

SERUM OSTEOCALCIN, SHALL WE CONSIDER IT AS A BIOCHEMICAL MARKER FOR OSTEOPOROSIS IN POSTMENOPAUSAL WOMEN

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ABSTRACT: BACKGROUND: Disease of the bone, particularly osteoporosis, represents a major health care problem predominantly affecting women. There are various markers of bone turnover including markers for bone formation and bone resorption. Osteocalcin is a bone Gla protein synthesized by osteoblasts is found to increase in osteoporosis. In our study serum osteocalcin level was measured in pre and post menopausal women and also in osteoporotic women. **MATERIALS & METHODS:** Ninety individuals were included in this study and classified as three groups. Group A - 30 Pre menopausal women, Group B - 30 Post menopausal women and Group C - 30- Osteoporosis women. The above subjects were confirmed by Dexa Scan for osteoporosis. Serum sample was collected and osteocalcin were measured in all women by ELISA method. **RESULTS:** The serum Osteocalcin level was found to be increased in post menopausal and Osteoporotic individuals when compared to the pre menopausal women. **CONCLUSION:** The increased level of serum osteocalcin in post menopausal women may be a predictor for future risk for osteoporosis. To support this view, an extended study is absolutely essential.

INTRODUCTION: The ultimate challenge facing health care providers is the quest for comprehensive treatments for chronic diseases within a framework of reduced patient access and limited financial resources. Osteoporosis is one of those diseases. Osteoporosis is a disease characterized by low bone mass and by architectural deterioration of bone tissue, the two factors related to abnormalities of bone turnover¹. It is easy to diagnose osteoporosis in a patient with fracture. A more difficult but potentially more important approach is to diagnose osteoporosis before the occurrence of fracture.

Basic and molecular studies of skeletal remodeling system have produced a wealth of new information about the osteoporotic process. Clinical studies employing new bone specific agents have generated tremendous enthusiasm for newer therapeutic options as well as providing a greater understanding of the spectrum of metabolic bone diseases. This expanded knowledge base has set the stage for even greater technological thrusts aimed at earlier diagnosis and cost effective treatment².

Bone formation and bone resorption are altered in most metabolic diseases like osteoporosis. The abnormalities have been characterized by bone histo-morphometry on iliac crest biopsy which allows both quantitative assessment of bone turnover at the cell and tissue level. Until recently the only available markers were total serum alkaline phosphatase for monitoring bone formation and urinary hydroxyproline for monitoring bone resorption. Both of them are not specific for bone tissue, as they are unable to detect the small increase in bone turnover. To overcome these problems, efforts were made in recent years to develop new and more specific markers of bone turnover. The rate of bone formation or degradation can be assessed by measuring the enzyme activity of osteoblastic or osteoclastic cells or by measuring components of the bone matrix released

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into the circulation during formation (osteocalcin) or resorption (Pyrinolidine cross linker). Post menopausal women are prone to get osteoporosis because of hormonal deprivation as supported by various literatures^{3, 4, 5}.

Osteocalcin, a bone Gla protein of 49 amino acids is the major non collagenous protein of the bone matrix with a molecular weight of 5669 dalton. The 3 specific glutamyl residues are converted to gamma carboxy glutamyl residues by a post translational vitamin K dependent, enzymatic carboxylation. It is predominantly synthesized by osteoblasts, incorporated into the extra cellular matrix of bone. A fraction of the neo synthesized osteocalcin is released into the circulation, where it can be measured by immunoassay^{6, 7}. This study focuses the estimation of serum osteocalcin in pre-menopausal, post menopausal & osteoporotic individuals.

MATERIALS & METHODS: Ninety individuals were included in this study and were grouped as follows.

Group A – Pre menopausal women 30 in number with age ranging from 33-43 years.

Group B - Post menopausal women 30 in number with age ranging from 51-59 years.

Group C – Osteoporotic women 30 in no confirmed by DEXA Scan with age ranging 48-62 years.

After obtaining the institutional ethical committee clearance, the informed consent was obtained from all the participants. Blood samples were collected. EDTA plasma was the sample for osteocalcin estimation which was separated with the help of refrigerated centrifuges and stored at -20°C.

