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Research Article

Assessment of IL-6, TGF-β1 and CTX-II in the diagnosis of early Post-Traumatic Osteoarthritis of knee

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Abstract

Objectives: Osteoarthritis is an inflammatory and degenerative disorder characterized by the degradation of the extracellular matrix. It commences with injuries to the knee that activate inflammatory pathways. Trauma may predispose to osteoarthritis, called post-traumatic osteoarthritis (PTOA). The disease is diagnosed in the later stages with (KL grade>2) as seen by X-ray; measuring the biomarkers present in the body fluids holds significant promise for the early detection and evaluation of PTOA. The study aimed to establish the role of inflammatory and collagen markers such as serum interleukin 6 (IL-6), transforming growth factor beta 1 (TGF-β1), and urine C-terminal cross-linked telopeptide of type II collagen (CTX-II) in the diagnosis of early post-traumatic osteoarthritis of the knee.

Methods: This case-control study was conducted among 80 participants, of which 40 were apparently healthy individuals, and 40 were cases with a history of trauma to the knee joint in the past ten to 12 weeks. Baseline characteristics, body mass index (BMI), Visual Analog Score (VAS), and Western Ontario McMasters Universities Osteoarthritis Index (WOMAC) were collected from all the participants. X-ray and MRI were done in the cases. Serum IL-6 and TGF-β1, and urine CTX-II were analyzed by ELISA. Statistical analysis was done with SPSS version 16. A P value ≤ 0.05 was considered statistically significant.

Results: The mean serum IL-6, TGF-β1, and urine CTX-II levels were significantly higher in cases than in controls, with P values of 0.025, 0.033, and 0.040 respectively. IL-6 showed correlations with age, WOMAC score, and urine CTX-II values. TGF-β1 showed a positive correlation with VAS.

Conclusion: Individuals with previous knee joint trauma exhibited notably elevated serum IL-6, TGF-β1, and urine CTX-II levels. Among the three biomarkers, IL-6 seemed to be a potential biomarker of early post-traumatic osteoarthritis in patients with knee injuries.

Keywords: Collagen break down products, interleukins, post-traumatic osteoarthritis, pro-inflammatory markers

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Osteoarthritis (OA) is the predominant arthritis type, affecting millions of individuals globally. The Osteoarthritis Research Society International (OARSI) defines osteoarthritis as a disorder affecting movable joints characterized by cell stress and degradation of the extracellular matrix (ECM). The process is triggered by micro- and/or macro-injuries to the knee joint that activate adaptive responses, which include inflammatory pathways of innate immunity [1]. Risk factors of the disease in-

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clude obesity, older age, joint injuries, genetics, and metabolic diseases [2]. Osteoarthritis following joint trauma is called post-traumatic osteoarthritis (PTOA). The prevalence of PTOA accounts for 12% of all symptomatic OA [3]. The injury can be in the form of a fracture, cartilage damage, ligament injury, or instability (or a combination). In many cases, injuries involve more than one structure in the knee. Pain, swelling, and instability are the most common features of knee osteoarthritis. Complications of osteoarthritis include difficult ambulation, decreased range of movement, and joint malalignment [2]. The time span required to clinically measure PTOA is highly variable, ranging from two years to decades depending on the severity of the joint injury [4]. The increasing incidence, age of presentation, rate of progression, and type of injury warrant a broader and in-depth approach to understanding PTOA. To date, X-ray is the investigation of choice, and the disease is classified into four grades by Kellgren Lawrence [5]. However, the radiographic techniques and the physical examination identify only a fraction of the individuals, especially in the later stages of Kellgren Lawrence (KL) grading. The radiographic features are found to have low inter-rater reliability. It has been shown that when the disease becomes radiographically apparent, significant joint damage could have occurred. As the molecular derangements appear much earlier in the course of the disease, analysis of the biomarkers, which indicate the molecular derangements, may have diagnostic and prognostic potentials, as well as serve as therapeutic targets. The biomarkers may be inflammatory, oxidative stress, collagen breakdown, genetic, and epigenetic markers. These markers may be measured in the blood, synovial fluid, urine, and the involved joint tissues. The markers have the potential to identify the different pathological processes involved in the causation of PTOA.

