Comparison of Urinary CTX-II Levels in Postmenopausal Women with Knee Osteoarthritis with or without Osteoporosis

Osteoporozu Olan ve Olmayan Diz Osteoartritli Postmenopozal Kadınlarda İdrar CTX-II Düzeylerinin Karşılaştırılması

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ABSTRACT Objective: In this study, we investigated a cartilage degradation marker; cross-linked C-terminal telopeptide of collagen, CTX-I (serum) and CTX-II (urine) along with other laboratory variables in postmenopausal women with osteoarthritis (OA) of the knee with/without associated osteoporosis (OP). In addition, we aimed to investigate whether CTX-II which is released into the synovial fluid and systemic circulation and excreted by urine was an effective marker for the diagnosis of OA. Furthermore, we evaluated a possible relation of CTX-II with radiological grades of OA. **Material and Methods:** Postmenopausal women without any history of OA or OP costituted the control group (n=21). Study Group I consisted of 30 post-menopausal women with primary OA of the knee, and Group II consisted of 14 postmenopausal women with primary OA of the knee along with OP. Group I and Group II were categorized by radiological stages according to Kellgren-Lawrence system as stage 2, stage 3 and stage 4. **Results:** The control group was not statistically significantly different from Group I, II, or radiologically 2nd, 3rd, 4th stage of OA groups for age, body mass index, duration of menopause or levels of alkaline phosphatase, erythrocyte sedimentation rate, C- reactive protein, osteocalcin, CTX-I and CTX-II. The results of CTX-II values (ng/mmol) of the control and staged groups were as follows; median (25th-75th) level:341 (216-850); 609.34 (363-1072); 492 (319-927); 928 (400-1245) respectively; (p=0.210). Percentage change of median values between the control group and group I, II and radiologically 2nd, 3th, 4th stage of OA groups were 61%+, 80%+, 78%+, 44%,+ 172%+, respectively. **Conclusion:** CTX-II levels of the control group were similar to those of the knee osteoarthritis patients with or without osteoporosis as well as radiologically graded subgroups, and statistical analysis did not show statistically significant differences. , However, percentage change of median values between the control group and group I, II, and between the control group and radiologically 2nd, 3th, 4th stage of OA groups were significant.

Key Words: Postmenopause; osteoporosis; osteoarthritis, knee; collagen type I trimeric cross-linked peptide

ÖZET Amaç: Bu çalışmada osteoporozun (OP) eşlik ettiği veya etmediği diz osteoartritli (OA) postmenopozal kadınlarda diğer laboratuvar değişkenlerinin yanı sıra kollagen çapraz bağlı C-terminal telopeptidi CTX-I (serum) ve CTX-II (idrar) olarak adlandırılan kıkırdak parçalanma belirtecini araştırdık. Sinovyal sıvı ve sistemik dolaşıma salınan ve idrarla atılan CTX-II'nin OA tanısı için etkili bir belirteç olup olmadığını araştırmayı da amaçladık. Ayrıca CTX-II ile OA'in radyolojik dereceleri arasında olası bir ilişkiyi değerlendirdik. **Gereç ve Yöntemler:** OA veya OP öyküsü olmayan postmenopozal kadınlar kontrol grubunu oluşturdu (n=21). Çalışma grubu I primer diz OA olan 30 postmenopozal kadından oluşurken, Grup II OP'nin yanı sıra primer diz OA'sı olan 14 kadından oluşuyordu. Grup I ve Grup II Kellgren-Lawrence sistemine göre evre 2, evre 3 ve evre 4 olarak radyolojik olarak kategorize edildi. **Bulgular:** Kontrol grubu ve Grup I, II ve radyolojik olarak 2., 3., 4. evre OA grupları arasında özellikler ve yaş, beden kitle indeksi, menopoz süresi ve alkalen fosfataz, eritrosit sedimentasyon hızı, C-reaktif protein, osteokalsin, CTX-I ve CTX-II düzeyleri gibi hasta verileri bakımından istatistiksel olarak anlamlı fark bulmadık. Kontrol ve evre gruplarının CTX-II değerleri (ng/mmol) sonuçları aşağıdaki gibiydi: Ortanca olarak sırasıyla (25-75): 341 (216-850); 609,34 (363-1072); 492 (319-927); 928 (400-1245); (p=0,210). Kontrol grubu ve grup I, II ve radyolojik olarak 2., 3., 4. evre OA grupları arasında ortanca değerlerin yüzde değişimi sırasıyla %61+, %80+, %78+, %44+, %172+ idi. **Sonuç:** Osteoporozu olan ve olmayan diz osteoartriti hastaların ve radyolojik olarak derecelendirilmiş alt grupların CTX-II düzeyleri kontrol grubuna göre istatistiksel olarak anlamlı fark göstermedi. Fakat kontrol grubu ve grup I,II arasında ve kontrol grubu ve radyolojik olarak 2., 3., 4. evre OA grupları arasında ortanca değerlerin yüzde değişimi belirgindi.

