

Analysis of Serum Vitamin D3 and Calcium Levels with Bone Mineral Density Measurement to Assess Osteoporosis in the Human Body

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Annotation: This cross-sectional study examined the correlation between serum vitamin-D3 level and calcium, site specific BMD and BMC as obtained by DEXA in pre-and postmenopausal women from Pakistan. Serum concentrations of 25-hydroxyvitamin D3 (25(OH)D3) 1,25dihydroxyvitamin D3 [(OH 2 1,25(OH)2D3], calcium, and DEXA measurements of the femoral neck, lumbar spine (L2-L4), and other areas of the skeleton were measured in 100 adults (39 women, 61 men).

The relationships between serum 25(OH)D3 and BMD were in general inverse at the different sites, most pronounced in women, and the ratio 1,25(OH)2D3/25(OH)D3 correlated with makers of bone turnover. Women presented lower BMD and higher osteoporosis frequency at lumbar and femur sites. For men, however, the correlations were weaker and nonsignificant. The findings imply that sex-specific and site-specific aspects may need to be considered for the evaluation of bone health, and serum levels of vitamin D3 and calcium could be a practical serum marker for predicting the risk of osetoporosis in a clinical setting. Futher long-term longitudinal studies are required to elucidate causal relationships and inform patient-specific treatment strategies.

Keywords: Dexa, Vitamin D3, BMD, Calcium, Osteoporosis, DXA.

Introduction

Such diseases are increasingly common as populations age, and have significant clinical and economic implications. Bone mineral density (BMD) is a critical determinant of bone strength and fracture risk and its most precise evaluation is performed using the dual energy x-ray absorptiometry (DEXA) technique [1]. A number of factors influencing both the BMD as well as the serum D3 and Ca levels are of crucial significance as the basic metabolic bone parameter.

The absorption of calcium from the gastrointestinal tract is stimulated by vitaminD3 which acts directly on bone resorption through its active metabolite [2]1,25dihydroxyvitamin D3.[' _5and calcium is a major structural component of bone matrix. While a significant amount of work has been completed in this area, the link between vitamin D3, calcium and BMD is not completely clear, particularly when considering site specific skeletal responses and the gender specific effects. Previous research has not been consistent, as the relationship between vitamin D status and BMD has bee n reported to be strong in some studies (26), but weak or absent in others 34). In addition, the hormonal status, especially postmenopausal women, might be a modifier between vitamin D3 and calcium on bone density, and sex-specific analysis should be required. [3]

Methodology Study Design

The study design It was a cross-sectional observational study carried out on both sexes in apparently healthy adult volunteers from 1 st Jan to 17 th May 2025. The study was carried out in the Department of X-Ray and ethical clearance was obtained from institutes' ethical review board. Informed consent was obtained from all participants before the study.

Participants

One hundred participants (39 females, 61 males) aged 20- to 55- years were included. Their exclusion criteria were history of metabolic bone disease, chronic renal or hepatic disease and malignancy, any other apparent reasons for the establishment of osteoporosis of the present case.

inflammatory diseases or use of calcium or bone metabolism-modifying drugs (e.g. corticosteroids, biphosphonates, calcitriol).

Data Collection

Demographic data was recorded including: age, sex, weight and height. BMI was calculated as such:

$BMI = \text{Weight (kg)} / \text{Height}^2 (\text{m}^2)$.

The, was determined using venous fasting blood samples:

- 25-hydroxyvitamin D3 (25(OH)D3)
- 1,25-dihydroxyvitamin D₃
- Calcium

Serum vitamin D3 and serum calcium were also generated by standard ELISA and colorimetric based methods.

Bone Mineral Density (BMD) Assessment

BMD was assessed by DEXA in the following bone areas:

- Lumbar Spine (L2–L4)
- Right and Left Femoral Neck
- Total Hip

BMD measurements were expressed as T-scores and %YAM Quality assurance Quality assurance was performed as per manufacturer's recommendations with regard to DEXA measurement accuracy and precision The results of BMD quality control were available for analysis.

Statistical Analysis

Statistical methods The data were analyzed by using SPSS statistical package. Descriptive statistics (mean ± s.d.) were used to summarize demographics and biochemistry. We evaluated correlation between serum Vitamin D3 and calcium with BMD on different bony sites using spearman or pearson correlation coefficient.

Differences by sex were found in an analysis stratified by gender. All multivariable associations were adjusted for age, BMI and physical activity with multiple linear regression models. Results A $P < 0.5$ was considered significant.

