

The relationship between subclinical hypothyroidism and vitamin D

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ABSTRACT

Aim: Vitamin D (vitD) is primarily responsible for bone formation and mineralization. However, in recent years, it has been suggested that vitD may play a role as an immune modulator in the development of numerous diseases, including autoimmune diseases. It has been observed that there is an association between chronic autoimmune thyroiditis (AIT) and vitD levels. This study aims to investigate whether there are differences in the levels of 25-hydroxy vitamin D [25(OH)D], calcium, and phosphorus in patients with subclinical hypothyroidism (SCH) due to AIT, in patients with antibody-negative subclinical hypothyroidism (ANSCH), and in healthy control subjects.

Material and Method: Data from 50 newly diagnosed patients with SCH (35 of whom AIT) and 50 euthyroid and antibody-negative healthy controls who presented to the Department of Endocrinology and Internal Medicine at our hospital between 2018 and 2020 were retrospectively reviewed. Calcium, phosphorus, and 25(OH)D levels of patients and controls were compared.

Results: Serum 25(OH)D levels were significantly lower in patients compared to controls (16.2±7.8 ng/ml and 20.4±8.2 ng/ml, respectively; p=0.024). Serum levels of calcium (p=0.081) and phosphorus (p=0.712) did not differ between groups. In a subgroup analysis, patients with AIT had significantly lower 25(OH)D values than controls (p=0.009). Compared to controls, 25(OH)D levels were comparable in the ANSCH group (p=0.096). 25(OH)D level was higher in the AIT group than in the ANSCH group (p=0.01).

Conclusion: Our results show that patients with SCH have lower 25(OH)D levels than healthy controls. However, this difference is significant in patients with AIT. It is recommended to screen for vitD deficiency in patients with SCH due to AIT.

Keywords: Chronic autoimmune thyroiditis, 25-hydroxy vitamin D, subclinical hypothyroidism.

INTRODUCTION

Vitamin D (vitD) deficiency is a common health problem in our country and around the world (1). VitD is a steroid hormone whose main function is the formation and mineralization of bone and the balance of calcium (Ca) and phosphorus (P) in the body. However, in recent years, vitD has been shown to play a role as an immune modulator in the development of numerous diseases such as autoimmune diseases, heart disease, cancer, inflammatory bowel disease, diabetes, and rheumatic diseases (2,3). In addition, patients with autoimmune thyroid disease (AITD), including Graves' disease and autoimmune thyroiditis (AIT) have been reported to have lower vitD levels (4). Both vitD and thyroid hormones bind to similar steroid hormone receptors and vitD receptor gene polymorphism has been found to be

associated with AITD (5). Studies have demonstrated vitD deficiency in hypothyroidism (4,5). It is suggested that the associated mechanism is inadequate uptake of vitD from the gut, or the body may not activate vitD properly (5) and also autoimmunity in AITDs (4,6). The vit D status of an individual is usually determined by measuring the 25-hydroxyvitamin D level [25(OH)D] because it has a long half-life and its level is regulated within a very narrow range by parathyroid hormone, Ca, and P. If the 25(OH)D level is below 20 ng/ml, vitD deficiency exists (7-9). A serum level of thyroid stimulating hormone (TSH) above the established upper limit of the reference range and a serum level of free thyroxine (fT4) within the reference range is termed subclinical hypothyroidism (SCH) (4). Globally, subclinical hypothyroid dysfunction is more common than overt disease (10). Limited studies discussed the vitD levels in SCH (11). This study aims to investigate

whether there are differences in 25(OH)D, Ca, and P in patients with SCH due to AIT, in patients with antibody-negative subclinical hypothyroidism (ANSCH), and in healthy controls. In addition, we aimed to observe whether there is a relationship between anti-thyroid peroxidase (anti-TPO) and vitD levels.

MATERIAL AND METHOD

This retrospective study was conducted in University of Health Sciences Antalya Training and Research Hospital, Department of Internal Medicine and Endocrinology. The Ethics Committee of the University of Health Science Antalya Training and Research Hospital approved the study protocol (Date 27.05.2021, Decision No. 7/2). All procedures were performed in accordance with the ethical rules and principles of the Declaration of Helsinki.