Anahtar Kelimeler: Postmenopoz; osteoporoz; osteoartrit,diz; kollajen tip I üç parçalı çaprazlayan peptid

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In general, the diagnosis and progression of osteoarthritis (OA) are monitored by the radiological measurement of changes in joint space width (JSW) although it is an indirect measure of cartilage integrity. Alternatively, biochemical markers of bone, synovium and cartilage turnover have been suggested for the diagnosis, prognosis, and treatment monitoring of OA.[1] One of these biomarkers is CTX-II, a six-amino acid (EKGPDP) fragment of C-terminal telopeptide region arising from degradation of type 2 collagen by collagenase. It is released into the synovial fluid and systemic circulation and excreted by urine. In some studies, CTX-II levels are shown to be increased in the patients with OA and rheumatoid arthritis (RA) when compared to healthy controls.[2] In 1972, Foss and Byers reported relatively rare occurrence of OA among patients with femoral neck fracture, suggesting a possible inverse relation between OA and OP.[3] So far, a number of studies have shown that these two conditions are in negative association in clinical practice; OA and OP are rarely found together in the same patient and thus, OP may have a protective or delaying effect on OA.[4-10]

Based on the hypothesis suggesting that CTX-II is a marker of type 2 collagen breakdown, we aimed to investigate whether CTX-II was a valuable marker for the diagnosis of OA, and to find a possible relation of CTX-II with radiological grades of OA.

MATERIAL AND METHODS

This study was performed between August 2008 - February 2009 in Physical Medicine and Rehabilitation (PMR) outpatient clinics of Turkish Ministry of Health, Istanbul Education and Research Hospital. This research was funded by the Istanbul Education and Research Hospital. The Institutional Review Board of Istanbul Education and Research Hospital approved this study (May 26, 2009/ 27). We evaluated 143 subjects at the beginning of the study. All participants gave their written informed consents. With exclusion of 78 participants, we finally evaluated a total of 65 subjects in our study. Our exclusion criteria were as follows: use of biphosphonates, calcitonin or selective estrogen receptor modulators or presence of a chronic diseases. Furthermore, the major exclusion criteria in our study were secondary OA, history of the knee surger, and use of intraarticular glucocorticoids. Patients admitted to PMR outpatient clinics were evaluated according to the American College of Rheumatology (ACR) diagnostic criteria.[11,12] Demographical information and data on age, duration of menopause and the side of the knee pain were collected from the patients' charts. If there was pain in both knees, clinical and radiological assessments were performed on the right knee. Control group was included 21 postmenopausal healthy women, and a total of 44 patients with a diagnosis of primary knee osteoarthritis were included in the patient groups. Patients with only primary OA of the knee formed the first group (Group I, n=30), while patients with primary OA along with OP formed the second group (Group II, n=14). Age, body mass index (BMI) values and duration of menopause of the study group are shown in Table 1. Postmenopausal women admitted to the PMR outpatient clinics were staged radiologically as mild [stage 2 (n=19)], moderate [stage 3 (n=18)], or severe [stage 4 (n=7)] by physical medicine specialists using Kellgren-Lawrence system. Grading was not performed for the control group (n=21) and a score of '0' was assigned to these patients. Radiological stage of the patients was evaluated by Kellgren-Lawrence index and a personal difference scale in the context of the study.[13] Bone mineral density (BMD) of the patients and control group was measured using DXA (Dual Energy X-Ray Absorptiometer) at the following sites: femoral wards, femoral neck, lumbar total, and lumbar L1-L4. Patients with T scores ≥2.5 as assessed by DXA were diagnosed with OP.

