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Assessment of Serum Vitamin D3 and Vitamin B12 Levels in Iraqi Women with Osteoporosis A Cross-Sectional Study

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Annotation: Background: Osteoporosis is a degenerative bone disease marked by a significant reduction in bone mineral density and a heightened susceptibility to fractures, particularly among postmenopausal women. Nutritional deficiencies, including those of vitamin D3 and vitamin B12, have been implicated in the pathogenesis of osteoporosis. The present study seeks to evaluate serum levels of vitamin D3 and vitamin B12 in postmenopausal women with osteoporosis compared to healthy controls and explore their correlation with age and BMI.

Material and Methods: This cross-sectional case-control investigation enrolled 68 postmenopausal women, stratified equally into osteoporotic and control cohorts according to their DEXA-derived T-scores. Serum levels of 25-hydroxyvitamin D3 and vitamin B12 were measured using CLIA and ECLIA, respectively. Statistical analyses included t-tests and Pearson correlation, with significance set at $p < 0.05$.

Results: Osteoporotic women had significantly lower mean serum vitamin D3 (16.8 ± 5.7 ng/mL) and vitamin B12 levels (221.3 ± 49.6 pg/mL) compared to controls (26.1 ± 6.4 ng/mL and 287.9 ± 56.8 pg/mL, respectively; $p < 0.001$ for both). Vitamin D3 and B12 levels showed positive correlations with BMI ($r = 0.44$

and r = 0.41) and negative-correlations with age (r = –0.36 and r = –0.33). A moderate positive-correlation was detected between vitamin D3 and B12 levels (r = 0.39, p = 0.003).

Conclusion: Postmenopausal women with osteoporosis exhibit significantly-lower levels of vitamin D3 and B12. Their deficiency correlates with lower BMI and advancing age, emphasizing the importance of routine nutritional assessment and targeted supplementation to mitigate osteoporosis risk.

Keywords: Bone health; Osteoporosis; Postmenopausal women; Vitamin B12; Vitamin D3.

Introduction

Osteoporosis is a chronic, progressive skeletal disorder typified by reduced bone mineral density (BMD), compromised bone microarchitecture, and heightened fracture susceptibility [1]. As one of the most common metabolic bone diseases, it constitutes a major global health concern, particularly in aging populations and postmenopausal women, where the natural decline in estrogen accelerates bone resorption [2]. The World Health Organization (WHO) operationally defines osteoporosis as a "T-score of ≤ –2.5 on dual-energy X-ray absorptiometry (DEXA) scans", denoting BMD values 2.5 standard deviations below those of young, healthy adults [3]. Worldwide, osteoporosis affects an estimated 200 million women, and it is responsible for millions of fractures annually, particularly of the hip, spine, and wrist [4].

Bone integrity is preserved through a continuous remodeling process mediated by osteoblasts and osteoclasts, under the influence of hormonal, nutritional, and mechanical stimuli [5]. Among the various regulators of bone metabolism, vitamins D3 (cholecalciferol) and B12 (cobalamin) have emerged as pivotal contributors. Their roles extend beyond classical functions, with growing evidence suggesting that deficiencies in either may adversely impact bone homeostasis, structure, and strength particularly in postmenopausal women, who are already vulnerable due to hormonal insufficiency [6,7].

Vitamin D3, a corticosteroid hormone, undergoes hepatic hydroxylation following its synthesis to produce 25-hydroxyvitamin D [25(OH)D], the principal circulating biomarker for evaluating vitamin D status [8]. This is further hydroxylated in the kidneys to generate 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], the biologically active form. The physiologically active form is responsible for promoting calcium and phosphate absorption in the intestine, facilitating bone mineralization, and suppressing parathyroid hormone (PTH) secretion [9].

Insufficient levels of vitamin D3 impair calcium absorption, stimulate secondary hyperparathyroidism, and increase bone resorption all of which accelerate bone loss and fracture risk [10]. Globally, vitamin D deficiency is widely prevalent, especially among older women, due to limited sun exposure, skin aging, reduced dietary intake, and certain sociocultural practices [11]. A multitude of observational studies have documented negative-correlations between circulating 25-hydroxyvitamin D [25(OH)D] concentrations and both-fracture rates and markers of bone turnover, while interventional trials have demonstrated that vitamin D3 supplementation, particularly when paired with calcium, reduces the risk of osteoporotic fractures [12].

Vitamin B12, a water-soluble coenzyme essential for DNA synthesis, methylation reactions, and neurologic function, is primarily acquired from animal-derived foods [13]. Its absorption is contingent on gastric production of intrinsic factor and terminal ileal uptake. B12 deficiency is common among elderly individuals due to conditions such as atrophic gastritis, Helicobacter pylori infection, prolonged use of acid-suppressive medications, and poor dietary intake [14]. Although historically associated with hematologic and neurologic disorders, vitamin B12 has recently gained attention in the context of bone health.

