | 0.270 |
| | P | 0.073 |
| CRP | $r_s$ | 0.256 |
| | P | **0.049*** |
| ESR | $r_s$ | 0.366 |
| | P | **0.013*** |

rs: Spearman's correlation.
P: Probability.
*: High numerically (P less than 0.05).
WOMAC: McMaster and Western Ontario Universities.
BMI: stands for body mass index.
CRP: stands for C-reactive protein.
ESR: stands for erythrocyte sedimentation rate.

**Table 5:** Predictive value of urinary CTXII level in identifying cases from control group

| **Diagnostic criteria** | **Urinary CTXII level** |
|---|---|
| AUC | 0.962 |
| Cut off point | > 85.25 |
| P | **< 0.001*** |
| Sensitivity | **91.1 %** |
| Specificity | **86.7 %** |
| PPV | **92.2 %** |
| NPV | **88.4 %** |
| Accuracy | **90.1 %** |

AUC: stands for area under the curve.
P: stands for Probability.
PPV: stands for positive predictive value.

NPV: stands for negative predictive value.

| RATE YOUR PAIN WHEN... | NONE | SLIGHT | MODERATE | SEVERE | EXTREME |
|---|---|---|---|---|---|
| Walking | 0 | 1 | 2 | 3 | 4 |
| Climbing stairs | 0 | 1 | 2 | 3 | 4 |
| Sleeping at night | 0 | 1 | 2 | 3 | 4 |
| Resting | 0 | 1 | 2 | 3 | 4 |
| Standing | 0 | 1 | 2 | 3 | 4 |
| **RATE YOUR STIFFNESS IN THE...** | **NONE** | **SLIGHT** | **MODERATE** | **SEVERE** | **EXTREME** |
| Morning | 0 | 1 | 2 | 3 | 4 |
| Evening | 0 | 1 | 2 | 3 | 4 |
| **RATE YOUR DIFFICULTY WHEN...** | **NONE** | **SLIGHT** | **MODERATE** | **SEVERE** | **EXTREME** |
| Descending stairs | 0 | 1 | 2 | 3 | 4 |
| Ascending stairs | 0 | 1 | 2 | 3 | 4 |
| Rising from sitting | 0 | 1 | 2 | 3 | 4 |
| Standing | 0 | 1 | 2 | 3 | 4 |
| Bending to floor | 0 | 1 | 2 | 3 | 4 |
| Walking on even floor | 0 | 1 | 2 | 3 | 4 |
| Getting in/out of car | 0 | 1 | 2 | 3 | 4 |
| Going shopping | 0 | 1 | 2 | 3 | 4 |
| Putting on socks | 0 | 1 | 2 | 3 | 4 |
| Rising from bed | 0 | 1 | 2 | 3 | 4 |
| Taking off socks | 0 | 1 | 2 | 3 | 4 |
| Lying in bed | 0 | 1 | 2 | 3 | 4 |
| Getting in/out of bath | 0 | 1 | 2 | 3 | 4 |
| Sitting | 0 | 1 | 2 | 3 | 4 |
| Getting on/off toilet | 0 | 1 | 2 | 3 | 4 |
| Doing light domestic duties (cooking, dusting) | 0 | 1 | 2 | 3 | 4 |
| Doing heavy domestic duties (moving furniture) | 0 | 1 | 2 | 3 | 4 |

**Figure (1):** Western Ontario and McMaster Universities (WOMAC) Index of Osteoarthritis. A series of 24 questions

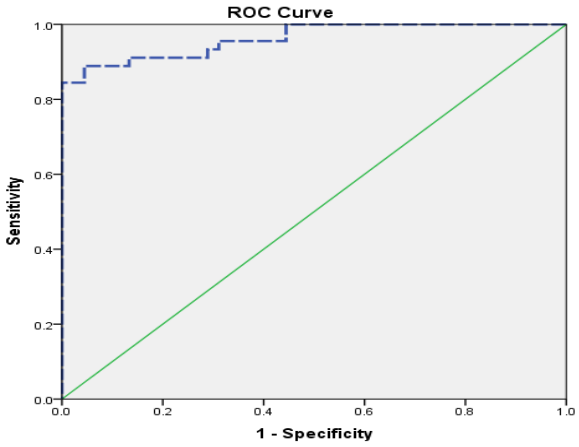


**Figure (2):** ROC curve of urinary CTXII level in identifying cases in the RA group.

## DISCUSSION

Osteoarthritis (OA) is the most prevalent persistent joint condition that causes joint discomfort and impairment. Patients with OA suffer from both physical and emotional health problems [14]. OA has lately been defined as a multi-joint illness characterized by articular cartilage deterioration, subchondral bone enlargement, synovial inflammation, ligament deterioration, and joint capsule hypertrophy. The condition develops because of a disparity among joint tissue healing and breakdown [15]. The better the prognosis, the earlier OA is recognized and treated. As a result, there is an urgent need to create procedures in clinical practice that are more effective in early diagnosis than radiography [16].

