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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

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Abstract:

Background: Work-related musculoskeletal disorders (WRMSDs) are serious occupational health problems among workers worldwide. Aim of the study: evaluate urinary C-terminal telopeptide of collagen type II (CTX-II) as a biomarker for early diagnosis of osteoarthritis and compare results with those obtained by the routinely used methods. Subjects and methods: One hundred and eighty workers from the outpatient clinics of rehabilitation center in Cairo performing physically demanding and office jobs. One hundred and twenty three workers diagnosed with knee OA (Group I) and 57 workers were healthy (Group II). Clinical examination, X-rays and questionnaire were done. Erythrocyte sedimentation rate, high sensitive C reactive protein and human CTX-II were measured. Results: No statistical significant difference between CTX-II in osteoarthritis workers and age, residence, smoking status and sport practice. Marked increase of urinary CTX-II level was found in osteoarthritis workers compared to healthy group. A high significance difference between CTX-II level and Western Ontario and Mcmaster Universities Arthritis Index (WOMAC) index scores in osteoarthritis workers, in addition high levels were found among grade 4 osteoarthritis. Mean urinary level of CTX-II in osteoarthritis workers increased with increased work duration and total working hours. Conclusion: Urinary CTX-II can predict clinical diagnostic criteria and x-ray progression in osteoarthritis, so it can be used as a tool for diagnosis of knee OA

Keywords: WRMSDs; osteoarthritis; WOMAC index scores; X-ray; urinary CTX-II

1. Introduction

Work-Related Musculoskeletal disorders (WRMSDs) appear to be the most common reportable work-related musculoskeletal damage affecting millions of workers. It encompasses a broad area of inflammatory and degenerative conditions that include muscles, tendons, ligaments, joints and peripheral nerves. Examples of WRMSDs are carpal tunnel syndrome, tendinitis, thoracic outlet syndrome, osteoarthritis, and tension neck syndrome [1]. The evaluation of WRMSDs is based mainly on patients' reports of symptoms and pain as well as a physical examination by a physician which usually depend on medical history, occupational risks, pain severity to confirm source of the pain, and sometimes laboratory investigations; X-rays or MRI scans [2].

Osteoarthritis (OA), a degenerative joint disease and the commonest form of arthritis (commonly appears at the knees, hips, and hands) is usually classified either by clinical or radiological methods [3]. The most commonly used clinical classification criteria for OA are those suggested by the American College of Rheumatology (ACR) [4]. Radiological criteria are based on the presence of osteophytes, narrowing of the articular space, and bone sclerosis. The most common radiological criteria used are those declared by the World Health Organization (WHO), which are based on the Kellgren–Lawrence (KL) classification [5]. Although in clinical practice the diagnosis of OA depends on using clinical examination confirmed by radiological findings, it is widely acceptable that there is no significant correlation between clinical features and radiological changes [6].

Characteristic features of OA include degeneration of articular cartilage (AC), with loss of proteoglycans and type II collagen, two of the most common components of cartilage, sclerosis of subchondral bone, bone formation, synovial enlargement and activation. OA has long been considered a wear and tear of the articular cartilage degenerative disease. Moreover, it is

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actually described as an organic disease that affects all joint structures, i.e., cartilage, subchondral bone (SB), synovium, capsule, ligaments, articular cartilage, and other structures surrounding the joint [7].

Articular cartilage consists of a unique, specialized, avascular, and aneural connective tissue, consisting of chondrocytes surrounded by an extracellular matrix and mainly containing water and two main important organic components: type II collagen and aggrecans (8). During the early stages of osteoarthritis, chondrocytes secrete tissue inhibitors of metalloproteinases (TIMPs) trying to increase synthesis of proteoglycans to match the degradative process, though this restoration process is not enough. Loss in equilibrium leads to a decrease in the amount of proteoglycans even with an increase in synthesis, an increase in the water content, a disorganized pattern of collagen, and finally a loss of elasticity of the articular cartilage. Macroscopically, these changes result in cracking and fissuring of the cartilage and lastly erosion of the articular surface [9].

