Original Article

Bone Alkaline Phosphatase and Urine Hydroxyproline Assay in Pre and Postmenopausal Women in the State of Sikkim and its Correlation with Bone Mineral Density

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ABSTRACT

Introduction: Osteoporosis could be viewed as a metabolic disease. The WHO guidelines for diagnosing osteoporosis reflect structural damage only and not the metabolic imbalance that leads to it. Biochemical markers of bone turnover have been shown to provide valuable information for diagnosing and monitoring metabolic bone disease. The present study analyzed bone-specific alkaline phosphatase (BALP) and urinary hydroxyproline in pre- and postmenopausal women and correlated them with changes in bone mineral density (BMD) in the state of Sikkim. The study also intended to know the ethnicity-based disease burden in Sikkim. **Materials and Methods:** A hospital-based cross-sectional study was done at a tertiary hospital in Sikkim. Blood and 24-h urine samples from 50 premenopausal and 50 postmenopausal women were analyzed for total alkaline phosphatase (ALP), BALP, and Urine Hydroxyproline. BMD was measured using the quantitative ultrasound technique by Achilles densitometer. **Results:** There was a statistically significant increase in serum calcium (P = 0.01), ALP (P = 0.01), and urine hydroxyproline (P = 0.03) levels in postmenopausal women as compared to premenopausal women. Although ALP was higher in postmenopausal women, BALP isoform was more elevated in premenopausal women (P = 0.001). BMD was significantly lower in postmenopausal women (P < 0.001). It was also noted that there was a significant difference in BMD between tribal and nontribal populations (P = 0.003). Total ALP and BALP as the bone formation marker and urine hydroxyproline as a bone resorption marker added statistically significant r to BMD prediction (P < 0.05). **Conclusion:** In this study, BALP combined with Urine Hydroxyproline was helpful as a screening biomarker to predict osteoporosis in postmenopausal women.

Keywords: *Bone alkaline phosphatase, bone mineral density, bone turn over markers, osteoporosis, postmenopausal women, premenopausal women, urine hydroxyproline*

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Introduction

Osteoporosis, considered a "silent disease," is characterized by reduction of bone mass and alteration of bone architecture, resulting in increased bone fragility, thus increased fracture risk. This deterioration of bone structure is caused by an imbalance in bone remodeling with increased osteoclasts' activity and decreased osteoblasts' activity.[1] Hence, osteoporosis could be viewed as a metabolic disease. The WHO guidelines for the diagnosis of osteoporosis is based on measuring bone mineral density (BMD) using dual-energy X-ray absorptiometry scan and

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defined as a BMD that lies 2.5 standard deviations (SD) or more below the average value for young, healthy women (a T-score of <2.5 SD).[2] The drawbacks of this have been that the definition reflects the physical damage (i.e., loss of density only) and not the metabolic imbalance that leads to it. Moreover, the diagnosis is specific to a particular population group only, and using a specific device reduces access to timely detection for early management. Hence, the need to analyze markers for bone metabolism imbalance has been sought after repeatedly.

Biochemical markers of bone turnover have been shown to provide valuable information for diagnosing and monitoring metabolic bone disease.[3] They reflect the rates of bone resorption (Resorption Markers) and bone formation (bone formation markers). Therefore, they provide a more representative index of overall skeletal bone loss than that obtained by measuring the rates of changes in the BMD at specific sites.[4]

The present study was conducted to analyze bone-specific alkaline phosphatase (BALP) and urinary hydroxyproline, the two common bone turnover markers (BTM), in pre and postmenopausal women and correlate them with BMD in population native to the state of Sikkim. Owing to the different ethnic groups in the Himalayan region, the study was also intended to know the ethnicity-based disease burden for the first time in Sikkim.

Materials and Methods

Study sample

A hospital-based cross-sectional study was done at a tertiary hospital in Sikkim. Fifty premenopausal and 50 postmenopausal women who satisfied inclusion and exclusion criteria and voluntarily participated in the study were included. Women with thyroid disease, skeletal tuberculosis, smokers, alcoholics, women on steroids or hormone replacement therapy, and premenopausal women with pregnancy were excluded from the study.

Marker assay

Each participant collected 5 ml of random blood sample in a plain vacutainer. Serum was separated immediately by centrifuging at 3000 rpm for 10 min and analyzed for total calcium, phosphorus, albumin, BALP, creatinine. The 24 h sample of urine was collected in a sterilized sealed plastic bottle and analyzed for hydroxyproline. BMD was measured using the quantitative ultrasound (QUS) technique by Achilles densitometer.