Biomarkers of OA have recently gained attention. Each biomarker's role in OA diagnosis, disease load, OA progression, and intervention efficacy was assessed [17]. OA is characterized by the breakdown of the extracellular matrix in articular cartilage. The most abundant and specific matrix protein in articular cartilage is type II collagen. It has already been demonstrated that cartilage degradation, particularly type II collagen deterioration, is an important phase in the course of knee osteoarthritis [18].

The urinary CTX-II (UCTX-II) is a type II collagen cross-linked C-terminal telopeptide (CTX-II) and has a dimeric-hexapeptide antigen (EKGPDP) with a porphyrin circle as a binder that is broken by matrix metalloproteinases. CTX-II diffuses from the joint to the bloodstream and is eventually eliminated in the urine [18]. As a result, the current study was carried out to evaluate the role of UCTX-II as a diagnostic marker in cases of osteoarthritis

and its relationship to disease severity. The current study comprised 45 patients with osteoarthritis and 45 seemingly healthy people who were matched for age and gender and recruited from Mansoura university hospitals.

In the current study, the mean age in the cases group was 44.93 ± 3.94 years and the mean age in the control group was 46.42 ± 7.74 years, with no statistically significant distinction between both categories (p= 0.253). There were 8 guys (17.8%) and 37 females (82.2%) in the cases group while there were 12 males (26.7%) and 33 females (73.3%) in the control group with no statistically significant distinction between both categories (p=0.310).

This increased frequency in females can be attributed to a variety of underlying factors, including postmenopausal hormonal substances changes that result in early bone loss and osteoporosis, both of which impact the mechanical architecture of the joints. Another possible explanation for this discrepancy could be that males have typically greater support for joints since they have larger muscle mass and more powerful ligaments. When compared to men, these variations can result in more traumatic effects and higher joint stress in women [19]. Women also have a greater body mass index than men, which is one of the most significant controllable disparities among the sexes. Having this in thoughts, weight-loss prevention programs should be more personalized and more tailored to persons with a high body mass index [20].

In the current study, the mean BMI in the cases group was 36.01 ± 6.36 Kg/m2 and the mean BMI in the control group was 32.06 ± 4.95 Kg/m2 with statistically significant difference between the two groups (p= 0.001). This was supported by Thigah and Khan [21] who discovered that people with a higher BMI are more likely to develop knee OA.

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) is widely regarded as the most sensitive, condition specific tool [9]. WOMAC is a disease-specific self-report questionnaire used to assess hip and knee OA symptoms. The WOMAC has been shown to be reliable, valid, and sensitive to changes in the health status of patients with knee OA [10]. It's a three-part questionnaire. Each section evaluates a particular factor, such as discomfort, stiffness, and functional limitation in activities of daily living (ADL). In the current study, the mean WOMAC score in the cases group was 16.96 ± 9.08 and the median score was 16 with range between 4 and 40. The current study's score was much lower than that of Mohammed et al who included 60 patients with primary knee OA who met the Arthritis Rheum 1986 OA categorization criteria. They discovered that the mean WOMAC score was 74.26±14.07, with a range of 45 to 96, and that radiological Kellgren-Lawrence (KL) grading revealed that only 5% of cases had KL grade I, whereas all cases in the current study had KL grade I [22].

In the current study, the mean Visual analogue scale (VAS) at rest score in the cases group was 0.67 ± 1.11 and the median score was 0 with range between 0 and 4. The mean VAS at activity score in the cases group was 3.67 ± 1.83 and the median score was 3 with range between 1 and 8. This was also lower than Radwan and Borai's [23] study, which comprised 165 patients with primary knee OA. They discovered that the mean VAS at rest was 3.81 ± 1.92 (range: 0-10), while the mean VAS at exercise was 5.53 ± 2.07 (range: 2-10) The difference could be attributed to the selection of instances of early knee OA.

Diabetes (DM) was found in 15.6% of cases and 22.2% of controls in the present investigation, where there is not a statistically significant distinction between the two sets (p= 0.419).

On the contrary, Nieves-Plaza et al [24] found that DM patients were more likely than non-diabetic people to have knee OA. Diabetes patients showed a 2.18-fold higher incidence of knee OA than non-diabetic controls. Furthermore, Fadhil et al [25] discovered a possible link between type 2 diabetes and the development of knee osteoarthritis.