The main scene in OA pathophysiology is the damage in the Type II collagen network which is the most important structural element of the joint cartilage; accounts for 80%–90% of the total collagen in articular cartilage. Thus, the objective of investigations is detecting OA-specific biochemical markers which have been focused on Type II collagen. There is an agreement that Type II collagen degradation products can be used as markers in the diagnosis and follow-up of OA [10].C-terminal telopeptide of collagen type II (CTX-II) the most abundant protein in cartilage and interleukin-1β (IL-1β) are engaged in articular cartilage degeneration. CTX-II, can be used to preliminary decide the degree of joint degeneration and deterioration in OA and can reflect the degradation degree of articular cartilage before radiological changes appear. Hence, urinary CTX-II can be used as an important indicator of cartilage damage and one of the potential OA biomarkers [11].

The diagnostic challenge in diagnosing OA of the knee is early diagnosis. Radiographic evidence of OA is a probable late sign which irreversible joint damage may have already happened. Hence, there is an urgent need for accurate methods that can detect joint changes in a sensitive, quantitative, and reliable manner to detect pre-osteoarthritic changes before the onset of irreversible changes [12].

The present study aimed to evaluate urinary CTX-II as a biomarker for early diagnosis of osteoarthritis and compare results with those obtained by the routinely used methods.

Subjects and methods

Study design and population:

The present study is a cross-sectional study that included 180 male workers divided into two groups: 123 workers suffering from knee OA and 57 healthy workers. They were randomly selected from outpatient clinics for Physical Medicine and the Rehabilitation Center in Cairo. Participants were performing two types of jobs, physically demanding and office jobs (≥8 hours per day and ≥ 6 days a week). Duration for working years was classified to (<10 years, 10-20 years, > 20 years).

Inclusion criteria:

- Workers age ≥ 21.
- Workers who were suffering from osteoarthritis.
- Occupational exposure for at least 5 years.

Exclusion Criteria:

Workers with a previous history of:

- Trauma or accident.
- Connective tissue diseases that affect musculoskeletal joints as rheumatoid arthritis (RA) and systemic lupus erythematous (SLE).
- Gouty arthritis: secondary knee OA.
- Diabetes Mellitus.
- Workers who have been treated with corticosteroids for a more than 1 year.

Sample size:

A sample of 180 suspected workers were required to estimate area under the curve (AUC) for urinary biomarker (CTX-II) in diagnosing musculoskeletal disorders [using early rheumatoid arthritis (RA) as an indicator] = 0.81 with precision of 0.2, Alpha =0.05 and design effect =2 will provide a power of 80% [13].

Methods:

Clinical examination

Was done for all workers for musculoskeletal disorders at the centre by 2 physicians.

Questionnaire

-All studied workers were interviewed face to face using well-structured questionnaire [14], including personal data and socio-demographic characteristics, detailed occupational history, determination of the mode of onset, duration, pattern and progression of the musculoskeletal complaints, medical history including (pattern of joint involvement, recording the severity of disorder), clinical symptoms of knee OA (pain, stiffness, knee swelling, muscle weakness, deformed joints, reduced range of motion and cracking and creaking).

-All workers were asked about past history of specific medical disorders that could have a significant association with the joint disorder as neurological disorders. Medications used in the past, with

emphasis on dosages, duration of treatments. Any present drug taken. Surgical procedures on joints, including date of the surgery. All workers also were asked about that as emotional and physical stress exacerbate symptoms of musculoskeletal disorders (Anxiety, depression, insomnia) and a recorded similar family history of a related musculoskeletal condition.

-Assessment of current functional ability: This was done in a question-and-answer format and quantified with the use of functional instrument: WOMAC score (The Western Ontario and McMaster Universities Arthritis Index): [15]

All studied workers fulfilled the WOMAC index score which is an easy 12 minutes' questionnaire to be completed, and can be written on a paper, by the telephone or by computer. Goal of WOMAC is for the evaluation of knee or hip Osteoarthritis. It is widely used and it can be completed by the patient himself and containing 24 items with 3 subscales:

- Pain is 5 items which are, during walking, using stairs, in bed, sitting or lying, and standing upright (Maximum pain score= 20).
- Stiffness is 2 items which are, after first waking and later in the day (Maximum stiffness score= 8).
- Physical Function is 17 items which are, using stairs, rising from sitting, standing,

bending, walking, getting inside or outside a car, shopping, putting on or taking off socks, rising and lying in bed, getting in and out of bath, sitting, getting on or off toilet, heavy and light domestic duties (Maximum physical function score = 68).