The data obtained were analyzed, and the difference in the mean of various parameters among the two groups was compared using the "independent samples *t*-test." Statistical analysis was performed using software IBM® SPSS® Modeler 17.0 windows (Statistical Packages for the Social Sciences, Chicago, IL, USA).

Results

Demographic data

The study population consisted of 50 premenopausal and 50 postmenopausal women with a mean age of 38.8 ± 3.7 years and 58.6 ± 3.9 years. Subjects belonged to tribal (8 in pre and 20 in postmenopausal group) and nontribal (42 in pre and 30 in postmenopausal) communities. Mean BMI was noted to be 27 ± 6 kg/m^2 and 28 ± 6.5 kg/m^2 in pre and post-menopausal groups, respectively. The demographic data are summarized in Table 1.

Table 1: Comparison between premenopausal and postmenopausal group

	Mean±SD (*n*=50)		Statistical significance (*P*)
	Premenopausal group	Postmenopausal group	
Age (years)	38.8±3.7	58.6±3.9	
BMI (kg/m^2)	27±6	28±6	0.04*
Biochemical assay			
Calcium (mg/dl)	9.7±0.37	9.9±0.35	0.01*
Phosphorus (mg/dl)	4.1±0.57	4.1±0.52	NS
Albumin (g/dl)	4.1±0.41	4.1±0.45	NS
Creatinine (mg/dl)	0.95±0.21	0.98±0.26	NS
ALP (U/L)	71±23	81±6	0.01*
BALP (U/L)	44±24	27±17	0.001**
Hydroxy proline (mg/24 h)	32±15	41±15	0.03*
BMD analysis, *n* (%)			
Normal	19 (38)	12 (24)	<0.001**
Osteopenia	27 (54)	28 (56)	
Osteoporosis	4 (8)	10 (20)	

*P<0.05, **P<0.01. Independent *t*-test analysis comparing the means between two groups. BMI: Body mass index, ALP: Alkaline phosphatase, BALP: Bone specific ALP, SD: Standard deviation, BMD: Bone mineral density, NS: Not significant

Biochemical analysis

Blood samples were tested for serum calcium and phosphate level, bone alkaline phosphatase (ALP) isoenzyme. The urine sample was tested for hydroxyproline. It was seen that there was a statistically significant increase in serum calcium (P = 0.01), ALP (P = 0.01), and urine hydroxyproline (P = 0.03) levels in postmenopausal women as compared to premenopausal women. Although ALP was higher in postmenopausal women, BALP isoform was more elevated in premenopausal women (P = 0.001) [Table 1].

Bone quality assessment

BMD was determined by the QUS method and was classified into normal (T score +1 to −1 SD), osteopenia (T score −1 to −2.5 SD), and osteoporosis (T score <−2.5 SD). The results show that among premenopausal women, 19 (38%) had normal BMD, 27 (54%) had osteopenia, and 4 (8%) had osteoporosis. Among postmenopausal women, 12 (24%) were normal, 28 (56%) had osteopenia, and 10 (20%) had osteoporosis. BMD was significantly lower in the postmenopausal group as compared to the premenopausal group (P < 0.001) [Table 1]. It was also noted that there was a significant difference in BMD between tribal and nontribal populations (P = 0.003). Among the tribal population, 42% had osteopenia, and 13% had osteoporosis. In the nontribal population, 59.7% had osteopenia, and 16% had osteoporosis [Table 2].

Correlation between bone turnover markers and bone mineral density

Multiple regression was run to predict BMD from the bone formation markers (Total ALP, BALP) and the bone resorption marker (urine hydroxyproline). The assumptions of linearity, independence of errors, homoscedasticity, unusual points, and normality of residuals were met. These variables statistically significantly predicted BMD (F[7, 92] = 3.801, P < 0.001, adj R^2 = 0.165). Total ALP and BALP as the bone formation marker and urine hydroxyproline as the bone resorption markers added statistically significantly to BMD prediction (P < 0.05). Regression coefficients and standard errors are summarized in Table 3.

Discussion

Osteoporosis is a metabolic bone disorder manifesting as fragility fractures increasing the morbidity and mortality of both sexes globally. Increasing longevity and a more significant proportion of the Indian population over the age of 50 years are likely to increase the number of people affected by osteoporosis. The exact burden of osteoporosis in India is not known as the data on the prevalence of osteoporosis among women in India come from studies conducted in small groups spread across the country. Various factors such as low calcium intake with an extensive prevalence of Vitamin D deficiency, increasing longevity, sex inequality, early menopause, genetic predisposition, lack of diagnostic facilities, and poor bone health knowledge have contributed to the high prevalence of osteoporosis.

