

# Relation of BMI and Postmenopausal Duration With Antioxidant and Anti-Inflammatory Effects of Raloxifene Treatment in Women With Osteoporosis: A Preliminary Report

## Osteoporozlu Kadınlarda Raloksifen Tedavisinin Antioksidan ve Antiinflatuvar Etkilerininin VKI ve Menopoz Süresi ile İlişkisi

Yasemin AKÇAY, Sibel EYİĞÖR\*, Bilal İLANBEY\*\*, Muammer KARADENİZ\*\*\*, Yeşim KIRAZLI\*, Eser SÖZMEN  
Ege University, School of Medicine, Department of Biochemistry, Izmir, Turkey

\*Ege University, School of Medicine, Department of Physical Medicine and Rehabilitation, Izmir, Turkey

\*\*Yozgat State Hospital, Department of Biochemistry, Yozgat, Turkey

\*\*\*Ege University, School of Medicine, Department of Internal Medicine, Division of Endocrinology and Metabolism, Izmir, Turkey

### Summary

**Objective:** We have not identified any objective evidence in the literature about the effects of raloxifene treatment on the antioxidant and anti-inflammatory markers. We investigated the antioxidant/anti-inflammatory effects of raloxifene and the association of its antioxidant/anti-inflammatory effects with body mass index (BMI) and postmenopausal period in women with osteoporosis (OP).

**Materials and Methods:** Fourteen postmenopausal women with OP were enrolled in this study. Raloxifene hydrochloride 60 mg/day was administered for 6 months. The oxidant/antioxidant state was evaluated by measuring erythrocyte catalase (e-CAT) and paraoxonase (PON1) activities, levels of thiobarbituric acid (e-TBARS), serum diene, total antioxidant activity (AOA), trolox equivalent antioxidant capacity (TEAC), ferric reducing ability of plasma (FRAP) and anti-inflammatory effects were assessed by measuring tumor necrosis factor (TNF)- $\alpha$ , Interleukin (IL-6), IL-18, IL-10 and adiponectin levels. Plasma cytokine (TNF-alpha, IL-6, IL-10, IL-18) levels were measured by ELISA kits and antioxidant parameters were determined by spectrophotometry. Serum levels of all parameters were measured at baseline and end of the study.

**Results:** IL-6 level was significantly decreased but IL-10 and PON1 levels were significantly increased after the study treatment ( $p<0.05$ ). Raloxifene treatment significantly decreased IL-6 levels when postmenopausal period was longer than 10 years and BMI was  $<30$  kg/m<sup>2</sup> ( $p<0.05$ ).

### Özet

**Amaç:** Literatürde, raloksifen tedavisinin antioksidan ve antiinflatuvar belirteçler üzerine olan etkileri ile ilişkili yeterli veri olmaması nedeniyle, çalışmamızda hem raloksifenin antioksidan ve antiinflatuvar etkilerini hem de osteoporozlu kadınlarda raloksifenin bu etkilerinin, vücut kütle indeksi (VKI) ve menopoz süresi ile olan ilişkisini araştırdık.

**Gereç ve Yöntem:** Osteoporozlu 14 kadın çalışmaya dahil edildi. 60 mg/gün olacak şekilde 6 ay boyunca raloksifen hidroklorid verildi. Oksidan/antioksidan durum eritrosit katalaz (e-CAT) ve paraoksonaz aktiviteleri, eritrosit tiyobarbitürik asid (e-TBARS), serum diene düzeyleri, total antioksidan aktivite (AOA), trolox eşdeğeri antioksidan aktivite (TEAC), plazma demir indirgeme kapasitesi (FRAP) ölçülerek belirlendi. Antiinflatuvar etki ise tümör nekrozis factor (TNF- $\alpha$ ), interlökin 6 (IL-6), interlökin 18 (IL-18), interlökin 10 (IL-10) ve adiponiktin düzeyleri ile belirlendi. Plazma sitokin düzeyleri (TNF- $\alpha$ , IL-6, IL-10, IL-18) ELISA kiti ile antioksidan parametreler spektrofotometrik olarak hem çalışmanın başında hemde sonunda ölçüldü.

**Bulgular:** Tedavi sonrası IL-6 düzeyi anlamlı düşük, IL-10 ve PON1 düzeyi anlamlı yüksek olarak saptandı ( $p<0,05$ ). Menopoz süresi 10 yıldan uzun ve VKI $<30$  kg/m<sup>2</sup> olan olgularda raloksifen tedavisi sonrası, IL-6 düzeyleri anlamlı düşük olarak saptandı ( $p<0,05$ ).

