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Vitamin D Level in Graves' Disease and Effect of Vitamin D Supplements on Associated Autoimmunity

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ABSTRACT

Background: Graves' disease (GD) is the most frequent cause of hyperthyroidism. There is debate about the role of vitamin D deficiency (VDD) in thyroid autoimmunity and the effect of vitamin D (VD) administration on thyroid autoimmunity.

Objective: This work aimed to study if vitamin D insufficiency (VDI) in patients with GD was linked to elevated thyroid autoantibody titer levels and how vitamin D supplementation affects Thyrotropin receptor antibody (TRAb), anti-thyroid peroxidase antibody (TPO Ab), and Anti-thyroglobulin antibody (anti Tg) titers in patients who have both VDI or VDD and GD.

Patients and Methods: A total of 30 patients with GD and 30 matched control individuals were involved in our study. Serum from these patients and controls was tested for vitamin D (25[OH]D) and thyroid profile. Following evaluation, patients (n=16) with GD and insufficient or deficient vitamin D were given cholecalciferol. An evaluation was conducted six months after the vitamin D treatment.

Results: Cases with GD were associated with a higher prevalence of VDI than the control group (P<0.001). VD values in Graves cases revealed a negative association with both TRAb (P<0.001) and age (P<0.001). The results of multivariate regression analysis indicated that the most significant predictors of Graves were TRAb (P=0.002), anti-TPO (P=0.006) and VDD (P=0.013).

Conclusion: Low VD level is linked to elevated TRAb titers in GD, and the level of thyroid autoantibodies were decreased after Vitamin D administration, pointing to a potential connection between elevated thyroid autoimmunity in GD patients and their vitamin D levels.

Keywords: Vitamin D deficiency, Graves' disease, TRAb.

INTRODUCTION

The most common etiology of hyperthyroidism is GD. It is an autoimmune disease (AID) that affects only certain organs and causes excess thyroid hormone production [(1)]. Antibodies against thyroid hormone receptors are frequently seen in GD patients [(2)]. Clinical symptoms are linked to both the autoimmune and hyperthyroidism processes. Several different physiological systems are impacted by excess thyroid hormones. Thus, warning indications including the severity of GD symptoms can vary widely and have a big impact on general health [(3)].

Fat-soluble vitamin D is important for maintaining Ca^{+2} homeostasis and bone metabolism. The nuclear vitamin D receptor (VDR), found in most tissues and cells, including immune cells, attaches to the active form of vitamin D and controls the expression of approximately 200 genes comprised in cell division, proliferation, and death. Numerous studies on vitamin D's non-skeletal effects have been conducted in recent years, and evidence supports a connection between VD insufficiency (VDI) and a number of illnesses, including cancer, metabolic syndromes, and autoimmune diseases [(4)].

According to multiple studies, the physiologically active form of vitamin D exerts strong immunomodulatory actions on the immunity. By triggering VDR, which reduces CD4+, Th1, Th2, and Th17 cell over activity and cytokine formation, vitamin D inhibits pro-inflammatory pathways [(5)].

Vitamin D deficiency (VDD) is considered as a significant problem for public health globally. There is growing evidence relating low vitamin D values to increased risks of several non-skeletal illnesses, such as autoimmune, heart disease, cancer, and infections. However, the fundamental mechanisms are still unknown [(5)].

The majority of the literatures reported a link between VDD and a higher propensity for development and/or greater titers of antibodies related to GD. However, there have been several studies that contradict such links, making it difficult to come to a consensus [(6)]. The efficacy of VD intake in VDD Graves cases is debatable. Certain researches have recorded that vitamin D supplementation decreases thyroid autoantibody titers, while others have shown no effect [(7)].

Information about the connection between GD and vitamin D is less abundant. **Misharin *et al.*** [(8)] used immunization of two BALB/c and C57BL/6 mouse strains fed either a vitamin D-sufficient or -depleted diet to study the response to TRABs induction. Compared to C57BL/6 strains, BALB/c strains showed a decreased capability for conversion of 25(OH)D to 1,25(OH)2D and a greater susceptibility to disease induction. Depending on the diet given, the immunological response of BALB/c mice varied marginally, according to the authors' findings, the primary difference was the increased risk of chronic hyperthyroidism [(8)].