The skeletal effects of B12 deficiency are largely mediated through its role in homocysteine metabolism [15]. In the absence of sufficient B12, homocysteine accumulates in the plasma. Elevated homocysteine levels are deleterious to the skeletal system; they disrupt collagen cross-linking in the bone matrix, reduce bone tensile strength, and stimulate osteoclastic bone resorption while inhibiting osteoblastic bone formation [16]. Epidemiological studies have observed that hyperhomocysteinemia correlates with increased risk of vertebral and non-vertebral fractures, independent of BMD, highlighting the qualitative damage to bone structure [17].

The complexity of bone health lies not only in BMD measurements but also in the biochemical quality and structural integrity of the bone, which are influenced by a multitude of metabolic pathways. Despite advances in osteoporosis research, there is limited exploration of the combined impact of vitamin D3 and B12 deficiencies on bone metabolism. Most studies tend to assess each nutrient in isolation, often neglecting their interactive roles, particularly through homocysteine-related mechanisms. Moreover, few studies have simultaneously evaluated serum levels of both vitamins in osteoporotic women, creating a gap in current knowledge.

Identifying modifiable nutritional factors associated with osteoporosis is crucial, especially in low- and middle-income regions where diagnostic and treatment resources are often limited. Early detection and correction of vitamin D3 and B12 deficiencies may offer a cost-effective approach to mitigating bone loss, reducing fracture risk, and improving patient outcomes [18]. Furthermore, regular monitoring of these vitamins in high-risk groups could inform targeted supplementation strategies and enhance comprehensive osteoporosis care. This study is aimed to assess serum levels of vitamin D3 and vitamin B12 in postmenopausal women with osteoporosis compared to healthy controls and explore their.

Material and Methods:

This research was structured as a cross-sectional, case-control study and was carried out between January 2024 and May 2025. Informed consent was obtained from all participants prior to inclusion in the study.

A total of 68 postmenopausal women, aged between 45 and 70 years, were enrolled and divided equally into two groups according to their bone-mineral density (BMD) status as Osteoporotic Group (n = 34) including women diagnosed with osteoporosis, as indicated by a T-score ≤ -2.5 at either the lumbar spine or femoral neck, based on DEXA results. Second group as Control Group (n = 34) including healthy, age-matched postmenopausal-women with normal BMD (T-score ≥ -1.0) and no clinical signs of bone pathology. Inclusion criteria including women aged 45–70 years, postmenopausal status (defined as the absence of menstruation for at least 12 months) and osteoporotic status confirmed by DEXA for the case group.

Exclusion criteria including presence of secondary osteoporosis (e.g., endocrine disorders, renal insufficiency, malignancies), Recent or current use (within the past six months) of medications known to affect bone metabolism (e.g., corticosteroids, bisphosphonates, vitamin D or B12 supplements), history of gastrointestinal surgery or malabsorptive disorders, neurological or hematologic conditions associated with vitamin B12 deficiency, and ngoing acute illness or chronic inflammatory disease at the time of sampling.

Bone Mineral Density Assessment

Bone mineral density was assessed using dual-energy X-ray absorptiometry (DEXA), specifically focused on the lumbar spine (L1–L4) and femoral neck anatomical sites [19]. T-scores were interpreted according to the WHO classification:

T-score ≤ –2.5: Osteoporosis

T-score ≥ –1.0: Normal bone density

The DEXA scanner underwent regular calibration, and strict adherence to quality control protocols was maintained throughout the measurement process.

Blood Sample Collection and Laboratory Analysis

A 5 mL venous blood sample was collected from each participant under aseptic conditions. Following coagulation, the samples were centrifuged at 3000 rpm for 10 minutes to isolate the serum, which was subsequently aliquoted and preserved at –20°C until further biochemical analysis [20].

Serum 25-hydroxyvitamin D [25(OH)D] concentrations were measured via chemiluminescent immunoassay (CLIA). Vitamin D3 status was classified according to established thresholds: deficiency (<20 ng/mL), insufficiency (20–30 ng/mL), and sufficiency (>30 ng/mL) [21].

Serum Vitamin B12: Measured via electrochemiluminescence immunoassay (ECLIA). Values below 200 pg/mL were considered indicative of deficiency [22].

Statistical Analysis

Data were processed using IBM SPSS Statistics software, version [Insert Version, e.g., 25.0]. Continuous variables—such as age, BMI, serum vitamin D3, and vitamin B12—were expressed as mean ± standard deviation (SD). The Shapiro–Wilk test was used to verify normality of distribution.

Group-comparisons for continuous-variables were performed using the "independent-samples t-test" [23]. Relationships between variables were analyzed using Pearson's correlation coefficient. A significance threshold of $p < 0.05$ was applied throughout.