Blood samples were collected into separate evacuated tubes (Beckton Dickinson) for alkaline phosphatase (ALP), erythrocyte sedimentation rate (ESR), C- reactive protein (CRP9, osteocalcin, and CTX-1 measurements following at least eight hours of fasting, and the laboratory results were recorded. Morning urine samples were taken from the same patient group in order to measure CTX-II and creatinine levels These samples were stored at -20°C

TABLE 1: Comparison and descriptive data of patients in the three groups.

Variables	Control Group (n= 21)	Group I (n= 30)	Group II (n= 14)	p
Age, years	58.6 ± 6.7	58.6 ± 4.6	60.2 ± 5.6	> 0.05*
Duration of menopause, years	11 (5-19)	11 (6.75 - 17.25)	14 (6-22)	> 0.05**
BMI, kg/m^2	29.3 ± 3.8	31.5 ± 3.6	30.7 ± 4.03	> 0.05*

* p value was calculated using one-way ANOVA.
** p value was calculated using Kruskal-Wallis test.
Control group: Post-menopausal women
Group I: Post-menopausal women with the knee osteoarthritis
Group II: Post-menopausal women with the knee osteoarthritis plus osteoporosis
BMI: Body Mass Index

for four months. Only one freeze-thaw episode was applied for each sample. ALP level was measured by colorimetric p-nitrophenylphosphate method, and urine creatinine levels in the spot urine samples were measured by colorimetric Jaffe method (Abbott Aeroset 2.0; Abbott Diagnostics, USA). ESR was measured by infrared barrier method (Electo Lab, Vital Diagnostics, Italy). CRP level was measured nephelometrically (Immage; Beckman Coulter, USA). Osteocalcin level was measured by RIA (Biosource, USA). Serum CTX-I (ng/mL) level; was measured by an electrochemiluminescence assay (Roche Diagnostics; Germany).

Urine CTX-II measurements were done by a sandwich ELISA assay (Nordic Biosciense Diagnostics, UK). This kit measures C-terminal telopeptide levels from collagen degradation products. Measurements were done using spot urine samples and expressed as micrograms per liter. CTX-II levels were adjusted to urine creatinine concentrations using the following equation: adjusted CTX-II (ng/mmol)= [1000 X urine CTX-II (μg/L)]/[Creatinine (mmol/L)]. Absorbance readings were done at 450 nm and 620 nm on the ELISA reader (GDV Reader-Washer; Italy). Intra-assay and inter-assay CV's were 7.8% and 12.2% (n=20), respectively.

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS 11.5 software. Normality of continuous variables was tested by Kolmogorov-Smirnov test. Variables with Gaussian distribution were expressed as mean ± SD, whereas variables with non-Gaussian distribution were expressed as median (25^{th}-75^{th} percentile). Intergroup comparison of normally distributed, logarithmic and square-root transformed variables was done by one-way ANOVA. Kruskal-Wallis test was used for intergroup comparisons of variables that do not exhibit normal distribution after transformation. Correlations between variables were examined by using Spearman's correlation coefficients (r_s) and Pearson's correlation coefficients (r). Associations were declared as statistically significant if the two-tailed P value was <0.05. Percentage change of median values between the control group and group I, II and radiologically 2^{nd}, 3^{th}, 4^{th} stage of OA were also calculated. The associations among radiologic grade and demographical data and laboratory results were evaluated with multiple regression analysis for Group I and II.

