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 turnover markers, osteoporosis, postmenopausal women, premenopausal women, urine hydroxyproline
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)**                                            |
| **Premenopausal group**                                       |
| **Postmenopausal group**                                     |
| Statistical significance (P)                                 |
| Age (years)                                                  | 38.8±3.7 | 58.6±3.9 | 0.04* |
| BMI (kg/m$^2$)                                                | 27±6 | 28±6 | NS |
| 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) | NS |
| Osteoporosis                                                  | 4 (8) | 10 (20) | NS |

*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 isofrom 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. 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. 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. The present study found that 54% had osteopenia among premenopausal women, and 8% had osteoporosis. Moreover, among postmenopausal

| Bone quality assessment | Tribal (n=58) (%) | Nontribal (n=62) (%) |
|------------------------|------------------|---------------------|
|                        | Lepcha | Bhutia | Sherpa | Subba | Tamang | Sharma | Pradhan | Chetri | Rai | Manger | Bhuje | Others |
| Normal                 | 47.4   |        |        |        |        | 24.2    |         |        |      |        |       |        |
| Osteopenia             | 42.1   |        |        |        |        | 59.7    |         |        |      |        |       |        |
| Osteoporosis           | 13.2   |        |        |        |        | 16.1    |         |        |      |        |       |        |

Table 2: Bone quality assessment amongst the tribal and nontribal population with percentage-wise distribution ranging from normal to osteoporotic findings
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. Studies have reported that Asian women have a higher predisposition for osteoporosis than their Caucasian counterparts. Reasons attributed for lower BMD in Indians include possible genetic differences, nutritional deficiencies, and smaller skeletal size. 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. 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.

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

| Variable                    | B    | SE_n | 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 hydroxyproline        | -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 co-efficient, SE: Standard error of the co-efficient, ALP: Alkaline phosphatase, BALP: Bone specific ALP, BMD: Bone mineral density, BTM: Bone turnover marker.

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 ± 23 U/L). The mean value of BALP in premenopausal women (44 ± 24 U/L) was significantly higher than in postmenopausal women (27 ± 17 U/L). This contradicts what has been demonstrated by various studies that BALP has a positive correlation with age after menopause. Seifert-Klauss et al. 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, 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.

