



## CLINICAL UTILITY OF BIOCHEMICAL MARKERS ON OSTEOPOROSIS IN PRE AND POSTMENOPAUSAL WOMEN

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### ABSTRACT

The awareness of osteoporosis has grown worldwide in recent years. This silently progressing metabolic bone disease is widely prevalent in India, and osteoporotic fractures are a common cause of morbidity and mortality in adult Indian men and women. Rapid bone loss occurs in postmenopausal women due to hormonal factors which lead to increased risk of fractures. A cross-sectional study of 200 pre- and postmenopausal women was carried out at SLIMS, Pondicherry. Bone formation markers (Total Calcium, Ionised calcium, Alkaline phosphatase), and bone resorption markers (Urinary Hydroxyproline) were analysed in pre and postmenopausal women. Bone formation markers, Total and Ionised calcium were significantly decreased ( $p < 0.001$ ) and Alkaline phosphatase was significantly increased ( $p < 0.001$ ) in postmenopausal women compared to premenopausal women. Bone resorption markers, Urinary hydroxyproline excretion was significantly increased ( $p < 0.001$ ) in postmenopausal women. Measurement of bone turnover through urinary hydroxyproline, Alkaline phosphatase and Calcium could form a tool available to assist health care professionals to predict fracture risk. Early prediction of osteoporosis has important public health and clinical implications, as osteoporosis usually has no symptoms until the occurrence of fractures.

**KEYWORDS :** Bone turnover, Hydroxyproline, Alkaline phosphatase, Osteopenia, Osteoporosis

### INTRODUCTION:

The word 'menopause' is derived from two Greek words, 'meno' (month) and 'paus' (to stop). Clinically, menopause is said to have occurred when menstruation has ceased for twelve months (1). Physiologically, menopause is defined as the permanent cessation of menses resulting from reduced ovarian hormone secretion that occurs naturally or is induced by surgery, chemotherapy, or radiation (2). The postmenopausal stage in women is essentially an oestrogen-deficient state (1). Both menopause and aging are associated with an accelerated loss of bone mass. Menopause occurs when the balance between bone formation and resorption is upset and resorption is excessive, resulting in a negative remodelling balance (3). Osteoporosis is an important public health problem in middle-aged and older women. Until recently, the diagnosis and monitoring of treatment for osteoporosis has been confined to clinical assessment, radiography and bone densitometry. However, in recent years biochemical markers of bone formation and resorption have been developed to quantify bone turnover and remodelling, with possible applications in clinical practice (1-3)

Osteoporosis is a major health and economic problem. An International consensus development conference has stated that osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitect deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. This silently progressing metabolic bone disease is widely prevalent in India, and osteoporotic fractures are a common cause of morbidity and mortality in adult Indian men and women (4). Osteoporosis has been linked to an increased fracture risk and subsequent mortality in the later life. Previous prediction models have focused on osteoporosis in postmenopausal women; however, a prediction tool for osteopenia is needed. Osteoporosis is a serious global health issue due to the rapid increase in the size of the aging population. (5-6) Osteoporosis-related fractures have been found to be associated with significant costs, increased morbidity and mortality, reduced quality of life, and loss of independence (5-6). The prevalence of osteoporosis increases with age for all sites, and by WHO

definition up to It is important to think clearly about the 2 principle determinants in adult bone health (a) Maximum attainment of Peak bone mass (PBM) in young adulthood, and (b) the rate of bone loss with advancing age. With the onset of menopause, rapid bone loss occurs which is believed to average approximately 2% to 3% over the following 5 to 10 yrs, being greatest in the early postmenopausal years (7). Life time losses may reach 30% to 40% of the peak bone mass in women and 20% to 30% in men. The pathogenesis of postmenopausal osteoporosis involves the interplay of many factors- Nutritional, Environmental, Genetic factors. (8)

Biochemical markers of bone turnover have been shown to provide valuable information for the diagnosis and monitoring of metabolic bone disease (9). They reflect the whole body rates of bone resorption (Resorption markers) and bone formation (Formation markers). Therefore they may provide a more representative index of the overall skeletal bone loss than would be obtained by measuring the rates of change in Bone Mineral Density (B.M.D) at specific skeletal sites (10). Osteoporosis is more common in post-menopausal women and not only gives rise to morbidity but also markedly diminishes the quality of life in this population. There is lack of information regarding the risk factors of osteoporosis in developing countries (11). The occurrence of Osteoporosis in postmenopausal women is very common problem especially in India who are exposed to many of the risk factors like Family h/o osteoporosis, history of anorexia or bulimia, prolonged amenorrhea, low calcium diet, lack of exercise, Vitamin D deficiency. But there are very few Indian studies regarding the prevalence of osteoporosis in postmenopausal women and also regarding the biochemical markers which indicate bone turnover in our setup.