RESULTS

Statistical analyses revealed no significant differences among the groups with regard to age, BMI, duration of menopause and ALP, ESR, CRP, and osteocalcin levels (p>0.05) (Tables 1 and 2). In addition, no statistically significant difference was present for the levels of CTX-II and CTX-I among the groups (p>0.05). Group I and Group II were categorized by radiological stages according to Kellgren-Lawrence system as stage 2, stage 3, and stage 4. P value was calculated using Kruskal-Wallis test and presented as median (25^{th}-75^{th}). The results of CTX-II values of the control and staged groups were as follows: 341 (216-850); 609.34 (363-1072); 492 (319-927); and 928 (400-1245) respectively; (p=0.210). A moderate correlation was found between the levels of CTX-I and ALP (r=0.520; p=0.016), and the levels of CTX-I and osteocalcin (r=0.649; p=0.001) in the control group by Spearman correlation analysis (Table 3).

TABLE 2: Comparison of the variables among the groups.

Variables	Control Group (n= 21)	Group I (n= 30)	Group II (n= 14)	p
ALP, U/L	83 (60-106.5)	86.5 (75.75-101.5)	83.5 (72-97.75)	>0.05**
ESR, mm/1h	16 (11.50-19)	19 (15.5-29)	14 (7.25-21)	= 0.051**
CRP, mg/dL	0.35 (0.23-0.71)	0.41 (0.18-0.65)	0.44 (0.27-0.73)	> 0.05**
Osteocalcin, ng/mL	12.3 (11.95-14.95)	12.5 (11.45-14.25)	11.4 (11.15-12.6)	= 0.062*
C-TX I, ng/mL	0.32 (0.23-0.50)	0.45 (0.33-0.54)	0.34 (0.22-0.50)	> 0.05**
CTX-II, ng/mmol	341 (216-850)	549 (345-963)	615 (226-1538)	>0.05†

* p value was calculated using one-way ANOVA
** p value was calculated using Kruskal-Wallis test
† p value was calculated using one-way ANOVA after logarithmic transformation
ALP : Alkaline phosphatase
ESR : Erytrocyte Sedimantation Rate
CRP: C-Reactive Protein
C-TX I: Cross-linked C-terminal telopeptide I
CTX-II: Cross-linked C-terminal telopeptide II

In Group I, a moderate correlation was present between the levels of CTX-I and osteocalcin (r=0.433; p= 0.017), and between the levels of CTX-II and ALP (r=0.395; p= 0.031). In Group II, a moderate correlation was found between CTX-II and CRP levels (r=0.543; p=0.045). Additionally, a strong correlation was determined between CRP and BMI levels (r=0.705; p=0.005) (Table 3). Percentage change of median values between the control group and Group I, II (61%+, 80%+, respectively), and control group and radiologically 2nd, 3rd, 4th stage of OA groups (78%+, 44%+, 172%+, respectively) were calculated. Multiple regression analysis was used to analyze independent determinants of radiologic grade. According to multiple regression analysis of Group I and II, CTX-I (β±SE: 3.237±0.730; p=0.011), ALP levels (-0.042±0.010; p=0.013) and duration of menopause (-0.137±0.049; p=0.048) were independently associated with radiologic grading only in Group II (p<0.05). No significant relation was present between radiologic grades with regard to demographical data and laboratory test results (Table 4).

TABLE 3: Correlation coefficients for pairs of demographical and biochemical variables.