The scores for each subscale were summed up, with a score range of 0-20 for Pain, 0-8 for Stiffness, and 0-68 for Physical Function. A sum of the scores for all three subscales gives a total WOMAC score. Total WOMAC score = 96.

It is performed for all workers to assess activities of daily living, functional mobility, gait, general health, and quality of life. WOMAC questions were scored on a scale of 0-4, which coincide to: None (0), Mild (1), Moderate (2), Severe (3), and Extreme (4). The knee pain score on the WOMAC scale is a widely validated tool and is applied to assess patient-reported pain. Higher scores on the WOMAC was referred to worse pain, stiffness, and functional limitations [16]

- Assessing functional ability at home by independence or reliance on help from family members and others. And at work by transportation and job requirements and limitations. Questions to define actions that cause and exacerbate knee OA. Also questions about recreational and social activities as limitations and extent.

Radiographic Assessment:

Knees X- rays were done for all workers participated in the study. X-Rays views were taken in standing anteroposterior (AP) and standing lateral in extension. The Kellgren-Lawrence (KL) [17,18] grading system use the following four radiographic features for classification of knee OA, which are:

- Narrowing of Joint space (JSN)
- Osteophytes
- Subchondral sclerosis
- Subchondral cysts

Based on those radiographic features, the severity of OA is given a grade from 0 to 4, with grade 0 indicating no presence of OA and grade 4 indicating severe OA [19].

- KL Grade I: doubtful significance with minute osteophyte
- Grade II: mild changes in joint with definite osteophyte and normal joint space.
- Grade III: moderate changes with moderate joint space reduction.
- Grade IV: severe changes with joint space greatly reduced, and subchondral sclerosis.

- **Diagnosis of knee OA:**

- ACR Clinical classification criteria of knee OA [20]:

- Age more than 50 years' old
- Morning stiffness less than 30 minutes
- Crepitus during knee movement

Bony tenderness

- Bony enlargement
- No palpable warmth
- Findings as bony tenderness which is mainly pain found by palpation at the joint line, crepitus which is crackling or grinding sound in the joint during weight bearing, bony enlargement at joint line and palpable warmth of the knee joint, were scored as yes or no which means present or absent respectively.

- ACR Clinical & Radiographic criteria of knee OA:

- Applied by using history, physical examination and radiographic findings. It depends on the presence of knee pain together with at least one of the following three items together with osteophyte in knee X-Ray proved diagnosis of knee OA:
- Age more than 50 years' old
- Morning stiffness less than 30 minutes
- Crepitus during knee movement

Blood and urine sample:

Five ml of blood was collected aseptically from each participant into 2 vacationer tubes, 2.5 ml for sodium citrate (for ESR) and 2.5 ml plain tubes for serum separation to estimate high sensitive C-reactive protein test (hsCRP). Also, a morning urine sample was collected at the centre and stored at 2-8°C until the analysis for CTX-II and creatinine determination was performed. Some of the potential changes in levels of

urinary CTX-II may depend on sample time and analysis, and to control this alteration, an adjustment of urinary levels of CTX-II by urinary creatinine concentrations is done.

-Determination of Erythrocyte sedimentation rate (ESR): **[21]**

Laboratory investigations:

-Quantitative Determination of High Sensitivity C-reactive protein (CRP): [22]

-Determination of the Urinary biomarker: Human Cross Linked C-telopeptide of Type II Collagen (CTX-II) by ELISA method :(WKEA MED SUPPLIES, China) [23]. The kit assay Human CTX-II level in the sample, use purified Human CTX- II antibody to coat microtiter plate wells, make solid-phase antibody, then add CTX- II to wells, combined CTX- II which with enzyme labeled, become antibody-antigen-enzyme-antibody complex, after washing completely, add substrate, substrate becomes blue color. At HRP enzyme-catalyzed, reaction is terminated by the addition of a sulphuric acid solution and the color changes is measured spectrophotometrically at a wavelength of 450nm. The concentration of CTX- II in the samples is then determined by comparing the O.D. of the samples to the standard curve.

