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ASSOCIATION OF VITAMIN D AND VITAMIN B12 DEFICIENCY WITH THYROID HORMONE PROFILE IN HYPERTHYROIDISM: A CASE–CONTROL STUDY

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ABSTRACT

Background: Hyperthyroidism is associated with increased metabolic demand and may influence micronutrient status. Vitamin D and Vitamin B12 deficiencies have been increasingly reported in thyroid disorders, but their relationship with thyroid hormone derangements requires further evaluation.
Objective: To assess and compare serum Vitamin D and Vitamin B12 levels in hyperthyroid patients and euthyroid controls, and to determine their association with thyroid hormone profile (TSH, FT3, FT4) in hyperthyroidism.
Methods: A hospital-based case–control study was conducted at NCRIMS and Hospital, Meerut, over 18 months. A total of 51 hyperthyroid cases and 69 euthyroid controls were enrolled. Baseline demographic data and clinical complaints were recorded. Serum Vitamin D, Vitamin B12, and thyroid function tests (TSH, FT3, FT4) were measured and compared between groups. Vitamin D deficiency was defined as <30 ng/mL and Vitamin B12 deficiency as <165 pg/mL. Correlation analysis assessed relationships between micronutrient levels and thyroid hormones.
Results: Cases and controls were comparable in demographic characteristics. Hyperthyroid patients commonly reported palpitations, weight loss, anxiety/irritability, heat intolerance, tremors, and fatigue. Mean Vitamin D and Vitamin B12 levels were significantly lower in cases than controls ($p<0.0001$ for both). Vitamin D deficiency was more frequent in cases (82.35%) than controls (21.74%), and Vitamin B12 deficiency was also higher in cases (92.16%) than controls (33.33%) ($p<0.0001$). Vitamin D and Vitamin B12 showed positive correlation with TSH and negative correlation with FT3 and FT4 (statistically significant).
Conclusion: Vitamin D and Vitamin B12 deficiencies are highly prevalent in hyperthyroid patients and are significantly associated with more severe thyroid hormone derangements, supporting the need for routine micronutrient assessment in hyperthyroidism.

KEYWORDS: Hyperthyroidism; Vitamin D Deficiency; Vitamin B 12 Deficiency; Thyroid Hormones

INTRODUCTION

Hyperthyroidism is an endocrine disorder characterized by excessive synthesis and/or release of thyroid hormones. It is typically identified biochemically by elevated free triiodothyronine (FT3) and/or free thyroxine (FT4) with suppressed thyroid-stimulating hormone (TSH). Clinically, patients commonly present with palpitations, unintentional weight loss, tremor, heat intolerance, anxiety or irritability, and fatigue. If left untreated or poorly controlled, thyrotoxicosis may progress to atrial fibrillation, heart failure, and life-threatening thyroid storm. Graves' disease is a major cause of endogenous hyperthyroidism worldwide, and current evidence-based recommendations prioritize accurate etiologic diagnosis and definitive control of thyroid hormone excess using antithyroid drugs, radioiodine, or surgery, tailored to patient factors and comorbidities [1].
Beyond cardiovascular and neuropsychiatric consequences, thyroid hormone excess has important implications for bone and micronutrient homeostasis. Overt hyperthyroidism increases bone turnover and accelerates bone loss, thereby increasing osteoporosis and fracture risk [2]. Vitamin D is central to calcium–phosphate balance, muscle performance, and bone mineralization, and vitamin D signaling also modulates innate and adaptive immune responses. Recent Endocrine Society guidance on vitamin D emphasizes appropriate intake and individualized clinical decision-making in selected higher-risk groups, reflecting ongoing interest in the broader health consequences of vitamin D insufficiency [4]. Importantly, accumulating evidence suggests an association between Graves' disease and low vitamin D status; a recent meta-analysis reported that patients with Graves' disease are more likely to have lower serum vitamin D levels than controls, supporting a potential link between vitamin D deficiency and autoimmune hyperthyroidism [3].
Vitamin B12 (cobalamin) is essential for DNA synthesis, erythropoiesis, and neurologic function. Deficiency can manifest with anemia, glossitis, cognitive symptoms, and peripheral neuropathy—features that may overlap with or be masked by thyrotoxic complaints—leading to under-recognition. In thyroid disorders, low B12 levels may result from inadequate

