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Original Research Article

Retrospective Evaluation of Serum Vitamin D Levels and Their Association with Bone Mineral Markers in Patients Attending a Tertiary Care Hospital

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Conflict of interest: Nil

Abstract:

Background: Vitamin D deficiency is a common nutritional disorder associated with impaired bone metabolism and an increased risk of osteoporosis and related skeletal complications. Assessment of serum vitamin D levels along with bone mineral markers is essential for early identification of individuals at risk.

Aim: To retrospectively evaluate serum vitamin D levels and their association with bone mineral markers among patients attending a tertiary care hospital.

Methodology: A retrospective cross-sectional study was conducted over 3 months at Netaji Subhas Medical College and Hospital in Bihta, Patna, Bihar, India. Medical records of 95 patients who underwent serum 25-hydroxyvitamin D [25(OH)D] estimation and bone mineral marker evaluation were reviewed. Serum calcium, phosphorus, alkaline phosphatase (ALP), and parathyroid hormone (PTH) levels were analyzed. Statistical analysis was performed using SPSS version 26.0, with $p < 0.05$ considered statistically significant.

Results: The mean age was 48.9 ± 13.2 years, and 55.8% of participants were females. Vitamin D deficiency and insufficiency were observed in 41.1% and 33.7% of patients, respectively. Serum vitamin D showed a significant positive correlation with calcium ($r = 0.461$) and phosphorus ($r = 0.352$), while significant negative correlations were observed with ALP ($r = –0.418$) and PTH ($r = –0.589$) ($p < 0.05$).

Conclusion: Vitamin D deficiency was highly prevalent and significantly associated with altered bone mineral markers, emphasizing the importance of routine vitamin D assessment for early detection and prevention of metabolic bone disorders.

Keywords: Vitamin D, 25-hydroxyvitamin D, Bone mineral markers, Calcium, Parathyroid hormone, Alkaline phosphatase, Osteoporosis, Retrospective study.

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Introduction

Osteoporosis and other metabolic bone diseases are significant public health problems globally due to their link with morbidity, disability, poor quality of life and health-care cost. Osteoporosis is a systemic skeletal disease defined by decreased bone mass, a disruption of the microarchitecture of bone, increased fragility of the bone and an increased risk of fracture, particularly in the elderly [1]. Fragility fractures of the hip, spine and wrist are often linked to high morbidity and mortality rates and the early identification of those at risk is therefore important in public health. While age is a non-modifiable risk factor for bone loss, other non-modifiable factors such as hormonal status, physical activity, nutrition and genetics as well as modifiable factors such as environmental factors, all play roles in bone health and osteoporosis risk [2]. Vitamin D is one of the most important of these, involved in regulating calcium and phosphate levels, both crucial for proper bone mineralization and bone integrity.

Vitamin D helps the kidneys regulate calcium and phosphorus uptake, inhibits overproduction of parathyroid hormone (PTH), and controls bone remodeling. Low vitamin D levels lead to low calcium absorption, which leads to secondary hyperparathyroidism, high bone turnover, accelerated bone loss, osteomalacia, osteoporosis and high fracture risk

[3]. Thus, measurement of serum vitamin D has become a routine part of the assessment of those patients with a suspected metabolic bone disorder. The serum 25-hydroxyvitamin D [25(OH)D] has been accepted as the most reliable biochemical marker for assessing vitamin D status since it serves as an indicator of vitamin D acquired through dietary intake, supplementation, and cutaneous synthesis after sun exposure [4]. Presently, international recommendations suggest that serum 25(OH)D levels lower than 20 ng/mL are a sign of vitamin D deficiency, levels of 20 to 29 ng/mL are considered insufficient, and anything over 30 ng/mL is considered adequate for skeletal health [5]. Although vitamin D deficiency is common in tropical countries like India, due to low mobility, skin pigmentation, urbanization, lifestyle changes, low intake of vitamin D rich foods, obesity and the lack of knowledge about sun exposure it is prevalent in all age groups [6].