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This study aimed to check if vitamin D insufficiency (VDI) in patients with GD was linked to elevated thyroid autoantibody titer levels and how vitamin D supplementation affects thyrotropin receptor antibody (TRAb), anti-thyroid peroxidase antibody (TPO Ab), and anti-thyroglobulin antibody (anti Tg) titers in patients who have both VDI or VDD and GD.

SUBJECTS AND METHODS

The study took place at Mansoura Specialized Medical Hospital, Endocrinology Outpatient Clinic, 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 comprised 30 patients with GD and 30 controls. The two groups were matched for age and sex. The second part of the research was a prospective study that included patients with Graves' disease and VDI or VDD.

Inclusion criteria: All participants were aged between 18 and 65.

Exclusion criteria: comprised patients with severe cardiovascular, liver & kidney disease, fat malabsorption syndromes, a history of other AIDs, thyroidectomy, nephrotic syndrome, parathyroid disorders and patients who took Ca^{++} supplementation and drugs, which could affect serum values of 25 (OH) vitamin D.

All patients were provided a thorough medical history including age, gender, duration of Graves' disease and medical treatment. They were subjected to complete physical examination and anthropometric measurements such as weight, height & BMI. Patients and controls were assessed for thyroid function (TSH, Free T4 and Free T3), Serum anti-TPO antibodies, anti-thyroglobulin antibodies, serum thyrotropin receptor antibody (TRAb), and 25 hydroxyvitamin D (25(OH) D). After assessment, patients with Graves' disease and VDD or VDI received 50000 IU of cholecalciferol every week as a single dose for two months followed by once per month for the following four months. After 6 months of giving vitamin D, reassessment was done.

Ethical approval: Mansoura Faculty of Medicine Ethics Committee gave its approval to this study. All participants gave written consents after receiving all information. The Helsinki Declaration was followed throughout the study's conduct.

Statistical analysis

The collected data were coded, processed, and analysed using the SPSS V. 29.0. Qualitative data were described as percentages and numbers, while quantitative data were described as means ± SD for parametric variables or medians (IQR), for nonparametric variables, as suitable. To evaluate the normal distribution of variables, Kolmogorov–Smirnov test was utilized. To compare between two groups, t-test was used for normally distributed variables, while Mann Whitney test was used for non-normal distribution of variables. Chi-square test was used for comparing between qualitative variables. Spearman's rank correlation was utilized to assess the relationship of multiple variables with vitamin D levels. Binary univariate and multivariate logistic regression analysis was used to recognize significant associates of Graves. Wilcoxon test was utilized to compare variables, prior to and following VD intake. In terms of all the formerly mention tests, the results were considered significant when P-value ≤ 0.05.

RESULTS

This study included 30 patients with Graves' disease with mean age of 27.33 ± 5.13 years. 22 (73.3%) were females in addition to 30 age- and gender-matched subjects (control group). No significant difference with age, gender, marital status, residency, smoking, and BMI status between the two groups (p values= 0.362, 0.774,1, 0.774, 1 & 0.171 respectively). WC and DBP were significantly lower in Graves than in control group (p=0.016 & 0.006 respectively) (Table 1).

Table (1): Sociodemographic, medical, and therapeutic data among studies groups

	Graves	Control	P value
Age	27.33±5.13	29.03±8.74	0.362
Gender: Male Female	 8(26.7%) 22(73.3%)	 9(30%) 21(70%)	0.774
Marital status: Single Married	 5(16.7%) 25(83.3%)	 5(16.7%) 25(83.3%)	1
Residency: Rural Urban	 22(73.3%) 8(26.7%)	 21(70%) 9(30%)	0.774
Smoking status: Smoker Non-smoker	 2(6.7%) 28(93.3%)	 2(6.7%) 28(93.3%)	1
BMI (kg/m^2)	23.99±3.71	25.57±5.02	0.171
WC (cm)	54.5(48.75-70)	79(52.75-88.50)	**0.016**
SBP	120(110-122)	120(110-120)	0.572
DBP	65(60-80)	80(70-80)	**0.006**
Duration of disease	3(1.38-5)		
Dose of carbimazole	30(20-40)		

As shown in table (2), TSH was significantly less in Graves' patients compared to controls (p<0.001). Free T3 and free T4 were significantly increased in GD patients compared to the controls (p value <0.001).

