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Assessment Of Vitamin D3 And Calcium Status In Patients With Graves' Disease In Babylon Province

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Abstract

Background:
Graves' disease is a leading cause of autoimmune thyroid disease, and environmental factors, play a significant role in the development of autoimmune thyroid disease. Recent studies have suggested a potential link between vitamin D deficiency and autoimmune thyroid diseases, including graves' disease. Graves' disease leads to increased thyroid hormone levels triiodothyronin and thyroxine , which can alter calcium homeostasis through several mechanisms. ***Objective:*** *This study aimed to investigate whether vit D3 and calcium levels are associated with the presence of Graves' disease and potential alterations in thyroid function. This study showed low-level vitamin D3 (17.66 nmol/L), while control group vitamin D3 level was (35.48 nmol/L), with (p-value<0.001) indicates for highly statistically significant. In the same time serum calcium level in patients was significantly lower (1.89 mmol/L) than in controls (2.24 mmol/L), with a P-value(< 0.001), indicating a statistically highly significant difference.* ***Conclusion:*** *This study indicates a significant link between vitamin D3 and calcium deficiencies and the development of graves' disease. We recommend further research on the role and impact of calcium and vit D3 in the pathogenesis and progression of Graves' disease .*

Keywords: *Graves' disease ,Vit. D3, Calcium, thyroxine .*

INTRODUCTION

Graves' disease (GD) is an autoimmune condition affecting the thyroid gland, with a worldwide prevalence of approximately 2% in women and 0.5% in men [1,2,3]. GD is characterized by a breakdown in immune tolerance, which results in infiltration of T lymphocytes into the thyroid gland. This triggers the activation of B lymphocytes, prompting them to produce autoantibodies that target the thyroid-stimulating hormone receptor (TSHR), also known as TRAb [1,2] . Individuals with overt hyperthyroidism are at a significantly higher risk of death, experiencing a 35–400% increase in all-cause mortality and a 20% increase in cardiovascular-related deaths compared to those with normal thyroid function. In severe cases, such as thyroid storm, the risk of death, with mortality rates ranging from 3.5% to 17%. Although the exact mechanisms that initiate these effects are not fully understood, they are believed to stem from heightened endothelial dysfunction and increased hypercoagulability, both of which are frequently observed in individuals with hyperthyroidism [2,3,4]. Environmental factors play a significant role in the development of autoimmune thyroid diseases (AITD), and specific micronutrients, including iodine and selenium, are especially important. These nutrients are closely linked to thyroid function and immune regulation, making them key contributors to AITD risk and progression. However, emerging evidence suggests the involvement of other micronutrients, such as zinc and iron [5,6,7]. Vitamin D regulates bone metabolism and the calcium and phosphorus homeostasis. The active form of vitamin D binds to the nuclear vitamin D receptor (VDR) and controls the expression of over 200 genes responsible for the regulation of cell

proliferation, differentiation, and apoptosis in most tissues and cells, including immune cells. Non-skeletal actions of vitamin D have been studied over the past few decades, and evidence suggests that there is a relationship between vitamin D deficiency and various diseases, such as autoimmune diseases [8]. Cardiovascular Disease and cancer [9]. In recent years, the extraskeletal effects of vitamin D have been studied extensively. Vitamin D deficiency is linked to a variety of autoimmune disorders, including autoimmune thyroid disease [10]. Genetic variation in the genes involved in vitamin D metabolism has been associated with several autoimmune disorders, including autoimmune thyroid disease [11]. Several studies have suggested that individuals with graves disease (GD) have lower vitamin D levels than the general population [10]. However, data on the relationship between vitamin D levels and clinical parameters in GD [12] or therapeutic issues [13] are limited. Graves' disease leads to hyperthyroidism, which accelerates bone turnover and can result in decreased bone mineral density (BMD). Elevated thyroid hormone levels increase osteoclastic activity (bone resorption) and, to a lesser extent, osteoblastic activity (bone formation), leading to a net bone loss [14]. Patients with Graves' disease are at an increased risk of osteoporosis and fractures owing to prolonged hyperthyroidism and its effects on calcium metabolism. Calcium and vitamin D supplementation, along with antiresorptive therapies (e.g., bisphosphonates), are often recommended to mitigate bone loss in hyperthyroid patients [15]. Hyperthyroidism can lead to mild hypercalcemia due to increased bone resorption and release of calcium into the bloodstream. This is often asymptomatic but can exacerbate symptoms such as fatigue, muscle weakness, and cognitive disturbances. Severe hypercalcemia is rare in Graves' disease but may occur in cases of coexisting primary hyperparathyroidism or other conditions [16]. Calcium and vitamin D are essential for maintaining bone health in patients with graves' disease, especially in those undergoing treatment for hyperthyroidism. Antithyroid medications, radioactive iodine therapy, or thyroidectomy can normalize thyroid hormone levels; however bone recovery may take time. Supplementation helps prevent further bone loss and supports restoration of bone density [17].