	Variable pairs	Correlation coefficient	p
Control Group	CTX-I- ALP	r_S= 0.520	<0.05
(n= 21)	CTX-I-Osteocalcin	r_S= 0.649	<0.001
Group I	CTX-1-Osteocalcin	r_S= 0.433	< 0.05
(n= 30)	CTX-II-ALP	r_S= 0.395	<0.05
Group II	CRP-BMI	rs= 0.705	< 0.005
(n=14)	CRP-CTX-II	rs= 0.543	< 0.05

r: Pearson's correlation coefficient.
r_S:Spearman's correlation coefficient.
Control group: Post-menopausal women.
Group I: Post-menopausal women with the knee osteoarthritis.
Group II: Post-menopausal women with the knee osteoarthritis plus osteoporosis.
ALP: Alkaline phosphatase; CRP: C-Reaktive Protein;
C-TX I: Cross-linked C-terminal telopeptide I;
CTX-II: Cross-linked C-terminal telopeptide II; BMI: Body Mass Index.

DISCUSSION

Osteoarthritis (OA) which is a common joint disease in all over world is defined as a group of conditions with joint symptoms related to the structural impairment of joint cartilage and changes in neighboring bones by American College of Rheumatology. Radiography which is used in diagnosis and progression of OA indicates the changes in the subchondral bone, and indirectly measures alterations in cartilage. For this reason, biochemical markers of bone, synovium or cartilage have been proposed for the diagnosis, prognosis and treatment of OA. Type II collagen is the most abundant protein in cartilage and highly specific for this tissue. Fragments that are released during its degradation could be used as markers of cartilage degradation. Urinary CTX-II level is one of them and the most frequenly studied marker in OA.

Findings of various studies performed by different investigators support the use of urinary CTX-II levels as an indicator of rapid joint cartilage degeneration in osteoarthritis patients.[14,15] In a number of studies, high levels of CTX-II in urine and synovial fluid were found in both inflammatory arthritis and OA when compared to the

TABLE 4: Multipl regression analysis for Group I and Group II.

Group I	β	SE (95% CI)	p value	Group II	β	SE	p value
Constant value	-3.512	2.467 [(-8.657)-1.634]	p>0.05		-4.998	2.917 [(-13.096)-3.100]	p>0.05
Logarithmic Urine-CTX-II	0.917	0.488 [(-0.101)-1.936]	p>0.05		1.273	0.478 [(-0.053)-2.599]	p=0.056
CTX-I	-1.722	1.238 [(-4.304)-0.859]	p>0.05		3.237	0.730 [(-1.211)-5.262]	p=0.011
CRP	-0.633	0.462 [(-1.598)-0.331]	p>0.05		-1.453	1.005 [(-4.243)-1.336]	p>0.05
Age	0.023	0.040 [(-0.062)-0.107]	p>0.05		0.151	0.057 [(-0.007)-0.310]	p>0.05
BMI	0.079	0.041 [(-0.07)-0.165]	p>0.05		0.040	0.057 [(-0.118-0.199]	p>0.05
Duration of menopause,	0.028	0.027 [(-0.028)-0.083]	p>0.05		-0.137	0.049 [(-0.272)-(0.002]	p=0.048
Osteocalcine	0.032	0.063 [(-0.100)-0.164]	p>0.05		-0.177	0.068 [(-0.364-0.011]	p>0.05
Sedimentation	-0.007	0.015 [(-0.038)-0.024]	p>0.05		0.068	0.033 [(-0.023)-0.159]	p>0.05
ALP	0.004	0.008 [(-0.011)-0.020]	p>0.05		-0.042	0.010 [(-0.069-(-0.015]	p=0.013

Dependent variable: Radiologic grade; β: Regression coefficient; SE: Standard error; 95% CI: 95% confidence Interval.
ALP: Alkaline phosphatase; CRP: C-Reaktive Protein; C-TX I: Cross-linked C-terminal telopeptide I; CTX-II: Cross-linked C-terminal telopeptide II; BMI: Body Mass Index.

healthy controls.[16-22] In our study, although the change in the percentages of median values of urine CTX-II levels in Group I and Group II were found as 61%+ and 80%+ respectively when compared to the control group, the difference was not statistically significant.

