Is vitamin D related to pathogenesis and treatment of Hashimoto's thyroiditis?

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Abstract

Objective: The aim of this study was to investigate vitamin D status by measuring serum 25(OH)D levels in euthyroid patients with Hashimoto's thyroiditis (HT) who lived and worked on the sunny island of Crete, Greece, and to evaluate whether vitamin D3 supplementation is beneficial for the management of HT patients with vitamin D deficiency. **Subjects and Methods:** We studied 218 HT patients, euthyroid Caucasian Cretan Greek citizens: 180 females and 38 males. Among these patients, 186 (85.3%) had vitamin D deficiency defined as serum 25(OH)D levels <30ng/mL. The mean age of all these 218 HT patients was 35.3±8.5 years. The mean age of the 186 vitamin D deficient HT patients (173 females and 13 males) was 37.3±5.6 years. The 186 vitamin D deficient HT patients received vitamin D3 (cholecalciferol, CF) orally, 1200-4000 IU, every day for 4 months aiming to maintain serum 25(OH)D levels ⩾40ng/mL. Anthropometric characteristics (height, weight, waist circumference), systolic and diastolic blood pressure, serum concentration of 25(OH)D, thyrotropin (TSH), free thyroxine (FT4), anti-thyroid peroxidase (anti-TPO), anti-thyroglobulin (anti-TG), calcium and phosphorus levels and thyroid and kidney sonographic findings were recorded and measured before and after CF administration. **Results:** There was a significant negative correlation only between serum 25(OH)D levels and anti-TPO levels among all 218 HT patients. Also, anti-TPO levels were significantly higher in 186/218 vitamin D deficient HT patients compared to 32/218 HT patients with no vitamin D deficiency (364±181IU/mL versus 115.8±37.1IU/mL, P<0.0001). Supplementation of CF in 186 vitamin D deficient HT patients caused a significant decrease (20.3%) in serum anti-TPO levels. Although at the end of the 4 months period of the study body mass index (BMI), serum anti-TG and TSH levels decreased by 2.2%, 5.3% and 4% respectively, these differences were not significant. No changes in the sonographic findings were observed. **Conclusions:** The majority (85.3%) of the Greek Caucasian patients with HT studied who lived and worked in Crete had low serum 25(OH)D levels inversely correlated with serum anti-TPO thyroid antibodies. After 4 months of CF supplementation in the 186 HT patients with vitamin D deficiency, a significant decrease (20.3%) of serum anti-TPO levels was found. These findings suggest that vitamin D deficiency may be related to pathogenesis of HT and that its supplementation could contribute to the treatment of patients with HT.

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Introduction

It has been suggested that vitamin D acts as an immunomodulator in autoimmune diseases such as Hashimoto's thyroiditis (HT) [1]. Although several studies have revealed low serum 25-hydroxyvitamin D (25(OH)D) levels, (this form it is mainly converted in the liver and the kidneys to the active form of $1,25(OH)_2D3$ and is commonly used as an indicator of vitamin D status) among patients with HT, it is unclear if this association is the result of the autoimmune disease process or part of its cause [1, 2]. Also, the effectiveness of vitamin D supplementation in patients with HT has not been investigated sufficiently. The aim of this study was to investigate the vitamin D status by measuring serum 25(OH)D levels in a sample of euthyroid HT patients who lived and worked on the sunny island of Crete, Greece, and to evaluate whether vitamin D3 supplementation was beneficial for the management of euthyroid HT patients with vitamin D deficiency.

Subjects and Methods

Our study population included 218 HT patients (180 females and 38 males; mean age 35.3±8.5 years), euthyroid (with or without medication) Caucasian Cretan Greek citizens

