



Association of Vitamin D and Vitamin B12 Deficiency with Thyroid Hormone Profile in Hypothyroidism: A Case–Control Study

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KEYWORDS

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ABSTRACT:

Background: Hypothyroidism is a frequent endocrine disorder characterized by reduced thyroid hormone production and metabolic disturbances. Micronutrient deficiencies, especially vitamin D and vitamin B12, have been implicated in its pathogenesis, but Indian data are limited. This study evaluated vitamin D and B12 levels in hypothyroid patients versus healthy controls and examined their correlation with thyroid hormone profile.

Methods: This hospital-based case–control study was conducted at NCRIMS and Hospital, Meerut, over 18 months. A total of 138 participants were enrolled, comprising 69 clinically diagnosed hypothyroid patients and 69 age- and sex-matched controls. Serum free triiodothyronine (FT3), free thyroxine (FT4), and thyroid-stimulating hormone (TSH) were measured using commercial immunoassay kits. Serum vitamin D and vitamin B12 levels were estimated using Enzyme Linked Fluorescent Assay technique on Minividas analyzer, respectively.

Results: Hypothyroid patients had significantly lower serum vitamin D (15.85 ± 8.35 ng/mL vs. 40.97 ± 21.52 , $p < 0.001$) and vitamin B12 (151.47 ± 80.31 pg/mL vs. 259.03 ± 110.59 , $p < 0.001$) compared with controls. Vitamin D deficiency was observed in 94.2% of cases versus 21.7% of controls, while vitamin B12 deficiency was found in 88.4% versus 33.3% ($p < 0.001$). Correlation analysis revealed negative associations of vitamin D ($r = -0.242$, $p = 0.048$) and vitamin B12 ($r = -0.301$, $p = 0.003$) with TSH, and positive correlations of vitamin B12 with FT3 and FT4 ($p < 0.05$).

Conclusion: Vitamin D and vitamin B12 deficiencies are highly prevalent in hypothyroid patients and significantly associated with deranged thyroid hormone profile.

INTRODUCTION:

Hypothyroidism represents one of the most frequent endocrine disorders, arising from inadequate production of thyroxine and leading to a wide range of clinical manifestations that extend from being clinically silent to presenting with severe, life-threatening complications [1]. Circulating thyroid hormones mainly include thyroxine (T4), triiodothyronine (T3), and their

biologically active free fractions—free T4 (fT4) and free T3 (fT3) [2]. Their secretion and plasma concentrations are regulated by the hypothalamic–pituitary–thyroid axis through a tightly controlled negative feedback loop [3]. In overt primary hypothyroidism, reduced serum fT4 is typically accompanied by elevated thyroid-stimulating hormone (TSH) concentrations [4]. A milder form, termed subclinical hypothyroidism, is defined by elevated TSH



with preserved T4 values [5]. Data from the National Health and Nutrition Examination Survey (NHANES III, 1988–1994) reported a prevalence of hypothyroidism of 4.6% in the U.S. population, including 0.3% with overt disease and 4.3% with subclinical disease [5]. While iodine deficiency accounts for most cases in hilly or mountainous regions, autoimmune thyroiditis (Hashimoto's thyroiditis) is the leading cause in iodine-sufficient areas [6].

Vitamin D is a fat-soluble prohormone that requires sequential hydroxylation in the liver and kidney to form the biologically active metabolite, calcitriol (1,25-dihydroxycholecalciferol) [7]. Vitamin D status is clinically assessed using serum 25-hydroxy vitamin D concentrations. It exists in two principal forms: vitamin D₂, derived from plant sterols, and vitamin D₃, synthesized in the skin from cholesterol following ultraviolet exposure [7]. In addition to maintaining calcium homeostasis and bone health, vitamin D has significant roles in immune regulation and may offer protection against malignancies [8]. Despite its importance, deficiency is a global public health concern. A threshold of <25 nmol/L of serum 25-hydroxy vitamin D is generally accepted as diagnostic of deficiency [9]. In the United States, its prevalence is approximately 41.6%, with the highest frequency observed in Black and Hispanic populations [10].