MATERIALS AND METHODS:

This case-control study was conducted from the first of April 2024 to November 2024. Eighty elbow venous blood samples were collected from patients with GD, and 50 samples were collected as a control group. All patients were evaluated at Merjan Medical City Babylon, Iraq. Demographic and clinical data, including age, sex, smoking status, place of residence, and Body Mass Index (BMI), were obtained using structured questionnaires. Serum blood used for measuring biochemical parameters including serum FT3, FT4, TSH , TRAB and Vit D3, were measured using commercial test kits and Biomeriux Minividas ,France, while calcium was measured using the FUJI device, Japan.

STATISTICAL ANALYSIS:

For all statistical analyses, SPSS version 26.0 for Windows was used (Chicago, IL, USA). One-way analysis of variance (ANOVA) was used to assess differences in continuous variables between groups, whereas the Kruskal-Wallis test used skewed data. The crosstab test was used to examine categorical variables. Statistically significance set at $p < 0.05$.

RESULTS

General Characteristics of the Participants:

Table 1 shows the demographic and clinical characteristics of the study participants,; with significant differences ($p < 0.05$) in weight, sex and body mass index (BMI) among GD groups and healthy control. Moreover, the differences in age, height and smoking were not statistically significant among the GD and control groups. Patients with GD were older and most were females.

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Table 1: Sociodemographic and clinical data for healthy controls (HC) and patients with graves' disease

Characteristic	Healthy control n = 50	Patients with CD n = 80	P-Value
Age (years)			
Mean ±SD	42.3 ± 9.9	45.34 ± 11.7	0.24 NS
Range	28– 59 years	25–67 years	
Gender			
Male, n (%)	32(64%)	24(32%)	<0.001 HS
Female, n (%)	18(36%)	56(68%)	
Weight (kg)			
Mean ±SD	76 ± 10.34	60.35 ± 10.09	<0.01 HS
Range	55– 103 kg	45–95 kg	
Height (Cm)			
Mean ±SD	160.04 ± 9.18	162.50 ± 9.61	0.151 NS
Range	140– 183 Cm	143–185 cm	
Smoking			
Positive, n (%)	14(28%)	25(26%)	0.684 NS
Negative, n (%)	36(72%)	55(74%)	
Body mass index (BMI) kg/m2			
Mean ± SD	29.76 ± 4.43	22.54 ±3.73	<0.001 HS
Range	19.71– 37.5	16.22– 31.79	

N: number of cases; **CI,** confidence interval (CI); **HS,** Highly significant at P ≤ 0.05, **NS,**not significant

Comparison means of parameters between healthy control groups and patients with GD:

Table 2 shows the differences between the means of TSH, Free triiodothyronine(FT3), free thyroxine(FT4), Anti-TSH Receptor Antibody (anti-TRAb), Vit.D3 and calcium levels between patients with GD and healthy control groups. Means of TSH level in control p was (0.32 ng/dL) within the normal range for FT3(0.23-0.43 ng/dL) while patient was (0.48 ng/dL), the mean of FT4 was (0.98 ng/dL) within the normal range for FT4 (0.8–1.8 ng/dL) while patients mean was (1.76 ng/dL), Controls showed low-levelof Anti-TRAb (mean = 1.06 IU/L) while patients showed a mean Anti-TRAb level (2.32 IU/L).Patients showed low-level vitamin D3 (17.66 nmol/L) on the other hand control group showed vitamin D3 level of (35.48 nmol/L), with (p-value<0.001). The mean serum calcium level in patients was significantly lower (1.89 mmol/L) than that in controls (2.24 mmol/L), (P< 0.001), indicating a statistically significant difference.

Table 2: Serum TSH,FT3,FT4,Anti TRAb, Vit D3 and Calcium levels in healthy control subject and patients with graves' disease (GD).