Interleukin 6 (IL-6) is a pro-inflammatory and a soluble mediator having a pleiotropic impact on inflammation, immune response, and hematopoiesis. Studies have shown that circulating levels of IL-6 are increased in osteoarthritis and cartilage loss in the knee joint occurring three to fifteen years after the injury [6]. Transforming growth factor beta 1 (TGF-β1) regulates cell proliferation, differentiation, apoptosis, and synthesis and degradation of the extracellular matrix (ECM). High levels of TGF-β1 alter the cartilage metabolism, as found in cases of PTOA [7]. Elevated levels of C-terminal cross-linked telopeptide of type II collagen (CTX-II) in urine have been observed to correlate with osteoarthritis, and increased concentrations are linked to the advancement of the disease [8]. The hypothesis of the study is that alterations in the levels of inflammatory and collagen biomarkers help in the diagnosis of early post-traumatic osteoarthritis of the knee. The study aimed to establish the role of inflammatory markers IL-6 and TGF-β1 in serum and collagen breakdown product, CTX-II in urine, in the diagnosis of early post-traumatic osteoarthritis (PTOA) of the knee.

Materials and Methods

This case-control study consisted of 80 participants in the age group of 20 to 50 years of both genders. The study was conducted in the Departments of Sports Medicine and Biochemistry at Sri Ramchandra Institute of Higher Education and Research. Ethics approval was obtained from the institutional ethics committee, SRIHER (IEC-N1/21/FEB/77/25). Participants satisfying inclusion criteria were inducted after obtaining written informed consent. The participants were administered VAS and WOMAC questionnaires [9, 10]. Cases included 40 participants with a history of trauma to the knee joint, for which they were treated conservatively or underwent surgery depending on the degree of trauma. They were subjected to X-ray of the knee to obtain the KL grading with a history of trauma to the knee joint in the past ten to 12 weeks. Controls included age- and sex-matched 40 apparently healthy individuals, with no history of trauma to the knee joint in the past two years. Individuals with osteoarthritis, autoimmune disorders, post-menopausal women, osteomyelitis, tumors, and septic arthritis of the knee, metabolic/systemic illnesses, participants on anti-resorptive therapy for bone or joint disorders, anticancer drugs, anti-metabolite drugs, hormone replacement therapy, oral contraceptive pills, calcium, vitamin D, intraarticular steroids, viscosupplementation in the past three months, and a history of previous surgery to the knee joint were excluded from the study.

Laboratory methods

Venous samples were collected for IL-6 and TGF-β1 by trained phlebotomists and were centrifuged for 15 minutes at 2000 X g; the serum was separated and stored at –80°C until analysis. Spot urine was collected for analysis of CTX-II; it was centrifuged at 1500 Xg for 10 minutes, and the supernatant was stored at –80°C until analysis. The levels of IL-6 and TGF-β1 in serum and CTX-II in urine were analyzed by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. As urine markers are usually expressed as a ratio to urinary creatinine concentration, the concentration of CTX-II in the urine was corrected for urine creatinine with the following formula:

$$\text{Corrected CTX II (ng/mmol)} = \frac{\text{CTX-II from ELISA (ng/mL)}}{\text{Con from creatinine (mmol/L)}} \times 1000 \text{ [11]}$$

Statistical analysis

Data analysis was performed using SPSS version 16. The variables were tested for normality of distribution using the Kolmogorov-Smirnov test. Continuous variables were expressed as mean and standard deviation or median and interquartile range. Categorical variables were expressed as frequency and percentage. The unpaired Student's t-test and Mann-Whitney U test were used to compare the variables. Pearson's and Spearman correlation coefficients were used to test the correlation between the variables. Receiver operating characteristic (ROC) curves were plotted to compare the discriminatory strengths of different mediators in the serum and urine

