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Role of Glutathione Peroxidase 1 and Sex Hormones in Iraqi Patients with Osteoporosis

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Abstract

Osteoporosis is a bone disorder often linked to hormonal disturbance and oxidative stress. Glutathione peroxidase 1 (GPX1) enzyme, along with sex hormones such as estradiol (E2) and testosterone, may play a role in bone metabolism and the development of osteoporosis. The current study aimed to evaluate the serum concentration of GPX1, E2, and testosterone hormones in addition to anthropometric measurements in patients with primary osteoporosis. The study included 210 individuals, including both osteoporosis patients and healthy controls. The levels of GPX1 were measured using an enzyme-linked immunosorbent assay (ELISA), and testosterone and E2 were measured through an automated analyzer Cobas E 411 for both patients and healthy individuals. The serum levels of GPX1 were significantly lower in osteoporosis patients than in healthy controls. Similarly, E2 and testosterone levels were significantly lower in osteoporosis patients than in healthy controls.

Key words: Estradiol E2, Glutathione peroxidase 1, Osteoporosis, Oxidative stress, testosterone

دور الكلوتاثايون بيروكسيداز 1 والهورمونات الجنسية لدى المرضى العراقيين المصابين بهشاشة العظام

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الخلاصة

هشاشة العظام هي اضطراب عظمي غالبا ما يرتبط باضطرابات هورمونية و الاجهاد التأكسدي. يلعب إنزيم الكلوتاثايون بيروكسيداز 1 (GPX1) الى جانب الهورمونات الجنسية مثل هورموني الاستراديول (E2) والتستوستيرون دورا في ايض العظام و تطور هشاشة العظام . هدفت الدراسة الحالية الى تقييم انزيم الكلوتاثايون بيروكسيداز 1 (GPX1) في المصل بالاضافة الى هورموني الاستراديول (E2) و والتستوستيرون فضلا عن القياسات الانثروبومترية لدى مرضى هشاشة العظام الاولى. شملت الدراسة 210 افراداً، منهم مرضى هشاشة العظام و اصحاء كعينة ضابطة. تم قياس مستويات إنزيم GPX1 باستخدام تقنية الفحص المناعي المرتبط بالإنزيم بينما تم قياس مستويات هورموني التستوستيرون و الاستراديول باستخدام جهاز المحلل الآلي Cobas E 411 للمرضى والأفراد الأصحاء. كانت مستويات الكلوتاثايون بيروكسيداز

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1 (GPX1) في مصل مرضى هشاشة العظام اقل بشكل ملحوظ عن المجموعة الضابطة. و بالمثل كانت مستويات الإستروجين والتستوستيرون في مصل مرضى هشاشة العظام اقل بشكل ملحوظ عن المجموعة الضابطة.

1. Introduction

Osteoporosis is a metabolic bone disease that causes a change in bone formation and abnormality in bone mass [1]. The diagnosis of osteoporosis is made mainly via the dual X-ray absorptiometry (DEXA) scan, which is used to evaluate bone mass by determining bone mineral density (BMD) [1, 2]. Age, sex, hormones, oxidative stress, and unhealthy lifestyle are considered the main risk factors for osteoporosis [1,2]. Osteoporosis is becoming a public health concern; evidence from populations from the Eastern Mediterranean region indicates a substantial occurrence of osteoporosis, reflecting regional patterns of bone health concern [3]. Moreover, research highlighted that osteoporosis occurred in both sexes, with a greater risk for older adults, especially postmenopausal women [4]. Glutathione peroxidase GPX1 is an antioxidant enzyme in the mitochondria and cytosol ubiquitously and plentifully expressed in blood [5]. In 1957, GPX1 was identified for the first time by Mills as an enzyme in erythrocytes that acts against oxidative stress [5]. Glutathione peroxidase catalyzes the reduction of hydrogen peroxide (H_2O_2) to eliminate or prevent harmful stress that disease causes, in addition to removing harmful components [6,7]. This antioxidant enzyme is implicated in the metabolism of bone and in eliminating the effect of oxidative stress, consequently causing bone diseases and a high risk of osteoporosis [6, 8]. Estradiol (E2) is the most effective estrogen hormone that is produced naturally in the human body [9]. Studies have demonstrated that E2 has a positive effect on bone health by decreasing bone destruction and minimizing osteoclast cell activity, which is crucial for keeping skeletal integrity and preventing osteoporosis [9,10]. Additionally, E2 plays a critical role in regulating vertebral trabecular bone mass, which shows its vital function in keeping bone microarchitecture [10]. Testosterone is a sex hormone that is essential for regulating bone mass and muscle strength, produced primarily in the testes in men and in smaller amounts in the ovary and adrenal gland in women [11]. Studies have shown testosterone is vital to keep it in sufficient quantities for both men and women, which is essential for maintaining musculoskeletal health and may also support vascular and brain function [11,12]. This study aims to evaluate GPX1, E2 and testosterone serum levels along with anthropometric measurements in osteoporosis patients and healthy controls. Additionally, the goal is to identify potential biomarkers that may aid in the early diagnosis and risk assessment of osteoporosis.