Parameter	Status	N	Mean level	Std. Deviation	95% Confidence Interval for Mean		P-Value
					Lower Bound	Upper Bound	
TSH Level (lU/L)	Control	50	2.69	1.56	2.24	3.13	<0.001 HS
	Patient	80	0.07	0.05	0.06	0.08	
FT3 Level (ng/dL)	Control	50	0.32	0.06	0.30	0.34	<0.001 HS
	Patient	80	0.48	0.04	0.47	0.49	
FT4 Level (ng/dL)	Control	50	0.98	0.14	0.94	1.02	<0.001 HS
	Patient	80	1.76	0.36	1.67	1.84	
Anti TRAb level (IU/L)	Control	50	1.06	0.34	0.97	1.16	<0.001 HS
	Patient	80	2.32	0.33	2.24	2.39	
Vit. D3 Level (nmol/L)	Control	50	35.48	7.19	33.435	37.524	<0.001 HS
	Patient	80	17.66	7.56	15.978	19.346	
Mean Calcium (mmol/L)	Control	50	2.24	0.14	2.20	2.28	<0.001 HS
	Patient	80	1.89	0.17	1.85	1.93	

N: number of cases; **CI:** confidence interval (CI); **HS:** Highly significant at P ≤ 0.01

Correlation between place of residence and other variables:

Table 3 shows the differences between the means of serum TSH, FT3, FT4, Anti TRAb, Vit.D3 and calcium levels in healthy control subjects and patients with graves' disease (GD) according to the place of residence in Babylon Province(Hilla, Mahawil, Musayab, and Hashimiyah). For TSH levels ,in the Hilla control group, the mean TSH level is (2.82) and for the patient group, the TSH level is (0.06). In Mahawil for the control group, the TSH level was (3.13), whereas in the patient group, the TSH level was (0.07). The TSH level in the control group in Musayab was (2.34), and the patient group had a TSH level (0.05). The Hashimiyah TSH level was (0.08) compared to the control group (2.53). All locations showed low TSH levels in patients compared to the control group, with high significancet value (p-value <0.001). The FT3 levels were lower ,in Hilla the

control group FT3 level was lower (0.312) than in the patient group (0.479). In Mahawil the FT3 levels was (0.327), while patient group level was (0.487). In addition, the FT3 level inof the control group in Musayab was (0.318) lower than that in the patient group (0.480). Finally, the Hashimiyah FT3 level in the patient group was (0.485) compared to the control group (0.275). All locations showed high FT3 levels in patients compared to the control group, with a highly significant value (p-value <0.001). The FT4 level ,in the control group FT4 level was lower (1.00) than that in the patient group (1.78). In Mahawil, for the control group, the FT4 level was (0.99), while the patient group level was (1.70). The FT4 level of the control group in Musayab was (1.00) was lower than that of the patient group (1.71). Finally, the patient group in Hashimiyah FT4 level was (1.81) compared to the control group (1.01). All locations showed high FT4 levels in patients compared to the control group, with a highly significant value (p-value <0.001). For anti TRAb levels were lower ,in the control group (1.11) than in the patient group (2.28). In Mahawil, for the control group, the anti- TRAb level was (1.06), while the patient group level was (2.43). In addition, the anti- TRAb level inof the control group in Musayab was (0.92) lower than that in the patient group (2.33). Finally, the Hashimiyah anti- TRAb level in the patient group was (2.33) compared to the control group (1.09). All locations showed low anti- TRAb levels in controls compared to thepatients group with high significance value (p<0.001). For Vit D3 levels, in each location, the control groups exhibited significantly higher mean Vitamin D3 levels than that in the patient groups, with p-values indicating high statistical significance ($p < 0.001$), except in Hashimiyah, where $p = 0.045$. In Hilla, the mean of vitamin D3 level in the control group was low (35.24 nmol/L) than that in the patient group (17.75 nmol/L), whereas the control group showed significantly higher vitamin D3 levels than to the patients group ($p < 0.001$). Mahawil's control group had a mean of vitamin D3 level (36.58 nmol/L), while the patient group in Mahawil had a mean level (16.86 nmol/L). This results showed a highly significant difference between the control and patient groups with (p-value<0.001). Musayab showed the same pattern of vitamin D levels (16.58 nmol/L) as the control group (36.77 nmol/L). This led to a highly significant difference ($P < 0.001$) in patients compared with theo control group. Finally, Hashimiyah showed vitamin D levels (30.75 nmol/L) in the control groups and (20.25 nmol/L) in the patient groups, but without a significant difference ($P < 0.045$) between the control and patients with GD. Regarding Calcium level, in Hilla, the mean calcium level in patients with Graves' disease (1.87 mmol/L) is significantly lower than in the healthy control group (2.26 mmol/L), with a P-value (< 0.001), indicating a statistically significant difference. In Mahawil, there is also a significant difference in mean calcium levels between the control (2.24 mmol/L) and patient (1.95 mmol/L) groups, with a P-value (< 0.001), again highlighting a statistically significant reduction in calcium levels in the graves' disease patients. In Musayab, the data showed significantly lower calcium levels in patients with Graves' disease (1.90 mmol/L) compared than into the control group (2.18 mmol/L), with a P-value (< 0.001). Finally, in Hashimiyah, the control group had a mean calcium level of (2.25 mmol/L), while the patient group had a mean level of (1.90 mmol/L), with a (P-value = 0.008), indicating a significant difference between the two groups.