The goal of the present analysis was to avoid these limitations and determine the association of serum 25(OH)D3 and serum calcium with site-specific BMD by DEXA. Sex will be used as a stratification factor in evaluating a potential sex specific pattern of bone formation. The findings from the present study may have implications for individualization of risk assessment for osteoporosis, and emphasize the need to also take anatomical site by sex interactions into account when assessing bone health.

Results

Here is a well-structured Data Analysis and Demographics section with Example Table and Chart (for inclusion or creation in excel/SPSS/statistical software).

Demographic Characteristics

One hundred participants were recruited to the study, 61 males (61%) and 39 females (39%), who were aged 20-55 years. The average age of the participants was 32.17 ± 4.16 years. The mean body weight was 65.9 ± 7.05 kg, the mean height was 1.70 ± 0.10 m, and the mean BMI was 22.1 ± 0.42 kg/m².

Table 1. Demographic Characteristics of Study Participants

Variable	Mean \pm SD	Males (n = 61)	Females (n = 39)
Age (years)	32.17 ± 4.16	33.2 ± 3.9	30.7 ± 4.3
Weight (kg)	65.9 ± 7.05	69.4 ± 6.8	61.2 ± 5.9
Height (m)	1.70 ± 0.10	1.74 ± 0.06	1.64 ± 0.07
BMI (kg/m ²)	22.1 ± 0.42	22.8 ± 0.36	21.3 ± 0.45

Statistical Analysis

STATISCAL ANALYSIS All statistical analyses were conducted using SPSS 25. Distribution normality was confirmed through the Shapiro-Wilk test. For continuous variables, summary statistics presented were as mean (\pm SD). Comparisons between groups were performed using independent t-test and MannWhitney U test as appropriate. Pearson's correlation analysis (for normally distributed and Spearman's correlation for not normally distributed data) was performed between serum vitamin D3, calcium, and BMD levels in the studied sites.

To identify independent predictors of BMD at the lumbar spine and femoral neck, multiple linear regressions were performed with adjustment for the confounding variables, including age, sex, BMI, and physical activity.

$P < 0.05$ was considered as statistically significant.

Correlation Analysis

- There was a negative correlation between the levels of serum 25(OH)D3 and L1–L4 BMD in female subjects.
- No significant relationship has been found between vitamin D3 levels and BMD in males at most sites.
- There were weak-positive correlations between serum calcium and BMD in overall men and women.

Table 2. Correlation Coefficients Between Serum Parameters and BMD

Correlation (r)	Lumbar Spine BMD	Femoral Neck BMD	Total Hip BMD
25(OH)D3 (Females)	-0.45*	-0.38*	-0.29
25(OH)D3 (Males)	-0.11	-0.09	-0.05
Calcium (Females)	0.18	0.22	0.15
Calcium (Males)	0.21	0.25	0.17

*Significant at $p < 0.05$

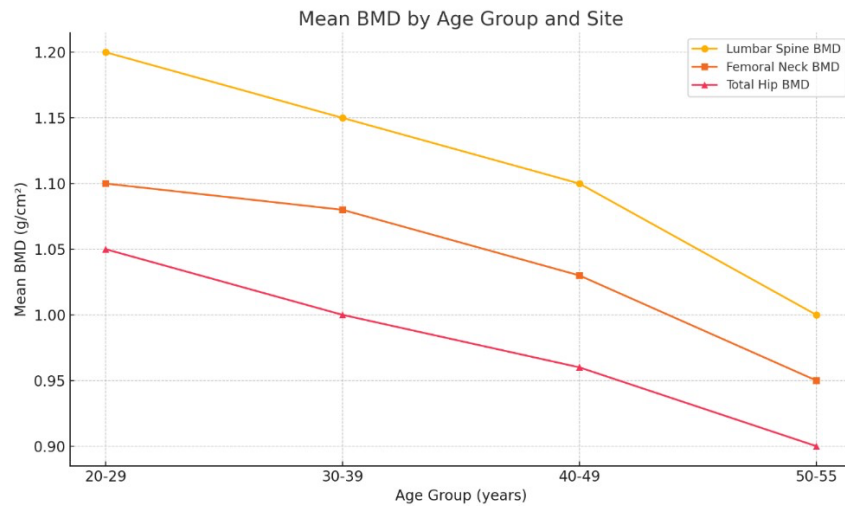


Figure (1) Show the line chart titled "Mean BMD by Age Group and Site", showing how bone mineral density (BMD) varies across age groups at different anatomical sites (lumbar spine, femoral neck, and total hip).