**Sonuç:** Raloksifen, osteoporozlu kadınlarda proinflatuvar sitokin IL-6'yı anlamlı düşürürken, antiinflatuvar sitokin IL-10'u ve antioksidan PON1'i

**Conclusion:** Raloxifene resulted in a significant decrease in pro-inflammatory cytokine IL-6 and significant increases in anti-inflammatory cytokine IL-10 and antioxidant PON1 levels in women with OP. BMI and postmenopausal period are both associated with anti-inflammatory and antioxidant effects of raloxifene in postmenopausal period. *Turk J Phys Med Rehab 2012;58:29-35.*

**Key Words:** Raloxifene, anti-inflammatory, antioxidant effect

anlamli artirdi. Postmenapozal dönemde Raloksifenin hem antiinflamatuvar hem de antioksidan etkileri, VKI ve menopoz süresi ile ilişkilidir. *Türk Fiz Tip Rehab Derg 2012;58:29-35.*

**Anahtar Kelimeler:** Raloksifen, antiinflamatuvar, antioksidan etki

## Introduction

The pathophysiology of postmenopausal osteoporosis (OP) has been investigated for many years. Recently, it has been suggested that cytokines might have a role in the mechanism of postmenopausal OP (1,2). Estrogen deficiency induces increases in the levels of some pro-inflammatory cytokines, namely interleukin (IL)-1, IL-6, IL-7, macrophage colony-stimulating factor and tumor necrosis factor (TNF)- $\alpha$ . These cytokines may be involved in bone resorption by facilitating the recruitment and maturation of osteoclast precursors.

It is known that, estrogen administration to postmenopausal women is associated with a decrease in bone resorption (3). Raloxifene, a selective estrogen receptor modulator (SERM) that belongs to the benzothiophene class, is used for the treatment of OP and it has estrogen-agonist effects on bone and lipids. Raloxifene modulates bone resorbing-forming cells by two mechanisms: inhibition of osteoclastogenesis and osteoclastic activity and induction of osteoblastic activity (4,5). Preclinical data show that osteoclastic differentiation and activity require certain factors, such as proinflammatory cytokines, especially TNF- $\alpha$  and IL-6 (6). The influence of raloxifene on cytokines, such as IL-6 and TNF- $\alpha$  has been shown in in vitro studies (7). However, in vivo studies evaluating the effects of raloxifene on levels of cytokines, that can influence osteogenic turnover and anti-resorptive effects, are not very numerous.

It has been shown that, estrogen has antioxidant effects independent from the receptor because there is a hydroxyl group at the C3 position on the steroid A ring (8). Estrogen exerts its beneficial actions by suppressing ROS (8). Thus, as a SERM, raloxifene prevents bone loss by enhancing oxidant defenses in bones. Blood contains many antioxidant enzymes and molecules which prevent free radical reactions, such as catalase (CAT), paraoxonase (PON1), arylesterase (ARE), glutathione, ceruloplasmin, albumin, uric acid and bilirubin (9). There are some techniques to assess total antioxidant activity because of the inherent difficulty in measuring each antioxidant component separately. Ferric reducing ability of plasma (FRAP) and trolox equivalent antioxidant capacity (TEAC) are simple methods that directly measure total antioxidant activity of plasma (5,6,8).

Adiponectin is a peptide which is secreted from the adipocytes and correlates negatively with obesity. It has anti-inflammatory properties by decreasing the production of pro-inflammatory cytokines such as TNF and IL-6 (10). In addition, it has been reported that adiponectin and its receptors have been identified in bone-forming cells (11) which suggest that, there may be a relationship between adiponectin and OP. On the other hand, the effect of adiponectin on the postmenopausal OP is not clear;

adiponectine can stimulate pro-inflammatory cytokines from macrophages and affect development of OP (10-12).

In the light of these findings and recent literature, one can conclude that there are no objective data and evidence in the literature about the effects of raloxifene treatment on the antioxidant and anti-inflammatory parameters.

The objectives of the present study were: (1) to investigate the antioxidant and anti-inflammatory effects of raloxifene treatment and, (2) to assess the relationship of its antioxidant/anti-inflammatory effects with BMI and postmenopausal period in older women with OP.

