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"Comparative Analysis of Endocrine Function in Sample of Iraqi Patients with Sufficient and Deficient Vitamin D Levels."

Research Project submitted to

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BY

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Abstract:

Introduction: Vitamin D deficiency is a global health concern with potential implications for thyroid function. This study investigated associations between vitamin D status and specific endocrine disorders, especially thyroid function, in Iraqi adults from four governorates.

Methods: A cross-sectional observational study was conducted on 200 Iraqi adults aged 18-65. Participants were categorized into vitamin D sufficient (VDS) and vitamin D deficient (VDD) groups based on serum 25(OH)D levels. Thyroid function tests, parathyroid hormone (PTH), and serum calcium levels were measured. Statistical analyses included independent t-tests, Mann-Whitney U tests, correlation analyses, and risk estimation.

Results: Significant differences were observed between VDS and VDD groups in thyroid-stimulating hormone (TSH) ($p = 0.005$), free thyroxine (FT4) ($p < 0.001$), PTH ($p < 0.001$), and serum calcium levels ($p = 0.015$). VDD participants showed higher TSH and PTH levels and lower FT4 and serum calcium levels than VDS participants. Vitamin D levels were weakly but significantly correlated with TSH ($r = -0.177$, $p=0.012$), FT4 ($r = 0.140$, $p=0.048$), PTH ($r = -0.166$, $p=0.018$), and serum calcium ($r = 0.239$, $p=0.001$). The prevalence of vitamin D deficiency was higher in females (67.20%) than males (59.40%). All cases of subclinical hypothyroidism ($n = 6$) were observed in the VDD group. The risk estimate was 0.953 (95% CI: 0.917-0.99), indicating a slightly lower probability of adequate thyroid function in those with low vitamin D levels.

Conclusion: This study highlights a complex relationship between vitamin D status and thyroid function in Iraqi adults. The findings suggest that vitamin D deficiency may be associated with altered thyroid function, particularly subclinical hypothyroidism. Further research is needed to elucidate these associations' mechanisms and clinical implications.

Keywords: Vitamin D deficiency, thyroid function, subclinical hypothyroidism, parathyroid hormone, calcium homeostasis.

Introduction

Vitamin D is a critical regulator of human health, as it is a hormone that affects several physiological processes and organ systems in the body. Vitamin D (this fat-soluble secosteroid) plays a major role in endocrine functionality and general health[1, 2]. Vitamin D greatly influences parts of the endocrine system, particularly regulates calcium homeostasis and bone metabolism, influencing glands such as the thyroid, parathyroid, and pancreas, among others[3]. Vitamin D has a classic endocrine role. Maintaining calcium and phosphate homeostasis is essential for bone health and cellular functions[4]. It works by helping the intestine absorb more calcium and also helps keep it in the kidneys when there is a deficiency in mobilization from bone. It acts with parathyroid hormone (PTH) to maintain serum calcium within a narrow concentration[5]. Several studies did find associations between vitamin D status and levels of thyroid hormones, but results have been inconsistent[6].

Vitamin D deficiency is a major health problem in Iraq, and studies have recently reported high prevalence rates among different population groups. A cross-sectional study conducted on the general population in Duhok province, Iraq, in 2021 reported a high prevalence of vitamin D deficiency among the target group, which was about (41.1%), and statistically significant higher deficiency rates were observed for females (43.03%) compared to male (37.8%; $P = 0.014$)[7]. In particular, a 2023 study from Sulaymaniyah Governorate observed that only 25% of all adults had adequate vitamin D levels (>30 ng/ml)[8]. Sadly, the case is more alarming among pregnant women in Iraq. In 2023, a study on pregnant women in Sulaimaniyah City reported a remarkable hypovitaminosis D prevalence of 71.3%[9]. Such a high prevalence is worrisome, especially due to the potential adverse effects of vitamin D deficiency on maternal and infant health.

