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## Relationship Between Osteopontin Biochemical Parameters and BMD Status in Iraqi Postmenopausal Women with Osteoporosis

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### Abstract

Osteopontin hormone (OPN) is an extracellular matrix protein that is expressed in bone cells such as osteoblasts and osteoclasts and associated with bone turnover and bone mineral density (BMD) in postmenopausal women with osteoporosis.

The aim of the study is to investigate serum levels of circulating OPN and its relationship with biochemical parameters and BMD in postmenopausal women with osteoporosis in Iraq. Serum samples from fifty postmenopausal women were selected from patients attending two educational hospitals in Baghdad, which are AL Wasy Educational Hospital and Baghdad Educational Hospital, during the period from November 2018 to March 2019. Twenty five postmenopausal healthy women were included as a control group. The studied subjects' ages were in the range of 45-65 years. Dual energy X-ray absorptiometry (DEXA) was the device used to measure bone mineral density and diagnose osteoporosis in both groups. Blood samples were collected from each participant for measuring the serum levels of biochemical parameters (P, Alp, Ca, and OPN)

The results of the demographic parameters showed a significant ( $P \leq 0.05$ ) increase in mean values of age, menopause duration, and duration of productive life in the patients as compared to the control group. Also, strong positive correlations between patients and the control were recorded in age and duration of productive age, with a weak inverse correlation in menopause duration. While a significant ( $P \leq 0.05$ ) decrease in the mean value of body mass index (BMI), BMD, and T-score as compared to the control. The correlation in BMI was weak significant positive while in the other two parameters it was weak significant inverse.

The results of the present study showed non-significant differences ( $P \geq 0.05$ ) between the patients and control group for serum biochemical parameters. It was also noticed that there was a significant ( $P \leq 0.05$ ) increase in the mean value of osteopontin hormone level in the patients as compared to the control, with a weak significant inverse ( $p \leq 0.05$ ) correlation.

**Key words:** Osteoporosis, postmenopause, Osteopontin, phosphorus, Calcium

العلاقة بين الاوستيوبونتين والمعلومات الكيموحيوية وكثافة كتلة الجسم في عينه من النساء  
العراقيات بعد سن اليأس المصابات بهشاشة العظم

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### الخلاصة

تهدف الدراسة إلى التحري عن العلاقة بين مستويات هرمون اوستيوبونتين وبعض المعلمات البايوكيميائية وال BMD في عينة العراقيات النساء المصابات بهشاشة العظام بعد سن انقطاع الطمث. جُمعت عينات مصل الدم من 50 امرأة عراقية في سن اليأس مصابات بهشاشة العظام ممن يترددون على مستشفى بغداد التعليمي ومستشفى الواسطي التعليمي للفترة من شهر تشرين الثاني سنة 2018 الى شهر اذار 2019 كمجموعة المرضى و25 امرأة غير مصابات كمجموعة سيطرة تراوحت اعمارهم بين(45-65) سنة كل المجاميع شخصت سريريا .

اظهرت نتائج التحليل ان هناك زيادة معنوية ( $P \leq 0.05$ ) في معدل العمر ،فترة سن الياس وفترة سن الاخصاب في مجموعة المرضى مقارنة بمجموعة السيطرة وكان معامل الارتباط موجب قوي في العمر وسن فترة الاخصاب

وعكسي في فترة انقطاع الطمث.بينما كان هناك نقصان معنوي ( $P \leq 0.05$ ) في قيمة مؤشر كتلة الجسم (BMI),(BMD) و T-score مقارنة بمجموعة الكونترول ,وبمعامل ارتباط موجب ضعيف في ال BMI وعكسي بالنسبة للمعلمات الباقية.

اظهرت نتائج الدراسة انه لا توجد فروق معنوية ( $P \geq 0.05$ ) بين مجموعة المريضات ومجموعة السيطرة في جميع المعلمات البايوكيميائية مستوى P,Alp وCa ولوحظ في دراستنا الحالية ان هناك زيادة معنوية ( $P < 0.05$ ) في مستوى هرمون الاوستيوبونتين في المريضات مقارنة بمجموعة السيطرة وكان معامل الارتباط عكسي ضعيف .

