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Status of serum calcium and vitamin D: a comparative study between premenopausal and postmenopausal women

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Abstract

Introduction: Nowadays, most of the postmenopausal women are experiencing deficiencies in calcium and vitamin D, which has led to an increased risk of osteoporotic fractures. Prompt estimation of serum calcium, phosphorus, vitamin D, and alkaline phosphatase (ALP) can aid in preventing complications and providing proper treatment. This study aims to compare serum calcium, phosphorus, vitamin D, and ALP levels in premenopausal and postmenopausal women.

Method: This was a quantitative cross-sectional study conducted in orthopaedic department at Kathmandu Medical College between 01 July – 31 December 2024. Ethical clearance was taken from Institutional Review Committee. Using consecutive sampling, participants' serum samples were analysed for calcium, phosphorus, and ALP activity using an automated analyser, and vitamin D was analysed using chemiluminescence immunoassays (CLIA) methods. T-test, Anova, Pearson's correlation and Multiple Linear Regression analysis were calculated using SPSS.

Result: Out of 244 participants, Body mass index (24.08±1.51 kg/m2), ($p<0.001$) vitamin D (24.11±9.27 ng/ml), ($p=0.039$), phosphorus (3.58±0.78 mg/dl), ($p=0.232$) and ALP (90.81±26.48 IU/L), ($p<0.001$) were higher in postmenopausal women, while serum calcium (9.14±0.50 mg/dl), ($p=0.009$) level was higher in premenopausal women. Vitamin D shows an insignificant positive correlation with serum calcium and ALP, while a negative correlation with phosphorus.

Conclusion: The study concludes that postmenopausal women had a higher BMI. A decline in serum calcium and a rise in ALP levels among postmenopausal women were observed, indicating bone turnover and bone loss. Regular assessment and supplementation of calcium and vitamin D may be necessary to support bone health and prevent bone-related complications.

How to cite

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Introduction

Calcium is an essential mineral that is vital for several physiological processes like cell signalling, muscle contraction, blood coagulation and along with phosphate contributes bone and teeth mineralization.[1,2] The need for calcium for healthy bones changes with age. Its demand is high during adolescence, slightly low in the twenties, and stays relatively stable through early and middle adulthood.

However, at menopause, the bone resorption rate increases and bone mass declines, associated with a fall in oestrogen production.[3] Similarly, postmenopausal women are at high risk for developing osteoporosis, cardiovascular diseases (CVD), and reproductive cancers.[4,5] Additionally, nutritional disturbances or especially deficiencies of trace elements and vitamins, which are essential to maintain health and well-being, are likely to occur during the postmenopausal period.[6]

Nepalese postmenopausal women are experiencing a deficiency in both vitamin D and calcium.[7] Hypocalcaemia in elderly women was reported to be 24%.[8] While the hypovitaminosis D ranges from 32.0% to 73.6%, with a higher incidence detected in the female population.[9] Calcium and vitamin D are often discussed together for promoting bone health. Their abnormality in menopausal women could be a useful tool for healthcare professionals for the treatment and follow up of the cases. With this perspective, the present comparative cross-sectional study was undertaken.

Method

This quantitative cross-sectional study was conducted in Orthopaedic in and outpatient department at Kathmandu Medical College from 01 Jul 2024 to 31 Dec 2024 after obtaining ethical approval from institutional review committee (IRC), Ref:08122023/01 Study population was grouped as premenopausal (30-45 years) and postmenopausal women with their age ranging from 46-60 years. In this study menopausal status was determined based on participant's history of amenorrhea for ≥12 consecutive months. Menopause typically occurs between the ages of 45 and 55, but including women up to 60 years of age captures a significant portion of the immediate postmenopausal period, which is crucial for studying the pronounced hormonal changes during this time.

Grouping of cases, based on menopausal period, 244 cases were arbitrarily divided into 3 groups, i.e., premenopausal comprising of participants aged 30-45 years, postmenopausal having 46-55 years and ≥56 years