Although vitamin D and thyroid function have been widely studied, there are still many gaps in knowledge, such as a lack of data on the prevalence of Vitamin D deficiency alongside its correlation with thyroid function tests in the multidimensional governorates in Iraq, an insufficient understanding of vital mechanisms linking vitamin D deficiency to subclinical hypothyroidism across different populations, absence of a cross-sectional study investigating the relationship between Vitamin D deficiency thyroid hormones Parathyroid hormone and Calcium levels in one group; Finally, gender differences associated with Vitamin D Deficiency as well as its impact on Thyroid status have not been subjected to adequate research projects among Middle Eastern States.

We aim to overcome these limitations by delivering aspects of vitamin D status related to thyroid function in a healthy, multi-governorate sample from Iraq, exploring the possible link between vitamin D deficiency and subclinical hypothyroidism in a population with a very high prevalence of vitamin D deficiency, examining the potentially complex interactions between vitamin D with parathyroid hormone and calcium levels, and investigating gender differences with vitamin D status for tailoring effective interventions & public health recommendations.

Materials and Methods

Study Design and Participants

A cross-sectional observational study was carried out throughout four governorates of Iraq, namely Karbala, Hilla (Babylon), Diwaniyah (Al-Qadisiyyah), and Salah al-Din governorate. The present study focused on older adults aged 18–65; 200 adults were divided into two groups based on their vitamin D status.

Population of the study and size of the sample

The current study included 200 Iraqi adults who were recruited. Sample size was calculated using G*Power 3.1 Software ($\alpha = 0.05$; power $(1-\beta) = 0.80$; medium effect size ($d = 0.5$); two-tailed t-test).

Criteria for Inclusion and Exclusion

Inclusion criteria:

1. Iraqi adults between the ages of 18–65 who resided in Karbala, Hilla, Diwaniyah, and Salah al-Din
2. Available to give informed consent
3. At present, no vitamin D supplementation

Exclusion criteria:

1. Pregnant or lactating women
2. Subjects already identified with malabsorption syndromes, liver, and kidney disease.
3. Patients receiving vitamin D antagonistic medications
4. Those with a history of hypothyroidism or hyperthyroidism
5. Diabetes Mellitus confirmed cases

Data Collection

Participants were recruited among attendees of outpatient clinics in the four mentioned governorates. Informed consent was taken then.

Laboratory Analysis

Blood samples were collected, and serum was prepared within 30 min after collection and stored at − 80°C until determination. The serum concentrations of thyroid-stimulating hormone (TSH), total triiodothyronine (T3) and free thyroxine (FT4), parathyroid hormone (PTH), and 25-hydroxyvitamin D (vitamin D3) were measured quantitatively by the MAGLUMI chemiluminescence immunoassay (CLIA) system on a fully automated MAGLUMI analyzer (Snibe Diagnostic, Shenzhen, China)[(10)].

A colorimetric assay based on the Arsenazo III method was used for serum calcium determination. The analysis was performed with a commercial kit (Spinreact, Spain) in an automated clinical chemistry analyzer[(11)]. This well-established method is a conventional technique used in clinical laboratories. Results were expressed in mg/dL, and quality control materials were run with each batch of patient samples to confirm measurement accuracy and precision. A high-performance liquid chromatography (HPLC) was used for the measurement of HbA1c (Bio-Rad D-10; Bio-Rad Laboratories, Hercules, CA, USA)[(12)].

Patient Classification

Participants were categorized into two groups according to their serum 25(OH) D levels,

Group A: Vitamin D Sufficient (VDS) (≥30 ng/mL)

Group B: (<30 ng/mL): Vitamin D Deficiency (VDD). This classification is based on the broadly accepted criteria for vitamin D status[(13, 14)].

Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics, version 26.0. Descriptive statistics were reported as means ± standard deviations for continuous variables and frequencies (percentages) for categorical variables. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess the normality of data distribution. Independent t-tests for normally distributed continuous variables (Free T4) and Mann-Whitney U tests for non-normally distributed continuous variables (TSH, Total T3, PTH, Serum Calcium, and HbA1c) were performed to compare VDS and VDD groups. A correlation analysis was made to find the correlation between the vitamin D level and the other variables. Due to a nonnormal distribution, Spearman's rho was used for TSH, Total T3, HbA1c, PTH, and Serum Calcium, whereas Free T4 was estimated using Pearson's correlation. Outcomes Risk estimate (odds ratio) with 95% confidence intervals for vitamin D deficiency to thyroid function. All analyses were declared statistically significant at $p < 0.05$. Results of the correlation analysis are notated with * for $p<0.05$ and ** for $p<0.01$ significance levels.