As mentioned above, we composed patient and control groups from postmenopausal women. There were no statistically significant differences among the ages of control and patient groups in our study. Additionally, we gathered the cases in the control and patient groups according to the criteria of the American College of Rheumatology (ACR) instead of using radiologic methods. In Framingham OA study, Felson et al. detected knee osteoarthritis in asymptomatic patients on X-ray and suggested that there was no correlation between clinical and radiological findings in knee osteoarthritis, particularly at earlier phases of the disease in asymptomatic patients.[23] We did not perform X-ray examinations of the control group. It is stated that prevalence of stage 1 OA in the vertebrae and other joints was higher particularly during the postmenopausal period.[1,15,16] However, this condition was not excluded in our study and this condition might have caused high urinary CTX-II levels; hence the contribution of the knee joint to urinary CTX-II levels may be too low. In line with this finding, we may not definitely exclude the possibility of knee osteoarthritis in the control group and the possibility of the other joint s' osteoarthritis in the control and patient groups. This situation might have caused a minor difference in the urinary CTX-II levels of the control and the patient groups.

On the other hand, there were only a few studies that indicated no association between urine CTX-II levels and radiologic OA.[24,25] It was reported that knee cartilage defect severity measured by magnetic resonance imaging showed a close relationship with urine CTX-II levels.[1] However, the relation between OA and the knee cartilage defect remained unclear. It is argued whether these cartilage defects lead to OA, or vice versa.[25,26] Longitudinal studies require describing causal directions. We did not find any statistically significant relationship between control and patient groups for radiological stage (stage 2, stage 3, stage 4) of OA. The dramatic percentage change of median values (78%, 44% and 172%, respectively), although not statistically significant, suggests that as the radiologic stage of OA increases also do the urine CTX-II levels. However, we did not evaluate the severity of cartilage defect by MRI.

We aimed to investigate predictive values of biochemical and demographic parameters on the radiological grades. For this reason, we developed two separate models for multiple regression analysis in which the radiologic grades in patient groups were incorporated as dependent variables, and urine CTX-II level, serum levels of CTX I, CRP, and ALP, age, ESR, duration of menopause and BMI as

independent variables, separately. Serum levels of CTX I, ALP, and duration of menopause were found as predictors for radiological severity of OA in only group II. In our opinion, in addition to scoring of OA by radiologic criteria, CTX-I, and ALP levels and duration of menopause might be considered for the evaluation of severity and prognosis of disease.

The other issue that we searched for was the presence or absence of the protective effect of osteoporosis from osteoarthritis. Integrity of cartilage is known to depend on the integrity of the underlying bone bed. Increase in the bone mass is thought to result in harder subchondral bone that promotes cartilage damage, hence contributing to the pathogenesis of OA. There are several studies suggesting that the peak mechanical stress impending over cartilage during loading would increase with increasing bone mineral density.[22,27-29] In this study, we found that the percentage change of the median value of CTX-II level was higher in the group with osteoporosis (Group II) than the group without osteoporosis (Group l).

It was reported that BMD measurements show variations at different skeletal sites in the same individual. They claimed that bone measurements of the heel might be more strongly related to the knee OA and hand BMD measurements might be more strongly related to the hand OA.[30] We used the femoral wards, femoral neck, lumbar total, and lumbar L1-L4 for BMD measurements in OA. This approach might also have affected the study results.

It was mentioned that immobility of subjects who suffered from OA for a long time was likely to affect bone mass.[31] Therefore, osteoporosis might have developed because of immobilization. Further studies are needed for the evaluation of relationship between OP and OA.

This study did not show any statistically significant differences with regard to parameters we investigated in a postmenopausal control group, postmenopausal knee osteoarthritis group, postmenopausal knee osteoarthritis plus osteoporosis group, and different radiological stages of osteoarthritis. We considered that the percentage changes of median values in Group I and Group II, and the Groups according to radiological grades, although did not reach statistical significance, were dramatic and remarkable.

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