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Association of vitamin D status with ferritin and TSH levels across age and sex groups: a cross-sectional study

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ABSTRACT

Vitamin D deficiency has been implicated in disturbances of thyroid function and iron metabolism, but data from Iraqi clinical populations remain limited. We performed a hospital-based cross-sectional study (August 2024-January 2025) of 99 adults (15-45 years; 49 males, 50 females) attending Mosul General Hospital to examine associations between serum 25-hydroxyvitamin D status and two biochemical markers: thyroid-stimulating hormone (TSH) and ferritin. Participants were classified as vitamin D deficient (≤10 ng/mL, n = 37), insufficient (11-20 ng/mL, n = 31), or sufficient (>20 ng/mL, n = 31). Mean TSH was highest in the deficient group (5.09 ± 0.77 µIU/mL) and lowest in the sufficient group (1.90 ± 0.13 µIU/mL; $p < 0.001$). Mean ferritin was lowest in the deficient group (42.8 ± 3.07 ng/mL) and higher in the sufficient group (60.26 ± 7.3 ng/mL; $p = 0.03$). Correlation analysis showed a significant inverse association between vitamin D and TSH ($r = -0.488$, $p < 0.001$) and a positive association between vitamin D and ferritin ($r = 0.212$, $p = 0.035$). These findings indicate that lower vitamin D status is associated with higher TSH and lower iron stores in this hospital cohort. Consideration of vitamin D and iron status may aid the interpretation of abnormal TSH values and warrants prospective investigation to test whether nutritional correction modifies thyroid-related outcomes.

Keywords: *Ferritin, Mosul hospital, Thyroid-stimulating hormone, Vitamin D*

1 INTRODUCTION

Thyroid hormones, triiodothyronine (T3) and thyroxine (T4), are central regulators of metabolism, growth, and development, and their secretion is controlled by thyroid-stimulating hormone (TSH) from the pituitary gland [1]. Routine clinical assessment of thyroid function commonly relies on TSH and circulating thyroid hormone measurements to detect dysfunction and guide management [2]. In addition to iodine, thyroid physiology and peripheral hormone metabolism are influenced by several micronutrients and metabolic factors; deficiencies and imbalances in these elements can alter hormone synthesis, peripheral conversion, and overall endocrine homeostasis [3, 4]. Beyond classical micronutrients, metabolic biomarkers, such as branched-chain amino acids, have also been explored for their associations with hepatic and lipid parameters, highlighting a broader metabolic network that can interact with nutritional and endocrine status [5].

Iron has a direct biochemical role in thyroid hormone biosynthesis because thyroid peroxidase is an iron-dependent enzyme; therefore, iron deficiency and reduced ferritin (the principal intracellular iron-storage protein) may impair thyroid hormone production and peripheral conversion [4, 6]. Several hospital-based reports have described altered ferritin and vitamin D concentrations in patients evaluated for thyroid-related concerns, support-

ing concurrent assessment of these markers in clinical samples [6, 7]. At the population level, iron deficiency and anemia continue to represent major public-health issues that can confound clinical and biochemical associations [8], while vitamin D insufficiency/deficiency is widely recognized and has been discussed as a global health problem with potential extra-skeletal effects [9].

Epidemiological studies have reported associations between lower serum 25-hydroxyvitamin D and altered thyroid indices, including higher TSH levels or increased odds of hypothyroidism in cross-sectional analyses [10]. Local and regional clinical series have similarly documented concurrent reductions in vitamin D and ferritin among patients assessed for thyroid abnormalities [7, 11]. Investigations of trace elements in relation to thyroid disease and treatment further emphasize that mineral status may modify biochemical thyroid markers [12, 13]. Genetic and epigenetic factors, such as skewed X-chromosome inactivation, have been proposed as contributors to the female predominance of many thyroid disorders and may be relevant when interpreting sex-stratified relationships between micronutrients and thyroid indices [14].

Mechanistically, vitamin D receptors are expressed in thyroid tissue, and vitamin D exerts immunomodulatory effects that could influence autoimmune processes affecting the thyroid [7]. Iron availability, as reflected by ferritin levels, is required for optimal thyroid peroxidase activity and thus for effective thyroid hormone biosynthesis [15]. Oxidative stress and altered antioxidant defenses observed in experimental hypothyroidism may further perturb iron handling and ferritin expression [16]. Clinical conditions with altered iron handling (e.g., major thalassemia) illustrate how iron overload or chelation can affect thyroid parameters, underscoring the complexity of iron–thyroid interactions [17]. Recent hospital and observational studies continue to report variable ferritin and vitamin D profiles among patients investigated for thyroid complaints, indicating the need for locally relevant biochemical data [17, 18].