Results:

This study was designed to explore associations between a range of biochemical and hormonal parameters and blood concentrations of vitamin D in 200 individuals. The results show differences between VDS and VDD groups, and correlations with vitamin D levels are statistically significant.

Status and Biochemical Parameters of Vitamin D

Significant differences in biochemical and hormonal parameters between VDS and VDD groups are shown in Table 1. The VDD group had a significantly higher TSH level (1.96 ± 0.98 mIU/L) than the VDS group (1.52 ± 0.62 mIU/L, p = 0·005).

Compared to the VDS group, free T4 levels in the VDD group were significantly lower (1.11 ± 0.10 ng/dL vs. 1.33 ± 0.11 ng/dL; $p < 0.001$). The VDD group showed a significantly higher level of PTH (55.71 ± 19.13 pg/mL) than the VDS group (44.11 ± 16.68 pg/mL, $p < 0.001$). When compared to the VDS group (9.44 ± 0.30 mg/dL, $p = 0.015$), serum calcium levels were significantly lower in the VDD group (8.87 ± 1.52 mg/dL). Interestingly, Total T3 ($p = 0.101$) and HbA1c ($p = 0.671$) did not differ significantly between the VDS and VDD groups as shown in Table 1.

Vitamin D levels among gender groups

Vitamin D classification by gender data based on Table 2 The percentages of males and females who were vitamin D deficient were 59.40% and 67.20%, respectively, indicating a higher prevalence of vitamin D deficiency among females than in males in our study population.

Correlation with Vitamin D Levels and Scatter Plot

The correlation analysis (Table 3, Figures 1–4) showed that vitamin D levels were significantly associated with the following parameters: As illustrated in Figure 1, TSH had a weak negative correlation with VITD3 ($r = -0.177$; $p = 0.012$). Similarly, as seen in Figure 3, PTH had a weak negative association with VITD3 ($r = -0.166$; $p = 0.018$). As depicted in Figure 4, serum calcium correlated weakly and positively with VITD3 ($r = 0.239$, $p = 0.001$). Figure 2 shows a weak positive correlation between VITD3 and free T4 ($r = 0.140$, $p = 0.048$). As demonstrated in Table 3, it should be acknowledged that neither Total T3 ($p = 0.072$) nor HbA1c ($P = 0.684$) correlated significantly with VITD3.

Association Between Serum 25-Hydroxy Vitamin D Deficiency and Thyroid Function

Thyroid function analysis in conjunction with vitamin D status is shown in Table 4. Among 200 patients, 194 had normal thyroid function, and six subclinical hypothyroidism. Interestingly, all cases of subclinical hypothyroidism were found among vitamin D-deficient persons. We applied the criteria for thyroid dysfunction as defined by the American Family Physician (AAFP) guidelines and the European Thyroid Association (ETA) guidelines. Primary hypothyroidism was defined as elevated TSH (>4.0 mIU/L) with low Free T4 (<0.8 ng/dL), while subclinical hypothyroidism was characterized by TSH between 4.0-10.0 mIU/L with normal Free T4[15, 16].