TRAB titers were significantly higher in Graves' patients compared to the controls (p value < 0.001). Anti TPO, Anti Tg titers were significantly higher in Graves' group than in the controls (p<0.001). Patients with VDD were significantly more in Graves than in control group (p value = 0.003).

Table (2): Basal laboratory data among studies groups

	Graves	Control	P value
TSH (mIU/L)	0.02(0.006-0.425)	2.30(1.75-3.13)	**<0.001**
Free T3 (ng/dL)	6(4.20-10.43)	2.75(1.90-3.00)	**<0.001**
Free T4 (ng/dL)	3.50(1.78-4.75)	1.80(1.49-1.93)	**<0.001**
TRAB	6.80(4.50-8.43)	1.68(1.43-2.69)	**<0.001**
Anti-TPO	46.30(29.75-60.67)	8.44(5.92-13.43)	**<0.001**
Anti-TG	50.00(25.25-100)	12(8.90-25.68)	**<0.001**
Vitamin D (ng/mL)	26(15.93-59)	42.45(34.25-58.5)	0.060
Vitamin D deficiency (ng/mL)	16(53.3%)	5(16.7%)	**0.003**
Triglycerides (mg/dl)	110(80-150)	105(100-120)	0.590
Total cholesterol (mg/dL)	141.5(104.5-162.5)	130(120-153.5)	0.594
LDL (mg/dL)	77.50(70-90.50)	80(78-86.5)	0.238
HDL (mg/dL)	50(40-55.88)	46(40.75-50)	0.225

Data are expressed by median (IQR): Nonparametric test.

TSH- thyroid stimulating hormone, TRAB-Thyroid Stimulating Hormone Receptor Antibody, Anti-TPO-anti–thyroid peroxidase antibody, Anti-Tg- Antithyroglobulin antibody

Patients with VDD were significantly older than those without (P<0.001). Females were significantly more in VDD group than in the other group (P=0.012). TRAB was significantly higher in VDD group than in the other group (P<0.001) (Table 3).

Table (3): Comparison of parameters among Graves' disease individuals divided as per vitamin D deficiency

	Non-VDD group (n=14)	VDD group (n=16)	P value
Age	22.71±2.33	31.38±2.96	**<0.001**
Gender: **Male** **Female**	 7(50%) 7(50%)	 1(6.3%) 15(93.8%)	**0.012**
Marital status: **Single** **Married**	 1(7.1%) 13(92.9%)	 4(25%) 12(75%)	0.336
Residency: **Rural** **Urban**	 10(71.4%) 4(28.6%)	 12(75%) 4(25%)	1.000
Smoking status: **Smoker** **Non-smoker**	 2(14.3%) 12(85.7%)	 0 16(100%)	0.209
Body Mass Index (kg/m^2)	23.31±4.01	24.59±3.44	0.353
Waist circumferences	53(47.5-72.5)	55(49.25-60)	0.918
Systolic blood pressure	110(100-122.5)	120(110-127.5)	0.423
Diastolic blood pressure	65(60-80)	62.50(60-77.5)	0.608
Duration of disease	2.25(0.83-5.50)	3.50(2.00-4.75)	0.334
Dose of carbimazole	30(18.75-42.50)	20(20-30)	0.377
TSH (mIU/L)	0.01(0.005-0.075)	0.225(0.01-1.00)	0.052
Free T3 (ng/dL)	7.85(4.42-10.90)	5.50(3.70-8.00)	0.193
Free T4 (ng/dL)	3.95(1.95-5.03)	3.14(1.33-3.68)	0.101
TRAB	4.40(3.95-5.33)	8.40(7.93-9.30)	**<0.001**
Anti-TPO	36.50(39.80-60.67)	49.50(23.80-61.04)	0.142
Anti-TG	49.50(34.33-100)	69.70(23.67-123.47)	0.637
Triglycerides (mg/dl)	100(77.5-142.50)	110(92.50-150)	0.377
Total cholesterol (mg/dL)	150(104.5-180)	127.5(102.5-150)	0.313
Low density Lipoprotein	85(70-100)	71.5(70-80)	0.058
High density Lipoprotein	52.5(40-58.88)	50(36.25-51.75)	0.498

Data are expressed by median (IQR): nonparametric test.

