

## The Beneficial Role of Some Bone Markers in Evaluating Women with Osteoporosis under Different Therapeutic Regimens

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### Abstract

Osteoporosis is a systemic disease of the skeleton, characterized by low bone mass and alteration in the micro-architecture of the bone tissue that lead to an increase in brittleness with the ensuing predisposition to bone fracture. Global statistics shows that women are more exposed to this disease than men and in particular at menopause. This study was designed to evaluate the use of some bone markers: serum osteocalcin (Ost), alkaline phosphatase (ALP), as bone formation markers, also parathyroid hormone (PTH), calcium and inorganic phosphate level, for the assessment of patients with osteoporosis and to evaluate their role in monitoring of several types of therapeutic interventions (such as bisphosphonates, hormonal replacement therapy, and Ca and vit.D) in postmenopausal women. This study comprised of 36 women (age  $51.67 \pm 5.14$  years) those diagnosed to have osteoporosis, to be allocated randomly into three groups according to the type of therapy to be given as;

**group A:** received bisphosphonates (sodium alendronate 10mg/day) for twelve weeks (N=12).

**group B:** treated with hormonal replacement therapy (conjugated estrogen 0.625mg/day) for twelve weeks (N=12).

**group C:** received Ca and vit. D (Ca1500mg/day and cholecalciferol 1000IU/day) for twelve weeks (N=12).

In addition to 15 perimenopausal healthy women to serve as a control group (age  $51.13 \pm 7.62$  years). The studied parameters were measured in serum obtained before starting treatment and after 12 weeks of therapy. Result indicated that the baseline values of both serum Ost and ALP were significantly higher in postmenopausal patients as compared to controls and serum Ost showed a significant reduction after treatment with alendronate compared to those treated with either HRT or Ca and vit. D. From this study we recommend estimating the baseline bone markers (Ost and ALP) status for newly diagnosed osteoporotic patients to be used as a guide for deciding the initial therapeutic intervention, and detection of non responder instead of waiting until patients develop further fracture while they are on therapy.

**Key words:** Osteoporosis, Postmenopause, Osteocalcin, Alendronate

### الخلاصة

يعتبر مرض هشاشة العظام "ترقق العظام" من الأمراض التي تصيب الهيكل العظمي وتسبب خللاً في النسيج العظمي مما يؤدي لنقص في كثافة العظام وتغيرات دقيقة في النسيج العظمي والتي تؤدي إلى زيادة هشاشة العظام وزيادة احتمالية التعرض لكسر العظام. وتدل الإحصائيات العالمية إن معدل إصابة النساء بهذا المرض أكثر من الرجال خاصة بعد سن اليأس. يهدف البحث إلى تقييم فعالية استخدام بعض مؤشرات العظام في تشخيص المرض منها الاستيوكلسين ومحلل الفوسفات القاعدي كمؤشرات لبناء العظام. إضافة إلى قياس مستوى هرمون الباراثيرويد إلى جانب الكالسيوم والفوسفات اللاعضوية وفي متابعة المرض باستخدام علاجات مختلفة مثل الفوسفونات الثنائية وعلاج الهرمونات التعويضي والكالسيوم وفيتامين دي. تضمنت الدراسة 36 من النساء اللاتي يعانين من مرض هشاشة العظام وتم تقسيمهن إلى ثلاثة مجاميع وفقاً للعلاج الذي أعطي لهن لمدة اثنا عشر أسبوعاً. (بمعدل أعمار  $51.67 \pm 5.14$  سنة).