## Materials and Methods

### Study Group and Design

After being evaluated for the exclusion criteria, 19 patients were taken into the study. During the treatment period, five of them did not complete the study for personal reasons. Fourteen postmenopausal osteoporotic women aged between 50 and 75 (mean age: 58.8 $\pm$ 7.6 years) years were enrolled. Participants were amenorrheic for at least 12 months or had a history of hysterectomy, and had a either Lumbar spine or femur bone mineral density (BMD) T-score less than -2.5 SD. Women with the following conditions were excluded from the study: any bone disease other than primary OP, an acute or chronic infection, severe postmenopausal symptoms requiring estrogen replacement therapy, history of or suspected breast carcinoma, any history of other cancer within the previous 5 years, abnormal uterine bleeding, a history of deep venous thrombosis or tromboembolic disorders, endocrine diseases, an acute or chronic hepatic disorder, chronic renal disease, history of stroke, Parkinson's disease or psychiatric disorders, cardiovascular diseases, excessive alcohol consumption, drug abuse, prior use of calcitonin, hormone replacement therapy or bisphosphonates within the previous 6 months, intake of vitamin K2 or ipriflavone within the previous 3 months, use of lipid lowering medications, use of fluoride therapy for more than 3 months during the previous 2 years, use of nonsteroid anti-inflammatory drug within the previous 6 months, systemic corticosteroid therapy for more than one month in the past year or anti-seizure drugs and, warfarin therapy.

The patients were given a questionnaire in order to obtain demographical data. After the completion of a medical history questionnaire, the participants have undergone a physical examination by the same physician.

All subjects were informed about the study and treatment protocol and gave written informed consent. The study was approved by the local Ethics Committee and conducted in accordance with the 1975 Helsinki Declaration, as amended in 1983.

### Measurement of Bone Mineral Density

BMD values of the lumbar spine (L1-L4) and proximal femur were measured by dual-energy X-ray absorptiometry (DXA-Hologic). OP was defined as a T-score which is lower than -2.5 SD according to the WHO criteria (13).

### Clinical Laboratory Measurements

Blood and urine samples were collected from patients before treatment and sixth month after treatment at the same time points as the BMD measurement. Plasma levels of TNF- $\alpha$  (Biosource, Cat.: KHC3011, USA), IL-6 (Biosource, KHC0061, USA), IL-10 (Biosource, Cat.: KHC0101, USA), IL-18 (Biosource, Cat.: KHC0181, USA), and adiponectine (LINCO Research, Cat.: EZHADP-61K, USA) were measured by immunoassay using commercial ELISA kit. After preparing hemolysates, levels of tiobarbituric acid reactive substance (TBARS) and catalase (eCAT) activities were measured in erythrocytes by spectrophotometry. Serum total antioxidant (AOA) activity, ferric reducing ability of plasma (FRAP), Trolox equivalent antioxidant capacity (TEAC) serum diene levels, serum arylesterase and paraoxonase (PON1) activities were measured spectrophotometrically.

Commercially available kits were used (Biosource, LINCO Research) to measure TNF- $\alpha$ , IL10, IL6, adiponectine, osteocalcin and urine deoxyypyridinoline as it has been known that all kits have already completed inter- and intra-assay CV values. The methods that are explained by us and others in the literature (14-18) were used to determine the other parameters (eCAT, e-TBARS, AOA, FRAP, TEAC).

All patients included in the study were treated by raloxifene hydrochloride 60 mg/day (Evista; Lilly Pharmaceutical Co. USA) for 6 months. All study participants also received daily supplements of 1000 mg calcium and 400 IU of vitamin D.

### Statistical Analyses

Statistical analyses were performed using SPSS, version 13.0 for Windows (SPSS, Chicago, IL). Evaluation of paired analyses for the parameters was performed before and sixth months after treatment (matched values of the same subject) by Wilcoxon signed-ranks test. Unmatched values were excluded from the statistical analyses. A p-value of <0.05 was considered statistically significant.

## Results

*The effect of raloxifene treatment on the antioxidant and anti-inflammatory parameters:*

The effect of raloxifene treatment on the antioxidant markers, cytokines and adiponectine levels are shown in Table 1.

