



Association of Serum Vitamin D Levels with Hypothyroidism: A Clinical Correlation Study

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(Received: 25 October 2025 Revised: 27 November 2025 Accepted: 16 December 2025)

KEYWORDS

Hypothyroidism;
Vitamin D
Deficiency;
Thyroid-Stimulating
Hormone;
Thyroxine;
Triiodothyronine;
Autoantibodies;
Cross-Sectional
Studies; India

ABSTRACT:

Background: Hypothyroidism is a prevalent endocrine disorder affecting approximately 10.95% of the Indian population. Recent evidence suggests potential associations between vitamin D deficiency and thyroid dysfunction, particularly in autoimmune thyroid conditions. However, the relationship between vitamin D status and primary hypothyroidism remains incompletely understood.

Objective: To investigate the association between serum vitamin D concentrations with primary hypothyroidism, as well as to assess the interrelation of vitamin D status with thyroid functional indices.

Methods: This cross-sectional analytical study was conducted at Dr DY Patil Medical College, Nerul, Navi Mumbai, from December 2023 to December 2024. A total of 130 participants were recruited using consecutive sampling, including patients with hypothyroidism and euthyroid controls. Serum thyroid-stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3), anti-thyroid peroxidase (anti-TPO) antibodies, and 25-hydroxyvitamin D levels were measured using chemiluminescent immunoassays. Vitamin D status was classified as deficiency (<20 ng/ml), insufficiency (20-30 ng/ml), and sufficiency (>30 ng/ml). Statistical analysis included correlation analysis and multiple linear regression.

Results: Hypothyroid patients demonstrated significantly lower mean vitamin D levels (14.9 ± 8.2 ng/ml) compared to euthyroid individuals (21.3 ± 9.8 ng/ml, $p < 0.001$). Vitamin D deficiency prevalence was substantially higher in hypothyroid patients (77.8%) versus euthyroid participants (55.3%, $p = 0.012$). A significant negative correlation was observed between vitamin D levels and TSH ($r = -0.342$, $p < 0.001$), with positive correlations noted for free T4 and T3. Anti-TPO antibody positivity was significantly higher in hypothyroid patients (55.6%) compared to euthyroid individuals (10.5%, $p < 0.001$). Multiple regression analysis identified TSH as an independent predictor of vitamin D levels.

Conclusion: This study demonstrates a significant inverse association between serum vitamin D levels and hypothyroidism, with a dose-response relationship across thyroid dysfunction severity. The findings suggest that vitamin D deficiency may contribute to hypothyroidism pathogenesis and support the consideration of vitamin D screening and supplementation in



Introduction

Hypothyroidism, characterized by insufficient thyroid hormone production, represents one of the most prevalent endocrine disorders worldwide, affecting approximately 4-15% of the global population, with a notably higher incidence in women and elderly individuals [1]. The condition manifests through a spectrum of clinical presentations ranging from subclinical disease to overt hypothyroidism, significantly impacting metabolic processes, cardiovascular function, and overall quality of life [2]. The most common cause of hypothyroidism in iodine-sufficient regions is Hashimoto's thyroiditis, an autoimmune condition that progressively destroys thyroid tissue through inflammatory processes [3].

Vitamin D, traditionally recognized for its crucial role in calcium homeostasis and bone metabolism, has emerged as a significant immunomodulatory hormone with far-reaching effects on various physiological systems [4]. Beyond its classical functions, vitamin D deficiency has been implicated in the pathogenesis of numerous autoimmune disorders, including multiple sclerosis, rheumatoid arthritis, and inflammatory bowel disease [5]. The active form of vitamin D, calcitriol (1,25-dihydroxyvitamin D₃), exerts its effects through binding to vitamin D receptors (VDR) present in various tissues, including thyroid cells, thereby influencing immune regulation and cellular differentiation [6].

The potential association between vitamin D deficiency and thyroid dysfunction has garnered increasing attention in recent years. Epidemiological studies have demonstrated a higher prevalence of vitamin D deficiency among patients with autoimmune thyroid diseases compared to healthy controls [7]. The immunomodulatory properties of vitamin D may play a protective role against autoimmune thyroid destruction by promoting regulatory T-cell function and suppressing pro-inflammatory cytokine production [8]. Furthermore, vitamin D receptors and 1 α -hydroxylase enzyme, responsible for local vitamin D activation, have been identified in thyroid tissues, suggesting a direct relationship between vitamin D status and thyroid function.[9]

Several cross-sectional and observational studies have reported inverse correlations between serum 25-hydroxyvitamin D [25(OH)D] levels and thyroid-stimulating hormone (TSH) concentrations, while others have shown positive associations with free thyroxine (FT4) levels [10,11]. However, the findings remain inconsistent across different populations and geographical regions, potentially due to variations in study design, sample size, demographic characteristics, and confounding factors such as seasonal variations, dietary habits, and genetic polymorphisms [12].