### Introduction

Bone is a very complex structure that is essential for providing mobility, support, and protection for the human body, as well as serving as a reservoir for storing essential minerals. There are two main components of bone strength, which are BMD (mineral content in grams per area) and bone quality (bone architecture, bone turnover, damage accrual to the bone, matrix properties, and mineralization) [1].

Menopause is defined as the state of an absence of menstrual periods for 12 months due to the inability of the ovary to release estrogen. This can lead to bone loss and risk factors for osteoporosis [2].

Osteoporosis is described by the World Health Organization (WHO) as a “progressive systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture [3].

OPN is one of the bone resorption markers and a major component of the non-collagenous bone matrix produced by osteoblasts and osteoclasts. It was found to be associated with bone strength and remodeling (Morinobu et al., 2003). Osteopontin plays important roles in bone resorption by facilitating osteoclast attachment to the bone matrix [4].

### Materials and Methods

#### Participants

Blood samples were collected from fifty postmenopausal women attending AL Wasity Educational Hospital and Baghdad Educational Hospital during the period from November 2018 to March 2019, as a patients group, and twenty five postmenopausal healthy women, as control group. The age range was from 45-65. Dual energy X-ray absorptiometry (DEXA) was the device used to measure bone mineral density and diagnose osteoporosis in both groups. Medical history of each subject was recorded. Subjects with any medical condition, which might cause changes in bone metabolism, were excluded. Five milliliters (ml) of venous blood was collected from all subjects using a gel tube, allowed to clot at 37°C for 10-15 minutes, and centrifuged for 5 min. Serum was reserved at -20°C until used.

#### Demographic parameters

A questionnaire form was included for all studied groups and contained information about age, BMI, BMD, menopause duration, duration of production life, and T-score.

#### Biochemical parameters determination in serum

##### Determination of serum phosphorous

Using FUJI DRI-CHEM ANALYZER, serum phosphorus concentration was measured depending

on optical reflection density [5].

#### Determination of serum alkaline phosphatase (ALP)

ALP activity in the serum was measured as it is directly proportional to the rate of change in absorbance [6].

#### Determination of serum Calcium

The method used is based on the metallochromogen Arsenazo III. Bichromatically, the absorbance of the Ca-ArsenazoIII complex was measured, which is directly proportional to calcium concentration in the sample [6].

#### Determination of Osteopontin (OPN)

ELISA was used to measure osteopontin quantitatively in human serum, depending on the affinity tag-labeled capture antibody and a reporter-conjugated detector antibody which immunocapture the sample analyte in solution [7].

#### Statistical analysis

To assess the influence of variables in this sample, t-tests were used to compare two groups while analysis of variance (ANOVA) was used to compare more than two groups. The differences between means was explained by significant differences (LSD) at  $p \leq 0.05$ , where the results were expressed as mean  $\pm$  SEM. Also the correlation coefficient for parameters was found and the correlation state was determined. Statistical analysis was carried out with the social sciences statistical system (SPSS 21.0) and Microsoft Excel 2013 [8, 9].

