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Original Article

Serum Vitamin D and B12 Deficiencies as Predictors of thyroid Dysfunction: A Cross-Sectional Analysis

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Abstract

Background and Objectives: Thyroid disorders, including hypothyroidism and hyperthyroidism, are frequently associated with disturbances in micronutrient balance, particularly involving vitamin D and B12. In this study we sought to explore how the serum levels of these vitamins relate to thyroid functions in patients with thyroid disorders

Methods: A cross-sectional study was performed between January 2023 and June 2024 at a tertiary care hospital. In total 250 participants were enrolled, including patients with hyperthyroidism (n=52), hypothyroidism (n=99) and healthy control groups (n=99). Serum TSH, FT4, FT3, Vitamin D, and Vitamin B12 levels were measured.

Results: Patients with hyperthyroidism were older on average (77.5± 17.9 years) compared with those who have hyperthyroidism (43.1 ± 14.8 years) and the control group (38.8 ± 15.9 years). Correlation analysis showed a weak but statistically significant inverse association between TSH levels and both vitamin D and vitamin B12 levels. In logistic regression analysis, lower vitamin D levels were associated with an increased likelihood of hypothyroidism (OR= 1.17) indication 17 % rise in risk for each unit decrease. Correspondingly, reduce vitamin B12 levels were also coupled to hypothyroidism (OR= 1.08), corresponding to an 8% increase risk per unit decrease.

Conclusion: The study found an inverse relationship between serum vitamin D and vitamin B12 levels and TSH. Higher concentration of these vitamin were associated with lower TSH values.

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1. Introduction:

Thyroid diseases cover a spectrum of problems affecting the thyroid gland, which plays an important role in regulating metabolism via the production of thyroid hormones, particularly triiodothyronine (T3) and thyroxine (T4). The thyroid gland regulates hormone production through the action of thyroid-stimulating hormone (TSH) released from the pituitary gland. The body develops thyroid disease when this regulatory process is disrupted (1). Approximately 200 million individuals worldwidely have been diagnosed with thyroid disorders (2).

Alteration in thyroid hormone synthesis or signaling, as observed in hypothyroidism and hyperthyroidism, are often frequently linked to micronutrient deficiencies, especially vitamins

D and B12 (3). Vitamin D (Vit-D) and B12 (Vit-B12) are involved in immune regulation and metabolic functions, both of which may influence thyroid activity (4, 5). Vitamin D has also been associated to the regulation of TSH levels and thyroid hormones production (6). A systematic review reported reduced Vit-D levels in individual with hypothyroidism, autoimmune thyroid diseases (AITD), and Hashimoto's thyroiditis (HT) compared to healthy control groups (7). Vitamin B12 play a key role in the neurotransmitter synthesis and maintenance of the nervous system (8), which may indirectly affect thyroid function (9). One study found that Vit-B12 deficiency is common among patient with autoimmune hypothyroidism, suggesting a connection between Vit-B12 status and normal functioning of the thyroid gland (10).

The clinical manifestations of thyroid diseases are diverse. Fatigue, weight gain, and cold intolerance is often associated with hypothyroidism (11), while weight loss, palpitations, and heat intolerance are commonly seen in hyperthyroid patients (12). Untreated or undiagnosed, both result in long-term adverse health outcomes that can have serious complications, including cardiovascular disease (13), osteoporosis (14), and cognitive impairments (15). Thyroid derangements can be exacerbated by vitamin deficiencies. Changes in vitamin D and Vitamin B12 status were described as a modifiable factor that may influence thyroid physiology. Accumulating evidence suggests that both vitamins are involved in the synthesis of thyroid hormones and immune modulation, which may serve as potential risk factors for THDs (3). Yet, their clinical and biochemical relevance in the setting of everyday thyroid diseases is not well defined.

However, the association of Vit-D and Vit-B12 with thyroid status is not very clear as evidenced by a few available studies regarding their associations. Thus, it is important to have more evidence-based literature in diverse populations. Despite many previous studies on the individual effects of these vitamins, there is no report about their concurrent effects on thyroid diseases. Thus, this study was an attempt to assess the relationship among Vit-D and Vit-B12 with thyroid status in patients with thyroid disorders.

2. Materials and Methods:

1. Methods and Materials

1.1. Study design and setting

This was a single-center cross-sectional comparative study conducted in the clinical laboratory of a tertiary care hospital in the Kurdistan Region of Iraq between January 2023 and June 2024.