Postmenopausal women up to 60 are still relatively early in their postmenopausal years, making them ideal subjects for investigating the initial effect of serum calcium and vitamin D levels. Beyond 60, other age-related factors and comorbidities increasingly influence these levels, potentially confounding results. Out of 244 women participating in the study, 122 participants were in premenopausal group and the rest were in the postmenopausal group. Selections of participants were done on the basis of inclusion and exclusion criteria as mentioned below. The participants who wish to take part in the study were enrolled after explaining the nature of study and its objective. A written consent was obtained and structured questionnaire were provided to gather the relevant information on demographics and medical history. Participants were enrolled in groups by using non-probability sampling methods with consecutive sampling technique. Study subject was instructed to perform the biochemical tests, such as serum calcium, phosphorous, vitamin D and alkaline phosphatase (ALP) following the procedure which were routine investigations. Under aseptic conditions, a venous blood sample was collected in a vial. Serum was separated after centrifugation and specific biochemical tests such as serum calcium, phosphorous and ALP were analysed using automated analyser and vitamin D was analysed by chemiluminescence immunoassays (CLIA) method. The obtained results were recorded. The value of normal serum calcium is 8.4–10.4mg/dL,[10] serum

phosphorus is 3.5-5.5mg/dL,[11] and Alkaline phosphatase (ALP) is 46-116U/L.[12] But the reference range of vitamin D is categorized as: deficient ≤20ng/ml, insufficient 20-29ng/ml and sufficient >30ng/ml.[13]

Inclusion criteria for premenopausal group were reproductive age of 30-45 years with a normal menstrual cycle, and postmenopausal group age of 46 – 60 years menopause women with one year of amenorrhea and were not receiving any hormonal replacement therapy.

Exclusion criteria were women with menstrual disorders like- irregular menses and menorrhagia, with any bone fracture in previous one year, on hormonal replacement therapy, oral contraceptives, smoker, alcoholic, under any oestrogen therapy or any supportive treatment for menopausal symptoms for at least 6 months before study, diabetes, hypertension, malabsorption and any bone diseases were excluded from the study based on history, clinical examination by clinicians and by laboratory criteria (fasting plasma glucose ≥126mg/dl, BP ≥140/90mmHg, or current treatments), similarly malabsorption was excluded via history of chronic diarrhoea, weight loss and relevant laboratory results (low serum albumin, abnormal stool fat). Bone disease was excluded by clinical history, examination for skeletal deformities/tenderness, and laboratory evaluations (Serum ALP, calcium, and phosphorus) that contradict the result findings.

The prevalence of vitamin D deficiency observed in females was 81.51%.[14]

The sample size was calculated by using the formula, $n=z^2pq/e^2$, where z=1.96 at 95% Confidence Interval (CI), P=prevalence of Vitamin D deficiency=81.51%, q=(1-p), e=permissible error at 5% with degree of assurance as 95% confidence level. So, n=(1.96)2x0.8151x0.1849/0.0025=231.59. For a non-response rate of 5%, n=231.59x5=11.57, 231.59+11.57=243.16. So, the required sample size for this study was 244.

The sample size calculation was initially based on the prevalence formula, which does not align with the comparative design. However, a post-hoc analysis comparing premenopausal calcium 9.14±0.50 mg/dl vs. postmenopausal calcium 8.96±0.55 mg/dl. The calculated effect size was ~0.34, yielding a statistical power of ~76%. This is slightly below the ideal 80% threshold, and suggests moderate confidence in detecting the observed difference.

Data were entered in an Excel sheet and analysed using SPSS version 21. Descriptive statistics were represented as mean±standard deviation (SD) with 95% confidence intervals for continuous data (age, height, weight, body mass index (BMI) based on WHO guidelines, serum calcium, phosphorus, vitamin D and ALP) and categorical data (gender, religion) were depicted as frequency numbers. The Shapiro-Wilk test indicated that variables such as age and BMI deviated significantly from a normal distribution ($p \leqslant 0.05$). However, given the large sample size (n=244), the Central Limit Theorem ensures that the sampling distribution of the mean is approximately normal, justifying the use of parametric tests. Therefore, the independent sample T-test was considered appropriate.

Statistical analysis was done to compare mean serum calcium, phosphorus, vitamin D and ALP levels, which were evaluated by using independent t-tests and Anova. Assessment of correlation was done by Pearson's correlation coefficient to analyse the relation between calcium, phosphorus, vitamin D and ALP in pre- and postmenopausal women, and multiple linear regression analysis of predictor of serum calcium in postmenopausal women in status of calcium and vitamin D was done with statistical significance assumed at p≤0.05.

Result

Out of a total of 244 participants, premenopausal women (30-45 years old) and postmenopausal (46-60 years old), the average age was 37.13 and 52.58 years, respectively.

The average age of menopause was 46.42 years. Most were Hindu, Table 1.

The average height(cm) was 153.84±4.38 and 154.10±4.52 (p=0.645), weight (kg) was 54.96±4.39 and 57.16±4.01 p<0.001). Similarly, BMI was 23.16±1.58 and 24.08±1.51 (p<0.001) in pre- and postmenopausal women, respectively, Table 2.