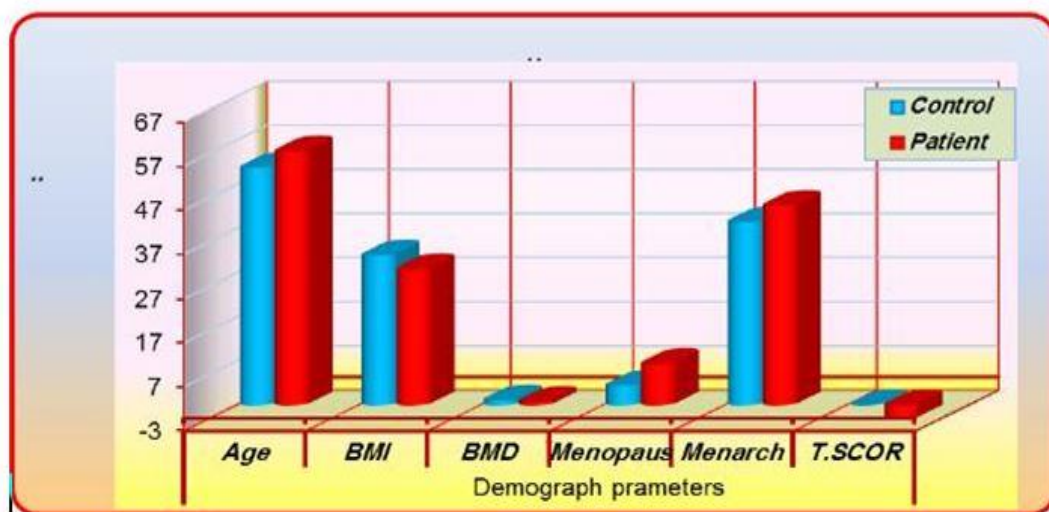
### Results and Discussion

#### Demographic studies in osteoporotic postmenopausal women and control

**Table 1**-demographic studies in osteoporotic postmenopausal women and control and their correlation coefficient values

Parameters	Patient n=50	Control n=25	P-Value	Significant $P \leq 0.05$	Correlation
Age (years)	57.46 $\pm$ 0.69	53.76 $\pm$ .11	0.00*	S	0.89
BMI (kg/m <sup>2</sup> )	30.76 $\pm$ 0.76	33.89 $\pm$ 0.71	0.01*	S	0.16
BMD (g/cm <sup>2</sup> )	0.72 $\pm$ 0.01	1.09 $\pm$ 0.02	0.00*	S	-0.23
Menopause duration	9.34 $\pm$ 0.87	4.48 $\pm$ 0.64	0.00*	S	-0.04
Duration of production life	45.10 $\pm$ 0.73	41.44 $\pm$ 1.05	0.00*	S	0.78
T-score (Lumbar spine)	-3 $\pm$ 0.07	0.02 $\pm$ 0.16	0.00*	S	-0.21

\*= Significant at  $P \leq 0.05$



**Figure 1**-Results of demographic studies in osteoporotic postmenopausal women and control.

Table-1 and Figure-1 revealed a significant ( $P \leq 0.05$ ) increase in mean values of age, menopause duration, and duration of productive life in patients ( $57.46 \pm 0.69$  year,  $9.34 \pm 0.87$  and  $45.10 \pm 0.73$  respectively) compared with the control ( $53.76 \pm 1.11$  year,  $4.48 \pm 0.64$  and  $41.44 \pm 1.05$ , respectively). A statistically significant strong positive correlation between patients and control was recorded in age and duration of productive life ( $r=0.89$  and  $0.78$  respectively), while menopause duration showed a weak inverse correlation ( $r=-0.04$ ).

Through the results significant ( $P \leq 0.05$ ) decreases were noticed in the mean values of BMI, BMD, and T-score in patients ( $30.76 \pm 0.76$  Kg/m<sup>2</sup>,  $0.72 \pm 0.01$  g/cm<sup>2</sup> and  $-3 \pm 0.07$ , respectively) in comparison to the control ( $33.89 \pm 0.71$  Kg/m<sup>2</sup>,  $1.09 \pm 0.02$  g/cm<sup>2</sup> and  $0.02 \pm 0.16$ , respectively). The correlation in BMI was significant weak positive ( $r=0.16$ ) while in the other two parameters, the correlation was significant weak inverse ( $r=0.23$ , and  $r=-0.21$ , respectively).

While osteoporosis is a well-recognized major public health problem associated with increased morbidity, mortality, and health care cost amongst elderly, there is no sufficient epidemiological or demographic data on the magnitude of the problem in Iraq. Moreover, osteoporosis is often underdiagnosed and undertreated in Iraq due to poor availability of central DXA machines, although there are recently 15 DXA machines which are only available in urban centers [10].