Total alkaline phosphatase activity in serum has been used commonly as a biochemical marker of osteoblast function, but lacks specificity because of the contribution of activity derived from the liver, in particular. Human alkaline phosphatases (ALP) are a group of enzymes of similar specificity coded for by at least four different gene loci that catalyse the hydrolysis of phosphate esters at an alkaline pH. (11) The gene for tissue

non-specific ALP encodes the isoenzymes expressed in liver, bone and kidney. In healthy individuals about half the activity of alkaline phosphatase in serum is derived from bone and the remainder from liver. The isoforms differ only in the degree of sialylation and glycosylation, reflected in differences in electrophoretic mobility, heat stability and precipitation by lectin. Methods to separate and quantify bone ALP in the presence of liver ALP, based on these properties, have not had sufficient specificity or sensitivity to be useful clinically. In the USA, the prevalence of osteoporosis in postmenopausal women aged 50 years has been found to be 15.8% in non-Hispanic whites, 7.7% in non Hispanic blacks, and 20.4% in Mexican Americans.(12) Among postmenopausal women, the average bone mineral density (BMD) was found to be highest among African Americans, followed by among Hispanics, native Americans, and Asians (13). In Taiwan, the prevalence of osteoporosis among women aged > 40 years has been estimated at 10.1% and 7.5% based on the BMD of the spine and femoral neck, respectively (14). Despite the availability of diagnostic tools and treatment protocols, osteoporosis remains underdiagnosed and undertreated (15). Women reach peak bone mass between the ages of 20 years and 30 years. Then, BMD decreases gradually and continues to decline rapidly after menopause (16). Therefore, predicting the risk of osteoporosis at an earlier age (e.g., premenopausal) or a preclinical phase (i.e., osteopenia) is crucial for early prevention of osteoporosis.

A recent Canadian study used a weight of > 70 kg, 1 postmenopausal years, a history of a fragility fracture after the age of 40 years, and an age of > 51 years to predict the risk of low BMD (T-score < -2) in women aged 40-60 years.(17) Other risk factors not included in these prediction tools but known to be related to the risk of osteoporosis include anthropometric measures (e.g., body mass index and waist circumference), calcium and vitamin D supplements, physical inactivity, cigarette smoking, alcohol consumption,(18) low-density lipoprotein cholesterol level,(19) hypertension,(20) and renal dysfunction.(21) Despite the fact that there are several prediction tools for osteoporosis, none has been developed for osteopenia probably because the factors incorporated into previously developed tools are less sensitive for predicting osteopenia. Therefore, a tool that includes easily accessible factors may be a plausible approach to predict the risk of osteopenia. Some biomarkers (e.g., blood or urine markers) can easily be obtained during routine health checkups and improve the sensitivity in predicting osteoporosis at the preclinical phase (i.e., osteopenia). However, biomarkers are rarely included in the currently available prediction tools. Therefore, this study aimed to develop a simple and accurate prescreening tool for identifying premenopausal and early postmenopausal women (aged 40-55 years) with a high risk of osteopenia via the incorporation of biomarkers.

Thus, the aim of the present study is carried out to assess the clinical utility of biochemical markers of bone turnover, such as Total Calcium, Ionised calcium, Alkaline phosphatase in pre- and post menopausal women and to evaluate the risk of accelerated bone mass loss by assessing bone markers, such as alkaline phosphatase (ALP) and serum calcium, in postmenopausal women

**MATERIAL AND METHODS:**

We performed a cross-sectional study of 200 pre- and post menopausal women, at Department of Biochemistry, SLIMS, Pondicherry. The Institutional Ethical Committee approved the study and Informed consent was obtained from each participant in the study. The study group consisted of 100 Postmenopausal women in the age group of 46-65 years and 100 Premenopausal women in the age group of 25-45 years. All the participants were non smokers, non alcoholic and ambulatory. The women were neither pregnant nor on

oral contraceptive pills. None of the postmenopausal women had suffered any fracture in the previous 1 year nor were they on Hormone replacement therapy or any other medication that might affect bone turnover. Based on time since menopause, 100 postmenopausal women

were categorized into 2 groups. 30 women were in their early postmenopausal period (<5 years) and remaining 70 women were in their late postmenopausal period (>5 years). Height and Weight of all the participants were noted and Body mass index (BMI) was calculated using the formula = Weight (Kg) / Height<sup>2</sup> (m).