Results and Discussion:

As shown in Table 1, the study evaluated 68 postmenopausal women divided equally into osteoporotic and control groups, comparing key demographic and biochemical parameters.

Table 1: Comparison of Clinical and Biochemical Parameters between Groups

Parameter	Osteoporotic Group (n = 34)	Control Group (n = 34)	p-value
Age (years)	63.2 ± 5.7	60.7 ± 5.2	0.027
BMI (kg/m²)	24.6 ± 3.2	27.1 ± 3.5	0.004
Vitamin D3 (ng/mL)	16.8 ± 5.7	26.1 ± 6.4	<0.001
Vitamin B12 (pg/mL)	221.3 ± 49.6	287.9 ± 56.8	<0.001

The mean age of participants in the osteoporotic group was 63.2 ± 5.7 years, significantly-higher than the control group (60.7 ± 5.2 years), with a statistically significant difference ($p = 0.027$). This result highlights age as a significant factor linked to osteoporosis within the study sample. It is consistent with existing knowledge that advancing age contributes to bone density loss, driven by hormonal shifts, decreased calcium uptake, and changes in bone metabolism [24]. The older age observed in the osteoporotic group likely reflects the cumulative impact of these age-related factors, increasing the likelihood of osteoporosis. This significant difference underscores the importance of accounting for age when evaluating osteoporosis risk and developing targeted interventions for postmenopausal-women [25].

Body mass index (BMI) was significantly-lower in the osteoporotic group (24.6 ± 3.2 kg/m²)

compared to the control group (27.1 ± 3.5 kg/m², p = 0.004). This suggests that lower BMI may be associated with an increased risk of osteoporosis, which is consistent with previous research indicating that higher body weight can have a protective effect on bone density, possibly due to greater mechanical loading and increased estrogen production from adipose tissue [26]. The finding highlights the importance of maintaining a healthy BMI to potentially reduce the risk of osteoporosis in postmenopausal women.

The results revealed that serum 25(OH) vitamin D3 concentrations (Figure 1) were markedly lower in women with osteoporosis (16.8 ± 5.7 ng/mL) compared to the control group (26.1 ± 6.4 ng/mL), with a statistically significant difference (p < 0.001). Vitamin D3 deficiency, defined as levels under 20 ng/mL, was found in 76.5% of osteoporotic women, whereas only 26.5% of the control group exhibited deficiency, this underscores a significant association between diminished vitamin D levels and osteoporosis. Given vitamin D's critical function in facilitating calcium absorption and regulating bone metabolism, its insufficiency can compromise bone integrity and strength [27]. A positive association between vitamin D3 levels and BMI (r = 0.44, p = 0.001) was observed, suggesting that individuals with higher body mass tend to have better vitamin D status. In contrast, vitamin D3 levels negatively correlated with age (r = –0.36, p = 0.004), reflecting the natural decline of vitamin D as people get older, possibly due to decreased skin synthesis and other age-related factors [28]. These findings underscore the necessity of vitamin D assessment and supplementation, particularly for older women with lower BMI, to help prevent or manage osteoporosis.

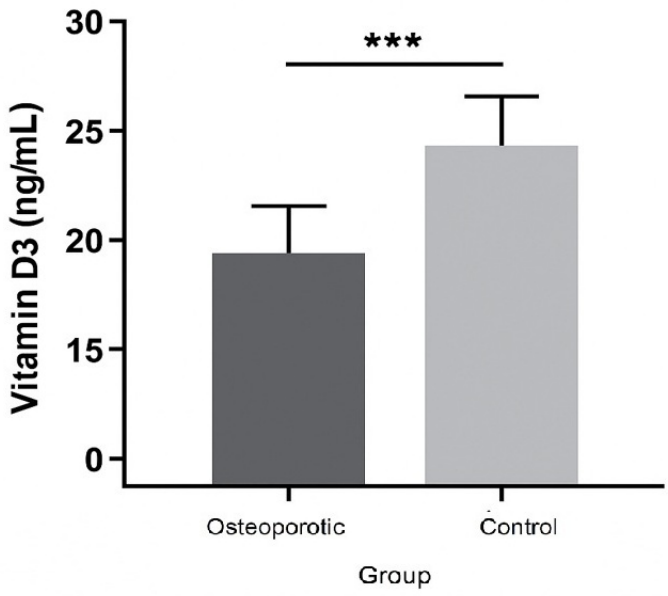

Figure 1: Comparison of Serum Vitamin D3 Levels Between Osteoporotic and Control Groups

