Research Article

Relationship of urinary C terminal telopeptide of type II collagen (uCTX-II) with radiographic severity of primary knee osteoarthritis in Maharashtrian population: A Case-control study.

Namrata Ghatge[1*], Abhay Jagtap[1], Ashutosh Bhosale[2], Ashok Shinde[3]

[1]Department of Biochemistry, BJ Government Medical College and Sassoon General Hospital, Pune, Maharashtra, India
[2]Department of Orthopaedics, Krishna Institute of Medical Sciences, Karad, Maharashtra, India.
[3]Department of Biochemistry, Sinhgad Dental College, Pune, Maharashtra, India
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Corresponding Author: ***Namrata Ghatge.*** Email: namrata.abhibhosale@gmail.com

ABSTRACT

Introduction: Knee Osteoarthritis (OA) is diagnosed based on clinical features, functional assessment, and radiographic evaluation. Recently, biochemical markers of bone and cartilage have been studied for early disease detection and monitoring its progression. This study aims to measure one of the biochemical marker, urinary C-terminal telopeptide of type II collagen (uCTX-II), and evaluate how its levels vary between healthy individuals and patients with different radiological severities of knee osteoarthritis.

Methodology: After clinical and radiographic examination, 50 patients with primary knee osteoarthritis and 50 controls of age group 40-60 are included in the study. Levels of urinary C terminal telopeptide of type II collagen (uCTX -II) are measured using a competitive enzyme-linked immunosorbent assay. The Kellgren-Lawrence grading scale is used for radiological grading.

Results: The mean uCTX-II levels (pg/ml) in OA patients (280.02 ± 257.89) are significantly higher than in healthy controls (125 ± 72.2) with $p<0.05$.When the control group mean is compared with disease severity grades, the mean differences between the control group and Grades 1 and 2 are statistically significant.

Conclusion: This study found that uCTX-II levels are significantly high in osteoarthritis (OA) patients compared to healthy individuals. Subgroup analysis revealed that uCTX-II levels are elevated in early-stage knee OA (Grade 1 and Grade 2) compared to advanced stages (Grade 3 and Grade 4). These findings suggest that uCTX-II can be a useful marker for monitoring cartilage turnover in early OA and can help in early diagnosis, particularly when radiographic grading is unclear or subjective.

Keywords: : KOA, Primary Knee Osteoarthritis, Urinary C terminal telopeptide of type II collagen.

1. INTRODUCTION

Osteoarthritis is a chronic degenerative joint disease characterised by articular cartilage degeneration, subchondral bone sclerosis and osteophyte formation, loss of joint space which affects the quality of life. The primary tool used to define these features of OA in clinical practice and in research is by taking clinical presentations and radiographs. Currently, radiological changes are mostly detectable only when the disease has reached to an advanced stage [1, 2]. During the tissue turnover process biochemical markers originated from connective tissue matrices are released into biological fluid which can be used to diagnose the OA in the early stage [3, 4]. The CTX-II is a crosslinked C-terminal telopeptide of type II collagen with a pyridine ring, made up of dimeric-hexapeptide epitope (EKGPDP), which is broken down by the action of matrix metalloproteinases (MMPs) [5, 6]. Diffusion of CTX II from joint to blood and it finally gets released into urine. The uCTX-II increased in knee OA patients as compared to healthy subjects

[4, 7]. However, this diagnostic approach is not widely used in Asian populations, likely due to the limited number of studies conducted in Asia [8-10]. The present study aims to measure uCTX-II levels in patient of knee osteoarthritis and healthy controls, diagnosed through clinical and radiological assessments, and to access the association between uCTX-II levels and OA to determine its potential as an early diagnostic tool.

2. Material and Methods:

The study is carried out in the out-patient Department of a tertiary care hospital after obtaining ethical clearance from the research institute. From all the participant prior informed consent is obtained. The study includes 50 newly diagnosed primary knee osteoarthritis (KOA) patients aged 40–60 years, selected based on the American College of Rheumatology (ACR) clinical criteria [11]. Patients with secondary osteoarthritis, hepatic, kidney or malignant diseases, or any other conditions affecting the joints are excluded. Additionally, 50 age-matched controls, preferably first-degree relatives of the cases with no signs or symptoms of KOA, are included in the study.