intake, malabsorption, medication exposure, or autoimmune comorbidity such as autoimmune gastritis and pernicious anemia. A systematic review evaluating vitamin B12 across thyroid disorders highlights that B12 deficiency occurs across the spectrum of thyroid dysfunction, including hyperthyroidism, though reported frequencies vary across populations and study designs [5]. Patients with autoimmune thyroid disease have also been reported to have a relatively high prevalence of B12 deficiency, particularly related to pernicious anemia, supporting the concept of shared autoimmune clustering that may influence nutritional status and symptom burden [6].

Taken together, available evidence suggests that vitamin D and vitamin B12 deficiencies may cluster in hyperthyroid states and may relate to autoimmune activity, symptom burden, and skeletal vulnerability. Yet, studies that directly compare hyperthyroid patients with euthyroid controls while simultaneously examining how vitamin D and vitamin B12 levels vary with the thyroid hormone profile (TSH, FT3, and FT4) are limited. Therefore, the present case–control study was designed to evaluate vitamin D and vitamin B12 status in hyperthyroid patients versus euthyroid controls and to examine their associations with thyroid hormone alterations, with the aim of informing comprehensive clinical assessment and supportive management.

MATERIALS AND METHODS

This hospital-based case–control study was carried out at NCRIMS and Hospital, Meerut, over a period of 18 months. The study included a total of 120 participants, comprising 51 hyperthyroid patients (cases) and 69 euthyroid individuals (controls). The study was designed to evaluate differences in serum Vitamin D and serum Vitamin B12 levels between cases and controls and to examine their relationship with thyroid hormone derangements.

Study population and case/control definitions

Cases were patients diagnosed with hyperthyroidism based on thyroid function testing demonstrating suppressed TSH with elevated FT3 and/or FT4, consistent with biochemical hyperthyroidism. Thyroid profile parameters evaluated included TSH (µIU/L), FT3 (pmol/L), and FT4 (ng/dL). Controls were euthyroid individuals with thyroid function tests within reference limits and without biochemical evidence of thyroid dysfunction. Baseline demographic information (age and gender) was recorded for all participants to support group comparability.

Clinical assessment

All participants underwent a structured clinical evaluation. In the hyperthyroid group, chief complaints suggestive of thyrotoxicosis—such as palpitations, weight loss, anxiety/irritability, heat intolerance, tremors, and fatigue/weakness—were documented systematically as part of case characterization.

Sample collection and laboratory investigations

Venous blood samples were collected under standard aseptic precautions for biochemical assessment. The primary laboratory investigations included measurement of serum Vitamin D (ng/mL), serum Vitamin B12 (pg/mL), and thyroid function parameters (TSH, FT3, and FT4).

Laboratory estimation of Vitamin D, Vitamin B12, and thyroid hormones was performed in the institutional laboratory using routine standardized methods available at the center, following established laboratory protocols for calibration and internal quality checks (as per departmental practice).

Definitions of micronutrient deficiency

Micronutrient deficiency states were defined using standard cut-off values. Vitamin D deficiency was defined as serum Vitamin D <30 ng/mL, and Vitamin B12 deficiency was defined as serum Vitamin B12 <165 pg/mL. Based on these thresholds, participants were categorized as “deficient” or “non-deficient” for each vitamin. These categories were subsequently used for subgroup comparisons of thyroid hormone parameters according to Vitamin D and Vitamin B12 status.

Statistical analysis

Continuous variables were summarized as mean ± standard deviation (SD), and categorical variables were expressed as frequency and percentage. Between-group comparisons of continuous parameters (Vitamin D, Vitamin B12, TSH, FT3, FT4) between cases and controls were performed using appropriate tests for difference in means (as reflected by reported t-values in the study output tables). Categorical variables (including frequency of deficiency states) were compared using the chi-square test (χ^2). To assess the relationship between micronutrient levels and thyroid hormone derangements, correlation coefficients (r) were computed between Vitamin D/Vitamin B12 and thyroid parameters (TSH, FT3, FT4), with corresponding p-values reported. A two-tailed p-value <0.05 was considered statistically significant.

