Alnakhla
Journal of Science (AJOS)

Print ISSN: 3007 – 0198
Online ISSN: 3007 – 0201

www.alnakhlajos.com

Evaluation vitamin D_3 and calcium in osteoporosis patients

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Received: March 27st 2025
Accepted May 30th 2025
Published: June 4th 2025
DOI:
https://doi.org/10.63799/AJOS/14.1.69

OPEN ACCESS

ABSTRACT

Osteoporosis and other bone disorders represent major public health concerns, frequently associated with deficiencies in essential nutrients such as vitamin D_3 (25-hydroxyvitamin D) and calcium. This comparative cross-sectional study aimed to analyze the relationship between serum vitamin D_3 and calcium levels and their impact on bone health. The study included 40 participants, stratified into 28 patients with suspected bone disorders (case group) and 12 healthy individuals (control group). The average age of participants was 33 ± 16 years. Data were collected at Al-Shatrah General Hospital, Dhi Qar Governorate, between November 1st 2023, and December 20th 2023. Serum vitamin D_3 was measured using the I-Chroma immunoassay and calcium levels by colorimetry. Among the case group, 78.6% had vitamin D_3 deficiency and 60.7% exhibited hypocalcemia, while all controls had normal vitamin D_3 and calcium levels. The overall deficiency rate of vitamin D_3 was 55%, and hypocalcemia was found in 42.5% of participants. Mean vitamin D_3 levels were significantly lower in cases (16.55 ± 7.80 ng/mL) than in controls (32.6 ± 2.10 ng/mL, $P < 0.05$). Calcium differences were not statistically significant ($P > 0.05$), yet a potential clinical relevance is noted. Vitamin D_3's broader immunological roles, though not assessed here, underscore the importance of addressing deficiency. Only 3% reported regular supplementation. These findings recommend improved screening, public education, and further studies accounting for gender, BMI, and sunlight exposure.

Keywords: Vitamin D_3 deficiency, Hypocalcemia osteoporosis, Bone health markers, Case-control study.

Introduction

Vitamin D: Vitamin D is a crucial fat-soluble vitamin for animals, playing a vital role in the metabolism of calcium (Ca^{+2}) and phosphorus (P), and ensuring normal skeletal health. It is involved in various biological processes, including the absorption of Ca^{+2} and P, regulation of parathyroid hormone, bone mineralization and mobilization, and controlling the risk of bone disorders (Adhikari et al., 2020). The three main sources of vitamin D are sunlight (ultraviolet radiation), diet, and supplements. The skin produces about 90% of the body's vitamin D requirements through exposure to solar radiation (Aguilar et al., 2020).

Vitamin D deficiency: Vitamin D has become a major focus in scientific research, clinical practice, and daily life discussions. Over recent years, extensive research has shown that widespread vitamin D deficiency affects more than just the human skeletal system (Zmijewski., 2019). Low levels of vitamin D, affecting approximately 30%-50% of the population, are recognized as a global health issue (Nakashima et al., 2016). Vitamin D is essential for preventing and treating nutritional rickets in infants and children, and it plays a role in both bone health and other bodily functions. While it's agreed that severe vitamin D deficiency (with serum 25-hydroxyvitamin D levels below 30 nmol/l) needs treatment (Bouillon et al., 2020). There's a noted association between low serum 25-hydroxyvitamin D levels and a higher risk of immune-related diseases and disorders such as psoriasis, type 1 diabetes, multiple sclerosis, rheumatoid arthritis, tuberculosis, sepsis and respiratory infections (Charoenngam and Holick, 2020). Vitamin D deficiency is closely linked to bone demineralization, increasing the risk of fractures (Zmijewski, 2019).