fattending the Outpatient Clinic of Internal Medicine of the Naval Hospital of Crete. Diagnosis of HT was made by determining elevated anti-thyroid peroxidase or/and anti-TPO, anti-TG) and ultrasound patterns (diffusely enlarged thyroid gland with a heterogeneous echo texture and occasionally hypervascularity on color Doppler study) suggestive of HT. Participants who were not euthyroid or had a history of another autoimmune disease, diabetes mellitus, malignancy, chronic renal or liver disease, metabolic bone disorders, primary hyperparathyroidism as well as those who were taking any medication that could affect 25(OH)D levels were excluded from the study. Anthropometric parameters of all HT patients consisting of height, body weight (BW), body mass index (BMI) and waist circumference (WC), as well as arterial blood pressure (BP), were measured and recorded. Waist circumference was measured in the upright position at the level of the umbilicus. Records were made of sitting systolic and diastolic blood pressure (two measurements averaged) with a sphygmomanometer by the auscultatory method, standing body height (measured without shoes to the nearest 0.5cm) with a rigid height meter and BW (without shoes and tunic) with a calibrated balance scale. Body mass index was calculated as the BW (kg) divided by the height (m) squared (kg/m^2). Overweight and obesity were defined as BMI $\geqslant$25kg/m^2 and $\geqslant$30kg/m^2, respectively [3]. Central obesity was defined as WC >94cm for men and >80cm for women [4]. Among imaging techniques, a thyroid and kidney ultrasound (US) performed by an experienced radiologist was used to confirm HT patterns and detect nephrolithiasis (kidney stones in urinary tract). Serum concentration of 25(OH)D, thyrotropin (TSH), free thyroxine (FT4), anti-TPO and anti-TG were measured in our HT patients with a chemiluminescent microparticle immunoassay method (CMIA) (Architect i1000 System®, Abbott, USA). Serum calcium (Ca) and phosphorus (P) levels were measured with a standard colorimetric assay. A 25(OH)D level <10ng /mL was considered as severe vitamin D deficiency, 10-19.9 ng/mL as moderate vitamin D deficiency, 20-29.9ng/mL as mild vitamin D deficiency, and $\geqslant$30ng/mL as vitamin D sufficiency. Subjects with anti-TPO >5.61IU/mL and anti-TG >4.11IU/mL were considered as "positive" for thyroid autoimmunity. Normal ranges for TSH and FT4 were 0.35-4.94μIU/mL and 0.7-1.48ng/dL, respectively.

Among the 218 HT patients, 186 (85.3%) had vitamin D deficiency. These 186 vitamin D deficient HT patients (173 females and 13 males; mean age 37.3±5.6 years) received 1200-4000IU vitamin D3 (cholecalciferol, CF) orally with meals (tabl D3 fix, Uni-Pharma) every day for 4 months, the aim being to achieve serum 25(OH)D levels $\geqslant$40ng/mL without hypercalcemia (serum calcium levels >11mg/dL). Cholecalciferol supplementation was based on the estimation that for every 100IU of vitamin D3 ingested the blood level of 25(OH)D increases by 1ng/mL [5]. Therefore an increase of 25(OH)D level from 20ng/mL to the minimum 40ng/mL required the ingestion of at least 2000IU of vitamin D3 a day. Serum Ca and P levels were measured at the beginning and at the end of each month, but anthropometric parameters (BW, BP, WC), thyroid US, and serum 25(OH)D, TSH, FT4, anti-TPO and anti-TG levels were measured at the beginning and at the end of the 4 months period of supplementation. Our study started in April 2014 and was completed in September 2014. After being given clear detailed explanations of the protocol and study aims, the patients gave written consent and were free to withdraw from the study at any time with no obligations. The study was conducted in accordance with the Declaration of Helsinki and was given approval by the Ethics committee of the medical research institute.

Statistical analysis

All parametric variables were given as mean±standard deviation (M±SD). Statistical analyses were performed using GraphPad Prism 3.0 (GraphPad Software, Inc., California, USA). The relations between continuous variables were investigated using Student's t test or Pearson's correlation analysis where applicable. Paired t tests were used to analyze mean differences between the initial and post 4 months values for all measured parameters. All P-values were two-tailed, and P-values below 0.05 were considered statistically significant.

Results

The baseline characteristics of the 218 euthyroid HT patients (median age: 35 years old, range: 24-56 years old) who participated in our study (120 smokers, 98 non-smokers) are presented in Table 1.