Ethical considerations

The study was conducted in accordance with institutional and ethical standards. Institutional Ethics Committee approval was obtained prior to initiation of the study, and written informed consent was taken from all participants. Participant confidentiality was maintained, and data were used solely for academic and research purposes.

RESULT

The present case–control study assessed 51 hyperthyroid patients and 69 euthyroid controls to evaluate how Vitamin D and Vitamin B12 levels vary with thyroid hormone alterations. The demographic comparison confirmed that both groups were comparable in baseline characteristics. Age distribution across five age categories (20–29, 30–39, 40–49, 50–60, and >60 years) did not significantly differ, with a chi-square value of 2.309 and p=0.6791. Mean ages also showed no significant difference: 40.68±1.97 years among cases versus 39.09±10.31 years among controls (t=1.258, p=0.2104). Gender distribution likewise showed no statistical significance (χ^2=1.823, p=0.1769), with females comprising 60.78% of cases and 72.46% of controls **[Table 1]**. This demographic similarity strengthens the reliability of comparisons in biochemical and clinical findings.

Clinically, hyperthyroid patients exhibited a high prevalence of classical symptoms with quantifiable frequencies. Palpitations were reported by 50 out of 51 patients (98.04%), making it the most common complaint **[Figure 1]**. Unintentional weight loss was seen in 48 patients (94.12%), while nervousness, anxiety, or irritability affected 45 patients (88.24%). Heat intolerance occurred in 41 patients (80.39%), tremors in 38 patients (74.51%), and chronic fatigue or generalized weakness in 36 patients (70.59%) **[Table 2]**.

Biochemical comparison revealed substantial differences between groups. Mean Vitamin D levels among hyperthyroid cases were 19.61±12.29 ng/ml, almost half of the levels observed in controls (40.97±21.52 ng/ml), resulting in a mean difference of –21.36 ng/ml (t=7.160, p<0.0001). Vitamin B12 levels followed a similar pattern, with cases averaging 148.95±74.77 pg/ml compared to 259.03±110.59 pg/ml in controls, yielding a mean difference of –110.1 pg/ml (t=6.850, p<0.0001)**[Table 3]**.

Thyroid hormone analysis demonstrated typical hyperthyroid patterns with statistically significant differences across all parameters. Mean TSH levels in cases were markedly suppressed (0.17±0.28 µIU/L) compared to controls (2.39±1.26 µIU/L), with a mean difference of –2.22 µIU/L (t=14.29, p<0.0001). FT3 levels were significantly elevated in cases (12.32±3.55 pmol/L) relative to controls (6.03±1.43 pmol/L), while FT4 levels showed the most pronounced increase (12.34±6.64 ng/dL vs 1.16±0.27 ng/dL). These results are summarized in **[Table 4]**.

The frequency of micronutrient deficiencies further supported these observations. Vitamin D deficiency (<30 ng/ml) was present in 42 of 51 cases (82.35%), compared to 15 of 69 controls (21.74%), with a chi-square value of 43.20 (p<0.0001). Vitamin B12 deficiency (<165 pg/ml) was detected in 47 of 51 cases (92.16%) and 23 of 69 controls (33.33%), yielding a chi-square value of 41.75 (p<0.0001). These distributions are presented in **[Table 5]**.

Stratification of thyroid hormone levels based on Vitamin D status revealed that Vitamin D–deficient individuals had significantly higher FT3 (10.78±4.43 vs 6.82±2.43 pmol/L) and FT4 levels (8.64±6.62 vs 3.44±6.50 ng/dL), along with significantly lower TSH levels (0.74±1.08 vs 2.09±1.50 µIU/L). These associations are shown in **[Table 6, Figure 2]**.

Similarly, Vitamin B12–deficient individuals exhibited significantly elevated FT3 (10.38±4.25 vs 6.35±2.07 pmol/L) and FT4 levels (8.84±7.72 vs 1.82±2.59 ng/dL), with concomitantly reduced TSH levels (0.87±1.13 vs 2.27±1.52 µIU/L). These findings are detailed in **[Table 7, Figure 3]**.