Hashimoto's thyroiditis is the prototypical autoimmune cause of hypothyroidism in iodine-replete settings, characterized by lymphocytic infiltration of the thyroid gland and gradual destruction of thyroid follicles [11]. Clinical expression ranges from normal thyroid function to subclinical or overt hypothyroidism, sometimes accompanied by goiter. Circulating antibodies to thyroid peroxidase (TPO) and thyroglobulin (Tg) are hallmarks of the disease, as both are central to thyroid hormone synthesis and storage [11]. Epidemiologically, it occurs more often in women and is relatively less frequent among smokers and individuals who consume alcohol [12]. The pathogenesis involves both genetic predisposition and environmental triggers, with micronutrient deficiencies, particularly of vitamin D and selenium, increasingly implicated.

Vitamin D exerts immunomodulatory and anti-inflammatory actions by influencing dendritic cell differentiation, shifting T-helper cell balance toward

Th₂ predominance, and enhancing the activity of regulatory T lymphocytes [13]. Consequently, low vitamin D has been associated with an increased risk of autoimmune conditions such as systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, multiple sclerosis, type 1 diabetes mellitus, and autoimmune thyroid disorders [14]. A meta-analysis confirmed that patients with Hashimoto's thyroiditis and Graves' disease exhibit lower vitamin D levels compared with healthy controls [15]. On the other hand, some studies failed to establish any consistent association between vitamin D deficiency and autoimmune thyroid disease [16].

Given the heterogeneity in existing evidence, it becomes important to explore the interplay between micronutrient deficiencies and thyroid dysfunction. Alongside vitamin D, vitamin B₁₂ deficiency has also been suggested to occur more frequently in hypothyroid patients, potentially exacerbating metabolic and clinical outcomes. Therefore, the present study was undertaken to assess vitamin D and vitamin B₁₂ levels in hypothyroid individuals in comparison with healthy controls, and to examine their relationship with thyroid hormone parameters.

MATERIALS AND METHODS

This research was designed as a hospital-based case-control study and was conducted in the Department of Biochemistry at NCRIMS and Hospital, Meerut (U.P.), over a period of 18 months following approval from the Institutional Ethical and Scientific Committees. A total of 138 participants were enrolled, consisting of 69 clinically diagnosed hypothyroid patients and 69 age- and sex-matched healthy controls.

Patients aged 25–65 years of either sex who were clinically diagnosed with thyroid disorders and who provided written informed consent were included. Individuals were excluded if they had coexisting systemic illnesses such as diabetes mellitus, cardiac diseases, carcinoma, or renal disorders; neurological or psychiatric conditions including depression; subclinical hypothyroidism or hyperthyroidism; suspected or confirmed pancreatic pathologies; and pregnancy, lactation, or other gynecological disorders.

Serum thyroid function tests were performed for all participants. Free triiodothyronine (FT₃), free thyroxine



(FT4), and thyroid-stimulating hormone (TSH) were measured using a commercially kit based ELFA technique on Minividasanalyzer. The kit reference ranges were TSH: 0.25–4.5 $\mu\text{IU/mL}$, FT4: 0.77–1.51 ng/dL, and FT3: 4–8.3 pmol/L. Hypothyroidism was defined as decreased or normal free hormone levels with a TSH concentration greater than 7 $\mu\text{IU/mL}$. Samples with TSH levels above 60 $\mu\text{IU/mL}$ were diluted appropriately using the manufacturer's diluent (1:2 or 1:4) before measurement.

Serum vitamin D levels were estimated as 25-hydroxy vitamin D3 using a commercially available kit based on ELFA technique on the Minividasanalyzer, with deficiency defined as levels below 30 ng/mL and sufficiency considered between 30 and 100 ng/mL, according to kit reference standards. Vitamin B12 concentrations were assessed using commercially available kit based on ELFA technique on the Minividasanalyzer. The normal values defined as 167–585 pg/mL as per the manufacturer's reference range.