REFERENCES

1. Florencio-Silva R, Sasso GR, Sasso-Cerri E, Simões MJ, Cerri PS. Biology of bone tissue: Structure, function, and factors that influence bone cells. Biomed Res Int 2015;2015:421746.
2. Cosman F, de Beur SJ, LeBoff MS, Lewiecki EM, Tanner B, Randall S, et al. Clinician’s guide to prevention and treatment of osteoporosis. Osteoporos Int 2014;25:2359-81.
3. Woitge HW, Scheidt-Nave C, Kissling C, Leidig-Bruckner G, Meyer K, Grauer A, et al. Seasonal variation of biochemical indexes of bone turnover: Results of a population-based study. J Clin Endocrinol Metab 1998;83:68-75.
4. Delmas PD, Eastell R, Garnero P, Seibel MJ, Stepan J; Committee of Scientific Advisors of the International Osteoporosis Foundation. The use of biochemical markers of bone turnover in osteoporosis. Committee of Scientific Advisors of the International Osteoporosis Foundation. Osteoporos Int 2000;11 Suppl 6:S2-17.
5. Shatruga V, Kulkarni B, Kumar PA, Rani KU, Balakrishna N. Bone status of Indian women from a low-income group and its relationship to the nutritional status. Osteoporos Int 2005;16:1827-35.
6. Marwaha RK, Tandon N, Garg MK, Kanwar R, Narang A, Sastry A, et al. Bone health in healthy Indian population aged 50 years and above. Osteoporos Int 2011;22:2829-36.
7. Rajan R, Paul J, Kapoor N, Cherian KE, Paul TV. Postmenopausal osteoporosis – An Indian perspective. Curr Med Issues 2020;18:98.
8. Vaidya R, Shah R. Bone mineral density and reference standards for Indian women. J Midlife Health 2010;1:55.
9. Paul T, Asha HS, Mahesh DM, Naik D, Rajaratnam S, Thomas N, et al. The diagnosis of osteoporosis among subjects of Southern Indian origin above 50 years of age - The impact of the Indian council of medical research versus Caucasian bone mineral density reference standards. Indian J Endocrinol Metab 2012;16:S514-24.
10. Leslie WD. Clinical review: Ethnic differences in bone mass – Clinical implications. J Clin Endocrinol Metab
11. Khadilkar AV, Mandalik RM. Epidemiology and treatment of osteoporosis in women: An Indian perspective. Int J Womens Health 2015;7:841-50.
12. Mithal A, Bansal B, Kyer CS, Ebeling P. The Asia-Pacific regional audit-epidemiology, costs, and burden of osteoporosis in India 2013: A report of International Osteoporosis Foundation. Indian J Endocrinol Metab 2014;18:449-54.
13. Christenson RH. Biochemical markers of bone metabolism: An overview. Clin Biochem 1997;30:573-93.
14. Bauer DC, Sklarin PM, Stone KL, Black DM, Nevitt MC, Ensrud KE, et al. Biochemical markers of bone turnover and prediction of hip bone loss in older women: The study of osteoporotic fractures. J Bone Miner Res 1999;14:1404-10.
15. Kress BC, Mizrahi IA, Armour KW, Marcus R, Emkey RD, Santora AC 2nd. Use of bone alkaline phosphatase to monitor alendronate therapy in individual postmenopausal osteoporotic women. Clin Chem 1999;45:1009-17.
16. Behnke B, Altrogge H, Delling G, Kruse HP, Müller-Wiefel DE. Bone mineral density in pediatric patients after renal transplantation. Clin Nephrol 1996;46:24-9.
17. Seifert-Klaus V, Fillenberg S, Schneider H, Luppa P, Mueller D, Kiechle M. Bone loss in premenopausal, perimenopausal and postmenopausal women: Results of a prospective observational study over 9 years. Climacteric 2012;15:433-40.
18. Ingle BM, Machado AB, Pereda CA, Eastell R. Monitoring alendronate and estradiol therapy with quantitative ultrasound and bone mineral density. J Clin Densitom 2005;8:278-86.
19. International Osteoporosis Foundation. The Asia-Pacific Regional Audit – Epidemiology, costs and Burden of Osteoporosis in 2013; 2013. Available from: https://www.osteoporosis.foundation/educational-hub/files/asia-pacific-regional-audit-2013. [Last accessed on 2021 Sep 10].
20. Mészáros S, Tóth E, Ferenz V, Csúpor E, Hosszú E, Horváth C. Calcaneal quantitative ultrasound measurements predicts vertebral fractures in idiopathic male osteoporosis. Joint Bone Spine 2007;74:79-84.
21. Ross PD, Knowlton W. Rapid bone loss is associated with increased levels of biochemical markers. J Bone Miner Res 1998;13:297-302.
22. Zhang XY, He JW, Fu WZ, Liu YI, Zhang ZL. Associations of serum osteocalcin and polymorphisms of the osteocalcin gene with bone mineral density in postmenopausal and elderly Chinese women. J Nutrigenet Nutrigenomics 2016;9:231-42.
23. Vestergaard P, Hermann AP, Gram J, Jensen LB, Eiken P, Abrahamsen B, et al. Evaluation of methods for prediction of bone mineral density by clinical and biochemical variables in perimenopausal women. Maturitas 2001;40:211-20.
24. Lumachi F, Orlando R, Fallo F, Basso SM. Relationship between bone formation markers bone alkaline phosphatase, osteocalcin and amino-terminal propeptide of type I collagen and bone mineral density in elderly men. Preliminary results. In Vivo 2012;26:1041-4.