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Association of vitamin D3 deficiency with autoimmun hypothyroidism in Southern Iraqi patients: A regional study from Thi-Qar Province

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Abstract: Autoimmune hypothyroidism is immune-mediated destruction of thyroid tissue. Emerging evidence highlights D3 immunomodulatory role in autoimmune diseases, although its association with thyroid autoimmunity in Iraq remains understudied. Objective: This study aimed to evaluate vitamin D3 levels in Hashimoto's patients compared to controls and assess the correlations between vitamin D3, thyrotropin hormone (TSH), and anti-thyroid peroxidase (TPO-Ab). A case-control study at Al-Refai Hospital and Al-Haboubi Teaching Hospital/ Iraq comprised 50 Hashimoto's patients and 50 age- and sex-matched healthy controls. TSH, TPO-Ab, and 25-hydroxyvitamin D3 were assessed using fluorescence and chemiluminescence assays. Patients exhibited significantly lower vitamin D3 levels (21.27 ± 7.6 ng/mL) compared to controls (35.45 ± 11.8 ng/mL; $p<0.01$). Patients had elevated TSH (10.25 ± 3.7 vs. 1.89 ± 0.81 µIU/mL) and TPO-Ab (58.6 ± 17.4 vs. 6.2 ± 5.3 IU/mL) ($p<0.01$). Weak, non-significant negative correlations were found between vitamin D3 and TSH ($r = -0.04$) and TPO-Ab ($r = -0.099$). ROC analysis confirmed that TSH and TPO-Ab were highly sensitive biomarkers for Hashimoto. Vitamin D3 deficiency is strongly associated with autoimmune hypothyroidism in southern Iraqi patients. These findings suggest potential therapeutic benefits of vitamin D3 supplementation in managing the condition, warranting further clinical trials.

Keywords: *Autoimmune hypothyroidism, Hashimoto's thyroiditis, TPO, Vitamin D3.*

1. Introduction

Hypothyroidism is a health issue associated with an endocrine disorder accompanied by low serum thyroid hormone levels, leading to a clinical spectrum ranging from no signs and symptoms to life-threatening complications [1]. Autoimmune hypothyroidism, or Hashimoto's disease, has been reported to be more common than iodine deficiency in areas with adequate iodine intake [2] and is considered the cardinal cause of hypothyroidism, with an incidence rate of 0.3–1.5 cases per 1,000 people in developed countries [3]. Women are more susceptible to this disorder than men, and its incidence increases with age [4].

Pathologically, Hashimoto's disease, characterized by a person's immune system, produces lymphocytic cells, mainly T helper lymphocytes that infiltrate into thyroid gland cells, and B cells that produce thyroid auto-antibodies (anti-TPO) that attack the thyroid gland antigens and cause progressive fibrosis, which clearly reflects the extent of lymphocytic infiltration within the thyroid gland [5, 6]

Hashimoto's thyroid disease develops slowly; therefore, diagnosis is often difficult and may not occur until late in the disease course. Signs and symptoms, along with blood test results that measure thyroid hormones, levels of thyroid-stimulating hormone (TSH) produced in the pituitary gland, and levels of thyroid autoantibodies, are used to diagnose Hashimoto's disease [7]. A significant role of vitamin D in the progression of Hashimoto's thyroiditis has recently been pointed out. Vitamin D is

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involved in regulating the calcium and phosphate balance and bone metabolism, in addition to maintaining the balance of the immune system, which attacks the thyroid gland when it is imbalanced. These findings indicate a fundamental role of vitamin D in Hashimoto's thyroiditis and its progression to hypothyroidism [8].