The incidence of osteoporosis and bone loss increases with age. The results of our study showed a significant difference ( $P \leq 0.05$ ) between patient and control groups in age. The mean value of age was higher in the osteoporosis group, with a strong positive statistical correlation. This is in agreement with previous reports [11, 12] which found the same relationship between the mean values of age in patients and control. These results were approved by many studies indicating that age is an important factor in the progression of osteoporosis. For example, an earlier work [13] found a negative effect of increasing age on the BMD, while another study [14] conducted in Baghdad found that older age women had significantly lower BMD.

After the age of 50 years, BMD level drops indicating increased bone resorption and a decrease in bone formation, resulting in osteoporosis [15][11].

The results showed a lower value of BMI in patients than that in the control. This is in line with the findings of an earlier study [13] which also found that the mean value of BMI was lower in the osteoporotic group than in the control. The results of another study [12] were also consistent with those of the current study regarding the value of BMI. However, another study [16] did not find significant differences between the patients and control, and the value of BMI in the patients was very slightly lower than that in the control. On the other hand, our results showed a significant weak positive correlation between the two groups. However, both of them had BMI values higher than 30, thus considered as obese people. This may be explained as the body weight is usually considered as a strong predictor of bone mass, but large - scale epidemiological studies have shown that increased body weight is positively correlated with a higher bone mineral density and a lower risk of fragility fractures in the elderly women, associated with lower BMI have a greater risk of hip fracture before the body adjust the BMD [17].

The prevalence of both obesity and osteoporosis have been rapidly increasing, leading to increased morbidity and mortality in both genders [18]. Several studies have investigated the relationship between obesity and osteoporosis, however, there is still not a consensus regarding this subject. The structure of the bone is influenced by many different factors such as age, gender, race, genetics, reproductively, calcium intake, BMI and exercise [19]. Among these, BMI is probably the most controversial. The increased BMI has multifactorial effects on bone metabolism. It is generally accepted that increasing body weight mechanically reinforces bone production [20]. Also, the adipose tissue is an important source of estrogen for the postmenopausal women. It is known that estrogen, the levels of which increase in the peripheral tissues in the case of obesity, inhibits osteoclasts causing, in other words, bone resorption [21]. The adipocytes also produce leptin hormone, which regulates appetite and body weight. It is suggested that leptin may decrease bone formation among obese women. It was recently suggested that obesity leads to inflammation and that the proinflammatory markers stimulate osteoclastic activity which leads to accelerate osteoporosis. Osteoporosis is a systemic skeletal disease characterized by a low BMD and/or microarchitectural deterioration, resulting in an increased bone fragility and hence an increased fracture risk. The decrease in bone mass in humans starts to occur around the age of 40 years. If no preventive action is taken, this process

may contribute to the development of osteoporosis [22]. BMD is the most used indicator for analyzing bone mass and for diagnosing osteoporosis [23].

BMD was significantly decreased in the patients group, with a negative correlation compared to the control, which agrees with previous findings [12, 24]. Even though multiple regression analysis showed significant effects on BMD of the lumbar spine, logistic regression analysis showed that this effect was not enough to affect the status of osteoporosis among tested subjects.

Several factors are effecting bone health, including estrogen which maintains calcium hemostasis of the bone and is eventually decreased in postmenopausal women, as expressed by the positive correlation obtained.

As shown in Table-1, both menopause duration and the duration of productive life manifest higher values in patients, showing a weak negative correlation with menopause duration and a strong positive one with other.

A higher value of menopause duration agrees with the results stated earlier [25], where the value of menopause duration was greater in postmenopausal women than that in the control.

There is a direct relationship between the lack of estrogen during peri menopause and menopause and the development of osteoporosis after menopause, because this hormone inhibits the bone resorption. Bone cells have estroid receptors for estrogen, and the osteoclast apoptosis is regulated by estrogens. Therefore, with estrogen deficiency, the osteoclasts live longer and are able to resorb more bone [17].