Data from patients treated between January 2018 and January 2020 were studied.

The inclusion criteria were as follows:

- 18-70 years old
- Patients with newly diagnosed SCH
- Patients whose history, physical examination, and laboratory tests including TSH, fT4, fT3 (free triiodothyronine), thyroid antibodies, 25(OH)D, Ca, and P were accessible
- Patients in whom thyroid ultrasound had been performed
- Patients who had no history of cardiovascular disease, cancer, osteomalacia, other bone diseases, diabetes mellitus, obesity, inflammatory bowel disease or rheumatologic diseases
- Patients who had no history of levothyroxine use.
- Patients who were not taking Ca or vitD supplements

The exclusion criteria were as follows:

- <18 years old or >70 years old
- Patients with known pre-existing conditions such as cardiovascular disease, malignancies, osteomalacia, other bone diseases, diabetes mellitus, obesity, chronic inflammatory diseases including inflammatory bowel disease, rheumatologic diseases etc.
- Patients with overt hypothyroidism
- Patients who have already been diagnosed with SCH and treated with levothyroxine
- Patients taking Ca or vitamin D supplements
- Smokers or alcoholics

Because the upper limit for TSH in our hospital is 5.6 uIU/ml, SCH was defined as a TSH level of 5.6-10 mU/l when the fT4 concentration was normal. The diagnosis of AIT was based on the positivity of thyroid antibodies (anti-TPO and, if negative, with anti-thyroglobulin antibodies

(anti-TG)) and typical ultrasound findings including pseudonodular appearance and hypoechogenic pattern. When anti-TPO is positive, we do not routinely measure anti-TG; therefore, only anti-TPO results were collected and recorded in these patients. Patients with SCH, in whom both anti-TPO and anti-TG were negative, were included in the ANSCH group. Fifty patients with newly diagnosed SCH (35 of whom had AIT) who met the inclusion and exclusion criteria were included in the study. Fifty control subjects aged 18-70 years with no acute or chronic disease, no medications, and no history of thyroid disease with normal TSH, fT3, fT4, anti-TPO, and anti TG levels were included in the study. The TSH, anti-TPO, fT3, fT4, 25(OH)D, Ca, P, and albumin levels of patients and controls were recorded. Because all patients with AIT had anti-TPO positivity, we could not obtain their anti-TG results, so anti-TG was not included in our analysis. For patients with low serum albumin levels, Ca levels were corrected according to the following formula: Corrected Ca (mg/dL)=measured total Ca (mg/dL) + 0.8 (4-patient albumin). The cut-off value for anti-TPO antibodies was assumed to be 10 IU/mL according to the cut-off value of our laboratory. Ca, P, albumin, and other biochemical assays were analyzed by conventional spectrophotometric methods using commercial kits from Beckman Coulter on a Beckman Coulter AU5800 (Beckman Coulter Inc. CA, USA) autoanalyzer. 25(OH) D, TSH, fT3, fT4, and other necessary hormone tests were analyzed on a Beckman Coulter DxI800 (Beckman Coulter Inc. CA, USA) using chemiluminescence methods.

Statistical Analysis

Descriptive statistics were used to determine continuous variables (mean±standard deviation and median, minimum and maximum). The Shapiro-Wilk test was used to determine if the parameters were normally distributed. Statistical analysis was performed using Student's t test and one-way test ANOVA, followed by Tukey's test for multiple comparisons. The Spearman correlation test was used for correlation analyzes. A 'p' value of less than 0.05 was considered statistically significant. Data analysis was performed using IBM SPSS version 20.