The prevalence of hypertension was26.7% and 11.1% in the cases and the control group respectively, with no statistically significant difference between the two groups (p= 0.059).On the other hand, the study of Li et al. [26] discovered that the prevalence of hypertension was considerably greater in osteoarthritis patients than in the control population, and that other risk factors such as obesity, dyslipidemia, and metabolic syndrome were also higher in osteoarthritic patients .

In the current study, all the cases had Joint crepitus on palpation. There were 41 cases (91.1%) with knee tenderness, 30 cases (66.7%) with Knee pain induced by movement and there were 3 cases (6.7%) with limited range of movement. This agreed with Ellaithy et al [27].

In the current study, the median UCTXII level in the case group was 224.3 pg/mL (range: 59.4_362.8) and in the control group was 55.4 pg/mL (range: 0-141.5). The level of CTXII was statistically much greater in the group of cases (p < 0.001). The results of the current study were consistent with those of Xin et al. [28] Guilin Medical College's Review Board authorized the

study. The authors demonstrated that the concentration of UCTXII in the case group was (261.235±39.944) pg/mL and in the control group was (218.341±22.270) pg/mL, which is statistically substantially higher than CTX-II in the non-intervention cohort (P<.001) (Xin et al., 2017).

This was also in agreement with Arunrukthavon et al [14] who enrolled seventy-eight individuals with osteoarthritis of the knee who were beyond the age of 40 and met the diagnostic requirements for osteoarthritis of the knee specified with the 1986 ACR. The results showed that CTX-II urine concentration was higher in the experimental group than in the control group (p< 0.001) Furthermore, this was consistent with a recent Egyptian study by Ellaithy et al [27] which demonstrated a significant rise in UCTX-II amounts in patients with osteoarthritis contrasted to a normal control group. In our research, we found a statistically substantial positive connection between UCTXII with WOMAC, VAS score. The current findings supported the results of Ellaithy et al [27] who showed that there existed a substantial distinction between CTX-II level and WOMAC index ratings in osteoarthritis individuals.

In the same context, the current findings matched with those of Arunrukthavon et al [14] who found a favorable association between CTX-II levels in the urine and WOMAC index (r = 0.367, p< 0.001). CTX-II levels in the urine and KL grades had a statistically substantial positive connection (r = 0.405, p <0.001). There was no association between UCTX-II concentrations and weight, height, or BMI.

García-Alvarado et al [29] also conducted a cross-sectional investigation with 155 women who had knee OA. Urine samples were collected to determine biomarker levels UCTX-II ng/mmol using the Enzyme-linked immunosorbent assay (ELISA) technique, and the WOMAC scale was employed for pain classification. UCTX-II biomarker levels associated with the WOMAC index, which indicated the severity of OA.

Some meta-analyses also found that UCTX-II levels in patients with severe knee OA were consistently higher than in those with light knee OA [30, 31]. In another study, Wang and his colleagues [32] revealed in another study that blood CTX-II levels in the research group were favorably connected with WOMAC score (r=0.357, P<0.001)

In our research, we found a statistically substantial positive connection between UCTXII with ESR and CRP which were done with routine diagnosis However, there was no statistically significant correlation between UCTXII with age and BMI.

This was consistent with Saberi Hosnijeh et al [33] who discovered a substantial association between cartilage turnover marker levels and the inflammatory marker C-reactive protein While, Keenan et al. [34] discovered a very weak connection with disease activity.

Because greater CTX-II concentrations were linked to higher disease activity scores, UCTX-II might be a valuable measure for evaluating the course of OA illness and its treatment. Catabolization of type II collagen was increased when the serum CTX-II content was greater. The higher the disease activity score, the more serious the articular cartilage deterioration. As a result, continuous measurement of serum CTX-II concentrations may indicate the degree of OA articular cartilage lesions, facilitating early OA evaluation and clinical therapy prediction [14].

One of the drawbacks of employing UCTX-II as a diagnostic biomarker for knee OA is the volatility in the appropriate cutoff level. A recent meta-analysis found that mean UCTX-II levels in the healthy population ranged from 129 to 345 ng/mmol creatinine [31, 35].

In a previous study, Wang and his colleagues 2019 demonstrated that the Area under the curve (AUC) of blood CTX-II in the determination of OA was 0.886 [95% CI: 0.930 to 0.942], the optimal cut-off value for evaluation of OA was 0.70, testing empathy was 84%, and particularity was 86% [32].