Table 3 Comparison of Serum TSH,FT3,FT4,Anti TRAb, Vit D3 and Calcium levels in healthy controls and patients with (GD) according to place of residence

Parameter	Place of Residence	Status	N	Mean Level	Std. Deviation	95% CI (Lower)(Upper)	P-Value
TSH Level (lU/L)	Hilla	Control	25	2.82	1.56	2.42-3.22	<0.001 HS
	Hilla	Patient	45	0.06	0.04	0.05-0.07	
	Mahawil	Control	12	3.13	1.67	2.55-3.71	<0.001 HS
	Mahawil	Patient	15	0.07	0.05	0.05-0.09	
	Musayab	Control	9	2.34	1.45	1.81-2.87	<0.001 HS
	Musayab	Patient	12	0.05	0.04	0.03-0.07	
	Hashimiyah	Control	4	2.53	1.32	1.78-3.28	<0.001 HS
	Hashimiyah	Patient	8	0.08	0.06	0.05-0.11	
FT3 Level (ng/dL)	Hilla	Control	25	0.312	0.060	(0.296-0.328)	<0.001 HS
	Hilla	Patient	45	0.479	0.038	(0.468-0.490)	
	Mahawil	Control	12	0.327	0.069	(0.300-0.354)	<0.001 HS
	Mahawil	Patient	15	0.487	0.037	(0.476-0.498)	
	Musayab	Control	9	0.318	0.064	(0.288-0.348)	<0.001 HS
	Musayab	Patient	12	0.480	0.040	(0.465, 0.495)	
	Hashimiyah	Control	4	0.275	0.053	(0.230-0.320)	<0.001 HS
	Hashimiyah	Patient	8	0.485	0.034	(0.470-0.500)	
FT4 Level (ng/dL)	Hilla	Control	25	1.00	0.04	(0.990-1.027)	<0.001 HS
	Hilla	Patient	45	1.78	0.37	(1.671-1.897)	
	Mahawil	Control	12	0.99	0.05	(0.962-1.030)	<0.001 HS
	Mahawil	Patient	15	1.70	0.36	(1.499-1.904)	
	Musayab	Control	9	1.00	0.05	(0.964-1.046)	<0.001 HS
	Musayab	Patient	12	1.71	0.31	(1.511-1.913)	
	Hashimiyah	Control	4	1.01	0.02	(0.979-1.045)	<0.005 HS
	Hashimiyah	Patient	8	1.81	0.43	(1.451-2.171)	
Anti TRAb level (IU/L)	Hilla	Control	25	1.11	0.34	(0.974-1.260)	<0.001 HS
	Hilla	Patient	45	2.28	0.33	(2.181-2.385)	
	Mahawil	Control	12	1.06	0.32	(0.856-1.275)	<0.001 HS
	Mahawil	Patient	15	2.43	0.35	(2.238-2.634)	
	Musayab	Control	9	0.92	0.35	(0.655-1.196)	<0.001 HS
	Musayab	Patient	12	2.33	0.30	(2.139-2.520)	
	Hashimiyah	Control	4	1.09	0.34	(0.535-1.644)	<0.001 HS
	Hashimiyah	Patient	8	2.33	0.36	(2.027-2.632)	
Vitamin D3 Level (nmol/L)	Hilla	Control	25	35.24	6.37	(32.606-37.873)	<0.001 HS
	Hilla	Patient	45	17.75	7.79	(15.415-20.096)	
	Mahawil	Control	12	36.58	9.08	(30.814-42.352)	<0.001 HS
	Mahawil	Patient	15	16.86	7.37	(12.781-20.951)	
	Musayab	Control	9	36.77	7.94	(30.667-42.888)	<0.001 HS
	Musayab	Patient	12	16.58	6.58	(12.399-20.766	

