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Vitamin D level in Hashimoto thyroiditis: A mere coincidence or a real relationship and the effect of Vitamin D supplement on Hashimoto thyroiditis associated antibodies

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Abstract

With a prevalence of approximately 10–12% and an increasing incidence, Hashimoto's thyroiditis (HT) is one of the thyroid illnesses and is thought to be the most common thyroid condition globally**.** Nonetheless, there is debate over vitamin D, and it is unclear whether taking supplements of the vitamin is associated with a decrease in thyroid autoantibodies and an improvement in thyroid function. To assess the relationship between vitamin D level and HT and the impact of vitamin D supplementation on thyroid antibody titers (anti-TPO, anti-Tg, and TRAb) in patients with HT and vitD deficiency or insufficiency. our study included 30 patients with Hashimoto thyroiditis and 30 healthy controls. The two groups were matched for age and gender. All subjects (n = 60) were evaluated for thyroid hormones, thyroid autoantibody levels, fasting lipid profile, and 25OHD level. After assessment, patients with HT and Vitamin D deficiency or insufficiency (n=22) received 50,000 IU of cholecalciferol once a week for an initial 8 weeks, followed by once a month for the next 4 months. After 6 months of giving vitamin D, reassessment was done. Vitamin D deficiency was more common among patients with Hashimoto compared to control (P<0.001). Vitamin D levels in Hashimoto patients were negatively correlated with duration of disease (rho=-0.588, P<0.001), anti-TPO (rho=-0.727, P<0.001) and anti-Tg (rho=-0.515, P0.004). Multivariate regression analysis showed that anti-TPO and vitamin D deficiency were significant predictors of Hashimoto. After supplemental vitamin D in patients with vitamin D deficiency, vitamin D, free T3, Free T4 were significantly increased while TSH, anti-TPO and anti-TG were significantly decreased. Vitamin-D levels were low in Hashimoto patients, its supplementation decreased thyroid antibodies titers, and there was significant reduction in serum TSH and increased in free T3 and ft4. We can propose that one of the possible contributing elements to the pathophysiology of Hashimoto disease is a vitamin D deficiency.

Keywords: vitamin D, Hashimoto thyroiditis, TRAB, anti-TPO, and anti-Tg.

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1. Introduction

Among autoimmune thyroid diseases, HT is the most common. About 20–30% of patients develop hypothyroidism as a result, which results in a chronic inflammation of the thyroid tissue. HT, also known as chronic lymphocytic or autoimmune thyroiditis, is an autoimmune thyroid condition characterized by an enlarged thyroid, parenchymal lymphocyte infiltration, and the presence of antibodies directed against thyroid antigens. HT is currently the most common cause of hypothyroidism [1]. Also, HT patients are more susceptible to malignant neoplasms and cardiovascular problems [2]. In the past 30 years, the incidence of HT has quickly increased. HT is currently one of the most prevalent thyroid illnesses with an incidence of 0.3 to 1.5 cases per 1000 people. Around 2% of women have clinical symptoms and more than 10% of women have positive antibodies. One-tenth of this frequency is males. While HT is uncommon in Pacific Islanders, it is more common in the white race than the black one. Disease incidence increases with age [3]. Vitamin D, usually described as a fat-soluble vitamin, is a proven steroid hormone with its key role in calcium and phosphorus homeostasis and the regulation of bone metabolism. Two substances are referred to as "vitamin D": ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3) [4]. According to multiple studies, the physiologically active form of vitamin D exerts potent immunomodulatory effects

on both the innate and adaptive immune systems. By triggering VDR, which reduces CD4+, Th1, Th2, and Th17 cell overactivity and cytokine production, vitamin D inhibits pro-inflammatory pathways [5]. Most of the data are consistent with a connection between vitamin D and HT, despite considerable differences between the research's conclusions thus far. There are other ways, nevertheless, to interpret this connection. The most widely reported theory is that 1, 25(OH)2D deficiency patients diminished 1,25(OH)2D immunomodulatory role related to the development of AID. However, most of the information gathered thus far is from cross-sectional research, which makes it impossible to identify causal correlations [6].