Statistical Analysis

Data were compiled and analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0. Continuous variables such as vitamin D, vitamin B12, TSH, FT3, and FT4 were expressed as mean \pm standard deviation (SD), and comparisons between cases and controls were performed using the independent Student's *t*-test. Categorical variables such as vitamin deficiency status were presented as frequencies and percentages, and differences between groups were assessed using the Chi-square test. Pearson's correlation coefficient was applied to evaluate the relationship between serum vitamin levels and thyroid hormone parameters. A *p*-value of <0.05 was considered statistically significant.

RESULTS

A total of 138 participants were studied, including 69 hypothyroid cases and 69 age- and sex-matched controls. The mean age of cases was 41.01 ± 12.00 years versus 39.09 ± 10.31 years in controls ($t = 0.958$, $p = 0.684$). Age subgroup distribution (20–29 years: 18.84% vs. 36.23%; 30–39: 26.09% vs. 17.39%; 40–49: 26.09% vs. 24.64%; 50–60: 26.09% vs. 21.74%; >60 : 2.90% vs. 0%) showed no significant difference ($\chi^2 = 7.29$, $p = 0.121$). Females predominated in both groups

(78.26% vs. 72.46%), with comparable gender distribution ($\chi^2 = 0.356$, $p = 0.553$). [Table 1]

Among hypothyroid cases, the most frequent complaint was fatigue and lethargy (72.5%), followed by weight gain (60.9%) and cold intolerance (55.1%). Other symptoms included dry skin (31.9%), hair loss (29.0%), constipation (26.1%), and facial puffiness/swelling (21.7%). [Table 1]

Biochemically, cases had significantly lower vitamin D (15.85 ± 8.35 ng/mL) compared with controls (40.97 ± 21.52 ng/mL), mean difference -25.15 ($t = 8.983$, $p < 0.001$).

Vitamin B12 levels were also reduced in cases (151.47 ± 80.31 pg/mL) versus controls (259.03 ± 110.59 pg/mL), mean difference -107.56 ($t = 6.490$, $p < 0.001$). [Table 2]

Thyroid hormones showed marked derangements: TSH was significantly elevated in cases (52.13 ± 109.91 $\mu\text{IU/L}$) compared with controls (2.39 ± 1.26 $\mu\text{IU/L}$), mean difference $+49.73$ ($t = 3.765$, $p < 0.001$). In contrast, FT3 (3.21 ± 0.53 vs. 6.03 ± 1.43 pmol/L, mean difference -2.81 , $t = 15.221$, $p < 0.001$) and FT4 (0.62 ± 0.12 vs. 1.16 ± 0.27 ng/dL, mean difference -0.54 , $t = 15.260$, $p = 0.0015$) were significantly lower in cases. [Table 2]

Deficiency prevalence was striking: vitamin D deficiency (<30 ng/mL) occurred in 94.20% of cases vs. 21.74% of controls ($\chi^2 = 71.409$, $p < 0.001$), while vitamin B12 deficiency (<200 pg/mL) was found in 88.41% vs. 33.33%, respectively ($\chi^2 = 41.649$, $p < 0.001$). [Figure-1] Subgroup analysis revealed that vitamin D-deficient patients had lower FT3 (3.74 ± 1.33 vs. 5.84 ± 1.57 , mean difference $+2.10$, $t = 2.618$, $p = 0.072$) and FT4 (0.71 ± 0.25 vs. 1.13 ± 0.30 , mean difference $+0.42$, $t = 2.742$, $p = 0.065$), though not significant, but significantly higher TSH (44.69 ± 102.79 vs. 3.22 ± 3.47 , mean difference -41.47 , $t = -3.223$, $p = 0.002$). [Table 3] Vitamin B12-deficient patients showed significantly lower FT3 (4.04 ± 1.64 vs. 5.52 ± 1.58 , mean difference $+1.48$, $t = 2.480$, $p = 0.035$) and FT4 (0.78 ± 0.34 vs. 1.06 ± 0.26 , mean difference $+0.28$, $t = 2.753$, $p = 0.020$), along with higher TSH (42.71 ± 100.73 vs. 3.23 ± 2.48 , mean difference -39.48 , $t = -3.054$, $p = 0.003$). [Table 3]



