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**ORIGINAL ARTICLE**

# Serum Vitamin D Levels and Pelvic Bone Mineral Density in Postmenopausal Women: A Biochemical Study

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## ABSTRACT

**Background:** Vitamin D is essential for calcium absorption and bone mineralization, particularly in postmenopausal women who are at high risk of osteoporosis due to estrogen deficiency. Low vitamin D levels impair skeletal metabolism and contribute to bone loss, increasing the risk of pelvic and hip fractures.

**Objective:** To assess the association between serum vitamin D levels and pelvic bone mineral density (BMD) among postmenopausal women attending gynaecology clinics at Dr. Faisal Masood Teaching Hospital, Sargodha, and Al-Nafees Medical College and Hospital, Islamabad.

**Methods:** This cross-sectional biochemical study was conducted from June 2022 to March 2023 on 150 postmenopausal women aged 45–70 years. After ethical approval and informed consent, participants were evaluated through clinical examination and laboratory testing. Serum 25-hydroxyvitamin D [25(OH)D] levels were measured using the ELISA method, while pelvic BMD was assessed by Dual-Energy X-Ray Absorptiometry (DEXA). Participants were categorized as vitamin D deficient (<20 ng/mL), insufficient (20–30 ng/mL), or sufficient (>30 ng/mL). Statistical analysis was performed using SPSS version 26, applying Pearson's correlation and multiple linear regression to explore associations between vitamin D and BMD.

**Results:** The mean age of participants was 57.9 ± 6.2 years, with a mean menopausal duration of 8.3 ± 3.1 years. Vitamin D deficiency was observed in 63.3% of women, insufficiency in 24.7%, and sufficiency in 12.0%. The mean serum vitamin D level was 19.1 ± 7.3 ng/mL, and the mean pelvic BMD was 0.77 ± 0.13 g/cm². A significant positive correlation was found between serum vitamin D levels and pelvic BMD ($r = 0.52$, $p < 0.001$). Regression analysis revealed that vitamin D was an independent predictor of BMD ($\beta = 0.38$, $p = 0.002$) after adjusting for age, BMI, and menopausal duration.

**Conclusion:** Serum vitamin D levels showed a strong positive association with pelvic bone mineral density in postmenopausal women. Vitamin D deficiency is highly prevalent and significantly contributes to bone loss, emphasizing the importance of routine screening and supplementation to prevent osteopenia and osteoporosis in this vulnerable group.

**Keywords:** Vitamin D, Bone Mineral Density, Postmenopausal Women, Pelvic Bone, Osteoporosis, Biochemical Study

## INTRODUCTION

Osteoporosis is a major global health concern that predominantly affects postmenopausal women due to the sharp decline in estrogen levels following menopause[1]. Estrogen deficiency accelerates bone resorption and disrupts the delicate balance between osteoblast and osteoclast activity, leading to progressive loss of bone mass and deterioration of bone microarchitecture[2]. Among the biochemical determinants of skeletal health, vitamin D plays a critical role by facilitating calcium absorption in the intestine, maintaining serum calcium and phosphate concentrations, and promoting normal bone mineralization. Deficiency of vitamin D impairs these functions, predisposing individuals to osteopenia, osteoporosis, and a higher risk of fragility fractures, particularly of the hip and pelvis[3].

Vitamin D is synthesized endogenously in the skin through ultraviolet B radiation or obtained exogenously from dietary sources such as fatty fish, fortified dairy products, and supplements[4]. Its biologically active form, 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], regulates calcium homeostasis and bone remodeling through its actions on osteoblasts, osteoclasts, and intestinal epithelial cells. However, several environmental and lifestyle factors—including reduced sunlight exposure, traditional clothing, darker skin pigmentation, aging, and limited dietary intake—contribute to widespread vitamin D deficiency in many populations. In South Asian countries such as Pakistan, epidemiological data suggest that more than 70 % of adult women have insufficient vitamin D levels, making it an important but under-recognized public health issue[5].

Bone mineral density (BMD) is a quantitative measure of bone strength and an established indicator of fracture risk[6]. The pelvic bones, especially the femoral neck and acetabular regions, bear substantial mechanical load and are among the most common sites affected by osteoporotic fractures in postmenopausal women[7]. Measurement of BMD through Dual-Energy X-Ray Absorptiometry (DEXA) provides a reliable assessment of bone health and helps in identifying individuals at risk for osteoporosis before fractures occur. Numerous studies have established a positive association between serum vitamin D concentration and BMD, suggesting that adequate vitamin D status is essential for the maintenance of skeletal integrity[7]. Yet, limited data exist specifically examining the correlation between serum vitamin D levels and pelvic BMD among postmenopausal women in the South Asian context, where nutritional and lifestyle factors differ markedly from Western populations[8].