*The effect of menopausal period on raloxifene treatment:*

The mean age of the patients with the duration of menopause shorter than 10 years ( $6.0 \pm 2.2$  years) and longer than 10 years ( $12.9 \pm 2.8$  years) were  $52.5 \pm 3.8$  and  $61.9 \pm 6.1$  years, respectively. At the baseline of the study (pretreatment data), the levels of all parameters showed no statistically significant difference according to menopausal period (differences between shorter than 10 years and longer than 10 years). Raloxifene treatment significantly decreased IL-6 ( $p=0.046$ , Figure 1) and slightly decreased TNF- $\alpha$  when postmenopausal period was longer than 10 years and increased IL-10 ( $p=0.028$ , Figure 2) independent from postmenopausal period and significantly decreased IL-18 ( $p=0.046$ , Figure 3) when

postmenopausal period was shorter than 10 years. When antioxidant activities have been evaluated according to the postmenopausal period, serum TEAC, FRAP and e-CAT and specifically PON1 activity ( $p=0.016$ ) increased significantly after treatment if it was started in the first 10 years of menopause. The effect of postmenopausal period on raloxifene treatment is shown in Table 2.

*The effect of BMI on raloxifene treatment:*

The mean age of the subjects with BMI <30 kg/m<sup>2</sup> (mean weight:  $61.1 \pm 8.1$  kg) was  $58.6 \pm 8.0$  years, while that of the subjects with BMI >30 kg/m<sup>2</sup> (mean weight:  $84.3 \pm 4.2$  kg) was  $60.7 \pm 5.9$ .

At the baseline of the study (pretreatment data), the levels of all parameters showed no statistically significant difference according to BMI (differences between smaller than 30 kg/m<sup>2</sup> and bigger than 30 kg/m<sup>2</sup>). IL-6 level was significantly lower in patients with a BMI <30 kg/m<sup>2</sup> compared to those with a BMI >30 kg/m<sup>2</sup> following treatment ( $p=0.041$ , Figure 4). The effect of BMI on raloxifene treatment is shown in Table 3.

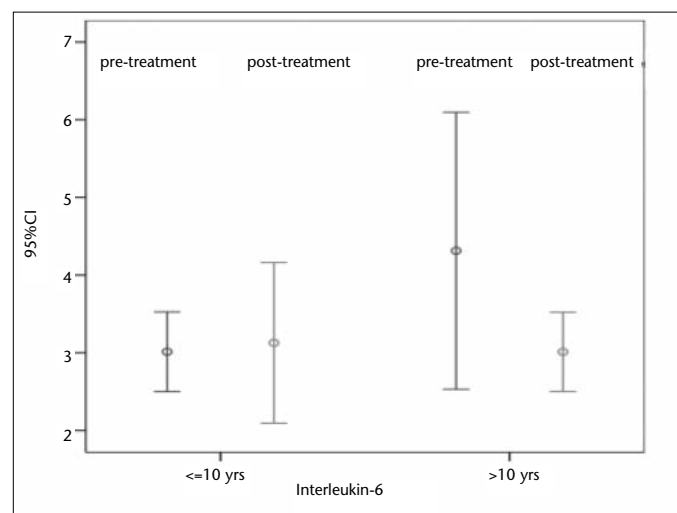


Figure 1. The effect of raloxifene treatment on IL-6 in relation to postmenopausal period.

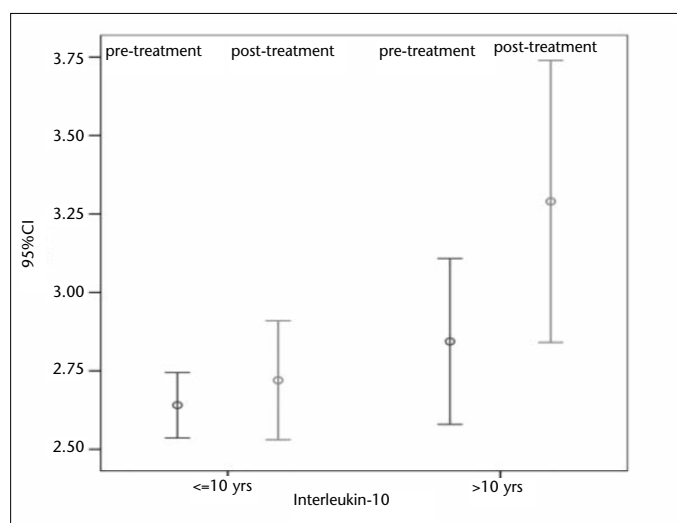


Figure 2. The effect of raloxifen treatment on IL-10 in relation to postmenopausal period.

## Discussion

Our data demonstrated that raloxifene treatment resulted in a significant decrease in pro-inflammatory cytokine IL-6 levels and significant increases in anti-inflammatory cytokine IL-10 and antioxidant PON1 levels.