In the current study, the best cutoff point of UCTXII to identify osteoarthritis cases from the control was > 85.25. This point showed high degree of sensitivity and specificity, with high statistically significant value (p < 0.001). No previous studies have revealed the best cutoff point of UCTXII in identifying cases with OA from controls. This could provide an easy non-invasive diagnostic biomarker for cases with OA.

## CONCLUSION

According to our findings, urine C-telopeptide fragments of type II collagen (UCTXII) are an effective, safe, and promising method for detecting early knee OA. This study has some limitations, such as the small sample size and the fact that it is a single-center study, which may limit the power of the conclusions reported. The study's cross-sectional nature is also one of its drawbacks, and further prospective studies should be conducted.

## REFERENCES

[1] **Issa SN, Sharma L**. Epidemiology of osteoarthritis: an update. Curr. Rheumatol. Rep. 2006; 8(1): 7-15.

[2] **Blagojevic M, Jinks C, Jeffery A, Jordan K**. Risk factors for onset of osteoarthritis of the

knee in older adults: a systematic review and meta-analysis. Osteoarthr. Cartil. 2010; 18(1): 24-33.

[3] **Huang M, Zhao J, Huang Y, Dai L, Zhang X.** Meta-analysis of urinary C-terminal telopeptide of type II collagen as a biomarker in osteoarthritis diagnosis. J. Orthop. Transl. 2018; 13: 50-57.

[4] **Garnero P, Delmas PD**. Biomarkers in osteoarthritis. Curr. Opin. Rheumatol. 2003; 15(5): 641-646.

[5] **Abramson SB, Attur M**. Developments in the scientific understanding of osteoarthritis. Arthritis Res. Ther .2009; 11: 1-9.

[6] **Lotz M, Martel-Pelletier J, Christiansen C, Brandi ML, Bruyère O, et al**. Republished: Value of biomarkers in osteoarthritis: current status and perspectives. Postgrad. Med. J. 2014; 90(1061): 171-178.

[7] **Røtterud JH, Reinholt FP, Beckstrøm KJ, Risberg MA, 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 Musculoskelet. Disord. 2014; 15(1): 1-7.

[8] **Mouritzen U, Christgau S, Lehmann H, Tanko L, Christiansen C**. Cartilage turnover assessed with a newly developed assay measuring collagen type II degradation products: influence of age, sex, menopause, hormone replacement therapy, and body mass index. Ann. Rheum. Dis. 2003; 62(4): 332-336.

[9] **Escobar A, Quintana J, Bilbao A, Arostegui I, Lafuente I, et al.** Responsiveness and clinically important differences for the WOMAC and SF-36 after total knee replacement. Osteoarthr. Cartil. 2007; 15(3): 273-280.

[10] **Hmamouchi I, Allali F, Tahiri L, Khazzani H, Mansouri LE, et al**. Clinically important improvement in the WOMAC and predictor factors for response to non-specific non-steroidal anti-inflammatory drugs in osteoarthritic patients: a prospective study. BMC Res. Notes .2012; 5(1): 1-9.

[11] **Salehi-Abari I**. 2016 ACR revised criteria for early diagnosis of giant cell (temporal) arteritis. Autoimmune Dis Ther Approaches 2016; 3(1): 1-4.

[12] **Kellgren JH, Lawrence JS**. Radiological assessment of osteo-arthrosis. Ann Rheum Dis 1957; 16(4): 494-502.

[13] **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 in patients with osteoarthritis of the hip or knee. J. Rheumatol 1988; 15(12): 1833-1840.

[14] **Arunrukthavon P, Heebthamai D, Benchasiriluck P, Chaluay S, Chotanaphuti T, et al**. Can urinary CTX-II be a biomarker for knee osteoarthritis? J. Arthroplasty. 2020; 2(1-7.

[15] **Vina ER, Kwoh CK**. Epidemiology of osteoarthritis: literature update. Curr. Opin. Rheumatol. 2018; 30(2): 160.

[16] **Bai B, Li Y**. Combined detection of serum CTX-II and COMP concentrations in osteoarthritis model rabbits: an effective technique for early diagnosis and estimation of disease severity. J. Orthop. Surg. Res. 2016; 11: 1-7.

[17] **Van Spil W, DeGroot J, Lems W, Oostveen J, Lafeber F**. Serum and urinary biochemical markers for knee and hip-osteoarthritis: a systematic review applying the consensus BIPED criteria. Osteoarthr. Cartil .2010; 18(5): 605-612.

[18] **Luo Y, He Y, Karsdal M, Bay-Jensen A-C**. Serological CTX-II does not measure the same as urinary CTX-II. Osteoarthr. Cartil. Open 2020; 2(3): 100082.