Risk of Vitamin D Insufficiency for Subclinical Hypothyroidism

The risk estimate of 0.953 is smaller than 1.0, as is its confidence interval, which ranges from 0.917 to 0.990, indicating that the probability of having adequate thyroid function is slightly lower in those with low vitamin D levels, as shown in Table 5; however, further statistical tests are needed to determine whether the association is statistically significant. Table 6 displays the Chi-Square and Fisher's Exact tests used to determine a relationship between Thyroid Function and Vitamin D Levels. The p-value in the Pearson Chi-Square test was 0.059, meaning there is no statistically significant association between variables at a 5% significance level (that means our null hypothesis cannot be rejected at a significance level of 0.05, which is a conventional value). Nonetheless, this is nearly significant and may represent a relationship worth pursuing. Results described in Table 6 suggest an association of Thyroid Function with Vitamin D Levels (Likelihood Ratio statistics: p-value=0.018). Fisher's Exact Test, more appropriate given low expected cell counts, yielded a two-sided p-value of 0.088 and a one-sided p-value of 0.063. These figures do not reach a 5% level of statistical significance but may suggest a potential trend that warrants further investigation. Table 6 shows that, with a p-value of 0.060 obtainable from the Linear-by-Linear Association test result, this also suggests no evidence against a linear relationship between them.

Table 1: Biochemical and Hormonal Parameter Comparison of Vitamin D Sufficient (VDS) and Vitamin D Deficient (VDD) Groups.

Variable	VDS (n=71)	VDD (n=129)	p-value
TSH (mIU/L)	1.5(1)	1.8(1.30)	0.005†
Total T3 (ng/dL)	120(31)	116(17)	0.101†
Free T4 (ng/dL)	1.33 ± 0.11	1.11 ± 0.10	<0.001*
PTH (pg/mL)	44(20)	51(28)	<0.001†
Serum Calcium (mg/dL)	9.3(0.9)	9.2(0.91)	0.015†
HbA1c (%)	6.3(1.70)	6.1(2)	0.671†

Notes: Data Presentation: Values are presented as median(IQR) for non-normally distributed variables; Free T4 is presented as mean ± SD due to normal distribution

Statistical Analysis:

*indicates variables with a normal distribution ($p > 0.05$ in both Kolmogorov-Smirnov and Shapiro-Wilk tests), analyzed using a t-test

† indicates variables with non-normal distribution ($p < 0.05$ in one or both normality assessments), analyzed using Mann-Whitney U test

Abbreviations:VDS: Vitamin D Sufficiency, VDD: Vitamin D Deficiency, IQR: Interquartile Range, SD: Standard Deviation

Table 2: Distribution of VDS and VDD by Gender.

Gender	VDS Count	VDS Percentage	VDD Count	VDD Percentage
Males (M)	28	40.60%	41	59.40%
Females (F)	43	32.80%	88	67.20%

Table 3: Correlation Between Vitamin D (VITD3) and Other Variables

Variables	TSH (mU/L)	Total T3 (ng/dL)	HbA1c (%)	PTH (pg/mL)	Serum.Ca (mg/dL)	Free T4 (ng/dL)
Correlation Coefficient	*-0.177	0.127	-0.029	*-0.166	**0.239	*0.140
Sig. (2-tailed)	0.012	0.072	0.684	0.018	0.001	0.048

Significance levels: *: Significant at the 0.05 level. **: Significant at the 0.01 level.

Spearman's rho was employed for TSH, Total T3, HbA1c, PTH, and Serum Calcium due to the potential non-normality of these variables, but Pearson's correlation was utilized for Free T4, implying a normal distribution for this variable.

Table 4: Crosstabulation of Thyroid Function and Vitamin D3 Status

Thyroid Function	Vitamin D Deficient	Vitamin D Sufficient	Total
Normal	121	73	194
Subclinical Hypothyroidism	6	0	6
Total	127	73	200

Table 5: Risk Estimate for Vitamin D Deficiency Group

Description	Value	95% Confidence Interval (Lower)	95% Confidence Interval (Upper)
For cohort Thyroid Function = Normal	0.953	0.917	0.990
N of Valid Cases	200		

Table 6: Chi-Square and Fisher's Exact Test Results for the Association Between Thyroid Function and Vitamin D Levels.