Recently, raloxifene, a SERM, overshadowed estrogen replacement therapy in the treatment and prevention of OP (19-21). It is known that estrogen deficiency stimulates OP by releasing some pro-inflammatory cytokines which lead to bone resorption (1). It has been shown that raloxifene modulates osteoclastic and osteoblastic activity by decreasing the levels of a number of pro-inflammatory cytokines, such as TNF- $\alpha$  and IL-6 (22). In a study by Ozmen et al. (23), IL-6 and TNF- $\alpha$  levels did not change significantly after three months treatment with raloxifene. Our study showed that raloxifene treatment decreased pro-inflammatory IL-6 levels significantly and TNF- $\alpha$  and IL-18 slightly and, increased the anti-inflammatory cytokine IL-10 significantly. These findings are similar with the studies advocating the anti-inflammatory effects of raloxifene (2,3,19,20,22). Although the role

of cytokines in the pathogenesis of OP is not well understood, there is increasing evidence that IL-1, IL-6, TNF- $\alpha$  and IL-11, IL-2, IL-8 and IL-10 may play roles in the pathogenesis of OP (24-26). It has been suggested that IL-10 suppresses some inflammatory cytokines, such as IL-1a, IL-1b, TNF-a, IL-6, IL-8, IL-2 (27). Therefore, raloxifene may be involved in bone formation and resorption by suppressing production of these cytokines.

In addition, studies showed that adiponectin increases the levels of anti-inflammatory cytokine IL-10 and IL-1 receptor agonists and pro-inflammatory TNF- $\alpha$  (28). Our results show that there were no significant changes in serum levels of adiponectin after 6 months of raloxifene treatment. This can be explained by the fact that patients received raloxifene treatment only briefly, for 6 months. In our study, adiponectin levels were slightly higher in women with a postmenopausal period longer than 10 years and BMI lower than 30 kg/m<sup>2</sup> before raloxifene treatment. Although there was a slight decrease in adiponectin levels after treatment irrespective of the postmenopausal period and BMI, these results were not statistically significant. The effect of raloxifene on adiponectin secreted from adipocytes remains unclear and requires further studies.

Studies showed that the inhibitory effect of estrogen on pro-inflammatory cytokines is abolished because of the decreased estrogen levels in postmenopausal women. It is believed that it stimulates osteoclast formation, resulting in bone resorption (29,30). Pratelli et al. (31) demonstrated that cytokines leading to bone resorption, especially IL-6, increased with aging and

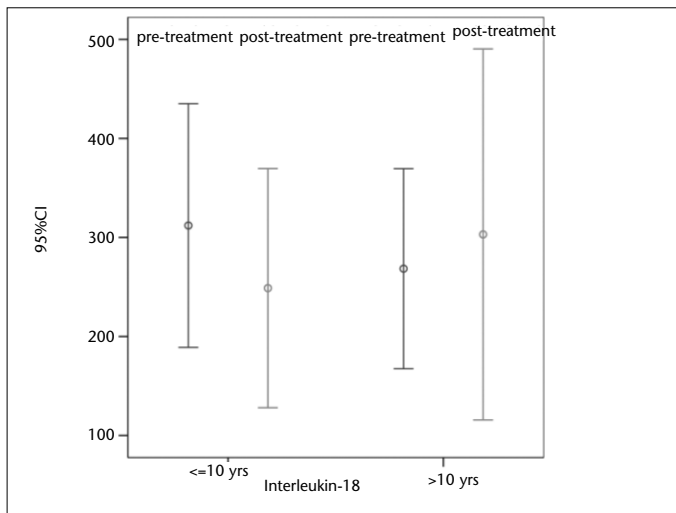


Figure 3. The effect of raloxifene treatment on IL-18 in relation to postmenopausal period.