**المجموعة أ-** أعطيت عقار الاندرونيت 10 ملغم/اليوم لمدة اثنا عشر أسبوعاً وتضمنت 12 مريضة.

**المجموعة ب-** أعطيت علاج الهرمونات التعويضي من الاستروجين (0.625 ملغم/اليوم) لمدة اثنا عشر أسبوعاً وتضمنت 12 مريضة.

**المجموعة ج-** أعطيت الكالسيوم مع فيتامين دي (1500 ملغم و 1000 وحدة دولية يومياً) وتضمنت 12 مريضة. إضافة إلى خمسة عشر من النساء الأصحاء كمجموعة ضابطة بمعدل عمر ( $51.13 \pm 7.62$  سنة). تم قياس المؤشرات قيد الدراسة في مصل المريضة قبل وبعد بدء العلاج بأثنا عشر أسبوعاً. أظهرت النتائج بان المستويات البدائية للاوستيوكلسين ومحلل الفوسفات القاعدي في المصل كانت مرتفعة في النساء في سن انقطاع الطمث والمصابات بترقق العظام مقارنة بمجموعة الضابطة وانخفض مستوى الاوستيوكلسين في مصل المريضة بعد علاجهن بالاندرونيت. من نتائج الدراسة يمكننا التوصية بتقييم مستوى مؤشرات العظام للمريضات حديثات التشخيص بترقق العظام لتكون دليلاً على اختيار العلاج المناسب وكذلك للكشف عن عدم الاستجابة بدلاً من الانتظار لحدوث الكسور خلال استمرارهم على العلاج.

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## Introduction

Bone consists of an extracellular matrix, the organic phase of which is composed of type I collagen, proteoglycans, and noncollagenous proteins including: osteocalcin, bone sialoprotein, osteonectin, thrombospondin, and osteopontin. Bone matrix also contains growth factors and cytokines that have an important regulatory role in bone remodeling<sup>(1)</sup>. Bone modeling prevents the occurrence of damage by adapting bone structure-and hence strength-to its loading circumstances<sup>(2)</sup>. Bone in the adult skeleton is renewed continuously in response to a variety of stimuli by the process of bone remodeling. This involves removal of trenches or tunnels of bone from the surfaces of trabecular and cortical bone, respectively, by osteoclasts (bone resorbing cell) and osteoblasts that subsequently fill in these trenches by laying down new bone matrix in them<sup>(3)</sup>. In normal adult bone, the processes of resorption and formation are coupled both in space and time, thus bone resorption always precedes formation<sup>(4)</sup>. Bone loss is the result of an imbalance in bone turnover with bone resorption occurring at a faster rate than new bone formation<sup>(5)</sup>. Osteoporosis most commonly affects postmenopausal women, placing them at a significant risk of fracture. Hip fractures are important causes of mortality and morbidity among postmenopausal women. Approximately 20% of patients with hip fractures die within a year, most of the deaths occurring within the first 6 months after a fracture. Among the survivors, 30%-50% never regain their prefracture functional status<sup>(6)</sup>. Within geriatric population in most countries, disorders of bone and mineral metabolism are becoming increasingly relevant to every day clinical practice. Consequently, the interest in, and the need for effective measures to be used in the screening, diagnosis and follow-up of such pathologies has markedly grown. Together with the clinical and imaging techniques, biochemical tests could play an important role in both the assessment and differential diagnosis of osteoporosis<sup>(7)</sup>, but yet have not been practically used in osteoporosis<sup>(8)</sup>. In recent years, a panel of new simple to use kits to determine bone markers had become available for the diagnosis, monitoring, of osteoporosis, and other metabolic diseases of bone tissue<sup>(9)</sup>.

This study was designed to evaluate:

1. The role of some biochemical bone formation markers, such as, Osteocalcin, Parathyroid hormone, Alkaline phosphate, serum Calcium and serum

Inorganic phosphatase, in the assessment of osteoporosis status in postmenopausal women.

2. The effect of three therapeutic interventions applied in osteoporosis (i.e. Bisphosphonates, supplement of vit. D and Ca and hormonal replacement therapy) on the studied bone formation parameters after 12 weeks of starting their therapies.

## Subjects and Methods

This study comprised 36 postmenopausal women with newly diagnosed osteoporosis by a senior physician based on (x-ray and sign and symptoms), according to WHO diagnostic criteria for osteoporosis<sup>(10)</sup> with a mean age of  $51.67 \pm 5.14$  years. They were classified into three main groups to be treated for twelve weeks as follows:

**GROUP A;** include 12 patients to be treated with alendronate 10 mg/day.

**GROUP B:** include 12 patients to be treated with hormone replacement therapy (HRT) (conjugated estrogen 0.625mg /day).