We found the mean value of serum calcium level in premenopausal (9.14±0.50 mg/dl) and postmenopausal women (8.96±0.55 mg/dl) was compared where p<0.009 which was statistically significant, similarly mean vitamin D level in premenopausal was 21.83±7.79 ng/ml and postmenopausal women was 24.11±9.27 ng/ml was also statistically significant p=0.039 and mean serum phosphorous level was 3.46±0.81 mg/dl in premenopausal women and 3.58±0.78 mg/dl in postmenopausal women which was statistically insignificant p=0.232. The mean value of ALP in premenopausal women was 74.91±17.68 IU/L and 90.81±26.48 IU/L in postmenopausal women was statistically significant p<0.001, Table 3.

We compared the mean value of serum calcium level in premenopausal was 9.14±0.50 mg/dl, postmenopausal (46-55 years) was 8.94±0.49 mg/dl and postmenopausal (>56 years) was 9.01±0.66 mg/dl which were statistically significant with p<0.026. Similarly, mean vitamin D levels in pre, postmenopausal (46-55 years) and (>56 years) were 21.83±7.79 ng/ml, 23.72±8.77 ng/ml and 25.00±10.41 ng/ml which were statistically insignificant with p=0.089 respectively. Likewise, mean serum phosphorous level in pre, postmenopausal (46-55 years) and (>56 years) were 3.46±0.81 mg/d, 3.50±0.80 mg/dl and 3.75±0.69 mg/dl respectively which were statistically insignificant with p=0.134. Furthermore, the mean value of ALP in premenopausal was 74.91±17.68 IU/L, postmenopausal (46-55 years) was 85.99±17.59 IU/L, and postmenopausal (>56 years) was 101.89±38.13 IU/L, and were statistically significant with p<0.001, Table 4.

It was observed that vitamin D shows an insignificant positive correlation with calcium and ALP, while it shows a negative correlation with phosphorous. Similarly, calcium shows insignificant negative correlation with phosphorus and ALP, Table 5.

Although the regression model showed a positive coefficient for vitamin D, the association with serum calcium was statistically insignificant (p>0.05) after adjusting for BMI and age. This suggests that, within this population, higher vitamin D levels were not a strong independent predictor of calcium status when potential confounders were considered. The observed positive trend may reflect biological plausibility- vitamin D supports intestinal calcium absorption-but the lack of significance indicates that other factors, such as hormonal status, dietary intake, or homeostatic mechanisms, may play more dominant role, Table 6.

Vitamin D level was sufficient in 17 premenopausal and 21 postmenopausal women, while the rest had deficiency or insufficiency, Figure 1.

Table 1. Socio-demographic characteristics of premenopausal and postmenopausal women participants in a study on status of serum calcium and vitamin D, n=244

Variables	n(%)	
	Premenopausal, 122	Postmenopausal, 122
Age in year, Mean±SD	37.13±4.70	52.58±4.10
Menopause age, Mean±SD		46.42±1.58
Religion		
Hindu	97(79.5%)	94(77%)
Buddhist	18(14.8%)	22(18%)
Christian	5(4.1%)	6(4.9%)
Muslim	2(1.6%)	--

Table 2. Anthropometric measurement of premenopausal and postmenopausal women participants in a study on status of serum calcium and vitamin D, n=244

Variables	Premenopausal (n=122)	Postmenopausal (n=122)	p-value (t-test)
Weight kg,Mean±SD	54.96±4.39	57.16±4.01	<0.001
Height cm,Mean±SD	153.84±4.38	154.10±4.52	0.645
BMI kg/m^2Mean±SD	23.16±1.58	24.08±1.51	<0.001
BMI normal, n(%)	106(86.9%)		90(73.8%)
Overweight, n(%)	16(13.1%)		31(25.4%)
Grade-I obesity,n(%)	-		1(0.8%)

Table 3. Comparison of various parameters of premenopausal and postmenopausal women participants in a study on status of serum calcium and vitamin D, n=244

Parameter (Sr.)	Group	n	Mean±SD	p-value (t-test)
Calcium(mg/dl)	Premenopausal	122	9.14±0.50	0.009
	Post-menopausal		8.96±0.55	
Vitamin D(ng/ml)	Premenopausal		21.83±7.79	0.039
	Post-menopausal	122	24.11±9.27	
Phosphorous(mg/dl)	Premenopausal		3.46±0.81	0.232
	Post-menopausal	122	3.58±0.78	
ALP(IU/L)	Premenopausal	122	74.91±17.68	<0.001
	Post-menopausal		90.81±26.48	

