

# Evaluation of Serum Osteocalcin Level in Iraqi Postmenopausal Women with Primary Osteoporosis

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**Summary:**

**Background:** Osteoporosis (OP) is a chronic and a progressive disease characterized by low bone mass and micro-architectural deterioration of bone tissue, resulting in an increased risk of fracture. Osteocalcin is a non-collagenous protein synthesized and secreted by osteoblasts. Its main physiological functions are calcium ion homeostasis, maintain the normal bone mineralization rate, inhibit the abnormal formation of hydroxyapatite crystal, and to be involved in bone remodeling through a negative feedback mechanism.

**Objective:** This study was planned to evaluate serum level of bone formation marker osteocalcin (OC) in postmenopausal women with and without primary osteoporosis; and study the correlation between serum osteocalcin level with women's age.

**Subjects and Methods:** Forty four (44) postmenopausal women were included in this study with age range (51-68 years). Subjects were divided into two groups: group A: twenty three (23) women with primary osteoporosis and group B: twenty one (21) women without primary osteoporosis (serve as controls). Patients were diagnosed as osteoporosis and controls as normal by measuring bone mineral density (BMD), using dual energy x-ray absorptiometry (DXA). In addition, serum calcium, phosphorous and alkaline phosphatase measured by spectrophotometer, while serum osteocalcin measured by enzyme linked immuno sorbent assay (ELISA).

**Results:** Mean serum osteocalcin level in postmenopausal women with primary osteoporosis was significantly higher than controls ( $P < 0.0001$ ). Moreover, a positive correlation between serum osteocalcin level with age for both patients ( $r = 0.86$ ,  $P < 0.0001$ ) and controls ( $r = 0.71$ ,  $P < 0.001$ ).

**Conclusion:** Increasing serum osteocalcin level in postmenopausal women with osteoporosis plays an important role in development of primary osteoporosis.

**Keywords:** Osteoporosis, postmenopausal women, osteocalcin.

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**Introduction:**

Osteoporosis (OP) is a chronic and a progressive disease characterized by low bone mass and micro-architectural deterioration of bone tissue, resulting in an increased risk of fracture (1).

Osteoporosis occurs three times in women more than in men because women have a lower peak bone mass and hormonal changes that occur at the menopause. Estrogens have an important function in preserving bone mass during adulthood, and bone loss occurs as levels decline (2).

Osteocalcin (OC) also known as bone gamma-carboxy glutamic acid-containing protein (BGLAP), is 49 residue polypeptide with 5.8 kDa. In humans, the osteocalcin is encoded by the BGLAP gene (3).

Osteocalcin is a non-collagenous protein synthesized and secreted by osteoblasts. Its main physiological functions are calcium ion homeostasis, maintain the normal bone mineralization rate, inhibit the abnormal formation of hydroxyapatite crystal, and to be involved in bone remodeling through a negative feedback mechanism (4). Osteocalcin has a high affinity for calcium and exhibits a compact calcium

dependent  $\alpha$  helical conformation, in which the gamma-carboxy glutamic acid (Gla) residues binds and promote absorption to hydroxyapatite in bone matrix, in this way mineralization of bone takes place (5).

**Subjects and Methods:**

Forty four (44) postmenopausal women were included in this study with age range (51-68 years); Menopausal status was defined by the absence of menses for more than one year in a woman 50 years of age and over. All women attended to Rheumatology and Rehabilitation outpatient clinic in Baghdad Teaching Hospital during the period from July 2012 to September 2012. Subjects were divided into two groups: group A: twenty three (23) women with primary osteoporosis and group B: twenty one (21) women without primary osteoporosis (serve as controls).

Patients were diagnosed as osteoporosis and controls as normal by measuring bone mineral density (BMD), using dual energy x-ray absorptiometry (DXA) according to World Health Organization (WHO) diagnostic guidelines:

- T-score  $\geq -1.0$  is "normal".
- T-score between  $-1.0$  and  $-2.5$  is "osteopenia".

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•T-score -2.5 or below is “osteoporosis” (6).

Serum investigations included calcium, phosphorous and alkaline phosphatase measured by spectrophotometer, all these parameters were normal in controls and patients with primary osteoporosis to distinguish osteoporosis from other metabolic bone disease such as (Osteomalacia and Paget disease). Also serum osteocalcin measured by enzyme linked immuno sorbent assay (ELISA) using kit manufactured by (SIGMA , USA).