Test	Value	df	p-value (2-sided)	p-value (1-sided)
Pearson Chi-Square	3.555*	1	0.059	-
Continuity Correction	2.117	1	0.146	-
Likelihood Ratio	5.556	1	0.018	-
Fisher's Exact Test	-	-	0.088	0.063
Linear-by-Linear Association	3.538	1	0.06	-

*2 cells (50.0%) have an expected count of less than 5

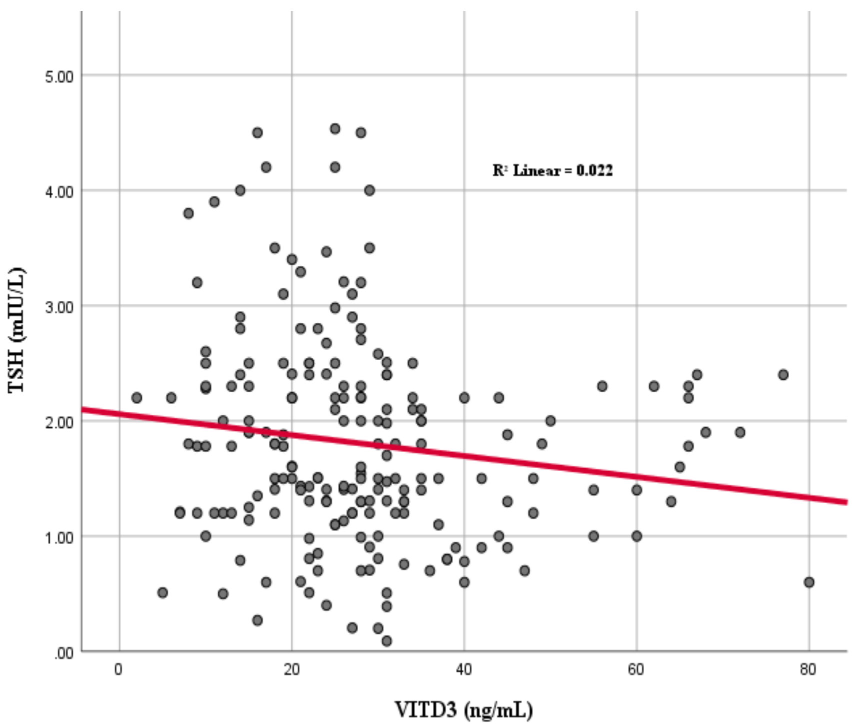

Figure 1: Simple Scatter Plot with Fitted Line of TSH (mIU/L) vs VITD3 (ng/mL)

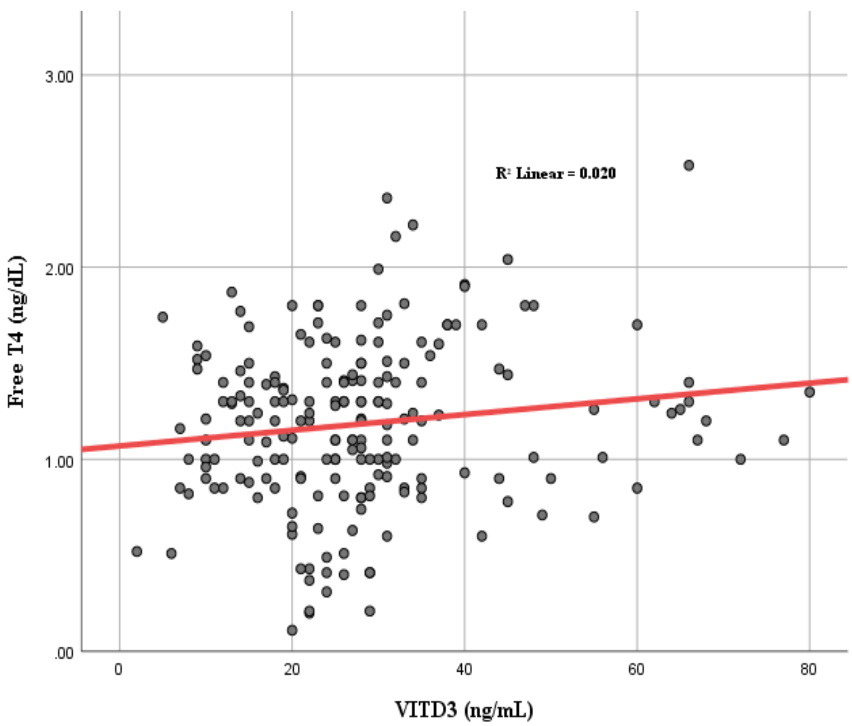

Figure 2: Simple Scatter Plot with Fitted Line of Free T4 (ng/dL) vs VITD3 (ng/mL)