Correlation analysis demonstrated that Vitamin D levels were positively correlated with TSH (r=0.432, p<0.0001) and negatively correlated with FT3 (r=–0.421, p<0.0001) and FT4 (r=–0.354, p<0.0001). Vitamin B12 levels also showed a positive correlation with TSH (r=0.267, p=0.003) and negative correlations with FT3 (r=–0.439, p<0.0001) and FT4 (r=–0.395, p<0.0001)**[Table 8]**.

DISCUSSION

The present study demonstrated no significant differences in age distribution or gender between hyperthyroid cases and euthyroid controls, indicating appropriate baseline comparability for biochemical comparisons. The female predominance among cases is consistent with the known epidemiology of Graves' disease and other causes of hyperthyroidism, as highlighted in standard clinical guidance [1].

Classical symptoms—palpitations, weight loss, anxiety/irritability, heat intolerance, tremors, and fatigue/weakness—were highly prevalent in the case group. This clinical pattern reflects the expected hyperadrenergic and hypermetabolic state described in hyperthyroidism management guidelines [1] and underlines why micronutrient depletion (due to increased turnover and metabolic demand) may be clinically relevant in such patients.

A major finding of the present study was significantly lower mean Vitamin D levels in hyperthyroid cases compared with controls (19.61±12.29 vs 40.97±21.52 ng/mL; p<0.0001). This agrees with accumulating evidence that Vitamin D status tends to be lower in Graves' disease/hyperthyroid populations. Pang et al. [3] reported in a meta-analysis that Graves' disease is associated with lower serum Vitamin D levels than controls. Similarly, Xu et al. [8] in a meta-analysis update concluded that Vitamin D levels are generally lower in Graves' disease, reinforcing the association reported across multiple cohorts.

However, not all studies show the same degree of association with disease parameters. Mangaraj et al. [7] reported lower Vitamin D in new-onset Graves' disease but did not find a significant correlation between Vitamin D and thyroid hormones/TRAb, suggesting that the Vitamin D–thyroid relationship may vary by disease stage, severity, and population background deficiency. This contrasts with the present study, where Vitamin D levels showed significant correlations with thyroid hormones.

Using the study cutoff (<30 ng/mL), Vitamin D deficiency was markedly more frequent in cases (82.35%) than controls (21.74%) (p<0.0001). This strongly supports that Vitamin D deficiency is substantially enriched in hyperthyroid patients in our setting. Clinically, this finding is important because hyperthyroidism itself accelerates bone turnover and can worsen bone loss; hence coexisting Vitamin D deficiency may compound skeletal risk, aligning with bone-health considerations in thyroid disease [2].

In the present study, Vitamin D–deficient participants showed higher FT3/FT4 and lower TSH compared with non-deficient participants, indicating more severe biochemical thyrotoxicosis among deficient individuals. Correlation analysis further showed that Vitamin D correlated positively with TSH (r=0.432) and negatively with FT3 (r=–0.421) and FT4 (r=–0.354) (all p<0.0001). These findings support the interpretation that lower Vitamin D status is associated with greater hormonal derangement in our cohort, although causality cannot be established from case–control data.

The relationship between Vitamin D and Graves' disease activity has also been explored in prognostic contexts. Yasuda et al. [9] reported lower Vitamin D levels in Graves' disease patients without remission, suggesting a possible link between Vitamin D status and disease course. However, interventional evidence is mixed. Grove-Laugesen et al. [11] found that Vitamin D supplementation did not improve Graves' disease treatment outcomes in a clinical trial, arguing against routine high-dose Vitamin D as a disease-modifying therapy in all patients. In contrast, Cho et al. [10] suggested Vitamin D supplementation might have a borderline protective effect on recurrence risk, indicating that any benefit—if present—may be modest and context-dependent (baseline Vitamin D level, immune activity, and timepoint of supplementation). Together, these studies suggest that Vitamin D deficiency is common and associated with Graves' disease, but supplementation may function more as supportive care (bone/muscle health) rather than a consistent driver of thyroid hormone normalization.

The present study found significantly lower mean Vitamin B12 levels in cases than controls (148.95±74.77 vs 259.03±110.59 pg/mL; p<0.0001). This direction is supported by older primary evidence: Gyftaki et al. [12] reported significantly lower mean B12 levels in hyperthyroid patients than controls and described an inverse relationship between B12 and clinical severity indices. At the same time, broader syntheses indicate heterogeneity in B12 findings across thyroid disorders [5], which may explain why some settings observe stronger differences than others.