Taken together, the literature indicates a complex, potentially bidirectional relationship among vitamin D status, iron stores (as reflected by ferritin), and TSH, with heterogeneous findings across study designs and populations [4, 17, 18]. Broader prospective and mechanistic work underscores the systemic importance of adequate vitamin D and justifies examining its associations with endocrine markers [19], while regional clinical and metabolic studies provide additional local context relevant to this investigation [20].

Accordingly, the present study aimed to evaluate the association between categorized serum vitamin D status (deficiency, insufficiency, sufficiency) and two biochemical markers, TSH and ferritin, across age and sex subgroups using hospital laboratory records. The objective was descriptive and associative: to characterize biochemical relationships that may inform hypotheses for future prospective or interventional research.

2 MATERIALS AND METHODS

2.1 Study design and setting

This hospital-based cross-sectional study was conducted at Mosul General Hospital, Nineveh Health Directorate, Mosul, Iraq. The study utilized existing laboratory records and associated demographic information obtained from the hospital laboratory database. Data collection covered the period August 2024 to January 2025.

2.2 Study population and eligibility criteria

A total of 99 subjects aged 15–45 years were identified from the laboratory records. The sample included 49 males and 50 females. Subjects were eligible if they had recorded serum values for thyroid-stimulating hormone (TSH), 25-hydroxyvitamin D (hereafter referred to as "vitamin D"), and ferritin measured during the study period. Participants were excluded if their records indicated chronic smoking, current vitamin D supplementation, or pregnancy at the time of testing. No further clinical interventions were performed during this study.

2.3 Classification of vitamin d status and subgrouping

Vitamin D status was categorized according to predefined cutoffs:
Deficiency: ≤10 ng/mL
Insufficiency: 11–20 ng/mL
Sufficiency: >20 ng/mL
For subgroup analyses, subjects were further stratified by age (15–30 years, >30 years) and sex (male, female).

2.4 Laboratory assays and specimen handling

All measurements were obtained as part of the hospital's standard diagnostic protocol. Venous blood samples were collected into plain tubes, allowed to clot, and centrifuged at 2,000 rpm for 10 minutes. Serum concentrations of TSH, vitamin D, and ferritin were measured using the Mini VIDAS® system (bioMérieux, France) with enzyme-linked immunofluorescent assay (ELFA) methodology. Recorded units were:

TSH: µIU/mL
Vitamin D: ng/mL
Ferritin: ng/mL

2.5 Data extraction and study variables

From the laboratory database, the following variables were extracted: age, sex, serum vitamin D, serum ferritin, and serum TSH. Vitamin D categories and age strata were applied as defined above for all statistical comparisons.

2.6 Statistical analysis

SPSS for Windows, version 27, was utilized for data analysis. Continuous variables are shown as mean ± standard error (SD), while categorical variables are expressed as frequencies and percentages. The Kruskal-Wallis test was employed for multiple comparisons to identify differences among the three vitamin D categories. The t-test for normally distributed data or the Mann-Whitney U test for abnormally distributed data were used for subgroup comparisons. Spearman's rank correlation coefficient assessed the relationship between vitamin D and TSH, as well as between vitamin D and ferritin. A two-sided p-value ≤0.05 was deemed statistically significant. Scatter plots were created to visualize the directions of the observed correlations.

3 RESULTS

Table 1 illustrates the demographic characteristics of the study participants. The age distribution shows that 49.49% of subjects were 15–30 years old, while 50.5% were older than 30 years. Regarding sex, the study included 49 males and 50 females.