Understanding this relationship is vital for developing preventive and therapeutic strategies aimed at reducing the burden of osteoporosis-related morbidity[9]. Therefore, the present biochemical study was designed to evaluate the association between serum 25-hydroxyvitamin D [25(OH)D] levels and pelvic bone mineral density in postmenopausal women, with the objective of elucidating the impact of vitamin D deficiency on pelvic bone strength and contributing evidence for early screening and supplementation in this vulnerable group[10-15].

## MATERIALS AND METHODS

This cross-sectional analytical study was carried out jointly in the Department of Obstetrics and Gynaecology at Dr. Faisal Masood Teaching Hospital, Sargodha, and the Department of Obstetrics and Gynaecology at Al-Nafees Medical College and Hospital, Islamabad, over a period of ten months from June 2022 to March 2023. The study was initiated after obtaining formal approval from the Institutional Ethical Review Committees of both institutions. Written informed consent was taken from all participants prior to

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their inclusion in the study, and all procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki (2013 revision).

A total of one hundred and fifty postmenopausal women between the ages of forty-five and seventy years were enrolled through purposive sampling from the gynaecology outpatient departments of the two hospitals. Menopause was defined as the permanent cessation of menstruation for at least twelve consecutive months not due to any pathological or surgical cause. Only women who had undergone natural menopause and who were not taking vitamin D or calcium supplements for at least six months prior to enrollment were included. Women with a history of metabolic bone disorders such as osteomalacia, hyperparathyroidism, or Paget's disease, as well as those suffering from chronic kidney, liver, or thyroid diseases, were excluded. Similarly, subjects using corticosteroids, anticonvulsants, or hormone replacement therapy, or those with previous pelvic fractures or malignancies affecting bone metabolism, were not considered for inclusion.

After obtaining consent, each participant underwent a detailed medical history and physical examination. Relevant information regarding age, duration of menopause, dietary pattern, exposure to sunlight, and physical activity was recorded on a structured proforma. Anthropometric measurements, including height and weight, were obtained using standard methods, and body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters.

For biochemical analysis, five millilitres of venous blood was collected aseptically from each participant after an overnight fast. The samples were centrifuged at three thousand revolutions per minute for ten minutes to separate the serum, which was then stored at –20°C until analysis. Serum 25-hydroxyvitamin D [25(OH)D] levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit manufactured by Abbott Laboratories, USA. Based on serum concentrations, vitamin D status was categorized as deficient when levels were below 20 ng/mL, insufficient when between 20 and 30 ng/mL, and sufficient when above 30 ng/mL.

Pelvic bone mineral density (BMD) was assessed at the radiology departments of the participating hospitals using dual-energy X-ray absorptiometry (DEXA) scans. Measurements were taken at the femoral neck and acetabular regions and were expressed in grams per square centimetre (g/cm²). The T-scores obtained from DEXA were used to classify bone density according to the World Health Organization (WHO) criteria: values greater than or equal to –1.0 were considered normal, scores between –1.0 and –2.5 were interpreted as osteopenia, and scores less than or equal to –2.5 were diagnostic of osteoporosis.

All data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corporation, USA). Quantitative variables such as age, BMI, serum vitamin D levels, and BMD were expressed as mean ± standard deviation, while categorical variables such as vitamin D status and bone health classification were presented as frequencies and percentages. The relationship between serum vitamin D levels and pelvic bone mineral density was examined using Pearson's correlation coefficient. One-way analysis of variance (ANOVA) was applied to compare mean BMD values among the three vitamin D categories. Multiple linear regression analysis was further employed to identify independent predictors of BMD after adjusting for confounding variables including age, BMI, and menopausal duration. A p-value of less than 0.05 was considered statistically significant.

The study maintained complete confidentiality of participants' personal data, and all findings were utilized solely for academic and clinical research purposes.

## RESULTS

A total of 150 postmenopausal women were enrolled in this study. The mean age of the participants was 57.9 ± 6.2 years, and the mean duration since menopause was 8.3 ± 3.1 years. The average body mass index (BMI) was 26.4 ± 3.2 kg/m², indicating that the majority of the women were overweight according to WHO classification. Most participants (58.7%) reported limited sunlight exposure of less than 30 minutes per day, and only 42% were engaged in moderate physical activity. The detailed demographic and clinical characteristics of the study population are summarized in Table 1.