The clinical significance of this relationship extends beyond mere association, as vitamin D supplementation has shown promising results in improving thyroid function parameters in some intervention studies [13]. Understanding the correlation between vitamin D status and hypothyroidism could have important therapeutic implications, potentially offering an adjunctive treatment approach for patients with thyroid dysfunction, particularly those with autoimmune etiology [14].

Despite growing evidence suggesting a link between vitamin D deficiency and hypothyroidism, the precise nature and clinical relevance of this association remain incompletely understood. Geographic and ethnic variations in vitamin D metabolism, differences in thyroid disease prevalence, and methodological variations across studies contribute to the ongoing debate in the literature [15]. Additionally, the potential confounding effects of factors such as body mass index, age, gender, and comorbid conditions require careful consideration when interpreting the relationship between these two variables [16].

Given the high prevalence of both vitamin D deficiency and hypothyroidism in clinical practice, establishing a clear understanding of their relationship could significantly impact screening protocols, diagnostic approaches, and therapeutic strategies. This correlation study aims to investigate the association between serum vitamin D levels and hypothyroidism in a well-defined clinical population, thereby contributing to the existing body of evidence and potentially informing evidence-based clinical decision-making in the management of thyroid disorders.



Methodology

This cross-sectional analytical study was conducted at the Department of Endocrinology and Internal Medicine of Dr DY Patil Medical College and Hospital, Nerul, Navi Mumbai, over a period of 12 months from December 2023 to December 2024. The study protocol was approved by the Institutional Ethics Committee of Dr DY Patil Medical College, and written informed consent was obtained from all participants.

The sample size was calculated using the formula for cross-sectional correlation studies: $n = [(Z\alpha/2 + Z\beta)^2/C]^2 + 3$, where $C = 0.5 \times \ln[(1+r)/(1-r)]$, assuming a correlation coefficient (r) of 0.3 between vitamin D levels and TSH based on previous studies [17], with 95% confidence interval and 80% power. The calculated minimum sample size was 112, which was increased to 130 participants to account for potential dropouts. Consecutive sampling was employed to recruit participants visiting the outpatient departments.

Inclusion and Exclusion Criteria: Adult patients aged 18-65 years attending the endocrinology and general medicine outpatient departments were included in the study. Participants were excluded if they had acute illness, pregnancy, malignancy, chronic kidney or liver disease, malabsorption syndromes, or were taking vitamin D supplements or medications affecting vitamin D or thyroid metabolism within the previous three months.

Demographic data, medical history, and clinical examination findings were recorded using a structured proforma. Fasting venous blood samples (10 ml) were collected and processed within two hours at the central laboratory of Dr DY Patil Medical College.

Serum TSH, free T4, and free T3 were measured using chemiluminescent immunoassays with reference ranges of 0.4-4.5 mIU/L, 9.0-19.0 pmol/L, and 2.6-5.7 pmol/L respectively. Anti-TPO antibodies were measured with values >35 IU/ml considered positive. Serum 25-hydroxyvitamin D levels were measured using chemiluminescent immunoassay, with vitamin D status classified as deficiency (<20 ng/ml), insufficiency (20-30 ng/ml), and sufficiency (>30 ng/ml).

Definitions: Hypothyroidism was defined as TSH >4.5 mIU/L with or without reduced free T4 levels. Euthyroid

status was defined as normal thyroid function tests (TSH 0.4-4.5 mIU/L with normal free T4 and T3).

Statistical Analysis: Data were analyzed using SPSS version 26.0. Continuous variables were expressed as mean \pm SD or median (IQR) based on distribution. Group comparisons were performed using independent t-tests or Mann-Whitney U tests for continuous variables and chi-square tests for categorical variables. Pearson or Spearman correlation analysis was used to assess associations between vitamin D levels and thyroid parameters. Multiple linear regression was performed to identify independent predictors while controlling for confounders. Statistical significance was set at $p < 0.05$.