2. Materials and Methods

The study took place at Mansoura Specialized Medical Hospital. Endocrinology Outpatient Clinic of Mansoura University. Over period of 22 month from October 2021 to august 2023. The first part of the research was a case control study that included 30 patients with Hashimoto thyroiditis and 30 healthy controls. The 2 groups were matched for age and sex. The second part of the research was a prospective study (descriptive longitudinal) that included patients with HT and vitamin D deficiency or insufficiency. An informed consent was taken from all subjects in this study. All participants were aged between 18 and 65. exclusion criteria included patients with severe cardiovascular, liver, and renal disease, a history of other autoimmune disease, thyroidectomy, fat malabsorption syndromes, nephrotic syndrome, lymphomas, parathyroid disorders, patients who took calcium supplements and medications that may interfere with serum levels of 25 (OH) vitamin D (e.g., antiepileptic, steroids, methotrexate, isoniazid, thiazides, antacids, calcium channel blockers, and anticonvulsants). All patients were provided a thorough medical history including age, gender, duration of Hashimoto disease and medical treatment. They were subjected to complete clinical examination and anthropometric measurements including weight, height, BMI. Patients and controls were assessed for thyroid function (TSH, Free T4 and Free T3), Serum anti-thyroid peroxidase antibodies, anti-thyroglobulin antibodies, serum Thyrotropin receptor antibody (TRAb), and 25 hydroxyvitamin D (25(OH) D). After assessment, patients with HT and Vitamin D deficiency or insufficiency received 50,000 IU of cholecalciferol once a week for an initial 8 weeks followed by once a month for the next 4 months. After 6 months of giving vitamin D, reassessment was done.

2.1. Statistical analysis

The collected data were coded, processed, and analyzed using the Statistical Package for Social Science (SPSS) version 29 for Windows on personal computers. Qualitative data were described as percentages and numbers, while quantitative data were described as means [± standard deviation (SD)] for parametric variables or medians (interquartile range; IQR), for nonparametric variables, as suitable. To assess the normality of distribution of variables, Kolmogorov–Smirnov test was used. For comparing between two groups, t-test was used for normally distributed variables, while Mann Whitney test was used for nonnormally distributed variables. Chi-square test was used for comparing between qualitative variables. Spearman's rank correlation was used for correlation of multiple variables with vitamin D levels. Binary univariate and multivariate logistic regression analysis was used to recognize significant associates of Hashimoto. Wilcoxon test was used to compare variables, before and after supplementation of vitamin D. The level of significance was considered at 5% ($P \leq 0.05$).

2.2. Ethics

Informed consent was obtained from each participant.

3. Results and discussion

As shown in table 1 Significant increase in BMI, WC, SBP in Hashimoto patients than control group (p values<0.001). DBP was significantly higher in Hashimoto group than control group (p= 0.002). As shown in Table (2) TSH was significantly higher in Hashimoto group than control group (p <0.001). Free T3 and free T4 were significantly lower in Hashimoto group than control group. (p<0.001). Anti TPO titers and Anti Tg titers were significantly higher in Hashimoto patients than control group(p<0.001). Patients with vitamin D deficiency were significantly more in Hashimoto group than Control group(p<0.001). TG, total cholesterol, and LDL were significantly higher in Hashimoto than control group. (p<0.001). No significant difference between the groups as regard HDL (P=0.471). In table 3 Patients with VDD had significantly longer duration of disease than other group (P<0.001). TSH, anti-TPO, anti-Tg, and total cholesterol were significantly higher in VDD than other group (p=0.035, p<0.001,p<0.001,p=0.006, respectively). No significant difference as regard age, gender, marital status, residency, smoking, BMI, WC, SBP, DBP, dose of Eltroxin, FT3, FT4, TRAB, triglyceride, LDL, HDL between vitD and non vitD deficiency patients (p value 0.901, 1.00, 1.00, 0.682, 1.00, 0.548, 0.909, 0.597, 0.872, 0.629, 0.565,0.836,0.475,0.801,0.344,0.730) respectively. In table 4 the patients with HAHSIMOTO disease, there was negative correlation between vit D blood level and duration of disease (R=-0.588, p = <0.001). There was negative correlation between vit D blood level and Anti-TPO, and Anti-Tg titers (R=-0.727, p <0.001; R=-0.515, P=0.004) respectively. No significant correlation between vit D level and other variables as age, gender, residency, BMI, WC, SBP, DBP, duration of Hashimoto disease, dose of Eltroxin, TSH,FT3,FT4, TRAB, triglyceride, total cholesterol, LDL, HDL (R=-0.060, P=0.753; R=0.101, p=0.595; R=0.106, P=0.576; R=-0.048, P=0.803; R=-0.086, P=0.651; R=-0.137, P=0.469; R=-0.167, P=0.377; R=-0.035, P=0.852; R=-0.264, P=0.158;R=-0.293, P=0.116; R=0.045, P=0.813, R=-0.079, P=0.678; R=0.096, P=0.612; R=-0.334, P=0.071; R=0.085, P=0.657;R=-0.073, P=0.700) respectively. In table 5 the univariate regression analysis, Hashimoto patients were positively correlated with BMI (OR=1.334, P<0.001), WC (OR=1.086, P<0.001), SBP (OR=1.129, P=0.001), DBP (OR=1.119, P=0.005),. Anti-TPO(OR=1.286, P=0.022), anti-TG (OR=1.087, P<0.001), triglycerides (OR=1.054, P<0.001), total cholesterol (OR=1.034, P=<0.001), LDL (OR=1.084, P=<0.001),and