Calcium was estimated by Arzenazo III method in semi automated Analyzer. Phosphorous was estimated by modified by Wang et al of Daly and Ertingshose method in semi automated analyzer.

Osteocalcin was estimated by (Bio-Source Europe SA) a solid phase enzyme amplified sensitivity immunoassay (EASIA) using Monoclonal Antibodies detected against distinct epitopes of human osteocalcin. Standards and samples react with the captured monoclonal Ab (Mab1) coated on the microtiter well and with Mab 2, assessed with horse radish peroxidase. After an incubation in period allowing the formation of a sandwich (Coated Mab1- human osteocalcin -Mab2 –HRP) the microtiter plate is washed to remove the unbound enzyme labeled Ab. Bound enzyme assessed Ab is measured through a Chromogenic reaction. Chromogenic solution (TMB) is added and incubated. The reaction is stopped with the addition of stop solution and the microtiter plate is then read at the appropriate wave length. The amount of substrate turnover is determined colorimetrically by measuring the absorbance which is proportional to the human osteocalcin concentration. A standard curve is plotted and human osteocalcin concentration in a sample is determined by interpolation from the standard curve.

The Bone Mineral Density was assessed by Dual Energy X-ray Absorptiometry scan (DEXA Scan) at Bharath Scan private Limited Chennai. For women four general diagnostic categories have been proposed by WHO and modified by the international osteoporosis foundation for assessment done with DEXA. Normal hip BMD greater than 1 SD below the young adult female reference mean T score >-1.

Low bone mass (osteopenia) hip BMD greater than 1 SD below the young adult female , mean , but less than 2.5 SD below this value (T-Score <-1 and >-2.5).

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Osteoporosis - hip BMD 2.5 SD or more below the young adult female mean (T score < - 2.5).

Based on above score osteoporosis was considered.

EXCLUSION CRITERIA: Chronic renal failure, Diabetes Mellitus, liver disorder and patients with drug intake like anti convulsants and calcium supplementation was excluded from the study.

RESULTS: The analysis of the results obtained from the present study has revealed a significant increase of serum osteocalcin levels in osteoporotic individuals as evidenced by the Table 1. Analysis was done by ANOVA with P value <0.05 was considered as statistically significant. However the mean value of calcium and phosphorous were not statistically significant between the three groups.

DISCUSSION: In almost all cases of osteoporosis, bone formation remains at least partially coupled to bone resorption, even though the resorption rate can far exceed formation. Therefore during states of high turnover, markers of bone formation should be increased. Osteocalcin is synthesized during bone formation and released during resorption, which is considered a marker of bone turnover; the clinical utility may be substantial for monitoring tightly coupled formation/ resorption process. When formation and resumption are uncoupled, osteocalcin is considered a marker of osteoblastic activity. The analysis has also revealed that serum osteocalcin values are significantly increased in postmenopausal women as evidenced by Table 1 with a significant p value. The increased level in post menopausal group is attributed to be due to oestrogen deficiency, a finding correlating to similar studies indicating in principle, a clear correlation between oestrogen deficiency and these markers of bone turnover especially serum osteocalcin^{3,4,8}. This group of patients is more prone to get fractures. The occurrence of Osteoporosis in Indian Population is 15-20 yrs. earlier than in Caucasian population, which is attributed to deficient intake of calcium. In a study conducted at Kanpur, fracture neck of femur occurs at all ages, but occurring earlier in Indians. 50% fracture following mild trauma, 22% following moderate trauma⁹. The study reveals that there are no significant variation in serum calcium levels and serum phosphorous levels among premenopausal, postmenopausal women, and osteoporotic patients.

Bone remodeling is a highly integrated process of resorption and successive formation of bone tissue that results in maintenance of skeletal mass with renewal of the mineralized matrix^{10, 11}. This is accomplished by focal cell mediated degradation and regeneration of bone tissue without comprising the overall architecture of the anatomy of bones.