-Determination of urinary Creatinine: [24]

Statistical analysis:

Data was analyzed using Statistical Package for Social Sciences (SPSS)IBM Corp., Armonk, N.Y., USA version 21. Categorical variables were presented as frequency and percentage. Numerical data were described using mean and standard deviation or median and range, as appropriate. Data was tested for normality using Kolmogrov-Smirnov test and Shapiro-Wilk test, p-value less than 0.05 was considered significant. All tests are 2 tailed.

Tests used were:

- Independent T test: comparison between two independent groups of normal distributed variables were done.
- F-test (ANOVA): comparison between three independent groups of normal distributed variables were done.
- Mann–Whitney (U test): comparison between two or three independent groups of non- normally distributed variables were done.
- Kruskal–Wallis test: comparison between three independent groups of not normally distributed variables were done.
- Spearman coefficient: for correlating between two distributed abnormally quantitative variables.
- Receiver operating characteristic curve (ROC) curve analysis was performed to estimate sensitivity and specificity.

Results:

- Study included 180 male workers. After applying all of ACR Clinical & Radiographic criteria of knee OA we found that 123 workers demonstrated to have knee OA, and who met the diagnostic criteria for knee OA prescribed by the American College of Rheumatology (ACR) with X-rays of grade 2, 3, or 4 (Group I). While 57 workers demonstrated that healthy participants without clinical or radiological evidence of OA of the knee with a score of 0 or 1 X-ray (Group II).

--Regarding diagnosis of knee OA (according to ACR diagnostic criteria) among workers (n=180), the present study shows that 123 (68%) worker's complaint from knee pain, 102 (56.7%) workers suffered morning stiffness < 30 minutes, 107(59.4%) workers suffered from crepitus on knee motion, 39 (21.7%) workers were> 50 years old, 75 (41.7%) workers complain from bony tenderness, 123 (68.3%) workers complain from bony enlargement, and no palpable warmth was found in 103 (57.2) workers.

Table (1): The age of all the workers range from 23 to 60 years, the mean age ± SD of osteoarthritis diagnosed workers (group I) was 42.2 ± 8.2 years, and the mean age ± SD of healthy workers (group II) was 41.6 ± 9.4 years. No significant difference was reported between the two groups (p=0.693) concerning age. Also no statistical significant differences were found between the two groups regarding residence, marital status, educational level, smoking status and sport practice (p=0.769), (p=0.258), (p=0.517), (p=0.087), (p=0.917) respectively.

Fig (1): box plots of urinary CTX-II among osteoarthritis workers with median of 366 pg. /mg creatinine and a range from 207 to 1558 pg. /mg creatinine ((mean, 456.32 ± 243.5 pg. /mg creatinine) which is higher compared to healthy workers with median of 198 pg. /mg creatinine and a range from 91 to 633 pg. /mg creatinine (mean, 246.37 ± 143.05 pg. /mg creatinine). A high statistical significant difference in urinary CTX-II levels between the two groups (p=< 0.001*).

Table (2) demonstrated the comparison between Urinary CTX-II concentrations in different WOMAC score among group I (osteoarthritis studied workers). Total WOMAC score (96) was calculated by combining scores of pain (20), stiffness (8) and physical function (68). In our study the cut point of WOMAC score was (63). High WOMAC score were found in 87(70.7%), while low WOMAC score were found in 36 (29.3%) in group I. The mean ± SD of

urinary CTX-II for high and low WOMAC score were 536.14 ± 248.7 and 263.4 ± 24.7 respectively. A High statistical significant difference was found between urinary CTX-II of group I and WOMAC score (U=67.5, p=< 0.001*).

Table (3) demonstrated correlation between concentrations of urinary CTX-II of group I (osteoarthritis workers) and WOMAC pain subscale which ranges from 0-20, WOMAC stiffness subscale which ranges from 0-8 and WOMAC physical function subscale which ranges from 0-68 and uCTX-II. Significant positive correlation was found between pain subscale and physical function with levels of urinary CTX-II (r_s = 0.641* and r_s = 0.560*, p = < 0.001* respectively). While no significant correlation was found between urinary CTX-II and stiffness subscale (r_s = 0.097 and p = 0.285).