The association between vitamin D status and autoimmune thyroid disease (AITD) remains unclear. Prior investigations have analyzed the impact of serum vitamin D deficiency on autoimmune thyroid conditions [9]. sought to address this gap in knowledge. Accumulating evidence underscores the immunomodulatory role of vitamin D in autoimmune hypothyroidism, with deficiency implicated in elevated prevalence rates of thyroid autoimmunity across diverse demographic cohorts, such as pediatric, adolescent, and obese populations [10]. Although the interplay between vitamin D and AITD has garnered increasing attention, the underlying pathophysiological mechanisms remain unclear. Initial explorations were exemplified by a comparative analysis of serum 25-hydroxyvitamin D (25(OH)D) concentrations in patients with Hashimoto's thyroiditis versus healthy controls [11, 12]. According to what was explained above, Vitamin D deficiency is increasingly recognized as a factor in various autoimmune diseases. Investigating its potential role in autoimmune hypothyroidism can enhance our understanding of disease mechanisms and progression; therefore, the present study was undertaken to estimate the levels of TSH and TPO-AB in the context of vitamin D in autoimmune Hashimoto's patients in the Thi-Qar province and their role in the prognosis of this disease.

2. Subjects and Methods

This case-control study was conducted between September and December 2023 at Al-Rifai General Hospital and Al-Haboubi Teaching Hospital in Thi-Qar Governorate, Iraq. A total of 100 Iraqi participants aged 18–60 years were enrolled, comprising 50 patients diagnosed with autoimmune hypothyroidism (Hashimoto thyroiditis) and 50 age- and sex-matched healthy controls. Patients were recruited from endocrinology outpatient clinics, while controls were selected from individuals attending routine health checkups with no history of thyroid disorders, autoimmune diseases, or chronic illnesses. Inclusion and Exclusion Criteria, Patients: Diagnosis of autoimmune hypothyroidism was confirmed by elevated (TSH >4.2 μIU/mL), reduced free thyroxine (fT4 <0.8 ng/dL), and positivity for anti-thyroid peroxidase antibodies (anti-TPO >35 IU/mL). Exclusion criteria were pregnancy, recent thyroid surgery, iodine contrast exposure, or concurrent autoimmune conditions (e.g., type 1 diabetes, lupus). Controls: Individuals with normal thyroid function (TSH 0.4–4.0 μIU/mL, fT4 0.8–1.8 ng/dL), negative anti-TPO antibodies (<35 IU/mL), and no family history of autoimmune thyroid disease. Venous blood samples (5 mL) were drawn from all participants after a 12-hour fast. Serum was separated via centrifugation at 3,000 rpm for 10 min, aliquoted into sterile cryovials, and stored at −20°C. To minimize degradation, samples were analyzed within four weeks of collection to avoid freeze-thaw cycles.

2.1. Laboratory Assays

Thyroid function tests, including "thyroid stimulating hormone (TSH), triiodothyronine hormone (T3), and thyroxine hormone (T4)," were performed utilizing AFIAS-10 apparatus' automated fluorescence immunoassay. Anti-TPO antibody and 25-hydroxyvitamin D (25[OH]D) levels were measured using a Cobas e411 chemiluminescence immunoassay (Roche Diagnostics, Germany).

2.2. Statistical Analysis

Data were analyzed using SPSS version 26. Continuous variables are expressed as mean ± standard deviation (SD) and compared using independent Student's t-tests (assuming normality, confirmed by Shapiro-Wilk tests). Categorical variables (e.g., sex and anti-TPO positivity) and Pearson's correlation coefficient (r) were used to assess the relationships between vitamin D, TSH, and anti-TPO. Receiver

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operating characteristic (ROC) curves were used to evaluate diagnostic accuracy. Statistical significance was set at $p < 0.05$.

2.3. Ethical Considerations

The study protocol was approved by the Iraqi Ministry of Health (Document No. 2024/255), and adhered to the Declaration of Helsinki. Written informed consent was obtained from all the participants. Confidentiality was maintained by anonymizing data, and participants received no financial compensation.

3. Results

A description of the investigated groups according to sex is shown in Figure (1). This study included 100 individuals classified into two categories: the first category included 50 individuals suffering from autoimmune hypothyroidism [16 males and 34 females] and the second was healthy people as a control that included 50 individuals [15 males and 35 females]. The majority of patients participating in the study were female (68%) compared to males (32%), and the female-to-male ratio was 2.13:1.

