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UNCORRECTED PROOF

Association of serum 25-hydroxyvitamin D levels with fatigue severity in adults: A cross-sectional study

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ABSTRACT

OBJECTIVE: The objective of the study is to evaluate the association between serum 25-hydroxyvitamin D (25[OH]D) levels and fatigue severity.

METHODS: This single-center, cross-sectional study included 228 participants aged 18–65 years who completed the fatigue severity scale (FSS) questionnaire. Individuals who had received vitamin supplementation within the previous 3 months, had known chronic disease, used regular medication, or had a history of antidepressant/anxiolytic use for depression/anxiety were excluded. Serum 25(OH)D, hemoglobin, ferritin, iron, magnesium, vitamin B12, and folate values were retrieved from the hospital information system. The Mann–Whitney U-test was used for comparisons between genders, and the Spearman correlation test was used for relationships between FSS and biochemical parameters. P<0.05 was considered statistically significant.

RESULTS: A total of 228 participants (57 males and 171 females) were included in the study. Most variables were not normally distributed; therefore, non-parametric analyses were performed. Spearman correlation analysis demonstrated a weak but significant negative correlation between FSS scores and both hemoglobin (p=0.003) and ferritin levels (p=0.001). No significant correlations were found between FSS and vitamin D, vitamin B12, folate, magnesium, or serum iron levels (p>0.05). In group comparisons based on fatigue severity, ferritin levels were significantly lower in the high-fatigue group compared to the low-fatigue group (p<0.001). Hemoglobin levels were also lower in the high-fatigue group (p=0.043). In contrast, vitamin B12 levels were significantly higher in the high-fatigue group (p=0.032). No significant differences were observed between groups in terms of vitamin D, folate, magnesium, or serum iron levels (p>0.05).

CONCLUSION: Serum 25(OH)D levels were not associated with fatigue severity. The observed relationship between fatigue severity and hematological markers (hemoglobin and ferritin) suggests that these parameters should be considered in the clinical evaluation of fatigue.

Keywords: Endocrinology; family medicine; internal medicine; public medicine.

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Vitamin D is a steroid hormone primarily involved in calcium–phosphorus homeostasis and bone mineralization. In 1967, the metabolism of vitamin D was elucidated for the first time, and vitamin D was subsequently defined as a steroid hormone responsible for maintaining calcium and phosphorus homeostasis and regulating bone mineralization [1]. More than 80% of vitamin D in the human body is synthesized in the epidermis from 7-dehydrocholesterol as a prehormone under the influence of ultraviolet radiation from sunlight. In addition, vitamin D can be obtained through dietary intake from animal (vitamin D_3, cholecalciferol) and plant (vitamin

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D_2, ergosterol) sources, after which it is converted to its biologically active forms in the liver and kidneys [2]. Recent studies have demonstrated the presence of vitamin D receptors in nearly all tissues, suggesting that vitamin D is not limited to calcium–phosphorus metabolism and bone turnover but also plays a role in multiple biological systems, including malignancies, autoimmune, rheumatologic, neurologic, cardiovascular, psychiatric disorders, and diabetes mellitus [3–9].

Fatigue is a common and clinically important complaint, often described as a lack of energy, tiredness, exhaustion, and weakness, and it may substantially impair occupational performance and social functioning. While it can occur as a consequence of physical or mental activity, fatigue may also arise from a wide range of organic conditions, including infections, psychiatric disorders, rheumatologic diseases, malignancies, anemia, and medication use [10]. Accordingly, it is a symptom frequently encountered across multiple medical specialties during a comprehensive clinical history. Fatigue is also among the most common reasons for presentation to internal medicine outpatient clinics. Because fatigue is subjective and has a broad differential diagnosis, its evaluation and management can be challenging and often necessitate extensive clinical assessment and laboratory testing, resulting in a considerable burden in terms of workload and healthcare resource utilization [11].

The pathophysiology of fatigue has not been fully elucidated. It is generally hypothesized to involve multiple mechanisms, including elevated circulating pro-inflammatory cytokines that may trigger oxidative stress and disrupt glial function (astrocytes and microglia), activation of the hypothalamic–pituitary axis leading to increased cortisol levels, and alterations in serotonergic and melatonergic pathways that adversely affect sleep quality [12–14].