Menopause is characterized by the loss of estrogen production by the ovaries. This may occur by natural means or by the surgical removal of both ovaries. This loss of estrogen accelerates bone loss for a period ranging from 5 to 8 years. In terms of bone remodeling, the lack of estrogen enhances the ability of osteoclasts to absorb bone. Since the osteoblasts are not encouraged to produce more bone, the osteoclasts win and more bone is lost than is produced. This drop can lead to a significant bone loss and, over time, to low bone density. In our results, the statistical analysis showed this situation by the negative correlation, that may be due to the increased duration of menopause which women cross during their life along with the incidence of this disease. In the other words, earlier age at menopause is associated with increased osteoporosis risk [17].

Menarche is the hallmark of puberty and researchers showed that it may potentially play a critical role in the etiology of complex diseases developed by women later in life, such as osteoporosis [26]. Many previous studies found that age at menarche of 11 years or less was associated with a reduced incidence of osteoporosis. Also, a shorter number of menstrual years was associated with an increased risk of osteoporosis, supporting the hypothesis that lifetime cumulative exposure to estrogens is protective against osteoporosis [27]. Studies showed that early menarche has a protective effect on osteoporosis and some results showed that late menarche has adverse effects on menarche, which implies that it decreases BMD [28, 29]. This is the opposite to the results of our study which found that the long production life (i.e., years of menstruation) correlates with osteoporosis. This agrees with some studies which demonstrated no direct correlation between the age at menarche and the osteoprosis [30]. One explanation of this difference may be the use of self-reported data on the duration of production. All the subjects within the two groups were restricted to an older population with an average age of 60 years, without being compared to younger subjects. In addition, there is a need for a larger sample size. Moreover, osteoporosis may be common in some bones in body but not in all. In this study, we did not investigate the type and location of osteoporosis. In some previous studies, it was proved that delayed puberty decreases bone mass, and hence, the possibility of fractures in pre- and post-menopausal age increases [31]. Estrogen is a very important hormone for bone health. Therefore, after menopause, when there is a decreased or no production of estrogen, the bones become very susceptible to fractures. Apart from menopause, many events which affect circulating estrogen levels, such as menarche, pregnancy, lactation, and use of oral contraceptive pills, also affect bone health and increase the risk of osteoporosis, but these factors are inconsistent [31]. Menarche causes many physiological changes and it includes secretion of estrogen, which makes menarche a very important associating factor for osteoporosis [32]

Clarke and Khosla proved that the onset of gonadal sex steroid secretion at puberty is the major factor responsible for skeletal longitudinal and radial growth. Also, a significant gain in bone density, until the peak of bone density, is achieved in the third decade of life. In their research, they also concluded that at menopause, gonadal sex steroid production decreases which leads to rapid bone loss

[33]. Vikho and Apter proved that early menarche also causes higher estrogen production, both during and even after menarche [26].

The T-score of bone density shows how much the bone mass of a subject differs from the that of an average healthy 30 year old adult. A significantly low T-score in osteoporotic postmenopausal women, with and without vertebral fracture, was found compared with healthy postmenopausal women, indicating bone loss with age and menopause. A rapid bone loss is commonly seen in elderly individuals and tends to be worsen with advancing age [34].