India has been identified as one of the countries with high prevalence of vitamin D deficiency, ranging from 50% to 90% of apparently healthy population and patients with chronic diseases [7]. It is especially worrying that hypovitaminosis D is so common, as this can lead to poor muscle function, recurrent falls, fractures, cardiovascular diseases, diabetes mellitus, autoimmune disorders and other chronic diseases [8]. Hence, early diagnosis of vitamin D deficiency is a critical point for the early intervention and prevention of long-term skeletal complications. The assessment of bone health is usually made using a combination of biochemical markers and radiological measurements. Bone markers such as serum calcium, serum phosphorus, alkaline phosphatase (ALP), parathyroid hormone (PTH) and bone turnover markers are useful to understand the bone metabolism and bone remodeling activity. Raised bone turnover markers may be a sign of increased bone resorption and weaker bones, whereas changes in calcium-phosphate metabolism may be a sign of disturbances in vitamin D metabolism. Multiple clinical studies have shown that low levels of vitamin D are correlated with high levels of PTH, high bone turnover and low bone mineral density (BMD), and therefore higher risk of osteoporosis and fractures [9]. Many studies have been conducted on the association between vitamin D and bone health, but the results are inconsistent.

There have been several studies reporting a positive relationship between serum 25(OH)D levels and bone mineral density, indicating that sufficient vitamin D levels lead to stronger bones and reduced fracture risk. However, a few studies have not shown any clear relationship between circulating vitamin D levels and BMD once age, BMI, ethnicity, physical activity and genetics were considered [10]. Additionally, randomized controlled trials have shown mixed results for the effects of vitamin D on bone mineral density and fracture rates, suggesting that vitamin D is not the sole cause of these differences in bone health. These differences indicate that several biologics, genetic, nutritional and environmental factors are interacting to affect skeletal metabolism [11]. Additionally, advances in molecular epidemiology have been made to examine the causal relationship between vitamin D and bone metabolism with Mendelian randomization studies. Such studies employ genetic variants associated with vitamin D metabolism to reduce the potential of confounding and reverse causation found in traditional observational studies [12].

Some Mendelian randomization studies have challenged the direct association between vitamin D deficiency and decreased BMD but have consistently found increased bone turnover (measured through biochemical markers) and elevated parathyroid hormone in those with low vitamin D status. So, the serum vitamin D level is still clinically relevant with bone mineral markers to assess metabolic bone health. Factors such as sunlight exposure, dietary practices, socioeconomic and cultural factors and genetics and the resulting vitamin D deficiency burden are different between geographic regions. The relationship between serum Vitamin D level and bone mineral marker is limited among patients attending tertiary care centres in eastern India, especially in Bihar. This is crucial, as the understanding of this relationship could be used to target high risk individuals to institute preventive and/or therapeutic interventions. Furthermore, a convenient, cost-effective way to estimate the prevalence of vitamin D deficiency and its effects on bone metabolism in the real world is to conduct a retrospective study using routinely collected information from hospital laboratory records [13].

Hence, the present study was conducted to review and compare the serum vitamin D levels and its relationship with bone mineral markers in the patients of a tertiary care hospital in Bihta, Patna, Bihar, India in a retrospective manner. The results of this study will help to advance our knowledge of vitamin D deficiency and its association with biochemical markers of bone health and will facilitate diagnosis, risk stratification and good management of patients at risk of metabolic bone disease.

Methodology

Study Design: This study was a retrospective cross-sectional observational study conducted to evaluate serum vitamin D levels and their association with bone mineral markers among patients attending a tertiary care hospital.

Study Area: The study was carried out in the Department of Biochemistry in collaboration with the Department of Orthopaedics and General Medicine at Netaji Subhas Medical College and Hospital in Bihta, Patna, Bihar, India

Study Duration: The study was conducted over a period of 3 months.

Sample Size: A total of 95 patients fulfilling the eligibility criteria were included in the study.

Sample Population: The study population consisted of adult patients who attended the tertiary care hospital during the study period and underwent laboratory evaluation for serum vitamin D [25-hydroxyvitamin D; 25(OH)D] along with bone mineral markers as part of their routine clinical assessment. Patients with complete demographic, clinical, and biochemical records were included for retrospective analysis.

Data Collection: Data were collected retrospectively from hospital medical records and laboratory information systems after obtaining ethical approval. Demographic variables including age and gender, along with clinical history and laboratory investigations, were recorded using a structured data collection form. Laboratory parameters included serum 25-hydroxyvitamin D [25(OH)D], serum calcium, phosphorus, alkaline phosphatase (ALP), and parathyroid hormone (PTH), wherever available. All biochemical investigations had been performed in the hospital's central clinical laboratory using standardized analytical methods with regular internal and external quality control procedures to ensure accuracy and reliability of results.

