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RESEARCH ARTICLE

Role of Estrogen in the Oxidation Process in Postmenopausal Osteoporosis

Elaf Mahmood Shihab[1]*, Mustafa G. Al-Abbassi[2], Deleen Abd. Al Wahab Al Shukri[2], Inas Taha Ahmad[3]

1. *Department of Pharmacology, AL-ESRAA University College.*
2. *Department of Pharmacology and Toxicology, College of Pharmacy, Al-Mustansirya University, Baghdad, Iraq.*
3. *Al-Elwyia Maternity Teaching Hospital, Baghdad, Iraq.*

***Corresponding Author E-mail:** *Phelafmahmood86@yahoo.com*

Abstract

Introduction: Bone exerts important functions in the body, such as locomotion, support and protection of soft tissues, calcium and phosphate storage, and harboring of bone marrow. Osteoporosis is a common metabolic bone disease characterized by reduced bone mass, micro architectural deterioration of bone tissue, and an increased risk of fragility fracture. After menopause, the period of time after a woman has experienced 12 consecutive months without menstruation called postmenopausal period. Estrogen showed to be the most important sex steroid in preventing osteoporosis in women, so estrogens modulate bone growth and turnover in vivo. Total antioxidant capacity (TAC) is the measure of the amount of free radicals scavenged by a test solution, being used to evaluate the antioxidant capacity of biological samples. Estrogens are steroid hormones that have been shown to affect numerous tissues, including reproductive tissues (such as the breast, endometrium, and vagina) and the skeletal, cardiovascular, and central nervous systems(11).The aim of the study was to evaluate the effect of estrogen therapy on the level of total antioxidant capacity in serum of postmenopausal osteoporotic women. Materials and Methods: Randomized, single blind clinical trial carried out at the Rheumatology outpatients department of Baghdad Teaching Hospital / Medical City Complex in Baghdad, Iraq from the 10th of Jan to the 12th of June 2016.The study was carried out on 24 postmenopausal women, with amenorrhea for more than 12 consecutive months. Venous blood samples (10 ml) were drawn from each patient by venipuncture .Blood samples were taken from participants at day zero of treatment as baseline and after 90 days of treatment to obtain serum for measurement of total antioxidant capacity .Total Antioxidant Capacity (TAC) Bio Assay ™ Elisa Kit manufactured by US Biological. The manufacturer instructions were strictly adhered during the laboratory procedure. Results: A total of 24 osteoporosis woman patients were enrolled in this study and they consisted of two studied groups: Group A:12 postmenopausal osteoporotic women were treated by conjugated equine estrogens tablet 0.625mg (PREMARIN ®) once daily for 90 days. Group B:12 postmenopausal osteoporotic women were treated by placebo one capsule (filled with starch) daily for 90 days and served as a control group. The mean age of the patients was 56.3 ± 6.14 (range:47 – 65). The mean Body Mass Index (BMI) of the participants was 26.86 ± 2.1 (range: 22.3 – 29.4) kg/m^2. On the other hand, the comparison of mean age and BMI across the two studied groups revealed no statistically significant differences, (P>0.05) .Statistics had shown that there was no significant changes between pre and post treatment in Group A or B with baseline increase from (476.35 ± 112.79) to (484.11 ± 117.67) and (471.87 ± 165.59) to (472.78 ± 184.12) respectively (P value > 0.05).Conclusion: daily dose of 0.625 mg of estrogen therapy for 90 days duration was not proven to be effective in lowering the oxidation level following menopause in osteoporotic women.

Keywords: *Estrogen, Postmenopausal osteoporosis, Antioxidant.*

Introduction

Bone exerts important functions in the body, such as locomotion, support and protection of soft tissues, calcium and phosphate storage, and harboring of bone marrow [1].

Osteoporosis is a common metabolic bone disease characterized by reduced bone mass, micro architectural deterioration of bone tissue, and an increased risk of fragility

fracture [2].Osteoporosis is called the "silent disease" because bone loss by itself does not cause any symptoms. Osteoporosis often presents as a clinically evident fracture. A low trauma fracture (following a fall from standing height or less) in someone aged over 45 should trigger the suspicion of osteoporosis. In other cases, osteoporosis may present as backache, height loss and spinal deformity such as thoracic kyphosis [3] .

