

## Serum osteocalcin levels in postmenopausal osteoporotic women receiving alendronate

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Osteoporosis is characterized by low bone mass, changes in the microscopic structure of bone, and increased bone fragility. Monitoring response to treatment is critical for the appropriate management of osteoporosis. Serum osteocalcin has become known as a bone formation biomarker for the evaluation of treatment response in postmenopausal osteoporosis. The present study assessed the effect of alendronate on serum osteocalcin levels in patients with postmenopausal osteoporosis. Thirty-four women with postmenopausal osteoporosis diagnosed by DEXA received alendronate 10 mg/day for 3 months. Serum osteocalcin, calcium, phosphorus, and alkaline phosphatase (ALP) were measured at baseline and after 45 and 90 days. Mean age and duration of menopause were  $57.91 \pm 7.68$  and  $9.37 \pm 8.43$  years, respectively. Mean serum osteocalcin at baseline was  $15.27 \pm 3.7$  ng/ml, which decreased significantly after 45 and 90 days of treatment with alendronate ( $p=0.009$  and  $p<0.001$ , respectively). The levels of serum osteocalcin at days 45 and 90 in patients aged  $< 60$  and  $\geq 60$  years were not significantly different. The levels of serum osteocalcin at days 45 and 90 in patients with a menopause duration of  $< 5$  and  $\geq 5$  years were not significantly different. No significant difference was observed in serum osteocalcin levels at days 45 and 90 in patients with osteoporosis only in the femoral neck ( $n=7$ ), only in the lumbar spine ( $n=20$ ), and in both femoral neck and lumbar spine ( $n=7$ ). Measurement of serum osteocalcin is a less expensive, more available tool for monitoring the results of treatment in osteoporotic patients. It provides a practical suggestion about the effectiveness of treatment earlier than densitometry. As expected, osteocalcin levels decreased after treatment of osteoporosis.

**Keywords:** alendronate, osteocalcin, osteoporosis.

### Introduction

Osteoporosis is the most prevalent skeletal disorder characterized by low bone mass, changes in the microscopic structure of bone, and increased bone fracture. Osteoporotic fractures most commonly happen in the wrist, hip, pelvis, and spine [1]. Osteoporosis is the second most common healthcare problem in the world after cardiovascular disease [2]. Osteoporosis mainly affects women after the menopause [1]. It has been suggested that menopause is associated with bone formation, resorption imbalance, and finally bone loss [3,4]. The prevalence of osteoporosis and its associated complications is increasing with life expectancy and may become an important health concern. Based on a meta-analysis, 17% and 35% of Iran's general population aged more than 30 years have osteoporosis and osteopenia, respectively [1].

Currently, there are many medications for the treatment of osteoporosis [5,6]. Alendronate, which is a

second-generation bisphosphonate, has been used to treat osteoporosis for many years. The therapeutic effect of alendronate is through the direct inhibition of osteoclasts [7]. According to Black et al. [8], an increase of about 6% in bone mineral density (BMD) and a decrease of 50% in vertebral fracture rate were observed after a three-year treatment with alendronate [8]. Interestingly, the BMD increment was 32% more in the lumbar spine than in the hip, but the decrease in fracture risk at both areas was approximately the same [8].

Monitoring response to treatment is critical for the appropriate management of osteoporosis. DEXA is a valuable tool for the diagnosis and monitoring of response to treatment in osteoporosis; however, despite its importance, its value is limited because of the time it takes to assess the effect of treatment with DEXA [9]. To overcome this problem, the measuring of biochemical markers of bone formation and degradation has been suggested. Serum osteocalcin has emerged as a promising bone formation biomarker for the evaluation

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of response to treatment in postmenopausal osteoporosis. Osteocalcin is produced by osteoblasts during bone formation [7,10], and it binds to the  $\alpha$ -carboxyglutamic acid (Gla) residues because of its high affinity for calcium. This phenomenon promotes the absorption of calcium to the hydroxyapatite in bone matrix and leads to the mineralization of bone. Decreases in bone mineralization in osteoporotic postmenopausal females causes free osteocalcin to circulate in the blood [11]. Previous studies have indicated the prognostic value of osteocalcin for the evaluation of postmenopausal osteoporosis [12-15]. Jagtap et al. [11] reported a significant decrease in serum osteocalcin levels after alendronate treatment in women with postmenopausal osteoporosis. In another study on postmenopausal osteoporotic women [16], alendronate led to a significant decrease in serum osteocalcin levels compared to baseline and the placebo group. Moreover, Aonuma et al. [17], Sarioglu et al. [18], and Tascioglu et al. [19] indicated that treatment with alendronate led to a significant decrease in serum osteocalcin levels in postmenopausal osteoporotic women. The objective of the present study was to assess the effect of alendronate on serum osteocalcin levels in patients with postmenopausal osteoporosis.