**GROUP C:** include 12 patients to be treated with Ca and vit .D (Ca1500 mg and cholecalciferol 1000IU /day).

In addition to a control group of 15 perimenopausal of apparently healthy women with a mean age of  $51.13 \pm 7.62$  years.

A fasting 5 ml venous blood sample was withdrawn from each subject before and twelve weeks after treatment. Serum was separated and divided into aliquots to be used for immediate determination of total ALP<sup>(11)</sup>. Other aliquots were stored frozen (-20°C) for determination of osteocalcin<sup>(12)</sup>, PTH<sup>(13)</sup>, serum total Ca<sup>(14)</sup>, and serum inorganic phosphate levels<sup>(15)</sup>. Statistical analysis was performed using Microsoft excel 2007 in order to estimate mean  $\pm$  SEM, as well as, the estimation of paired t-test values, considering  $p < 0.05$  to be significant.

## Results

### 1. Effect of treatment with either alendronate, HRT, or calcium and vitamin D on serum level of osteocalcin in women with postmenopausal osteoporosis.

Figure (1) showed that the Serum levels of osteocalcin in osteoporotic women were significantly elevated ( $p < 0.05$ ) as compared to controls by 29.18% ,19.33% ,and 40.33%, respectively in the three groups A,B and C .There was a significant reduction ( $P < 0.05$ ) in serum osteocalcin levels after treatment with

alendronate by -33.03%, in comparison with corresponding values before treatment. Meanwhile, alendronate administration induced a significant decrease in serum osteocalcin in comparison with HRT, and (Ca and vit. D) treated groups.

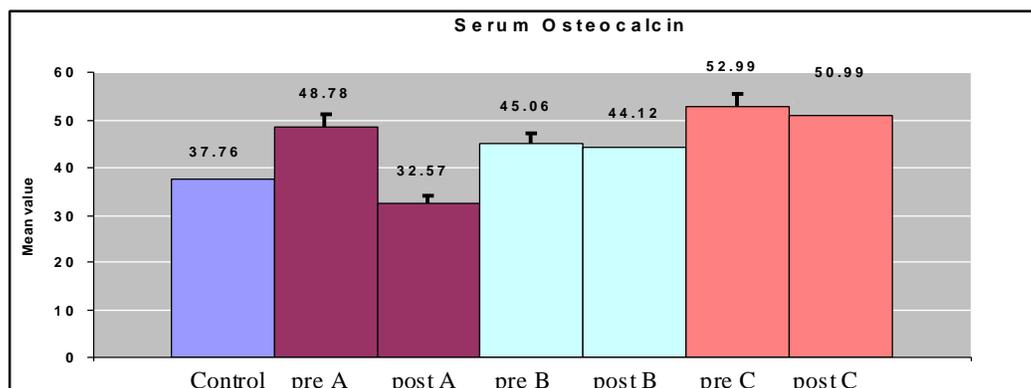
**2. Effect of treatment with either alendronate, HRT, or calcium and vitamin D on serum level of ALP in women with postmenopausal osteoporosis.**

Table(1) indicates a significant increase (p<0.05) in serum total ALP activity before starting treatment with alendronate. Whereas, non significant alteration in serum

level of ALP(P>0.05) were observed following treatment with the three tested therapies in comparison with their pre-treatment values.

**3. Effect of treatment with either alendronate, HRT, or calcium and vitamin D on serum level of PTH in women with postmenopausal osteoporosis.**

Table (1) showed that the serum PTH levels were non-significantly altered baseline values in comparison to the control group. Also non significant changes in serum levels of PTH were observed following the treatment with either one of the three tested therapies in comparison with their pre-treatment values.



**Figure 1: Effect of alendronate(10mg/day), HRT(0.625 mg/day conjugated estrogen), and Ca and vit. D (Ca and vit. D 1500 mg/day and 1000 IU/day respectively), for twelve weeks on serum osteocalcin level in post menopausal osteoporosis women .**

Group A= Treated with alendronate.

