

**How to Cite:**

Ali, S. S., & AL-Attabi, M. R. S. (2022). Evaluation of osteocalcin and osteopontin among women with osteoporosis in Wasit province, Iraq. *International Journal of Health Sciences*, 6(S6), 7480–7486. <https://doi.org/10.53730/ijhs.v6nS6.12095>

## **Evaluation of osteocalcin and osteopontin among women with osteoporosis in Wasit province, Iraq**

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**Abstract**---Background: Osteoporosis (OP) is one of the most common metabolic diseases, characterized by low bone mass and abnormal bone microarchitecture leading to the development of fractures and subsequent disability. Disruption of bone remodeling by osteoporosis results in an imbalance between bone formation by osteoblasts and resorption by osteoclasts. Aims: This study was aimed to investigate relationship between Osteocalcin and Osteopontin, with estimating their effects on osteoporosis in women and the benefits in diagnosis. Materials and method: In this study, 50 women who were referred to DEXA unit in Al-Karama Teaching Hospital to diagnosed with Osteoporosis. They were compared with 40 healthy individuals who did not have any clinical symptoms. Blood samples were used after separating them by a centrifuge for the purpose of examining levels of vital indicators were detected by Enzyme linked immunosorbent assay (ELISA). Results: The results of the current study showed a decrease in the level of Osteocalcin, increasing in the levels of Osteopontin, with significant differences between the study groups at  $P \leq 0.0001$  compared to the control group. Conclusion: Through the study it is clear that osteoporosis is an age-related disease, Osteocalcin and Osteopontin play a role in their effect on the bones leading to fragility, and therefore can be used to detect and evaluate the disease.

**Keywords**---osteoporosis, osteocalcin, osteopontin.

## **Introduction**

Osteoporosis is a metabolic bone disease of the skeleton caused by bone metabolism disorders, and is the most common bone disease in humans (1). It is characterized by a decrease in bone mass and increased bone fragility (2, 3). The mechanism of osteoporosis is mainly the excessive bone resorption caused by the increased osteoclast, and the decreased bone formation caused by the decreased osteoblast (4). Although it is seen in all age groups, gender, and races, it is more common in Caucasians (white race), older people, and women. Studies have shown that bone loss starts from the age of 30-40 years in both men and women (5). Sex is a well-established non-modifiable factor for osteoporosis. The risk of osteoporosis is greater in women than in men and is a major health issue because the occurrence of certain types of fractures (hip, vertebra, upper humerus, pelvis, upper leg, several simultaneous and spine due to accelerated bone turnover secondary to estrogen deficiency (6, 7). With an aging population and longer life span, osteoporosis is increasingly becoming a global epidemic. The most commonly used technique for measuring bone mineral density is dual energy X-ray absorptiometry (DEXA) (8). Also various parameters are now available that allow a specific and sensitive assessment of the rate of bone formation and bone resorption of the skeleton. The biomarkers of bone metabolism are proteins, which are synthesized by the osteoblast and osteoclast and are released into the bloodstream or circulation (8). Osteocalcin and Osteopontin are among the most important of these biochemical markers.

Osteocalcin also referred to as bone Gla protein or (BGP). is a small protein a 46–50 amino acid, 5.6 kDa, is contains three glutamic acid (Glu) residues (9,10). OC synthesis is essentially specific to osteoblasts with only small amounts being produced by odontoblasts during bone formation phase of bone remodeling (11). and is excreted by the kidneys and is one of the most abundant non-collagenous proteins in bone. It is also released during bone resorption (12). OC it is a vital constituent of the bone matrix, binding with calcium to form hydroxyapatite crystals (13). Higher serum OC rates have been found to be fairly well associated with rises in BMD during osteoporosis treatment with anabolic bone formation drugs. Serum OC is used as biomarker for osteoblast function for evaluation of bone formation rate in osteoporosis (14,15).

Osteopontin is a highly phosphorylated and glycosylated sialoprotein (16). composed of 314 amino acids with a molecular weight ranges from 44 to 75 kDa (17). is produced by a variety of cell types, such as B and T cells, macrophages, neutrophils, bone cells (osteoblasts and osteocytes). (18). also produced by kidneys. Plasma osteopontin levels are elevated in many pathologic conditions, including cancer, autoimmune disease, obesity and cardiovascular disease. OPN is reportedly involved in bone resorption and bone formation (19).

## **Materials and Methods**

### **Study samples**

The study samples included 90 samples of females from different age groups ranging from (25-65) years (osteoporotic women), as they were divided into two

groups, a group of Post-menopausal osteoporosis patients from (45-65) years , and a group of Pre-menopausal osteoporosis patients from (25-45) years and compared with the control group: 40 samples of apparently healthy people were taken into account, in terms of age for the group of patients.

### Sample collection

Samples were collected from Al-Karama Teaching Hospital and private laboratories in Wasit Governorate for the period from November 2021 to January 2022 after taking information from patients and healthy people and filling out the form for each person. After the area of blood drawn was sterilized with cotton and diluted alcohol 70%, 5 ml of venous blood is withdrawn for patients and healthy people, then placed in glass tubes container on gelatin in order to separate the clotted part of the serum from the blood. Leave for 1-2 hours at room temperature for complete coagulation and gloat formation. Then the blood samples are separated using a centrifuge at 3000 rpm for (10 minutes )The serum was then transferred to test tubes (Eppendorf tubes) by micropipette and stored at (-20°C ) until biochemical tests were performed using the enzyme-linked immunosorbent assay (ELISA).