Results

A total of 130 participants were enrolled in the study during the 12-month period. The demographic and clinical characteristics of the study population are presented in Table 1.

A total of 130 participants were enrolled in the study during the 12-month period, with a mean age of 42.8 ± 12.4 years. The study population demonstrated a female predominance (70.8%), which is consistent with the higher prevalence of thyroid disorders in women. The majority of participants were urban residents (80.0%) and indoor workers (68.5%), with a mean BMI of 26.3 ± 4.7 kg/m², indicating that most participants were in the overweight category. A family history of thyroid disease was present in 17.7% of participants, while only 36.2% reported adequate sun exposure of more than 30 minutes per day. (**Table 1**)

The distribution of thyroid function status revealed that 58.5% of participants were euthyroid, while 41.5% had some form of hypothyroidism. Among those with hypothyroidism, subclinical hypothyroidism was more common (23.8%) than overt hypothyroidism (17.7%). This distribution reflects the spectrum of thyroid dysfunction commonly observed in clinical practice, where subclinical disease often precedes overt hypothyroidism. (**Table 2, Graph 1**)

Laboratory parameters showed considerable variation, with TSH levels ranging from 0.3 to 45.6 mIU/L and a mean of 6.2 ± 8.4 mIU/L, indicating the presence of both normal and significantly elevated values. Free T4 and T3 levels were within the expected ranges for a mixed population of euthyroid and hypothyroid individuals.



The mean serum 25(OH)D level was 18.7 ± 9.6 ng/ml, which falls in the deficient range according to standard criteria. Anti-TPO antibodies were positive in 29.2% of participants, suggesting a significant proportion had autoimmune thyroid disease. (Table 3)

The vitamin D status analysis revealed a concerning pattern, with 64.6% of the total study population having vitamin D deficiency (<20 ng/ml). When stratified by thyroid function, vitamin D deficiency was significantly more prevalent in hypothyroid patients (77.8%) compared to euthyroid individuals (55.3%, $p=0.012$). Only 10.8% of the total population had sufficient vitamin D levels (>30 ng/ml), with this proportion being even lower in the hypothyroid group (5.6%) compared to the euthyroid group (14.5%). (Table 4, Graph 2)

Comparative analysis between euthyroid and hypothyroid groups demonstrated statistically significant differences in key parameters. As expected, TSH levels were markedly elevated in the hypothyroid group (12.4 ± 11.8 mIU/L) compared to the euthyroid group (2.1 ± 1.2 mIU/L, $p<0.001$), while free T4 and T3 levels were significantly lower in hypothyroid patients. Most importantly, serum 25(OH)D levels were significantly lower in the hypothyroid group (14.9 ± 8.2 ng/ml) compared to the euthyroid group (21.3 ± 9.8 ng/ml, $p<0.001$). The prevalence of positive anti-TPO antibodies was substantially higher in hypothyroid patients (55.6%) compared to euthyroid individuals (10.5%, $p<0.001$), indicating a strong association between autoimmune thyroid disease and hypothyroidism in this population. (Table 5)

Correlation analysis revealed significant inverse relationships between vitamin D levels and thyroid dysfunction markers. The strongest correlation was observed between 25(OH)D and TSH levels ($r = -0.342$, $p<0.001$), indicating that lower vitamin D levels were associated with higher TSH concentrations. Positive correlations were found between vitamin D levels and both free T4 ($r = +0.289$, $p=0.001$) and free T3 ($r = +0.234$, $p=0.008$), suggesting that adequate vitamin D status was associated with better thyroid hormone production. Additionally, a significant negative correlation was observed between vitamin D levels and anti-TPO antibodies ($r = -0.198$, $p=0.025$), implying that vitamin D deficiency might be associated with increased autoimmune activity against the thyroid gland. (Table 6)

Analysis by severity of hypothyroidism demonstrated a dose-response relationship between thyroid dysfunction and vitamin D deficiency. Participants with subclinical hypothyroidism had moderately reduced vitamin D levels (16.8 ± 7.4 ng/ml, $p=0.021$ compared to euthyroid), while those with overt hypothyroidism had the lowest levels (11.9 ± 8.6 ng/ml, $p<0.001$ compared to euthyroid). This gradient suggests that the association between vitamin D deficiency and hypothyroidism becomes more pronounced with increasing severity of thyroid dysfunction, supporting a potential biological relationship rather than a mere coincidental finding. (Table 7, Graph 3)