All participants underwent a detailed history, clinical examination, and knee radiographs to assess structural knee damage. Anteroposterior weight-bearing radiographs of both knees are taken and classified using the K-L grading system into Grades 1, 2, 3, and 4 [9]. Morning urine samples (5-10 ml) are collected, and centrifuged at 3000 rpm for 15-20 minutes, and the supernatant is transferred to a centrifuge tube and stored at -20°C for further analysis. Urinary CTX-II levels (pg/ml) are measured using a competitive enzyme-linked immunosorbent assay (Human C-teloPeptide of Type 2 collagen, CTX-2 GENLISA™ ELISA : KBH1382, Krishgen Biosystem, Maharashtra, India). Data are statistically analyzed using SPSS version 21.0.

3. Results:

The study includes 100 subjects, comprising 50 patients and 50 controls. The demographic data is summarized in Table 1. The patient group has 15 males and 35 females, while the control group has 18 males and 32 females. The patient group has a significantly higher mean age (51.68 ± 5.68 years) compared to the control group (48.42 ± 5.11 years) (p = 0.004). The mean height is comparable between the patient group (156.94 ± 8.56 cm) and the control group (155.98 ± 8.73 cm), with no significant difference (p = 0.56). Similarly, the mean weight did not differ significantly between the patient group (64.49 ± 11.46 kg) and the control group (61.18 ± 11.35 kg) (p = 0.17). However, the mean BMI is significantly higher in the patient group (26.21 ± 4.12 kg/m²) compared to the control group (24.88 ± 3.73 kg/m²) (p = 0.05).

Table 1: Demographic data of different parameters in both groups

Demographic Data (N=100)	Range	Total Subjects (N=100)	Control (N=50)	Patients (N=50)	p-value
Age (year)	40-60	50.05 ± 5.67	48.42 ±5.11	51.68 ± 5.68	0.004*
Male (%)	33	33(33.0%)	18(36.0%)	15(30.0%)	0.53
Female (%)	67	67(67.0%)	32(64.0%)	35(70.0%)	
Height (cm)	143-183	156.46 ± 8.67	155.98 ±8.73	156.94 ±8.56	0.56
Weight (Kg)	38-92	62.84 ± 11.58	61.18 ±11.35	64.49 ±11.46	0.17
BMI (kg/m²)	16.9-35	25.55 ± 4.00	24.88 ±3.73	26.21 ±4.12	0.05*

N: Sample size; SD: Standard deviation, p - value * < 0.05 is statistically significant

Patient distribution according to K-L grade by radiograph

On radiographic assessment, the number of patients in different K-L grades are given in Table 2 as follows: Grade 1 (22%), Grade 2 (46%), Grade 3 (20%), and Grade 4 (12%).

Table 2: Distribution of patient group by K-L grading scale

		Patients (N=50)
K-L grading	**Grade 1**	11 (22%)
	Grade 2	23 (46%)
	Grade 3	10 (20%)
	Grade 4	6 (12%)

N: Sample size; K-L grads as - Grade 1, Grade 2, Grade 3, Grade 4.

Comparison of uCTX-II levels between KOA patients and healthy controls

The mean level of uCTX-II in the control group is 125 ± 72.2 pg/ml; in the patient group is 280.02 ± 257.89 pg/ml, and the difference is highly

significant (<0.006). The comparison is shown in Table 3.

Table 3: Comparison of uCTXII level in controls and patients

	Mean SD Contol (N=50)	Mean SD Pateints (N=50)	p Value
uCTXII pg/ml	125 ± 72.2	280.02 ± 255.30	0.006*

N: Sample size; SD-Standard deviation; uCTXII - Urinary C terminal telopeptide of type II collagen, pg/ml picogram per milliliter, p-value * < 0.05 - statistically significant.

Comparison of uCTX-II levels and different radiographic categories of OA severity

Comparison of control with disease severity by one-way ANOVA test is done to analyse levels of uCTX-II (pg/ml) between different grades which shows the mean of control (125 ± 72.2) as compared to the mean of Grade 1 (445 ± 277.2), mean of Grade 2 (270 ± 278.1), mean of Grade 3 (211 ± 158.9) and mean of Grade 4 (130 ± 125.6) is statistically highly significant (0.006) shown in Table 4.

Table 4: Comparison of uCTXII (pg/ml) with severity (K-L grading) of disease

	Xray Grading by K-L grading scale	N	Mean	SD	SE	F	p-value
uCTXII (pg/ml)	Normal	50	125	72.2	10.2	5.04	0.006*
	Grade 1	11	445	277.2	83.6		
	Grade 2	23	270	278.1	58		
	Grade 3	10	211	158.9	50.3		
	Grade 4	6	130	125.6	51.3		

uCTXII -Urinary c terminal telopeptide of type II collagen, pg/ml picogram per milliliter N: sample size; SD: Standard deviation; SE: standard error, p-value * < 0.05 is statistically significant.