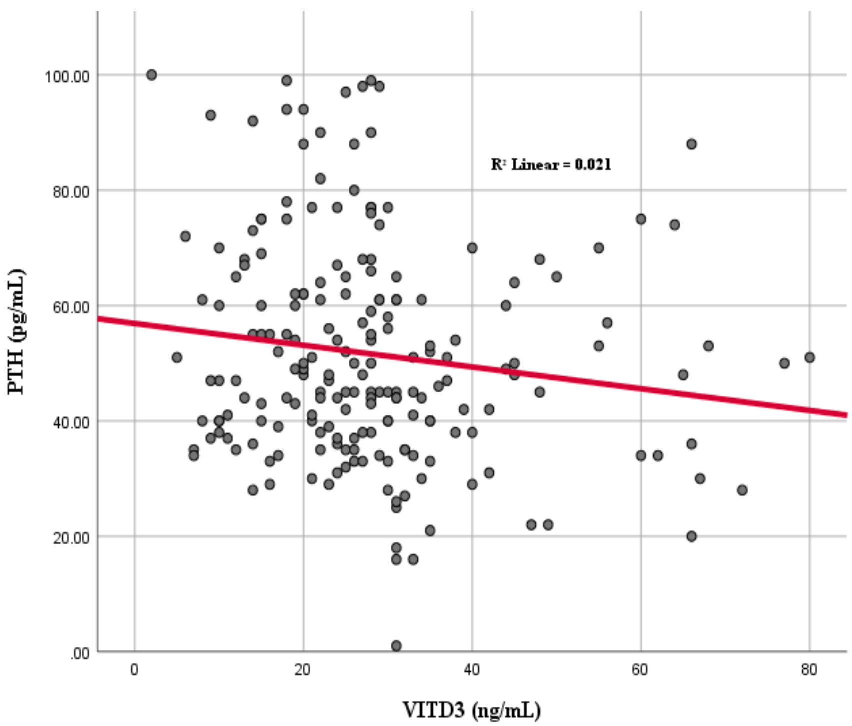

Figure 3: Simple Scatter Plot with Fitted Line of PTH (pg/mL)vs VITD3 (ng/mL)

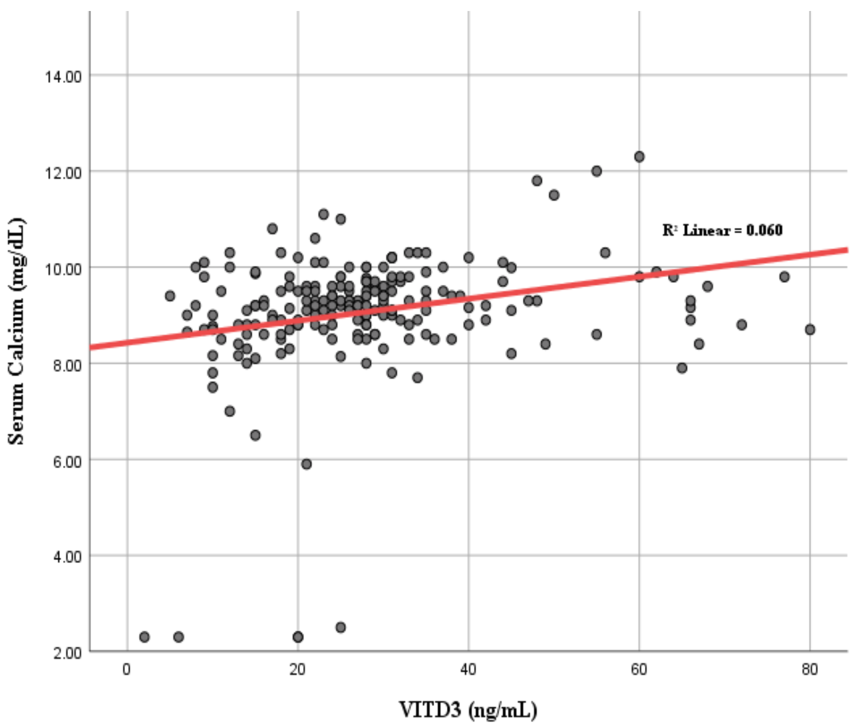

Figure 4: Simple Scatter Plot with Fitted Line of Serum Calcium (mg/dL) vs VITD3 (ng/mL)