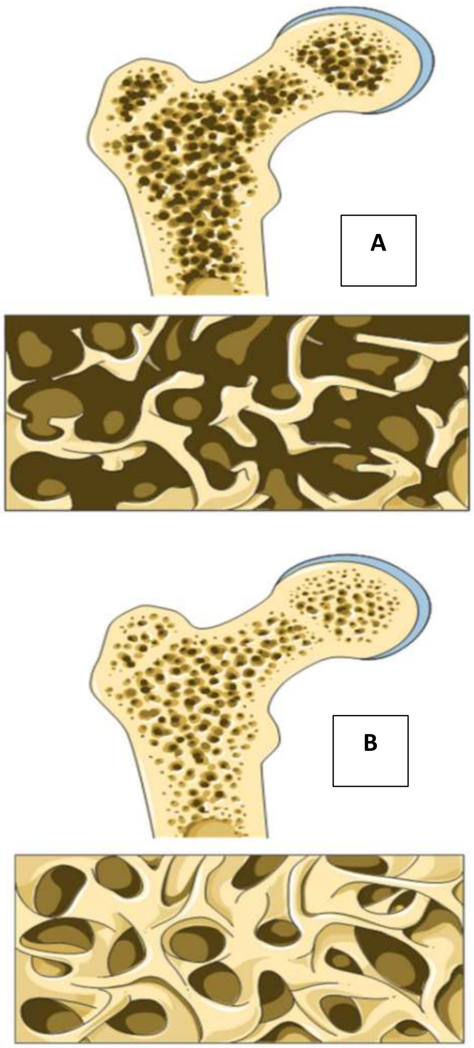

Figure (1): A: Schematic structure of a healthy bone; dense and loadable bone structure. B: Schematic structure of an osteoporotic bone; decreased bone mass and pathological changes in microarchitecture (Wintermeyer et al., 2016).

Calcium: Calcium is the nutrient found in the largest quantity within the human body. The vast majority, over 99% of it (around 1.2 to 1. kg), is stored in bones and teeth. In contrast, less than 1% of the body's calcium is present in extracellular serum. When adults ingest calcium through food or supplements, on average, about 30% of it is absorbed. However, this absorption rate can differ significantly depending on various factors. For instance, during pregnancy, the body increases its calcium absorption rate to meet the higher calcium needs of the developing fetus. Calcium levels in the blood are typically assessed through a venous sample. Normal ranges are 8.8 to 10. mg/dL for total calcium and .7 to 5.2 mg/dL for ionized calcium. It's important to adjust total calcium measurements based on the albumin level, as albumin is a key carrier protein for calcium. Serum calcium levels remain stable and don't vary with changes in dietary intake. Even a slight decrease in serum calcium below the normal range triggers an immediate response from the body. To maintain normal serum calcium levels and avoid hypocalcemia, the body can transfer calcium from other sources, usually within minutes, utilizing one of three organ systems (Bonjour et al., 2013).

Calcium deficiency: The three primary groups most susceptible to dietary calcium deficiency are women, especially during certain life stages or conditions; individuals with milk allergies or lactose intolerance; and those with a higher risk of dietary deficiencies, like adolescents and the elderly. Specifically, female adolescents are at a critical risk because this is a key time for bone growth and development. This risk continues into later life, especially if they didn't develop strong bones during adolescence. Women with eating disorders or those who are highly physically active, such as those with female athlete triad syndrome, are also at a significant risk. Additionally, postmenopausal women are closely studied for calcium deficiency risk due to hormonal changes that can impact bone mineralization (Bonjour et al., 2013).

Materials and Methods

Subjects: The study enrolled a total of 0 participants, comprising 28 patients diagnosed with bone diseases (e.g., osteoporosis) and 12 healthy controls. Participants were selected through simple random sampling from outpatient referrals to the Department of Laboratory Medicine at Al-Shatrah General Hospital, Dhi Qar, Iraq. The cohort had a mean age of 33 ± 16 years, with baseline serum vitamin D_3 levels averaging 20.57 ± 9.80 ng/mL and calcium levels of 8.9 ± 0.9 mg/dL. Inclusion criteria for the case group required confirmed clinical/radiological evidence of bone disorders and serum vitamin D_3 levels below 30 ng/mL, while controls exhibited normal vitamin D_3 levels (≥30 ng/mL) and no history of bone or metabolic diseases.

Exclusion criteria for both groups included chronic kidney/liver dysfunction or recent use of vitamin D_3/calcium supplements
(≤3 months). Written informed consent was obtained from all participants prior to enrollment.