vitamin D deficiency (OR=13.750, P<0.001). Multivariate analysis shows that Anti-TPO (OR=1.51, P=0.04). and vitamin D deficiency (OR=10.4, P=0.004). are statistically significant predictors of Hashimoto. As shown in table 6 Data are expressed by median(IQR) + P value was computed by Wilcoxon signed-rank test. Significant increase in vitamin D level, FT3, and FT4 after vitamin D supplementation (p value <0.001,=0.008, <0.001) respectively. Significant decrease in TSH, Anti-TPO, and Anti-Tg after vitamin D supplementation (P value <0.001, <0.001, <0.001) respectively. No significant change in TRAB (P value=0.306). As shown in table 7 there was a decrease in patients with vitamin D deficiency after vitamin D supplementation. This study included 30 patients with HT with mean age27.63±6.23. 22.(80%) were females in addition to 30 age and gender matched subjects (control group). The current study demonstrated that, Patients with vitamin D deficiency were significantly more in Hashimoto group than Control group. These outcomes were comparable to those of a meta-analysis involving 25 research with 2695 cases and 2263 controls revealed that Vitamin D levels were lower in HD patients than in the control groups, however there was a considerable amount of heterogeneity across the studies (Cohen's D = 0.62; 95% CI 0.89–0.34; p = 1.5 x 10−5) [7]. Those results were in agreement with meta-analysis conducted by Taheriniya and his colleagues who reported that Lower vitamin D levels were correlated with AITDS (p = 0.013), hypothyroidism (p = 0.03), and Hashimoto's thyroiditis (p < 0.001) after reviewing 42 data base met their inclusion criteria [8]. Similar findings were reported in Wang et al.'s meta-analyses [9]. Chao and his colleagues have reported that The HT group's vitamin D3 concentration was significant lower than that of the non-HT group (p = 0.014) [10]. On the other hand, Hanna et al., Cvek et al., reported that no significant differences in vitamin D levels between HT patients and controls [11], [4]. D deficient patients have a decreased immunomodulatory role, which contributes to the development of AITD. Nevertheless, the majority of the data collected thus far come from cross-sectional research, which makes it impossible to establish causal relationships. Thus, it is critical to assess alternate explanatory hypotheses. Some authors have suggested that the evidence supporting VitD's role in HT may really be a result of the disease rather than its cause. Due to malabsorption, decreased sunshine exposure, incapacitation, and corticosteroid treatment [12]. In addition, the hypothyroidism-induced increase in fat mass in HT may be a contributing factor to the deficiency [13], [6]. The use of several assays for measuring serum 25(OH)D levels, heterogeneity of study group, various definition for declaring vitamin D deficiency and the confounding effects of gender, age, obesity, sun exposure, dietary habits, and smoking are factors that could explain the considerable variability between the studies. The waist circumference (WC), body mass index (BMI), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were statistically significant higher in Hashimoto patients than control group (p values <0.001. <0.001, <0.001, = 0.002) respectively. This was in agreement with (He et al., 2021) who have demonstrate that, BMI, waist circumference, SBP and DBP in the subclinical and overt hypothyroidism groups were significantly higher than the euthyroid group in women (P ＜ 0.01) [14]. Additionally, Yan et al. discovered that when thyroid autoantibodies were present, obesity was linked to subclinical hypothyroidism [15]. Thyroid hormones (THs) mostly regulate basal metabolism, which accounts for roughly two-thirds of daily energy expenditure. Furthermore, THs works with a mediator known as sterol regulatory element-binding proteins to regulate the metabolism of glucose and lipids [16]. On studying the effect of HT on lipid metabolism, we observed that the level of TG, total cholesterol, and LDL were significantly higher in Hashimoto than control group (p< <0.001). Such outcomes were consistent with research conducted by Ejaz et al. that examined the lipid profiles of people with and without subclinical hypothyroidism (SCH) and found that those with SCH had significant higher levels of TC and LDL than those without SCH [15]. dyslipidemia in hypothyroidism (either subclinical or overt hypothyroidism) is mainly due to a reversible reduction in the low-density lipoprotein (LDL) receptor number and activity [17]. Also a diminishing control by tri- iodothyronine (T3) of sterol regulatory element-binding protein 2 (SREBP-2), which modulates cholesterol biosynthesis, and a role of 3,5-diiodothyronine (T2), a natural thyroid hormone derivative, may also be involved in lipid catabolism and lipogenesis, via a different pathway than that of T3 [18]. The current study demonstrated that Hashimoto patients with vitamin D deficiency had longer duration of disease, higher level of TSH, anti-TPO, anti_Tg, and total cholesterol than non VDD group (P<0.001, p=0.035, p<0.001, p=0.006, respectively). This came in accordance with Unal and his colleagues who have demonstrated that anti-Tg and anti-TPO levels were significantly high in the vitamin D deficient(n = 183)group when compared with sufficient (n = 98) HASHIMOTO patients (p = 0.02 and p = 0.003 respectively) [19]. Significant negative correlation between vitamin D level and AntiTPO, and Anti-Tg titers in Hashimoto patients (R=-0.727, p <0.001; R=-0.515, P=0.004, respectively) were reported in the current study. Those results were in agreement with Sulejmanovic and his collagues, Aktas and his colleagues, and Hosny and his collagues who documented that Vitamin D levels and thyroid antibodies(anti-Tg and anti-TPO) had a statistically significant negative connection [20-22]. Our results are concordant with Feng et al. his study found significant positive correlation between vitamin D deficiency and anti-TPO and anti-Tg in 36 patients with Hashimoto disease(odds ratio:2.428, 95% confidence interval: 1.383–4.261) [23]. On the other hand, Sönmenzgöz and his colleagues discovered no relationship between anti-TPO levels and vitamin D levels [24]. Two population-based investigations conducted in Thailand [25] and China [26] likewise found no link between the levels of 25(OH)D, anti-Tg, and anti-TPO. In the univariate regression analysis in our research, HT patients were positively correlated with BMI, WC, SBP, DBP, Anti TPO, anti Tg, total cholesterol, LDL, and vitamin D deficiency but multivariate analysis shows that Anti TPO (OR=1.51, p=0.04), and vitamin D deficiency (OR=10.4, P=0.004) are statistically significant predictors. These results were in agreement with Chao and his colleagues who found that HT patients were positively correlated with BMI, WC, SBP, DBP, and 25(OH)D in univariate regression .On the other side they found that vitamin D was not independently correlated with increased prevalence of HT in multivariate regression [10].