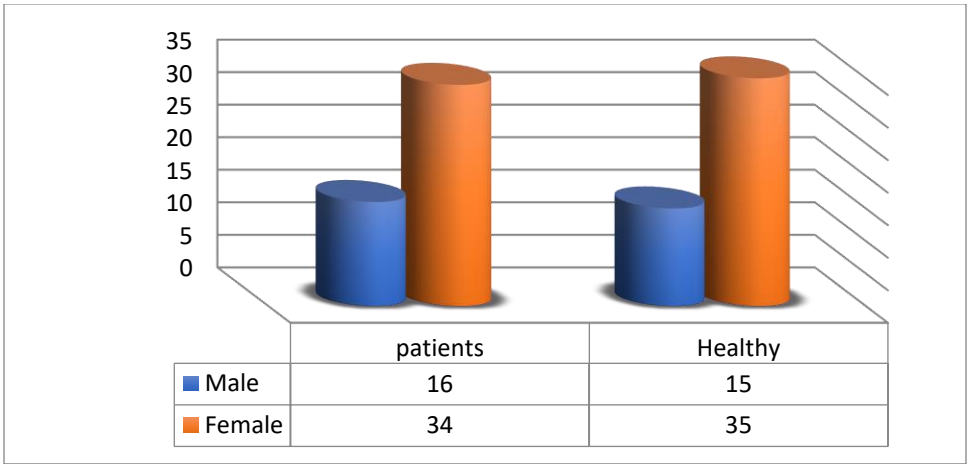

Figure 1.
Represent the prevalence of Hashimoto's disease in women compared to men.

Thyroid hormone values were estimated to diagnose patients with hypothyroidism, and TPO antibody values were used to confirm autoimmune hypothyroidism, as listed in Table 1. The results showed that the positivity rate of the TPO antibody was 37/50 (74%) in patients. Only two healthy individuals were positive for the TPO antibody 2/50 (4%), and the TPO-Abs mean values were higher in patients than in healthy individuals. According to the levels of vitamin D in the studied groups, patients had reduced levels compared with healthy individuals.

Table 1.
Represent the means of levels of thyroids hormones, vitamin D, TPO-Abs, and BML of the patients group compared to control group using T-test.

P value	T test	Control Mean± SD	Patients Mean± SD	Parameters
0.00**	15.3	1.89± 0.81	10.25±3.7	TSH
0.00**	-13. 66	66.02 ±19.5	25.73±7.28	T4
0.01**	-8.001	2.39±0.6	1.51± 0.5	T3
0.01**	20.4	6.2±5.3	58.6±17.4	TPO-Abs
0.01**	-7.4	35.45±11.8	21.27±7.6	VD
0.01**	7.32	24.4±1.8	28.3±3.3	BMI

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Pearson's correlation showed a non-significant negative association between vitamin D and TSH and anti-TPO levels. Figures (2) and (3).