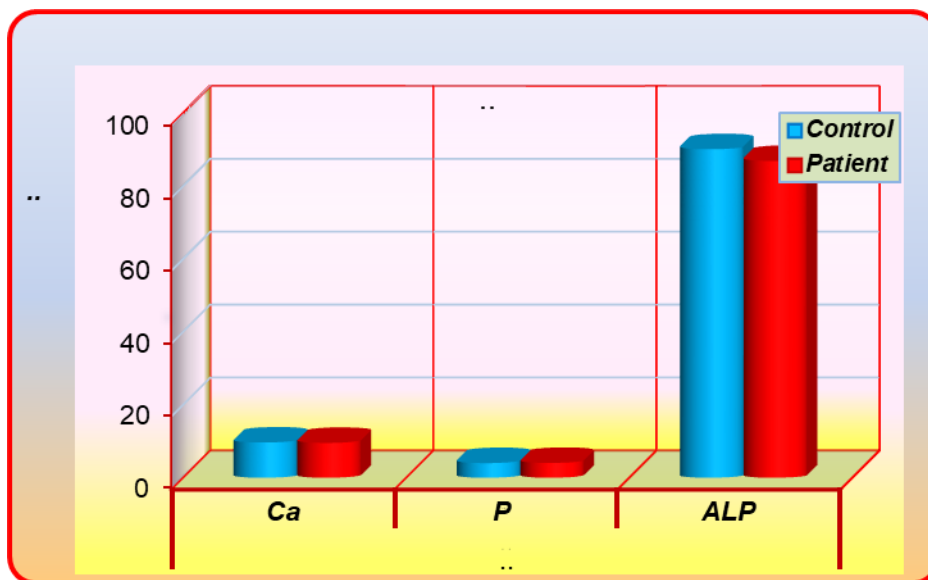
The result of T-score in the present study show a lower mean value in the patients than that in the control. These results are in line with those of a previous study [35].as well as with other works [12, 36] which reported a significantly higher T-score in healthy women compared with osteoporotic women. This indicates bone loss with age and menopause. However, T-score in this study was more related to osteoporosis.

### Biochemical parameters and osteopontin levels.

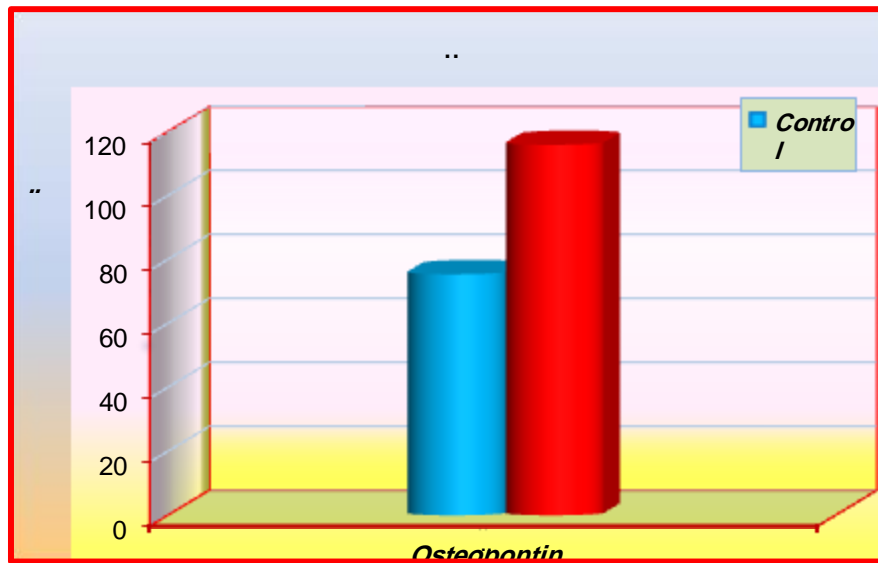
**Table 2-**Biochemical and hormonal parameters in osteoporotic menopausal women and control and there correlation coefficient

Parameters	Patient N=50	Control N=25	P-value	Significant $p \leq 0.05$	Correlation
PO <sub>4</sub> (mg/dL)	4.06±0.07	4.02 ±0.13	0.77	NS	0.25
ALP (U/L)	86.9±5.31	90.1 ±4.02	0.64	NS	0.39
Ca (mg/dl)	9.56±0.06	9.66 ±0.07	0.28	NS	-0.08
Osteopontin (ng/ml)	115.86±10.24	75.38±11.18	0.02*	S	-0.13

\*= Significant of ( $P \leq 0.05$ ), NS: Non-significant.