TSH- thyroid stimulating hormone, TRAB-Thyroid Stimulating Hormone Receptor Antibody, Anti-TPO-anti–thyroid peroxidase antibody, Anti-Tg- Antithyroglobulin antibody.

In patients with Graves' disease, there was significant negative correlation between vit D level and TRAB titers (R=-0.773, p <0.001). There was significant negative correlation between age and vitamin D levels (R=-0.720, P<0.001). In addition, vitamin D blood level was correlated with gender (R=-0.362, p =0.049) (Table 4).

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Table (4): Correlations of vitamin D levels with different variables of patients with Graves

Age	**Rho**	**-0.720**
	P value	**<0.001**
Gender	Rho	-0.362
	P	**0.049**
Residency	Rho	-0.087
	P	0.646
BMI (kg/m^2)	Rho	-0.191
	P	0.313
WC (cm)	Rho	0.114
	P	0.547
SBP	Rho	-0.139
	P	0.463
DBP	Rho	0.123
	P	0.517
Duration of disease	Rho	-0.178
	P	0.347
Dose of carbimazole	Rho	0.177
	P	0.350
TSH (mIU/L)	Rho	-0.312
	P	0.093
Free T3 (ng/dL)	Rho	0.332
	P	0.073
Free T4 (ng/dL)	Rho	0.304
	P	0.103
TRAB	Rho	-0.773
	P	**<0.001**
Anti-TPO	Rho	-0.039
	P	0.836
Anti-TG	Rho	-0.162
	P	0.392
Triglycerides (mg/dl)	Rho	-0.213
	P	0.259
Total cholesterol (mg/dL)	Rho	0.137
	P	0.469
LDL (mg/dL)	Rho	0.211
	P	0.264
HDL (mg/dL)	Rho	0.105
	P	0.581

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.

In the univariate regression analysis, Graves' patients had a positive correlation with TRAB (OR1.3, P=0.001), Anti-TPO (OR=1.144, P**<0.001**), anti-Tg (OR=1.059, P**<0.001**), and vitamin D deficiency (OR=5.741, P=**0.004**). While, they were negatively correlated with waist circumference (OR=0.962, P=**0.008**) and diastolic blood pressure (OR=0.921, P=**0.008**). Multiple regression analysis showed that the most significant predictors of Graves were TRAB (OR=1.10, P=0.002), anti-TPO (OR= 0.829, P=0.006) and vitamin D deficiency (OR=57.355, P=0.013). But not with waist circumference (OR =1.041, P= 0.263), diastolic blood pressure (OR=1.061, P=0.398) and anti-TG (OR=0.968, P=0.237) (Table 5).

Table (5): Univariate and multivariate binary logistic regression analysis of Graves vs control

	Univariate analysis				Multivariate analysis			
	B	OR	P value	CI	B	OR	P value	CI
Age	-0.035	0.966	0.360	0.896-1.041				
Male gender	-0.164	0.848	0.775	0.276-2.612				
Urban	-0.164	0.848	0.775	0.276-2.612				
BMI (kg/m^2)	-0.084	0.919	0.174	0.814-1.038				
WC (cm)	-0.038	0.962	**0.008**	0.936-0.990	0.041	1.041	0.263	0.970-1.118
SBP	-0.018	0.983	0.472	0.937-1.031				
DBP	-0.083	0.921	**0.008**	0.866-0.978	0.059	1.061	0.398	0.925-1.217
TRAB	1.25	1.3	**0.001**	1.1-5.6	1.15	1.10	**0.002***	1.02-3.68
Anti-TPO	0.135	1.144	**<0.001**	1.072-1.221	-0.187	0.829	**0.006**	0.726-0.947
Anti-Tg	0.058	1.059	**<0.001**	1.024-1.096	-0.033	0.968	0.237	0.916-1.022
Triglycerides (mg/dl)	0.011	1.011	0.266	0.992-1.030				
Total cholesterol (mg/dL)	-0.002	0.998	0.803	0.984-1.013				
LDL (mg/dL)	-0.004	0.996	0.860	0.954-1.040				
HDL (mg/dL)	0.001	0.999	0.980	0.957-1.046				
Vitamin D deficiency (ng/mL)	1.743	5.741	**0.004**	1.724-18.944	4.049	57.355	**0.013**	2.318-1419.001

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. COR:Crude odds ratio , AOR:Adjusted odds ratio

Significant increase in vitamin D level after vitamin D supplementation (p value <0.001). Significant decrease in FT3, TRAB, Anti-TPO, and Anti-Tg after vitamin D supplementation (P value =0.020, <0.001, 0.039 & 0.007 respectively). No significant change in TSH, and FT4 after vitamin D supplementation. (P value=0.328 & 0.267 respectively) (Table 6).