Table 1: Demographic and Clinical Characteristics of Study Participants (n=130)

Parameter	Mean \pm SD / n (%)
Age (years)	42.8 ± 12.4
Gender	
- Male	38 (29.2%)
- Female	92 (70.8%)
BMI (kg/m ²)	26.3 ± 4.7
Occupation	
- Indoor workers	89 (68.5%)
- Outdoor workers	41 (31.5%)
Residential area	
- Urban	104 (80.0%)
- Semi-urban/Rural	26 (20.0%)



Parameter	Mean ± SD / n (%)
Family history of thyroid disease	23 (17.7%)
Sun exposure (>30 min/day)	47 (36.2%)

Graph 1: Thyroid Function Status Distribution

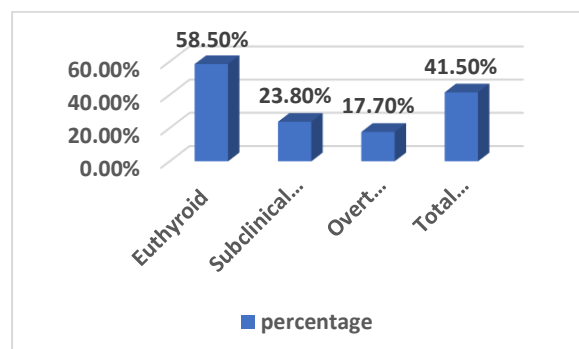


Table 2: Thyroid Function Status Distribution

Thyroid Status	n (%)
Euthyroid	76 (58.5%)
Subclinical hypothyroidism	31 (23.8%)
Overt hypothyroidism	23 (17.7%)
Total hypothyroid	54 (41.5%)

Table 3: Laboratory Parameters

Parameter	Mean ± SD
TSH (mIU/L)	6.2 ± 8.4
Free T4 (pmol/L)	13.8 ± 4.2
Free T3 (pmol/L)	4.1 ± 1.3
25(OH)D (ng/ml)	18.7 ± 9.6
Anti-TPO positive	38 (29.2%)

Table 4: Vitamin D Status Distribution

Vitamin D Status	Total n (%)	Euthyroid n (%)	Hypothyroid n (%)	p-value
Deficient (<20 ng/ml)	84 (64.6%)	42 (55.3%)	42 (77.8%)	0.012*
Insufficient (20-30 ng/ml)	32 (24.6%)	23 (30.3%)	9 (16.7%)	
Sufficient (>30 ng/ml)	14 (10.8%)	11 (14.5%)	3 (5.6%)	