5 ml of random blood sample was collected in a plain bulb from each participant. Serum was separated immediately by centrifuging at 2000rpm for 15min and analysed for Total calcium, Ionised Calcium and Alkaline phosphatase. Random sample of urine was collected at the same time in a clean plastic bulb and analysed for Hydroxyproline and Creatinine immediately.

**Statistical analysis :**

The data were presented as Mean ± SD. Statistical analysis were done by using Microsoft Excel and SPSS for windows version 11.5 (SPSS, Inc., Chicago). P value <0.05 was considered statistically significant.

**Results:**

**Table. 1 : Comparison of markers of bone turnover in pre- and post Menopausal women (values expressed as mean ± SD)**

Parameters	Pre menopausal women( n=100)	Post menopausal women (n=100)
Age	33.28±6.12	57.76±7.89**
Years after Menopause		11.69±7.89
BMI	23.42±4.57	25.68±5.76*
Alkaline phosphatase	2.22±0.54	3.29±1.08**
T.Serum Calcium (m.mol/lit)	2.221±0.23	2.099±0.19**
I. Serum Calcium (m.mol/lit)	1.29±0.21	1.18±0.22**
Urinary Hydroxy Proline (mg /g crt)	10.28±3.23	22.42±10.92**

\*p<0.01 (significant), \*\*p<0.001 ( highly significant), (mg /g crt=milly grams per grams creatinine)

Table. 1. shows the comparison of all the biochemical parameters estimated as their mean values.

**Table. 2 : Comparison of markers of bone turnover in Early (<5yrs) and Late (>5 yrs) post menopausal period (values expressed as mean ± SD)**

Parameters	Early menopausal Period ( n=30)	Post menopausal Period (n=70)
Age	49.9±4.47	59.9±12.4**
Years after enopause	3.18±1.53	14.93±4.36***
BMI	25.8±4.38	25.73±5.79*
Alkaline phosphatase	3.63±1.14	3.15±1.35*
T.Serum Calcium (m.mol/lit)	2.081±0.17	2.16±0.48**
I. Serum Calcium (m.mol/lit)	1.07±0.15	1.17±0.25*
Urinary Hydroxy Proline (mg /g crt)	25.17±13.68	21.23±14.26*

\*(not significant), \*\*p<0.05, \*\*\*p<0.001 ( highly significant)

Table. 2. shows the comparison of all the biochemical markers of bone turnover on early and late postmenopausal periods.

**DISCUSSION:**

Current guidelines from the US Preventive Services Task Force recommend that women younger than 65 years be screened for osteoporosis using dual-energy X-ray absorptiometry (DXA) if their 10-year risk of a major osteoporotic fracture is greater than or equal to that of a 65-year-old white woman without additional risk factors (22). Biochemical parameters can give an idea as to the rates of bone formation and resorption. High rate of bone turnover correlates with a low bone mass. Calcium salts in bone are embedded in collagen fibrils, 13% of which is mainly hydroxyproline. During bone loss, collagen fibrils are broken down and hydroxyproline is thus excreted in the urine. Urinary hydroxyproline(OHPr) is thus considered as an index of bone resorption and a major determinant of bone status (23). In this study urinary hydroxyproline is expressed as mg of hydroxyproline per gram of creatinine, because creatinine is excreted in the urine in relatively constant amounts proportional to an individual's muscle mass, thus serving as a reference standard (23-24). In the present study there was a significantly increased urinary excretion of hydroxyproline (mg/g Cr) in postmenopausal women when compared to premenopausal women.

Menopause is known to be associated with numerous physiological and biochemical changes affecting bone mineral metabolism. The results of various case control studies in pre- and post-menopausal women have shown that changes in the serum calcium levels in post-menopausal women are not statistically significant (25-27); however, in the present study, we found that the serum calcium levels were significantly reduced in the post-menopausal group ( $p < 0.001$ ) when compared to the pre-menopausal group ( $p < 0.0001$ ) Ashuma et al. reported that aging and menopause lead to a decline in oestrogen and progesterone production, which has been implicated in the increased calcium levels of post-menopausal women (25). The response of markers of bone turnover may inform the physician about patient compliance, absorption of the bisphosphonate and the effect of treatment. It has been suggested that markers of bone turnover could be used to predict the response of an individual (in terms of BMD) to bisphosphonate therapy. In general, women lose about 1% of their bone density per year during and after menopause. However, nearly 35% of women lose bone at a faster rate during the late perimenopausal period. Biochemical markers can detect women who are considered "rapid losers" that is, those who lose 3% to 5% of bone per year (28).