### Statistical Analysis

All obtained data were documented using the Microsoft Office Excel (2016), and analyzed statistically by the GraphPad Prism (2012) software. One-Way Analysis of Variance (ANOVA) and t-test were applied to detect significant differences between values of the measured marked as well as among different age groups. Significant differences were represented as  $P < 0.05$  (\*),  $P < 0.01$  (\*\*),  $P < 0.001$  (\*\*\*), and  $P < 0.0001$  (\*\*\*\*).

### Results

The results of the current study showed, as shown in Table (1) the Mean and Standard Deviation (SD) of the level (Osteocalcin and Osteopontin) in the study groups. The level of Osteocalcin in the patients group was  $(4.516 \pm 0.1115)$  and in the control group  $(5.7 \pm 0.1674)$ . The difference was significant ( $p < 0.0001$ ) between study groups. The level of Osteopontin in the patients group was  $(3.018 \pm 0.101)$  and  $(1.425 \pm 0.075)$  in the control group with a significant difference ( $p < 0.0001$ ).

Table 1  
Levels of Osteocalcin and Osteopontin in the study groups

Parameters	OP Patient (N=50)	Controls (N=40)
	Mean $\pm$ SD	Mean $\pm$ SD
OC (ng/ml)	4.516 $\pm$ 0.1115 B	5.7 $\pm$ 0.1674 A
OPN (ng/ml)	3.018 $\pm$ 0.101 A	1.425 $\pm$ 0.075 B
Variation in horizontal large letters refers to significant differences at $P < 0.05$ .		

Concerning age, Osteocalcin reduced significantly ( $P < 0.05$ ) in P2 comparison with the values of P1 as well as the values of C1 and C2. Also, there was a significant decrease in value of P1 when compared to those of C1 and C2. As The findings of Osteopontin Significantly, were showed an increases ( $P < 0.05$ ) among the individuals of P1 and P2 in comparison with those of C1 and C2. Also, there were significant increases in values of C2 when compared to values of C1, as well as in values of P2 when compared to those of P1. (Table 2; Figures 1 and 2).

Table 2  
Association between age and levels of Osteocalcin and Osteopontin

Parameters	P1 (N=25)	P2 (N=25)	C1 (N=20)	C2 (N=20)
OC (ng/ml)	4.824 ± 0.146 C	4.208 ± 0.147 D	5.945 ± 0.235 A	5.455 ± 0.232 B
OPN (ng/ml)	2.744 ± 0.115 C	3.292 ± 0.148D	1.515 ± 0.0419A	1.665 ± 0.0544 B
Variation in horizontal large letters refers to significant differences at $P < 0.05$ .				

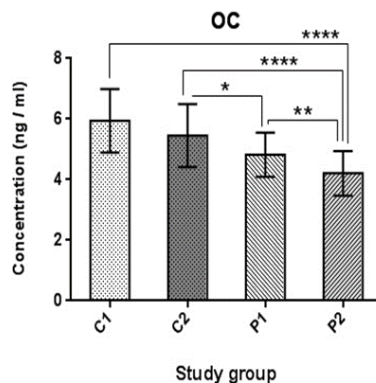


Figure 1. Association between the age and the levels of OC in control and patient groups

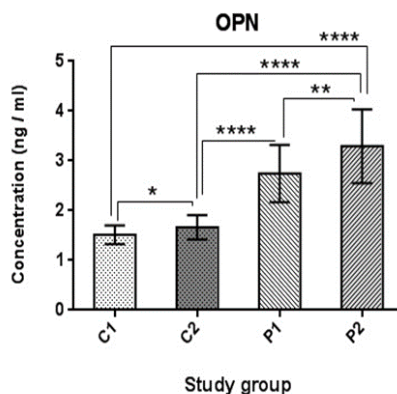


Figure 2. Association between the age and the levels of OPN in control and patient groups

## Discussion

The data of this study showed significant difference in the level of serum Osteocalcin between osteoporosis group and control group, it was found that serum osteocalcin was significantly lower in patients than in controls, this came in conformity with what previous studies have reached. statistically there is previous studies disagreed with our results, finding increase in serum osteocalcin level in patient group, such as Hamdi (20), Singh et al (21). Singh et al (21) see that in osteoporosis generally there is a deficiency of calcium level and since osteocalcin is a calcium dependent biomarker and has a strong affinity with bone matrix (hydroxyapatite) responsible for mineralization of bone. Thus, OP leads to decreased hydroxyapatite crystal formation and results increase in serum osteocalcin levels.

Since osteocalcin is produced only by osteoblasts, it is often used as a marker for the process of bone formation. Higher serum osteocalcin levels have been observed to be relatively well correlated with increased bone mineral density (BMD) during osteoporosis therapy with bone formation drugs. (22) The bone remodeling biomarker of serum OC may be useful for the assessment of osteoporosis and for the prediction of the fracture risk in elderly persons, especially in women. The current study showed significant difference in the level of serum Osteopontin between osteoporosis group and control group. the results of our study is agreement with previous studies conducted by Wei et al (23); Allah et al (24); Chan et al (25) which found a significant increase in the level of Osteopontin in relation to postmenopausal osteoporosis, thus indicating that OPN levels in plasma can be used as a vital marker for early diagnosis of osteoporosis in postmenopausal women. Some studies have shown that individuals with increased signs of bone circulation lose their bones at a faster rate than normal or low bone turnover markers (26). The increases in OPN level in plasma, which increase destructive bone reflux and reduce anabolic bone formation in postmenopausal women, this makes us suggesting that OPN may play a pivotal role in bone formation in postmenopausal women . In addition, a relationship between OPN plasma levels and the severity of the disease was observed.

## Conclusion

We concluded from this study a Positive strong correlation appears clearly in this study between age and osteoporosis and this indicate that it is an age- associated diseases. Osteocalcin and Osteopontin play a role in their effect on the bones leading to fragility, and therefore can be used to detect and evaluate the disease.

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