Table (1): Sociodemographic Characteristics among both OA and healthy groups

Sociodemographic Data		Group I (n =123)		Group II (n =57)		P
		No.	%	No.	%	
Age (years)	Mean ± SD	42.2 ± 8.2		41.6 ± 9.4		0.693
Residence	Urban	59	48.0	26	45.6	0.769
	Rural	64	52.0	31	54.4	
Marital Status	Married	78	63.4	40	70.2	0.258
	Single	18	14.6	10	17.5	
	Divorced	15	12.2	6	10.5	
	Widow	12	9.8	1	1.8	
Educational level	Illiterate	12	9.8	6	10.5	0.517
	Read & Write	3	2.4	0	0	
	Primary	7	5.7	4	7	
	Preparatory	14	11.4	8	14	
	Secondary	54	43.9	30	52.6	
	University	32	26.0	8	14	
	Post graduated	1	0.8%	1	1.8	
Smoking Status	Smoker	48	39.0%	30	52.6	0.087
	Non Smoking	75	61.0%	27	47.4	
Sport Practice	No	68	55.3%	31	54.4	0.917
	Yes regular	28	22.8%	12	21.1	
	Yes sometimes	27	22.0%	14	24.6	

Table (2): Comparison between urinary CTX-II levels among group I (osteoarthritis studied workers) and WOMAC score

group I (n=123)	High WOMAC Scores (> 63/96) N=87 (Mean ± SD)	Low WOMAC scores (<=63/96) n=36 (Mean ± SD)	Test of Sig.	P
Urinary CTX-II (pg./mg Creatinine)	536.14 ± 248.7	263.4 ± 24.7	U=67.5	< 0.001*

cut point of WOMAC 96 in the study is 63 (65.6%) U: Mann Whitney test*

Table (3): Correlation between Urinary CTX-II concentrations and WOMAC pain, stiffness and physical function subscales

Urinary CTX-II (pg./mgCreatinine)With WOMAC score subscales	Test of sig.	P
Pain Subscale	r_s = 0.641*	< 0.001*
Stiffness Subscale	r_s = 0.097	0.285
Physical Function Subscale	r_s = 0.560*	< 0.001*

r_s: spearman Coefficient

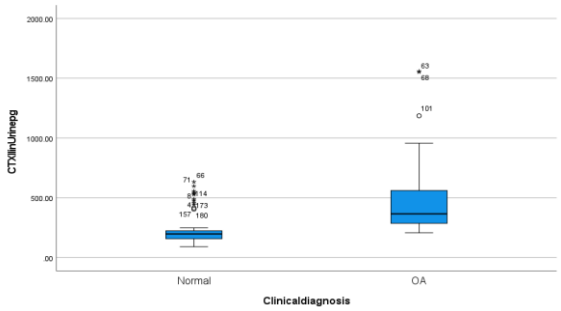

Figure (1) Comparison of the box plots of urinary CTX-II between knee OA workers and normal workers

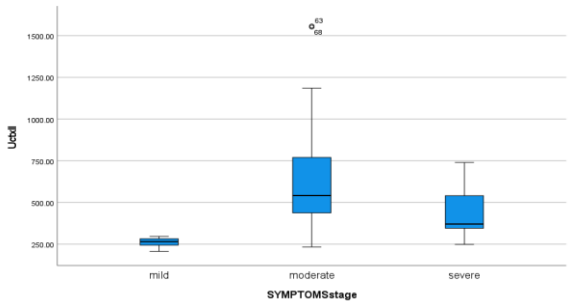

Figure (2) Comparison of the box plot of urinary CTX-II among OA workers (group I) with OA stages

Figure (2) demonstrated the comparison between levels of urinary CTX-II among Group I OA workers with different symptoms & signs severity (mild, moderate and severe). Higher level of urinary CTX-II was found in moderately staged OA workers [mean ± SD (630.27 ± 290.2)] followed by severe stage [mean ± SD (439.1 ± 138.1)] while lower level was observed in mild stage [mean ± SD (263.9 ± 24.5)]. A high statistical significant difference was found between OA stages and CTX-II in urine in OA workers group (p=< 0.001*).