The most important consequence of osteoporosis is fragility fracture, which incurs a high degree of morbidity and mortality. Hence, diagnosis, treatment, and monitoring of osteoporosis treatment are critical. A significant challenge in this regard is that osteoporosis is asymptomatic until presenting with a fracture; thus, clinical diagnosis and subsequent treatment rely on radiologic and laboratory testing in "patients at risk" based on clinical history and demographics.

Disease burden

Approximately 30% of all postmenopausal women in the United States and Europe and 25%–62% of postmenopausal women in India have osteoporosis.[5-7] These studies have used the manufacturer's white Caucasian reference database. The Indian Council for Medical Research (ICMR) carried out a large multicenter study to generate an India-specific database, which showed that Indians have lower BMD than their North American counterparts.[8] Paul T *et al.*, in their study to assess the effect of the newly generated ICMR database (ICMRD) on the diagnosis of osteoporosis, concluded that a more significant proportion of patients were diagnosed as having osteoporosis using the Hologic reference database, as compared to the ICMRD.[9] The present study found that 54% had osteopenia among premenopausal women, and 8% had osteoporosis. Moreover, among postmenopausal

Table 2: Bone quality assessment amongst the tribal and nontribal population with percentage-wise distribution ranging from normal to osteoporotic findings

Bone quality assessment	Tribal (n=38) (%)					Nontribal (n=62) (%)						
	Lepcha	Bhutia	Sherpa	Subba	Tamang	Sharma	Pradhan	Chettri	Rai	Manger	Bhujel	Others
Normal			47.4						24.2			
Osteopenia			42.1						59.7			
Osteoporosis			13.2						16.1			

Table 3: Correlation between bone mineral density and bone turnover markers

Variable	*B*	SE_B	Standardized co-efficient	*P*
Total ALP	−0.010	0.005	−0.224	0.048
Bone specific ALP (BALP)	0.011	0.005	−0.224	0.024
Urine hydroxy proline	−0.027	0.007	−0.373	0.0001

Correlation between BMD and BTM using multiple regression model. $P<0.05$ Statistically significant. *B*: Unstandardized regression coefficient, SE_B: Standard error of the co-efficient, ALP: Alkaline phosphatase, BALP: Bone specific ALP, BMD: Bone mineral density, BTM: Bone turnover marker

women, 56% had osteopenia, and 20% had osteoporosis. It was also noted that there was a significant difference between osteopenia (42.1% tribal, 59.7% nontribal) and osteoporosis (13% tribal, 16.1% nontribal) among the tribal and nontribal populations. Such difference in BMD among people of different ethnicity has been well documented in studies.[10] Studies have reported that Asian women have a higher predisposition for osteoporosis than their Caucasian counterparts.[11] Reasons attributed for lower BMD in Indians include possible genetic differences, nutritional deficiencies, and smaller skeletal size.[12] Tribal women in Sikkim have a different lifestyle and body build-up owing to difficult terrain conditions, which probably explain a lesser incidence of osteoporosis as compared to nontribal inhabitants of Sikkim.

Role of bone turnover markers

The mainstay for the diagnosis of osteoporosis remains radiographic techniques such as DXA scan. However, as osteoporosis emerges directly from alterations in the number of osteoblasts and osteoclasts, it follows that biomarkers of the activity of these cells reflect current levels of bone turnover. Bone formation markers are produced by osteoblastic cells or derived from procollagen metabolism, whereas resorption markers are the degradation products of osteoclasts or collagen degradation.[13] Bauer *et al*.,[14] in their study to predict hip bone loss using BTM, stated that a clear relationship had been documented between perimenopausal BTM levels and subsequent bone loss. The association between BTM levels and subsequent bone loss in older women is less obvious. As the positive predictive value of altered BTM levels for accelerated bone loss in elderly white women is modest, they suggested that, in assessing BTM, one bone formation marker and one bone resorption marker are to be combined for an accurate representation of bone metabolism.