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 Graves' disease and vit D deficiency.

	Pre	Post	P value
Vitamin D (ng/mL)	16.60(12.40-20.63)	35(30.25-45)	**<0.001**
TSH (mIU/L)	0.05(0.01-1)	0.1(0.01-1.2)	0.328
Free T3 (ng/dL)	6(4-8)	4.5(4-6)	**0.020**
Free T4 (ng/dL)	3.14(1.40-3.70)	2.20(2-3.40)	0.267
TRAB	8.40(7.93-9.30)	6(4.63-7.00)	**<0.001**
Anti-TPO	49.50(23.80-61.04)	30.80(20.75-47.65)	**0.039**
Anti-Tg	69.70(23.68-123.48)	30.15(16-113.75)	**0.007**

Data are expressed by median (IQR): non parametric test

There was an increase in the patients without VDD over the study period. In contrast, there was a reduction in patients with VDD (Table 7).

Table (7): Status of vitamin D in Graves' patients and baseline and 6 months after vit D supplementation

	Pre vitamin D treatment	Post vitamin D treatment
Normal	14(46.7%)	27(90%)
Mild VDD	4(13.3%)	3(10%)
Moderate VDD	9(30%)	0
Sever VDD	3(10%)	0

DISCUSSION

According to the current study, there were substantially more vitamin D-deficient patients in the GD group than in the controls. These results are similar to a meta-analysis by **Khozam *et al.*** [9], which based on most of the reviewed literature, concluded that patients with GD and HT were associated with a reduction in VD levels or VDI**.** Additionally, **Yasuda *et al.*** [10] found that GD participants had a statistically greater prevalence of VDD (65.4%) than in the controls (32.4%) ($p < 0.05$). Vit D levels in 292 cases with recent-onset GD and 2305 controls were measured by **Planck and colleagues** [11] who found that the Vit D values in the GD group were considerably lower (55 ± 23.2 nmol/L) than in the healthy group (87.2 ± 27.6 nmol/L), ($p < 0.001$)**. Ma and his colleagues** [12] also found that cases with AITD had reduced VD values, and that HT and GD morbidity decreased 1.55 and 1.62 times for every 5 nmol/L increases in serum VD values**.** This is in line with research by **Unal *et al.*** [13]**,** which showed that 65% of AITD patients had a VDD.

In contrast, some research disproved the relationship between AITD and a vitamin D3 deficit. According to **Botelho *et al.*** [14] there was no significant variation in vitamin D3 values between the cases with GD and the control group ($p = 0.1024$). Furthermore, **Ke *et al.*** [15]**, Kim** [16] and **Jyotsna *et al.*** [17] noted that no connection between the concentration of VD and GD has been found. The significant variation between the studies may be explained by the use of multiple assays to measure serum VD levels, the heterogeneity of the study group, different criteria for describing VDD, and the confounding effects of gender, smoking, age, obesity, dietary practices, sun exposure, and the season when the samples were collected.

According to the current study, the VDD group had a considerably greater number of female patients than the other group (P=0.012), and Graves cases with VDD were significantly older than those without (P<0.001). These findings corroborate with the findings of **Sulejmanovic *et al.*** [18]**, Chao *et al.*** [19] and **Naeem *et al.*** [20] who noted that women had much lower vitamin D levels than men. Additionally, 20% of males and 40% of females with Graves' disease who were euthyrotic were demonstrated to have inadequate VD levels in a Japanese study ($p < 0.005$) [21]**.**

In the current study, TRAB titers were significantly higher in Graves' cases with VDD than in the non-VDD group (P<0.0001). Such results are in the same line with **Zhang *et al.*** [22] who demonstrated a relationship between GD patients' vitamin D status and TRAb level?

The current research demonstrated a significant negative association (R=-0.773, $p <0.001$) between the TRAB titers and vitamin D level in patients with Graves. The current results are in accordance with those of **Li *et al.*** [23] and **Zhang *et al.*** [22] who found a negative relationship between vitamin D values and TRAB titers?