Inclusion Criteria

- Patients aged 20 years and above.
- Patients who underwent serum 25(OH)D estimation along with one or more bone mineral marker investigations.
- Patients with complete demographic and laboratory records.
- Patients attending the tertiary care hospital during the study period.

Exclusion Criteria

- Patients with chronic liver disease or chronic kidney disease.
- Patients with known metabolic bone disorders other than osteoporosis.
- Patients receiving vitamin D supplementation, corticosteroids, anticonvulsants, or medications affecting bone metabolism.
- Pregnant and lactating women.
- Patients with incomplete laboratory or clinical records.

Study Procedure: Following hospital laboratory records were screened to identify eligible patients according to the predefined inclusion and exclusion criteria. Demographic details, relevant clinical information, and biochemical investigation reports were extracted from medical records using a standardized data collection form. Serum vitamin D levels were classified as deficient (≤20 ng/mL), insufficient (21–29 ng/mL), and sufficient (≥30 ng/mL) according to internationally accepted guidelines. Bone mineral markers, including serum calcium, phosphorus, alkaline phosphatase, and parathyroid hormone, were evaluated and compared across different vitamin D categories to determine their association. The collected information was verified for completeness before statistical analysis.

Statistical Analysis: The data collected were entered into Microsoft Excel 2021 and analyzed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages. The Shapiro–Wilk test was used to assess the normality of data distribution. Differences between vitamin D status groups were analyzed using one-way analysis of variance (ANOVA) with appropriate post hoc tests for continuous variables, whereas categorical variables were compared using the Chi-square test. The association between serum vitamin D levels and bone mineral markers was assessed using Pearson's correlation analysis and multiple linear regression analysis after adjusting for relevant demographic variables. A p-value <0.05 was considered statistically significant throughout the analysis".

Result

Table 1 presents the demographic profile of the study participants. A total of 95 patients were included in the study. The majority of participants belonged to the 50–64 years age group (32.6%), followed by 35–49 years (30.5%), 20–34 years (18.9%), and ≥65 years (17.9%). The mean age of the participants was 48.9 ± 13.2 years. Female participants constituted 55.8% of the study population, whereas males accounted for 44.2%, indicating a slight female predominance among patients evaluated for serum vitamin D levels and bone mineral markers.

Table 1: Demographic Characteristics of the Study Participants (N = 95)		
Variable	**Frequency (n)**	**Percentage (%)**
Age Group (years)		
20–34	18	18.9
35–49	29	30.5
50–64	31	32.6
≥65	17	17.9
Mean Age (years)	**48.9 ± 13.2**	
Gender		
Male	42	44.2
Female	53	55.8

Table 2 shows the distribution of serum vitamin D status among the study participants. The mean serum vitamin D level was 22.8 ± 8.6 ng/mL. Vitamin D deficiency (≤20 ng/mL) was the most common finding, observed in 41.1% of patients, followed by vitamin D insufficiency (21–29 ng/mL) in 33.7% of patients. Only 25.2% of participants had sufficient vitamin D levels (≥30 ng/mL). Overall, nearly three-fourths of the study population had suboptimal vitamin D status, indicating a high prevalence of hypovitaminosis D among patients attending the tertiary care hospital.

Table 2: Distribution of Serum Vitamin D Status Among Study Participants (N = 95)			
Vitamin D Status	**Serum 25(OH)D (ng/mL)**	**Frequency (n)**	**Percentage (%)**
Deficient	≤20	39	41.1
Insufficient	21–29	32	33.7
Sufficient	≥30	24	25.2

Table 3 compares the mean bone mineral marker levels according to vitamin D status. Patients with vitamin D deficiency had the lowest mean serum calcium (8.4 ± 0.6 mg/dL) and phosphorus levels (3.2 ± 0.5 mg/dL), whereas patients with sufficient vitamin D levels had significantly higher calcium (9.3 ± 0.4 mg/dL) and phosphorus (3.8 ± 0.4 mg/dL) concentrations. Conversely, alkaline phosphatase (ALP) and parathyroid hormone (PTH) levels were highest among vitamin D-deficient patients (154.7 ± 32.5 U/L and 67.5 ± 14.2 pg/mL, respectively) and progressively decreased with improving vitamin D status. The differences in all bone mineral markers across the vitamin D categories were statistically significant ($p < 0.05$), suggesting a strong association between serum vitamin D levels and bone metabolism.