Table 1 Demographic features of patients

Features Total No. 99		Frequencies	Percentage (%)
Age years	15–30	49	49.49%
	> 30	50	50.5%
Gender	Male	49	49.49%
	Female	50	50.5%

Table 2 summarizes mean ± SE values of serum vitamin D, TSH, and ferritin across the three vitamin D categories. As expected, serum 25-hydroxyvitamin D differed markedly between groups (deficiency: 8.50 ± 0.01 ng/mL; insufficiency: 16.90 ± 0.58 ng/mL; sufficiency: 33.04 ± 1.1 ng/mL; p < 0.001, Kruskal–Wallis). Mean TSH was highest in the deficiency group (5.09 ± 0.77 µIU/mL) and lowest in the sufficiency group (1.90 ± 0.13 µIU/mL) (p < 0.001, Kruskal–Wallis). Mean ferritin also differed across vitamin D categories (deficiency: 42.8 ± 3.07 ng/mL; insufficiency: 58.54 ± 4.5 ng/mL; sufficiency: 60.26 ± 7.3 ng/mL; p = 0.03, Kruskal-Wallis).

Table 2 Classification of Subjects According to Vitamin D Levels

Parameters (Mean ± SE)	Deficiency Vit D No.=37	Insufficient Vit D No.=31	Sufficiency Vit D (optimal value) No.=31	P-value
Vit D (ng/mL)	8.5 ± 0.10	16.9 ± 0.58	33.04 ± 1.1	$< 0.001^{*K}$
TSH (μIU/ml)	5.09 ± 0.77	2.56 ± 0.30	1.9 ± 0.13	$< 0.001^{*K}$
Ferritin (ng/ml)	42.8 ± 3.07	58.54 ± 4.5	60.26 ± 7.3	0.03^{*K}

*TSH, thyroid-stimulating hormone; K, Kruskal–Wallis test; *, significant level of difference when p value ≤ 0.05.*

Table 3 shows age-stratified (15–30 vs >30 years) mean ± SE values and within-category comparisons. Participant counts were roughly balanced between age strata within each vitamin D category. For TSH, a significant age effect was observed only in the vitamin D deficiency group: older participants (>30 years) had higher TSH than younger participants (6.90 ± 1.30 vs 3.08 ± 0.24 µIU/mL; p = 0.02, Mann–Whitney U). No significant age differences in TSH were observed in the insufficiency or sufficiency groups (p = 0.545 and p = 0.953, respectively). For ferritin, a significant age difference was found in the insufficiency group (older >30 years: 66.7 ± 7.2 ng/mL vs younger 45.9 ± 4.8 ng/mL; p = 0.045), whereas ferritin differences in the deficiency and sufficiency groups did not reach significance (p = 0.663 and p = 0.163, respectively).

Table 3 Impact of Age Groups on TSH and Ferritin Concentrations Among Patients with varying Vitamin D Levels.

Parameters (Mean ± SE)	Deficiency Vit D ≤ 10 ng/ml		Insufficiency Vit D 11–20 ng/ml		Sufficiency Vit D > 20 ng/ml	
Age (years)	**15–30**	> 30	**15–30**	> 30	**15–30**	> 30
Frequencies	18 (18.18%)	19 (19.19%)	16 (16.16%)	15 (15.15%)	15 (15.15%)	16 (16.16%)
TSH (μIU/ml)	3.08±0.24	6.9 ± 1.3	2.7 ± 0.35	2.68±0.57	1.84±0.19	1.95±0.18
P-value	0.02^{*m}		0.545^{m}		0.953^{m}	
Ferritin (ng/ml)	38.5 ± 2.7	46.5 ± 5.3	45.9 ± 4.8	66.7 ± 7.2	46.8 ± 5.1	74 ± 12.7
P-value	0.663^{m}		0.045^{*m}		0.163^{m}	

*TSH, thyroid-stimulating hormone; m, Mann–Whitney U test; * significant level when p value ≤ 0.05.*

Table 4 displays sex-stratified mean ± SE values for TSH and ferritin within each vitamin D category. For TSH, no statistically significant sex differences were

observed in any category: deficiency (male 4.25 ± 0.87 vs female 5.88 ± 1.20; p = 0.118), insufficiency (male 2.23 ± 0.39 vs female 2.90 ± 0.49; p = 0.216), and sufficiency (male 1.86 ± 0.19 vs female 1.95 ± 0.18; p = 0.732). For ferritin, a statistically significant sex difference was observed only in the sufficiency group (male 72.06 ± 11.8 ng/mL vs female 49.18 ± 8.2 ng/mL; p = 0.02). Ferritin differences by sex in the deficiency and insufficiency groups did not reach statistical significance (p = 0.443 and p = 0.437, respectively).

Table 4 Impact of Gender Groups on TSH and Ferritin Concentrations Among Patients with Varying Vitamin D Levels.