Table 4. Comparison of various parameters of premenopausal and postmenopausal women participants in a study on status of serum calcium and vitamin D, n=244

Parameter (Sr.)	Group	n	Mean±SD	p-value, Anova
Calcium(mg/dl)	Premenopausal (30-45 y)	122	9.14±0.50	0.026
	Postmenopausal (46-55 y)	85	8.94±0.49	
	Postmenopausal (>56 y)	37	9.01±0.66	
Vitamin D(ng/ml)	Premenopausal (30-45 y)	122	21.83±7.79	0.089
	Postmenopausal (46-55 y)	85	23.72±8.77	
	Postmenopausal (>56 y)	37	25.00±10.41	
Phosphorous(mg/dl)	Premenopausal (30-45 y)	122	3.46±0.81	0.134
	Postmenopausal (46-55 y)	85	3.50±0.80	
	Postmenopausal (>56 y)	37	3.75±0.69	
ALP(IU/L)	Premenopausal (30-45 y)	122	74.91±17.68	<0.001
	Postmenopausal (46-55 y)	85	85.99±17.59	
	Postmenopausal (>56 y)	37	101.89±38.13	

Table 5. Correlation of serum calcium, phosphorus, ALP in postmenopausal women participants in a study on status of serum calcium and vitamin D, n=122

Parameter	Correlation coefficient r-value	p-value
Vitamin D v/s calcium	0.157	0.085
Vitamin D v/s phosphorus	-0.091	0.317
Vitamin D v/s ALP	0.022	0.812
Calcium v/s phosphorous	-0.085	0.352
Calcium v/s ALP	-0.032	0.723

Table 6. Multiple Linear Regression analysis of predictor of serum calcium in postmenopausal women in status of calcium and vitamin D, n=122

Predictor	B (unstandardized coefficient)	Std. Error	Beta (standardized coefficient)	t-statistics	p-value
Constant	7.804	1.003	-	7.785	<0.001
Vitamin D	0.008	0.005	0.141	1.533	0.128
BMI	0.032	0.033	0.088	0.967	0.335
Age	0.0040	0.012	0.288	0.288	0.774

Model statistics: R2=0.033, Adjusted R2=0.008, F(3,118)=1.344, p=0.263; B=unstandardized regression coefficient, represents the change in serum calcium(mg/dl) for each 1unit change in the predictor, holding other variable constant; Model explain (0.033X100=3.3%) 3.3% of the variance in serum calcium, F(3,118)=1.344, p=0.263- statistically insignificant, although the regression coefficients indicated a positive but weak relationship for both vitamin D and BMI; Beta=standardized coefficient, used to compare effect sizes across predictors; p<0.005 considered statistically significant

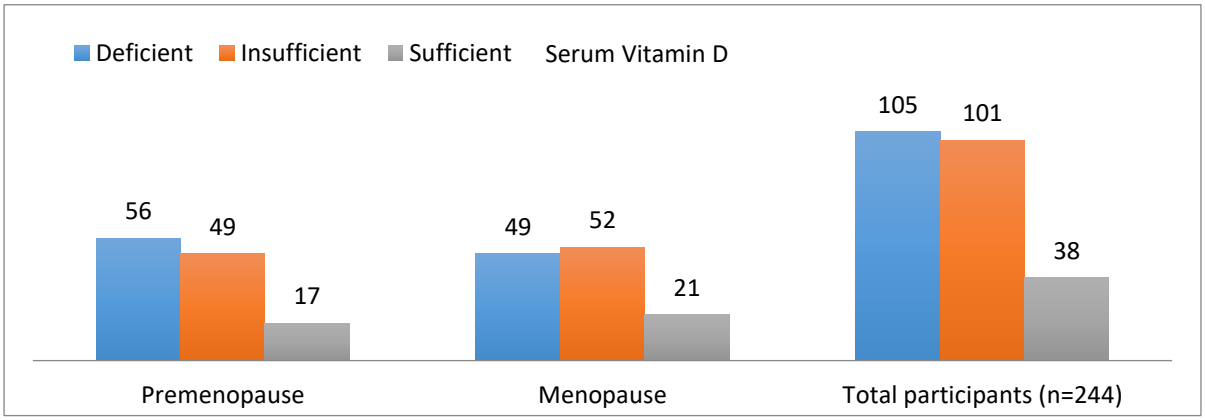

Figure 1. Cone diagram showing the serum vitamin D level in premenopausal and postmenopausal women participants in a study on status of serum calcium and vitamin D, n=244