	Hashimiyah	Control	4	30.75	2.98	(25.998-35.501)	0.045
	Hashimiyah	Patient	8	20.25	8.74	(12.937-27.562)	NS
Calcium Level(mmol/L)	Hilla	Control	25	2.26	0.14	(2.20-2.32)	<0.001
	Hilla	Patient	45	1.87	0.17	(1.82-1.92)	HS
	Mahawil	Control	12	2.24	0.14	(2.15-2.33)	<0.001
	Mahawil	Patient	15	1.95	0.18	(1.85-2.05)	HS
	Musayab	Control	9	2.18	0.15	(2.06-2.29)	<0.001
	Musayab	Patient	12	1.90	0.15	(1.80-1.99)	HS
	Hashimiyah	Control	4	2.25	0.14	(2.01-2.49)	0.008
	Hashimiyah	Patient	8	1.90	0.18	(1.74-2.05)	HS

Correlation between age groups and other variables:

Table 4 illustrates the differences between the means of serum TSH, FT3, FT4, Anti TRAb, Vit.D3 and calcium levels in healthy control subject and patients with graves' disease (GD) according to age groups. For TSH level ,in age group (<30 Years) the mean of TSH level in control group (1.38) and for patient group TSH level (0.03). In (30–50 years old), the TSH level of control group was (2.88), while patient group TSH level was (0.07). TSH level of control group in (>30 years old) was (2.82), and patient group TSH level (0.06). All three age groups showed low TSH levels in patients compared to control group with high significant value (p-value <0.001). For FT3 level ,in age group (<30 Years) the mean of FT3 level in control group (0.30) and for patient group FT3 level (0.50). In (30–50 years old), the FT3 level of control group was (0.31), while patient group FT3 level was (0.48). FT3 level of control group in (>30 years old) was (0.32), and patient group FT3 level (0.49). All three age groups showed high FT3 levels in patients compared to control group with high significant value (p-value <0.001) except (>30 years old) group (p-value <0.006). For FT4 level ,in age group (<30 Years) the mean of FT4 level in control group (1.04) and for patient group FT4 level (1.72). In (30–50 years old), the FT4 level of control group was (0.99), while patient group FT4 level was (1.80). FT4 level of control group in (>30 years old) was (1.00), and patient group FT3 level (1.68). All three age groups showed high FT4 levels in patients compared to control group with high significant value (p-value <0.001). Regarding anti TRAb level ,in age group (<30 years old) the mean of anti TRAb level in control group (0.99) and for patient group anti TRAb level (2.55). In (30–50 years old), the anti TRAb level of control group was (1.07), while patient group anti TRAb level was (2.33). Anti TRAb level of control group in (>30 years old) was (1.08), and patient group zinc level (2.25). All three age groups showed high anti TRAb levels in patients compared to control groups with high significant value (p-value <0.001). For Vit D3 level, In age group (<30 Years) the mean of Vitamin D level in serum of patients was significantly decreased (22.80 nmol/L) compared to age-matched controls (36.00 nmol/L) with statistically significant (P-value<0.003) Statistically significant. For (30–50 Years Old), Vitamin D level is significantly reduced in patients (18.59 nmol/L) compared to controls (34.40 nmol/L), with highly significant (P-value <0.001). Regarding (Age >50 Years Old), mean of Anti-TRAb in patients is (14.92 nmol/L), significantly higher than in controls (37.92 nmol/L) with (P-value <0.001). Calcium levels were : In the <30 years age group, the data shows that the mean calcium level in the patient group (2.02 mmol/L) is slightly lower than the control group (2.21 mmol/L), but the P-value (0.108) indicates no statistically significant difference between the two groups. In the 30-50 years age group, the mean calcium level in the patient group is significantly lower (1.90 mmol/L) compared to the control group (2.24 mmol/L), with a P-value (< 0.001), indicating a highly significant difference. In the >50 years age group, the mean calcium level in patients with graves' disease (1.86 mmol/L) is significantly lower than in the control group (2.25 mmol/L), with a P-value (< 0.001), indicating a highly significant difference.

Table 4 Comparison of Serum TSH,FT3,FT4,Anti TRAb, Vit D3 and Calcium levels in healthy controls and patients with (GD) according to Age Groups.