Regarding selection of subjects; both patients and controls with the following criteria were excluded from study:

- 1) Alcoholic.
- 2) Smoker.
- 3) They had history of disease that is known to affect bone metabolism such as:
  - Endocrine disorders: Including Cushing’s syndrome, hyperparathyroidism, thyrotoxicosis and diabetes mellitus.
  - Gastrointestinal tract diseases: Including ulcerative colitis, celiac disease and inflammatory bowel disease.
  - Liver diseases
  - Renal disease.
  - Hematologic disorders: Including multiple myeloma, mastocytosis, lymphoma and leukemia, etc...
  - Inherited disorders: including osteogenesis imperfecta, Marfan’s syndrome, hemochromatosis, etc...
  - Rheumatoid Arthritis and Ankylosing Spondylitis.

**Table (1): Mean value of age, BMI, serum calcium, phosphorous, alkaline phosphatase and osteocalcin levels for patients and controls.**

Parameters	Patients n=23		Controls n=21		P value
	Mean±SD	Range	Mean±SD	Range	
Age (years)	57.82±4.88	(51-68)	56.66±3.43	(52-65)	0.37 (NS)
BMI ( Kg/m <sup>2</sup> )	26.95±5.44	(20.7-40.1)	28.85±4.32	(20.3-36.6)	0.2 (NS)
Serum Calcium Normal (2.1-2.6 mmol/L)	2.19±0.11	(2-2.4)	2.24±0.14	(2.1-2.5)	0.14 (NS)
Serum Phosphorus Normal (0.8-1.6 mmol/L)	1.13±0.19	(0.9-1.5)	1.15±0.17	(0.9-1.5)	0.75 (NS)
Serum Alkaline phosphatase Normal (21-92 U/L)	65.43±12.91	(44-85)	64.28±13.5	(40-85)	0.77(NS)
Serum Osteocalcin Normal (15-45 ng/ml)	30.63±2.13	(28.2-37.1)	22.30±1.77	(19.7-24.5)	< 0.0001(S)

S= significant, NS= non-significant

4) They were taking medication known to affect bone turnover such as (Steroid therapy, thyroxine, heparin, barbiturates, phenytoin and Thiazolidinediones).

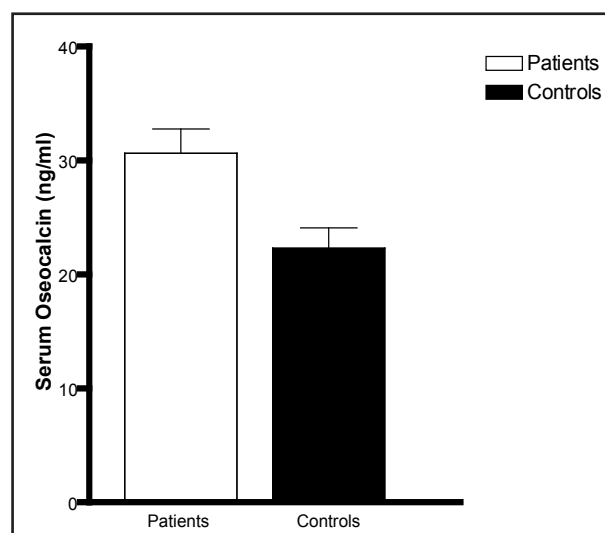
Statistical analysis:

Data were analyzed using graph pad prism version 3.02. The results were expressed as numbers, range and mean ± SD (standard deviation). Significance of difference was assessed using Student-t test for two independent means.

Correlation and regression were applied for the relationship between two quantitative variables, taking P≤0.05 lowest limit of significance.

**Results:**

Patients and controls were matching for age and body mass index (BMI), no significant difference in serum calcium, phosphorous and alkaline phosphatase levels were observed between patients and controls , however serum osteocalcin level was significantly elevated in patients compared to that of controls (table 1).