**Figure 2-**Serum levels of biochemical parameters in osteoporotic menopausal women and control



**Figure 3-**Serum levels of osteopontin in osteoporotic

### Menopausal women and control

The results of the present study, as shown in Table-2 and Figure-2, showed non-significant differences ( $P \leq 0.05$ ) between the patients and control group for all biochemical parameters. Although the level of serum phosphorus was higher and that of calcium was lower in the patients as compared to the control, this did not appear statistically.

As noted in Table-2 and Figure-3, a weak inverse significant ( $p \leq 0.05$ ) correlation of osteopontin hormone level was found in patients ( $115.86 \pm 10.24$  ng/ml,  $r = -0.13$ ) as compared to control ( $75.38 \pm 11.18$  ng/ml).

The results of the present study proved that there is no significant correlation in biochemical markers (P, ALP and Ca) in patients and control group. The analysis results are consistent with those of an earlier study [37], which did not find a significant correlation between the levels of serum calcium, phosphorous and alkaline phosphatase with the incident of osteoporosis.

This also agree with other reports [38, 39] which stated that non-significant results might be logical because bone formation markers are substances that are directly or indirectly produced by osteoblasts at each stage of osteoblast differentiation, given that all the participants were free from any metabolic disease. Because of multiple sources of origin, total ALP has not been widely used as a bone remodeling marker. Many investigations found that ALP is not a specific marker of bone turnover in the elderly persons because the serum pool of total ALP consists of several isozymes from various tissues, including liver, bone, intestine, kidney and placenta. In adults, about 50% of ALP activity in serum is from liver and the other 50% is from bone. Therefore, from this study and other previous studies, we can say that ALP is considered as a secondary indicator for bone loss. Also, serum calcium and phosphorous levels are tightly regulated and their homeostasis is maintained in serum regardless of their store in bone [40]

Bone turnover markers have important roles in the management of osteoporosis. Recently, the clinical application of BTMs has achieved a significant progress and the measurement of bone turnover markers provides better understanding of pathogenesis of osteoporosis [41].

Menopause and aging are correlated with accelerated loss of cortical bone, which happens when the balance between formation and resorption is deregulated and when the resorption is excessive [39]. Some studies proved that individuals with increased bone turnover markers lose their bones at a faster rate than normal people do. [42]. The explanation of these results agrees with the results of our study. Earlier studies [11, 43] found a significant increase in osteopontin level in osteoporotic postmenopausal women, which indicates that the process of bone resorption by the activity of osteoclasts is the major component of bone metabolism. According to another report [44], bone resorption markers seemed to be stronger predictors of future bone loss than markers of bone formation.

Different cell types may differ in their regulatory mechanisms of the OPN gene. OPN expression in bone occurs by osteoblasts and osteocytes, as well as osteoclasts. Hypocalcemia and hypophosphatemia which were indicated in this study, although not significantly, lead to increases in OPN transcription, translation and secretion. This is due to the presence of a high-specificity of vitamin D response in the OPN gene promoter. OPN serves to initiate the process by which osteoclasts develop their ruffled borders to begin bone resorption [45]. Also, some investigations proved that an increase in plasma OPN levels was observed in patients with idiopathic hip osteoarthritis. Furthermore, a correlation between OPN plasma levels and the severity of the disease was noted.

### Conclusion

After conducting the present study, we have reached the following conclusions:

1. Bone biomarkers hold a prospect in the diagnosis of BMI because they can be measured easily, relatively noninvasively, inexpensively and they can provide information on the status of BMI.
2. The biochemical bone markers are not useful in the diagnosis of postmenopausal osteoporosis but may have a role in monitoring the progress and response to treatment.
3. The increased serum OPN level in postmenopausal women with osteoporosis provides an evidence that determining serum OPN level can be used as a reliable marker in early diagnostic criteria of osteoporosis.
4. From the present data, we can conclude that early menopause age may represent a triggering factor of osteoporosis.

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