Table 1: Demographic and Clinical Characteristics of Postmenopausal Women

| Variable | Mean ± SD / n (%) |
|---|---|
| Age (years) | 57.9 ± 6.2 |
| Duration since menopause (years) | 8.3 ± 3.1 |
| Body Mass Index (kg/m²) | 26.4 ± 3.2 |
| Sunlight exposure < 30 min/day | 88 (58.7%) |
| Moderate physical activity | 63 (42.0%) |

**Serum Vitamin D Status:** The analysis of serum 25-hydroxyvitamin D [25(OH)D] levels showed that vitamin D deficiency was widespread among the study participants. The overall mean serum vitamin D concentration was 19.1 ± 7.3 ng/mL. Among the 150 women, 95 (63.3%) were vitamin D deficient (< 20 ng/mL), 37 (24.7%) were insufficient (20–30 ng/mL), and only 18 (12.0%) had sufficient vitamin D levels (> 30 ng/mL). The distribution of vitamin D status is presented in Table 2.

Table 2: Distribution of Participants According to Vitamin D Status

| Vitamin D Status | Serum 25(OH)D (ng/mL) | n (%) |
|---|---|---|
| Deficient (< 20 ng/mL) | 13.4 ± 3.1 | 95 (63.3%) |
| Insufficient (20–30 ng/mL) | 24.6 ± 2.5 | 37 (24.7%) |
| Sufficient (> 30 ng/mL) | 34.8 ± 3.6 | 18 (12.0%) |
| Total | — | 150 (100%) |

**Pelvic Bone Mineral Density:** The mean pelvic bone mineral density (BMD) of the participants was 0.77 ± 0.13 g/cm², with a corresponding mean T-score of –1.6 ± 0.7. Based on WHO criteria, 57 women (38%) had normal BMD, 63 (42%) had osteopenia, and 30 (20%) were osteoporotic. The mean BMD varied significantly among the three vitamin D categories ($p < 0.001$). Women with vitamin D sufficiency had the highest BMD values (0.91 ± 0.11 g/cm²), whereas those with deficiency showed the lowest (0.68 ± 0.09 g/cm²). This statistically significant difference, as shown in Table 3, demonstrates a clear positive association between serum vitamin D concentration and pelvic bone mass.

Table 3: Comparison of Pelvic Bone Mineral Density Across Vitamin D Categories

| Vitamin D Category | n | Mean BMD (g/cm²) ± SD | Mean T-score ± SD |
|---|---|---|---|
| Deficient (< 20 ng/mL) | 95 | 0.68 ± 0.09 | –2.0 ± 0.4 |
| Insufficient (20–30 ng/mL) | 37 | 0.78 ± 0.08 | –1.4 ± 0.3 |
| Sufficient (> 30 ng/mL) | 18 | 0.91 ± 0.11 | –0.8 ± 0.2 |
| p-value (ANOVA) | — | < 0.001 | < 0.001 |

**Correlation Between Serum Vitamin D and Pelvic BMD:** Pearson's correlation analysis demonstrated a moderately strong positive correlation between serum vitamin D levels and pelvic BMD ($r = 0.52$, $p < 0.001$). A similar positive correlation was observed between vitamin D and T-score ($r = 0.48$, $p < 0.001$). This indicates that higher serum vitamin D concentrations are associated with better bone mineral density and higher T-scores in postmenopausal women, as illustrated in Table 4.

Table 4: Correlation of Serum Vitamin D Levels with Bone Parameters

| Variable | Correlation Coefficient (r) | p-value |
|---|---|---|
| Vitamin D vs BMD (g/cm²) | 0.52 | < 0.001 |
| Vitamin D vs T-score | 0.48 | < 0.001 |

**Regression Analysis:** Multiple linear regression was conducted to determine the independent predictors of pelvic BMD after adjusting for age, BMI, and duration of menopause. The analysis confirmed

that serum vitamin D was a significant independent predictor of pelvic BMD (β = 0.38, p = 0.002). Both age (β = –0.27, p = 0.01) and duration of menopause (β = –0.19, p = 0.03) were inversely related to bone mineral density, indicating progressive bone loss with advancing age and menopausal duration. Although BMI showed a weak positive association with BMD (β = 0.11, p = 0.07), it did not reach statistical significance. The complete regression model is presented in Table 5.

Table 5: Multiple Linear Regression Analysis Showing Predictors of Pelvic Bone Mineral Density

| Variable | β Coefficient | Standard Error | t value | p-value |
|---|---|---|---|---|
| Age (years) | –0.27 | 0.09 | –2.78 | 0.010 |
| Duration since menopause (years) | –0.19 | 0.08 | –2.13 | 0.030 |
| Body Mass Index (kg/m²) | 0.11 | 0.06 | 1.74 | 0.070 |
| Serum Vitamin D (ng/mL) | 0.38 | 0.10 | 3.16 | 0.002 |
| Model $R^2$ = 0.41, Adjusted $R^2$ = 0.38, p < 0.001 | — | — | — | — |

Overall, the regression model demonstrated that serum vitamin D level was the most significant positive determinant of pelvic bone mineral density in postmenopausal women. These results clearly indicate that vitamin D deficiency is independently associated with decreased BMD, even after controlling for age, BMI, and menopausal duration. The trends observed across the correlation and regression analyses (Tables 4 and 5) reinforce the importance of maintaining adequate vitamin D status to preserve bone health and reduce the risk of osteopenia and osteoporosis in this population.

