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HASHIMOTO'S THYROIDITIS NOT ASSOCIATED WITH VITAMIN-D DEFICIENCY

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Running title: Vitamin-D & Hashimoto's Thyroiditis

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Abstract:

Objective: Vitamin-D deficiency is associated with several autoimmune diseases. This study assessed whether vitamin-D deficiency is associated with Hashimoto's thyroiditis (HT).

Methods: Two groups of patients were selected in which serum 25(OH)D levels had been measured: (I) a study group of patients diagnosed with HT as indicated by thyroid antibodies, and (II) a healthy control group. Each group was separated by sex and then controlled for age and BMI. Groups' mean 25(OH)D levels were compared by ANOVA, and percent frequencies of vitamin-D sufficiency, insufficiency, and deficiency were compared with a Z-test. The correlation between 25(OH)D levels and TgAb, TPO, or TSH levels was also tested using Spearman's correlation test.

Results: The mean 25(OH)D levels for the HT and control groups were significantly different in females (30.75 vs. 27.56 ng/mL respectively) but not in males (14.24 vs. 13.26). HT females had a higher rate of vitamin-D sufficiency (51.7% vs. 31.1%) and a lower rate of insufficiency (48.3% vs. 68.9%) relative to control females. No such differences were found in the male groups. None of the females were vitamin-D deficient, but almost all males were. In the males, a significant ($p=.016$) positive correlation ($r_s=.436$) between 25(OH)D and TgAb was observed.

Conclusion: HT is not associated with higher rates of vitamin-D deficiency relative to in a control group.

Conflicts of Interest:

The authors declare no conflicts of interest or affiliations that may inappropriately influence the content of this paper.

Keywords:

Hashimoto's thyroiditis; vitamin-D deficiency; thyroid autoimmunity.

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Abbreviations:

HT = Hashimoto's thyroiditis; **TgAb =** Thyroglobulin antibodies; **TSH =** Thyroid-stimulating hormone; **TPOAb =** Thyroid-peroxidase antibodies; **25(OH)D =** 25-hydroxyvitamin D**; VDRs** = Vitamin-D receptors.

Background:

Vitamin-D is a pro-hormone with widespread effects in the human body. Upon conversion to 25(OH)D and 1,25(OH)2D, it regulates the functions of numerous cells by binding to vitamin-D receptors (VDRs). Activation of VDRs plays a role in cellular growth and immune modulation, among other activities [1, 2]. Many immune cells possess VDRs and may alter their functions in response to serum levels of vitamin-D [3,4].

Growing evidence has shown vitamin-D deficiency is associated with certain autoimmune diseases [5]. These include rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, and type-1 diabetes mellitus [6]. Patients with such conditions have been shown to have lower serum vitamin-D levels relative to healthy subjects [5]. In addition, certain single-nucleotide polymorphisms in VDR genes have been associated with higher incidence of certain autoimmune disorders, including Hashimoto's thyroiditis (HT) [7,8].

Hashimoto's thyroiditis (HT) is a common, chronic autoimmune disorder of the thyroid and is characterized by the presence of thyroid antibodies in the serum and by lymphocytic infiltration of the thyroid. Few, conflicting studies have investigated its potential relationship to vitamin-D deficiency. Some studies have found a higher incidence of vitamin-D deficiency in patients with HT, correlating inversely with levels thyroid antibodies [9-12]. Others have found such associations limited or altogether absent [13,14]. Furthermore, to date no published studies have been conducted assessing these associations in a US population pool.

This study is planned to evaluate whether HT is associated with a higher prevalence of vitamin-D deficiency. Additionally, it is planned to assess the correlation between serum 25(OH)D and certain thyroid levels (i.e., TSH, TPOAb, and TgAb).

Methods:

An IRB-approved, retrospective chart review was assessed of about 3000 patients who visited Queens Hospital Center in New York, USA. Patients were either assigned to the study or control groups or excluded from the review.

Inclusion criteria in the study group were (1) patient age greater than 20 and (2) presence of HT diagnosis based on TgAb or TPOAb levels of more than 2 IU/mL. Factors for inclusion in the control group were (1) patient age greater than 20, (2) absence of thyroid disease, and (3) normal thyroid function, as indicated by TSH levels between 0.5-4 mIU/L. Laboratory results for thyroid function and autoimmunity had been determined with chemiluminescence immunoassays.

