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ORIGINAL ARTICLE

BMD and Serum Intact Osteocalcin in Postmenopausal Osteoporosis Women

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Abstract India seems to have the highest prevalence of osteoporosis. With growing awareness of osteoporosis and its impact on life span especially in India, special attention is being paid to early detection, management and treatment of postmenopausal osteoporosis in women. Measurement of BMD and osteocalcin are of value in estimating bone turnover rates. The aim of this study is (1) to measure the specific, sensitive bone formation marker such as osteocalcin and BMD in postmenopausal osteoporosis women and postmenopausal non-osteoporosis women; (2) the follow up study to evaluate the impact of specific antiresorptive therapy (alendronate + calcium + vitamin D) regimen in postmenopausal osteoporosis by assaying osteocalcin and BMD. Sixty clinically diagnosed postmenopausal osteoporosis patients and 60 normal subjects (postmenopausal non-osteoporosis women) were recruited as control. Mean bone mineral density *T* score and *Z* score was significantly decreased ($P < 0.001$) in postmenopausal osteoporosis patients as compared to controls. Highly significant increase in the mean score of BMD—*T* score and *Z* score from baseline to post therapy of 3 months was observed in postmenopausal osteoporosis women. Serum osteocalcin levels were significantly increased ($P < 0.001$) as compared to control group. Serum osteocalcin levels were decreased significantly ($P < 0.001$) from baseline to post therapy of 3 months in postmenopausal osteoporosis women. BMD is the best quantifiable predictor of osteoporotic fracture and osteocalcin is specific, sensitive, promising, currently used marker for better prognosis of osteoporosis and for monitoring responses to antiresorptive therapy.

Keywords Bone formation marker · Bone mineral density · Postmenopausal osteoporosis

Introduction

"Osteoporosis is a progressive systemic skeletal disorder characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture" [1]. This is a disease that may have a tremendous impact on the lives of many postmenopausal women. Osteoporosis and its potentially devastating sequelae of fracture are increasing as the population ages, and assessment of skeletal health is an important component of a women's routine care.

Osteoporosis is second only to cardiovascular disease as a leading health care problem, according to the World Health Organization. Worldwide, the lifetime risk for women to have an osteoporotic fracture is 30–40% [2]. Occurrence of osteoporosis is 10 years earlier in Indian people than in the West. It currently affects approximately one in three women and one in five men over age 50. Because of related morbidity, disability, diminished quality of life, and mortality, osteoporosis and fractures associated with it are major public health concern [3].

A lack of estrogen in postmenopausal women prevents the absorption and utilization of calcium and is the single most important factor in the development of osteoporosis in older women. Increase in life expectancy is another concept of formation of osteoporosis. Menopause and ageing is associated with accelerated loss of cortical bone. Bone loss occurs when the balance between formation and

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resorption is upset and resorption is excessive resulting in a negative remodeling balance [4, 5].

Treatment of the patient with osteoporosis frequently involves management of acute fractures as well as treatment of the underlying disease. It is known that alendronate has a direct action on osteoclast activity. A decline in resorptive markers can be ascertained after treatment with bisphosphonate. With a view of studying the effect of alendronate on the activity of osteoblasts, this study was designed in which osteocalcin was determined for assessment of osteoblastic activity.

Materials and Methods

Present study was conducted in the Department of Biochemistry, Government Medical College Miraj and P.V.P.Government Hospital Sangli. Study group included 60 postmenopausal women in the age group 45–60 years and diagnosed as osteoporosis by clinicians, based on clinical features and radiological evidence. The study group was given alendronate—70 mg/week, calcium citrate 1,200 mg and calcitriol—0.25 μg once a day. Control group included 60 postmenopausal non osteoporotic women in the age group 45–60 years.

The Institutional Ethical Committee approved the study and informed consent was obtained from each participant in the study. Patients taking HRT and anticonvulsants, having a chronic debilitating illness (cancer, AIDS), renal diseases, liver diseases, diabetes mellitus were excluded from this study.

Serum osteocalcin shows diurnal variation in circulating level, blood samples were collected at a specific time 10–11 a.m. Blood samples were collected from control group and study group at baseline level under aseptic conditions. In the follow up study blood sample was collected from study group after 3 months therapy. The bone mineral density was measured at the midshaft tibia by Quantitative Ultrasound method [6]. Estimation of intact osteocalcin by EASIA method [7]**.** EASIA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtiterplate. The assay uses monoclonal antibodies (MAbs) directed against distinct epitopes of human osteocalcin. Calibrators and samples react with the capture monoclonal antibody (MAb-1) coated on microtiter well and with a monoclonal antibody (MAb2) labeled with horseradish peroxidase (HRP), after an incubation period allowing the formation of a sandwich: coated MAb1–MAb2–HRP, the microtitre plate is washed to remove unbound enzyme labeled antibody. Bound enzyme-labeled antibody is measured through a chromogenic reaction. The amount of substrate turnover is determined ELISA reader by measuring the absorbance, which is proportional to the osteocalcin concentration.