### Materials and Methods

In a before-and-after investigation, 34 postmenopausal osteoporotic women were recruited from the rheumatology clinic of AJA University of Medical Sciences (simple selection of samples based on number of most previous studies). Considering a confidence level of 95% and power of 80%, the sample was determined with at least 25 cases. The sample size was increased to 34 cases after considering a possible dropout rate of 20%. Inclusion criteria were having an age of 45-70 years and T-score values  $\leq -2.5$  at the lumbar spine and/or neck of femur [20] measured by DEXA [(Bone mineral densitometry (BMD) for each patient was performed at baseline by dual-energy x-ray absorptiometry (Hologic QDR Explorer machine; Hologic, Inc.)]. Exclusion criteria were contraindications for treatment with alendronate, having secondary causes of osteoporosis, using medications that affect bone metabolism, suffering from any metabolic bone diseases, and having a history of hormone replacement therapy. Informed consent was gained from all patients before entering the study. All patients received alendronate 10 mg/day for 3 months (Osteofos 10mg-Cipla-India). Patients were visited at

baseline, 45 and 90 days after therapy initiation. Five mL samples of blood were obtained after overnight fasting in each visit. Serum osteocalcin was measured with platinum enzyme-linked immunosorbent assay (ELISA) kits (BioSource hOST-EASIA Kit, BioSource Europe S.A. Rue de l'Industrie, 8, B-1400 Nivelles, Belgium). Serum calcium, phosphorus, and alkaline phosphatase (ALP) were determined by Auto-Analyzer (Abbott Park, Illinois, USA).

### Statistical analysis

SPSS version 16.0 software (SPSS, Inc, Chicago, IL, USA) was used for statistical analysis. Normality was evaluated using the Kolmogorov-Smirnov test. Repeated measures analysis of variance was used to assess changes throughout the study, and assumptions of this analysis (e.g., homogeneity of variances and sphericity) were checked. Correlations were determined using Pearson's correlation coefficient, and  $p < 0.05$  was considered statistically significant.

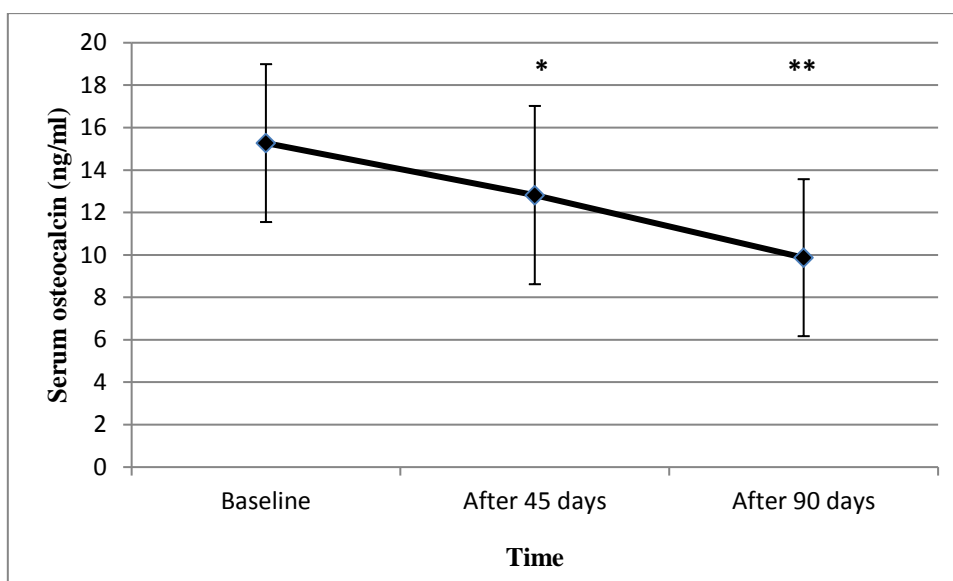
### Results

Thirty-four postmenopausal osteoporotic women entered this study, and all of them finished the study. Table 1 presents the demographic and clinical features of the participants.