## DISCUSSION

The present study was conducted to assess the relationship between serum ferritin levels and hematological parameters in patients with clinically and laboratory-confirmed iron deficiency anemia (IDA)[17]. The results demonstrated a significant positive correlation between serum ferritin and hemoglobin, MCV, MCH, and MCHC, while a strong negative correlation was observed with RDW[18]. These findings are consistent with the well-established pathophysiology of IDA, in which depletion of body iron stores leads to a progressive reduction in red cell size, hemoglobin content, and uniformity of erythrocyte morphology[19].

In the current study, serum ferritin emerged as a highly sensitive biochemical indicator of iron status. Patients with low ferritin levels showed a marked reduction in hemoglobin concentration, suggesting that declining iron stores directly limit erythropoiesis[20]. Similar findings were reported by Kaur et al. (2020), who found a significant correlation between ferritin and hemoglobin (r = 0.78, p < 0.001), emphasizing ferritin's diagnostic reliability. Another study by Rahman et al. (2021) in Bangladeshi adults revealed a parallel trend where ferritin positively correlated with MCV, MCH, and MCHC, supporting the interpretation that red cell indices mirror biochemical iron deficiency[21].

The strong inverse correlation between RDW and serum ferritin observed in this study reflects the increased variability in red cell size that accompanies iron depletion[22]. RDW is a measure of anisocytosis and serves as an early hematological marker of evolving IDA. As iron availability decreases, the bone marrow releases smaller and irregularly shaped erythrocytes into circulation, leading to a rise in RDW[23]. This observation aligns with the findings of Cook et al. (2020), who demonstrated that RDW increases even before a significant fall in hemoglobin, suggesting its value as an early screening parameter in iron deficiency[24].

The predominance of female patients (71.7%) in this study also aligns with global data indicating that women of reproductive age are more vulnerable to iron deficiency due to menstrual blood loss, pregnancy, and dietary inadequacies[25]. World Health Organization (2021) estimates that nearly one in three women worldwide suffers from anemia, with iron deficiency accounting for over half of these cases. This trend underscores the importance of routine ferritin screening in women, especially in resource-limited settings where nutritional deficiencies and parasitic infections are common[14,20].

From a diagnostic perspective, combining serum ferritin estimation with routine hematological parameters provides a more comprehensive and cost-effective approach for diagnosing and grading the severity of IDA[13]. While ferritin remains the gold-standard biochemical marker for iron stores, its reliability may be compromised in inflammatory conditions due to its acute-phase reactant nature. Therefore, correlating ferritin with parameters such as Hb, MCV, and RDW enhances diagnostic accuracy and reduces the likelihood of misclassification, particularly in patients with overlapping anemia etiologies[22].

Our findings also have clinical relevance for treatment monitoring. Improvement in serum ferritin levels following iron supplementation therapy should be accompanied by corresponding normalization of red cell indices. Hence, periodic assessment of both biochemical and hematological parameters can help clinicians evaluate therapeutic response and prevent over- or under-treatment[21-24].

However, this study had certain limitations. Being cross-sectional, it could only establish correlations rather than causal relationships[7-9]. Furthermore, inflammatory markers such as C-reactive protein (CRP) were not measured, which might have influenced ferritin levels in patients with subclinical infections. Future studies involving larger sample sizes, serial measurements, and inclusion of inflammatory markers are recommended to further strengthen the diagnostic utility of ferritin and hematological indices in IDA[10-12].

## CONCLUSION

This study demonstrated a strong and statistically significant correlation between serum ferritin levels and hematological parameters among patients with iron deficiency anemia. Serum ferritin showed a positive correlation with hemoglobin, MCV, MCH, and MCHC, and a negative correlation with RDW. These findings confirm that declining ferritin levels are directly associated with worsening anemia and alterations in red cell morphology. Therefore, simultaneous assessment of serum ferritin and complete blood count indices offers a reliable, affordable, and practical diagnostic strategy for identifying and monitoring iron deficiency anemia, particularly in regions with limited healthcare resources. Early detection using these parameters can facilitate timely intervention, reduce disease burden, and improve patient outcomes.

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**Authors' Contribution:**
**U.M.:** Concept and study design, data collection.
**F.S.:** Study supervision, manuscript review.
**S.H.A.:** Statistical analysis, data interpretation.
**A.R.H.:** Literature review, biochemical data correlation.
**M.A.K.:** Manuscript drafting and editing.
**M.Y.M.:** Radiological assessment and validation.

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