Table 1. Sociodemographic, medical, and therapeutic data among studies groups

	Hashimoto	**Control**	**P value**
Age	27.63±6.23	29.03±8.74	0.478
Gender: **Male** **Female**	 6(20%) 24(80%)	 9(30%) 21(70%)	0.371
Residency: **Rural** **Urban**	 20(66.7%) 10(33.3%)	 21(70%) 9(30%)	0.781
Smoking status: **Smoker** **Non-smoker**	 1(3.3) 29(96.7%)	 2(6.7%) 28(93.3%)	0.554
BMI	31.38±4.12	25.57±5.02	**<0.001**
WC	101(88.75-107)	79(52.75-88.50)	**<0.001**
SBP	130(120-130)	120(110-120)	**<0.001**
DBP	80(80-90)	80(70-80)	**0.002**
Duration of disease	2(0.76-3)		
Dose of thyroxin	100(100-150)		

BMI- body mass index, wc -waist circumferences, SBP-systolic blood pressure, DBP- diastolic blood pressure
The data are expressed as mean±SD, median (IQR), or n(%), as suitable
* P value was computed by One-way ANOVA
** P value was computed by Kruskall-Wallis test
*** P value was computed by Chi-squared test.
# P value was computed by Mann-Whitney test

Table 2. Basal laboratory data among studies groups

	Hashimoto	Control	P value
TSH	6.00(4.00-7.425)	2.30(1.75-3.13)	**<0.001**
Free T3	2(1.43-2.50)	2.75(1.90-3.00)	**<0.001**
Free T4	1.00(0.84-1.12)	1.80(1.49-1.93)	**<0.001**
Anti-TPO	95(57.50-137.06)	8.44(5.92-13.43)	**<0.001**
Anti-TG	90(57.5-129.50)	12(8.90-25.68)	**<0.001**
Vitamin D	24.8(17.68-37.98)	42.45(34.25-58.5)	**<0.001**
Vitamin D deficiency	22(73.3%)	5(16.7%)	**<0.001**
Triglycerides	185(121.5-230.5)	105(100-120)	**<0.001**
Total cholesterol	205.5(172.5-232.5)	130(120-153.5)	**<0.001**
LDL	100(90-122.5)	80(78-86.5)	**<0.001**
HDL	44.50(35-54)	46(40.75-50)	0.471

TSH- thyroid stimulating hormone, TRAB-Thyroid Stimulating Hormone Receptor Antibody, Anti-TPO-anti–thyroid peroxidase antibody, Anti-Tg- Antithyroglobulin antibody. 25(OH)Vit- D – 25 hydroxy vitamin D
The data are expressed as median (IQR), or n(%), as suitable
** P value was computed by Kruskall-Wallis test
*** P value was computed by Chi-squared test.
# P value was computed by Mann-Whitney test

Table 3. Comparison of parameters among Hashimoto disease subjects divided as per vitamin D deficiency.

