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THE SERUM LEVEL OF SUPEROXIDE DISMUTASE AND THE RISK OF OSTEOPOROSIS IN PREMENOPAUSAL WOMEN WITH ENDOMETRIOSIS

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ABSTRACT

Objective

The aim of this study is to evaluate the superoxide dismutase enzyme's level and its effects, in patients with premenopausal endometriosis, on the serum levels of bone formation markers, bone resorption markers and osteoporosis.

Subjects and Methods

In this case-control study, eighty female patients had endometriosis, at their reproductive age with standard criteria, were participated in this study. Eighty apparently healthy females were enrolled in this study as a control group for comparison. Assessments of the serum levels of; superoxide dismutase enzyme, alkaline phosphatase enzyme, carboxy terminal telopeptides of type I collagen, carboxyterminalpropeptide of type I procollagen, total calcium and inorganic phosphate were performed by the use of enzyme-linked immunosorbent assay and colori metric methods.

Results

The results revealed that the serum levels of;superoxide dismutase enzyme, alkaline phosphatase enzyme, carboxyterminaltelopeptides of type I collagen, carboxyterminalpropeptide of type I procollagen and total calcium were significantly changed in patients with premenopausal endometriosis as compared with the control group. The change of serum inorganic phosphate level was non-significant as compared to the control group.

Conclusions

The raised antioxidant activity of superoxide dismutase enzyme was an important indicator of the elevated oxidative stress status that may be involved in the development of osteoporosis in patients with premenopausal endometriosis as described by the serum levels of bone formation and resorption biomarkers.

KEYWORDS: Endometriosis, Osteoporosis, Superoxide Dismutase

INTRODUCTION

Free radicals and reactive oxygen species have essential role in many disease states such as diabetes, degenerative disorders and cancer through lipid peroxidation of membranes, protein cross linkage, DNA, and mitochondrial damage [1]. Oxidative stress related factors and lowered antioxidant status are important risk factors for osteoporosis along with other factors like race, sex, age, genetics, previous fractures, low body weight, calcium intake, hormones, medications, high lipid intake and nutritional deficiencies[2,3].

The weakness of an antioxidant defense or excessive production of oxygen's reactive species results in oxidant stress, which may also result from normal metabolic activity or other factors such as diet [4]. The major antioxidant systems in the body, provide additional defense against this stress are; superoxide dismutase(SOD), glutathione-S-transferases, glutathione-S-peroxidase, catalase, vitamin C, vitamin E, ß-carotene, selenium, lycopene, and polyphenols [5].

In bone, the higher osteoclastic activity and increased production of reactive oxygen radicals are related to many skeletal pathologies. The production of those radicals by normal osteoclasts could accelerate the destruction of calcified tissue and stimulate bone remodeling process[6]. The enhanced osteoclastic activity, observed in bone disorders, may be associated with increased production of superoxide anion ($O^{\cdot-}_2$), normally detoxified by SOD enzyme, with elevated levels of serum malondialdehyde (the end product of lipid peroxidation)[7]. The defective activities of the antioxidant enzymes, SOD and glutathione peroxidase, illustrated a defective defense mechanisms that may result from increased superoxide radical production by osteoclasts. Therefore, oxidative stress plays a key role in the inflammation and destruction of rheumatoid arthritis joints [8], and can be reduced by intake of certain dietary antioxidants, e.g. lycopene [9].

Some studies have proposed that endometriosis might be associated with oxidative stress[10,11]. In pelvic endometriosis; there might be activated macrophages in peritoneum associated with increased production of reactive oxygen and nitrogen species, cytokines, prostaglandins, growth factors and, therefore, generating lipid peroxidation and malondialdehyde which act as foreign bodies, leading to an antigenic response and antibodies' production [12]. This process may cause oxidative damage to red blood cells and to endometrial and peritoneal cells which would recruit and activate a larger number of mononuclear phagocytes with progressive damage to the pelvic environment [13]. Oxidative stress contribute to development and progression of endometriosis by compromising mesothelial cells and inducing adhesion sites for endometrial cells [14,15]. In women with adenomyosis and endometriosis, upregulated expression and activity of SOD was observed in the ectopic endometrium throughout the menstrual cycle [14].

The stress biomarkers have been surveyed in different tissues for assessing age related diseases such as osteoporosis. Therefore, the use of biomarkers to identify patients with oxidative stress may be helpful in managing osteoporosis [16].