Serum alkaline phosphatase is the most commonly used marker of bone formation. ALP is a ubiquitous enzyme that plays an important role in osteoid formation and mineralization. The total ALP serum pool consists of several dimeric isoforms which originate from various tissues such as liver, bone, intestine, spleen, kidney and placenta. In adults with normal liver function, approximately 50% of the total ALP activity in serum is derived from the liver, whereas 50% arises from bone (10). In our study the total ALP levels were significantly high in postmenopausal women in comparison to premenopausal women. Bonespecific ALP level is a bone turnover marker that is associated with low BMD and fracture risk (29). A previous Taiwanese study showed that serum total and bone-specific ALP levels slowly increased in women after the age of 40 years. (30) These two types of ALP have been found to have a linear association and a high level of correlation among people without bone diseases. (30) Therefore, total ALP serves as a surrogate for bone-specific ALP level. Finally, the number of years since menopause was an important predictor of osteopenia. ALP level reflects the physiological status in the body, which is more sensitive than using weight or age for predicting subtle changes in BMD during the preclinical phase of osteoporosis. a serum marker, total ALP level, which can be obtained during routine health

checkups, significantly increased the sensitivity of the model for osteopenia prediction compared with the existing tools. Early prediction of osteoporosis has important public health and clinical implications, as osteoporosis usually has no symptoms until the occurrence of fractures. Conversely, the serum alkaline phosphatase (ALP) levels were significantly increased in the post-menopausal group compared to the premenopausal group ( $p < 0.001$ ). It has also been shown that oestrogen deficiency, as occurs during menopause, induces the synthesis of cytokines by osteoblasts, monocytes, and T cells and thereby stimulates bone resorption by increasing osteoclastic activity. This action results in modification of the reabsorption, excretion, and resorption of calcium, which leads to increased circulating levels of this ion (31-34). Thus, we have reported a negative correlation between serum calcium and ALP levels in post-menopausal women. Several studies have reported no significant correlation between serum calcium levels and ALP when assessing various years since menopause (35-40). However, contrary to these findings, higher levels of calcium and ALP have been demonstrated in early post-menopausal women compared with late menopausal women ( $p < 0.001$ ) (34).

The ALP levels were high in women during their early postmenopausal period when compared to late postmenopausal period (Table II). Ionised calcium levels were found to be significantly decreased in Early postmenopausal women compared to late postmenopausal women. (Table II). This shows that the bone mass continues to decline with age but at a slower rate than during the early menopausal time. Annual change in ALP seems to indicate that bone resorption prevails on bone formation in early postmenopausal period. Total calcium and ionized calcium also the markers of bone formation were significantly decreased in postmenopausal women, when compared to premenopausal women.

The results from this study suggests that simple, easy, common biochemical markers such as age, years after menopause, urinary hydroxyproline, total serum ALP, total serum calcium and ionized calcium could be used as indicators of increased bone turnover, to enable early intervention so as to minimize fracture due to osteoporotic changes. These markers may help identify women at greatest risk for bone loss who would benefit most from therapeutic interventions. To the best of our knowledge, the OPAT is the first tool developed for predicting osteopenia in women aged 40 to 55 years. Integration of a serum marker, total ALP level, Calcium and urinary hydroxyproline which can be obtained during routine health checkups, significantly increased the sensitivity of the model for osteopenia prediction compared with the existing tools. More studies are warranted to validate this new tool for predicting osteopenia risk in women aged 40 to 55 years.

**CONCLUSION:**

We therefore conclude that bone ALP is useful in monitoring alendronate treatment of patients with osteoporosis. It has better clinical utility for following both treatment groups and monitoring individual patients. The biochemical markers of bone turnover provide dynamic measures of bone remodeling and thus potentially useful in predicting the course of changes in bone mass. The inclusion of serum total alkaline phosphatase level in the model, which is easy to obtain from routine health checkups, significantly enhanced the sensitivity for detecting osteopenia in women aged 40 to 55 years.

In normal post-menopausal women, an increase in bone turnover accelerates bone mass reduction. The present study reveals that serum calcium levels are significantly reduced in post-menopausal women, whereas serum ALP levels are significantly increased. In addition, a significant negative correlation was observed between serum calcium and ALP

levels in the experimental group. Measurement of bone turnover through urinary hydroxyproline, Alkaline phosphatase and Calcium could form a tool available to assist health care professionals to predict fracture risk. Early prediction of osteoporosis has important public health and clinical implications, as osteoporosis usually has no symptoms until the occurrence of fractures. The results from this study suggest that simple, easy, common biochemical markers can still be used to assess the bone turnover in postmenopausal women and hence their risk of developing osteoporosis and fractures.

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