*Chi-square test, p<0.05 considered significant



Graph 2: Vitamin D Status Distribution

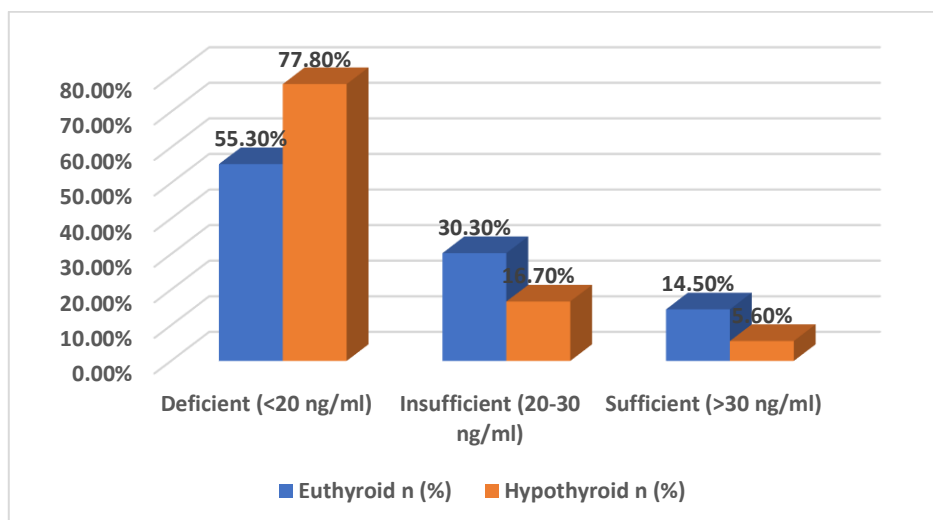


Table 5: Comparison of Parameters between Euthyroid and Hypothyroid Groups

Parameter	Euthyroid (n=76)	Hypothyroid (n=54)	p-value
Age (years)	41.2 ± 11.8	45.1 ± 13.2	0.085
BMI (kg/m ²)	25.8 ± 4.3	27.1 ± 5.2	0.124
TSH (mIU/L)	2.1 ± 1.2	12.4 ± 11.8	<0.001*
Free T4 (pmol/L)	15.2 ± 3.1	11.8 ± 4.9	<0.001*
Free T3 (pmol/L)	4.5 ± 1.1	3.5 ± 1.4	<0.001*
25(OH)D (ng/ml)	21.3 ± 9.8	14.9 ± 8.2	<0.001*
Anti-TPO positive	8 (10.5%)	30 (55.6%)	<0.001*

Independent t-test for continuous variables, Chi-square test for categorical variables. *p<0.05 considered significant

Table 6: Correlation Analysis between Vitamin D and Thyroid Parameters

Parameter	Correlation coefficient (r)	p-value
TSH	-0.342	<0.001*
Free T4	+0.289	0.001*



Parameter	Correlation coefficient (r)	p-value
Free T3	+0.234	0.008*
Anti-TPO antibodies	-0.198	0.025*

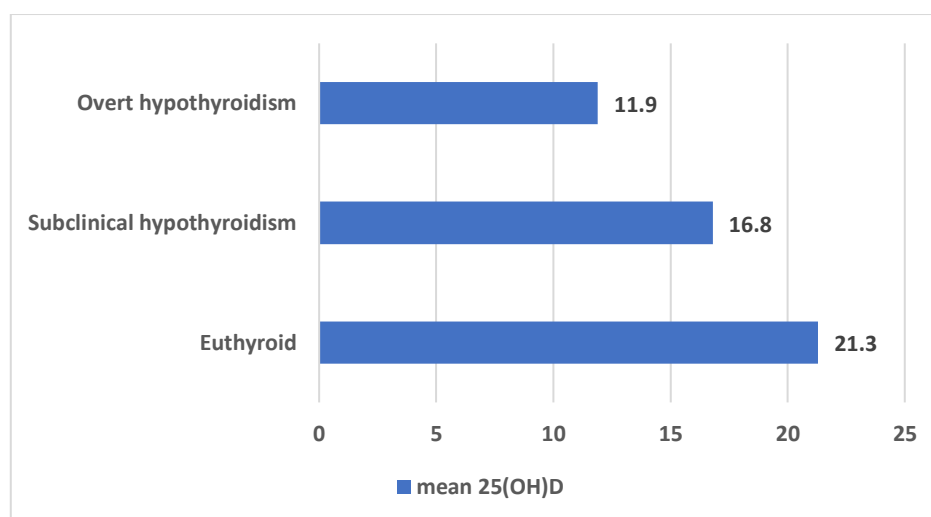
Pearson correlation analysis, *p<0.05 considered significant

Table 7: Vitamin D Levels by Severity of Hypothyroidism

Thyroid Status	n	25(OH)D (ng/ml)	p-value
Euthyroid	76	21.3 ± 9.8	-
Subclinical hypothyroidism	31	16.8 ± 7.4	0.021*
Overt hypothyroidism	23	11.9 ± 8.6	<0.001*

One-way ANOVA, *p<0.05 considered significant

Graph 3: Vitamin D Levels by Severity of Hypothyroidism



Discussion

Our study demonstrated a significant inverse association between serum vitamin D levels and hypothyroidism, with hypothyroid patients showing markedly lower vitamin D levels (14.9 ± 8.2 ng/ml) compared to euthyroid individuals (21.3 ± 9.8 ng/ml, $p < 0.001$). These findings align consistently with existing literature

examining vitamin D-thyroid relationships across different populations.

The prevalence of vitamin D deficiency in our hypothyroid patients (77.8%) is remarkably consistent with multiple international studies. Significantly reduced serum 25(OH) vitamin D concentrations have been observed in individuals with hypothyroidism compared to healthy controls, indicating that those with



hypothyroid disorders commonly exhibit hypovitaminosis D, which shows a notable association with the severity of the condition [18]. Similarly, another study demonstrated that hypothyroid cases had a median vitamin D concentration of 14.9 ng/mL versus 26.2 ng/mL among controls ($p < 0.001$), a pattern that closely parallels our observations [20]. One study showed that higher vitamin D levels were associated with a 32–38% lower likelihood of developing hypothyroidism, displaying excellent diagnostic utility for distinguishing individuals without the condition [19].