Table 1. Baseline characteristics of cases and controls

Variables	Case (n=40)		Control (n=40)		p
	n	%	n	%	
Age (years)#	31.05 (9.37)		27.58 (5.71)		0.2
Sex					<0.001**
Female	9	22	26	65	
Male	31	78	14	35	
Weight (kg)#	67.48 (12.13)		70.14 (15.88)		0.4
Height (cm)#	162.51 (8.35)		164.38 (8.58)		0.3
BMI (kg/m^2)					0.9
(≤23.4)	12	30	12	30	
(23.5 to 27.4)	16	40	14	35	
(≥27.5)	12	30	14	35	
VAS					<0.001**
No pain	6	15	39	98	
Mild pain	14	35	1	2	
Moderate pain	20	50	0	0	
WOMAC score#	76.55 (16.55)		100.00 (0.00)		<0.001**

#: Data expressed in mean and standard deviation. **: P value highly significant. BMI: Body mass index; VAS: Visual analog score; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index.

of study participants. Sensitivity, specificity, and area under the curve were calculated. A P value ≤0.05 was considered statistically significant.

Results

The study was conducted with 80 participants (40 cases, 40 controls) at Sri Ramchandra Institute of Higher Education and Research.

Age, gender, BMI, VAS, and WOMAC scores were the baseline characteristics. Sex, VAS, and WOMAC scores showed statistical significance between cases and controls (p<0.001). The mean age of the participants in the cases and controls were 31.05 (9.37) and 27.58 (5.71) years, respectively, with no statistical significance between the two groups (p=0.2). Among the cases, 50% experienced moderate pain, and 14% experienced mild pain, which was statistically different (p=0.001). The WOMAC score was found to be 76.5 in cases and 100 in controls with statistical significance (p<0.001). Isolated anterior cruciate ligament (ACL) injury accounted for 30%, and anterior cruciate ligament tear with medial meniscus tear injury accounted for 20%. Among the cases, 39 cases had grade 0, and one patient had grade 1 changes as seen in X-ray (Table 1).

The level of IL-6 was higher in cases when compared to controls with a P value of 0.025 and was statistically significant. Serum TGF-β1 levels were significantly higher in cases compared to that of the controls (p=0.033). Urine CTX-II was significantly higher in cases compared to controls (p=0.040) (Table 2).

Table 2. Serum and urine levels of biochemical mediators in cases and controls

Variable	Case (n=40)	Control (n=40)	p
Serum			
IL- 6 (pg/mL)#	10.96 (6.16–88.28)	8.36 (5.50–11.40)	0.025*
TGF-β1 (ng/mL)#	2.58 (1.92–3.19)	2.34 (1.59–2.66)	0.033*
Urine			
CTX-II (ng/mmol)#	6.91 (2.03–17.29)	3.15 (0.72–13.87)	0.040*

#: Expressed as median and interquartile range (IQR). *: P value significant. IL-6: Interleukin 6; TGF-β1: Transforming growth factor-β1; CTX-II: C -telopeptide fragments of type II collagen.

The correlation study indicated that VAS positively correlated with BMI (r=0.35, p=0.04) and negatively correlated with WOMAC scores (r=–0.45, p=0.007) with statistical significance. IL-6 showed a positive correlation with age (r=0.46, p=0.001) and a negative correlation with WOMAC scores (r=–0.24, p=0.04). TGF-β1 showed a positive correlation with VAS (r=0.32, p=0.04) and weight (r=0.21, p=0.04). Urine CTX-II showed a negative correlation with IL-6 (r=–0.11, p=0.34); however, it was not statistically significant (Table 3).