Fig. (3) showed box plot between urinary CTX-II concentrations with total working hours (3-a), and duration of work in years (3-b) among OA diagnosed workers. It was found that the level of urinary CTX-II is significantly higher among workers (working more than 8 hours/day) [mean ± SD (559.67 ± 320)] than workers (working <= 8 hours/day) [mean ± SD (422.98 ± 204.31)], (t= -2.74*, p=0.007*) and was significantly higher among workers more than 20 years' [mean ± SD (489.4 ± 256.7)] and workers from 10 to 20 years' [mean ± SD (462.5 ± 254.2)] than workers less than 10 years' [mean ± SD (368.7 ± 165.4)], ($X^{2=}$12.05*, p=0.002*). So, urinary CTX-II levels increase with increased duration of work years, and increased working hours.

Fig. (4) shows that higher levels of urinary CTX-II were found in (KL) grade 4 workers (50/123) [mean ± SD (676.66 ± 244.75)] followed by grade 3 (33/123) [mean ± SD (356.06 ± 30.54)] and lower levels were found in grade 2 workers (39/123) [mean ± SD (263.60 ± 24.77)]. Grade 1 was recognized only in 1 worker.

-No statistical significance difference was found between the two groups concerning ESR1sthr, ESR 2ndhr and hsCRP.

-There were no statistical significant correlation between CTX-II in urine and ESR1sthr, ESR2ndhr and hsCRP done for group I (r_s=0.058 and p=0.526), (r_s=0.085 and p=0.351) and (r_s=0.039 and p=0.668) respectively.

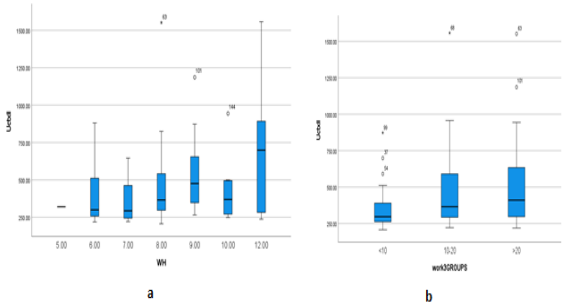

Figure 3 (a-b): Box plot between urinary CTX-II levels and (total working hours and work duration in years among OA workers)

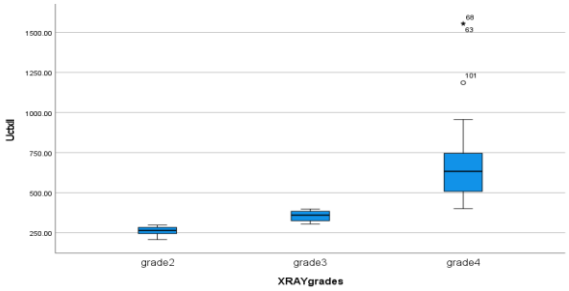

Figure (4): Comparison in urinary CTX-II levels in different radiographic OA knee (KL) grades among OA workers Stages

Using ROC curve to study the results of CTX-II in comparison to clinical diagnosis and x ray, it was found that AUC were 0.851 and 1.0 respectively (p<0.001) with cutoff (221pg./mg Cr. And 289.5pg./mg Cr. respectively). Sensitivity (98.4% , 100% respectively) and specificity (73.7% , 80% respectively). Regarding WOMAC score parameters with CTX-II; it showed that AUC for bone tenderness was (0.730) and it was the biggest area shown as compared to other parameter (p< 0.001*). This parameter showed high sensitivity (62.1%) and high specificity (85.7%) among other parameters.

Table (4): Agreement (sensitivity, specificity) for urinary CTX-II to predict osteoarthritis versus routine methods and different WOMAC score parameters:

Parameters	AUC	p value	Cut off	Sensitivity (%)	Specificity (%)
Routine diagnostic methods					
Clinical diagnosis	0.851*	< 0.001*	221pg./mg Cr.	98.4%	73.7%
X Ray	1.000*	< 0.001*	289.5pg./mg Cr.	100%	80%
WOMAC score					
Bone tenderness	0.730*	< 0.001*	-	62.1	85.7
Symptoms stage	0.652*	0.014*	-	76.8	53.6
WOMAC score	0.634*	0.032*	-	76.8	50
Decrease range of movement	0.377*	0.046*	-	32.6	42.9

p value: probability value

DISCUSSION:

Osteoarthritis (especially Knee and hip OA) represents the most common musculoskeletal disorder. Early diagnosis of knee osteoarthritis has been considered a diagnostic challenge due to the non-specific signs and symptoms that appear from the early stage of the disease. OA radiographic evidence is a potent late sign which irreversible joint damage have already occurred [25]. The role of several biomarkers for knee OA diagnosis had been evaluated previously, but the biomarkers of cartilage degradation appeared to be extensively investigated in comparison to other biomarker categories because articular cartilage degradation is a central feature of OA pathogenesis [26]. Therefore, we focused in this study on a biomarker of cartilage degradation (urinary CTX-II) because it is found that collagen type II degradation is one of the main pathologies of OA process and may reflects the process of joint degeneration and give a clear idea about what's going on inside the joint [27].

In the present study mean levels of urinary CTX-II in workers with knee OA were significantly higher than that in healthy workers. Our results were in compatible with the findings of Cheng et al., [29] which found significant rise in urinary CTX-II levels in the group of diagnosed knee OA compared with controls.

The current study shows significance in group I (OA workers) concerning levels of urinary CTX-II and WOMAC index scores. This was compatible with the study of Arunrukthavon et al. [30] who showed a significance between urinary CTX-II levels and WOMAC index scores ($r = 0.367$, $p < 0.001$). In contrast, the study of Wang et al. [31] found no association between levels of uCTX-II and WOMAC index scores.

Our study revealed that there is a significant positive correlation between pain and physical function subscales of WOMAC index and urinary CTX-II biomarker (p= < 0.001 and p= < 0.001respectively). While there was no significant correlation between stiffness subscale of WOMAC index and urinary CTX-II biomarker. This finding was in accordance to the cross sectional study conducted by Selistre et al. [32], who provide evidence of positive association in levels of uCTX-II with pain (r=0.49) and physical function (r=0.53), while Garnero et al. [33] found no significant correlation between urinary CTX-II levels and pain & physical function subscales of WOMAC index.

The present study shows significant association between severity of symptoms and signs of osteoarthritis in (Group I) and urinary CTX-II levels. This come in accordance with results of Joseph et al. [34] study who reported severity of symptoms and signs with increased urinary CTX-II levels, while, Rotterud et al., [35] found no relation between urinary CTX-II levels and patient-reported outcome scores.

In our study mean level of CTX-II in urine (Group I) was significantly higher among workers (>8 hours/day) than workers (≤8 hours/day). Moreover, a high statistical significance difference was found between years of duration of work and CTX-II in urine.

Moreover, in the present study higher mean levels of urinary CTX-II were found among (KL) grade 4 workers followed by grade 3 [mean ± SD (356.06 ± 30.54)] and lower levels were found in grade 2 workers [mean ± SD (263.60 ± 24.77]. Previous meta-analysis study also reported an increase in urinary CTX-II levels consistently in patients with severe knee OA on radiographic evaluation in comparison with those with mild knee OA (29). Subsequently, in osteophytosis progression, the increase in cartilage degradation defined by urinary CTX-II may refer to the invisible accompanying defects cartilage by plain radiography. So, urinary CTX-II can be used as a powerful tool for monitoring disease progression in OA. Urinary CTX-II levels may represent a predictor biomarker for subsequent OA progression, in several knee compartments, in case of its expression by combined osteophytosis [36]. The study of Waknine [37] showed that baseline urinary CTX-II concentrations in the highest quartile were associated with increased significant risk of prevalent radiographic knee OA (odds ratio [OR], 4.2; 95% confidence interval [CI], 2.2 - 7.0) compared with lowest-quartile levels and highest-quartile CTX-II levels were also associated with significantly increased risk of disease progression at the knee (OR, 6.0; 95% CI, 1.2 - 30.8) compared with lowest-quartile levels.

Concerning laboratory investigations in the present study no significant correlation was found

between urinary CTX-II levels and ESR and hsCRP (inflammatory markers) in OA workers (group I) which were done with routine diagnosis. This was in agreement with Keenan et al. [38] who found very weak correlation with disease activity. While, Hosnijeh et al. [39] found a significant correlation between the marker levels which reflect cartilage turnover urinary CTX-II and the inflammatory marker C-reactive protein.