All data were expressed as means ± SD. Statistical analysis was done by using '*z*' test and paired *t* test.

Results and Discussion

The hallmark of menopause is a reduction in skeletal mass caused by an imbalance between bone resorption and bone formation due to loss of ovarian function. Hence, loss of ovarian function is the most important factor in the development of postmenopausal osteoporosis.

The mean score of BMD in PMO was found to be significantly decreased as compared to control group ($P < 0.001$, Table 1). Findings of Neetakumar et al. [3] and Johannes et al. [8] support our result. In osteoporosis there may be exaggeration of the imbalance between bone formation and resorption. The change in activation frequency causes a transient bone loss until a new steady state between resorption and formation is achieved. The remodeling imbalance, however, results in permanent decrement in mass that can only be corrected by a remodeling event during which formation exceeds resorption. Thus significant increase in BMD scores from baseline to post therapy of 3 months (alendronate + calcium + Vitamin D) was observed in PMO (Table 1).

By suppressing bone turnover, this therapy prevents bone loss and preserves bone architecture and increase bone strength. Alendronate of this therapy can binds

Table 1 BMD and osteocalcin in control group and PMO pre and post therapy

S.no.	Bone mineral density and serum intact osteocalcin	Postmenopausal non osteoporosis women Controls, $n = 60$ Mean ± SD	Postmenopausal osteoporosis women	
			Baseline, $n = 60$ Mean ± SD	Post therapy, $n = 60$ Mean ± SD
1	*T*-score	0.345 ± 0.731	−3.233 ± 0. 752*	−2.083 ± 0.770*
	Z-score	0.366 ± 0.698	−2.735 ± 1.539*	−1.713 ± 0.417*
2	Intact osteocalcin (ng/ml)	11.467 ± 3.183	25.184 ± 4.974*	14.640 ± 4.475*

The statistical method used to compare data was *Z* test and paired *t* test

* $P < 0.001$ highly significant

hydroxyapatite crystals of bone with high affinity and inhibit bone resorption by decreasing osteoclastic activity and its growth. After the inhibition of resorption, these agents become affixed to the bone matrix, where they reside until the remodeling begins again. Thus it has very long retention in the skeleton and may exert long term effects. It inhibits farnesyl diphosphate synthase, a critical enzyme in the cholesterol mevalonic acid pathway that is also required for protein prenylation. When the activity of this enzyme is blocked, the cytoskeleton integrity and intracellular functioning of the osteoclasts is disrupted and apoptosis ensues. Thus antiresorptive therapy is effective in reducing the risk of fracture and should be considered for all patients with osteoporosis. Findings of Ones et al. [9] and Rhee et al. [10] support our results.

Mean level of serum osteocalcin was found to be significantly elevated in PMO when compared with controls ($P < 0.001$, Table 1). Osteocalcin is synthesized in the skeleton by osteoblasts, the cells responsible for the bone formation. Osteocalcin is a major and most thoroughly characterized non collagenous protein in mature human bone. It is a highly sensitive marker for bone formation. The advantage of using osteocalcin as a clinical index of bone turnover is its tissue specificity and it's relatively low within person's variation [11]. Elevated level of osteocalcin may be associated with increased activity of osteoblast.

Osteocalcin has a high affinity for calcium and exhibits a compact calcium dependent α helical conformation, in which the γ-carboxyglutamic acid (Gla) residues binds and promote absorption to hydroxyapatite in bone matrix, in this way mineralization of bone takes place. In osteoporotic women, deficiency of calcium and phosphorus 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. Assay of the specific marker of the osteoblastic activity i.e. osteocalcin, reveals the prognostic significance of osteocalcin for better management of PMO. Pino et al. [12] found that osteocalcin is a promising marker of bone turnover useful in the diagnosis and follow-up of high turnover osteoporosis. Similar observations were reported by a number of other studies Verit et al. [13], Cabrera et al. [14] and Rosenquist et al. [15].

Table 1 shows significant decrease in osteocalcin level from baseline to post therapy of 3 months (Alendronate + calcium + vitamin D) in PMO. This therapy suppresses osteoclast mediated bone resorption and indirectly and more slowly, it may decrease osteoblast activity and bone formation. Thus it acts as antiremodeling drug. Net positive calcium balance may be achieved during therapy, promoting binding of osteocalcin with calcium. Osteocalcin is involved in bone calcification; hence its level may be lowered after therapy. Our findings were also supported by the study of Johannes et al. [8], Ones et al. [9], Sambrook et al. [16] and Fardellone et al. [17].

To conclude, osteoblastic activity was ascertained by measurement of osteocalcin—a promising marker of bone turnover. It is shown to be increased in PMO. In post therapy it becomes normalized along with bone formation markers. BMD is the best quantifiable predictor of osteoporotic fracture, it provides a static picture of the skeleton, and the biochemical marker of bone turnover provide dynamic measure of bone remodeling and thus is potentially useful in predicting the course of changes in bone mass. The combined use of BMD and osteocalcin are of use in the evaluation of osteoporosis and for monitoring responses to the antiresorptive therapy.

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