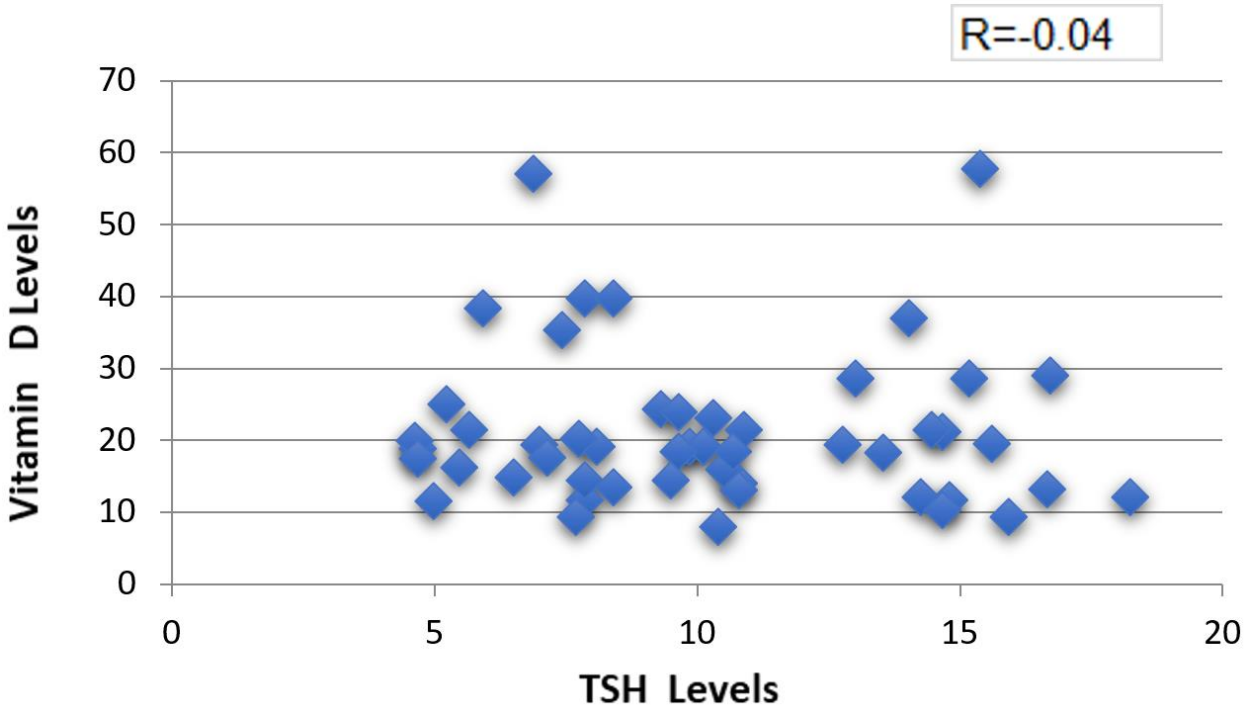

Figure 2.
Represent an inverse relationship between TSH &Vitamin D levels was identified in patients group using pearson's correlation coefficient.

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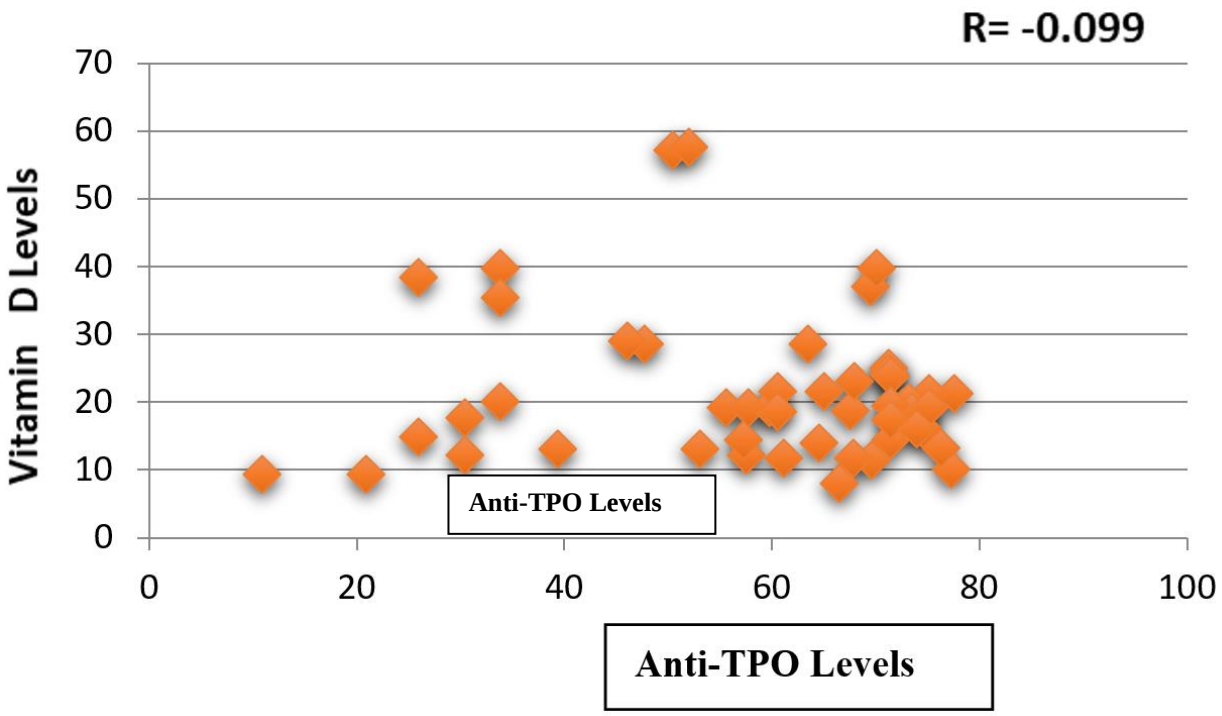