2. Materials and methods

Patients and control subjects

A total of 210 participants, women and men, grouped as 75 osteoporotic women and 75 healthy participants subdivided as 45 postmenopausal women and 30 premenopausal women in addition to 30 men with osteoporosis and 30 healthy men were enrolled in this study that was attended at Medical City – Baghdad teaching hospital and AlWasity teaching hospital in Baghdad province/Iraq, during the period from November 2023 until August 2024, approved by the Iraqi Ministry of Health and Environment [Ref.: 39489 on October 17, 2023]. After approval of the ethics committee of the University of Baghdad and written consent from each participant in this study, participants also filled out a questionnaire that included clinical history, chronic diseases, and physical exercise. Any individuals with chronic diseases who have recently taken drugs that impact bone metabolism and are thereby related to secondary osteoporosis were excluded.

BMD measurement

All participants underwent physical examination for anthropometric measurements. Dual-energy X-ray absorptiometry (DEXA) was utilized to measure BMD and T-scores at the lumbar spine. As per World Health Organization (WHO) criteria, osteoporosis patients with (-2.5) T score and lower were included; meanwhile, others were excluded from this study [1].

Blood collection

Three milliliters of blood samples by venipuncture were collected in a gel tube prepared and labelled, and the sample was allowed to clot at room temperature for about one hour. After that, by utilizing centrifugation at about $1790 \times g$ for five minutes, the serum was separated into equal parts in Eppendorf tubes and then frozen at -20°C to be used for the enzyme-linked immunosorbent assay (ELISA) procedure and other hormonal assays. Serum GPX1 levels were quantified for both osteoporosis patients and healthy controls using an ELISA kit, the Human Glutathione Peroxidase 1, GPX1 ELISA Kit (Cat No. E3921Hu) employs a sandwich ELISA method to measure GPX1 levels. According to the manufacturer's instructions, the serum hormone concentrations were calculated using the calibration graph. E2 and testosterone hormone levels were measured using kits through an automated analyzer machine (Roche Company, Germany) that was applied by kits on the Cobas E 411 system model to utilize Electro ChemiLuminescence (ECL) technology for immunoassay analysis, enabling the precise quantification of hormones. All reagent kits were stored and handled according to the manufacturer's instructions at the recommended temperature and adequately mixed. All materials utilized in the experiments included in the current study were sterilized at the optimum conditions.

Statistical analysis

The results reported in this study were expressed as mean $\pm$ SD and median. An independent t-test was used to test the difference between two parameters. One-way ANOVA was used to test the differences between study groups. Probability values less than 0.05 and 0.01 were considered significant and highly significant differences, respectively [13]. Categorical variables were expressed using numbers and percentages. All statistical analyses were performed using SPSS Statistics 27.0 (Armonk, NY: IBM Corp.). Graphs were plotted with Graph-Pad Prism 9.4.1 (San Diego, California, USA) [13].