Osteoporosis has been defined on the basis of Bone Mineral Density (BMD) assessment. According to the World Health Organization (WHO) criteria, osteoporosis is defined as a BMD that lies 2.5 standard deviation or more below the average value for young healthy women (a T- score of less than -2.5 SD).

This criterion provides both a diagnostic and interventional threshold. The most widely validated technique to measure BMD is dual energy X-ray absorptiometry (DEXA) and diagnostic criteria based on the T-score for BMD are recommended entry criterion for the development of pharmaceutical interventions in osteoporosis. Osteoporotic fractures are a major public health problem worldwide because of the associated morbidity, mortality and costs [4].After menopause, the period of time after a woman has experienced 12 consecutive months without menstruation called postmenopausal period.

Estrogen showed to be the most important sex steroid in preventing osteoporosis in women, so estrogen modulate bone growth and turnover in vivo. Estrogens have two forms of receptors and both of them have been detected in osteoblasts and osteoclasts. In general, estrogen is an inhibitor of bone desorption that decreases both osteoclast numbers and activity, also been showed that it promotes apoptosis; moreover it also has anabolic effects on osteoblasts. However, estrogen action on osteoclasts is superior in comparison with that on osteoblasts [5] .

The shift in the balance between oxidants and antioxidants in favor of oxidants is termed "oxidative stress." Regulation of reducing and oxidizing state is critical for cell viability, activation, proliferation, and organ function. Oxidative stress contributes to many pathological conditions and diseases such as neurological disorders and atherosclerosis [6] .Various studies have shown that, osteoporosis significantly correlate with the progressive loss of estrogen and its protective effects, combined with deficient antioxidant defense leading to a pronounced redox imbalance. Levels of TNF α and inflammatory cytokines have been established to be elevated in the presence of oxidative stress. Therefore, oxidative stress is present in increased amounts in postmenopausal women [7,8]. Total antioxidant capacity (TAC) is the measure of the amount of free radicals scavenged by a test solution [9] , being used to evaluate the antioxidant capacity of biological samples [10] .

Estrogens are steroid hormones that have been shown to affect numerous tissues, including reproductive tissues (such as the breast, endometrium, and vagina) and the skeletal, cardiovascular, and central nervous systems [11] .In women with sex steroid deficiency, the administration of estrogen reverses many of the effects of loss of ovarian function.

Bone cells have estrogen receptors [12].Estrogen effect on bone mineralization by:1-Promoting osteoblast proliferation, synthesis of osteoblastic proteins like Insulin such as growthfactor-1, transforming growth factor, and bone morphogenic protein-6 ;increasing osteocyte survival thus having an anabolic effect.2-Suppressing osteoclastic activity:

(a) By increasing its apoptosis (b) By reduced production of receptor activator of nuclear factor-kB ligand from osteoblasts which stimulate osteoclasts (c) By decreasing the production of IL-1, IL-6,TNFresponsible for osteoclast recruitment and activity (d) By increasing production of osteoprotegerin which prevents differentiation of osteoclast precursor to mature osteoclast [13].CEE is a complex 'natural' extract of pregnant mares' urine containing 10 different estrogens mainly estrone sulfate and equilin sulfate.

All estrogen components of CEE are antioxidants. The most prescribed dose of CEE is 0.625 mg/d because this was the dose that alleviates postmenopausal related symptoms in the majority of women that endure ovarian failure. Other forms of estrogen in HT include esterified estrogen, ethinyl estradiol,17β-estradiol, estradiol acetate, and estropipate [14] . The aim of the study was to evaluate the effect of estrogen therapy on the level of total antioxidant capacity in serum of postmenopausal osteoporotic women.

Materials and Methods

Randomized, single blind clinical trial carried out at the Rheumatology outpatients department of Baghdad Teaching Hospital/ Medical City Complex in Baghdad, Iraq from the 10th of Jan to the 12th of June 2016.The patients who attended the unit were screened for eligibility according to the following inclusion and exclusion criteria: The study was carried out on 24 postmenopausal women, with amenorrhea for more than 12 consecutive months, aged from (47-65) years checked by assessment of follicle stimulating hormone which was elevated (greater than 40 mIU/mL) and serum estradiol level was (below 30 pg/mL).