Group B= Treated with HRT

Group C= Treated with Ca and Vit.D

**Table 1 : Effect of alendronate (10 mg/day), HRT(0.625 mg/day conjugated estrogen),and Ca and vit. D (Ca and vit. D 1500 mg/day and 1000 IU/day respectively) for twelve weeks on serum total ALP and PTH levels in all patients.**

Marker	Drug	Control (N=15)	Before Treatment (N=12)	After Treatment (N=12)
ALP (U/L)	ALN	88.47 ±13.82	133.92±37.91*	125.58±44.21
	HRT		100.83±26.88	93.42± 20.89
	Ca and vit D		100.25±27.61	91.08±16.41
PTH (ng/ml)	ALN	27.77±14.55	27.93±17.19	28.92± 16.15
	HRT		32.77± 16.88	32.29±16.69
	Ca and vit D		33.37±10.8	31.17±10.0

values are presented as mean ± SEM.

\* significant difference from control at p < 0.05

# significant difference from before treatment at p<0.05

ALN= Alendronate

HRT= Hormone replacement therapy

**4. Effect of treatment with either alendronate, HRT, or calcium and vitamin D on serum level of calcium and inorganic phosphate in women with postmenopausal osteoporosis.**

Table (2) indicate that there was non significant fluctuation in serum total Ca

and inorganic phosphate levels in all patients groups in comparison with control group , nor after treatment with either alendronate ,HRT, or Ca and vit D or in comparison with their pre treatment values .

**Table 2:Effect of alendronate (10 mg/day) , HRT(0.625 mg /day conjugated estrogen) ,and Ca and vit. D(Ca and vit. D 1500 mg /day and 1000 IU /day respectively) for twelve weeks on serum total Calcium and inorganic phosphate levels in postmenopausal osteoporosis women.**

Marker	Drug	Control (N=15)	Before Treatment (N=12)	After Treatment (N=12)
Ca (mg/dl)	ALN	8.78±0.69	8.45±1.16	8.50±1.13
	HRT		8.32±1.12	8.21±1.07
	Ca and vit D		8.98±1.12	9.39±0.54
PO <sub>4</sub> <sup>-3</sup> (mg/dl)	ALN	3.85±0.38	4.13±1.61	4.47±1.70
	HRT		4.11±0.95	4.25±1.01
	Ca and vit D		3.67±0.51	3.73±0.47

values are presented as mean ± SEM.

ALN= Alendronate

HRT= Hormone replacement therapy

## Discussion

In our study the levels of serum Ost, was significantly higher in untreated patients with postmenopausal osteoporosis as compared to controls (Figure 1). These results are in agreement with (Aysan T et al, 2007 and Peipchl P et al, 2000) <sup>(16,17)</sup>. This could be attributed to the accelerated bone loss occurring within the first years following menopause <sup>(18)</sup>. Since during both menopause and aging, the coordinated balance to disturbance of bone formation and resorption can be disturbed, resulting in excessive bone loss and osteoporosis <sup>(19)</sup>. Thus serial measurement of Ost is indicated to be an excellent marker to assess the long term effects of antiresorptive therapy <sup>(20)</sup>. The significant reduction in serum Ost level of postmenopausal osteoporosis following alendronate administration in comparison with the baseline values. This result is confirmed by other studies. <sup>(21, 22, 23)</sup> And this is because aminobisphosphonates can decrease bone remodeling by decreasing osteoclast activity and by inducing osteoclast apoptosis. <sup>(24,25)</sup> The hypothesis that alendronate, like other nitrogen-containing bisphosphonates, inhibits a rate-limiting step in the mevalonic acid pathway that is essential for osteoclast function <sup>(26,27)</sup>, this could be explained that the first measurable effect of bisphosphonate is the decrease of the rate of bone resorption that is followed by a slower decrease of the rate of bone formation, due to the coupling of the two processes and the attainment of a new steady