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Exclusion criteria from either group were presence of (1) pre-existing cause of vitamin-D deficiency (e.g., celiac disease, IBD, malabsorption, or bone disease), (2) chronic renal and/or hepatic failure, (3) other autoimmune disease, or (4) use of contraceptives or anti-seizure medications. Presence or absence of levothyroxine therapy in the study group was not a factor for exclusion; therefore, the recency of HT diagnosis was not a selection criterion. The total data collected included the subjects' aforementioned thyroid levels, serum 25(OH)D, age, BMI, and sex.

As a control, females and males were analyzed in separate study and control groups. The female study group was matched with a female control group, similar in size, mean BMI, and age. The same was done for males.

To determine if the 25(OH)D means were significantly different in the HT and control groups, a one-way ANOVA was used. To ensure ANOVA assumptions were valid for this data, the following steps were taken. The Shapiro-Wilk test assessed the normality of the variables' distributions and was compared to a histogram for reassurance. Levene's test was used to confirm the equality of the variances. Non-normal 25(OH)D distributions were logarithmically transformed.

The percent frequencies of vitamin-D sufficiency, insufficiency, and deficiency were calculated for the HT and the control groups. 25(OH)D levels ≥ 30.0 ng/mL were classified as a vitamin-D sufficiency; those between 20 and 29.9 ng/mL, as an insufficiency; and those less than 20 ng/mL, as a deficiency. A multi-sample Z-test was then used to assess if the frequencies of vitamin-D sufficiency, insufficiency, or deficiency were significantly different in the study and control groups.

Spearman's correlation test was used to determine if 25(OH)D levels are associated with thyroid levels. This test was chosen as it can assess the relationships among the variables that are continuous and nonparametric (not assumed to conform to a normal distribution).

Finally, the ranges, means, and standard deviations were generated for age, 25(OH)D levels, thyroid antibodies, and TSH. A p-value of less than 0.05 was considered statistically significant. Data analysis was performed with SPSS 23.0 for Windows (SPSS Inc., Chicago, IL, United States).

Results:

A total of 185 individuals were included in the review. For the females, 60 were in the HT group and 45 were in the control group. Their mean age was 47.5±8.3 and mean BMI was 28.5±5.3. For the males, 37 were in the HT group and 43 were in the control group. Their mean age was 46.2±7.4 and mean BMI was 28.4±5.5 [Table 1].

For females, the mean 25(OH)D level for the HT group (30.8 ±7.5 ng/mL) was significantly higher (p=.013) relative to the control group (27.6±8.1). For males, there was no significant difference (14.2±4.2 vs. 13.3±4.3, respectively) [Table 2].

51.7% of female HT subjects were vitamin-D sufficient (>30 ng/mL), compared to 31.1% of the female control group. 48.3% of the female HT group and 68.9% of the control were insufficient

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(20-29.9 ng/mL). No females in either group were deficient (<20 ng/mL). These differences in sufficiency and insufficiency were statistically significant [Table 3, Figure 1].

For males, none were vitamin-D sufficient in the HT and control groups. 5.4% of the male HT group and 7.0% of the control were insufficient. 94.6% of the male HT group and 93.0% of the control group were deficient. These differences in deficiency and insufficiency were not statistically significant.

In the female HT group, 45 had TPOAb levels available and 38 had TGA levels available. TgAb ranged from 2-2194 IU/mL and TPOAb levels ranged from 6-3000 IU/mL. TSH levels in the control group ranged from 0.6-4 mIU/L [Table 4].

In the male HT group, 30 had TPOAb levels available and 19 had TGA levels available. TgAb levels ranged from 25-3000 IU/mL and TPOAb levels ranged from 32-3000 IU/mL. TSH levels in the control group ranged from 0.6-4 mIU/L.

In the control groups, there was no significant correlation between 25(OH)D and TSH levels. In the study groups, there were no significant correlations between 25(OH)D and TgAb. However, in the male study group, there was a significant ($p=.016$) positive correlation ($r_s=.436$) between 25(OH)D and TgAb.

Discussion:

The observation that the mean 25(OH)D level for the female HT group (30.8 ±7.5 ng/mL) was significantly higher than in the female control group (27.6±8.1) does not confirm previous studies on this topic. While some [13,14] have found limited to no association between HT and 25(OH)D levels, others [9-12] have found that HT subjects have significantly higher rates of vitamin-D deficiency. In contrast, it was observed that female HT subjects had both a higher rate of vitamin-D sufficiency (51.7% vs. 31.1%) and a lower rate of insufficiency (48.3% vs. 68.9%). This difference may be due to potentially increased vitamin-D supplementation in HT females. Nevertheless, the finding still suggests that HT is not associated with higher rates of vitamin-D deficiency. This is also indicated by the male groups, where the differences in mean 25(OH)D or vitamin-D classification were not significantly different.