Table 3: Mean Bone Mineral Markers According to Vitamin D Status				
Parameter	**Deficient (n=39)**	**Insufficient (n=32)**	**Sufficient (n=24)**	**p-value**
Calcium (mg/dL)	8.4 ± 0.6	8.8 ± 0.5	9.3 ± 0.4	**0.001**
Phosphorus (mg/dL)	3.2 ± 0.5	3.5 ± 0.4	3.8 ± 0.4	**0.003**
ALP (U/L)	154.7 ± 32.5	138.2 ± 28.4	119.8 ± 21.7	**0.002**
PTH (pg/mL)	67.5 ± 14.2	54.8 ± 11.6	42.3 ± 9.5	**<0.001**

Table 4 demonstrates the correlation between serum vitamin D levels and bone mineral markers. Serum vitamin D levels showed a moderate positive correlation with serum calcium ($r = 0.461$, $p = 0.001$) and serum phosphorus ($r = 0.352$, $p = 0.004$), indicating that higher vitamin D concentrations were associated with higher calcium and phosphorus levels. In contrast, serum vitamin D demonstrated a negative correlation with alkaline phosphatase ($r = –0.418$, $p = 0.002$) and a strong negative correlation with parathyroid hormone ($r = –0.589$, $p < 0.001$). These findings suggest that lower vitamin D levels are associated with increased bone turnover and secondary hyperparathyroidism.

Table 4: Correlation Between Serum Vitamin D Levels and Bone Mineral Markers (N = 95)		
Variable	**Correlation Coefficient (r)**	**p-value**
Serum Calcium	0.461	**0.001**
Serum Phosphorus	0.352	**0.004**
Alkaline Phosphatase	–0.418	**0.002**
Parathyroid Hormone	–0.589	**<0.001**

Table 5 presents the association between vitamin D status and abnormalities in bone mineral markers. Hypocalcemia was observed in 32.6% of patients and showed a significant association with vitamin D

deficiency (p = 0.006). Similarly, hypophosphatemia was present in 25.3% of participants and was significantly associated with lower vitamin D levels (p = 0.018). Elevated alkaline phosphatase was detected in 38.9% of patients (p = 0.001), while elevated parathyroid hormone was observed in 35.8% of patients and demonstrated the strongest association with vitamin D deficiency (p <0.001). These findings indicate that vitamin D deficiency is significantly associated with abnormalities in bone mineral metabolism and increased biochemical evidence of bone turnover among the study participants.

Table 5: Association Between Vitamin D Status and Bone Mineral Marker Abnormalities (N = 95)

Bone Mineral Marker	Abnormal n (%)	Normal n (%)	p-value
Hypocalcemia	31 (32.6)	64 (67.4)	**0.006**
Hypophosphatemia	24 (25.3)	71 (74.7)	**0.018**
Elevated ALP	37 (38.9)	58 (61.1)	**0.001**
Elevated PTH	34 (35.8)	61 (64.2)	**<0.001**

Discussion

This was a retrospective study to analyze the correlation of serum vitamin D level with bone mineral markers in the patients presented to the tertiary care hospital in Bihta, Patna, Bihar. The results showed that 25.2% of the participants had optimum vitamin D levels, 41.1% vitamin D deficiency and 33.7% vitamin D insufficiency. Mean serum 25(OH)D was 22.8 ± 8.6 ng/mL, which is very high in the present study population indicating hypovitaminosis D. The results are similar to those of the Aparna et al. study which reported almost 70–90% of the Indian population had vitamin D deficiency, even when they had adequate exposure to sunlight. Likewise, Amrein et al. [14] pointed out that vitamin D deficiency is a worldwide public health issue especially in South Asian countries, because of lifestyle changes, reduced sun exposure, dietary inadequacies and urbanization. Patients with vitamin D deficiency had significantly lower serum calcium level (8.4 ± 0.6 mg/dL), phosphorus level (3.2 ± 0.5 mg/dL), and serum alkaline phosphatase (ALP) level (154.7 ± 32.5 U/L) and a significantly higher level of parathyroid hormone (PTH) (67.5 ± 14.2 pg/mL) (p < 0.05)".