Collection of specimens: Peripheral blood (5 mL) was collected with sterile syringes into gel-clot tubes. After clotting (30 min, room temperature), samples were centrifuged (1500 rpm, 5 min). Serum was aliquoted, stored at −20°C, and analyzed for vitamin D_3 (immunoassay) and calcium (colorimetry).

Kits: Table (1) explain kits used in this study and their companies.

Table (1): Kits used in this study

Kit	Company
I-Chroma Vitamin D Neo kit	Boditech Med Inc, South Korea
Calcium kit	LiNEAR, Spain

Statistical Analysis: Descriptive statistics (means ± standard deviations, frequencies, and percentages) were employed to characterize the demographic and clinical profiles of the study population. Comparative analyses between the case (n=28) and control (n=12) groups were conducted for age (continuous variable), medical history (categorical variables: diabetes mellitus, hypertension, rheumatism), and laboratory parameters (serum vitamin D_3 and calcium levels). Normality of data distribution was assessed using the Shapiro-Wilk test. Between-group differences were evaluated via the independent samples t-test for normally distributed continuous variables and the chi-square test (or Fisher's exact test, where appropriate) for categorical variables. Pearson's correlation coefficient was applied to examine associations between vitamin D_3 and calcium levels. Statistical significance was defined as a two-tailed p-value <0.05. All analyses were performed using SPSS version 28 (IBM Corp., USA), with results visualized using Graph Pad Prism 9.

Results and Discussion

Tables 2, 3 and 4 revealed that among the 28 patients (cases), 78.6% (22 individuals) exhibited vitamin D_3 deficiency, while 60.7% (17 individuals) had hypocalcemia. All 12 control subjects demonstrated normal levels of both vitamin D_3 and calcium. The overall prevalence of vitamin D_3 deficiency in the study population was 55% (22 out of 40), and hypocalcemia was found in 42.5% (17 out of 40). Mean serum vitamin D_3 levels were significantly lower in the case group (16.55 ± 7.80 ng/mL) compared to the control group (32.6 ± 2.10 ng/mL, $P < 0.05$). Serum calcium levels averaged 8.7 ± 1.0 mg/dL in cases and 9.2 ± 0.6 mg/dL in controls, with no statistically significant difference ($P > 0.05$). It is worth noting that although differences in calcium levels were statistically insignificant, a high proportion of patients had clinical hypocalcemia. Additionally, Figure (2), which was referenced in the original document, was not included and therefore could not be evaluated.

Table (2): Correlation between vitamin D_3 and calcium levels (N=40)

Variable	Pearson r	P-value	Interpretation
Vitamin D_3 vs Ca^{2+}	0.189	> 0.05	No statistically significant correlation between Vitamin D_3 and Calcium levels

Table (3): Comparison of variables between cases (N=28) and controls (N=12)

Variable	Cases (N=28)	Controls (N=12)	P-value
Age (years)	33 ± 16	33 ± 17	> 0.05
Vitamin D_3 Level (ng/mL)	16.55 ± 7.80	32.6 ± 2.10	< 0.05 (*)
Calcium Level (mg/dL)	8.7 ± 1.0	9.2 ± 0.6	> 0.05
Vitamin D_3 Deficiency	22 (78.6%)	0 (0%)	< 0.01 (**)
Vitamin D_3 Insufficiency	6 (21.4%)	0 (0%)	—
Normal Vitamin D_3	0 (0%)	12 (100%)	—
Hypocalcemia	17 (60.7%)	0 (0%)	> 0.05
Normal Calcium	11 (39.3%)	12 (100%)	—
Diabetes Mellitus	1 (3.6%)	0 (0%)	> 0.05
Hypertension	4 (14.3%)	2 (16.7%)	> 0.05
Rheumatism	1 (3.6%)	0 (0%)	—
None (No Chronic Disease)	22 (78.6%)	10 (83.3%)	—

Table (4): Demographic and laboratory characteristics of the study (N=40)