Parameters (Mean ± SE)	Deficiency Vit D ≤ 10 ng/ml		Insufficiency Vit D 11–20 ng/ml		Sufficiency Vit D > 20 ng/ml	
Gender	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Frequencies	18 (18.18%)	19 (19.19%)	16 (16.16%)	15 (15.15%)	15 (15.15%)	16 (16.16%)
TSH (μIU/ml)	4.25±0.87	5.88 ± 1.2	2.23±0.39	2.9 ± 0.49	1.86±0.19	1.95±0.18
P-value	0.118^{m}		0.216^{m}		0.732^{T}	
Ferritin (ng/ml)	45.27±4.4	40.47±4.2	62.06±6.9	54.8±6.01	72.06 ± 11.8	49.18±8.2
P-value	0.443^{m}		0.437^{T}		0.02^{*m}	

*TSH, thyroid-stimulating hormone; T, independent t-test; m, Mann–Whitney U test; * significant level when p-value ≤ 0.05.*

Table 5 shows that vitamin D was negatively correlated with TSH in the patient group (r = -0.488, p < 0.001). In contrast, vitamin D levels were positively correlated with ferritin levels (r = 0.212, p = 0.035), indicating that higher vitamin D levels were associated with higher ferritin concentrations.

Table 5 Spearman's correlation coefficients of Vitamin D with TSH and Ferritin

Parameters	Correlation Coefficient	p value
TSH (μIU/ml)	-0.488	< 0.001*
Ferritin (ng/ml)	0.212	0.035*

*TSH, thyroid-stimulating hormone; *, Significant level when p-value ≤ 0.05.*

Figures 1 and 2 visually support these findings using scatter plots. Figure 1 displays a downward trend line, reflecting the inverse correlation between vitamin D and TSH. Figure 2 shows a slight upward trend line, consistent with the positive correlation between vitamin D and ferritin. The scatter plots show the distribution of individual data points and the direction of the correlations, further confirming the statistical outcomes presented in Table 5.

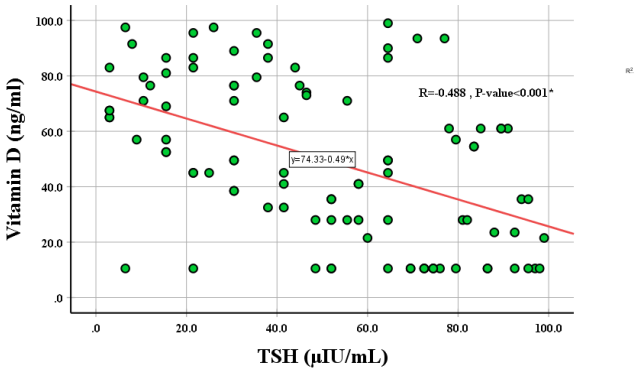

Fig. 1 Scatter plot to show Spearman's rank correlation of Vitamin D with TSH

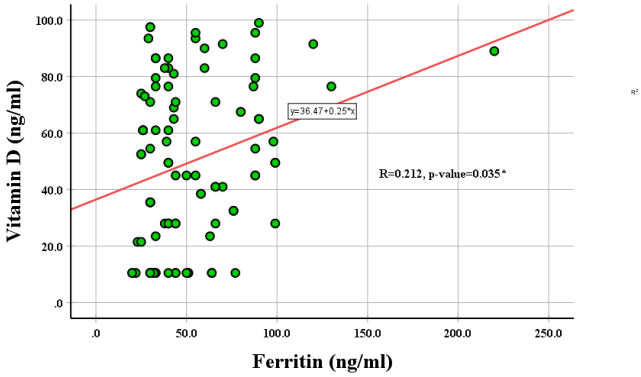

Fig. 2 Scatter plot to show the Spearman rank correlation of Vitamin D with Ferritin

4 DISCUSSION

This hospital-based cross-sectional study examined the relationship between vitamin D status categories and two clinically relevant biochemical markers, TSH and ferritin, among adults attending Mosul General Hospital. The principal finding is that lower vitamin D status co-occurs with a less favorable thyroid–iron profile: participants in the vitamin D-deficient category had higher mean TSH and lower mean ferritin than those with sufficient vitamin D. Age and sex stratification revealed that the association between low vitamin D and elevated TSH was more pronounced in older adults, while sex differences were most evident for ferritin concentrations. These observations indicate that vitamin D status covaries with

both thyroid regulation and iron stores in this clinical cohort.