Correlation analysis confirmed significant associations. Vitamin D showed a negative correlation with TSH ($r = -0.242$, $p = 0.048$) and positive but non-significant correlations with FT3 ($r = +0.182$, $p = 0.110$) and FT4 ($r = +0.165$, $p = 0.135$). Vitamin B12 demonstrated a stronger profile: negative correlation with TSH ($r = -0.301$, $p = 0.003$), and significant positive correlations with FT3 ($r = +0.251$, $p = 0.035$) and FT4 ($r = +0.268$, $p = 0.020$). [Table 4, Figure 2]

DISCUSSION

The present case–control study evaluated vitamin D and B12 in relation to thyroid hormone profile in hypothyroidism. Among 138 participants (69 hypothyroid patients and 69 matched controls), mean serum vitamin D and B12 levels were significantly lower in cases. Deficiency rates were strikingly higher in hypothyroid patients (94.2% vitamin D, 88.4% vitamin B12) versus controls (21.7% and 33.3%). Correlation analyses showed these deficiencies were linked to worsened thyroid derangements, including elevated TSH and reduced FT3/FT4.

Our findings of lower B12 levels align with Tripathi et al. (2019) [17] and Sinha MK et al. (2022) [18], who also observed reduced B12, folate, ferritin, higher TSH, and lower T3/T4 in hypothyroid patients. Since thyroid hormones regulate nutrient absorption and hematopoiesis, B12 deficiency may exacerbate hormonal imbalance, as confirmed in our cohort. Mallick et al. (2023) [19] similarly reported significant B12 and iron differences between hypothyroid cases and controls, advocating routine screening—supported by our finding that nearly 90% of cases were B12 deficient.

The association with vitamin D deficiency mirrors Mirhosseini et al. (2017) [20], who showed that serum $25(\text{OH})\text{D} \geq 125$ nmol/L reduced hypothyroidism risk by 30% and thyroid antibody risk by 32%. Aslan et al. (2023) [21] also found negative correlations between vitamin D and anti-Tg/anti-TPO in Hashimoto's thyroiditis, highlighting its immunomodulatory role. In contrast, Knutsen et al. (2017) [22] did not observe significant effects of vitamin D supplementation, possibly due to baseline and ethnic differences. Nonetheless, the high prevalence of vitamin D deficiency in our cohort suggests a key contributory role in thyroid dysfunction, particularly in the Indian setting.

Although most studies support our findings, some discrepancies exist. Adam et al. (2025) [29] found hypothyroid patients had lower ferritin and vitamin B12 but no significant vitamin D differences compared with controls, unlike our observation of near-universal vitamin D deficiency. Similarly, Gurbuz et al. (2023) [30] reported only 8% prevalence of B12 insufficiency versus our 88.4%. These contrasts likely reflect differences in geography, sunlight exposure, dietary practices, ethnicity, and study design, underscoring the need for region-specific studies.

The consistently high prevalence of vitamin D and B12 deficiencies in hypothyroid patients, as shown in our study and corroborated by others, carries important clinical implications. Routine screening for these micronutrients may enable early detection and correction, thereby improving biochemical control and outcomes. Supplementation strategies could serve as cost-effective adjuncts to thyroid hormone replacement. Mirhosseini et al. (2017) [20] demonstrated that optimal vitamin D levels reduced risk of hypothyroidism and thyroid autoantibody titers, suggesting supplementation may modify disease progression. Likewise, correcting B12 deficiency may alleviate anemia, fatigue, cognitive impairment, and overall quality-of-life deficits in hypothyroid patients.

Key strengths of our study include its case–control design, age- and sex-matched controls, and comprehensive assessment of both thyroid hormones and micronutrients. Few studies from North India have addressed vitamin D and B12 simultaneously in hypothyroidism, enhancing the regional relevance of our findings. However, limitations include the cross-sectional design, which precludes causal inference; omission of autoimmune markers (anti-TPO, anti-Tg); lack of dietary and sun exposure data; and a modest sample size limiting generalizability, though our results were highly significant.