Further subgroup analysis using a Post Hoc test shows comparison between uCTX-II levels of controls and different disease severity grades. The mean difference in uCTX-II levels (pg/ml) between controls and Grade 1 (-320, p=0.001) and Grade 2 (-145, p=0.015) is statistically significant. However, the mean difference between controls and Grade 3 (-85.7, p=0.639) and Grade 4 (-4.95, p=1) is not statistically significant. The mean difference between Grade 1 and Grade 3 (233.9, p=0.028) and between Grade 1 and Grade 4 (314.62, p=0.007) is statistically significant. Differences between other grades are not statistically significant (p>0.05), as shown in Table 5.

Table 5: Pairwise comparison of control with severity of disease

		Normal	Grade 1	Grade 2	Grade 3	Grade 4
Normal	**Mean difference**	-	-320	-145	-85.7	-4.95
	p-value	-	<0.001 *	0.015*	0.639	1
Grade 1	**Mean difference**		-	175	233.9	314.62
	p-value			0.066	0.028*	0.007*
Grade 2	**Mean difference**			-	59.2	139.93
	p-value			-	0.905	0.433
Grade 3	**Mean difference**				-	80.71
	p-value				-	0.905
Grade 4	**Mean difference**					-
	p-value					-

p-value * < 0.05 is statistically significant

4. Discussion:

Currently, to diagnose OA clinical history and X-rays are still the most dependable ways and helpful to measure the severity of the disease [12,13]. However, the destruction of joints caused by OA begins before it is diagnosed by radiographic changes [14]. Therefore, there is a need to create procedures in clinical practice that are more effective in early diagnosis than radiography [15]. Researchers face challenges in identifying biomarkers that enable the early diagnosis of osteoarthritis (OA) and predict its progression. Such biomarkers could help identify patients likely to experience disease progression, tell in advance interim structural changes or the evolution of pain and symptoms associated with OA pathophysiology, and assess the efficacy of treatments while monitoring their effects at an individual level [16].

In present study, the patient group has 15 males and 35 females, while the control group has 18 males and 32 females, which shows females are more prone to developed OA than men.Previous studies have shown similar results; the higher prevalence of osteoarthritis in females can be attributed to several factors. After menopause, hormonal changes particularly decreased estrogen levels can lead to early bone loss and increased risk of osteoporosis, which negatively affects joint stability and structure. Additionally, men generally have greater muscle mass and

stronger ligaments, providing better joint support. In contrast, women may experience greater joint stress and are more susceptible to joint injury, contributing to the increased risk of osteoarthritis [17]. Women tend to have a higher body mass index (BMI) than men, which is one of the most important modifiable differences between the sexes [18].

In the current study, most of the patients belong to the age group of 40-60 years, the mean age of the patients is 51.68 ± 5.68 years, showing an increased incidence after the age of 50 years. Similar findings were observed in the previous studies from India and reported that the mean age of patients with and without knee pain was 44.72 and 42.71 years, respectively (19), which show the age group of Indians to get KOA is much younger than the mean age in the studies conducted by other populations [20, 21]. Rossignol *et al.,* [22], observed that early onset of osteoarthritis (OA) was common among heavy workers, with nearly 40% of patients showing their first symptoms before the age of 50. Occupations that require repeated movements like squatting, kneeling, or lifting heavy objects can cause early knee osteoarthritis. These actions put constant stress on the knee joints, which speeds up cartilage damage over time. The mean BMI is significantly higher in the patient group (26.21 ± 4.12 kg/m²) compared to the control group (24.88 ± 3.73 kg/m²) with (p = 0.005). This finding aligns with Thigah *et al.,* [23], who reported an association between higher BMI and the development of knee osteoarthritis (KOA). Excess body weight increases the stress on weight-bearing joints, especially the knees. This additional load accelerates the wear and tear of joint cartilage, leading to its degeneration and the onset of osteoarthritis. Huaqing *et al.,* [24] showed that the risk of knee OA rises proportionally with body weight. However, in this study, no association is found between height, weight, or gender and uCTX-II levels in both patients and controls.

In this study, based on the K-L radiographic grading scale, the distribution of patients is as follows: Grade 1 (22%), Grade 2 (46%), Grade 3 (20%), and Grade 4 (12%). Similarly, Ram *et al.,* [17], reported that Grade 2 was the most common stage of knee osteoarthritis among Indian patients. In the current study, the mean of the uCTX-II in the patient group is (280.02 ± 255.30) and in the control group is (125 ± 72.2) with statistically significant (p=0.006) which can easily differentiate the knee OA patient from normal healthy individuals, his result aligns with findings from previous studies [25-28]. The increased uCTX-II pg/ml levels observed in KOA patients compared to the controls are attributed to increased enzymatic degradation of type II collagen, influenced by mechanical stress, inflammatory processes, and other factors contributing to cartilage breakdown [28-30]. So, uCTX-II is one of the specific marker for cartilage degradation [31] which can be used for diagnosing KOA patients.