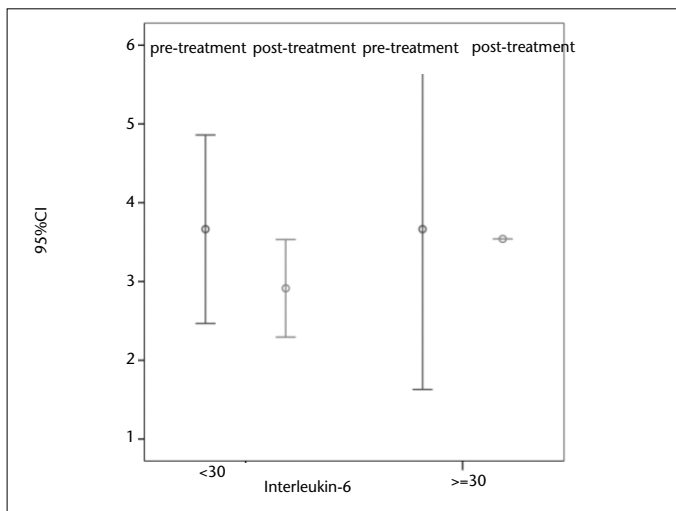


Figure 4. The effect of raloxifene treatment on IL-6 in relation to BMI.

Table 1. The effects of raloxifene treatment on anti-inflammatory and antioxidant markers.

	Pretreatment (mean $\pm$ SD)	Posttreatment (mean $\pm$ SD)
Cytokines		
TNF- $\alpha$ (pg/mL)	12.0 $\pm$ 12.8	11.3 $\pm$ 13.3
IL-6 (pg/mL)	3.6 $\pm$ 1.3	3.0 $\pm$ 0.7 *
IL-10 ( pg/mL)	2.7 $\pm$ 0.2	3.0 $\pm$ 0.4 *
IL-18 (pg/mL)	276.2 $\pm$ 105.3	267.7 $\pm$ 162.6
Oxidative/antioxidant parameters		
Serum TBARS (nmol/mL)	17.5 $\pm$ 2.5	18.7 $\pm$ 5.7
e-CAT (U/gHb)	12524 $\pm$ 2136	13537 $\pm$ 2780
e-TBARS (U/gHb)	694.8 $\pm$ 87.8	726.8 $\pm$ 159.6
Dienes ( $\mu$ mol/mL)	150.7 $\pm$ 43	154.2 $\pm$ 53
PON1 (U/L)	25.8 $\pm$ 23	37.1 $\pm$ 33 *
Arylesterase	59.3 $\pm$ 7.7	60.5 $\pm$ 12.7
TEAC ( $\mu$ mol/L)	5.7 $\pm$ 0.3	5.3 $\pm$ 1.3
FRAP ( $\mu$ mol/L)	1979 $\pm$ 308	2095 $\pm$ 244
Adiponectin (ng/mL)	14791 $\pm$ 5080	12728 $\pm$ 3054
Total AOA ( $\mu$ ml/L)	0.73 $\pm$ 0.4	0.85 $\pm$ 0.7

TNF- $\alpha$ : Tumor necrosis factor; IL-6: Interleukin-6; IL-10: Interleukin-10;  
IL-18: Interleukin-18; TBARS: Thiobarbituric acid; eCAT: Erythrocyte catalase;  
PON: Paraonase; TEAC: Trolox equivalent antioxidant capacity;  
FRAP: Ferric reducing ability of plasma, AOA: Antioxidant activity. \*p<0.05

menopause duration but TNF levels were decreased. However, there are some studies which found no correlations between cytokine levels and postmenopausal period (32). In our study, we investigated the relationship between menopause period and raloxifene treatment and suggest that estrogen decrease and associated bone resorption are more significant within the first 10 years. We found that raloxifene treatment significantly decreases IL-6 when postmenopausal period is longer than 10 years and increases IL-10 independent from postmenopausal period and significantly decreases IL-18 when postmenopausal period is shorter than 10

years. These results are similar with studies showing that raloxifene treatment decreases inflammatory cytokines and treatment is beneficial in postmenopausal OP (2,3,19,22). However, changes in the activity of proinflammatory cytokines triggered by menopause are emerging topic. The relationship between estrogen and proinflammatory cytokines is still an unsolved issue. In fact, contradictory data have been reported (33). The possible reason for this can be the limited sample size, but after this preliminary study, the effect of treatment on menopause period might possibly be investigated on more subjects.

Table 2. The effect of postmenopausal period on anti-inflammatory and antioxidant markers during raloxifene treatment.