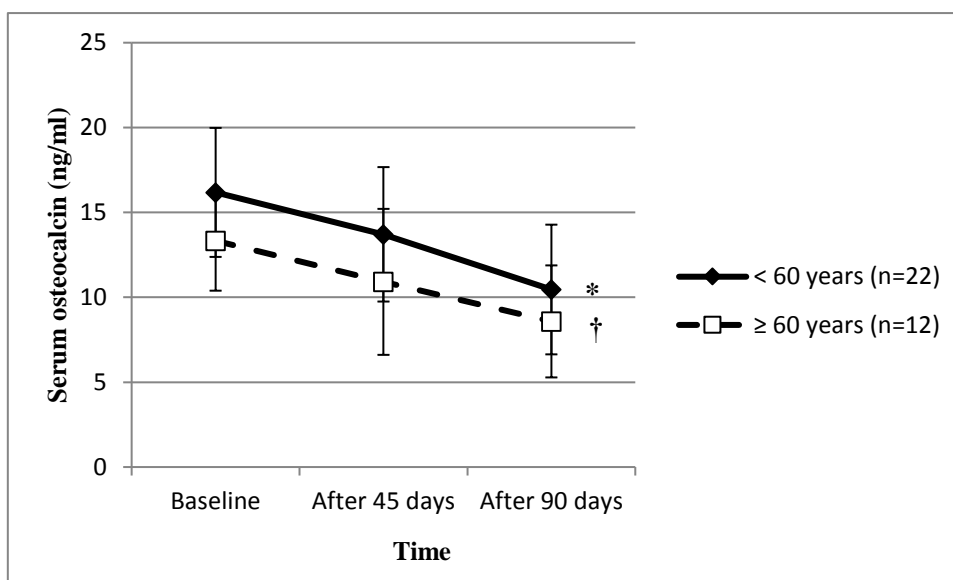
**Table 1.** Baseline characteristics of the studied patients (n=34)

Characteristics	Mean $\pm$ SD
Age (years)	57.91 $\pm$ 7.7
Menopausal duration (years)	9.37 $\pm$ 8.4
T-score	
Femoral neck	-2.15 $\pm$ 0.8
Lumbar spine	-2.78 $\pm$ 0.7
Serum calcium (mg/dl)	9.40 $\pm$ 0.5
Serum phosphorous (mg/dl)	3.77 $\pm$ 0.5
Serum alkaline phosphatase (mg/dl)	177.64 $\pm$ 70.9

Figure 1 presents the serum osteocalcin levels of before and after treatment with alendronate. Mean serum osteocalcin at baseline was 15.27 $\pm$ 3.7 ng/ml which decreased significantly after 45 and 90 days of treatment with alendronate ( $p=0.009$  and  $p<0.001$ , respectively). In patients aged  $< 60$  and  $\geq 60$  years, mean serum osteocalcin at baseline was 16.18 $\pm$ 3.8 ng/ml and 13.33 $\pm$ 2.95 ng/ml, respectively (Fig. 2). Serum osteocalcin levels decreased significantly in both groups after 45 and 90 days of treatment with alendronate ( $p<0.001$  and  $p=0.001$ , respectively), but the difference between the 2 groups was not significant (Fig. 2).



**Fig. 1.** Effect of alendronate treatment on serum osteocalcin levels (n=34)  
\* $p=0.009$  and \*\* $p<0.001$  based on the repeated-measures analysis of variance



**Fig. 2.** Serum osteocalcin before and after treatment with alendronate in postmenopausal osteoporosis women with respect to the age group  
\* $p<0.001$  and † $p=0.001$  compared to baseline

Furthermore, mean serum osteocalcin levels were  $15.53\pm 4.5$  ng/ml and  $15.15\pm 3.5$  ng/ml in women with a menopause duration of  $< 5$  and  $\geq 5$  years, respectively (Fig. 3). The decline in serum osteocalcin levels in both groups after 45 and 90 days of treatment with alendronate was significant ( $p=0.002$  and  $p<0.001$ , respectively), but the difference between the 2 groups was not significant (Fig. 3). In patients with osteoporosis only in the femoral neck (n=7), only in the