Figure 1 shows the ROC curves of IL-6, TGF-β1, and CTX-II. The area under the curve, sensitivity, and specificity for IL-6 were 0.55, 72%, and 52%, respectively. The area under the curve for TGF-β1 was 0.54, with a sensitivity and specificity of 68% and 49%, respectively. The area under the curve for CTX-II was 0.58, with sensitivity and specificity of 54% and 41%, respectively. They did not show statistical significance (Table 4).

Discussion

Trauma causes a cascade of biochemical reactions, leading to the deterioration of the structural stability of the joint, resulting in post-traumatic osteoarthritis (PTOA). This condition causes significant physical disabilities, such as an inability to perform routine physical activities, which further results in mental disturbances such as depression, anxiety, fatigue, and inadequate sleep [12]. Hence, it is imperative to understand the early biochemical changes in the joint, which would help in identifying the biomarkers that have diagnostic, prognostic, and therapeutic potential. The indicators of inflammation are demonstrated by the changes in the levels of markers found in different body fluids like blood, synovial fluid, and urine. The study aimed to determine the significance of inflammatory markers such as IL-6 and TGF-β1 in serum and collagen breakdown product, CTX-II in urine, in diagnosing early post-traumatic osteoarthritis (PTOA) of the knee. In a cohort study conducted by Gelber et al. [13], it is observed that middle-aged individuals who experienced knee injuries faced a significantly heightened risk of developing osteoarthritis in the future. Similarly, in the present study, participants were in the middle-aged group demographic population. Though females are more susceptible to OA development, an associ-

Table 3. Correlation of demographic details and biochemical parameters among study participants

	Expressed as correlation coefficient 'r' value and 'p' value								
	Demographic details				Pain score		Biochemical mediators		
	Age	Weight	Height	BMI	VAS	WOMAC	IL-6	TGF-β1	CTX-II
Demographic details									
Age	1	0.02 (0.89)	–0.2 (0.07)	0.12 (0.27)	0.22 (0.21)	–0.38 (<0.001)**	0.46 (0.001)*	–0.03 (0.79)	–0.01 (0.92)
Weight		1	0.36 (0.001)**	0.87 (0.00)	0.15 (0.39)	0.04 (0.72)	–0.05 (0.68)	0.21 (0.049)*	0.07 (0.51)
Height			1	–0.14 (0.23)	–0.29 (0.049)*	0.03 (0.79)	–0.12 (0.31)	0.15 (0.19)	0.02 (0.86)
BMI				1	0.35 (0.041)*	0.02 (0.85)	0 (0.96)	0.15 (0.17)	0.07 (0.55)
Pain score									
VAS					1	–0.45 (0.007)*	0.08 (0.69)	0.32 (0.048)*	–0.03 (0.87)
WOMAC						1	–0.24 (0.04)*	–0.1 (0.38)	0.07 (0.53)
Biochemical mediators									
IL-6							1	–0.09 (0.42)	–0.11 (0.34)
TGF-β1								1	0.07 (0.54)
CTX-II									1

*: P value significant; **: P value highly significant. BMI: Body mass index; VAS: Visual analog score; WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index; IL-6: Interleukin 6; TGF-β1: Transforming growth factor -beta 1; CTX-II: C -telopeptide fragments of type II collagen.

Table 4. Area under the curve and cut off value of IL-6, TGF- β and Urine CTX- II

Variables	AUC	SE	p	95% CI	Cut off value	Sensitivity (%)	Specificity (%)
IL-6	0.55	0.06	0.37	0.42–0.68	0.62	72	52
TGF-β1	0.54	0.06	0.51	0.41–0.66	2.36	68	49
CTX-II	0.58	0.06	0.21	0.45–0.70	1.44	54	41

IL-6: Interleukin 6; TGF-β1: Transforming growth factor β1; Urine CTX-II: Urine C -telopeptide fragments of type II collagen; AUC: Area under the curve; SE: Standard error; CI: Confidence interval.

ation between gender and disease prevalence in post-traumatic osteoarthritis (PTOA) is yet to be established [3]. In the present study, 22% were females and 78% were males. Among the sports activities, male skiers sustain more knee injuries than females [14]. Female athletes are more prone to anterior cruciate ligament (ACL) injuries than males [15]. The type and number of injuries depend on the type of routine work and also any sports activity involved.