	Non-VDD group (n=8)	VDD group (n=22)	P value
Age	27.88±6.17	27.55±6.39	0.901
Gender: **Male** **Female**	 2(25%) 6(75%)	 4(18.2%) 18(81.8%)	1.000
Marital status: **Single** **Married**	 0 17(100%)	 0 13(100%)	1.000
Residency: **Rural** **Urban**	 6(75%) 2(25%)	 14(63.6%) 8(36.4%)	0.682
Smoking status: **Smoker** **Non-smoker**	 0 8(100%)	 1(4.5%) 21(95.5%)	1.00
BMI	30.62±3.42	31.66±4.39	0.548
WC	102(97.75-103.75)	100(88-108)	0.909
SBP	130(120-137.5)	130(120-130)	0.597
DBP	80(80-90)	80(80-90)	0.872
Duration of disease	0.03(0.01-0.045)	2.75(1.88-3.25)	**<0.001**
Dose of Eltroxin	100(100-150)	100(100-150)	0.629
TSH	5.00(4.00-5.60)	6.91(4.00-8.00)	**0.035**
Free T3	1.85(1.20-2.38)	2.00(1.43-2.58)	0.565
Free T4	0.85(0.58-1.12)	0.95(0.84-1.22)	0.836
TRAB	1.10(1.08-2.00)	1.55(0.80-2.05)	0.475
Anti-TPO	47(40-50)	105(84-152)	**<0.001**
Anti-TG	44.10(33.52-60)	120(88.5-142.63)	**<0.001**
Triglycerides	189.5(120-243.5)	185(121.5-212.5)	0.801
Total cholesterol	155(130-210)	220(188.75-250)	**0.006**
LDL	100(92.5-115)	105(87.5-130)	0.344
HDL	42(36.25-48.75)	45(35-60)	0.730

BMI- body mass index, wc -waist circumferences, SBP-systolic blood pressure, DBP- diastolic blood pressure, BMI- body mass index, wc -waist circumferences, SBP-systolic blood pressure, DBP- diastolic blood pressure, TSH- thyroid stimulating hormone, TRAB-*Thyroid Stimulating Hormone Receptor* Antibody, Anti-TPO-anti–thyroid peroxidase antibody, Anti-Tg-*Antithyroglobulin antibody.* 25(OH)Vit- D – 25 hydroxy vitamin D

Table 4. Correlations of vitamin D levels with different variables of patients with Hashimoto

Age	**Rho**	-0.060
	P value	0.753
Gender	**Rho**	0.101
	P value	0.595
Residency	**Rho**	0.106
	P value	0.576
BMI	**Rho**	-0.048
	P value	0.803
WC	**Rho**	-0.086
	P value	0.651
SBP	**Rho**	-0.137
	P value	0.469
DBP	**Rho**	-0.167
	P value	0.377
Duration of disease	**Rho**	-0.588
	P value	**<0.001**
Dose of Eltroxin	**Rho**	-0.035
	P value	0.852
TSH	**Rho**	-0.264
	P value	0.158
Free T3	**Rho**	-0.293
	P value	0.116
Free T4	**Rho**	0.045
	P value	0.813
TRAB	**Rho**	-0.079
	P value	0.678
Anti-TPO	**Rho**	-0.727
	P value	**<0.001**
Anti-TG	**Rho**	-0.515
	P value	**0.004**
Triglycerides	**Rho**	0.096
	P value	0.612
Total cholesterol	**Rho**	-0.334
	P value	0.071
LDL	**Rho**	0.085
	P value	0.657
HDL	**Rho**	-0.073
	P value	0.700

BMI- body mass index, wc -waist circumferences, SBP-systolic blood pressure, DBP- diastolic blood pressure, BMI- body mass index, wc -waist circumferences, SBP-systolic blood pressure, DBP- diastolic blood pressure, BMI- body mass index, wc -waist circumferences, SBP-systolic blood pressure, DBP- diastolic blood pressure, TSH- thyroid stimulating hormone, TRAB-*Thyroid Stimulating Hormone Receptor* Antibody, Anti-TPO-anti–thyroid peroxidase antibody, Anti-Tg- *Antithyroglobulin antibody.*

Table 5. Univariate and multivariate regression analysis of Hashimoto

	Univariate analysis				Multivariate analysis			
	B	**OR**	**P value**	**CI**	**B**	**OR**	**P value**	**CI**
Age	-0.025	0.975	0.473	0.910-1.045				
Male gender	0.539	1.714	0.374	0.523-5.621				
Urban	0.154	1.167	0.781	0.393-3.467				
Smoking	-0.728	0.483	0.561	0.041-5.628				
BMI	0.288	1.334	**<0.001**	1.139-1.563				
WC	0.082	1.086	**<0.001**	1.037-1.137	-0.276	0.759	0.263	0.468-1.231
SBP	0.122	1.129	**0.001**	1.049-1.216				
DBP	0.113	1.119	**0.005**	1.035-1.211				
TRAB	1.614	5.023	0.992	1.598-15.789				
Anti-TPO	0.251	1.286	**0.022**	1.037-1.594	1.078	1.51	**0.04**	1.06-1.98
Anti-Tg	0.084	1.087	**<0.001**	1.039-1.138	0.241	1.273	0.427	0.702-2.307
Triglycerides	0.053	1.054	**<0.001**	1.022-1.087	0.305	1.356	0.311	0.752-2.444
Total cholesterol	0.034	1.034	**<0.001**	1.017-1.052				
LDL	0.081	1.084	**<0.001**	1.034-1.137	-0.433	0.649	0.391	0.241-1.743
HDL	0.005	1.005	0.838	0.961-1.051				
Vitamin D deficiency	2.621	13.750	**<0.001**	3.917-48.266	5.69	10.4	**0.004**	6.74-30.58