[19] **Geusens PP, van den Bergh JP**. Osteoporosis and osteoarthritis: shared mechanisms and epidemiology. Curr. Opin. Rheumatol. 2016; 28(2): 97-103.

[20] **Safiri S, Kolahi A-A, Smith E, Hill C, Bettampadi D, et al.** Global, regional and national burden of osteoarthritis 1990-2017: a systematic analysis of the Global Burden of Disease Study 2017. Ann. Rheum. Dis. 2020; 79(6): 819-828.

[21] **Thigah AA, Khan AA**. Prevalence of knee osteoarthritis among adult patients attending Al-iskan Primary Health Care Center, Makkah, Saudi Arabia. J. Clin. Anal. Med. 2020; 9(3): 271-278.

[22] **Mohammed WF, Mohamed FI, Ahmd GM, Abdelmagied RA, Mounir AM, et al.** Osteopontine in knee osteoarthritis. MJMR .2019; 30(1): 68-71.

[23] **Radwan A, Borai A.** Neuropathic pain in Egyptian patients with primary knee osteoarthritis: relationship with functional status and radiological severity. Egypt. Rheumatol. 2019; 41(4): 261-264.

[24] **Nieves-Plaza M, Castro-Santana LE, Font YM, Mayor AM, Vilá LM.** Association of hand or knee osteoarthritis with diabetes mellitus in a population of Hispanics from Puerto Rico. J Clin Rheumatol: practical reports on rheumatic & musculoskeletal diseases 2013; 19(1).

[25] **Fadhil AS, Hussain FY, Salow SA.** Type 2 Diabetes Mellitus As a Risk Predictor for Knee Osteoarthritis (A Case Control Series Study among Iraqi People at Mosul City). Med. J. Tikrit Univ. 2013; 19(2).

[26] **Li H, George DM, Jaarsma RL, Mao X**. Metabolic syndrome and components

exacerbate osteoarthritis symptoms of pain, depression and reduced knee function. Ann. Transl. Med . 2016; 4(7).

**[27] Ellaithy LS, Moselhi M, El Gazzar R, Amer NM, Mansour TA, et al.** Evaluation of urinary C-terminal cross-linked telopeptides of type II collagen CTX-II as a biomarker for early diagnosis of osteoarthritis in comparison to routine diagnostic methods. Egypt. J. Chem. 2022; 65(132): 707-717.

**[28] Xin L, Wu Z, Qu Q, Wang R, Tang J, et al.** Comparative study of CTX-II, Zn2+, and Ca2+ from the urine for knee osteoarthritis patients and healthy individuals. Medicine 2017; 96(32).

**[29] García-Alvarado FJ, González-Martínez MdR, Jaramillo-Rodríguez Y, Delgado-Aguirre HA.** Increased urinary concentration of C-terminal telopeptide of Type II collagen and pain by radiographic grade in women with knee osteoarthritis in Northeastern Mexico: a cross-sectional study. BioResearch Open Access 2020; 9(1): 7-12.

**[30] Huang Z, Chen J, Ma J, Shen B, Pei F, et al.** Effectiveness of low-level laser therapy in patients with knee osteoarthritis: a systematic review and meta-analysis. Osteoarthr. Cartil. 2015; 23(9): 1437-1444.

**[31] Cheng H, Hao B, Sun J, Yin M.** C-terminal cross-linked telopeptides of type II collagen as biomarker for radiological knee osteoarthritis: a meta-analysis. Cartil. 2020; 11(4): 512-520.

**[32] Wang P, Song J, Qian D**. CTX-II and YKL-40 in early diagnosis and treatment evaluation of osteoarthritis. Exp. Ther. Med. 2019; 17(1): 423-431.

**[33] Saberi Hosnijeh F, Siebuhr AS, Uitterlinden AG, Oei EH, Hofman A, et al.** Association between biomarkers of tissue inflammation and progression of osteoarthritis: evidence from the Rotterdam study cohort. Arthritis Res. Ther. 2016; 18(1): 1-10.

**[34] Keenan R, Swearingen C, Yazici Y.** Erythrocyte sedimentation rate and C-reactive protein levels are poorly correlated with clinical measures of disease activity in rheumatoid arthritis, systemic lupus erythematosus and osteoarthritis patients. Clin. Exp. Rheumatol. 2008; 26(5): 814.

**[35] Hao H, Zhang J, He Q, Wang Z.** Cartilage oligomeric matrix protein, C-terminal cross-linking telopeptide of type II collagen, and matrix metalloproteinase-3 as biomarkers for knee and hip osteoarthritis (OA) diagnosis: a systematic review and meta-analysis. Osteoarthr. Cartil. 2019; 27(5): 726-736.

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