Figure 3.
Represent an inverse relationship between TPOAbs&Vitamin D levels was identified in patients group using pearson's correlation coefficient.

The results of ROC listed in Tables (2) and (3) demonstrate the calculated results for testing the selected variables as well as to reveal the validity of these biomarkers in diagnosing Hashimoto's disease Figures 4 and 5.

Table 2.
Represent the calculated results for testing anti-TPO and TSH using ROC test for Patient and Healthy Subjects.

Area Under the Curve					
Test Result Variables	**Area**	**Std. Error**	**Asymptotic Sig.**	**Asymptotic 95% Confidence Interval**	
				Lower Bound	**Upper Bound**
TPO	.980	0.011	0.000	0.959	1.000
TSH	1.000	0.000	0.000	1.000	1.000

The results indicated that autoantibodies to thyroperoxidase(anti-TPO) and TSH have the most sensitive and specific values, making them the best biomarkers for detecting Hashimoto's disease.

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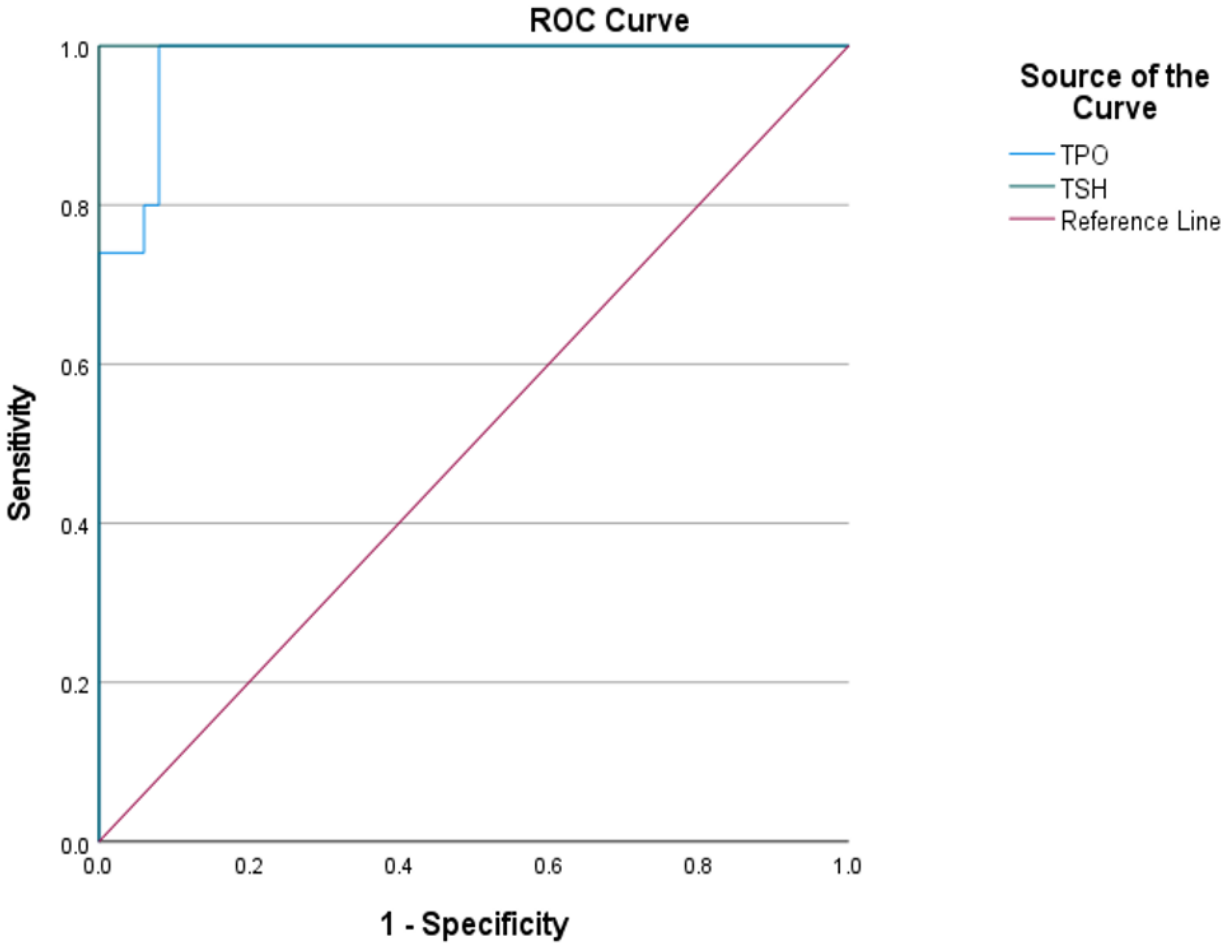