The observation that men and women differ so greatly in serum 25(OH)D confirms that sex is a confounding variable in this study, and directly controlling for its effects has not been implemented in past studies on this topic [9-12].

The female study and control groups had relatively higher rates of vitamin-D sufficiency than described in other studies; this may be because this study was conducted in the US as opposed to Hungary [9], Turkey [10, 11, 12], or India [13]. Furthermore, because this study had a larger sample size of vitamin-D sufficient individuals, it may have more accurately statistically tested vitamin-D's associations with HT and thyroid levels. Specifically, while 51.7% (n=31) of this study's female HT group was vitamin-D sufficient, only 1.9% (n=10) [9], 8.08% (n=12) [11], and <21% (n<6) [10] of other study groups were similar when defining sufficiency as 25(OH)D levels ≥ 30.0 ng/mL. The nevertheless limited proportion of vitamin-D sufficient individuals even in this study was expected given the wide prevalence of vitamin-D deficiency in the US and worldwide [15].

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The lack of correlations between TSH and TgAb with 25(OH)D levels resembles a previous study [12] but conflicts with others [9, 10]. This discrepancy may perhaps be due to this study's use of a larger sample of vitamin-D sufficient individuals and sex-segregated study groups, which may allow for more accurate measurements of statistical associations.

Though studies [10, 13] have found a negative correlation between TPOAb and 25(OH)D levels, this study observed a significant positive correlation (r_s=.436) in the male HT group but none in the female HT group. Because the male group was largely vitamin-D deficient, this suggests an association between increased 25(OH)D levels when deficient and slightly increased TPOAb levels in HT patients. While this may be a possibility, it does not necessitate that this correlation implies a causation.

The pathophysiology of autoimmune diseases, like HT, depend on the complex interplay of genes, hormones, and the environment. Current studies of the association of vitamin-D and HT have produced differing results in the sampled populations worldwide. Similarly, investigations into the association of VDR polymorphisms and HT have yielded both positive [7,8] and negative [16] results in different sample populations. Notably, a set of recent studies have found that vitamin-D deficiency may not affect the expression of VDRs in humans [17, 18]. Taken together, these findings may suggest that although VDRs may play a role in the development of HT, vitamin-D deficiency is neither an associated nor causal factor.

A potential limitation of this study is that the control group was not directly tested for thyroid autoimmunity, which would indicate the presence of HT. However, the TSH levels were seen as reliable indicators of normal thyroid function, and given the retrospective nature of this study, thyroid antibody tests were not indicated for otherwise healthy patients. Additionally, though potential confounding factors such as skin pigmentation may influence subjects' levels of vitamin-D deficiency, these variables were not controlled for directly. The study's large sample sizes may mitigate these random effects, because the occurrence of these demographic factors may be equally distributed between the study and control groups.

Further studies on this topic may clarify seeming discrepancies among findings and help better understand and treat HT.

Conclusion:

Subjects with HT did not have a higher incidence of vitamin-D deficiency relative to a control group. Rather, female HT subjects had both a higher rate of vitamin-D sufficiency and a lower rate of insufficiency. In male subjects, the mean 25(OH)D was not different in HT and control groups, and both were almost entirely vitamin-D deficient. 25(OH)D was not significantly correlated to TgAb or TSH levels, but it was positively correlated to TPOAb levels in males.

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Tables and Figures:

Demographic Characteristics of Subjects

	HT		Control		Total	
	Female	Male	Female	Male	Female	Male
Mean Age	48.7_a	47.2_a	46.0_a	45.2_a	47.5	46.2
Mean BMI	28.3_a	27.4_a	28.8_a	29.3_a	28.5	28.4
Count	60	37	45	43	105	80

Table 1: Mean age, mean BMI, and count of HT, control, and total subject groups. Values in the same row not sharing the same subscript are significantly different at $p<.05$.

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Serum Vitamin-D Levels

	HT		Control	
	Female	Male	Female	Male
25(OH)D Levels (ng/mL)	30.75_a	14.24_b	27.56_c	13.26_b

Table 2: Mean 25(OH)D levels in HT and control groups, separated by sex. Values in the same row not sharing the same subscript are significantly different at $p<.05$.