Para meter	Age Group	Status	N	Mean Level	Std. Deviation	95% CI (Lower)-(Upper)	P-Value
TSH Level (lU/L)	<30	Control	5	1.38	1.08	0.68-2.08	<0.001 HS
		Patient	5	0.03	0.03	0.01-0.05	
	30-50	Control	32	2.88	1.56	2.55-3.21	<0.001 HS
		Patient	49	0.07	0.05	0.06-0.08	
	>50	Control	13	2.82	1.56	2.42-3.22	<0.001 HS
		Patient	26	0.06	0.04	0.05-0.07	
FT3 Level (ng/dL)	<30	Control	5	0.30	0.04	(0.248-0.363)	<0.001 HS
		Patient	5	0.50	0.04	(0.449-0.562)	
	30-50	Control	32	0.31	0.06	(0.294, 0.338)	<0.001 HS
		Patient	49	0.48	0.03	(0.473, 0.491)	
	>50	Control	13	0.32	0.06	(0.295, 0.361)	<0.001 HS
		Patient	26	0.49	0.04	(0.481, 0.505)	
FT4 Level (ng/dL)	<30	Control	5	1.04	0.018	(1.02-1.06)	0.006 HS
		Patient	5	1.72	0.404	(1.22-0.2.22)	
	30-50	Control	32	0.99	0.046	(0.982-1.016)	<0.001 HS
		Patient	49	1.80	0.358	(1.701-1.906)	
	>50	Control	13	1.00	0.051	(0.978-1.040)	<0.001 HS
		Patient	26	1.68	0.378	(1.53-1.84)	
Anti TRAb level (IU/L)	<30	Control	5	0.99	0.261	(0.67-1.31)	0.001 HS
		Patient	5	2.55	0.419	(2.03-3.07)	
	30-50	Control	32	1.07	0.363	(0.943-1.205)	<0.001 HS
		Patient	49	2.33	0.310	(2.249-2.427)	
	>50	Control	13	1.08	0.324	(0.887-1.279)	<0.001 HS
		Patient	26	2.25	0.363	(2.104-2.398)	
Vitamin D3 Level (nmol/L)	<30	Control	5	36.00	5.00	(29.79-42.21)	0.003 HS
		Patient	5	22.80	5.167	(16.38-29.22)	
	30-50	Control	32	34.4063	7.237	(31.796-37.015)	<0.001 HS
		Patient	49	18.5918	7.705	(16.378-20.805)	
	>50	Control	13	37.92	7.620	(33.317-42.528)	<0.001 HS
		Patient	26	14.92	6.916	(12.129-17.716)	
Calcium Level (mmol/L)	<30	Control	5	2.21	0.11	(2.07-2.35)	0.108 NS
		Patient	5	2.02	1.75	(3.52-2.28)	
	30-50	Control	32	2.24	0.15	(2.19-2.30)	<0.001 HS
		Patient	49	1.90	0.16	(1.85-1.95)	

	>50	Control	13	2.25	0.13	(2.16-2.33)	<0.001 HS
		Patient	26	1.86	0.18	(1.78-1.93)	

N: number of cases; CI: confidence interval (CI); HS: Highly significant at P ≤ 0.01, NS:No Significant

DISCUSSION

Our results in this study showed that patients with GD had TSH levels that were significantly lower than those of healthy control (P< 0.001). which is in agreement with other studies. [18] Confirms that TSH is the most sensitive and specific marker for diagnosing hyperthyroidism. It states that a suppressed TSH level (<0.1 IU/L) is a key diagnostic criterion for hyperthyroidism, including graves' disease. [19] Illustrated the diagnostic utility of TSH levels in thyroid disorders, is illustrated, noting that low TSH levels are highly sensitive for detecting hyperthyroidism, though additional tests are needed to determine the underlying cause (e.g., Graves' disease vs. toxic nodular goiter). [20] Also showed the role of TSH as a primary screening tool for thyroid dysfunction, with low TSH levels being a reliable indicator of hyperthyroidism, including graves' disease. Table 3 showed highly significant (P<0.001) of serum FT3 in patients compared to controls. This result agrees with the results of other studies, [21] detailed how TSIs bypass normal TSH regulation, leading to excess T3 secretion. [22] Showed how TSIs activate TSH receptors, leading to autonomous thyroid hormone production. [18] Discussed effect of graves' disease and its role in thyroid hormone overproduction. Graves' disease is caused by autoantibodies that bind to and stimulate the thyrotropin receptor (TSHR), leading to uncontrolled thyroid hormone production this leads to excessive production and secretion of thyroid hormones T3 and T4 [23]. Our results appeared that the FT4 level significantly higher in patients compared to controls and this consistent with the results of other studies , FT4 is produced by the thyroid gland and is elevated in hyperthyroidism, especially graves' disease, due to TSH receptor antibody stimulation [24]. Table 3 showS that anti TRAb level high in significant value in patients compared to controls and this consistent with the results of other studies that anti-TSH receptor antibodies (anti-TRAb) are key immunological markers in graves' disease, acting as stimulating autoantibodies that bind to and activate the TSH receptor on thyroid follicular cells, leading to unregulated thyroid hormone production and hyperthyroidism according [25,22]. Table 2 shows patients have low-level vitamin D3 compared to control group with (p<0.001) indicates for highly statistically significant difference. These results are consistent with the results of other studies. Vitamin D plays an immunomodulatory role, particularly by inhibiting Th1 immune responses (which are pro-inflammatory) , promoting regulatory T cells (which help prevent autoimmune responses) and reducing antigen presentation by dendritic cells. Hence, low levels of vitamin D may contribute to the development or worsening of autoimmune diseases, including graves' disease. [26] In their study (Meta-analysis of the association between vitamin D and autoimmune thyroid disease) showed that vitamin D deficiency is significantly more common in graves' disease patients than in controls also found that lower vitamin D levels may correlate with disease severity. [27] Appeared in a their study (Low serum vitamin D is associated with anti-thyroid peroxidase antibody in autoimmune thyroiditis) on newly diagnosed graves' patients, vitamin D levels were significantly lower also proposed that vitamin D supplementation might help modulate immune response. Table 3 showed results of place of residence. Where in Hilla, control group shows significantly higher vitamin D3 levels compared to patients (p < 0.001). Many researches indicate that Vitamin D deficiency is prevalent in autoimmune thyroid diseases, including graves' disease. A study in Sweden (Vitamin D in Graves Disease: Levels, Correlation with Laboratory and Clinical Parameters, and Genetics) found significantly lower vitamin D levels in graves' disease patients compared to controls [28]. Mahawil's control group has vitamin D3 level higher than patient group, this result showed highly significant difference between control and patient groups with (p<0.001). This result aligns with international studies. A meta-analysis (Association between serum vitamin D level and graves' disease: a systematic review and meta-analysis.)