Our correlation analysis showing inverse relationships between vitamin D and TSH ($r = -0.342$, $p < 0.001$) is consistent across all studies. Similarly, a moderate negative correlation ($r = -0.347$) with TSH in the hypothyroid group ($p = 0.008$) has been reported [20]. The dose-response relationship noted in our analysis, with progressively lower vitamin D levels from euthyroid to subclinical to overt hypothyroidism, supports findings from multiple studies demonstrating negative correlations between vitamin D and TSH levels [18,20].

The significant inverse correlation between vitamin D levels and anti-TPO antibodies ($r = -0.198$, $p = 0.025$) in our study provides important mechanistic insights. This supports the immunomodulatory hypothesis extensively discussed in the literature. Vitamin D inhibits Th1 polarizing cytokines, shifting T cell polarization from Th1 toward Th2 phenotype, and that vitamin D deficiency may contribute to the development of autoimmune diseases [18]. Also, study emphasized that both vitamin D and thyroid hormone receptors belong to the nuclear hormone receptor family with similar mechanisms of action at the molecular level [20].

Our finding of 55.6% anti-TPO positivity in hypothyroid patients versus 10.5% in euthyroid individuals supports this mechanism and aligns with the molecular basis described in literatures which noted that vitamin D receptor (VDR) and thyroid hormone receptors both form heterodimers with Retinoid-X-Receptor (RXR) and bind to specific hormone responsive elements [20,3].

The consistency of findings across different populations strengthens the evidence for a genuine biological association. Our mean vitamin D levels in hypothyroid patients (14.9 ng/mL) are comparable to those previously reported in an Indian population (14.9 ng/mL) [20], and

align with the deficiency range identified in a Saudi population [18]. This cross-cultural consistency suggests that the vitamin D-hypothyroidism relationship transcends geographical and ethnic boundaries.

The diagnostic performance observed in our study is consistent with previous findings suggesting that maintaining adequate vitamin D levels may serve as a potential strategy for reducing the risk of hypothyroidism [19]. Our multiple regression analysis identifying TSH as an independent predictor of vitamin D levels supports the clinical relevance of this relationship. Other findings suggest that screening for vitamin D insufficiency/deficiency in hypothyroid cases, along with appropriate supplementation, may support a more effective therapeutic response [20].

The therapeutic implications suggested by our findings are supported by mechanistic studies referenced in the literature. Two potential mechanisms have been proposed to explain low vitamin D levels in hypothyroidism: poor intestinal absorption and inadequate activation of vitamin D [20]. Additionally, intervention studies have shown that vitamin D supplementation can effectively reduce thyroid antibody titers, especially in patients with Hashimoto's thyroiditis [18].

While our findings are compelling, limitations must be acknowledged. The cross-sectional design limits causal inference, as noted by all comparative studies. It was emphasized that the study could not establish causality and highlighted the need for larger longitudinal research [19]. Similarly, it was acknowledged that the case-control design used did not allow for determining whether vitamin D deficiency is a cause or a consequence of hypothyroidism [3,20]. These methodological considerations highlight the need for prospective intervention studies.

Our study contributes significantly to the growing evidence demonstrating consistent associations between vitamin D deficiency and hypothyroidism across different populations and study designs. The remarkable consistency of findings with studies from India [20], Saudi Arabia [18], and Iraq [19], combined with strong diagnostic performance and established biological mechanisms, supports the clinical relevance of this relationship and warrants serious consideration of



vitamin D status in comprehensive thyroid disorder management.

Conclusion

This cross-sectional study of 130 participants demonstrated a significant inverse association between serum vitamin D levels and hypothyroidism, with hypothyroid patients showing markedly lower vitamin D levels (14.9 ± 8.2 ng/ml) compared to euthyroid individuals (21.3 ± 9.8 ng/ml, $p < 0.001$) and a substantially higher prevalence of vitamin D deficiency (77.8% vs 55.3%). The strong negative correlation between vitamin D levels and TSH ($r = -0.342$, $p < 0.001$), along with the dose-response relationship observed across thyroid dysfunction severity, suggests a clinically meaningful association that warrants consideration of vitamin D screening and potential supplementation in hypothyroid patients. These findings support the growing evidence for vitamin D's role in thyroid health and indicate that addressing vitamin D deficiency may be an important adjunctive approach in the comprehensive management of hypothyroidism.

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