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Percent Frequencies of Vitamin-D Classifications

		HT		Control	
		Female	Male	Female	Male
Vitamin-D Classification	Deficient (<20 ng/mL)	0_b	35_a	0_b	40_a
		0.0%	94.6%	0.0%	93.0%
	Insufficient (20-29.9)	29_a	2_b	31_c	3_b
		48.3%	5.4%	68.9%	7.0%
	Sufficient (>30)	31_a	0_b	14_c	0_b
		51.7%	0.0%	31.1%	0.0%
Total		100.0%	100.0%	100.0%	100.0%

Table 3: Counts and percent frequencies of vitamin-D classifications in the HT and control groups, separated by sex. Values in the same row not sharing the same subscript are significantly different at $p < .05$.

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Thyroid Levels

		TgAb				TPOAb				TSH			
		Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
HT	Female	2	2194	304	484	6	3000	442	545	.	.	.	.
	Male	25	3000	500	873	32	1000	445	441	.	.	.	.
Control	Female	.	.	.	.	.	.	.	.	.60	4.00	1.76	.80
	Male	.	.	.	.	.	.	.	.	.57	4.00	1.63	.77

Table 4: Minimum, maximum, mean, and standard deviations of thyroid levels for the HT and control groups, separated by sex.

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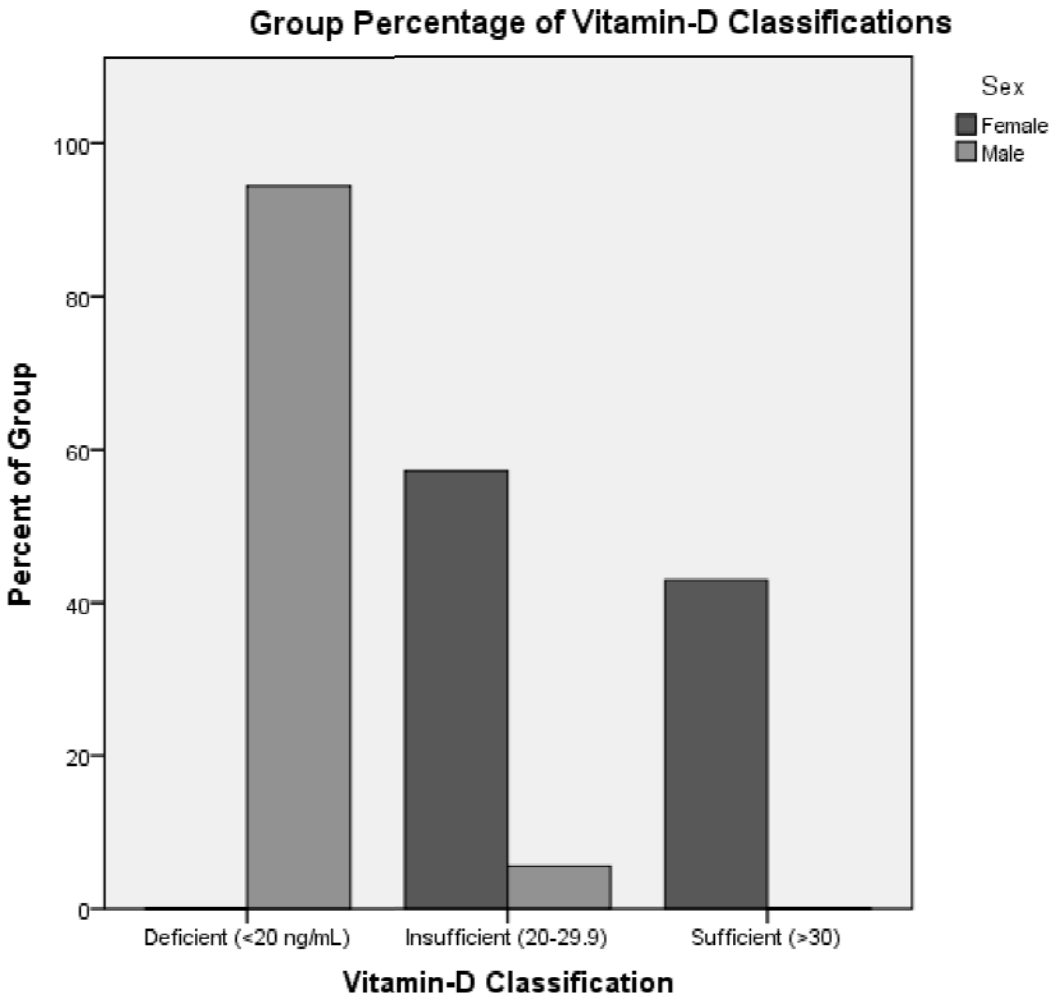

Figure 1: Group percentages of vitamin-D classifications, shown for sexes and not disease status.

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