demonstrated that patients with graves' disease are significantly more likely to have low vitamin D levels [29]. Musayab showed the same pattern of vitamin D levels in patients compared to control group . This led to give highly significant difference with (P < 0.001) in patients compared to control group. This result consistentes with findings from other regions. Research in India (Evaluation of Vitamin D Status and its Impact on Thyroid Related Parameters in New Onset Graves' Disease- A Cross-sectional Observational Study) reported significantly lower vitamin D levels in new-onset graves' disease patients compared to controls [30]. Finally in Hashimiyah showed high vitamin D levels levels in control groups and for patient groups but without significant difference (P < 0.045) between control and patients with GD. This study aligns with broader research indicating lower Vitamin D levels in autoimmune thyroid conditions. A study in Iraq (Assessment of Vitamin-D Levels Among Hashimoto and Hypothyroidism disease in Iraq, A Comparative Study) focusing on Hashimoto's thyroiditis found significantly lower Vitamin D levels in patients compared to controls, suggesting a potential link between Vitamin D deficiency and autoimmune thyroid diseases [31]. Table 4 appears differences the parameters according to age group. Where, in age group (<30 Years) the mean of Vitamin D level in serum of patients was significantly decreased compared to age-matched controls with statistically significant (P-value<0.003). In younger individuals, Vitamin D levels are already lower than older age groups, but a significant decline is observed in graves' patients. This could be related to changes in immune system regulation that appear early in life. Some studies suggest that Vitamin D plays a protective role against autoimmune diseases in youth. [32] In a study (Vitamin D status in children with Hashimoto thyroiditis) observed significantly reduced Vitamin D levels in children and adolescents with autoimmune thyroiditis, emphasizing its role early in life. For (30–50 Years Old), Vitamin D level is significantly reduced in patients compared to controls with highly significant (P-value <0.001). This age group shows a substantial drop in Vitamin D levels among patients. It is a critical age range where autoimmune thyroid conditions like Graves' disease are often diagnosed. Lower vitamin D levels may play a role in triggering or exacerbating autoimmunity during this period of life. [26] In his study (Meta-analysis of the association between vitamin D and autoimmune thyroid disease) conducted a meta-analysis and found a strong association between low serum Vitamin D and autoimmune thyroid diseases, especially in adults. Regarding (Age >50 Years Old), mean of Vit D3 in patients is significantly higher than in controls with (P-value <0.001). Older adults with graves' disease demonstrate the most profound drop in Vitamin D levels. This may reflect not only the disease impact but also age-related factors such as reduced skin synthesis of vitamin D, dietary insufficiency, and less sun exposure. Vitamin D is essential for immune modulation in older age, and its deficiency may worsen autoimmune responses. [33] In his study (Vitamin D deficiency in autoimmune thyroiditis) found marked Vitamin D deficiency in elderly patients with autoimmune thyroid diseases and suggested that age-related decline in Vitamin D may predispose to or worsen these conditions. Table 2 showed calcium level in patients was significantly lower than in controls with a P-value(< 0.001), indicating a statistically highly significant difference. This result aligns with other studies. Thyroid hormones stimulate osteoclastic bone resorption, releasing calcium into circulation, but over time, calcium may be lost via urine due to increased glomerular filtration rate and reduced tubular reabsorption. This can lead to hypocalcemia or relatively lower calcium levels despite increased bone resorption, [34] in his study (Serum calcium and phosphorus levels in patients with hyperthyroidism and hypothyroidism). Hyperthyroidism is associated with hypercalciuria, contributing to lower serum calcium levels [35]. In a study titled (Evaluation of calcium and phosphorus metabolism in hyperthyroid patients). There may be alterations in vitamin D metabolism in hyperthyroid patients, leading to decreased intestinal calcium absorption [36]. Table 3 in Hilla, appeared calcium level in patients with graves' disease is significantly lower than in the healthy control group with a P-value (< 0.001), indicating a statistically significant difference. This agree with other studies. [35] in his study (Evaluation of calcium and phosphorus metabolism in hyperthyroid patients) highlighted that hyperthyroid patients, especially those with graves' disease, exhibit hypocalcemia due to hypercalciuria and altered bone metabolism. In Mahawil, there is also a significant