We found that Graves' cases with VDI had a significant decrease in TRAB, anti-TPO, and anti-Tg titers after taking VD supplements for six months ($p<0.001$, 0.039, and 0.007, respectively). These results are in accordance with **Kravchenko and his colleagues** [24] who reported a significant decrease in TRAB titers in Graves' cases after vitamin D intake and came to the conclusion that, autoimmune thyroid illnesses can be prevented, or their clinical symptoms can be diminished by using vitamin D in optimal dose. There are a limited number of research on the actions of VD supplementation in GD, but those that are available seem to indicate that it has a positive impact. These results are similar to **Grove-Laugesen and his colleagues** [25] who reported that TRAB titers were lower in vitamin D-treated patients at antithyroid drug cessation than in those without vitamin D intake. Also, These results are similar to **Wang *et al.*** [26] results in a meta-analysis. Their study comprised six randomized controlled studies, only one of them included mixed population of Graves' patients (N=14) and Hashimoto patients (N=68). They found that taking vitamin D supplement seems to dramatically lower anti-TPO and anti-Tg levels (for treatments longer than six months) with no significant negative effects noted. Moreover, our results agree with **Simsek and his colleagues** [27] who studied 82 individuals with GD and HT; 46 received a daily vitamin D supplementation of 1000 IU, while the remaining 36 did not get any supplementation. Anti-TPO and anti-Tg titers were shown to be lower exclusively in the supplementation group, according to the researchers but they didn't measure TRAB titers [27]**.**

Conversely, **Behera *et al.*** [7] included patients with hyperthyroidism. For eight weeks, 23 of them received weekly dosages of vitamin D equal to 60,000 IU, and then the same amount was continued for an additional four months. TPO antibody levels were re-evaluated six months later. TPO antibody titers increased statistically significantly, from 746.8 ± 332 IU/mL to 954.1 ± 459.8 IU/mL (p=0.006). The exact correlation between thyroid autoimmunity and VDI is still unknown. It is claimed that VD has anti-inflammatory and immunomodulatory qualities. Immune system regulation is significantly impacted by

VD, which inhibits the adaptive immune system while promoting the innate immunological response. The majority of immune cells, comprising B cells, T cells, dendritic cells, and macrophages, express 1α-hydroxylase and the vit-D receptor [28].

In the current study, after receiving vitamin D for six months, Graves' patients showed a substantial drop in FT3 (p=0.020). TSH levels also increased and FT4 decreased, but the differences weren't statistically significant (p=0.328 & 0.267 respectively). These results are similar to **Kravchenko *et al.*** [24] who found that taking vitamin D supplement seems to improve thyroid function (increase in serum TSH values and a decline in thyroid volume) in Graves' patients**.** Also, **Sheriba and his colleagues** [29] studied 60 individuals with GD; 40 received a 30 mg of methimazole and 200000IU of vitamin D per month for 3 months, while the remaining 20 received methimazole alone and reported that Vitamin D administration has favorable effects on the degree of exophthalmos as well as on thyroid volume**.** In addition **Grove-Laugesen and his colleagues** [25] reported that vitamin D intake decreased the risk of GD relapse after antithyroid drug cessation.

In the context of GD in animal models, where mice were injected with an adenovirus encoding the α-subunit of the thyrotropin receptor, Vit D deficiency was linked to the maintenance of hyperthyroidism, recommending that Vit D could control thyroid functions [8]. These outcomes are consistent with the results of **Kawakami *et al.*** [30] who found that significant and rapid decrease in FT3 , FT4 and significant increase in TSH level in Graves' patients who treated with methimazole and vitamin D3 compared to patients treated by methimazole alone**.**

On the other side, 25 patients with GD were included in the **Purnamasari *et al.*** [31] trial. 12 of them got oral vitamin D in addition to propylthiouracil for 8 weeks and 13 patients got propylthiouracil and placebo and there was no difference in the percentage of FT4 level decline between the two groups. The short duration of vitamin D intake in that study could be the cause of the discrepancy in outcomes**.** Also, **Simsek and his colleagues** [27] reported that no significant changes in thyroid function after vitamin D intake to patients with AITDS (GD and HT)**.**

VD may affect TSH and thyroid hormone values through a number of poorly understood methods [32]. Nonetheless, additional experimental research is required to elucidate the fundamental mechanisms [33]. The improvement in thyroid hormone profile in Graves' patients may be due to factors other than the administration of vitamin D, such as improved patient compliance regarding treatment, due to weekly follow-ups rather than monthly ones and improved patient education regarding the disease and appropriate treatment (dosage and timing).