3. Results and discussion

Anthropometric and BMD measurements

The current study enrolled 105 patients diagnosed with primary osteoporosis and 105 healthy control (HC) subjects. The results of the characteristics are shown in Table 1. The mean $\pm$ SD for the age of primary osteoporosis patients was (55.0 ± 12.0), and for the HC was (56.0 ± 12.0), with no significant differences. Regarding age groups (32-49 years and 50-75 years), about 67.6 % of osteoporosis patients were sorted into the age group (50-75 years). In terms of gender, the number of men with osteoporosis was 30, with a percentage of 28.6 %, the same as 30 HC men (28.6%). The number of women with osteoporosis was 75, with a percentage of 71.4 %, the same as HC women. According to menopausal status for women, the number of premenopausal osteoporosis women was 30, with a percentage of 28.6%, and the number of postmenopausal osteoporosis women was 45, accounting for 42.9, with no significant differences. Regarding the height, weight, and BMI measurements, there were no significant differences. Regarding the BMD, the mean $\pm$ SD of primary osteoporosis patients and HC were (0.74 ± 0.04) and (0.89 ± 0.05), respectively, with a p value <0.001 which is considered highly significant. Together with the T score of DXA scan for the primary osteoporosis (-3.0 ± 0.3) and HC (0.1 ± 0.7). As shown in Table 1.

Table 1: Baseline characteristics of primary osteoporosis patients and healthy controls.

Characteristic; mean $\pm$ SD or n(%)		Patients; n = 105	HC; n 105	p-value
Age; years		55.0 $\pm$ 12.0	56.0 $\pm$ 12.0	0.950
Age group; years	32-49	34 (32.4)	33 (31.4)	0.883
	50-75	71 (67.6)	72 (68.6)	
Gender	Males	30 (28.6)	30 (28.6)	1.000
	Females	75 (71.4)	75 (71.4)	
Height; m		1.65 $\pm$ 0.08	1.65 $\pm$ 0.09	0.705
Weight; kg		77 $\pm$ 10	76 $\pm$ 11	0.507
BMD; g/cm ²		0.74 $\pm$ 0.04	0.89 $\pm$ 0.05	0.001
BMI; kg/m ²		28.4 $\pm$ 4.3	27.8 $\pm$ 3.7	0.294
	Normal (19-25)	35 (33.3)	37 (35.2)	
	Abnormal (> 25)	70 (66.7)	68 (64.8)	
T score		-3.0 $\pm$ 0.3	0.1 $\pm$ 0.7	0.001
	Normal density (-1 to +1)	0 (0.0)	105 (100.0)	
	Osteoporosis (< -2.5)	105 (100.0)	0 (0.0)	
Menopausal status for women	Premenopausal	30 (28.6)	30 (28.6)	1.000
	Postmenopausal	45 (42.9)	45 (42.9)	

SD: Standard deviation; HC: Healthy controls; BMI: Body Mass Index; BMD: Bone Mineral Density; *p*: Two-tailed probability (significant *p*-value is indicated in bold). Significance was assessed using a one-way analysis of variance test or Pearson's Chi-square test (categorical variable).

Abnormal weight affects the whole body and causes various diseases, including osteoporosis. Being underweight or overweight is identified as an independent risk factor for developing osteoporosis, which is necessary to maintain a healthy body and promote healthy bones [14]. Although the current study did not find a statistically significant difference concerning BMI between osteoporosis patients and healthy controls, this contrasts with findings from other research. Murat *et al.*, observed a positive association between BMI and bone mineral density. Similarly, Ma *et al.*, through a Mendelian randomization approach, identified a potential causal link between elevated BMI and greater bone mineral density. However, their genetic analysis did not find a significant association between BMI and fracture risk [15,16]. Bone is a connective tissue that is a structural framework for other body tissues due to its hardness and density. BMD level monitors bone health. Our results showed that the T score is higher in women and men with osteoporosis than in healthy controls. This agrees with De Martinis *et al.*, who found that mean T score values were significantly higher in osteoporosis patients and mainly in women [17]. Also, Salman and her colleagues showed significant differences between osteoporosis patients and healthy controls [18]. Consistent with Jafer and Ali's findings of no significant differences in height, weight, or BMI between diabetic osteoporotic women and healthy controls [16]. Our study likewise found that these anthropometric measures did not differ significantly between osteoporosis patients and controls. Conversely, BMD and T-score exhibited highly significant differences between the two groups [19]. Generally, when multiple risk factors are present in a patient, the likelihood of developing osteoporosis and experiencing fractures increases, compared to when only one or two risk factors are present in the individual [20].