Figure 4.
Receiver operator characteristic curve analysis of TSH & Anti-TPO for the calculation a possible diagnostic cut-off value in patients and healthy (TSH Cut off > 4.2 sensitivity 100%, specifity 100%, Anti-TPO Cut off > 9.5, sensitivity 100%, specifity 0.92%).

While , the results of ROC test for vitamin D demonstrated that the computed area under the curve is 0.759, reflecting a fair level of discrimination for vitamin D in hypothyroid diseases, but not conclusive ,with fair Sensitivity and Specificity Table 3.

Table 3.
Represent the calculated results for testing Vitamin D using ROC test for Patient and Healthy Subjects.

Area Under the Curve					
Test Result Variables	**Area**	**Std. Error**	**Asymptotic Sig.**	**Asymptotic 95% Confidence Interval**	
				Lower Bound	**Upper Bound**
Vitamin D	0.759	0.048	0	0.664	0.854

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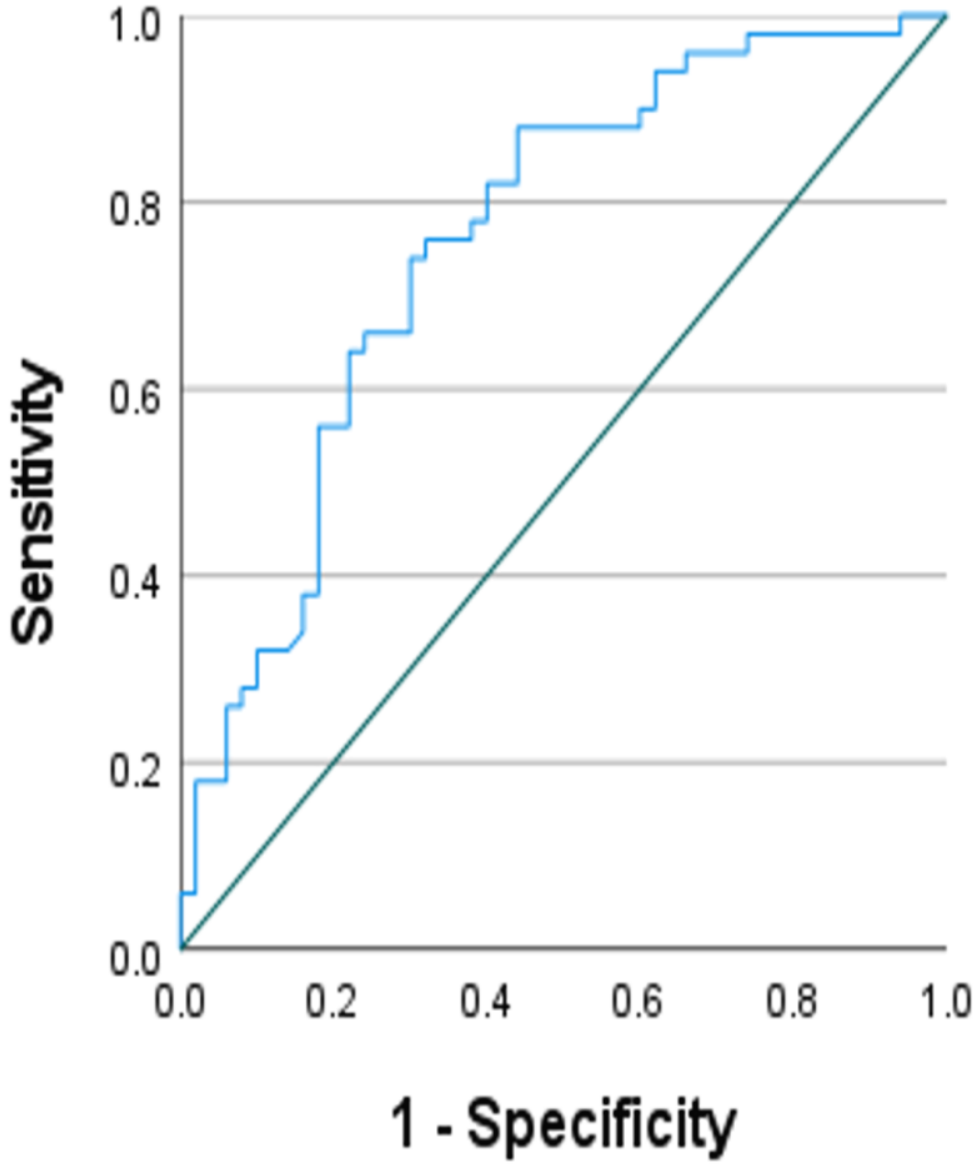