Discussion

This cross-sectional study of pre- and postmenopausal women showed, the average age for menopause 46.42±1.58 years. A higher BMI was found in postmenopausal women compared to that in premenopausal women (p<0.001). Similarly, vitamin D was deficient in both groups, more severe in the premenopausal group. Various physiological and biochemical changes are seen during bone metabolism which is linked with menopause.[15] A previous study also reported a similar type of finding.[16]

Religion was recorded to describe the study population comprehensively and to assess whether the sample represents the broader community or not.

Serum calcium level was found to be lower in postmenopausal women when compared to premenopausal women which was 8.96±0.55 mg/dl and 9.14±0.50 mg/dl respectively and was statistically significant (p=0.009). A similar finding was also reported in a previous study.[17]

The mean calcium in premenopausal, postmenopausal (46-55 years) and postmenopausal (>56 years) showed statistically significant as p=0.026.

We noted interesting observation that calcium was within the normal limit during premenopausal but as women reaches the postmenopausal (46-55 years) period the calcium level reached to subnormal level but during postmenopausal(>56 years) period, the levels of serum calcium reached normal state, which showed that calcium level got deteriorate in postmenopausal (46-55 years) state which could be due to hormonal changes and later other factor such as diet, supplement might regulatory mechanism get activated.

The progress of bone loss is most quickly during the first 8-10 years following menopause due to decline in oestrogen level, after which it continues at a slower, age-related rate.[18] A decrease in calcium level is also linked with other factors, such as hypovitaminosis D and reduced intestinal absorption of calcium.[19,20]

In present study, vitamin D was deficient in both groups; premenopausal women showed a lower level of vitamin D 21.83±7.79 ng/ml, compared to those in postmenopausal women (24.11±9.27 ng/ml), which was statistically significant (p=0.039). A similar finding was reported with a decrease in vitamin D levels in premenopausal women,[21] which appears counterintuitive, and could be due to reduced oestrogen levels and limited outdoor activity are generally associated with lower vitamin D status. This unexpected finding may reflect the influence of unmeasured or uncontrolled confounders, e.g., many postmenopausal women may have received vitamin D supplementation, calcium therapy, or may have frequent regular health examinations than premenopausal women. Additionally, differences in BMI, dietary habits, or sunlight exposure could have affected vitamin D synthesis and metabolism.

When potential confounders such as BMI and age were considered in multivariate analysis, the association between vitamin D and serum calcium remained statistically insignificant (p=0.089), suggesting that vitamin D alone may not be an independent determinant of calcium homeostasis in the study population.

Meanwhile, contradictory findings were reported in another study,[7] who observed lower vitamin D levels among postmenopausal women. Several factors may explain this discrepancy like, postmenopausal women in our study may have been more health conscious or more likely to take vitamin D or calcium supplements following menopause, sunlight exposure, diet or sampling period might have influenced the results so, these findings highlight the importance of considering potential confounders such as supplement use, BMI and age when interpreting vitamin D status across menopausal group.

In a study for Vitamin D in the middle-aged population (20 to 45 Years), the period including attainment of peak bone mass, typically by the age of 30, reported a widespread vitamin D deficiency or insufficiency (hypovitaminosis D), and rural populations had better vitamin D status compared to their urban counterparts, which might be due to longer sun exposure among rural residents, many of whom are agricultural labourers.[22] A deficiency in vitamin D in premenopausal women could be due to reduced sunlight exposure, dietary deficiencies, and advancing age.

Vitamin D is responsible for moderating bone growth and turnover, and its deficiency is most prevalent in postmenopausal women.[23] Vitamin D level is influenced by an age-related decrease in the cutaneous synthesis of vitamin D, a decline in renal activation of calcitriol, poor intestinal sensitivity to vitamin D absorption, and inadequate sun exposure.[18,19]

The level of serum phosphorus was slightly higher in postmenopausal women compared to that of premenopausal women, 3.58±0.78 mg/dl and 3.46±0.81 mg/dl, respectively and the difference was insignificant (p=0.232). Likewise, serum phosphorus was also insignificant in different menopausal group p=0.134 but serum phosphorous noted in postmenopausal (>56 years) was higher in comparison to another menopausal group. Parathyroid hormone (PTH) and calcitriol are two main hormones responsible for the regulation of phosphorus and calcium. Several studies also reported a rise in serum phosphorus in postmenopausal women compared to that in premenopausal women.[24,25]

In this study, serum ALP level was lower in premenopausal women 74.91±17.68 than in postmenopausal women 90.81±26.48 (p<0.001). Similar findings were obtained in various studies.[17,26] Serum ALP was statistically significant in the entire group where p=<0.001.