Variable	Value / Distribution	Frequency	Percentage (%)
Age (years)	33 ± 16	—	—
Vitamin D_3 Level (ng/mL)	20.57 ± 9.80	—	—
Calcium Level (mg/dL)	8.9 ± 0.9	—	—
Vitamin D_3 Deficiency (<20 ng/mL)	—	22	55.0
Vitamin D_3 Insufficiency (20–29 ng/mL)	—	6	15.0
Normal Vitamin D_3 (≥30 ng/mL)	—	12	30.0
Hypocalcemia (<8.8 mg/dL)	—	17	42.5
Normal Calcium (8.8–10 mg/dL)	—	23	57.5
Diabetes Mellitus	—	1	2.5
Hypertension	—	4	10.0
Rheumatism	—	1	2.5
None (No Chronic Disease)	—	34	85.0

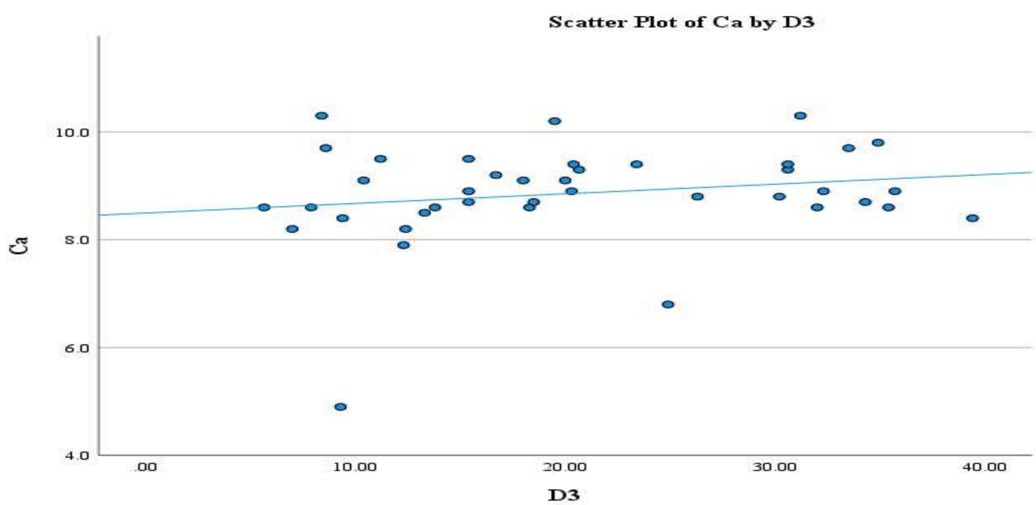

Figure (2): Correlation between Calcium levels and D_3

The study findings reaffirm the high prevalence of vitamin D_3 deficiency in individuals with suspected bone disorders, even in regions with ample sunlight exposure such as southern Iraq. The significantly lower mean vitamin D_3 level in the case group underscores the importance of this nutrient in bone health. Despite the statistically non-significant difference in serum calcium levels between cases and controls, the elevated hypocalcemia rate in the case group suggests a possible clinical relevance that warrants further investigation. The omission of key confounding variables, such as gender distribution, BMI, and exposure to sunlight, is a methodological limitation. These factors are known to influence vitamin D_3 status and could have provided additional insight. Furthermore, vitamin D_3 plays a role beyond skeletal function, including immunomodulation and anti-inflammatory effects, which were not explored in this study but remain critical in understanding systemic implications.

Conclusions and Recommendations

This study highlights a high prevalence of vitamin D_3 deficiency and hypocalcemia in patients with suspected bone disorders. The data support a significant association between vitamin D_3 deficiency and bone health impairment, while the relationship with calcium levels requires further exploration. These results advocate for the implementation of public health strategies focused on early screening, increased awareness, and supplementation especially for high-risk groups such as elderly individuals, women with limited sun exposure, and those with poor nutritional status. Future studies should incorporate broader variables such as gender, BMI, dietary intake, and sun exposure levels. Incorporating immunological markers may also shed light on the systemic effects of vitamin D_3 deficiency.

Acknowledgements

Feelings of love always extend to every person who participated and helped plant another flower in the field of our achievement.

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