	Menopause duration ≤10 years (mean ±SD) (n:5)		Menopause duration >10 years (mean ±SD) (n:9)	
	Pretreatment	Posttreatment	Pretreatment	Posttreatment
TNF-α (pg/mL)	15.5±17.5	13.6±18	8.3±4.8	9.6±5.9
IL-6 (pg/mL)	2.9± 0.45	3.0±0.9	4.3±1.6 <sup>*c</sup>	3.0±0.48 <sup>*b</sup>
IL-10 ( pg/mL)	2.6±0.09	2.7±0.23 <sup>*a</sup>	2.8±0.25	3.2±0.24 <sup>*b</sup>
IL-18 (pg/mL)	285±110	226±102 <sup>*a</sup>	235 ±73	253 ±169
PON1 (U/L)	36.1±32	52±46 <sup>*a</sup>	20.5±7.5	29.5±10
TEAC (μmol/L)	5.8±0.3	5.9±0.3	5.7±0.15	4.7±2.0
FRAP (μmol/L)	1880±266	2048±182	2200±181 <sup>*c</sup>	2115±333
e-CAT (U/gHb)	13170±2470	13648±2910	12167±1630 <sup>*c</sup>	13517±3135
Adiponectin (ng/mL)	17634±3761	12614±4593	12244±1032	12289±328

TNF-α: Tumor necrosis factor; IL-6: Interleukin-6; IL-10: Interleukin-10; IL-18: Interleukin-18; PON: Paraoxonase; TEAC: Trolox equivalent antioxidant capacity; FRAP: Ferric reducing ability of plasma, eCAT: Erythrocyte catalase; \*p < 0.05.  
<sup>a</sup>Significantly different compared to pre-treatment values when postmenopausal period is shorter than 10 years.  
<sup>b</sup>Significantly different compared to pre-treatment values when postmenopausal period is longer than 10 years.  
<sup>c</sup>Significantly different compared to pre-treatment values of patients with postmenopausal period is shorter than 10 years.

Table 3. The effects of BMI on anti-inflammatory and antioxidant markers during raloxifene treatment.

	BMI<30 kg/m <sup>2</sup> (mean ±SD) (n:9)		BMI≥30 kg/m <sup>2</sup> (mean ±SD) (n:5)	
	Pretreatment	Posttreatment	Pretreatment	Posttreatment
TNF-α (pg/mL)	9.3 ±2.6	8.7±5.4	9.9±0.8	6.7±3.7
IL-6 (pg/mL)	3.6±1.5	2.9±0.8 <sup>*a</sup>	3.6±0.8	3.5±0.0 d
IL-10 ( pg/mL)	2.7±0.2	2.8±0.3	2.8±0.3	3.3±0.5
IL-18 (pg/mL)	275±100	259±148	246±13.4	216±50
PON1 (U/L)	31.47±28	45.2±40 <sup>*a</sup>	18.6±7.4	26.8±10.7 <sup>*b</sup>
TEAC (μmol/L)	5.8±0.1	5.7±0.7	5.8±0.05	4.3±2.6
FRAP (μmol/L)	1940±305	2107±275	2243±72 <sup>c</sup>	2133±107
e-CAT (U/gHb)	12457±2189	13242±3185	12887±2368	14800±2561
Adiponectin (ng/mL)	14844±6095	12363±3266	12460±1353	11524±1066

TNF-α: Tumor necrosis factor; IL-6: Interleukin-6; IL-10: Interleukin-10; IL-18: Interleukin-18; PON: Paraoxonase; TEAC: Trolox equivalent antioxidant capacity; FRAP: Ferric reducing ability of plasma, eCAT: Erythrocyte catalase; \*p<0.05.  
<sup>a</sup>Significantly different compared to pre-treatment values when BMI is lower than 30 kg/m<sup>2</sup>.  
<sup>b</sup>Significantly different compared to pre-treatment values when BMI is higher than 30 kg/m<sup>2</sup>.  
<sup>c</sup>Significantly different compared to pre-treatment values and dpost-treatment values of patients with BMI is lower than 30 kg/m<sup>2</sup>.

It is proposed that high BMI prevents from OP by converting androgens to estradiol at adipose tissue by aromatase, leptine release and cushioning effect of fat (34). On the other hand, adipocytes secrete pro-inflammatory cytokines such as TNF, IL-6 which can affect bone remodeling (35). However, there is no data about the relation of BMI with respect to the effects on raloxifene treatment in postmenopausal OP. We found that IL-6 levels were lower in patients with a BMI<30 kg/m<sup>2</sup> compared to those with a BMI>30 kg/m<sup>2</sup> following treatment. In addition, raloxifene treatment resulted in slight decreases in pro-inflammatory cytokines IL-18 and TNF- $\alpha$  and a small increase in anti-inflammatory IL-10 levels irrespective of BMI values. Since it has been found that raloxifene treatment has a clear inhibitory effect on cytokines in postmenopausal women with OP with a BMI of <30 kg/m<sup>2</sup>, we might suggest that raloxifene exerts its anti-inflammatory effect by inhibiting IL-6 release from macrophages rather than adipose tissue.