Among the available BTM, BALP as bone formation marker and urine hydroxyapatite are easily accessible and cost-effective. The bone-specific isoform of ALP is directly released into the circulation in proportion with the number and differentiation state of osteoblasts.[15] Of the total ALP found in the circulation, 95% of the enzyme in blood originates from either the liver or bone. In health, the bone to liver isoforms ratio is approximately 1:1. Osteoblasts produce the bone isoform of ALP as a tetramer. BALP plays role in bone mineralization process by hydrolysis of phosphate esters at the osteoblast cell surface to provide a high phosphate concentration for bone remodeling. As a result, BALP levels represent active bone formation and bone growth periods. During life, there are two age-dependent physiological peaks of high BALP activity, during infancy and at the time of puberty, when the effects of sex steroids accelerate bone growth.[16]

In the present study, it was observed that total ALP was significantly higher in postmenopausal women (81 ± 6 U/L) as compared to premenopausal women (71 ± 23U/L). The mean value of BALP in premenopausal women (44 ± 24U/L) was significantly higher than in postmenopausal women (27 ± 17U/L). This contradicts what has been demonstrated by various studies that BALP has a positive correlation with age after menopause. Seifert-Klauss *et al*.,[17] conducted a study to evaluate bone loss in premenopausal, perimenopausal, and postmenopausal women. Interestingly, they found that although significantly higher levels of bone markers (osteocalcin, bone-specific ALP, c-terminal telopeptide cross-linked collagen type I) were measured in post menopause, the most significant increase in these markers was seen during the menopausal transition and thus concluded that women in the menopausal transition which extends for up to 10 years, lose trabecular bone at a rapid rate. This could explain the higher levels of BALP in premenopausal women noted in our study as the mean age of the premenopausal group was closer to this transition age of menopause.

Role of bone mineral density

The definition of osteoporosis, according to WHO, is based on the measurement of BMD using a DXA scan. However, this mode of measuring BMD is neither easily accessible nor affordable for osteoporosis screening in a large population in an economically developing country such as ours. According to an International Osteoporosis Foundation (IOF) survey, conducted in 11 countries, the number of people with osteoporosis may be underestimated in rural areas throughout the Asian countries,[18] as in most developing Asian countries access to DXA technology is neither easily available nor affordable relatively expensive and is not widely available in most developing Asian countries, especially in rural areas. In this IOF survey, it was seen that

countries such as China, India, Indonesia, Pakistan, the Philippines, Sri Lanka, and Vietnam are severely under-resourced, with less than 1 DXA machine per million population.[18]

QUS sonography is an alternative to measure the BMD in the appendicular skeleton, such as calcaneus, phalanges of the hand, and tibia. QUS parameters are highly correlated with structural and architectural parameters, such as trabecular volume, number, strength, and load-bearing capacity.[19] Several studies showed that the fracture prediction by QUS was equal and sometimes better than DXA.[20] These techniques are safe, easy to use, radiation-free, and portable devices, thus making them ideal for screening. Using this model in our study, we found that although osteoporosis was marginally higher in postmenopausal women than in premenopausal women, osteopenia was almost equally distributed in both groups. This also corroborates our finding of rapid bone loss in the perimenopausal age group, as demonstrated by BALP.

Correlation of bone mineral density and bone turnover markers

For osteoporosis screening in a vast population in remote areas, it is important to have easily accessible tools. BTM are sought to be a surrogate for bone loss and identify rapid bone losers to take preventive measures. The present study was one such attempt to find alternatives for screening. In this aspect, we found that Total Alp and BALP as the bone formation marker and hydroxyproline as the bone resorption marker added statistically significantly to BMD prediction.

Ross and Knowlton[21] in their study showed a continuous relationship between the measured levels of various BTM and the risk of rapid bone loss at the calcaneus: For each SD increase in serum BALP, serum osteocalcin, urinary-free pyridinoline, the odds of rapid bone loss (>2.2%/year) doubled. In a similar comparison among older Chinese women, Zhang *et al*.[22] found a positive correlation between osteocalcin and BALP but a negative correlation with total and sub-regional BMD (at the lumbar spine and total hip). Those authors also found that BALP was a valuable parameter for evaluating age-related changes in bone turnover. However, Vestergaard *et al.,* showed that serum osteocalcin, BALP, and Hydroxyproline are poor predictors of lumbar and hip bone loss in individual perimenopausal women.[23] Similarly, Lumachi *et al*.[24] evaluated BAP and other bone formation markers (osteocalcin, type I collagen, and BMD) in older men with no history of fractures; they found no relationship between BALP and bone density in this population.

Thus, the role of BALP and urine hydroxyproline in predicting bone density has not been defined precisely. However, our study showed that Total ALP and BALP as the bone formation marker and urine hydroxyproline as the bone resorption marker added statistically significantly to BMD prediction. It shows encouraging results for these BTMs to be applied as a predictor of BMD in the Sikkim population.

Conclusion

In this study, BALP combined with urine hydroxyproline was helpful as a screening biomarker to predict osteoporosis in postmenopausal women. However, the same may be tested in a larger population to establish its credibility as a simple screening biomarker for osteoporosis in the community.

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Conflicts of interest

There are no conflicts of interest.

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