1.2. Sample Size and Sampling Method

A convenience sampling technique was used. A Power analysis was conducted using G*Power software, with power set at 0.80, alpha at 0.05, and an estimated medium effect size ($r = 0.30$). This resulted in a sample size estimate of 220, to which 20% were added to account for dropout rates, providing us with a target sample size of 250 participants.

Inclusion Criteria contain Adults (≥18 years) with hypothyroidism, hyperthyroidism, or AITD diagnosed from clinical and laboratory findings. Subjects with recent measurements of serum Vit-D, Vit-B12, TSH, FT4 and anti-TPO antibodies. No Vit-D or B12 supplementation in the last six months.

Exclusion Criteria contain Pregnant or lactating women. Patients with malignancies, chronic kidney disease, liver dysfunction, or absorption syndromes. History of thyroidectomy or taking medications influencing vitamin metabolism.

1.3. Data Collection Methods

The structured questionnaire served to collect both demographic data and clinical information of participants including gender and age as well as medical history and medication details. The researchers obtained 5 ml of blood through venous draw from each subject who had fasted overnight before their samples were collected. Following centrifugation at 3000 rpm for 10 minutes to isolate serum, the researchers preserved the samples at -20°C until analysis was performed. Thyroid function testing consisted of analyzing TSH, FT4, FT3, T4 and T3 serum levels by chemiluminescent immunoassay (CLIA) on a Cobas e411 analyzer (Roche Diagnostics, Germany) (16). Additionally, Vit-D (25-hydroxyvitamin D) levels were quantified using enzyme-linked immunosorbent assay (ELISA) with the IDS-iSYS kit (Immunodiagnostic Systems, UK) (17). Vit-B12 levels were assayed using the ADVIA Centaur XPT system (Siemens Healthineers, Germany), employing a competitive chemiluminescent immunoassay method (18). A Vitamin D level below 75 nmol/L (or 30 ng/mL) is considered suggestive of Vitamin D insufficiency (19). Subclinical B12 deficiency is characterized by a blood B12 level below 400 ng/L, whereas normal levels are defined as 400 ng/L or above (20).

1.4. Ethical Considerations

The research protocol received approval from the ethic committee at College of Medicine University of Sulaimani with a reference (142) and was executed in compliance with the Declaration of Helsinki. Informed permission in writing was acquired from all subjects. Confidentiality was preserved by data anonymization, and those with vitamin deficits were directed for therapeutic intervention.

1.5. Statistical Analysis

Data were analyzed using IBM SPSS Statistics for Windows, Version 28.0 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean ± SD and categorical variables as frequencies and percentages. Between-group differences were tested with independent-samples t tests (continuous variables) or chi-square tests (categorical variables). Spearman's rank correlation was used to assess associations between vitamin D / vitamin B12 and thyroid measures (TSH, FT4, FT3, total T4, total T3). Binary logistic regression models were constructed to estimate the adjusted odds of hypothyroidism and of hyperthyroidism (each vs euthyroid control) as a function of vitamin D, vitamin B12, age, and sex. Statistical significance was defined as $p<0.05$. Missing data were handled with pairwise deletion.

2. Results

In terms of sex distribution among those with hyperthyroidism, 8 patients (15.4%) were male, and 44 patients (84.6%) were female. Among patients with hypothyroidism, 10 (10.1%) were male, and 88 (89.9%) were female, whereas the control group (CG) comprised 19 males (19.2%) and 80 females (80.8%). Patients with hypothyroidism were 43.11 ± 14.839 years old, whereas those with hyperthyroidism were 77.54 ± 17.928 years old. The control group had a mean age of 38.79 ± 15.879 years.

Table 1. Demographic characteristics of hyperthyroid, hypothyroid, and control group patients

Characteristics		Group			P-value***
		Hyperthyroidism Group (n=52)	Hypothyroidism Group (n=99)	Control Group (n=99)	
Sex	Male	8 (15.4%)*	10 (10.1%)	19 (19.2%)	0.340
	Female	44 (84.6%)	88 (89.9%)	80 (80.8%)	
Age		77.54 ± 17.928**	43.11 ± 14.839	38.79 ± 15.879	0.059

* Frequency (%), ** Mean ± SD, *** Statistical analysed by chi-square and t-test

Table 2 presents the clinical characteristics of patients with hyperthyroidism in comparison with the control, highlighting key biochemical parameters. Statistically significant differences were observed in thyroid function markers, including TSH (0.068 ± 0.091 vs. 1.941 ± 0.835 mU/ml, p=0.001), FT4 (24.243 ± 13.584 vs. 16.101 ± 2.855 ng/ml, p=0.001), FT3 (7.929 ± 6.808 vs. 5.266 ± 3.546 ng/ml, p=0.015), T4 (10.420 ± 3.725 vs. 8.498 ± 1.879 ng/dl, p=0.002), and T3 (1.516 ± 0.810 vs. 1.162 ± 0.507 ng/dl, p=0.013), indicating pronounced alterations in hormone levels among individuals with hyperthyroidism.