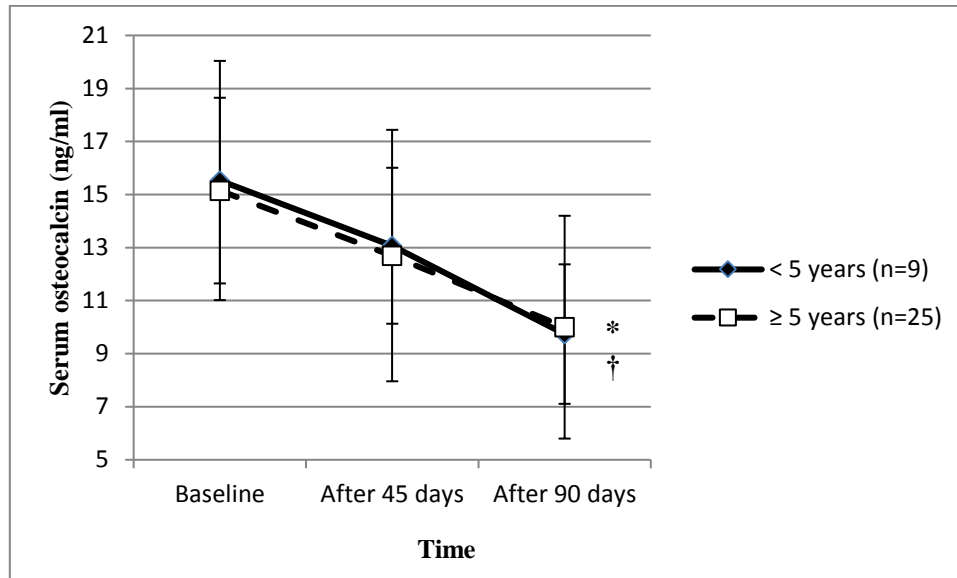
lumbar spine (n=20), and in both the femoral neck and the lumbar spine (n=7), mean serum osteocalcin levels at baseline were  $14.76\pm 1.4$  ng/ml,  $16.35\pm 4.3$  ng/ml, and  $13.20\pm 3.3$  ng/ml, respectively. The decrease in serum osteocalcin levels after 45 and 90 days of treatment in all 3 groups was significant ( $p=0.007$ ,  $p<0.001$ , and  $p=0.019$ , respectively), but the difference between the groups was not significant (Fig. 4).

As indicated in Table 2, the mean baseline serum

Postmenopausal osteoporotic women

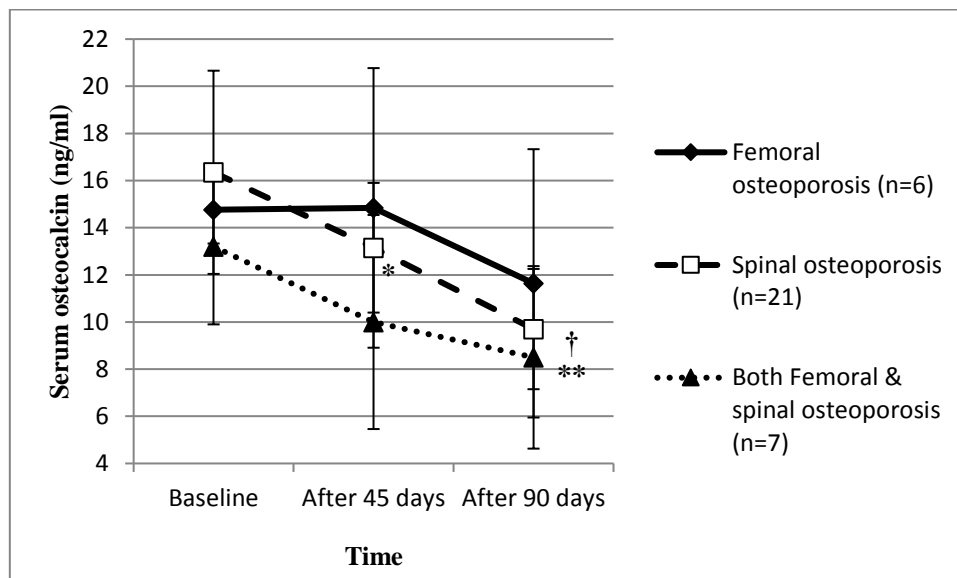
osteocalcin levels did not correlate significantly with serum calcium, phosphorous, ALP, or BMD. Furthermore, correlations between changes in serum

osteocalcin level and serum calcium, phosphorous, and ALP were not significant.