Glutathione peroxidase 1 (GPX1), E2 and testosterone measurements

The concentration of GPX1 (median and IQR) was significantly decreased in osteoporosis patients in comparison with HC (51.36 [32.3-65.19] vs 66.23 [47.3-87.80] mIU/ml; p-value <0.001). Regarding sex hormones, the testosterone levels (median and IQR) were significantly decreased in osteoporosis patients in comparison with HC 0.21 [0.10-1.78] vs. 0.24 [0.17-3.56] ng/ml; p-value <0.001). Likewise, E2 levels were significantly decreased in osteoporosis patients compared to HC (18.00 [13.20-21.80] vs. 50.70 [44.90-58.50] pg/dl; p-value = 0.001), as shown in Figure 1.

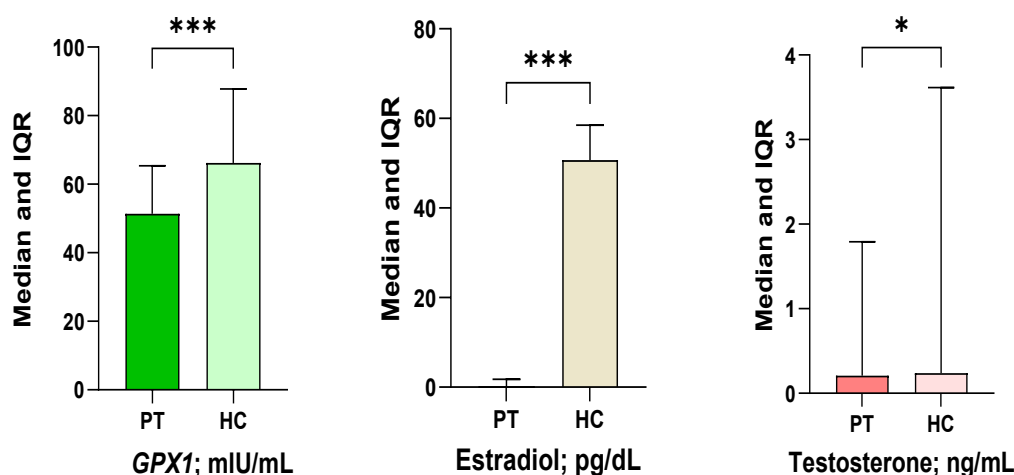


Figure 1: Serum level of *GPX1*, Estradiol (E2), and testosterone in osteoporosis (n = 105) patients and healthy controls (HC; n = 105). Values are given as mean and standard deviation. Significance was assessed using a one-way analysis of variance test (parametric variable). *GPX1*; (51.36 (32.3-65.19) vs. 66.23 (47.3-87.80) mIU/mL; p-value <0.001), Estradiol; 18.00 (13.20-21.80) vs. 50.70 (44.90-58.50) pg/dL; p-value = 0.001) and testosterone; 0.21 (0.10-1.78) vs. 0.24 (0.17-3.56) ng/mL; p-value <0.001).

The findings of significantly lower serum *GPX1* levels in osteoporosis patients than HC align with León-Reyes *et al.*, which emphasized that insufficient *GPX1* amount or function can disturb bone remodeling by impairing bone resorption and weakening osteoblast activity, causing severe bone mineral density loss, thereby driving disease pathology [21]. Free radicals are thought to play a role in the activation of osteoclast cells. Consequently, *GPX1*, a plentiful antioxidant enzyme, is an inhibitor against osteoclast cell differentiation and bone resorption [22]. Sex hormones are essential to keep bones healthy. Thus, lacking E2 and testosterone could impact bone structure and enhance osteoporosis [9, 11]. Besides, testosterone hormones in men are related to healthy bone status; thus, any level changes indicate bone disease [23-25]. Men often reach a more significant peak bone mass than women, and they do not go through a drastic shift in their reproductive function with ageing, unlike women who experience menopause. However, with regular, healthy ageing, circulating testosterone, growth hormone, and adrenal androgens drop [25-27].