People who suffer from OA undergo varying levels of pain and restricted mobility of the affected joint. Visual Analog Scales (VAS) and the Western Ontario and McMaster University (WOMAC) scale are the most commonly employed self-administered scales to assess the intensity of joint pain in patients with osteoarthritis of the knee or hip. The WOMAC pain subscale uses a five-item questionnaire, each about a different activity type (e.g., walking, standing, etc.), whereas VAS is based on a single-item questionnaire measuring any kind of pain specific to the index joint. The pain expressed by the VAS was categorized as no pain (score: 0), mild (score: 1–3), moderate (score: 4–6), and severe pain (score: 7–10) [9]. In the present study, among cases, 50% of the individuals experienced moderate pain, and 14% experienced mild pain, which was statistically significant ($p<0.001$) (Table 1). The Western Ontario and McMaster Universities Osteoarthritis (WOMAC) questionnaire consists of three subscales: pain, stiffness, and physical function. The extent of pain is noted to range from none to extreme. A score of 100 indicates no symptoms or functional disability, and 0 indicates extreme symptoms and functional disability [10]. As per Roos et al. [16], healthy individuals typically exhibit

a mean score ranging from 88 to 89, while the OA group usually demonstrates a mean score ranging from 52 to 84. Similarly, in the present study, the WOMAC score was notably lower among cases compared to controls with statistical significance ($p<0.001$). (Table 1) It showed a negative correlation with age among the participants ($r=-0.38$, $p<0.001$). (Table 3) This indicated that lower WOMAC scores in the advancing age groups are probably due to increasing pain thresholds with advanced age and also the variations in their daily physical activities.

In the present study, with regard to magnetic resonance imaging (MRI) findings, there were a maximum number of isolated ACL tears (30%). This is similar to a study by Maffulli et al. [17], which suggests that ACL tears are the most common type of knee injuries and can also be associated with other injuries, including meniscal tears, femoral condylar fractures, and cartilaginous loose bodies. X-ray is a commonly used investigation to assess KL grading, but it helps to diagnose the disease in later stages, especially from grade II onwards [18]. Hence, early diagnosis of the disease is possible by analyzing the molecular biomarkers, so that early management strategies can be implemented to slow down the progression of the disease.

Osteoarthritis is driven by inflammatory mediators in its initial stages, producing metalloproteinases that degrade the cartilage matrix. IL-6, a proinflammatory marker, has a crucial role in the pathogenesis of OA. Elevated levels of IL-6 in the serum or synovial fluid of osteoarthritis (OA) patients are linked to both the incidence and severity of the disease. It plays a significant role in the development of cartilage pathology by inducing matrix-degrading enzymes [6]. It increases sensitization to mechanical stimulation by activating the neurons through trans-signalling pathways; both peripheral and central sensitization can cause chronic pain [19]. In the present study, when the levels of IL-6 were compared between cases and controls, the mean levels were significantly higher in cases than in controls ($p=0.025$) (Table 2). IL-6 production is stimulated by IL-1 after trauma and is higher in the synovial fluid of ACL-deficient knees [20]. The injuries lead to high levels of inflammatory cytokines like IL-6, IL-8, interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α) in synovial fluid, reflecting local inflammation in the joint [21]. As reported by Beekhuizen et al. [22], there is an increased level of IL-6 in OA, implying the involvement of inflammatory processes. The levels in serum reflect the efflux of inflammatory cells into the systemic circulation. A cross-sectional study showed that IL-6 remained high for a maximum of 50 weeks after ACL injury [23].