During adult life, bone is lost from skeleton at a rate of approximately 1% of its peak mass per year. This overall bone loss is due to a small imbalance between the amount of bone resorbed and the subsequent amount formed in each episode of bone remodeling. In adults at the time of their peak bone mass, in each bone remodeling unit, an exactly similar amount of bone would be removed by the osteoclasts, as was subsequently replaced by the osteoblasts. Later in life, when that individual begins to lose bone, loss may either occur due to the osteoclast resorbing slightly more bone while the osteoblasts replace the same amount or the osteoblasts may reduce the amount of bone which they replace in each bone remodeling unit.

This difference between the amount of bone removed by the osteoclasts and that can be replaced by the osteoblasts in a single bone-remodelling unit is referred to as the remodeling imbalance¹⁰. For a given difference between the amount of bone resorption and bone formation at any individual bone remodeling unit, the rate of change of total skeletal mass will depend on the number of bone remodeling units taking place at any one time, that is the rate of bone turnover. This

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acceleration in the rate of bone loss may be caused simply by an increase in the rate of bone turnover. To some extent this explains the rapid phase of postmenopausal osteoporosis.

At menopause, there is a transient phase of bone loss, followed by a sustained bone loss. There is a steady decline in unbound estrogen levels with ageing which leads to elevation in the number of Basic Multicellular unit through increases activation frequency(no. of new remodeling units activated in each unit time) ultimately resulting in expansion of remodeling space, increased cortical porosity and enlarge the resorption area on trabecular surfaces^{9, 12}. Estrogen deficiency also prolong the resorption phase of remodeling cycle because of increased lifespan of osteoclasts due to reduced apoptosis ultimately resulting in increased erosion depth. Researches during the last decade have revealed that oestrogen regulates bone homeostasis apart from the direct effects on bone cells, also through the regulatory effects on the immune system and on oxidative stress. In our study a significant high level of osteocalcin noticed in postmenopausal women when compared to premenopausal women as evidenced by other studies. If the study would have been exclusively conducted in postmenopausal women, a cut off value for serum osteocalcin can be estimated before suggesting hormone replacement Therapy.

High value of serum osteocalcin noticed in postmenopausal women being closer to the value seen in osteoporosis group can pave the way of considering Serum Osteocalcin, a biochemical marker of bone turnover, a prognostic one before the occurrence of fracture.

MERITS: Elevated level of serum osteocalcin in post menopausal women can be considered as prognostic marker of osteoporosis. They can be treated with hormone replacement therapy to prevent or reduced the occurrence of fractures. Since osteocalcin is measured in serum, sample collection is easy and can be performed in various laboratories. Confirmation of osteoporosis by DEXA scans, even though a short duration procedure involves radiation exposure and quite expensive.

LIMITATION: To confirm serum osteocalcin a prognostic marker of post menopausal osteoporosis before the occurrence of fracture, the study should be performed exclusively in large number of post menopausal osteoporotic patients.

CONCLUSION: Osteocalcin, a bone Gla protein synthesized by osteoblasts, is a biochemical marker of bone turnover, seemed to be increased in osteoporotic patients.

It is easy to diagnose osteoporosis after the occurrence of fractures. An increased serum osteocalcin level provides a prognostic markers in postmenopausal women, may indicate a future risk of development of pathological fractures, for which calcium supplement /HRT can be considered. An increase of osteocalcin in majority of postmenopausal women indicates a future risk of fractures.

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ANOVA Between the three groups.

	PREMENOPAUSAL WOMEN Group A	POSTMENOPAUSAL WOMEN Group B	OSTEOPOROTIC PATIENTS Group C	
AGE	38 ± 5	54 ± 5	55 ± 7	P<0.05
CALCIUM	9.13 ± 1.28	8.40 ± 1.07	8.92 ± 1.36	NS
PHOSPHOROUS	4.68 ± 0.74	4.57 ± 0.71	4.56 ± 0.78	NS
OSTEOCALCIN	4.27 ± 3.02	15.97 ± 12.75	17.25 ± 6.39	P<0.05
BMD	0.831± 0.99	0.748 ±0.64	0.632 ± .91	P<0.05

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