BMI- body mass index, wc -waist circumferences, SBP-systolic blood pressure, DBP- diastolic blood pressure, BMI- body mass index, wc -waist circumferences, SBP-systolic blood pressure, DBP- diastolic blood pressure, BMI- body mass index, wc -waist circumferences, SBP-systolic blood pressure, DBP- diastolic blood pressure, TSH- thyroid stimulating hormone, TRAB-Thyroid Stimulating Hormone Receptor Antibody, Anti-TPO-anti–thyroid peroxidase antibody, Anti-Tg- Antithyroglobulin antibody.

Table 6. Depicts the change in values of vitamin D, thyroid hormones, TRAB, Anti TPOab, Anti Tgab. levels before and after 6 months of VITD administration among patients with Hashimoto disease.

	Pre	Post	P value
Vitamin D	19.20(13.08-26.43)	45(31-56.75)	**<0.001**
TSH	6.91(4.00-8.00)	3.25(2.45-4.05)	**<0.001**
Free T3	2(1.43-2.57)	2.2(1.9-3)	**0.008**
Free T4	0.95(0.84-1.22)	1.60(1.20-1.85)	**<0.001**
TRAB	1.55(0.80-2.05)	1.05(0.97-2.05)	0.306
Anti-TPO	105(84.50-152.5)	81.63(55-98)	**<0.001**
Anti-Tg	120(88.50-143.63)	87.50(62.60-110.98)	**<0.001**

N=22 ///25(OH)Vit- D – 25 hydroxy vitamin D, TSH- thyroid stimulating hormone, TRAB-Thyroid Stimulating Hormone Receptor Antibody, Anti-TPO-anti–thyroid peroxidase antibody, Anti-Tg- Antithyroglobulin antibody.

Table 7. Status of vitamin D in Hashimoto patients and baseline and 6 months after vitamin D supplementation

	Pre	Post
Normal	8(26.7%)	28(93.3%)
Mild vitamin D deficiency	9(30%)	2(6.7%)
Moderate vitamin D deficiency	8(26.7%)	0
Sever vitamin D deficiency	5(16.7%)	0

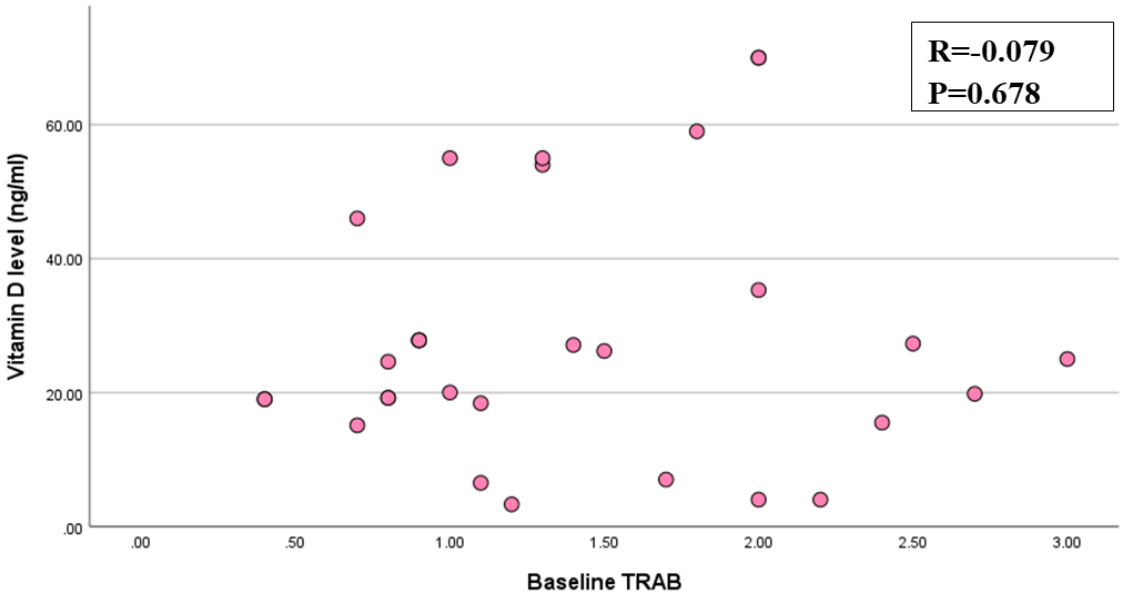

Figure 1. Scatter plot of correlation between vitamin D levels and baseline TRAB in Hashimoto group