All women underwent trans-vaginal ultrasound examination of the uterus and the endometrial thickness and all patients were concluded to have atrophic uterus. All participants were diagnosed as osteoporotic in which their bone mineral density which measured by dual energy X-Ray absorption me try was 2.5 Standard deviation or more below the young female adult mean (T-scoreless than or equal to –2.5 SD) and body mass index (BMI)>19.0kg/m^2 and <30 kg/m^2.

Exclusion criteria: History of Diabetes Mellitus, History of osteoporotic fracture, History of thyroid disease, Smokers, History of steroid therapy, liver disease, Cerebrovascular, Cardiovascular and thromboembolic diseases, Patient with undiagnosed vaginal bleeding, endometriosis, genital neoplasia, breast neoplasia and history of breast carcinoma in family, History of migraine, Rheumatoid arthritis, Renal impairment, Patients using supplements containing antioxidant such as vitamin A , C and selenium, History of using hormone replacement therapy.

The sample size was 24 patients divided into 2 groups, 12 patients for each group as the following: Group A: 12 postmenopausal osteoporotic women were treated by conjugated equine estrogens tablet 0.625 mg (PREMARIN ®)by Pfizer company/ United States once daily for 90 days. Group b: 12 postmenopausal osteoporotic women were treated by placebo one capsule (filled with starch) daily for 90 days and served as a control group (the patients were instructed to follow a well-balanced diet, half hour daily of sun exposure and to practice physical exercise on a daily basis).Venous blood samples (10 ml) were drawn from each patient by venipuncture by disposable plastic pyrogen free syringes and immediately transferred into gel tubes with anticoagulant. The clot was dispersed with glass rod and then centrifuged for 15-20 minutes at 2000 rpm, then the serum was separated in to eight pin tubes and stored in deep freeze (-20^{0}C-40^{0}C).

Blood samples were taken from participants at day zero of treatment as baseline and after 90 days of treatment to obtain serum for measurement of total antioxidant capacity .Total Antioxidant Capacity (TAC) Bio Assay ™ Elisa Kit manufactured by US Biological. The manufacturer instructions were strictly adhered during the laboratory procedure. The research was approved by local Committee from College of Pharmacy-Al Mustansiryah University. Also was reviewed and approved by Medical Ethics Committee at Medical city complex.

Each participant's gave informed consent to participate in the research. Data were entered using SPSS software (Statistical Package for Social Sciences (SPSS), Version 24, IBM, US, 2013.Descriptive statistics was reported as frequency, mean and standard deviation (SD). T-test was used to compare the difference in means between pre and post treatment of the two studied groups a. P value was set at less than or equal 0.05.

Results

A total of 24 osteoporosis woman patients were enrolled in this study and they consisted of two studied groups: Group A:12 postmenopausal osteoporotic women were treated by conjugated equine estrogens tablet 0.625 mg (PREMARIN ®) once daily for 90 days. Group B: 12 postmenopausal osteoporotic women were treated by placebo one capsule (filled with starch) daily for 90 days and served as a control group.

The mean age of the patients was 56.3 ± 6.14 (range:47-65). The mean Body Mass Index (BMI) of the participants was 26.86 ± 2.1 (range: 22.3-29.4) kg/m^2. On the other hand, the comparison of mean age and BMI across the two studied groups revealed no statistically significant differences, (P>0.05) as shown in Table (1).

Table 1: Age and body mass index mean values of the studied groups

Variable	Groups		P value
	Group (A)	Group (B)	
Age (in years)	57.1 ± 6.1	56.1 ± 6.4	0.91*
BMI (kg/m^2)	26.9 ± 2.1	26.5 ± 2.4	0.78*

Group A: 12 postmenopausal osteoporotic women were treated by conjugated equine estrogens tablet 0.625mg (PREMARIN ®) once daily for 90 days.

Group B: 12 postmenopausal osteoporotic women were treated by placebo one capsule (filled with starch) daily for 90 days and served as a control group.-* Not significant.

Statistics had shown that there was no significant changes between pre and post treatment in Group A or B with baseline increase from (476.35 ± 112.79) to (484.11 ± 117.67) and (471.87 ± 165.59) to (472.78 ± 184.12) respectively (P value > 0.05) as sown in Table 2 and Figure 1.