Our study showed that the most significant predictors of GD were TRAB (OR=1.10, P=0.002), anti-TPO (OR= 0.829, P=0.006) and vitamin D deficiency (OR=57.355, P=0.013). No other studies found similar or different results.

CONCLUSION

Low VD level is linked to elevated TRAb titers in GD, and the level of thyroid autoantibodies were decreased after vitamin D administration, pointing to a potential connection between elevated thyroid autoimmunity in GD patients and their vitamin D levels.

RECOMMENDATIONS

Additional research has to be conducted to assess VDD role and the effects of its correction in GD patients.

REFERENCES

1. **Antonelli A, Ferrari S, Ragusa F *et al.* (2020):** Graves' disease: Epidemiology, genetic and environmental risk factors and viruses. Best Practice & Research Clinical Endocrinology & Metabolism, 34 (1): 101387. doi: 10.1016/j.beem.2020.101387.
2. **Botello A, Herrán M, Salcedo V *et al.* (2020):** Prevalence of latent and overt polyautoimmunity in autoimmune thyroid disease: A systematic review and meta-analysis. Clinical Endocrinology, 93 (4): 375-89.
3. **De Leo S, Lee S, Braverman L (2016):** Hyperthyroidism. Lancet, 388(10047): 906–918.
4. **Cho Y, Chung Y (2020):** Vitamin D supplementation does not prevent the recurrence of Graves' disease. Scientific Reports, 10 (1): 16. DOI:10.1038/s41598-019-55107-9
5. **Miteva M, Nonchev B, Orbetzova M *et al.* (2020):** Vitamin D and autoimmune thyroid diseases-a review. Folia Medica, 62 (2): 223-9.
6. **Vieira I, Rodrigues D, Paiva I (2020):** Vitamin D and Autoimmune Thyroid Disease—Cause, Consequence, or a Vicious Cycle? Nutrients, 12 (9): 2791-95.
7. **Behera K, Saharia G, Hota D *et al.* (2020):** Effect of Vitamin D supplementation on thyroid autoimmunity among subjects of autoimmune thyroid disease in a coastal province of India: A randomized open-label trial. Nigerian Medical Journal, 61 (5): 237-42.
8. **Misharin A, Hewison M, Chen C *et al.* (2009):** Vitamin D deficiency modulates Graves' hyperthyroidism induced in BALB/c mice by thyrotropin receptor immunization. Endocrinology, 150 (2): 1051-60.
9. **Khozam S, Sumaili A, Alflan M *et al.* (2022):** Association between vitamin D deficiency and autoimmune thyroid disorder: A systematic review. Cureus, 14 (6): e25869. doi: 10.7759/cureus.25869.
10. **Yasuda T, Okamoto Y, Hamada N *et al.* (2012):** Serum vitamin D levels are decreased and associated with thyroid volume in female patients with newly onset Graves' disease. Endocrine, 42: 739-41.
11. **Planck T, Shahida B, Malm J *et al.* (2018):** Vitamin D in Graves disease: levels, correlation with laboratory and clinical parameters, and genetics. European Thyroid Journal, 7 (1): 27-33.
12. **Ma J, Wu D, Li C *et al.* (2015):** Lower serum 25-hydroxyvitamin D level is associated with 3 types of autoimmune thyroid diseases. Medicine, 94 (39): e1639. doi: 10.1097/MD.0000000000001639
13. **Unal A, Tarcin O, Parildar H *et al.* (2014):** Clinical immunology Vitamin D deficiency is related to thyroid

antibodies in autoimmune thyroiditis. Central European Journal of Immunology, 39 (4): 493-7.