Figure 5.
Receiver operator characteristic curve analysis of Vitamin D for the calculation a possible diagnostic cut-off value in patients and healthy(Vitamin D Cut off > 24.59 sensitivity 70%, specifity 74%).

4. Discussion

Hashimoto thyroiditis. is an immune-mediated injury that affects the thyroid gland, and women appear to be more susceptible to it Geetha, et al. [5]. The current data agree with a local study in Thi-Qar province that referred women who were more susceptible to hypothyroidism and hyperthyroidism [13]. The interpretation of prevalence Thyroid problems among females, It may be due to an imbalance of female sex hormones, especially estrogen, that increases during puberty and pregnancy in women, and the inactivation of the X chromosome in the thyroid gland and immune system, which in turn predisposes women to autoimmune thyroiditis [14]. Females disproportionately carry high-risk HLA alleles (e.g., HLA-DR3 and HLA-DR4) linked to thyroid autoimmunity. These alleles enhance antigen

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presentation of TPO and thyroglobulin fragments to T cells [15]. Females exhibit stronger innate and adaptive immune responses, increasing both pathogen defense and self-reactivity risk. Women with Autoimmune thyroid disease show elevated Th17 cells and reduced regulatory T cells, an imbalance exacerbated by estrogen [16].

Thyroid hormone levels were estimated to diagnose hypothyroidism. The increased TSH levels in patients with hypothyroidism compared to healthy controls were consistent with the findings of other studies Mousa and Zoori [13] and Ameen, et al. [17]. These high levels of TSH in patients with hypothyroidism may be a result of the pituitary gland releasing more TSH in an attempt to induce the thyroid gland to increase the production of more of the low thyroid hormones (T3, T4), which appeared low in patients compared to controls in the current study. This is consistent with the findings of several studies that have indicated low thyroid hormone levels in patients with hypothyroidism. These approve the criteria used to diagnose hypothyroidism and align with those reported in studies of thyroid hormone levels in patients with hypothyroidism [18, 19]