**Figure (1): Mean serum osteocalcin level for patients and controls.**

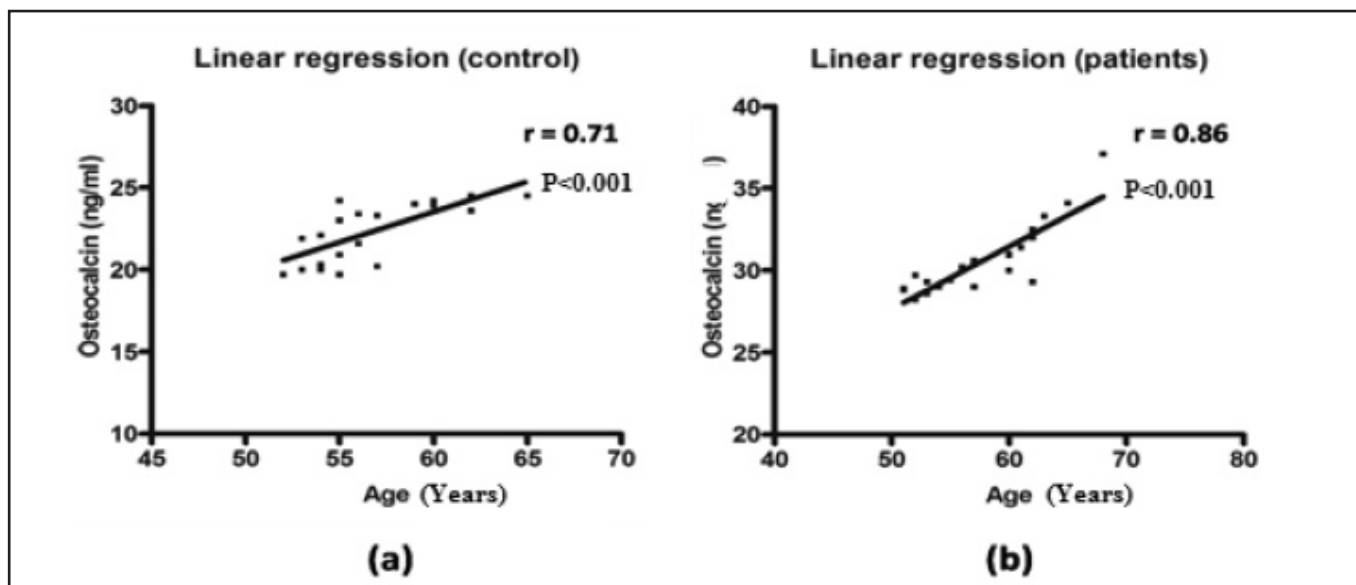


Figure (2): Correlation between serum osteocalcin level and women's age  
(a) Controls with  $r = 0.71$ ,  $P < 0.001$ , (b) Patients with  $r = 0.86$ ,  $P < 0.0001$ .

#### Discussion:

Biochemical markers represent the molecules directly connected to both the structure and function of bone tissue. The fact that changes in either the concentration or activity of these biochemical markers are reflecting dynamic status of bone metabolism. Markers of bone turnover, subdivided into markers of bone formation and bone disintegration, these markers are influenced by age, sex and menopausal status. Osteocalcin may be useful as a biochemical indicator of bone turnover; it has affinity for bone mineral constituents imply a role in bone formation (7).

This study revealed that serum osteocalcin level was significantly higher in postmenopausal women with primary osteoporosis comparing to controls. This result consists with a previous study which found that in osteoporotic women, deficiency of calcium and phosphorus in bone may lead to lowering of formation of hydroxyapatite crystals. Thus, in the state of decreased rate of bone mineralization, free osteocalcin may be available for circulation in the blood. This may explain the increased concentration of osteocalcin in the serum of osteoporotic postmenopausal women (5).

Another study showed that osteocalcin content in the blood of post-menopausal women with osteoporosis is significantly higher than that in osteopenia group and normal group; this explains that elevated level of serum osteocalcin may be associated with increased activity of osteoblasts, serum OC is considered a specific marker of osteoblast function, as its levels have been shown to correlate with bone formation rates. However, since it is also released from bone matrix during bone resorption, it reflects the overall turnover of bone and is considered as a bone turnover marker (8).

Pino et al. (9). found that osteocalcin is a promising marker of bone turnover and useful in the diagnosis and follow-up of

high turnover osteoporosis. Similar observations were reported by a number of other studies Rosenquist et al. (10), Cabrera et al. (11) and Verit et al. (12).

Further studies support these results; Garnero et al suggested that high levels of bone formation markers are associated with a greater bone loss (13). Stepan noted that markers of bone metabolism reflect the whole-body rates of bone formation and resorption and may therefore reliably predict the imbalance in bone turnover and the rate of bone loss (14).

Regarding the correlation between serum osteocalcin level and age for both osteoporotic women and controls, result of this study found that serum osteocalcin level was positively correlated with age for both groups similar to other published studies (9, 15–18) which showed increase serum osteocalcin level with aging.

The data of this study showed no significant changes in serum calcium, phosphorus and alkaline phosphatase (ALP) levels between patients and controls, these results agree with previous study which suggested that osteoblasts are rich in ALP, it is also associated with the plasma membrane of the cell in the liver, intestine and placenta, all of which may contribute to the total amount of ALP. Because of multiple sources of origin, total ALP has not enjoyed wide spread use as a bone-remodeling marker. Also serum calcium and phosphorous levels are tightly regulated and homeostasis is maintained in serum regardless of their store in bone (19).

#### Conclusion:

Increasing serum osteocalcin level in postmenopausal women with osteoporosis provide evidence that it plays an important role in development of primary osteoporosis thus determining serum osteocalcin may be used as a marker in diagnostic criteria of primary osteoporosis.

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