Diagnostic performance of serum *GPX1*, E2 and testosterone

Statistical analysis by the receiver operating characteristic (ROC curve) indicated that E2 hormone was a highly reliable biomarker in the diagnosis of primary osteoporosis patients from HC (AUC=0.987; p value< 0.001) with high sensitivity and specificity, suggesting its potential role in early diagnosis. In comparison, the ROC curve of serum *GPX1* showed

moderate diagnostic performance for detecting primary osteoporosis disease (AUC=0.649; p value< 0.001), indicating it could provide supportive value when used alongside conventional biochemical markers, such as serum calcium, phosphate, or alkaline phosphatase. Conversely, testosterone showed low diagnostic accuracy and limited clinical value as an independent diagnostic marker, as shown in Figure 2.

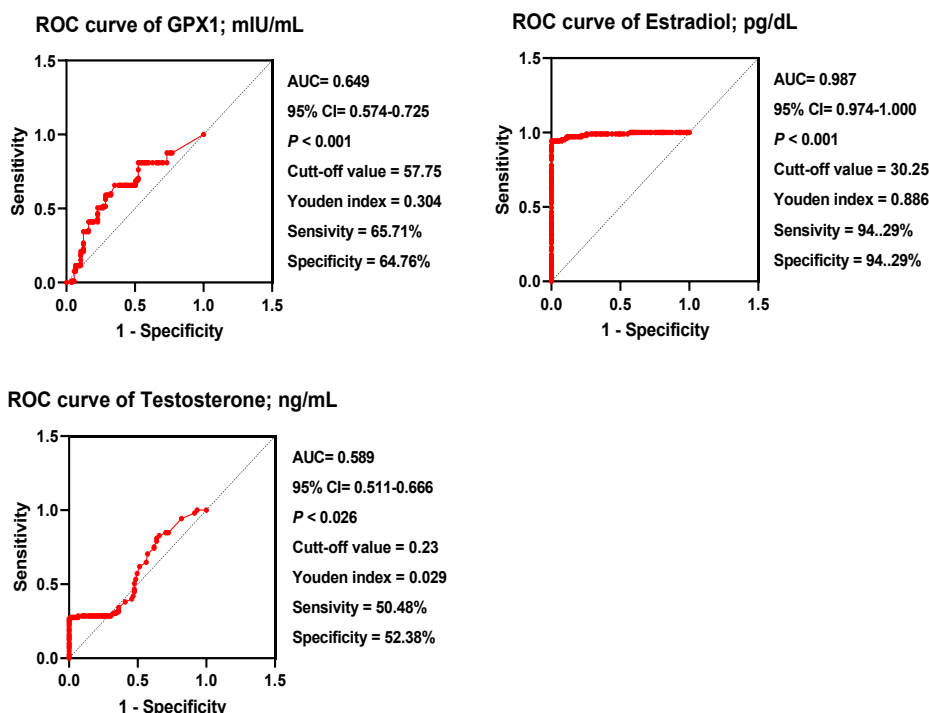


Figure 2: Receiver operating characteristic (ROC) curve analysis of GPX1, E2, and testosterone serum levels in osteoporosis patients ($n = 105$) versus healthy controls ($n = 105$).

The analysis assessed the ability of these parameters to distinguish between patients and controls. Area under the curve (AUC), 95% confidence interval (CI), probability (p), cut-off value (CV), sensitivity, and specificity are shown. The CV was adjusted with Youden index (YI). Among the measured parameters, E2 exhibited the highest diagnostic performance (AUC;0.987), followed by GPX1 (0.649), which showed fair diagnostic indication. In contrast, testosterone demonstrated limited diagnostic value (0.589).