state at a lower rate of bone turnover <sup>(28)</sup>. In the present study the level of Ost was non significantly lowered following twelve weeks of treatment with HRT therapy compared to their level before treatment (table1) this is in agreement with previous study <sup>(29,30)</sup>, indicated that serum Ost was able to decrease only after 6 months of treatment with HRT <sup>(17)</sup>. The Possible mechanism could include a decrease in osteoclastogenesis, osteoclast apoptosis and additional effect on calcium homeostasis <sup>(31)</sup>. Estrogens may affect osteoclasts directly, in addition estrogens certainly exerts effect on osteoclasts indirectly by suppressing the production of bone resorbing cytokines from osteoblast and bone marrow stromal cells <sup>(32)</sup>. The possible explanation to the lack of response of bone markers to treatment with calcium and vitamin D (Figure 1 and Table 1) may be simply because this treatment has a bone stimulant effect but no or very minimal anti-resorptive effect <sup>(33)</sup>. However, previous studies have shown some minimal improvement of bone mineral density (BMD) in patients treated with calcium and vitamin D but this did not correlate with any reduction of fracture risk <sup>(34)</sup>. (Shinchuk et al, 2005) had reported high prevalence of vitamin D deficiency in men and women with osteoporosis <sup>(35)</sup>. Therefore calcium and vitamin D is now considered as a complementary treatment to osteoporosis medications such as bisphosphonates rather

than a sole line of treatment by itself<sup>(36,37)</sup>, although (Palacios et al, 2005) had reported a significant reduction in serum bone markers after treatment with calcium and vitamin D<sup>(38)</sup>. This supports the notion found in (Figure 1 and Table 1) that alendronate is a stronger antiresorptive agent than both HRT<sup>(39,40)</sup> and calcium and vitamin D<sup>(41)</sup>. And that alendronate may have a very beneficial effect when added to HRT regimen in patients with postmenopausal osteoporosis<sup>(39)</sup>. Additionally total serum alkaline phosphatase in untreated postmenopausal women showed a significant increase in level as compared to control (Table 1). These results are in agreement with other studies<sup>(16, 42)</sup>. As this enzyme function in the metabolism of bone tissue, thus increased activity could be a way to compensate for lost calcium/phosphorus by recruiting more of these elements to build new bone tissue<sup>(16, 42)</sup>. However, no differences were noted in serum ALP levels following treatment with all tested medications in postmenopausal osteoporotic patients. This may be due to the fact that total ALP measurements is less specific to bone than measurements of the bone isoenzyme<sup>(7)</sup> and also may be due to short time for follow up period (twelve weeks) in this study. Meanwhile, PTH levels, in the current work, showed non significant changes in untreated postmenopausal osteoporosis groups in comparison with controls. Such finding could be explained through the changes that take place in calcium metabolism related to estrogen deficiency, which induces a decrease in the intestinal absorption of calcium and reduces the biological activity of vitamin D. It also enhances the renal calcium excretion and diminishes the ability of parathyroid hormone to reduce calciuria. Serum calcium remains unchanged or slightly increased due to compensatory increased bone resorption and PTH remains unchanged resulting in increased bone resorption and this is in agreement with our result<sup>(43)</sup>. Results in table 2 elucidated non significant fluctuations in serum calcium and inorganic phosphate before and after the treatment of postmenopausal women. Our results are in agreement with other studies<sup>(42,44,45,46)</sup>. As serum calcium is homeostatically controlled and the integrity of bone may be sacrificed to maintain serum calcium within the normal range<sup>(47)</sup>. Consequently, serum calcium is a poor predictor of histological features and is not indicative of bone resorption in osteoporotic patient<sup>(48)</sup>. Meanwhile non significant alteration in serum inorganic phosphate before and 12 weeks after treatment in postmenopausal women and this was in agreement with previous study<sup>(42,44,45,46)</sup>,

indicating that the determination of serum inorganic phosphate was of no significant values for the diagnosis and follow up of osteoporosis. Thus effective treatment with antiresorptive agents can show up significant changes in abnormal bone markers after treatment supporting the value of measuring them in the patients with osteoporosis. Hence bone markers (Ost) can be used for monitoring the response to therapy and also as a guide for patients compliance with treatment. Calcium and vitamin D supplementation is recommended as a complementary treatment even if dietary supply is sufficient. Thus we can recommend checking the baseline bone marker status (Ost) for all osteoporotic patients.

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