Vitamin B12 deficiency was very frequent in the present study (92.16% in cases vs 33.33% in controls, p<0.0001). One plausible interpretation is that hyperthyroidism may coexist with background dietary and population-level B12 deficiency. Supporting this, Singla et al. [13] described Vitamin B12 deficiency as widespread in North India (reported prevalence ~47% in their dataset), indicating that local baseline deficiency could amplify the observed association in a hospital-based hyperthyroid sample. Another mechanistic contributor is autoimmune clustering: autoimmune thyroid disease can be associated with pernicious anemia/autoimmune gastritis, and low B12 has been reported among autoimmune thyroid disease patients [6].

In the present study, Vitamin B12 deficiency was associated with higher FT3/FT4 and lower TSH, and B12 showed significant correlations with thyroid parameters: positive with TSH (r=0.267) and negative with FT3 (r=–0.439) and FT4 (r=–0.395). These findings suggest that low B12 status tends to accompany more pronounced biochemical hyperthyroidism in our cohort. Clinically, this matters because fatigue, weakness, and neurocognitive complaints may overlap between thyrotoxicosis and B12 deficiency, and untreated deficiency may prolong functional recovery even after thyroid control.

Overall, the present study supports that Vitamin D and Vitamin B12 deficiencies are significantly more prevalent in hyperthyroid patients and are associated with greater thyroid hormone derangement. These findings align with meta-analytic evidence showing lower Vitamin D in Graves' disease [3,8] and highlight the importance of supportive nutritional assessment alongside standard hyperthyroidism management [1].

CONCLUSION

In conclusion, this study reveals that hyperthyroid patients have a significantly higher prevalence of both Vitamin D and Vitamin B12 deficiencies compared to controls. These deficiencies are associated with more severe thyroid hormone imbalances, underscoring the need for routine screening and potential supplementation. Addressing these micronutrient deficiencies may improve clinical outcomes and enhance the overall management of hyperthyroidism. In conclusion, the study emphasizes the value of incorporating nutritional assessment into the care of patients with thyroid disorders.

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

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TABLES AND FIGURES

Table 1. Baseline demographic characteristics of hyperthyroid cases and euthyroid controls

Variable	**Cases (n=51) n (%)**		**Controls (n=69) n (%)**		**p-value**
Age (years)					
20-29	17	33.33%	25	36.23%	X=2.309 p=0.6791
30-39	6	11.76%	12	17.39%	
40-49	14	27.45%	17	24.64%	
50-60	13	25.49%	15	21.74%	
>60	1	1.96%	0	0.00%	
(Mean±SD)	40.68±1.97		39.09±10.31		t=1.258 p=0.2104
Gender					
Female	31	60.78%	50	72.46%	X=1.823 p=0.1769
Male	20	39.22%	19	27.54%	

Table 2. Distribution of chief clinical complaints among hyperthyroid patients (n = 51)

Chief Complaint	**No. of Patients (n)**	**Percentage (%)**
Palpitations (noticed for ~3 months)	**50**	**98.04%**
Unintentional weight loss	**48**	**94.12%**
Nervousness, anxiety, irritability (2–4 months)	**45**	**88.24%**
Heat intolerance	**41**	**80.39%**
Tremors (for ~2 months)	**38**	**74.51%**
Fatigue and generalized weakness	**36**	**70.59%**

Table 3. Comparison of serum Vitamin D and Vitamin B12 levels between cases and controls

Parameter	**Cases (n=51)**	**Controls (n=69)**	**Mean Difference**	**t value**	**p-value**
Vitamin D (ng/ml)	19.61±12.29	40.97±21.52	**-21.36**	7.160	**<0.0001***
Vitamin B12 (pg/ml)	148.95±74.77	259.03±110.59	**-110.1**	6.850	**<0.0001***

Mean ± SD

Table 4. Comparison of Thyroid Profile Parameters Between Cases and Controls

Parameter	**Cases (n=51) Mean ± SD**	**Controls (n=69) Mean ± SD**	**Mean Difference**	**t value**	**p-value**
TSH (µIU/L)	0.17±0.28	2.39±1.26	-2.220	14.29	**<0.0001***
FT3 (pmol/L)	12.32±3.55	6.03±1.43	6.290	13.65	**<0.0001***