Our results are consistent with a growing body of evidence linking micronutrient status to thyroid physiology. Several authors have emphasized that micronutrients, including iron, iodine, and other trace elements, are central to thyroid hormone synthesis and metabolism, and deficiencies can perturb thyroid function [3, 15]. In particular, iron is a cofactor for thyroid peroxidase, and iron deficiency (reflected by low ferritin) has been associated with impaired thyroid hormone synthesis and altered thyroid indices in systematic and primary studies [4, 15]. Likewise, clinical reports have documented concurrent reductions in vitamin D and ferritin among patients evaluated for thyroid disorders, suggesting interconnected pathways between vitamin D, iron status, and thyroid function [6, 7].

Epidemiological investigations have reported associations between lower 25-hydroxyvitamin D concentrations and adverse thyroid outcomes, such as higher TSH levels or increased odds of hypothyroidism, supporting the pattern observed here [10]. Mechanistically, vitamin D has recognized immunomodulatory properties, and vitamin D receptors are widely expressed in endocrine tissues; these features provide biologically plausible routes by which vitamin D status may influence thyroid homeostasis and immune-mediated thyroid disease processes [9, 10]. In addition, oxidative stress and altered antioxidant defenses reported in thyroid dysfunction can affect iron handling and ferritin expression, further linking the iron–thyroid–vitamin D triad [16].

The age-specific pattern we observed, higher TSH in older participants with vitamin D deficiency, warrants particular attention. Aging is accompanied by changes in endocrine regulation, nutritional status, and chronic disease burden that may amplify the biochemical consequences of micronutrient deficits. Age-related differences in iron stores and inflammation may also modify the relationship between vitamin D and thyroid markers, as suggested by higher ferritin in older participants within certain vitamin D strata [17]. These subgroup findings underscore the importance of stratified analyses: aggregate associations can mask clinically relevant heterogeneity across age and sex.

Sex differences in our dataset were largely confined to ferritin, with higher ferritin in males, a finding that aligns with known physiological dimorphism in iron metabolism and storage [18]. TSH did not show consistent sex-specific variation across vitamin D categories in this cohort, which suggests that sex modifies iron-related pathways more strongly than thyroid regulatory set points in the biochemical context studied. Genetic and epigenetic factors, including X-chromosome inactivation patterns linked to autoimmune thyroid disease susceptibility, may also contribute to observed sex differences and merit consideration in future mechanistic studies [14].

This study has several strengths worth highlighting. All three analytes (25-hydroxyvitamin D, ferritin, TSH) were measured in the same laboratory using standardized methods, and analyses included stratification by age and sex to identify subgroup patterns. The hospital-based setting provides locally relevant data for Nineveh, a population that has been underrepresented in prior reports on micronutrient–thyroid interactions [20].

Although the retrospective, cross-sectional design prevents causal inference, and uniformly measured anti-thyroid antibodies and free-hormone data were not available, the study reveals consistent associations between vitamin D, ferritin, and TSH in a locally representative hospital cohort. Clinically, these findings suggest that assessment of vitamin D and iron status should be considered when interpreting abnormal TSH, particularly in older patients or when multiple deficiencies are likely. Prospectively, the results justify longitudinal and mechanistic studies (including anti-TPO/anti-Tg, FT4/FT3, and inflammatory markers) and interventional trials to determine whether correcting vitamin D and/or iron deficits improves thyroid outcomes.

5 CONCLUSION

In conclusion, in this hospital cohort from Mosul, low vitamin D status was associated with higher TSH and lower ferritin, with age-specific effects on TSH and limited sex effects on ferritin. These observations strengthen the rationale for integrative nutritional–endocrine evaluation in patients with thyroid abnormalities and provide a rationale for future interventional and longitudinal investigations in similar populations.

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Author contributions

All authors contributed equally to the work.

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Data availability

N/A

DECLARATIONS

Conflict of interest

The authors declare no competing interests.

Consent to publish

N/A

Ethical approval

The study protocol was approved by the Scientific Committee of the Department of Medical Physics (Reference No. 4/74; 18/7/2024). As the study was based solely on anonymized laboratory records, individual patient consent was waived under institutional ethical guidelines.

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