The present study showed a positive correlation between IL-6 levels and age and a negative correlation with WOMAC scores among the participants (Table 3). With advancing age, the regulation of IL-6 gene expression becomes less effective, contributing to cartilage degradation and the pain associated with PTOA [24]. The cross-sectional study by Orita et al. [25] revealed a negative correlation between IL-6 levels and WOMAC scores. Elevated IL-6 levels in participants with trauma were directly related to pain and malfunction, with lower WOMAC scores indicating more severe symptoms and functional disability, thus establishing an inverse relationship between WOMAC score and IL-6 levels.

TGF-β1 is a growth factor that plays a significant role in the maintenance of homeostasis of articular cartilage. The isoforms and receptors are expressed in bone, cartilage, and synovial tissues, and the signaling by TGF-β1 is cell-dependent [26]. It stimulates chondrocyte proliferation and osteoblast terminal maturation, induces synovial tissue fibrosis, and plays a vital role in tissue formation, repair, and inflammation, essential for cartilage homeostasis [26]. The predominant form of TGF-β1 in articular cartilage is TGF-β1 (60–85% of total). High quantities in articular cartilage and synovial fluid indicate severe OA changes [27, 28]. In a study by Waly et al. [29], the levels of TGF-β1 are elevated in the OA group compared to the healthy individuals. It is suggested to be a helpful marker in assessing the prognosis of osteoarthritis. In post-traumatic osteoarthritis, there is an activation of TGF-β1 in the subchondral bone during osteoclast bone resorption and is found to be elevated in the synovial fluid of the knee joints [30]. In a study by Sarahrudi et al. [31], TGF-β1 levels are measured in patients with trauma in the form of long bone fractures and are higher in the early healing period. It started decreasing by two weeks and then returned to normal by eight weeks.

In the present study, serum TGF-β1 levels were significantly higher in cases compared to controls ($p=0.033$) (Table 2). Molloy et al. [32] describe the healing process of tendons and ligaments in five phases in sequence from the immediate post-injury phase, followed by inflammation, proliferation, reparation, and remodeling phases over 21 days. Levels of TGF-β1 are active in almost all stages of tendon healing. TGF-β1 mRNA levels increase after tendon injury and are found to be high in the third week, and the levels gradually decrease to normal levels by the end of the 14th week. In the present study, correlation studies of TGF-β1 with demographic variables showed a positive correlation with weight among the participants. TGF-β1 levels showed a positive correlation with the VAS score (Table 3). In line with the present study, Lin et al. [33] found a positive correlation between TGF-β1 and obesity in both men and women in the Japanese population. The study by Davidson et al. [34] shows that TGF-β1 levels are linked to pain and cartilage damage. Studies have shown that TGF-β1 decreases IL-6 receptor expression, which in turn decreases IL-6 signaling in chondrocytes and suppresses the action of IL-6 [35]. TGF-β1 levels in plasma have been found to correlate negatively with VAS in osteoarthritic patients [36]. When the cartilage is damaged, it releases TGF-β1, which can induce nerve growth factor (NGF). NGF is a neuron survival factor and a sensitizer of nociceptors that causes pain [37].

Proteases release C-telopeptide fragments of type II collagen from the matrix and cartilage. Type II collagen comprises the majority of the extracellular matrix. The levels of CTX-II can be detected in urine, offering a precise measure of the degradation