Fatigue may lead to a persistent sense of sleep deprivation, diffuse myalgias, and impaired attention. Consequently, quality of life can be substantially reduced, and individuals may experience difficulty performing even routine daily activities. Nevertheless, fatigue is a highly subjective complaint; therefore, its assessment commonly relies on standardized questionnaire-based instruments developed for general or specific populations. Among the most frequently used tools are the Fatigue Severity Scale (FSS) and the Fatigue Impact Scale (FIS). The Turkish version of the FSS was adapted by Armutlu et al. in 2007 [15, 16].

Highlight key points

- Fatigue severity was not associated with serum 25-hydroxyvitamin D [25(OH)D] levels in this cross-sectional cohort.
- Fatigue severity showed a significant negative correlation with hemoglobin and ferritin, even in non-anemic individuals.
- These findings suggest that subclinical iron deficiency may contribute to fatigue, independent of overt anemia.
- Vitamin D supplementation alone may have limited clinical impact on fatigue in the absence of other contributing factors.
- A multidimensional and holistic evaluation is essential in patients presenting with fatigue.

Although psychological factors are often considered the most common contributors to fatigue, a broad range of other etiologies should also be considered, including chronic fatigue syndrome, infections, malignancies, medication use, malnutrition, nutritional deficiencies, dehydration, pregnancy, and various systemic diseases [11]. In this study, we aimed to evaluate the association between serum 25-hydroxyvitamin D [25(OH)D] levels and fatigue severity as assessed by the FSS.

MATERIALS AND METHODS

Study Design and Ethical Approval

This study was designed as a single-center, cross-sectional study conducted in the Internal Medicine Outpatient Clinic of Ümraniye Training and Research Hospital. The study protocol was approved by the Ethics Committee on 10 July 2025 (Approval No.: 237). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Study Population

Based on the power analysis, a total of 228 participants aged 18–65 years who completed the FSS questionnaire were included in the study. Individuals who had received vitamin replacement/supplementation within the preceding 3 months, had a known chronic disease, used regular medication, or had a history of pharmacologic treatment for depression or anxiety were excluded. Data collection and laboratory sampling were performed between September 2025 and November 2025 (autumn season).

Individuals with previously diagnosed anemia under active treatment were also excluded. Anemia was defined according to World Health Organization criteria as hemoglobin levels <13 g/dL in men and <12 g/dL in women.

Table 1. Demographic and laboratory characteristics according to sex

Variables (unit)	Men (n=57)	Women (n=171)
Age (years)	33.37±9.79 (18.00–56.00)	33.42±10.39 (17.00–58.00)
FSS score (points)	37.63±15.62 (9.00–63.00)	43.37±14.12 (9.00–63.00)
25(OH)D (ng/mL)	17.67±7.77 (4.99–49.60)	15.26±9.58 (3.00–75.80)
Vitamin B12 (pg/mL)	309.95±98.53 (148.00–545.00)	313.28±130.74 (100.00–834.00)
Folate (ng/mL)	6.58±2.43 (3.10–13.60)	7.19±3.55 (1.70–20.00)
Hemoglobin (g/dL)	15.48±1.33 (11.30–17.70)	12.77±1.32 (7.20–16.30)
Ferritin (ng/mL)	111.03±106.78 (7.47–591.70)	31.56±31.73 (3.13–279.10)
Iron (µg/dL)	99.46±36.20 (16.00–176.00)	78.17±39.01 (9.60–182.00)

Data are presented as median (interquartile range, 25th–75th percentile). 25(OH)D: 25-hydroxyvitamin D; FSS: Fatigue Severity Scale.

Clinical and Laboratory Assessment

Serum levels of vitamin B12, folate, ferritin, hemoglobin, iron, magnesium, and (25[OH]D) were retrospectively retrieved from the hospital information system.

Fatigue Assessment

Fatigue severity was assessed using the FSS, which has demonstrated validity and reliability in the Turkish population [17, 18]. The FSS is a 9-item instrument, with each item rated on a 7-point Likert scale ranging from 1 ("strongly disagree") to 7 ("strongly agree"), designed to measure subjective fatigue severity. In this study, the total FSS score was calculated on a scale of 9–63, with higher scores indicating greater fatigue severity. Following data verification, no missing item responses were identified, and the minimum observed FSS total score was corrected to 9.