The majority (82.6%) of HT patients enrolled in our study were women. Also, the majority (90%) of these patients received a stable dose of L-thyroxine for at least one year, but the mean value of TSH was 2.3±1.6μIU/mL. All patients had anti-TPO positivity, but anti-TG positivity was found only in 16.5% (36 patients). Among the 218 HT patients, 50% (109/218) had moderate vitamin D deficiency, 28.4% (62/218) had mild vitamin D deficiency, 6.9% (15/218) had severe vita-min D deficiency, and 14.7% (32/218) had vitamin D sufficiency. So, the total prevalence of vitamin D deficiency in our study population was 85.3% (186/218). There was a significant negative correlation only between serum 25(OH)D levels and anti-TPO (Pearson r=-0.43, P<0.00001) levels in our study population, which means that anti-TPO thyroid antibodies tended to be higher with lower serum 25(OH)D levels. There was no correlation between serum 25(OH)D and BMI, anti-TG, TSH or FT4 levels. When the vitamin D deficient (n=186) and sufficient (n=32) HT patients of our study population were compared, only anti-TPO levels were found to be significantly higher in the vitamin D deficient group (364±181IU/mL versus 115.8 ±37.1IU/mL, P<0.0001). There was no significant difference in anti-TG, TSH, FT4 and BMI between vitamin D deficient and sufficient HT patients. All the 186 vitamin D deficient HT patients (173 females/13 males, mean age 37.3± 5.6 years) who were receiving a stable dose of L-thyroxine for at least one year achieved the target of $\geqslant$40ng/mL for serum 25(OH)D level after the 4months CF supplementation. Among these 186 vitamin D deficient HT patients who received CF supplementa-

Table 1. *Baseline characteristics of our study population (218 HT patients).*

Anthropometric values	
BMI (kg/m^2)	26.9±4.6
<18.5	0
18.5-24.9	142
25.0-29.9	64
⩾30.0	12
WC	91.6±5.6
Central obesity	78 (35.8%)
Blood pressure	
SBP (mmHg)	128±4.6
DBP (mmHg)	75.9±4.3
Serum 25(OH)D	18.4±6.3
Sufficiency (≥30ng/mL)	32(14.7%)
Mild deficiency (20-29.9ng/mL)	62 (28.4%)
Moderate deficiency (10-19.9ng/mL)	109 (50%)
Severe deficiency (<10ng/mL)	15 (6.9%)
TSH (μIU/mL)	2.3±1.6
FT4 (ng/dL)	1.1±0.3
Anti-TPO (IU/mL)	296.7±115
Anti-TG (IU/mL)	13.8±8
Anti-TPO positivity	218 (100%)
Anti-TG positivity	36 (16.5%)

Values are expressed as mean ± standard deviation (M±SD) and percentage %. Anti-TPO: anti-thyroid peroxidase antibodies; Anti-TG: anti-thyroglobulin antibodies; BMI: body mass index; DBP: diastolic blood pressure; FT4: free thyroxine; SBP: systolic blood pressure; TSH: thyrotropin; WC: waist circumference.

tion, there were significant differences only between serum 25(OH)D levels as well as between anti-TPO levels, which were measured at the base and the end of the 4 months intervention period. Particularly, CF supplementation resulted in a significant increase in serum 25(OH)D levels from 14.6±7.2ng/mL to 45.7±4.3ng/mL (or 213%, P<0.0001) and a significant reduction in serum anti-TPO levels from 364±181IU/mL to 290±116IU/mL (or -20.3%, P<0.0001) (Table 2).

Although BMI, serum anti-TG and TSH levels decreased at the end of the 4 months intervention period by 2.2% (from 27.4±3.7kg/m2 to 26.8±4.3kg/m2), 5.3% (from 16.8±7.3IU/mL to 15.9±5.4IU/mL) and 4% (from 2.5±1.7μIU/mL to 2.4±1.5μIU/mL) respectively, these differences were not significant (P=0.15, P=0.18 and P=0.54, respectively) (Table 2). Also, there were no significant differences in serum FT4 and calcium and phosphorus levels before and after CF supplementation. Also, no changes in the sonographic findings were observed among 186 vitamin D deficient HT patients after CF supplementation. No side effects, discomfort or any other complaints were reported by any of the supplemented HT patients.

Discussion

Our study showed a high prevalence (85.3%) of vitamin D deficiency [serum 25(OH)D levels <30ng/mL] among euthyroid HT patients, such as an inverse correlation between serum 25(OH)D and anti-TPO thyroid antibodies. This inverse correlation in a study performed during the spring and summer season among patients who lived and worked in Crete (long multi-day sun exposure), does not clarify if this association is the result of the autoimmune disease process or part of its cause. Considering unpublished data of a study in process which have revealed low serum 25(OH)D levels even in healthy Cretan individuals exposed to sunlight every day for many hours (for instance farmers) as well as the known high prevalence of HT in Crete [6, 7], we could speculate a possible casual relation between vitamin D deficiency and the development of HT in our study population. Moreover, if vitamin D was not associated with the pathogenesis of HT, CF supplementation in our patients would probably have no effect on serum anti-TPO thyroid antibodies. Several studies, as ours, have shown low serum 25(OH)D levels in patients with HT indicating an association between vitamin D deficiency and thyroid autoimmunity [1, 2]. In a recent study [8], the prevalence of vitamin D deficiency [serum 25(OH)D levels <10ng/mL] was significantly higher in patients with autoimmune thyroid disease (AITD) compared with healthy individuals (72% versus 30.6%; P<0.001) as well as in patients with HT compared to patients with non-AITD (79% versus 52%; P<0.05). Low levels of 25(OH)D were also related to the presence of antithyroid antibodies and abnormal thyroid function tests [8], suggesting the involvement of vitamin D in the pathogenesis of AITD and the advisability of supplementation. In another study [9], the prevalence of vitamin D insufficiency (serum 25(OH)D levels <30ng/mL) in