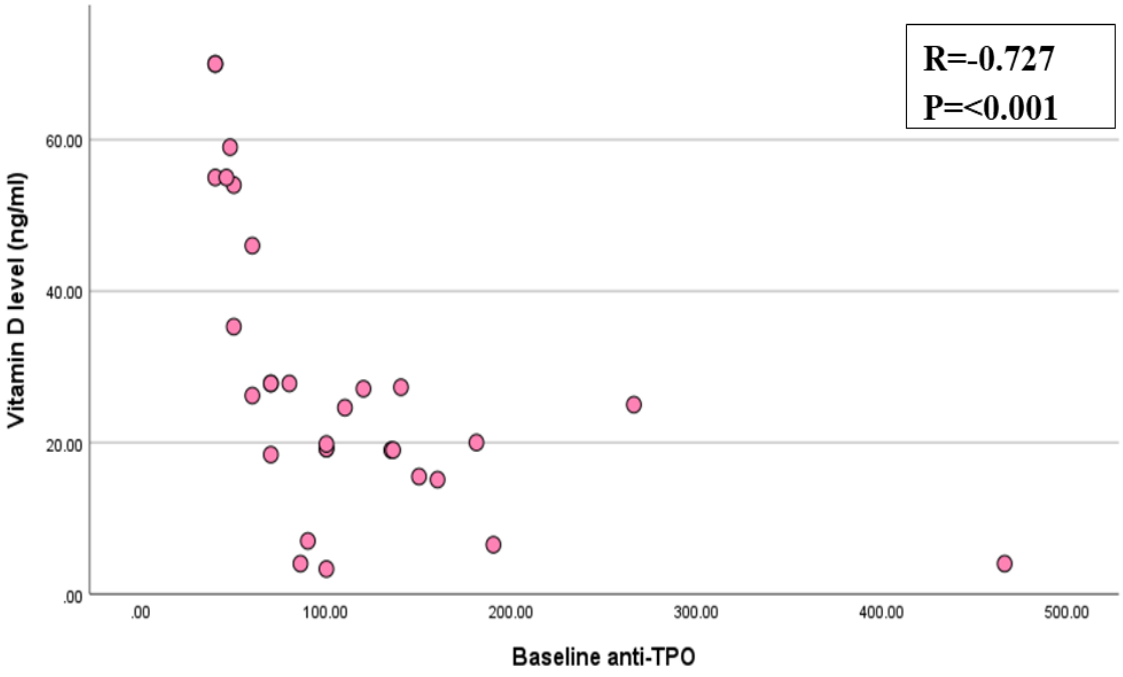

Figure 2. Scatter plot of correlation between vitamin D levels and baseline anti-TPO in Hashimoto group

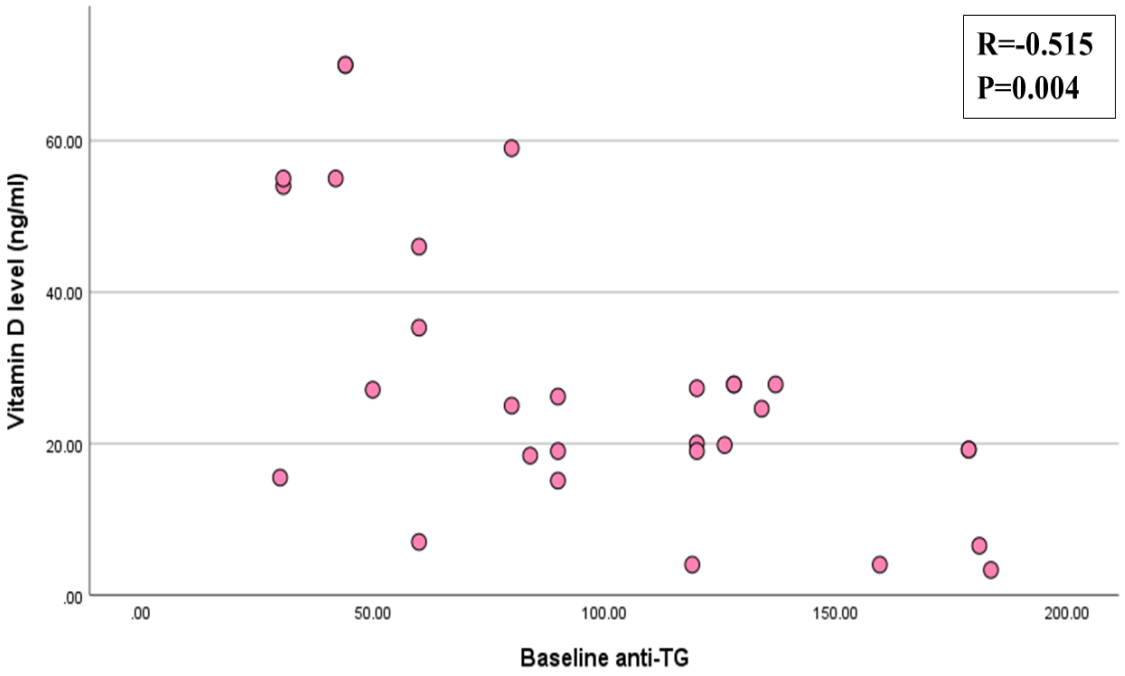

Figure 3. Scatter plot of correlation between vitamin D levels and baseline anti-Tg in Hashimoto group