Future research should focus on longitudinal and interventional trials to determine whether correcting vitamin D and B12 deficiencies improves thyroid function, reduces antibody titers, and mitigates disease progression. Multicentric studies across diverse Indian populations are also needed to capture geographical variation, while integrating hematological indices, dietary assessment, and autoimmunity profiling for a



more comprehensive understanding of the micronutrient–thyroid axis.

CONCLUSION

The present case–control study demonstrated a strong association between hypothyroidism and micronutrient deficiencies, particularly of vitamin D and vitamin B12. Patients with hypothyroidism had markedly reduced mean serum vitamin D (15.85 ± 8.35 ng/mL) and vitamin B12 levels (151.47 ± 80.31 pg/mL) compared with healthy controls, and these differences were highly significant. Thyroid function tests confirmed the classical biochemical profile of hypothyroidism, with substantially elevated TSH levels accompanied by significantly lower FT3 and FT4 values in cases relative to controls.

Deficiency analysis revealed that the vast majority of hypothyroid patients were vitamin D deficient (94.2%) and vitamin B12 deficient (88.4%), in contrast to much lower rates among controls, underscoring the high burden of these deficiencies in thyroid disease. Subgroup analysis further highlighted that deficiency of either micronutrient was linked with exaggerated hormonal disturbances—vitamin D deficiency was associated with significantly higher TSH, while vitamin B12 deficiency was associated with both higher TSH and lower FT3/FT4 levels. Correlation studies reinforced these findings, showing a negative relationship between vitamin D and TSH, and between vitamin B12 and TSH, while vitamin B12 exhibited positive correlations with FT3 and FT4.

In summary, the study establishes that vitamin D and vitamin B12 deficiencies are not only highly prevalent in hypothyroid patients but also significantly influence thyroid hormone status. These findings emphasize the importance of routine screening for micronutrient deficiencies in hypothyroid individuals and suggest that timely correction of vitamin D and B12 levels may have a beneficial role in optimizing thyroid function and improving overall patient outcomes.

Conflict of Interest: All authors declare no conflict of interest.

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TABLES AND FIGURES

Table 1. Baseline Characteristics and Chief Complaints of Study Participants

Variable	Cases (n=69) n(%)	Controls (n=69) n(%)	p-value
AGE (YEARS)			
20–29	13 (18.8%)	25 (36.2%)	$\chi^2 = 7.29,$ p = 0.121
30–39	18 (26.1%)	12 (17.4%)	
40–49	18 (26.1%)	17 (24.6%)	
50–60	18 (26.1%)	15 (21.7%)	
>60	2 (2.9%)	0 (0.0%)	
Mean ± SD	41.01 ± 12.00	39.09 ± 10.31	t = 0.958, p = 0.684
GENDER			



Female	54 (78.3%)	50 (72.5%)	$\chi^2 = 0.356,$ $p = 0.553$
Male	15 (21.7%)	19 (27.5%)	
CHIEF COMPLAINTS IN HYPOTHYROID CASES			
Fatigue & Lethargy	50 (72.5%)	--	--
Weight Gain	42 (60.9%)		
Cold Intolerance	38 (55.1%)		
Constipation	18 (26.1%)		
Dry Skin	22 (31.9%)		
Hair Loss	20 (29.0%)		
Facial Puffiness/Swelling	15 (21.7%)		

Table 2. Comparison of Vitamin D, Vitamin B12 and Thyroid Profile Parameters Between Cases and Controls

Parameter	Cases (n=69) Mean \pm SD	Controls (n=69) Mean \pm SD	Mean Difference	t value	p-value
Vitamin D (ng/ml)	15.85 \pm 8.35	40.97 \pm 21.52	-25.15	8.983	<0.001*
Vitamin B12 (pg/ml)	151.47 \pm 80.31	259.03 \pm 110.59	-107.56	6.490	<0.001*
TSH (μ IU/L)	52.13 \pm 109.91	2.39 \pm 1.26	+49.73	3.765	<0.001*
FT3 (pmol/L)	3.21 \pm 0.53	6.03 \pm 1.43	-2.81	15.221	<0.001*
FT4 (ng/dL)	0.62 \pm 0.12	1.16 \pm 0.27	-0.54	15.260	0.0015*