Table 2. Clinical characteristics of patients with hyperthyroidism and the CG

Variable	Group		P-value**
	Hyperthyroidism Group (n=52)	Control Group (n=99)	
TSH mU/ml	0.068 ± 0.091*	1.941 ± 0.835	0.001
FT4 ng/ml	24.243 ± 13.584	16.101 ± 2.855	0.001
FT3 ng/ml	7.929 ± 6.808	5.266 ± 3.546	0.015
T4 ng/dl	10.420 ± 3.725	8.498 ± 1.879	0.002
T3 ng/dl	1.516 ± 0.810	1.162 ± 0.507	0.013
Vitamin D ng/ml	24.946 ± 23.486	25.025 ± 30.533	0.986
Vitamin B12 pg/ml	433.271 ± 288.021	419.179 ± 295.864	0.834

* Mean ± SD, ** t-test

The TSH measurement of hypothyroidism were 15.586 ± 20.361 mU/ml compared with 1.941 ± 0.835 mU/ml in the control group, showing a strong statistical difference (P≤0.001). Measurements of mean FT4 showed patients with hypothyroidism presented values at 13.237 ± 4.342 ng/ml while the CG had values at 16.101 ± 2.855 ng/ml and a significant difference occurred (P≤0.001). Patients diagnosed with hypothyroidism presented a mean FT3 value of 4.181 ± 1.381 ng/ml while the CG measured 5.266 ± 3.546 ng/ml leading to a statistically meaningful result (P≤0.009).

Results showed that patients with hypothyroidism had a mean T4 level of 7.353

± 2.605 ng/dl and a CG mean T4 level of 8.498 ± 1.879 ng/ml with (P≤0.001) statistical significance. Hypothyroidism patients showed a mean T3 level at 1.013 ± 0.334 ng/dl but the CG demonstrated 1.162 ± 0.507 ng/dl having a statistically significant difference (P≤0.019).
The mean Vit-D level in patients with hypothyroidism was 18.460 ± 10.708 ng/dl, compared to 25.025 ± 30.533 ng/ml in the CG, with a statistically significant difference (P≤0.046). The mean Vit-B12 level in patients with hypothyroidism was 350.469 ± 227.597 pg/dl, compared to 419.179 ± 295.864 pg/ml in the CG, with no significant difference (P=0.135) (Table 3).

Table 3. Clinical characteristics of patients with hypothyroidism and the CG

Variable	Group		P-value**
	Hypothyroidism Group (n=99)	Control Group (n=99)	
TSH mU/ml	15.586 ± 20.361*	1.941 ± 0.835	0.001
FT4 ng/ml	13.237 ± 4.342	16.101 ± 2.855	0.001
FT3 ng/ml	4.181 ± 1.381	5.266 ± 3.546	0.009
T4 ng/dl	7.353 ± 2.605	8.498 ± 1.879	0.001
T3 ng/dl	1.013 ± 0.334	1.162 ± 0.507	0.019
Vitamin D ng/ml	18.460 ± 10.708	25.025 ± 30.533	0.046
Vitamin B12 pg/ml	350.469 ± 227.597	419.179 ± 295.864	0.135

* Mean ± SD, ** t-test

Research findings showed a weak and significant reverse link between TSH and Vit-D however FT4 FT3 T4 T3 and Vit-D had no correlation. The inverse relationship between TSH and Vit-B12 levels was weak yet significant while TSH showed no correlation with any of FT4, FT3, T4, T3, and Vit-B12 (Table 4).

Table 4. Correlation Vit-D and Vit-B12 with Thyroid tests

Variable	**Vitamin D**	**Vitamin B12**
TSH	(r=-0.128, P ≤0.04)*	(r=-0.075, P ≤0.03)
FT4	(r=0.102, P ≤0.128)	(r=-0.008, P ≤0.924)
FT3	(r=0.061, P ≤0.361)	(r=0.009, P ≤0.912)
T4	(r=0.125, P ≤0.054)	(r=0.092, P ≤0.251)
T3	(r=0.034, P ≤0.615)	(r=0.018, P ≤0.831)

* Spearman Rho Correlation coefficient

The results of the logistic regression analysis between Vit-D, Vit-B12, and age among patients with hyperthyroidism and hypothyroidism are shown in Table 5. Vit-D was a significant predictor of hypothyroidism (OR=1.17), indicating that each unit decrease in Vit-D increased the risk of hyperthyroidism by 17%. Similarly, Vit-B12 was also a significant predictor of hypothyroidism (OR=1.08), with each unit decrease in Vit-D increasing the risk of hyperthyroidism by 8%.