Table 2: Comparison of changes in Total antioxidant capacity levels among the studied groups

	(TAC) mean ± SD (ng/ml)	
	Group(A)	Group(B)
Pre treatment	476.35 ± 112.79	471.87 ± 165.59
Post treatment	484.11 ± 117.67	472.78 ± 184.12
Mean difference	7.76 ± 2.58	0.91 ± 0.302
Difference rate (%)	1.6%	0.2%
P value (pre vs. post) T test	0.14*	0.514*

Group A: 12 postmenopausal osteoporotic women were treated by conjugated equine estrogens tablet 0.625mg (PREMARIN ®) once daily for 90 days.

Group B: 12 postmenopausal osteoporotic women were treated by placebo one capsule (filled with starch) daily for 90 days and served as a control group.-*Not significant.

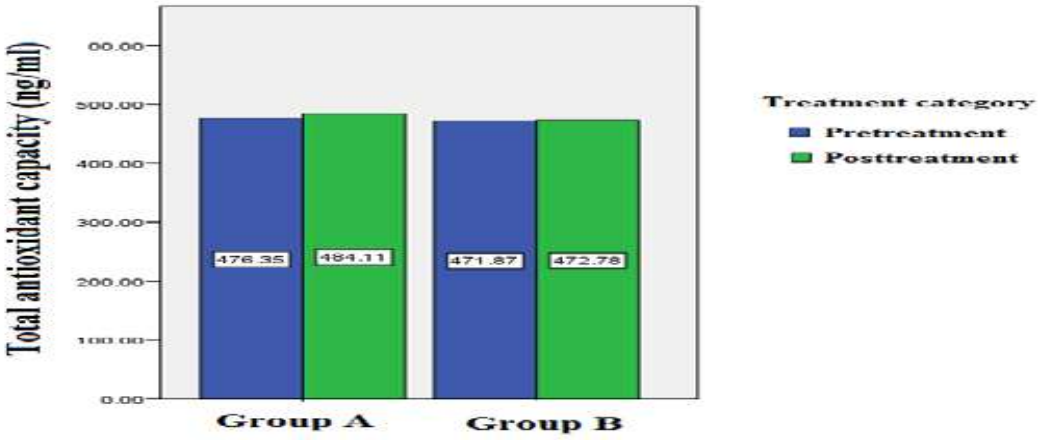

Figure 1: Comparison of mean difference in Total antioxidant capacity levels among the studied groups.

Discussion and Conclusion

Oxidative effects of free radicals are controlled by endogenous antioxidants and also by exogenous antioxidants. Plasma concentrations of antioxidants can be measured separately in the laboratory, but these measurements are time-consuming, labour-intensive and costly [15] . Antioxidant system has many components; a deficiency of any component can cause a reduction in the total antioxidant status of an individual. Therefore, it is more useful to measure the total antioxidant capacity of plasma using a single assay in a clinical laboratory [16]. Menopause is associated with significant change in antioxidant gene expression. Estrogens replacement therapy is able to prevent and counteract such change by acting as regulators of key antioxidant gene expression. Estrogens have been documented to have an antioxidant effect against oxidative

stress. Estradiol (E2) acts as an antioxidant and free radical scavenger [17]. In the current study it was found that the level of the total antioxidant capacity increased when the osteoporosis women were treated with estrogen, but with no significant differences between pre and post treatment groups. In contrast, a study done by Unfer T *et al*. determined that the plasma total antioxidant capacity was not affected by estrogen administration for 3 months [18] .

In agreement a recent study by Bellanti *et al* showed that in women there is a linear relationship between the level of circulating estrogen and the antioxidant status [17].Also Bednarek *et al* concluded ERT inhibit the generation of free radicals and raise antioxidant potential to the levels found in premenopausal women [19]. Moreover, Delibasi *et al* concluded that estrogen has an antioxidant effect following 3 months of hormone replacement therapy [16]. The antioxidant actions of estrogens probably result from at least two mechanisms: their chemical structure and also their regulatory effects on the activity of the system of natural antioxidant enzymes. Estrogens have a hydroxyphenolic structure in their molecule and may donate a hydrogen atom from their phenolic hydroxyl group to lipid peroxyradicals, terminating chain reactions. Also Estrogen may play an important role in the regulation of GSH-Px [19-20] .

Conclusion

Daily dose of 0.625 mg of estrogen therapy for 90 days duration was not proven to be effective in lowering the oxidation level following menopause in osteoporotic women.

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