The results of the ROC curve for serum GPX1 indicated that it could be assessed in diagnosis, aligned with other markers. Osteoblast cells synthesize the GPX1 to limit the cellular damage in bones [21, 28]. GPX1 is linked with causing osteoporosis due to increased oxidative stress [21, 28]. The mechanism of GPX1 regulation against oxidative stress in bone remodeling, which balances osteoblast and osteoclast, is influenced by many factors, including ageing and hormonal changes such as estrogen deficiency [29-30]. Comparing the results of E2 reveals a notable difference between osteoporosis patients, particularly among women, which can serve as a biomarker. This agrees with Camon and his colleagues, who mentioned the incidence of decreased hormone levels in osteoporosis patients, particularly postmenopausal women [31]. The AUC-ROC analysis demonstrated a suitable fit for the logistic regression model, indicating its effective identification capability was performed in this study to investigate the diagnostic performance for antioxidant enzyme GPX1, estradiol E2 and testosterone hormones serum measurements in primary osteoporosis disease. Our findings suggest that E2 and GPX1 offer greater diagnostic insight. E2 is directly linked to

bone loss mechanisms, especially in postmenopausal women, while GPX1 reflects the oxidative stress component of osteoporosis pathogenesis. Therefore, incorporating such markers may enhance early diagnosis and risk assessment beyond what traditional bone biochemistry alone can provide. The results of the current study indicated that E2 has strong diagnostic potential, suggesting utility in early detection of osteoporosis [2, 29,30]. Although GPX1 showed a moderate diagnostic value, its inclusion with other biomarkers may enhance overall assessment accuracy [24,25]. The inclusion of GPX1 and sex hormones may offer additional insight into the multifactorial nature of osteoporosis pathogenesis and assist in refining diagnostic approaches [24,25, 29,31].

Correlation between study parameters

To visualize a correlation matrix between the characteristic variables, Spearman's rank-order correlation analysis was performed between age, weight, height, BMI, BMD, T score, glutathione peroxidase1, E2, and testosterone. Age showed a positive correlation with BMI ($r_s=0.30$; $p<0.001$) and a negative correlation with T score ($r_s=-0.33$; $p<0.001$). BMD showed a positive correlation with height ($r_s=0.20$; $p<0.05$) and a negative correlation with E2 ($r_s=-0.04$; $p<0.001$). GPX1 showed a weak negative correlation with BMD and age. Consequently, testosterone positively correlated with T score ($r_s=0.33$; $p<0.001$), as shown in Figure 3.

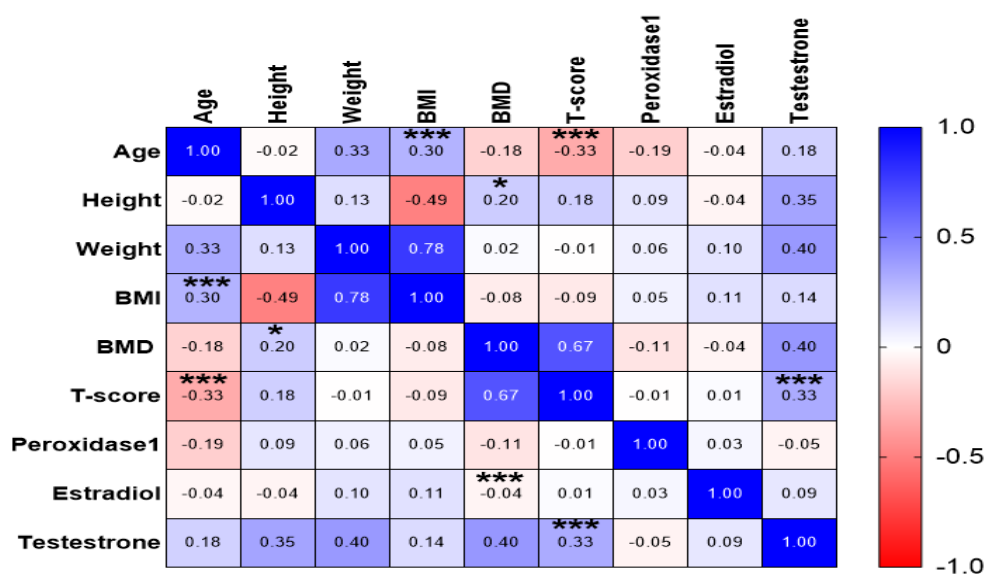


Figure 3: Heat map matrix of Spearman's rank-order correlation coefficient (r_s) between anthropometric measurements and serum levels of Glutathione Peroxidase1 (GPX1, Estradiol E2 and testosterone in osteoporosis patients. Values inside boxes indicate the correlation coefficient (r_s). Blue color indicates a positive correlation. Red color indicates a negative correlation. A significant correlation (two-tailed probability; p) is indicated by an asterisk (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