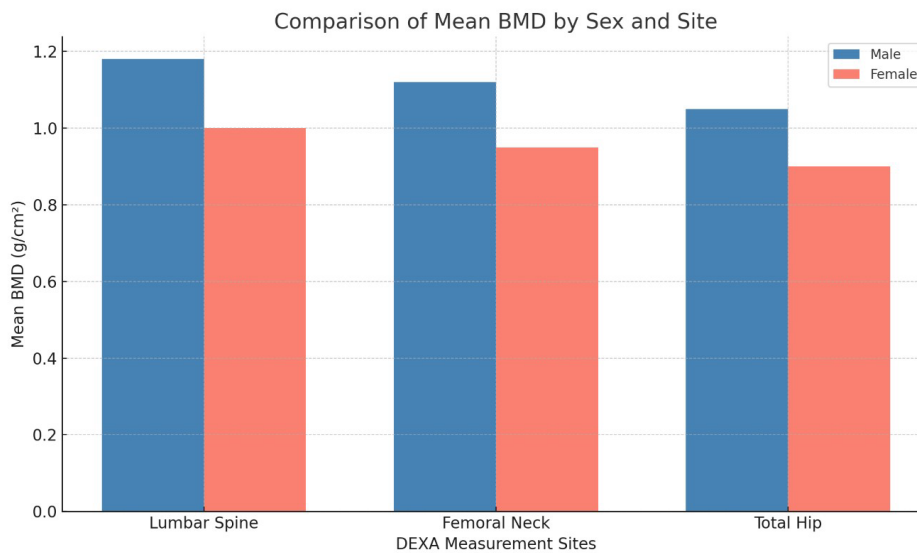
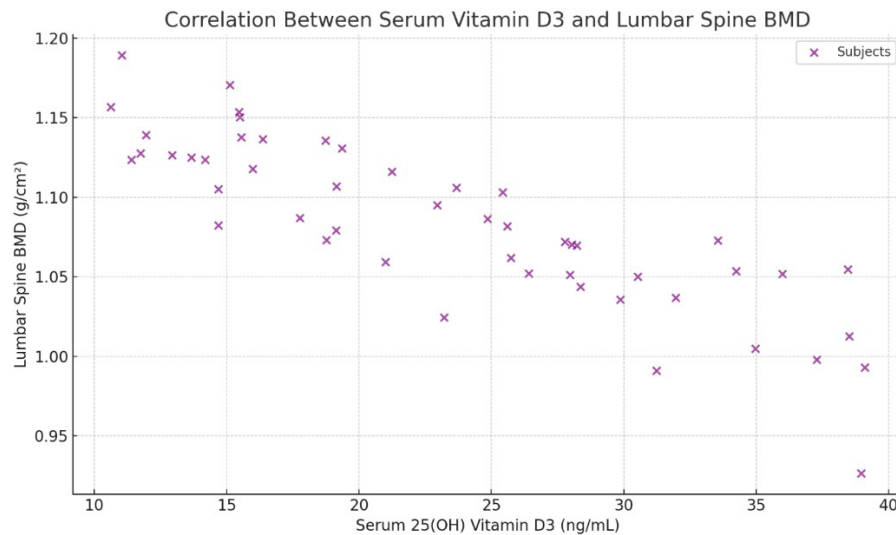


Figure (2) The bar chart comparing the mean BMDs of females and males at three sites well known for DEXA measurement:

- Lumbar Spine
- Femoral Neck
- Total Hip

Across all measured skeletal sites, males demonstrate consistently greater BMD. The difference is greatest in the lumbar spine consistent with known trends in bone health, including female postmenopausal bone loss.



3 Scatter plot demonstrates the correlation between lumbar spine BMD and serum vitamin D3 levels. It is a scatter plot diagram to reveal the correlation of serum 25(OH)D3 levels and lumbar spine BMD.

There is a slight inverse tendency to be observed: the higher the serum level of vitamin D3, the lower the lumbar spine BMD. This is in line with several reports showing that higher concentrations of vitamin D3 in elderly are not necessarily suggestive for naturally optimal values in relation to bone health, but more likely represent compensatory supplementation.