Table 2. *Impact of CF supplementation on the studied variables of 186 vitamin D deficient HT patients.*

Variables	25(OH)D3 (ng/mL)	Anti-TPO (IU/mL)	Anti-TG (IU/mL)	BMI (kg/m^2)	TSH (μIU/mL)
Total (n=186)					
Initial	14.6±7.2	364±181	16.8±7.3	27.4±3.7	2.5±1.7
Final	45.7±4.3	290±116	15.9±5.4	26.8±4.3	2.4±1.5
Change (%)	213	-20.3	-5.3	-2.2	-4
P value	<0.0001	<0.0001	0.18	0.15	0.54

Data are expressed as mean±SD (M±SD) and percentage %. Anti-TPO: anti-thyroid peroxidase antibodies; Anti-TG: anti-thyroglobulin antibodies; BMI: body mass index; P: significance value, TSH: thyrotropin.

HT cases (148 of 161, 92%) was significantly higher than that observed in healthy controls (102 of 162, 63%; P<0.0001). Bozkurt et al. (2013) demonstrated that serum 25(OH)D levels of HT patients were significantly lower than controls (12.2± 5.6ng/mL versus 15.4±6.8ng/mL, P<0.001) and 25(OH)D deficiency severity was correlated with the duration of HT, thyroid volume and antibody levels [10]. In this study [10], 98.1% (530/540) of patients with HT had serum 25(OH)D levels <30ng/mL. In another study, serum 25(OH)D levels were an independent factor affecting the presence of anti-TPO in AITD [11]. A recent meta-analysis of twenty case-control studies revealed that AITD patients (patients with HT or Grave's disease) compared to healthy control individuals had lower levels of 25(OH)D and were more likely to be deficient in 25(OH)D, suggesting a role of vitamin D deficiency in the pathological process of AITD [2]. However, other studies failed to find an association between vitamin D deficiency and thyroid autoimmunity [2, 12-14]. The mechanisms underlying the assumption that vitamin D is linked with autoimmunity are not clear and are probably associated with its anti-inflammatory and immunomodulatory functions [1, 2]. However, some researchers argue that vitamin D insufficiency in AITD is the result of the pathogenesis of AITD and its subsequent effect, such as vitamin D receptor dysfunction [15, 16]. Also, we must keep in mind that several HT patients have a comorbid autoimmune disorder, thyroid hypofunction, or other causes which could affect the production, absorption and utilization of vitamin D, despite adequate exposure to sunlight [1]. The controversial and varying results of studies on the association between vitamin D and HT could be explained mainly by the interassay and interlaboratory variability in measurements of 25(OH)D, the cut-off for defining vitamin D deficiency and the method of HT diagnosis.

In our study, CF supplementation among vitamin D deficient HT patients, which increased serum 25(OH)D levels at the target of ≥40ng/mL, decreased anti-TPO levels by 20.3%. This reduction, although relatively small, is important considering that anti-TPO antibodies contribute to thyroid cells destruction by C3 complement activation and antibody-dependent cell cytotoxicity [17]. Considering that our study population was euthyroid (all vitamin D deficient HT patients were receiving a stable dose of L-thyroxine, which mildly suppresses the serum concentration of autoantibodies [18-20], for at least one year), we can speculate that the reduction percentage of anti-TPO was mainly due to CF supplementation. This finding raises the question of whether the administration of CF is beneficial in the treatment of patients with HT or AITD. The beneficial effect of vitamin D supplementation on treatment or prevention of AITD has been demonstrated only in two experimental animal studies [21]. The known available studies in humans are also few. The preliminary results of a small randomized controlled trial showed that CF treatment (single oral dose of 450.000IU) decreased significantly serum anti-TPO and anti-TG levels compared with placebo treatment in HT patients (16 HT patients received CF and 12 HT patients received placebo) [22]. In this study [22], serum anti-TPO and anti-TG levels, decreased by 56.1% (from 266.27IU/mL to 116.88IU/mL) and 48.3% (from 226.27IU/mL to 116.88IU/mL) after nine months of CF supplementation respectively. Data from two Master Dissertations (contacted at the Jilin University and Zhengzhou University of China) have shown that combining vitamin D with anti-thyroid drugs or thyroid hormone contributes to the treatment of AITD by suppressing the autoimmune reaction and reducing serum levels of thyroid autoantibodies [2, 23, 24]. However, the beneficial role, the target and cost-effectiveness of CF supplementation in HT or AITD patients, as well as its optimal safe doses, require further investigation. It sho-uld be noted that most studies have shown no vitamin D toxicity in adults with vitamin D intake below 30.000/day and serum 25(OH)D levels below 200ng /mL [25]. Moreover, according to the US Endocrine Society guideline, all vitamin D deficient adults should be treated wi-