The current results showed that BMD and age factors are affected negatively by GPX1; the alterations in oxidative stress and antioxidants that cause imbalance are thought to contribute to diseases such as osteoporosis [7]. The cell aims to eliminate free radicals, maintain hemostasis, and prevent pathological conditions through antioxidant defense mechanisms in

mammals, particularly GPX1. This mechanism primarily involves the reduction of soluble H_2O_2 , which serves as a crucial antioxidant enzyme [7,32]. This antioxidant enzyme is implicated in the metabolism of bone and in eliminating the effect of oxidative stress, consequently causing bone diseases and a high risk of osteoporosis [7]. The positive correlation between BMD and T score, with the inverse correlation between both of them and age, has been reported by studies Ma *et al.*, and De Martinis *et al.*, [16-17]. In the study by Ma *et al.*, the importance of BMD and T score was emphasized as a fundamental determinant of skeletal strength and a key determinant in assessing fracture risk [16]. Similarly, De Martinis *et al.*, reported a progressive decline in T score with increasing age, highlighting the age-dependent nature of bone loss and the increased vulnerability to osteoporosis over time [17]. The serum estradiol levels were involved in the development of osteoporosis and associated with BMD and lower T scores in both genders [23,24]. The elevation in oxidative stress, specifically among postmenopausal women, is evidenced by reduced antioxidant levels. These changes are attributed to ageing and minimize estrogen and testosterone levels, which increase the susceptibility to various health issues and diseases such as osteoporosis [23,25]. Testosterone levels varied among men and women with osteoporosis and healthy control groups. Taha and his colleagues mentioned the association between testosterone hormones and T score in postmenopausal women that could impact osteoporosis [25]. According to men, studies suggested an association between increased testosterone levels and bone health and density [25,27]. Nevertheless, this relation is not fully discussed according to women who mentioned that high testosterone concentrations could play a role in protecting bones against osteoporosis. Hence, monitoring testosterone levels helps improve bone health in middle-aged adults, preventing bone-related disorders [26,27]. Sex hormones influence bone remodeling because bone cells (osteoblasts and osteoclasts) respond to signals from hormones, cytokines, and minerals. When these cells malfunction, the remodeling process becomes imbalanced. In osteoporosis, this imbalance results in bone resorption exceeding bone formation, leading to a net loss of bone density [26,27,31]. In line with this view, the results of the present study indicated that serum estradiol levels were involved in the development of osteoporosis and associated with lower T scores in both genders [9,33]. Although osteoporosis can occur at all ages, the downregulation of sex hormones, mainly after menstruation, leads to bone weakening and enhances the chance of osteoporosis; estrogen deficiency in older women substantially impacts the prevalence of osteoporosis by raising bone turnover [33].

4. Conclusion

The evaluation of BMD and T score alone may be insufficient to determine osteoporosis disease; therefore, additional clinical parameters are required as diagnostic tools to identify osteoporosis as complementary parameters together with DXA scan measurements. E2 showed a valuable diagnostic biomarker while GPX1 showed moderate utility as a supportive marker. Testosterone exhibited limited diagnostic prevalence. Correlation analysis further highlighted the association between age and T score, indicating a negative correlation that suggests bone density decline with age. Testosterone positively correlated with T score, suggesting that higher testosterone may be associated with improved bone health. Additionally, GPX1 exhibited weak negative correlations with both age and BMD, which may reflect the influence of oxidative stress on bone density and the aging process. Incorporating oxidative stress and hormonal biomarkers alongside DXA measurements may enhance diagnostic accuracy and guide more comprehensive clinical assessment to understand the multifactorial nature of osteoporosis.

Ethical approval

This is to certify that the work as mentioned above was approved by the Ethics Committee of the Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq (Ref.: CSEC/1023/0069 on October 30, 2023).

Conflict of Interest

The authors declare that they have no conflicts of interest.

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