After taking vitamin D supplements for six months, we discovered that Hashimoto patients with vitamin D deficiency had Significant decrease in Anti-TPO, and Anti-Tg titers over the study period (P value <0.001. Also, TRAB titers decreased over the study period however the difference wasn't statistically significant (p =0.306). Those results were in accordance with Koehler and his colleagues who examined retrospectively 933 patients with autoimmune thyroiditis and discovered that a subgroup of 58 individuals who saw an improvement in their originally low vitamin D level had a higher decrease in anti-TPO levels than a control group that kept its Vitamin D level below the cutoff. However, there was no statistically significant difference between the groups [27]. Parallel to this, 34 women with HT and normal vitamin D status who had taken levothyroxine for at least six months were split into two groups according to their preferences in a case-control study. While 18 of them received vitamin D supplements with a daily dose of 2000 IU for a period of six months, the other 16 were not administered vitamin D. Only those with sub-clinical hypothyroidism showed statistically significant decreases in antibody titers (mostly anti-TPO) in response to increases in 25 (OH)D after six months, and this effect depended on baseline antibody titers [28]. Also, 42 women with HT illness (aged 18–48) participated in a randomized, double-blind clinical trial in which participants were randomly assigned to one of two groups: the vitamin D group or the placebo group. Patients in the vitamin D and placebo groups took 50000 IU of vitamin D and placebo pearls for three months, respectively. The vitamin D group showed a significant decrease in anti-Tg antibody and TSH hormone when compared to the placebo group (p=0.08) [29]. contrary, Knutsen and his colleagues informed that Low dose vitamin D supplementation didn't significantly improve anti-TPO titers in a randomized, double-blind research by Knutsen et al. that included participants of African, Middle Eastern, and South Asian ethnicities with baseline vitamin D deficiency [30]. Four possible mechanisms that Vitamin D may contribute to the suppression of the immunological process in HT were described by Rui et al. : (1) blocking T-cell activation that is dependent on DCs; (2) Th17/Tregs ratio restoration; (3) impact on B-cells and plasma cell; and (4) thyroid down-regulation of HLA class II gene expression [31]. In the current study, Hashimoto patients exhibited significant decrease in TSH (P<0.001) and significant increase in FT3, FT4 after6 months of vitamin D administration (p=0.008, p<0.001 respectively). These outcomes were consistent with the findings of Pezeshki and his colleagues, who found that vitamin D supplementation significantly reduced TSH levels in 59 patients who had been diagnosed with subclinical hypothyroidism and vitamin D deficiency (P-value<0.001) [32]. Also, Krysiak and his colleagues, Chahardoli and his colleagues, reported significant reduction in TSH level after vitamin D supplementation to patients with Hashimoto thyroiditis but there were no changes in FT3 and FT4 level [29], [33-34]. On the other hand, a meta-analysis conducted by Mirhosseini and his colleagues reported that no change in

TSH, FT3,and FT4 after vitamin D administration for 258 patients with Hashimoto thyroiditis [35]. In addition, Chaudhary and his colleagues, Anaraki and his colleagues , and Nodehi and his colleagues reported no significant changes in thyroid hormone profile after vitamin D intake by patients with Hashimoto thyroiditis [36-38]. Better patient education about the disease and appropriate treatment (dosage and timing) as well as improved patient complaints regarding treatment due to weekly follow-ups instead of monthly ones may be the cause of the improvement in thyroid hormone profile in Hashimoto patients, independent of vitamin D administration. Experimental studies show that vitamin D directly affects Dio2, the enzyme needed in target tissues to convert T4 into T3. Vitamin D3 administration raises the expression levels of Dio2 in the brain and liver of diabetic rats, which in turn raises fT3 and lowers fT4 levels [39] a study employing rat pituitary cells, calcitriol administration was shown to increase TRH-induced TSH secretion [40]. In vitro studies revealed that calcitriol therapy reduced TSH-stimulated adenylyl cyclase activity and iodine uptake [41]. These results imply that vitamin D may have both central and peripheral effects on the release of thyroid hormone and TSH. However, more study that is experimental is needed to clarify the underlying mechanisms [42].

4. Conclusions

In this study, we found that patients with autoimmune thyroid disease present with lower vitamin D levels and Hashimoto patients have higher prevalence, an inverse correlation between vitamin D levels and thyroid antibody levels, and the level of autoantibodies were decreased after Vitamin D supplementation. It is therefore advised that the patients' serum vitamin D level be monitored on a regular basis. In the case that hypovitaminosis is detected, the patient should receive the proper therapy and preventive measure with vitamin D to prevent future VD deficiencies.

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