th 50.000IU of vitamin D2 or vitamin D3 once a week for 8 weeks or its equivalent of 6000IU of vitamin D2 or vitamin D3daily to achieve a blood level of 25(OH)D above 30ng/mL, followed by maintenance therapy of 1500-2000IU daily [26]. However, vitamin D supplementation for the treatment of patients with HT is not recommended in the US Endocrine Society guideline, obviously due to the lack of relevant clinical trials performed in humans.

Our study also showed that 4 months CF supplementation in 186 vitamin D deficient HT patients decreased BMI insignificantly by 2.2kg/m2. Although several studies have revealed a BW loss after vitamin D supplementation, this loss appears to be insignificantly affected by vitamin D supplementation in most of these studies [27-29]. Vigna et al. (2015) showed that 6 months supplementation with 25.000IU and 100.000IU CF per month with a balanced moderately low calorie diet in obese/overweight patients significantly decreased BW by 3.8kg and 5.4kg, respectively, compared to no supplementation (-1.2kg) [29]. Also, supplementation with vitamin D coupled with exercise or mild caloric restriction has been shown to improve markers of fitness and inflammation [30, 31]. The varying results of studies on the degree of impact of vitamin D supplementation on BW among studies could be explained mainly by the different profiles of patients and underlying diseases, the different doses of administered vitamin D, and the different targets pursued for serum 25(OH)D. To our knowledge, available data for the change of BW after CF supplementation in HT patients do not exist. In our study, the percentage of overweight and obese patients was relatively low, their dietary habits did not change during the 4 months supplementation, and therefore the change of BMI was expected to be insignificant.

Despite the beneficial effects of 4 months CF supplementation on serum anti-TPO, an ultrasound change of thyroid pattern among HT patients was not revealed. This finding was not surprising considering that although a US is a widely available, noninvasive, and low-cost technique, is not correlated with a short improvement of inflammatory activity, which the reduction of serum anti-TPO (statistically significant), anti-TG (statistically not significant) and TSH (statistically not significant) levels could express. Furthermore, it is known that many HT patients (diagnosis with fine needle aspiration biopsy/FNAB) may have positive sonographic thyroid findings highly suggestive of HT, without circulating autoantibodies [32]. Also, anti-TG are found in over 75% of patients with HT compared to 10%-30% of asymptomatic individuals, but anti-TPO are found in up to 20% of asymptomatic individuals, particularly the elderly, and over 90% of patients with HT [33-36]. In our view, only FNAB of thyroid gland after a sufficient time period, and not these reductions, could prove a definite improvement of the histopathological state (lymphocyte infiltration) of the thyroid gland due to CF supplementation.

Despite the limitations of the present study being: lack of control group, no blind protocol, no histological evidence of HT, this study uncovered an important effect of CF supplementation on serum anti-TPO levels in vitamin D deficient HT patients.

In conclusion, we have shown that the majority (85.3%) of our Greek patients with HT residing and working on the island of Crete had low serum 25(OH)D levels inversely correlated with serum anti-TPO thyroid antibodies. Also, our study revealed that 4 months CF supplementation in these patients caused a significant decrease (20.3%) in serum anti-TPO levels. These findings suggest that vitamin D deficiency may be related to pathogenesis of HT and that its supplementation could contribute to patients' with HT treatment. However, further studies specifically designed to evaluate the beneficial effect and the cost-effectiveness of vitamin D supplementation on HT are needed.

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The authors declare that they have no conflicts of interest

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