The mean circulating level of vitamin B12 in serum (Figure 2) was significantly lower in the osteoporotic group (221.3 ± 49.6 pg/mL) compared to the control group (287.9 ± 56.8 pg/mL), with a strong statistical significance (p < 0.001). Moreover, vitamin B12 deficiency defined as levels below 200 pg/mL was notably more prevalent among osteoporotic women (61.8%) than controls (17.6%), emphasizing a potential link between vitamin B12 insufficiency and osteoporosis. Vitamin B12 plays a crucial role in bone health through its involvement in DNA synthesis and regulation of osteoblast activity [29]. The positive-correlation between vitamin B12 and BMI (r = 0.41, p = 0.002) suggests that individuals with higher body mass may have better vitamin B12 status, while the negative correlation with age (r = –0.33, p = 0.007) reflects the tendency for vitamin B12 levels to decline as people grow older. These results underscore the critical need for regular assessment of vitamin B12 status in postmenopausal-women, especially those with lower BMI and advancing age, to support bone health and potentially reduce

osteoporosis risk [30].

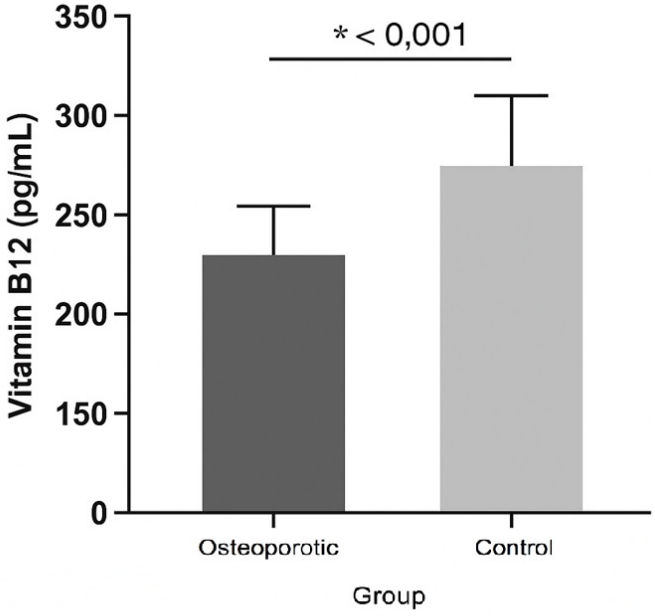

Figure 2: Comparison of Serum Vitamin B12 Levels Between Osteoporotic and Control Groups

A moderate positive correlation (Figure 3) was observed between serum vitamin D3 and vitamin B12 levels (r = 0.39, p = 0.003) as in Figure 3, indicating that higher vitamin D3 concentrations tend to be associated with higher vitamin B12 levels in the study population.

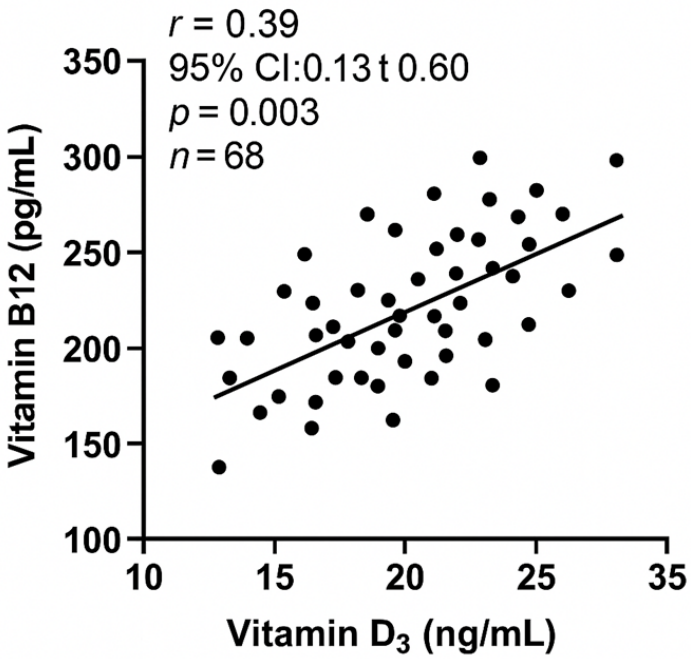

Figure 3: Correlation between Serum Vitamin D3 and Vitamin B12 Levels in Postmenopausal Women

Conclusion

This study demonstrates that postmenopausal women diagnosed with osteoporosis have

markedly reduced serum concentrations of vitamin D3 and vitamin B12 relative to their healthy counterparts. The observed positive associations of these vitamins with body mass index, alongside inverse relationships with increasing age, underscore their integral role in maintaining skeletal integrity. These results advocate for the incorporation of regular assessment and correction of vitamin D3 and B12 deficiencies within osteoporosis prevention and management protocols. Addressing these nutritional insufficiencies may represent a strategic, cost effective approach to mitigating bone fragility and lowering fracture incidence in this high-risk demographic.

Conflict of Interest

Authors declare there are no conflict of interest.

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