Table 3. Association of Vitamin D and Vitamin B12 Deficiency Status with Thyroid Hormone Parameters (FT3, FT4, TSH)

	Vitamin D deficiency status			t-value	p-value
	No (Mean \pm SD)	Yes (Mean \pm SD)	Mean Difference		
FT3 (pmol/L)	5.84 \pm 1.57	3.74 \pm 1.33	+2.10	2.618	0.072
FT4 (ng/dL)	1.13 \pm 0.30	0.71 \pm 0.25	+0.42	2.742	0.065
TSH (μ IU/L)	3.22 \pm 3.47	44.69 \pm 102.79	-41.47	-3.223	0.002**



	Vitamin D deficiency status			t-value	p-value
	No (Mean ± SD)	Yes (Mean ± SD)	Mean Difference		
FT3 (pmol/L)	5.52 ± 1.58	4.04 ± 1.64	+1.48	2.480	0.035*
FT4 (ng/dL)	1.06 ± 0.26	0.78 ± 0.34	+0.28	2.753	0.020*
TSH (μIU/L)	3.23 ± 2.48	42.71 ± 100.73	-39.48	-3.054	0.003**

Table 4. Correlation of Vitamin D, B12 with Thyroid Hormones.

Parameter Correlation	TSH (r, p)	FT3 (r, p)	FT4 (r, p)
Vitamin D	r = -0.242, p = 0.048*	r = +0.182, p = 0.110	r = +0.165, p = 0.135
Vitamin B12	r = -0.301, p = 0.003**	r = +0.251, p = 0.035*	r = +0.268, p = 0.020*

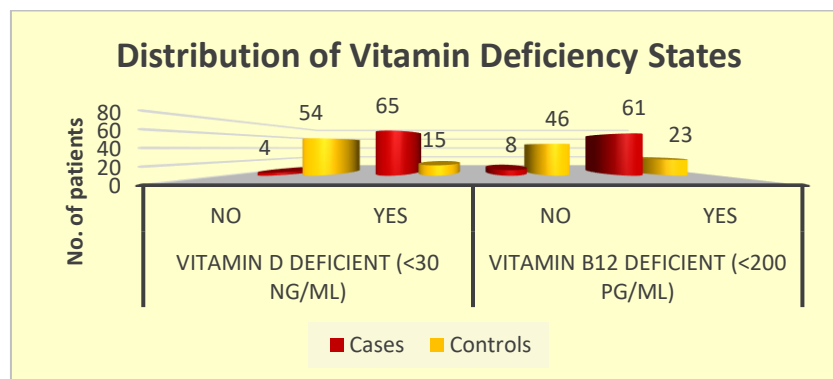


Figure: 1 Graphical representations of Frequency Distribution of Vitamin Deficiency States

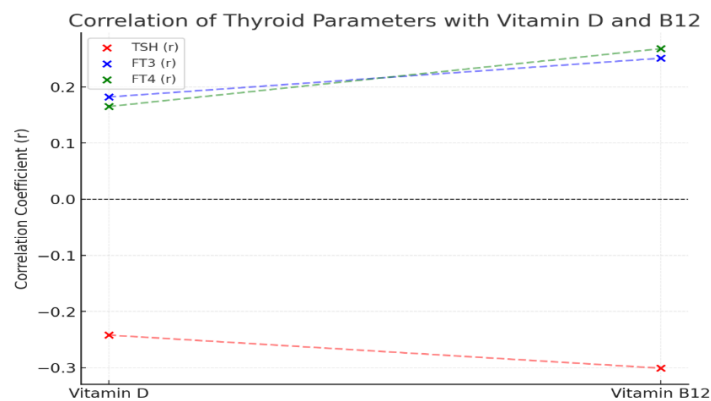


Figure: 2 Graphical representations of Correlation of Vitamin D, B12 with Thyroid Hormones.