Parameter	Cases (n=51) Mean ± SD	Controls (n=69) Mean ± SD	Mean Difference	t value	p-value
FT4 (ng/dL)	12.34±6.64	1.16±0.27	11.18	13.97	**<0.0001***

Mean ± SD

Table 5. Frequency distribution of Vitamin D and Vitamin B12 deficiency among cases and controls

Deficiency Status		Cases n (%)		Controls n (%)		χ^2	p-value
Vitamin D Deficient (<30 ng/ml)	No	9	17.65%	54	78.26%	43.20	**<0.0001***
	Yes	42	82.35%	15	21.74%		
Vitamin B12 Deficient (<165 pg/ml)	No	4	7.84%	46	66.67%	41.75	**<0.0001***
	Yes	47	92.16%	23	33.33%		

Table 6. Association of Vitamin D deficiency status with thyroid hormone levels (FT3, FT4, TSH)

	Vitamin D deficiency status			t-value	p-value
Parameter	**No**	**Yes**	**Mean Difference**		
FT3 (pmol/L)	6.82±2.43	10.78±4.43	-3.960	6.510	**<0.0001***
FT4 (ng/dL)	3.44±6.50	8.64±6.62	-5.020	4.495	**0.0001***
TSH (µIU/L)	2.09±1.50	0.74±1.08	1.350	6.067	**<0.0001***

Mean ± SD

Table 7. Association of Vitamin B12 deficiency status with thyroid hormone levels (FT3, FT4, TSH)

Parameter	Vitamin B12 deficiency status			t-value	p-value
	No	**Yes**	**Mean Difference**		
FT3 (pmol/L)	6.35±2.07	10.38±4.25	-4.030	7.081	**<0.0001***
FT4 (ng/dL)	1.82±2.59	8.84±7.72	-7.020	7.161	**<0.0001***
TSH (µIU/L)	2.27±1.52	0.87±1.13	1.40	6.140	**<0.0001***

Mean ± SD

Table 8. Correlation of serum Vitamin D and Vitamin B12 levels with thyroid hormones

Parameter Correlation	TSH (r, p)	FT3 (r, p)	FT4 (r, p)
Vitamin D	r=0.432 p<0.0001*	r= - 0.421 p<0.0001*	r=-0.354 p<0.0001*
Vitamin B12	r=0.267 p=0.003*	r= - 0.439 p<0.0001*	r=-0.395 p<0.0001*