The present study showed that the urinary CTX-II cut off value of 221 pg. /mg creatinine had a sensitivity of 98.4% and specificity of 73.7% and AUC (0.851*) for predicting clinical diagnostic OA criteria defined knee osteoarthritis, while the urinary CTX-II cut off value of 289.5 pg. /mg Creatinine had a sensitivity of 100% and specificity of 80% and AUC (1.000*) for predicting X-ray defined knee osteoarthritis. The AUC for X-ray (KL) grades was (1.000*) so this area was the biggest compared to clinical diagnostic criteria parameter, with high sensitivity (100%) and the highest specificity (80%), so it could be decided that values more than this point will be positive results and the values below this point will be negative. Accordingly, radiography could be recommended as the most sensitive and the most specific parameter for this study. Our result was in agreement with Jung et al. [40] study which demonstrated that value of urinary CTX-II 266 ng/ mmol creatinine showed a specificity of 87.5% and sensitivity of 75.5% for predicting X-ray defined knee osteoarthritis.

In accordance to the results of the present study, Song et al. [41] who proved that the prediction accuracy of urinary CTX-II (AUC 0.775) was more useful and significantly higher than that of the other biomarkers for assisting in the diagnosis of arthritis. While, Kalai et al. [42] found non-significant correlations between the CTX-II level and KL grade the cut off value of CTX-II is 210 µg/mol creatinine with the highest specificity and sensitivity (AUC 0.66, p = 0.001).

The current study reported that AUC for bone tenderness, Symptoms severity and WOMAC score were 0.730, 0.652, 0.634 respectively, while sensitivity was 62.1%, 76.8% and 77.8% respectively and the specificity was 85.7%, 53.6% and 50%. For those reasons our study concluded that urinary CTX-II may strongly aid in the early diagnosis of knee osteoarthritis in patients who have symptoms with no radiological evidence of osteoarthritis. This result was in accordance with Liem et al., [43] who noticed that Urinary CTX-II was shown to be strongly associated with clinical symptoms of OA as well as radiographic evidence of joint damage that could suggest urinary CTX-II to behave as the best-qualified biomarker for evaluation of OA.

Conclusion:

The current study concluded strong and consistent associations of urinary CTX-II with clinical symptoms of OA and radiographic evidence of joint damage as well. In accordance, urinary CTX-II can help in early OA diagnosis in symptomatic workers even with or without radiographic evidence of OA. Also it concluded that urinary CTX-II is a useful biomarker both clinically and investigatory and has high relevance for care planning and development of disease modifying drugs to decrease OA morbidity. Radiographic severity in addition to clinical features and demographic data suggested that OA development is not depending on single factor and that demographic factors and K&L grade in combination with urinary CTX-II can improve significantly the AUC for predicting OA pathology and diagnosis. So, urinary CTX-II biomarker could be used as a tool for early diagnosis in Egyptian male workers knee osteoarthritis before x-ray changes.

Recommendations:

- Urinary CTX-II biomarker is recommended to be used with routine investigations at periodic examination of workers for early detection of OA and early avoidance of any unwanted medicolegal issues.
- The field of study of OA diagnosis should pay more attention as a strategy for preventing or delaying the disease onset as there is no effective cure for OA at the advanced stage. Osteoarthritis biomarkers is an important area for additional investigations and further studies.
- Correct management of cases in early reversible stages is the main health and legal purpose to stop the disease progression and avoid expensive compensations.
- It is recommended to define more biomarkers of OA that can be applied on a larger scale from the very early stage to the final stage of OA, to design better management systems for patients with arthritis, to slow the progression and alter the natural course of the disease.

Ethical Considerations:

- Approval of the Ethics Committee of the High Institute of Public Health (286) was conducted.
- Approval of the Ethics Committee of National Research Centre (14-143) was conducted.
- An informed consent was taken from workers before participation in the study.
- The confidentiality of participant's information was considered and their identities were not mentioned.
- All samples were discarded after utilization in appropriate way.

Conflict of interest:
The authors declare no conflict of interest

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