**Fig. 3.** Serum osteocalcin before and after treatment with alendronate in postmenopausal osteoporosis women with respect to the menopausal duration

\* $p=0.002$  and † $p<0.001$  compared to baseline



**Fig. 4.** Serum osteocalcin before and after treatment with alendronate in postmenopausal osteoporosis women with respect to the osteoporosis

\* $p=0.007$ , † $p<0.001$  and \*\* $p=0.019$  compared to baseline

**Table 2.** Correlation between baseline levels of serum osteocalcin with serum calcium, phosphorous, alkaline phosphatase and BMD (n=34)

	Osteocalcin (ng/ml)	
	r	P*
Calcium (mg/dl)	-0.038	0.864
Phosphorous (mg/dl)	0.012	0.955
Alkaline phosphatase (mg/dl)	0.260	0.242
BMD (g/cm <sup>2</sup> )		
Femoral neck	0.175	0.321
Lumbar spine	0.111	0.532

\* Pearson's correlation coefficient

## Discussion

Based on the results of the current study, three months of treatment with alendronate was effective in reducing serum osteocalcin levels in postmenopausal osteoporotic women. Mean baseline serum osteocalcin levels did not correlate significantly with serum calcium, phosphorous, ALP, or BMD. Furthermore, correlations between changes in serum osteocalcin level and serum calcium, phosphorous, and ALP after 90 days of alendronate treatment were not significant. No correlation was observed between age, menopause duration, osteoporosis site, and changes in serum osteocalcin levels during treatment with alendronate.

Jagtap et al. [11] reported a significant decrease in serum osteocalcin levels after treatment with alendronate (70 mg/week for 3 months) in women with postmenopausal osteoporosis. In another study on postmenopausal osteoporotic women [16], 20 mg alendronate once a week for 3 months led to a significant decrease in serum osteocalcin levels compared to baseline and the placebo group. Consistent with the current study, Aonuma et al. [17] indicated that treatment with alendronate 5 mg/day for durations of both < and  $\geq 6$  months led to a significant decrease in serum osteocalcin levels in postmenopausal osteoporotic women. Moreover, Hirao et al. [21] demonstrated that alendronate 5 mg/day for 12 months decreased serum undercarboxylated osteocalcin significantly. In Sarioglu et al. [18] and Tascioglu et al. [19], treatment with alendronate (70 mg/week for 12 months and 10 mg/day for 24 months, respectively) led to a significant reduction in serum osteocalcin levels in postmenopausal osteoporotic women. Results of the current study were also consistent with those of Johannes et al. [22], Ones et al. [23], Sambrook et al. [24], and Ivaska et al. [25]. Consistent with the current study, Aonuma et al. [17] stated no significant correlation between BMD and changes in serum

osteocalcin levels after treatment with alendronate. Furthermore, Choi et al. [16] did not show a significant correlation between baseline lumbar spine BMD and changes in bone turnover markers after treatment with alendronate. In another study by Min et al. [26], baseline osteocalcin concentrations as well as changes after 3 and 6 months of therapy did not correlate with BMD after the study. It has been indicated that bone turnover markers may be useful when BMD changes are too small [27]. During antiresorptive therapy, short-term changes in bone turnover markers are related to long-term changes in BMD [28-30]. Moreover, greater reductions in bone turnover immediately after the initiation of treatment are associated with fewer bone fractures in the future [31, 32]. It has been indicated that alendronate regulates ephrinB1 gene expression in osteoclasts, which interacts with EphB1 or B3 receptors on osteoblasts to inhibit osteoblast function; therefore, it suppresses osteoblast differentiation indirectly. Furthermore, alendronate may affect osteoclast precursors, which then act on osteoblast precursors in bone marrow cells through ephrinB1-EphB interactions [33].

In the present research, the sample size was small and the duration of treatment with alendronate was short; these could be considered as limitations of the study. Furthermore, fracture risk was not evaluated.

## Conclusion

In conclusion, short-term prospective determinations of biochemical markers of bone turnover can be used in assessing early response to therapy, monitoring alendronate treatment, and predicting long-term change in bone mass. Verification of the study data by large-scaled longitudinal studies and the use of other bone formations and resorption markers plus confirmation of treatment response with BMD in one or two years will lead to better utilization of selected biochemical markers to guide practice for postmenopausal women with osteoporosis.

## Conflicts of interest

The authors declare no conflict of interest.

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