Discussion:

This study was conducted in a cohort of 200 subjects to determine the correlation between vitamin D levels and biochemical and hormonal parameters. The key findings include the difference in TSH, Free T4, PTH, and serum calcium level (VDS vs VDD). The prevalence of vitamin D deficiency is significantly higher in females (67.20%) than males (59.40%). By contrast, TSH ($r = 0.131$), PTH ($r = -0.085$), serum calcium ($r = 0.082$), and Free T4 ($r = -0.070$) had weak but significant correlations with vitamin D levels. The cases of subclinical hypothyroidism (6 / 200 participants) were all seen in the vitamin D deficient group.

These findings indicate a multifactorial relationship between vitamin D status and thyroid function. They found that the levels of TSH were statistically significantly higher, and Free T4 levels were statistically significantly lower in VDD, which may reflect an effect on thyroid functions by vitamin D deficiency. High PTH levels in the group with VDD are consonant, with the inverse association between vitamin D and PTH. It is also known that vitamin D deficiency could cause secondary hyperparathyroidism. The weak negative correlation with TSH and the weak positive correlation with Free T4 suggest the potential effects of vitamin D on thyroid hormone regulation. A corroborative relation is the known function of vitamin D in calcium homeostasis, as we observed a positive correlation of both vitamin D and serum calcium. The fact that females have higher vitamin D deficiency compared with males indicates gender is a possible risk factor for vitamin D deficiency, relying on the evidence of differences in lifestyle, sun exposure, or due to some hormonal factors. The risk estimate of 0.953 is smaller than 1.0, as is its confidence interval, which ranges from 0.917 to 0.990 indicates, The probability of having adequate thyroid function is slightly lower in those with low vitamin D levels; on the other hand, The Vitamin D sufficient group had a higher chance of having normal thyroid function. However, it is important to acknowledge that this estimation doesn't imply causation or statistical significance. The following statistical tests give us a bit of context about that. Results were near, but not statistically significant (at the $p < 0.05$) level with the Pearson Chi-Square test p=0.059 and the Fisher's Exact Test two-sided p = 0.088, one-sided p = 0.063 tests. There is, therefore, some suggestion of an association between vitamin D status and thyroid function, but these findings were not strong enough to draw firm conclusions. Thyroid Function and Vitamin D Levels; however, the Likelihood Ratio test gave statistically significant results (p=0.018). The different results from these statistical tests demonstrate the complexity of this relationship and warrant further investigation. These findings and the risk estimate that moved in the opposite direction of statistical significance demonstrate that vitamin D deficiency is possibly related to subclinical hypothyroidism. Still, our study lacks sufficient power to prove a true association conclusion. This highlights the need for larger studies to confirm this possible relationship.

The findings were consistent with several recent studies assessing associations between thyroid function and vitamin D status. The survey by Elamawy and Ameenis[17], consistent with our findings, showed that patients with subclinical hypothyroid dysfunction had markedly lower serum Vitamin D levels than controls. This concords with most of our clinical observations that the cases of subclinical hypothyroidism only appear in the vitamin D deficient group. Similarly, Wang et al. [18]. undertook a meta-analysis that corroborates our observations. Serum vitamin D was significantly lower in patients with autoimmune thyroid diseases, Hashimoto's thyroiditis, and hypothyroidism than in healthy controls. These data match the differences in thyroid function parameters between vitamin D-sufficient and deficient groups.