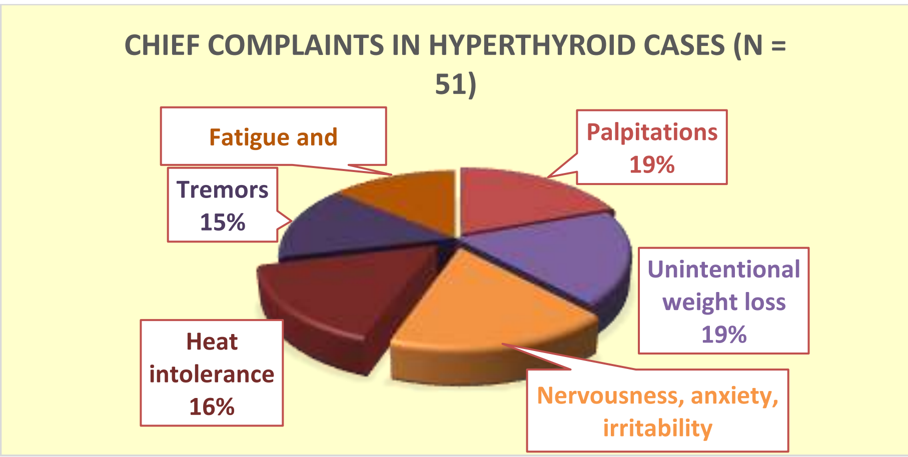

Figure-1: Distribution of chief clinical complaints among hyperthyroid patients

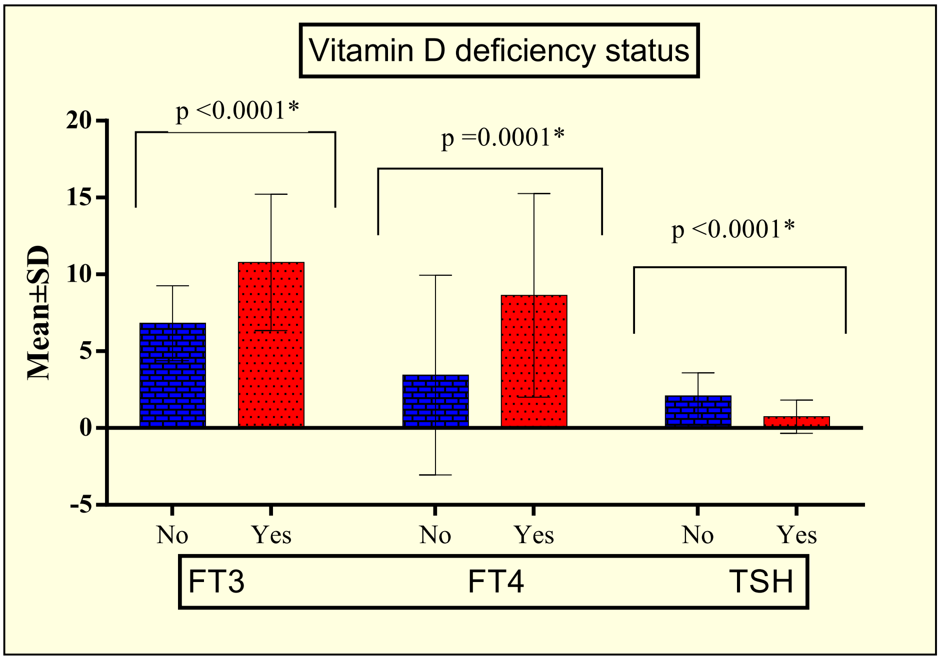

Figure-2: Comparison of mean serum Vitamin D and Vitamin B12 levels between cases and controls

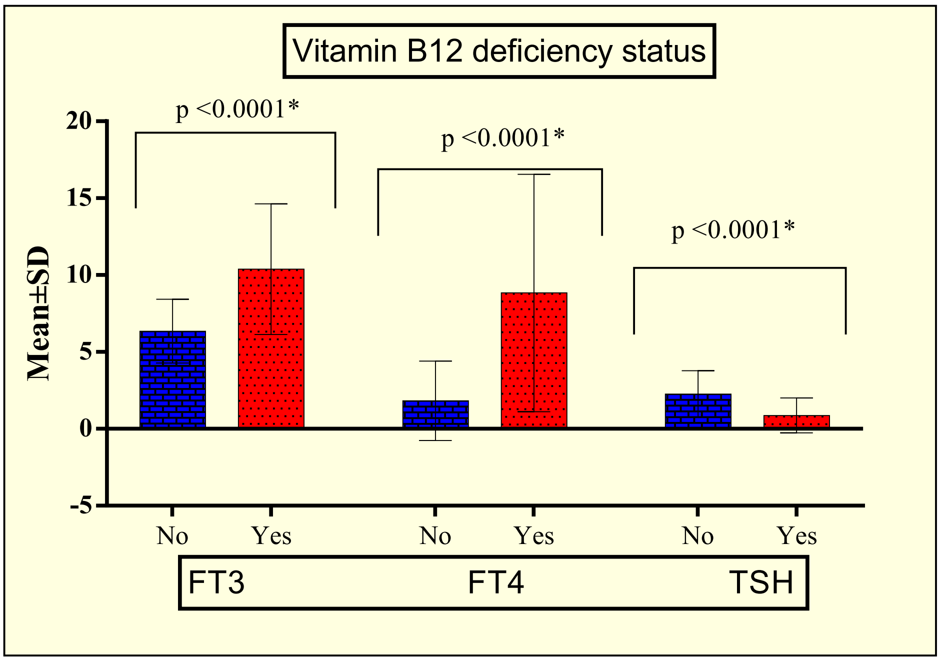

Figure 3: Correlation of serum Vitamin D and Vitamin B12 levels with thyroid hormone parameters