14. **Botelho I, Neto A, Silva C *et al.* (2018):** Vitamin D in Hashimoto's thyroiditis and its relationship with thyroid function and inflammatory status. Endocrine Journal, 65 (10): 1029-37.
15. **Ke W, Sun T, Zhang Y *et al.* (2017):** 25-Hydroxyvitamin D serum level in Hashimoto's thyroiditis, but not Graves' disease is relatively deficient. Endocrine Journal, 64 (6): 581-7.
16. **Kim D (2016):** Low vitamin D status is associated with hypothyroid Hashimoto's thyroiditis. Hormones, 15 (3): 385-93.
17. **Jyotsna V, Sahoo A, Singh A *et al.* (2012):** Bone mineral density in patients of Graves disease pre-& post-treatment in a predominantly vitamin D deficient population. The Indian Journal of Medical Research, 135 (1): 36-41.
18. **Sulejmanovic M, Begić A, Mujaric-Bousbia F *et al.* (2020):** The relationship between thyroid antibodies and vitamin D level in primary hypothyroidism. Medical Archives, 74 (5): 359-63.
19. **Chao G, Zhu Y, Fang L (2020):** Correlation Between Hashimoto's Thyroiditis–Related Thyroid Hormone Levels and 25-Hydroxyvitamin D. Frontiers in Endocrinology, 11: 4. doi: 10.3389/fendo.2020.00004
20. **Naeem Z, AlMohaimeed A, Sharaf F *et al.* (2011):** Vitamin D status among population of Qassim region, Saudi Arabia. International Journal of Health Sciences, 5 (2): 116-21.
21. **Yamashita H, Noguchi S, Takatsu K *et al.* (2001):** High prevalence of vitamin D deficiency in Japanese female patients with Graves' disease. Endocrine Journal, 48 (1): 63-9.
22. **Zhang H, Liang L, Xie Z (2015):** Low vitamin D status is associated with increased thyrotropin-receptor antibody titer in Graves disease. Endocrine Practice, 21 (3): 258-63.
23. **Li X, Wang G, Lu Z *et al.* (2015):** Serum 25-hydroxyvitamin D predict prognosis in radioiodine therapy of Graves' disease. Journal of Endocrinological Investigation, 38: 753-9.
24. **Kravchenko V, Rakov O, Kovzun O *et al.* (2021):** Relationship between vitamin D and autoimmune condition and thyroid function with newly onset Grave's disease. Acta Scientific Women's Health, 3 (8): 65-73.
25. **Grove-Laugesen D, Ebbehoj E, Watt T *et al.* (2023):** Effect of Vitamin D Supplementation on Graves' Disease: The DAGMAR Trial. Thyroid, 33 (9): 1110-1118.
26. **Wang S, Wu Y, Zuo Z *et al.* (2018):** The effect of vitamin D supplementation on thyroid autoantibody levels in the treatment of autoimmune thyroiditis: a systematic review and a meta-analysis. Endocrine, 59: 499-505.
27. **Simsek Y, Cakır I, Yetmis M *et al.* (2016):** Effects of Vitamin D treatment on thyroid autoimmunity. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences, 21: 85. doi: 10.4103/1735-1995.192501.
28. **Aktaş H (2020):** Vitamin B12 and vitamin D levels in patients with autoimmune hypothyroidism and their correlation with anti-thyroid peroxidase antibodies. Medical Principles and Practice, 29 (4): 364-70.
29. **Sheriba N, Elewa A, Mahdy M *et al.* (2017):** Effect of vitamin D 3 in treating hyperthyroidism in patients with graves' disease. The Egyptian Journal of Internal Medicine, 29: 64-70.
30. **Kawakami-Tani T, Fukawa E, Tanaka H *et al.* (1997):** Effect of 1α-hydroxyvitamin D3 on serum levels of thyroid hormones in hyperthyroid patients with untreated graves' disease. Metabolism, 46 (10): 1184-8.
31. **Purnamasari D, Djauzi S, Setiati S *et al.* (2017):** Effects of oral alfacalcidol on maturation of dendritic cells in graves' disease patients: a double-blinded randomized clinical trial. Asian J Pharm Clin Res., 10 (6): 100-104.
32. **Vassalle C, Parlanti A, Pingitore A *et al.* (2021):** Vitamin D, thyroid hormones and cardiovascular risk: Exploring the components of this novel disease triangle. Frontiers in Physiology, 12: 722912. doi: 10.3389/fphys.2021.722912.
33. **Babić Leko M, Jureško I, Rozić I *et al.* (2023):** Vitamin D and the Thyroid: A Critical Review of the Current Evidence. International Journal of Molecular Sciences, 24 (4): 3586. doi: 10.3390/ijms24043586.