SUBJECTS, MATERIALS & METHODS

This prospective study was carried out in Ibn-Gazwan hospital of gynecology and obstetrics in Al Basra city southern of Iraq. The work was started in November 2013 until April 2014. A total of 160 women (80 endometriotic patients and 80 healthy controls) were included in this study. The endometriotic patients (N=80) were females at their reproductive age with a mean age of 34.2 ± 0.59. The apparently healthy females, in the control group (N=80), were with regular menstrual cycles (28–30 days). The mean age of these subjects was 32.1 ± 0.97. The diagnosis of endometriosis was made by specialist gynecologists. It depends upon the presence of typical symptoms, physical examination and the diagnostic imaging, biopsy and laparoscopy [17,18]. Informed consents were approved and signed by the patients and healthy volunteers who were participated in this study. Disposable syringes and needles were used for blood collection. Fasting blood was aseptically collected at the mid-cycle (14–16th day) by venipuncture. About 7ml of venous blood The fresh serums were used for measuring the levels of; superoxide dismutase enzyme [19], carboxyterminaltelopeptides of type I collagen [CTXI][20], and carboxyterminalpropeptide of type I procollagen [PICP][21], by using ELISAkits and methods. The serum levels of total alkaline phosphatase [ALP][22], total calcium[23] and inorganic phosphate[24] were assessed by using

enzymatic kits and methods. The study protocol was approved by the institutional ethics committee in the Middle Eastern Arab countries [25]. The statistical analysis of the data was performed by the application of Microsoft Excel 2010[26]. The student's t-test, the regression analysis, and Pearson correlation (r)were used to determine the significant differences in results The results of analysis with (P) values less than 0.05 (P<0.05) were considered significant.

RESULTS

The Demographic Data of Endometriosis Patients and Controls

The demography of the patients with premenopausal endometriosis and the females in the control group were demonstrated in table (1).

Table 1: The Demographic Data of Endometriosis Patients & Healthy Controls. Data are Expressed as Mean ± Standard Error of Mean (SEM) or Percentages

Category	Controls (N = 80)	Patients (N = 80)
AGE (years)	**32.1 ± 0.97**	**34.2 ± 0.59**
Social Condition Single Married	 27 (33.8%) 53 (66.3%)	 18 (22.5%) 62 (77.5%)
Occupation: Employed Unemployed	 40 (50%) 40 (50%)	 36 (45%) 44 (55%)
Parity	1.7 ± 0.15	3.8 ± 0.29*
Menstrual cycle regularity Eumenorrhea Amenorrhea Menorrhagia	 79 (98.8%) 1 (1.3%) 0.0 (0.0%)	 21 (26.25%)** 24 (30%)** 35 (43.8%)**
Stage of Disease stage 1 Stage 2 Stage 3	 --- --- ---	 25 (31.25%) 23 (28.75%) 32 (40.0%)
Location of Disease Uterus Peritoneum& ovaries Tubes	 --- --- ---	 38 (47.5%) 40 (50.0%) 2 (2.5%)
Body Mass Index (kg/m2)	23.3 ± 0.18	28.8 ± 0.40*
Waist: Hip Ratio	0.8 ± 0.01	0.9 ± 0.00*

* Significantly different at (P<0.05) as compared to the control group values.
** Significantly different at (P< 0.001) as compared to the control group values.

Table (2) and figure (1) below revealed that the serum level of SOD, an antioxidant biomarker, was significantly elevated (P<0.001) in endometriosis patients (8.9 ± 0.86) than females in the control group (0.5 ± 0.03).

Table 2: Shows the Serum Levels of SOD Enzyme, Measured in IU/dl, in Control and Endometriotic Patients Groups; Data are Expressed as Mean ± SEM

Groups Parameters	Control (N = 80)	Patients (N = 80)	P value
SOD (IU/dl)	**0.5 ± 0.03***	**8.9 ± 0.86**	**p<0.001**

*Significantly different (p < 0.05) as compared to the control group values.

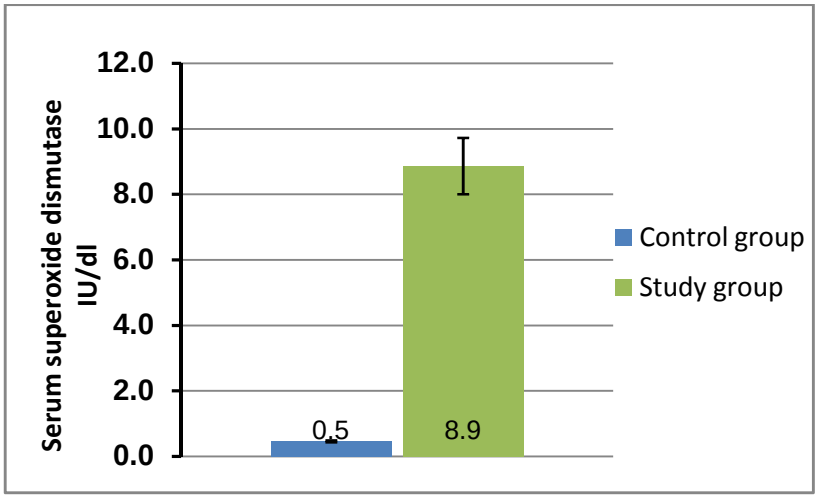

Figure 1: Histogram Shows the Difference in Serum Level of SOD, Measured in IU/dl, in Control and Study Groups