Most of the previous studies indicate that uCTX-II levels increase in KOA as disease progressed [32-35]. But Romi Singh *et al.,* (36) reported that uCTX-II levels increase in early OA and decrease in advanced stages, but the exact reason for this decline was not explained. According to their study, radiographs mainly detect structural changes rather than disease severity. Additionally, conventional radiography has limitations in evaluating knee compartments, including the lateral, medial, and patellofemoral regions [37, 38]. Similar findings are observed in the present study, where uCTX-II levels varied significantly with disease severity (p = 0.006). The mean levels of uCTX-II (pg/ml) are as, controls (125 ± 72.2), Grade 1 (445 ± 277.2), Grade 2 (270 ± 278.1), Grade 3 (211 ± 158.9), and Grade 4 (130 ± 125.6). Further subgroup analysis in the present study revealed statistically significant mean differences in uCTX-II levels between the following groups; Normal vs. Grade 1: -320 (p = 0.001), Normal vs. Grade 2: -145 (p = 0.015), Grade 1 vs. Grade 3: 233.9 (p = 0.028), Grade 1 vs. Grade 4: 314.62 (p = 0.007) comparisons between other grades, the differences in mean uCTX-II levels are not statistically significant (p > 0.05). This shows that uCTX-II is released more in Grade 1 & Grade 2 than Grade 3& Grade 4. Previous studies by Maheshwar *et al.,* [39] and Neuman *et al.,* [40] highlight that arthroscopic findings provide an accurate representation of the extent and

severity of pathology in osteoarthritis (OA). In early OA, as identified by plain radiographs (KL grades 1 and 2), arthroscopic evaluation often reveals cartilage damage that includes softening, superficial defects, partial-thickness cartilage loss, and surface irregularities, even though these changes may not be evident on radiographs. In contrast, advanced OA, characterized by higher KL grades (3 and 4) on plain radiographs, corresponds to more severe arthroscopic findings. These include full-thickness cartilage loss, exposure of subchondral bone, and significant intra-articular damage, reflecting the progression of the disease to its later stages. This correlation underscores the utility of arthroscopy in detecting structural changes, particularly in the early stages of OA where radiographic findings may underestimate the severity of cartilage damage. However, knee arthroscopy can not be used for diagnosis in every patient due to the financial burden on the economy and the risk involved during the surgical procedure. In this scenario, the uCTX-II level can be used as a better tool for diagnosis and progression of disease from Grade 1 to progressive severity of the disease. The level uCTX-II represents fragments of type II collagen, a major structural component of articular cartilage. It is released into the joint during cartilage breakdown and eventually excreted in urine [5-7]. Reduction in uCTX-II levels in grades 3 & 4 compared to grades 1 & 2 could be due to the extensive loss of cartilage in severe OA, leading to a diminished amount of type II collagen available for degradation and, consequently, lower uCTX-II levels in advanced OA. In the present study, findings suggest that early stages of KL Grading (1 and 2) are associated with measurable differences compared to normal knees, while advanced Grades (3 and 4) do not exhibit significant deviations in the measured parameter as compared to the controls. This observation is very important for clinicians, who can be able to pick up early OA of the knee in case of Grade 1 or doubtful cases and start remedial measures early.

5. Conclusion:

The current study demonstrated that urinary CTX-II levels can differentiate between healthy individuals and patients with knee osteoarthritis (KOA). The uCTX-II levels are found to increase in the early stages of OA (KL grades 1 and 2) and decrease in advanced stages (KL grades 3 and 4). This suggests that uCTX-II may serve as a useful biomarker for clinicians to monitor and diagnose cartilage turnover, particularly in early-stage OA. However, uCTX-II appears to be more valuable for diagnosing KOA rather than predicting disease progression. Further research with larger sample sizes is needed to better understand the role of uCTX-II in monitoring and managing osteoarthritis in the Indian population. Additionally, studies are required to determine whether uCTX-II can help to track the progression of the disease from stage 1 to stages 2, 3, and 4 which may require surgery.

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Conflict of Interest:

The authors declare that, there is no conflict of interest regarding the publication of this paper.

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Ethical Information:

The present study is ethically approved by Ethical committee on human research of BJ Government Medical College and Sassoon General Hospital, Pune.

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