This is in line with Sadat-Ali et al. who found a relationship between low serum vitamin D and lower calcium levels and higher PTH levels, which is indicative of poor bone mineralization and secondary hyperparathyroidism. Similarly, Hosseinpanah et al. [15] showed that lower levels of vitamin D were associated with higher levels of PTH and biochemical markers of higher bone turnover in healthy adults. In our study, moderate positive correlations were noted between serum vitamin D and calcium (r = 0.461) and between serum vitamin D and phosphorus (r = 0.352) and significant negative correlation between serum vitamin D and ALP (r = –0.418) and serum vitamin D and PTH (r = –0.589). These results corroborate the physiological actions of vitamin D in the regulation of calcium-phosphorus balance and in the inhibition of increased parathyroid hormone production. In line with this, Li et al. found an inverse relationship between serum 25(OH)D levels, PTH, and biochemical markers of bone metabolism. In their study, they found that vitamin D deficiency leads to secondary hyperparathyroidism and a rise in bone remodeling which ultimately results in osteoporosis and fractures [16].

In the present study, biochemical abnormalities were found in a high proportion of subjects, further underlining the clinical relevance of vitamin D deficiency. Thirty-two percent of patients had hypocalcemia, 25% hypophosphatemia, 38% elevated ALP, and 35% elevated PTH, with all showing statistically significant associations with vitamin D deficiency. These results correspond with Holick et al., who found vitamin D deficiency over time causes the intestinal calcium absorption to become compromised, elevation of PTH, increased bone turnover, and eventual loss of skeletal health. Biochemical changes found in our study thus reflect the initial metabolic changes that occur when vitamin D status is poor [17].

Direct bone mineral density (BMD) measures were not performed in the present study due to its retrospective nature, but disturbances in bone metabolism are indirectly suggested by changes in bone mineral markers that were seen in the vitamin D deficient patients. The biochemical markers and BMD studies done previously have yielded conflicting results. Arya et al. found that there was a significant positive relationship between serum vitamin D level and bone mineral density of the femur, which indicates that vitamin D deficiency lowers bone mass and increases fracture risk. Likewise, Villareal et al. [18] suggested that it was advisable to screen individuals with low vitamin D levels for osteoporosis since low BMD might be the first clinical sign of vitamin D deficiency. Other scientists, however, doubted the link between vitamin D and bone mineral density after taking other factors into account. However, while vitamin D was seen to have significant associations with PTH and bone turnover markers, it was only marginally stronger than these when direct associations with BMD were accounted for after adjustment for age, body mass index, and genetic influences [19].

Such opposite findings indicate that there are multiple factors in bone health, not only vitamin D but many others that have a biological and environmental impact. However, the strong link between vitamin D deficiency and alterations in BMM in the present study underlines the need for regular biochemical monitoring in routine practice. Although vitamin D deficiency was seen to be predominant in our hospital-based population, several factors may be responsible for this: less sun exposure, lack of physical activity, nutritional intake, older age, and poor knowledge of vitamin D supplements. Amrein et al. and Aparna et al. [20] have suggested similar reasons, which highlight the low vitamin D status in India despite the sunny climate due to various socioeconomic and behavioral factors. In the present study, the findings are from the east of India and thus the results are informative for conducting an epidemiological study on the burden of vitamin D deficiency in patients visiting tertiary healthcare facilities in the region. This study has a few limitations. This retrospective study restricted information on the dietary calcium intake, duration of sun exposure, levels of physical activity, body mass index, vitamin D supplementation history, and bone mineral density measurements. Additionally, the small sample size and single-center design may limit the applicability of the results. Notwithstanding these constraints, the study offers valuable insight into the relationship between serum vitamin D levels and biochemical parameters of bone metabolism in the patients of the study in a real-life scenario in a tertiary care hospital.

In view of this, future large scale prospective studies which involve assessment of bone mineral density, Nutritional assessment along with follow up studies are advocated to further elucidate the association between vitamin D deficiency and bone health in Indian population.

Conclusion

In the present retrospective study, it was observed that hypovitaminosis D was common among patients visiting tertiary care hospital in Bihta, Patna, Bihar, which indicates that there is a significant burden of hypovitaminosis D in the study population. There was a significant association between lower serum vitamin D levels and decreased serum calcium and phosphorus levels, increased alkaline phosphatase and parathyroid hormone levels, reflecting disturbances of bone metabolism and bone turnover rates. These results underscore the role of serum 25-hydroxyvitamin D as a useful biochemical marker for bone health and those at risk of metabolic bone disease. Serum vitamin D level should be routinely checked and its combination with bone mineral markers could help in early diagnosis, prompt therapeutic intervention and the prevention of osteoporosis and its associated skeletal complications. Further large-scale prospective studies with bone mineral density assessment and long term follow up are recommended to confirm the results and support the efforts of prevention and management of the vitamin D deficiency among the Indian population.

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