Table 5. Logistic regression test to investigate factors associated with Hyperthyroidism and hypothyroidism

Variable		OR	P-value (CI:95%)
Hyperthyroidism	Vitamin D	0.980	0.121 (0.955 – 1.005)
	Vitamin B12	0.999	0.217 (0.997 – 1.001)
	Age	1.008	0.6 (0.978 – 0.1.039)
Hypothyroidism	Vitamin D	1.17	0.02 (1.10 – 1.30)
	Vitamin B12	1.08	0.02 (1.02 – 1.18)
	Age	0.998	0.870 (0.977 – 1.02)

4. Discussion:

This research aimed to assess the levels of Vitamin D and Vitamin B12 in individuals with different thyroid diseases. The results indicated a significant difference in the mean levels of TSH, FT4, FT3, T4, and T3 between patients with hyperthyroidism and hypothyroidism. Mean Vit-D and Vit-B12 levels in hypothyroid patients were decreased compared with the CG. There was an inversely significant relationship of Vit-D and Vit-B12 with thyroid disorders; both the vitamins were significant predictors for hypothyroidism.

Differences in thyroid hormones were also noted among patients with the thyroid disorder in this study. Similarly, Feng et al. (2022) showed that patients with hyperthyroidism and hypothyroidism exhibited significantly different thyroid hormone levels (21). Zhang et al. (2023) also noted these differences in thyroid hormone levels among patients with thyroid disorders (22).

The importance of Vit-D is not limited to bone and mineral metabolism, with Vit-D deficiency having been shown to be associated with cardiovascular diseases, cancer, obesity, infectious diseases, and osteoporosis (23). Also, an association has been proven between Vit-D deficiency and autoimmune diseases (24).

The results of the present study showed that levels of Vitamin D were lower in both groups (hyper and hypothyroid patients, $P < 0.05$) as compared to controls, whereas it was highly significant in hypothyroid patients only. These conclusions are corroborated by several other publications emphasizing the role of Vitamin D deficiency in thyroid pathologies. Eldosouky et al. (2022) reported a strong correlation between Vit-D and hypothyroidism, including lower levels of Vit-D compared to healthy controls among patients (25). This relationship has also been documented in a study investigating Turkish patients, that found lower Vit-D levels in hypothyroid subjects than healthy controls (26).

Hypothyroidism may be associated with Vit-D deficiency through decreased intestinal absorption, or reduced activation of Vit-D (27). Vit-D and thyroid hormones share the same steroid receptors, Some polymorphisms of the Vit-D receptor are associated with susceptibility to autoimmune thyroid diseases (7).

The present research showed an inverse relationship between levels of TSH and Vitamin D. More specifically, TSH and Vit-D correlations showed that highest level of Vit-D is correlated significantly with the lowest level of TSH. Similarly, Leko et al. (2023) observed a relationship between Vit-D deficiency and increased TSH level among patient.(28). Studies conducted in India (29) and China (30), also showed that higher Vit-D levels are associated with reduced TSH levels.

The present study also identified a significant inverse relationship between TSH levels and Vit-B12 levels. Higher Vit-B12 levels were significantly associated with lower TSH levels. Gupta et al. (2023) reported that patient with hypothyroidism have reduced Vit-B12 levels and that increasing these levels leads to a significant decrease in TSH levels (31). In addition, a study by Aktaş (2020) in Turkey confirmed a significant association between Vit-B12 levels and TSH levels, with higher Vit-B12 levels resulting in a notable reduction in TSH levels (3).

3. Conclusions

The findings indicate that serum levels of Vit-D and Vit-B12 differ between patients with thyroid disorders and healthy individuals. Patients with thyroid disorders showed lower serum levels of both vitamins. A significant and inverse relationship was observed between serum levels of Vit-D and Vit-B12 levels and TSH , with higher vitamin levels associated with reduced TSH levels. Therefore, assessing serum Vit-D and Vitamin B12 levels in patient with thyroid disorder is important. Further studies are needed to evaluate the therapeutic effects of Vit-D and Vit-B12 supplementation on improving outcomes in patients with hypothyroidism.

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Data availability: Upon reasonable request, the data from the research may be obtained from the corresponding author.

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