Its level was increased during the postmenopausal (>56 years) period, suggesting bone deterioration. The ALP is a marker for bone metabolism, with several dimeric isoforms coming from different tissues like the liver, bone, intestine, spleen, kidney, and the placenta. In adults with normal liver function, about 50% of the total ALP activity is contributed by the liver and 50% is contributed by the bone.[27]

An increase in ALP results from hormonal changes and also due to the effect of parathyroid hormone on bone. It is the earliest bone marker in postmenopausal women as it plays an important role in bone formation and resorption.[25] This study showed an insignificant but a positive correlation between serum calcium and vitamin D (r-value 0.157, p=0.085) in postmenopausal women. It could be due to the involvement of vitamin D in the absorption of calcium from the gut.[28]

This study depicted an insignificant and negative correlation of vitamin D with serum phosphorus (r-value -0.091, p=0.317). Vitamin D increases the efficiency of intestinal absorption of calcium by 30-40% and phosphorus by approximately 80%.[29] We found a positive correlation of vitamin D with ALP, but it was statistically insignificant (r-value 0.022, p=0.812), while ALP showed a negative correlation with calcium (r-value -0.032, p=0.723). Similar findings were observed in various studies.[17,26]

This study found a negative, insignificant correlation between calcium and phosphorus (r-value -0.085, p=0.352). It could be due to decreased oestrogen and vitamin D in postmenopausal women, which in turn leads to decreased calcium and increased phosphorus levels. The hypovitaminosis D in Post-menopausal women in our study was 101 (82.8%), which included 40.2% as a deficiency and 42.6% as insufficiency. While another study reported an overall prevalence of 88.5%of hypovitaminosis D in Postmenopausal women.[30] To prevent further deterioration in bone mass, hormonal therapy, periodic dosing of calcium and vitamin D, foods rich in these nutrients, and sunlight may mitigate bone health-related problems and also help maintain normal levels of plasma vitamin D, calcium and phosphorus.

However, this study has some limitations. The sample size was estimated on a prevalence formula that does not align with the comparative design; however, a post-hoc analysis comparing pre- and postmenopausal calcium and effect size shows moderate confidence in detecting the observed difference. The study sample size estimation was not optimal, and post-hoc power analysis indicated low statistical power. Therefore, the lack of significant associations may partly due to insufficient sample size rather than a true null effect. We could not include bone density scan or dual energy x-ray absorptiometry (DEXA) scan, or sensitive marker like N-Terminal Telopeptide (NTx), intact parathyroid hormone (iPTH), total protein, osteocalcin, Follicular Stimulating Hormone, Luteinizing Hormone and oestradiol to confirm menopause status etc, because of the mostly out-of-pocket payment system. Women with prior hysterectomy and/or bilateral oophorectomy (surgical menopause) were not specifically analysed. Information that might affect bone mineral metabolism, such as dietary calcium and vitamin D intake, any supplements taken, exposure to sunlight etc were not analysed.

Conclusion

There was significant decrease in serum calcium and vitamin D levels in both pre- post-menopausal groups. A significant decrease in serum calcium level and an increase in ALP was observed in postmenopausal women, suggesting an increased bone turnover and bone loss. Similarly, mean serum calcium was lower in postmenopausal women while mean serum vitamin D, phosphorous, and ALP activities was higher suggesting accelerating bone remodelling after menopause due to hormonal changes which drives bone loss and compensatory mechanism attempt to maintain bone mineralization. The multiple linear regression analysis showed no statistically

significant association between serum calcium and the independent variables (vitamin D, BMI, and age) after adjustment for potential confounders ($p>0.05$). This indicates that within the present study population, none of these factors independently predicted calcium level. Although weak positive trends were observed for vitamin D and BMI were not significant suggesting that calcium homeostasis in postmenopausal women is influenced by multiple interacting factors rather than single determinants.

Author contribution

Concept design: AD, DB; Literature search: AD, AP, DB; Data collection: JMT, SD; Data analysis: AD; Draft manuscript: AD, AP; Final manuscript and accountability: All

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Conflict of interest

None

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Supplementary material

Data and supplementary material that support the findings of this study are available from the corresponding author upon reasonable request.

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