There are increasing evidence that show a close association between ROS and bone loss (24,36). While osteoclasts generate superoxide anion radicals, hydrogen peroxide, a product of superoxide anion in the superoxide dismutase enzyme pathway, might stimulate formation and differentiation of osteoclasts which are particularly important in the pathogenesis of OP due to lack of estrogen in postmenopausal period (36,37). The effects of raloxifene treatment mimic antioxidant features of estrogen on osteoblasts. Mann et al. (24) suggested that estrogen and its selective modulator raloxifene were capable of preventing hydrogen peroxide-induced apoptosis and resulted in viable osteocytes. We also investigated the effects of raloxifene treatment on the antioxidant status in relation to postmenopausal period and BMI. When antioxidant activities were compared by BMI levels, PON1 activity increased significantly and TEAC, FRAP, e-CAT activities insignificantly in both groups. When it has been evaluated in relation to postmenopausal period, serum TEAC, FRAP and e-CAT and specifically PON1 activity increased significantly after treatment, when the treatment was started within the first 10 years of menopause. It has been clearly known that aging is closely related with elevated oxidative stress and depleted antioxidant activity (38). Although the mechanisms underlying this reduced antioxidant status are not clearly defined, in elderly, it might be associated with a decrease in synthesis of antioxidant molecules due to aging. Raloxifene might probably stimulate protein synthesis of antioxidant molecules. Raloxifene treatment seems to be more effective when it is started in the early years of menopause and in patients with low BMI values. We believe that there is a need for further research to confirm this finding that would provide a basis to change the clinical practice.

However, it is obviously not possible to exclude OP while commenting on the possible anti-inflammatory and anti-oxidant effects of a medication used for the treatment of OP. As in many diseases, inflammatory factors play role in the pathogenesis of OP. Such inflammatory effects are implicated in the post-menopausal period that we addressed in the present study and BMI as well. These complex relationships become more prominent when we consider the diagnostic age group of OP. Therefore, we put forward the data obtained in the study and believed that it would serve as a guide for future studies. We presented the objective of the study i.e. the effects of raloxifene treatment on the antioxidant and anti-inflammatory parameters. In the introduction section, we tried to explain possible interactions between these parameters. Data obtained in this study suggest that it might be effective in the

pathogenesis of OP. However, it was not discussed since it would be very pretentious argument. Meanwhile, it is believed that pro-inflammatory cytokines could be used as a marker for bone resorption (36). On the other hand, it should be kept in mind that calcium and vitamin-D may have anti-inflammatory effects (37). Therefore, we believe that our study needs to be supported by studies on larger number of patients.

The strength of our study derives from the evaluation of anti-inflammatory and antioxidant features of raloxifene treatment within the scope of a single study. To our knowledge, this is the first study that investigated the antioxidant/anti-inflammatory effects of raloxifene in relation to both BMI and postmenopausal period in older women with OP who received raloxifene.

Also, our study has some limitations including a small sample size and lack of a randomized control group. Each different parameter to be looked at for anti-inflammatory and antioxidant assessment and each additional patient increase the cost of the study significantly. Many different parameters (catalase, paraoxonase activities, levels of thiobarbituric acid, serum diene, total antioxidant activity, trolox equivalent antioxidant capacity, ferric reducing ability of plasma, TNF- $\alpha$ , IL-6, IL-18, IL-10 and adiponectin levels) could be determined despite small number of patients. However, our aim was to share our preliminary results with the readers. Only postmenopausal women with OP were enrolled, but it was very difficult to obtain women without any systemic disease who could continue the study treatment for 6 months.

## Conclusion

Raloxifene treatment caused a significant decrease in pro-inflammatory cytokine IL-6 and significant increases in the levels of anti-inflammatory cytokine IL-10 and antioxidant PON1 in older women with OP. We suggest that BMI and postmenopausal period has an effect on the anti-inflammatory and antioxidant properties of raloxifene treatment during the postmenopausal period. Our preliminary data points out that the antioxidant and anti-inflammatory effects of raloxifene might be more potent in patients with low BMI values who are in the early phases of postmenopausal period. However, this preliminary study needs to be followed by further studies with more subjects in order to elucidate the treatment effects of raloxifene.

## Conflict of Interest:

Authors reported no conflicts of interest.

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