Table 3: Shows the Serum Levels of CTXI and PICP, Measured in ng/ml, in Control and Patients Groups; Data are Expressed as Mean ± SEM

Groups / Parameters	Control (N = 80)	Patients (N = 80)	P value
C-terminal telopeptide (CTXI) (ng/ml)	29.3 ± 5.32	103.0 ± 6.92*	p<0.001
C-terminal propeptide (PICP) (ng/ml)	18.4 ± 0.84	18.0 ± 0.75	0.7453

*** Significantly different (p < 0.05) as compared to the control group.**

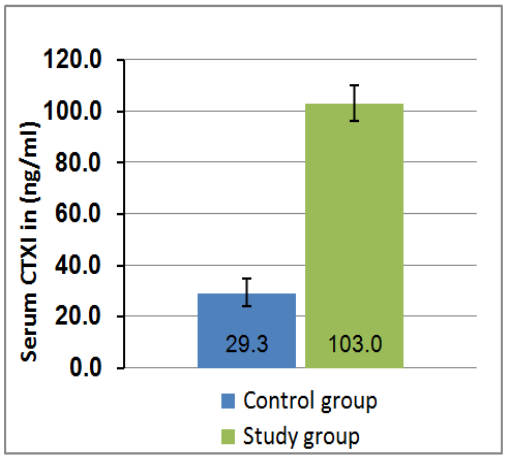

Figure 2: Histogram Shows the Difference in Serum Level of CTXI, Measured in ng/ml, in both Control and Study Groups

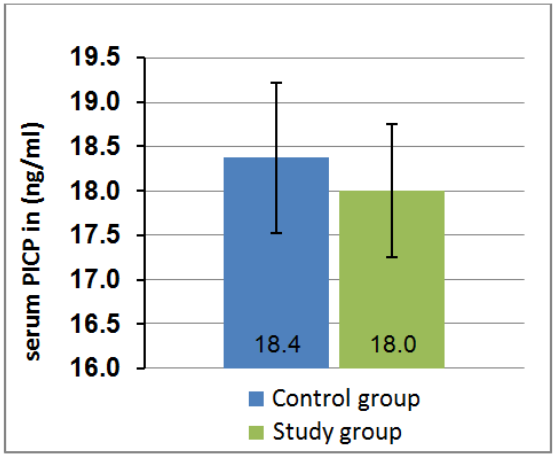

Figure 3: Histogram Shows the Difference in Serum Level of PICP, Measured in ng/ml, in Both Control and Study Groups

Table (4) and figures (4), (5) and (6) reveals the significant (P<0.001) and non-significant (P>0.001) differences among the serum levels of; total serum calcium, ALP, and inorganic phosphate, respectively.

Table 4: Shows the Serum Levels of Total Calcium, Inorganic Phosphate (Measured in mg/dl), and the Serum Activities of Total ALP (measured in IU/dl), in Control and Patients Groups; Data are Expressed as Mean ± SEM

Groups / Parameters	Control (N = 80)	Patients (N = 80)	P value
Total calcium (mg/dl)	8.1 ± 0.13	9.4 ± 0.06*	p<0.001
Inorganic phosphate (mg/dl)	4.36 ± 0.03	4.38 ± 0.03	0.6141
Total Alkaline phosphatase (IU/dl)	53.2 ± 1.31	65.6 ± 0.80*	p<0.001

* Significantly different ($p < 0.05$) as compared to the control group

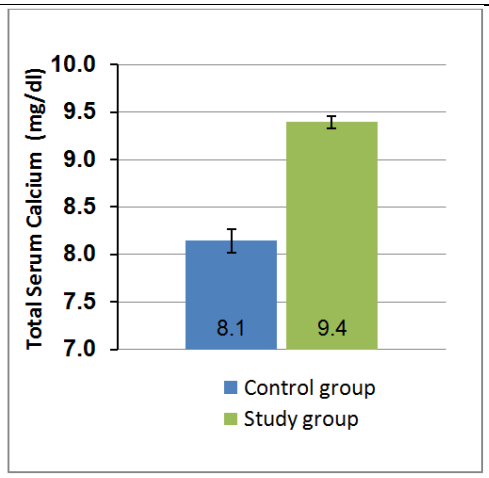

Figure 4: Histogram Shows the Difference in the Total Serum Calcium Level in both Control and Study Groups