Statistical Analysis

Statistical analyses were performed using IBM Statistical Package for the Social Sciences Statistics version 21.0 (IBM Corp., Armonk, NY, USA). Continuous variables were summarized as mean±standard deviation and minimum–maximum values and categorical variables as numbers and percentages. Normality of continuous variables was assessed using the Shapiro–Wilk test. Because the variables did not meet the assumption of normality, non-parametric statistical methods were applied. Sex-based comparisons were conducted using the Mann–Whitney U test. Associations between FSS scores and biochemical parameters were examined using Spearman's correlation test. $P<0.05$ was considered statistically significant.

Descriptive statistics were presented as mean±standard deviation for readability, whereas inferential analyses were performed using non-parametric methods due to non-normal data distribution.

RESULTS

The study included a total of 228 participants, comprising 57 males and 171 females. The mean age was 33.37±9.79 years in males and 33.42±10.39 years in females. The mean FSS score was 37.63±15.62 in males and 43.37±14.12 in females.

In descriptive analyses, mean vitamin D levels were 17.67±7.77 ng/mL in males and 15.26±9.58 ng/mL in females. Mean vitamin B12 levels were 309.95±98.53 pg/mL in males and 313.28±130.74 pg/mL in females, while folate levels were 6.58±2.43 ng/mL and 7.19±3.55 ng/mL, respectively. Hemoglobin levels were 15.48±1.33 g/dL in males and 12.77±1.32 g/dL in females. Ferritin levels were markedly higher in males (111.03±106.78 ng/mL) compared to females (31.56±31.73 ng/mL). Serum iron levels were 99.46±36.20 µg/dL in males and 78.17±39.01 µg/dL in females. Descriptive statistics are summarized in Table 1.

Normality analysis using the Shapiro–Wilk test demonstrated that most variables deviated significantly from normal distribution ($p<0.05$ for all variables except magnesium), and therefore, non-parametric tests were applied in subsequent analyses.

Spearman correlation analysis revealed a weak but statistically significant negative correlation between FSS scores and hemoglobin levels ($r=-0.197$, $p=0.003$), as

well as ferritin levels (r=-0.215, p=0.001). No significant correlations were observed between FSS and vitamin D (p=0.712), vitamin B12 (p=0.329), folate (p=0.587), magnesium (p=0.352), or serum iron levels (p=0.078). Correlation analysis data are summarized in Table 2.

Participants were divided into two groups according to the mean FSS item score. A mean FSS score of <4 was considered low fatigue, whereas a score of ≥4 was considered high fatigue. Comparative analysis using the Mann–Whitney U test showed that ferritin levels were significantly lower in the high-fatigue group compared to the low-fatigue group (44.09±67.98 vs. 65.26±69.11 ng/mL, p<0.001). Similarly, hemoglobin levels were significantly lower in the high-fatigue group (13.25±1.80 vs. 13.81±1.67 g/dL, p=0.043). Vitamin B12 levels were significantly higher in the high-fatigue group compared to the low-fatigue group (324.97±129.93 vs. 288.82±106.44 pg/mL, p=0.032).

No statistically significant differences were found between the low- and high-fatigue groups in terms of vitamin D (p=0.658), folate (p=0.258), magnesium (p=0.997), or serum iron levels (p=0.405).

A mean FSS item score of ≥4 was accepted as indicative of clinically significant fatigue, consistent with previous studies using the FSS.

Table 2. Spearman correlation analysis between FSS and biochemical parameters

Variables	ρ	p
FSS–hemoglobin (g/dL)	-0.20	0.003
FSS–ferritin (ng/mL)	-0.22	0.001
FSS–25(OH)D (ng/mL)	-0.03	0.712
FSS–vitamin B12 (pg/mL)	0.07	0.329
FSS–folate (ng/mL)	-0.04	0.587
FSS–magnesium (mg/dL)	-0.06	0.352
FSS–iron (µg/dL)	-0.12	0.078

Spearman's rank correlation coefficient (ρ) was used. A p<0.05 was considered statistically significant. FSS: Fatigue severity scale; 25(OH)D: 25-hydroxyvitamin D. TSH values were not included in the final analyses because complete laboratory data were unavailable for all participants.