It corresponds to the outcomes of Appunni et al. [19]. In their population-based study analysis using NHANES data, the authors found that vitamin D deficient patients (adjusted odds ratio [OR], 1.6; 95% CI: 1.4–1.9) and intermediate level of vitamin D was strongly associated in hypothyroidism development (OR, 1.7; 95% CI: 1.5–1.8) and findings of Donayeva A et al. [20], The second study focused on adolescents and similarly found that the group who were 25(OH)D-deficient had increased odds of subclinical hypothyroidism (OR 4.89, p=0.016) as well as clinical hypothyroidism (OR 4.3, p=0.013) compared to controls with normal vitamin D levels. This similarity supports a correlation between vitamin D deficiency and thyroid dysfunction. The weak but significant correlations we observed between vitamin D levels and various thyroid function markers align with Ibrahim's findings [21]. Their study also found significant positive correlations between vitamin D levels with free T4 and calcium and strong negative correlations with TSH; these illustrative studies agree with our findings and add to the mixed evidence base for vitamin D status either enhancing or impairing thyroid function. The tendency in our study, and others alike, to associate vitamin D deficiency with thyroid dysfunction- does lend some credence to this possible relationship, especially in the setting of subclinical hypothyroidism.

Implications of the findings

We draw several important implications from these findings:

1. Screening for vitamin D deficiency seems to be useful in routine practice, particularly among individuals with thyroid disorders or who may develop a functional thyroid disorder.
2. Therapeutic administration of vitamin D may serve as an adjunctive treatment for managing thyroid diseases in clinical practice, especially subclinical hypothyroidism.
3. Since sex differences in vitamin D deficiency are eminent, a focus on females may be needed to decrease this.

Limitations of the study

Furthermore, there are a few limitations to our study:

1. The sample size, especially for the cases of subclinical hypothyroidism, was modest and might have limited statistical power in some analyses.
2. Seasonal variations in vitamin D levels, dietary intake, and sunlight exposure were not considered.
3. Low potential generalizability due to the study population not representing the population as a whole.

Conclusions

This cross-sectional observational study on 200 Iraqi adults from four governorates provides valuable insights into the relationship between vitamin D status and thyroid function. The study revealed significant differences between vitamin D sufficient (VDS) and deficient (VDD) groups in thyroid-stimulating hormone (TSH), free thyroxine (FT4), parathyroid hormone (PTH), and serum calcium levels ($p < 0.05$).

VDD participants showed higher TSH and PTH levels and lower FT4 and serum calcium levels compared to VDS participants. Weak but significant correlations were observed between vitamin D levels and these parameters. Notably, all cases of subclinical hypothyroidism (n = 6) were found in the VDD group, and the risk estimate was 0.953 (95% CI: 0.917-0.99), indicating that the probability of having adequate thyroid function is slightly lower in those with low vitamin D levels. Vitamin D deficiency was higher in females (67.20%) than in males (59.40%). These findings highlight the complex relationship between vitamin D status and thyroid function, emphasizing the need for larger, longitudinal studies to elucidate the mechanisms underlying these associations and their clinical implications.

Recommendation

Based on these results and limitations, we recommend the following for future research:

1. Conduct longitudinal studies to establish the temporal relationship between vitamin D deficiency and thyroid dysfunction.
2. Larger, randomized controlled trials will be conducted to explore the impact of vitamin D supplementation on thyroid function.
3. Examine the gender difference in vitamin D deficiency and the evidence for its effect on thyroid function.
4. The association of vitamin D and thyroid autoantibodies highlights the potential role of vitamin D in autoimmune thyroid diseases.
5. Explore the possible confounding effects of other factors (e.g., iodine status, selenium) and environmental factors affecting the vitamin D-thyroid relationship.

Acknowledgments

We appreciate the roles of participants who volunteered in this study and medical staff in outpatient clinics during the data collection period at Karbala, Hilla, Diwaniyah, and Salah al-Din governorates. We also acknowledge the laboratory technicians who performed the biochemical analyses.

Conflict of Interest

The authors declare no conflict of interest exists in the context of this study.

Ethical approval

The study was approved by the Research Ethics Board of Al-Zahrawi University College (REBZ) on February 10, 2025 (REBZ Rec No. 12/2025). The study was conducted according to the institution's ethical standards and institutional guidelines, and informed consent was obtained from all participants, with all results being kept confidential.

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