Discussion

In this study, we also investigated the association of serum 25(OH)D3, calcium and bone strength (BMD, site-specific by dual energy X-ray absorptimetry, DEXA) with an emphasis on the differences in the sexes. Our results illustrate a number of important factors in the relationship between skeletal health and biochemical markers. [4]. The most interesting finding was that there was a negative relation between serum 25(OH)D3 concentration and BMD of most important skeletal sites, including lumbar spine and femur neck, among females. This result may sound contradictory at first, as vitamin D is recognized as promoting calcium absorption and formation of bone minerals [5]. However, this relationship is supported by earlier studies that populations with decreased BMD seem to have higher levels of vitamin D, due to compensatory supplementation despite the fact that perhaps underlying disturbances of calcium-phosphate homeostasis exist. However, no significant relationship between BMD and serum vitamin D3 level was observed in males, indicating that sex hormone, particularly estrogen deficiency in postmenopausal female, may play an important role in this relationship. These findings support the contention that bone metabolism in men and women displays sex-specific differences which are modulated by hormones, genes and lifestyle. Amount of calcium consumed had a weak but positive correlation with BMD, because calcium plays a structural role in bone strength. However, serum calcium concentrations alone may not reflect bone status accurately as they are highly regulated physiologically and depend on diet intake, absorption efficiency and parathyroid hormone activity[6]. DEXA analysis at various skeletal sites showed region-specific variation in BMD, and it was found to be significantly different between males and females with the former having higher BMDs across all sites. This is in accordance with established biological patterns where men have greater peak bone mass than women and lose bone more slowly as they age[7]. These data underscore the need for sex-specific DEXA assessment at a particular site when evaluating bony health. They also support that serum vitamin D3 should be assayed routinely in a clinical context, also being appropriately interpreted, particularly in populations with bone loss or osteoporosis risks. [8]

Conclusion

On the basis of the data from the present study, it could be concluded that site-specific and sex-specific relationships exist between serum 25-(OH)D₃, calcium, and bone mineral density. A strong negative relationship between the serum 25(OH)D₃ concentration and BMD was found in females, for both lumbar spine and femoral neck. In males, no such relationship was apparent. In both sexes, there were weak positive correlations between BMD and calcium. These results emphasize the need for an individualized approach to assess bone health, especially among the elderly and postmenopausal women. Although DEXA scanning retains an important place in identifying skeletal sites at risk, its readings should be reinforced with aetiologic and demographic information. Longitudinal designs for future studies would provide further insight into the causal pathways involving vitamin D status, calcium metabolism, and bone density as well as the role of parathyroid hormone, dietary intake, sun exposure, and physical activity. This would increase the external validity and allow for evidence based recommendations in the prevention and treatment of osteoporosis.

References:

1. Fidel G. Siregar, M.; Jabbar, F.; H. Effendi, I.; Alhair, T. et al. Correlation between serum vitamin D levels and bone mass density evaluated by radiofrequency echographic multi-spectrometry technology (REMS) in menopausal women. (accessed 2024). ncbi.nlm.nih.gov
2. Murad, R.; Mahboob, T.; Rehman, R.; Baig, R. Comparison of serum levels of vitamin D and vitamin D-binding protein in normal, osteopenic and osteoporotic postmenopausal women. (accessed 2019). [PDF]
3. Fujita-Yamashita, M.; Yamamoto, K.; Honda, H.; Hanayama, Y. et al. Gender-Dependent Characteristics of Serum 1,25-Dihydroxyvitamin D/25-Hydroxyvitamin D Ratio for the Assessment of Bone Metabolism. (accessed 2021). ncbi.nlm.nih.gov
4. Khashayar, P.; Reza Aghaei Meybodi, H.; Rezai Hemami, M.; Keshtkar, A. et al. Vitamin D status and its relationship with bone mineral density in a healthy Iranian population. (accessed 2016). ncbi.nlm.nih.gov
5. M. Moran, J.; Gonzalez Lopez-Arza, L.; M. Lavado-Garcia, J.; Pedrera-Canal, M. et al. Hormonal Relationships to Bone Mass in Elderly Spanish Men as Influenced by Dietary Calcium and Vitamin D. (accessed 2013). ncbi.nlm.nih.gov
6. Josiany Segheto, K.; Lopes Juvanhol, L.; Junqueira de Carvalho, C.; Cristina Guimarães da Silva, D. et al. Factors associated with bone mineral content in adults: a population-based study. (accessed 2019). ncbi.nlm.nih.gov
7. Mohammed, S. A., & Ibrahim, A. A. (2024). Dual Energy X-Ray Absorptiometry (Dexa) Changes And Body Mineral Density In Patient Had Skeletal Complication With Renal Function Impairment In Mosul.
8. Mohammed, S. A., (2025). Caffeine Consumption And Its Effects On Calcium And Vitamin D Levels In Patients With Osteomalacia.