difference in calcium levels between the control and patient groups, with a P-value (< 0.001), again highlighting a statistically significant reduction in calcium levels in the graves' disease patients. This finding aligns with the same mechanism seen in Hilla: thyroid hormones cause increased bone turnover and calcium loss through the kidneys. [36] In a study titled (Thyroid hormone action in the heart) discussed how thyroid hormones influence bone metabolism, leading to lower calcium levels in hyperthyroid patients. In Musayab, the data once again shows significantly lower calcium levels in patients with graves' disease compared to the control group with a P-value (< 0.001). This agree with the study of [34] in his study titled (Serum calcium and phosphorus levels in patients with hyperthyroidism and hypothyroidism).This further reinforces the impact of hyperthyroidism on calcium regulation, particularly renal calcium wasting and altered bone metabolism. Finally, in Hashimiyah, the control group had a mean calcium level of higher than patient group with a (P-value = 0.008), indicating a significant difference between the two groups. This result mirrors findings in other locations and supports the idea that hyperthyroidism leads to calcium dysregulation in the context of graves' disease [35] in his study (Evaluation of calcium and phosphorus metabolism in hyperthyroid patients). Table 4 appeared, in the <30 years age group, calcium level in the patient group was slightly lower than the control group but the P-value (0.108) indicates no statistically significant difference between the two groups, [35] found in his study (Evaluation of calcium and phosphorus metabolism in hyperthyroid patients) that calcium disturbances in hyperthyroidism are often more pronounced in older patients due to longer exposure to thyroid hormone imbalances and its effect on bone metabolism and [37] in their study (Fractures in patients with hyperthyroidism and hypothyroidism) found younger patients may compensate better due to higher bone density and renal function. In the 30-50 years age group, calcium level in the patient group is significantly lower compared to the control group with a P-value (< 0.001), indicating a highly significant difference. This result agree with study of [36] that titled (Thyroid hormone action in the heart) that explains thyroid hormone excess leads to increased bone resorption and calcium loss, which is especially prominent in middle-aged individuals as they have had more prolonged exposure to hyperthyroidism. In the >50 years age group, calcium level in patients with graves' disease is significantly lower than in the control group with a P-value (< 0.001), indicating a highly significant difference. [38,39] have noted in their studies (Serum calcium and phosphorus levels in patients with hyperthyroidism and hypothyroidism) and (Evaluation of calcium and phosphorus metabolism in hyperthyroid patients) respectively, that older individuals with hyperthyroidism tend to have lower serum calcium levels due to both osteopenia and altered renal calcium handling. The loss of bone mineral density is especially problematic in the elderly due to longer thyroid hormone exposure.

CONCLUSION

This study indicates a significant link between deficiencies in Vit D3 and calcium and the development of graves' disease (GD). We recommend further research into the role and impact of zinc and iron in the pathogenesis and progression of GD.

Ethical Approval:

This study was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki. Before sample collection, all the participants provided both verbal and written informed consent. The study protocol, along with the subject information and consent forms, was reviewed and approved by a local ethics committee in December 2023, under the document reference number 3342.

CONFLICT OF INTEREST: The authors have no conflicts of interest to this declare.

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