The TPO antibody values were used to confirm autoimmune hypothyroidism. The highly positivity mean of TPO antibody in patients compare to healthy groups with significant difference, compatible with the fact recorded by previous studies that referred that the cardinal sign of the autoimmune hypothyroidism is the auto-antibodies found against thyroid peroxidase "TPOAb" in thyroid [15]. According to the levels of vitamin D in the studied groups, patients had reduced levels compared with healthy people, corroborating prior observations of vitamin D insufficiency in autoimmune thyroid pathologies. Cross-sectional and case-control studies across diverse populations revealed a pronounced disparity, with HT cohorts exhibiting median 25(OH)D levels 20–40% lower than matched controls and deficiency prevalence (<20 ng/mL) exceeding 70% in the affected groups. Regional investigations highlight this trend even in high-insolation regions: Middle Eastern studies reported mean 25(OH)D values of 9.37±15.2 ng/mL in HT patients versus 11.95±28.1 ng/mL in controls ($p<0.01$). These findings align with meta-analytic evidence (OR=2.89, 95% CI: 1.92–4.35 for deficiency risk in HT) and support the putative role of vitamin D in modulating thyroid autoimmunity through VDR-mediated cytokine regulation. Mechanistic studies posit that suboptimal 25(OH)D levels (<30 ng/mL) may impair T-regulatory cell function, potentially exacerbating thyrocyte destruction in genetically susceptible individuals [20-23]. These disparities persisted even after adjusting for variables such as age, sex, and body mass index, suggesting an intrinsic link between hypovitaminosis D and autoimmune thyroid dysfunction.

The mechanistic interplay between vitamin D status and thyroid autoimmunity extends beyond simple deficiency. Observational studies noted inverse correlations between 25(OH)D levels and TPO-Ab titers, where every 1 ng/mL increase in vitamin D corresponds to measurable reductions in autoimmune activity [23, 24].

Regarding the relationship between auto-hypothyroidism and vitamin D, Pearson's correlation showed a negative non-significant association between vitamin D and TSH and anti-TPO levels, suggesting that vitamin D deficiency leads to elevated TSH and autoantibody levels. This result is in line with the Iraqi study by Raeef et al., who found the same negative correlation between vitamin D blood levels and TSH and anti-TPO levels, and other studies in Egypt Figures (2)(3) [25, 26].

Body mass index (BMI) has also been associated with hypothyroidism. The results revealed a significant increase in the mean BMI in patients with hypothyroidism compared to that in the healthy control group. This aligns with the results in [11]. This is consistent with the fact that hypothyroidism is linked to a lower metabolic rate , higher BMI, and greater prevalence of obesity, suggesting that subclinical hypothyroidism is associated with significant changes in body weight and is considered a risk factor for overweight and obesity [27]

The data were also subjected to ROC curve analysis to assess the validity and effectiveness of these diagnostic criteria for detecting Hashimoto's thyroiditis in the Thi-Qar. ROC analysis confirmed the validity of these markers for detecting Hashimoto's thyroiditis.

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The results indicated that autoantibodies to thyroperoxidase (anti-TPO) and TSH have the most sensitive and specific values, making them the best biomarkers to detect Hashimoto's disease, in line with Ameen, et al. [28]. The high levels of TSH and anti-TPO in patients compared to controls and the ROC curve result confirmed the validity of these parameters as exceptional biomarkers for the detection of Hashimoto's disease, which is usually not detected until advanced stages are associated with significant thyroid damage. From the other hand, the ROC analysis result for vitamin D showed that the sensitivity and specificity are not high enough to use this marker as a standalone diagnostic test this finding consistent with Iqbal and Samanje [29]. Instead, it is recommended to use it as a supportive tool alongside other clinical or laboratory indicators.

5. Conclusions

This study elucidates that vitamin D deficiency in patients aligns with emerging evidence linking hypovitaminosis D to thyroid autoimmunity, potentially exacerbating immune dysregulation through impaired T cell modulation. While the weak negative correlations between vitamin D and TSH/anti-TPO were not statistically significant, they underscore a plausible pathophysiological interplay, warranting further exploration. Additionally, the association between higher BMI and hypothyroidism highlights the metabolic implications of thyroid dysfunction. Finally, understanding how vitamin D insufficiency affects all the parameters associated with Hashimoto's disease opens the way for new therapeutic strategies, including the use of vitamin D supplements as part of the management of this disease.

Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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