DISCUSSION

Fatigue is a specific yet multidimensional symptom characterized by marked tiredness during daily activities, reduced energy to sustain routine tasks, and decreased performance. It is among the most common reasons for presentation to internal medicine and family medicine outpatient clinics, and its etiology is influenced by numerous factors, including hematologic, endocrine, metabolic, and psychosocial contributors [19, 20]. In a study by Dağ et al. [21] including 174 patients with iron deficiency anemia presenting to an internal medicine outpatient clinic, fatigue severity – assessed using a Visual Analog Scale for fatigue – was reported to be high (mean score, 6.2/10). Similarly, our study demonstrated significant associations between fatigue severity and hemoglobin and ferritin levels. However, because the primary aim of the present study was to evaluate the relationship between serum 25(OH)D levels and fatigue, participants with anemia were excluded to minimize the impact of overt hematologic disorders on the results [21]. Notably, the associations between fatigue and hemoglobin/ferritin persisted despite exclusion of anemic individuals, suggesting that fatigue may be related not only to overt anemia but also to reduced iron stores or subclinical states such as functional/early iron deficiency [22, 23]. Consistent with this interpretation, several studies in non-anemic individuals with low ferritin have reported that iron supplementation is associated with improvement in subjective fatigue outcomes, indirectly supporting our findings [22, 23].

In our study, no statistically significant association was found between serum 25(OH)D levels and fatigue severity. The literature on this topic remains inconsistent. Roy et al. [24]evaluated 174 patients presenting to an internal medicine outpatient clinic with fatigue and reported vitamin D deficiency in the vast majority; moreover, patient-reported fatigue decreased markedly after vitamin D supplementation. However, such findings may be influenced by selection bias and the subjective nature of post-supplementation assessment and may also reflect concurrent lifestyle changes and placebo effects. In a study by Knutsen et al. [25] including 572 patients of diverse ethnic backgrounds presenting with myalgia, fatigue, and headache, headache was reported to be more prominent than fatigue among those with low vitamin D levels, highlighting heterogeneity in symptom patterns. Conversely, a large-scale study conducted in the United Kingdom between 2006 and 2010 involving approximately 500,000 individuals did not demonstrate a meaningful association between vitamin D levels and self-reported fatigue and suggested that interventions aimed at increasing vitamin D levels are unlikely to have clinically significant effects on fatigue symptoms [26]. Differences

across studies may be attributable to variable control of confounding factors that can simultaneously contribute to both fatigue and low vitamin D status, such as obesity, physical inactivity, reduced sunlight exposure, dietary habits, sleep disturbances, and psychological conditions. In this context, the lack of comprehensive assessment of these potential confounders in the present study may have contributed to the absence of an observed vitamin D–fatigue association.

Although data collection was performed within a relatively limited seasonal period (autumn), future multicenter, prospective studies incorporating broader seasonal variation and detailed lifestyle variables may clarify the vitamin D–fatigue relationship more reliably. In addition, randomized controlled trials would provide higher-level evidence regarding the potential effect of vitamin D replacement on fatigue symptoms.

Study Limitations

This study has several limitations. First, its cross-sectional design precludes causal inference between vitamin D levels and fatigue severity. Second, the study was conducted at a single center with a predominantly female population, which may limit generalizability. Third, potential confounding variables including body mass index, physical activity, sleep quality, sunlight exposure, dietary habits, and inflammatory markers were not comprehensively evaluated. Finally, multivariable regression analyses adjusting for possible confounders such as age and sex were not performed.

Conclusion

In this study, no statistically significant association was found between serum 25(OH)D levels and FSS scores. In contrast, fatigue severity was associated with hematological parameters, particularly hemoglobin and ferritin. Given that fatigue is a multifactorial and subjective symptom, a comprehensive clinical evaluation of individuals presenting with fatigue should consider not only vitamin D status but also hematologic factors and other potentially contributing metabolic, psychological, and lifestyle-related determinants.

Ethics Committee Approval: This study was approved by The Ethics Committee of University of Health Sciences, Umraniye Training and Research Hospital on 10 July 2025 (Approval No: 237).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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