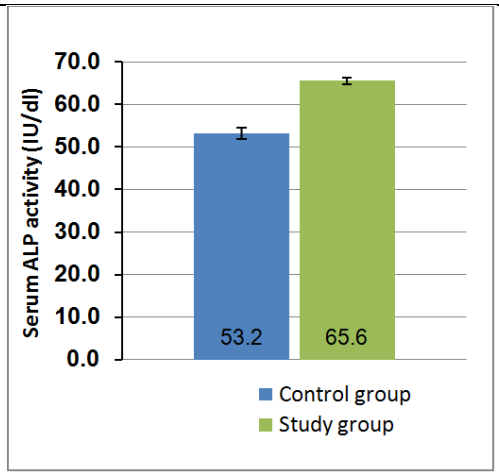

Figure 5: Histogram Shows the Difference in the Serum Activity of Total ALP, Measured in IU/dl, in Both Control and Study Groups

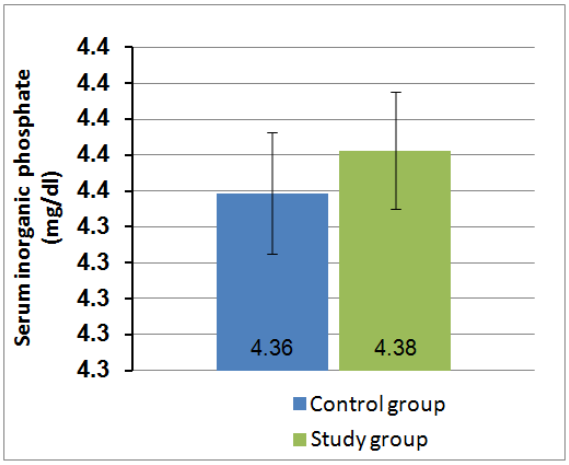

Figure 6: Histogram Shows the Difference in Serum Inorganic Phosphate Level, Measured in mg/dl, in Both Control and Study Groups

DISCUSSIONS

In this study, one of the stress biomarkers that have been surveyed in the serum of participants revealed that the serum concentration of SOD, an antioxidant parameter, was significantly elevated (P<0.001) in endometriosis patients in comparison with healthy women in the control group, as shown in table (2) and figure (1). Accordingly, The serum levels

of bone-specific biochemical turnover marker [CTXI] was significantly elevated (P<0.001), and the serum level of [PICP] was non-significantly decreased (P>0.05) in endometriosis patients than healthy females, as elucidated in table (3) and figures (2) and (3) respectively. Furthermore, the serum activity of total ALP was significantly elevated (P<0.001) in patients as compared to the healthy controls, as shown in table (4) and figure (5). Also in this study; the level of total serum calcium in the patients was significantly higher (p<0.001) as compared to the healthy women, as demonstrated in table (4) and figure (4). Whereas, the results of this study, as shown in table (4) and figure (6), revealed that the serum level of inorganic phosphate was non-significantly different (P>0.05) among endometriosis and control group women.

Some studies concerning the role of antioxidants in osteoporosis have been done and results found that there was a correlation between antioxidants and osteoporotic changes[2]. Other studies predict that certain antioxidants such as vitamin C, E, and ß-carotene may reduce the risk of osteoporosis[4,27,28]. The *in vitro* animal studies found that oxidant stress have important effects on osteoclast differentiation and function and the reactive oxygen radicals and antioxidant system which might be involved in the pathogenesis of bone loss. It was reported that oxidative stress markers are important indicators for bone loss in postmenopausal women [29].

Many studies propose that certain antioxidants (e.g. vitamin C, E, and ß-carotene) may reduce the risk of osteoporosis and counteract the negative effects of oxidative stress on bone [27,28]. Also it have been investigated that osteoporosis is associated with biochemical markers of oxidant stress, such as hydrogen peroxide, superoxide anion and urinary excretion of lactic acid, and also plasma antioxidants [30-33]. Superoxide radical is localized both intracellularly and at the osteoclast bone interface suggesting its participation in bone resorption [33]. Ozgocmen et al. (2007) found that SOD activity in women with postmenopausal osteoporosis is higher than the control, and antioxidant enzymes increase with OS and exercise training [28]. It seems that a decline in the level of antioxidants does not always occur in oxidative stress, and sometimes it will enhance by many ways like sports and physical activities.

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