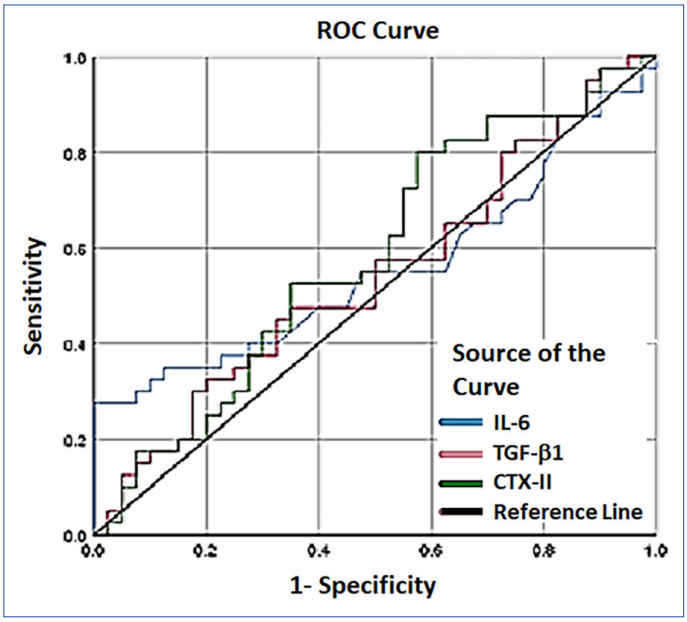

Figure 1. Receiver operating characteristics curve for the biochemical mediators IL-6, TGF-β1 and CTX-II.

ROC: Receiver operating characteristic; IL-6: Interleukin 6; TGF-β1: Transforming growth factor-β1; CTX-II: C -telopeptide fragments of type II collagen.

of type II collagen. Various studies indicate CTX-II levels in urine as a promising marker in the diagnosis of osteoarthritis [38, 39]. In the present study, urine CTX-II was significantly higher in cases compared to controls (p=0.040) (Table 2). In a study conducted by Arunrukthavorn et al. [11], the urine CTX-II levels are significantly elevated in the osteoarthritic (OA) group compared to that of the control group. These levels correlate with the radiological severity of OA. In patients with anterior cruciate ligament reconstruction, the levels of CTX-II are found to be significantly high through 16 weeks after adjusting for BMI [4]. In a study by Xin et al. [40], the level of urine CTX-II in grade-1 OA did not significantly differ from the control group, and there are significantly higher with higher grades of OA. In the present study, urine CTX-II showed a negative correlation with IL-6; however, statistical significance was not obtained, probably due to the small sample size (Table 3). This finding agrees with a study by Poree et al. [41], which concludes that IL-6 and sIL-6 R or a combination of both decrease the Sp1:Sp3 ratio and downregulate the expression of type II collagen at the transcriptional level.

In the present study, the measured biomarkers did not give statistically significant results in the ROC curve analysis. The area under the curve (AUC) for urine CTX-II was higher than other markers, but the sensitivity was 54%, which was the lowest compared to other parameters. IL-6 had better sensitivity than the other two variables, with the area under the curve being 0.55. However, the biomarkers did not show any statistical significance (Fig. 1). In the study by Panina et al. [42], the area under the curve for IL-6 is reported to be 0.753 with a sensitivity of 65.5% and specificity of 84.2%. According to Wang et al. [43], AUC for CTX-II is 0.886, with a sensitivity of 84% and specificity of 86%. All three mediators in the present study did not show statistical significance, probably due to the small sample size. It was a single-center trial with a small sample size. Measurement of the mediators in the synovial fluid could reflect the exact pathogenesis of the disease.

Limitations of the study

The sample size was small. As this study was part of a longitudinal cohort study, compiling the results at the end of the survey could be more informative and may shed more light on the diagnosis and prognosis of the disease. The future scope of the study can include long-term follow-ups with the participants to identify early biomarkers that are predictive of PTOA development and progression.

Conclusion

Post-traumatic osteoarthritis occurs after an injury to the joint. The disease occurs at an early age when compared to primary osteoarthritis. Since it goes undetected until advanced stages, early identification through analysis of biochemical alterations can forecast the onset of the disease. The study indicated that serum levels of interleukin-6 and serum transforming growth factor β1 and urine CTX-II were significantly higher in cases compared to controls. Interleukin-6 showed associations with age, WOMAC scores, and urine CTX-II levels while transforming growth factor β1 levels were associated with BMI and VAS scores. Among the three markers, IL-6 appeared to be a potential biomarker for the diagnosis of early arthritic changes in patients with knee injuries. Thus, these markers will help in initiating targeted therapies to prevent or slow down post-traumatic osteoarthritis and the associated morbidities.

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