RESULTS

The mean age of the patients was 36±8 years and 35 were female. The mean age of the control group was 34±9 years and 33 were female. There was no statistically significant difference between patients with SCH and the control group in terms of gender and age (p=0.741 and p=0.987, respectively). As expected, TSH level was significantly higher in the study population compared to the control group (7.1±1.4 µIU/ml and 2.1±0.7 µIU/ml, respectively;

$p=0.006$). The mean 25(OH)D level was 16.2 ± 7.8 ng/ml in the SCH group and 20.4 ± 8.2 ng/ml in the euthyroid control group. Serum 25(OH)D level was significantly lower in patients than in controls ($p=0.024$). The mean serum Ca level was 9.1 ± 0.6 mg/dl in the SCH patients and 9.3 ± 0.5 mg/dl in the controls ($p=0.081$). Serum P level did not differ between groups ($p=0.712$). The comparison of study parameters between the study and control groups is summarized in **Table 1**. Patients in the SCH group were divided into the AIT group ($n=35$) and the ANSCH group ($n=15$). All patients in the AIT group were anti-TPO positive. In the ANSCH group, both anti-TPO and anti-TG were negative. A one-way ANOVA test was used to compare these two subgroups and the control group (**Table 2**). There was no statistical difference between the groups in terms of sex ($p=0.860$), age ($p=0.145$), Ca ($p=0.126$), and P ($p=0.324$). There was a statistically significant difference between groups in terms of TSH ($p=0.005$), anti-TPO ($p=0.0001$) and 25(OH)D ($p=0.012$). According to post-hoc analyzes performed to determine the source of significance between groups, TSH level did not differ between AIT and ANSCH patients ($p=0.08$). TSH level was significantly higher in the AIT group ($p=0.001$) and in the ANSCH group ($p=0.006$) than in controls. The 25(OH)D level was similar between the control group and the ANSCH group ($p=0.096$). The 25(OH)D level was lower in the AIT group than in the control group ($p=0.009$). The 25(OH)D level was lower in the AIT group than in the ANSCH group ($p=0.01$) (**Table 2**). In the whole study group, there was an inverse correlation between 25(OH)D and TSH ($r=-0.241$, $p=0.004$) and anti-TPO ($r=-0.387$, $p=0.001$).

Table 1. Study Parameters between subclinical hypothyroid patients and control group

Parameters	Subclinical hypothyroid patients n (50)	Control group n (50)	p
Male n (%)	15 (30)	17 (34)	0.741
Female n (%)	35 (70)	33 (66)	
Age (yrs.)	36 ± 8	34 ± 9	0.987
TSH (μ IU/ml)	7.1 ± 1.4	2.1 ± 0.7	0.006*
fT3 (ng/dl)	2.9 ± 0.6	2.8 ± 0.3	0.843
fT4 (ng/dl)	1.3 ± 0.3	1.1 ± 0.2	0.921
25(OH)D (ng/ml)	16.2 ± 7.8	20.4 ± 8.2	0.024*
Calcium (mg/dl)	9.1 ± 0.6	9.3 ± 0.5	0.081
Phosphorus (mg/dl)	3.9 ± 0.3	3.8 ± 0.2	0.136

fT3: Free triiodothyronine; fT4: Free thyroxine; 25(OH)D: 25-hydroxy vitamin D, * $p<0.05$ is statistically significant

DISCUSSION

Our study showed that vitD levels were lower in patients with SCH due to AIT than in the control group. TSH levels were similar in the AIT and ANSCH groups, and vitD levels were lower in the AIT group than in patients with ANSCH. In addition, a negative correlation was observed between vitD levels and anti-TPO. Numerous

Table 2. Study Parameters between patients with subclinical hypothyroidism due to chronic autoimmune thyroiditis, patients with antibody negative subclinical hypothyroidism and control subjects

Parameters	SCH due to AIT n (35)	ANSCH n (15)	Control n (50)	P
Male n (%)	10 (28.5)	5 (33.3)	17 (34)	0.860
Female n (%)	25 (71.5)	10 (66.7)	33 (66)	
Age (yrs.)	36 ± 8	35 ± 10	34 ± 9	0.145
TSH (μ IU/ml)	7.3 ± 1.2	6.8 ± 0.7	2.1 ± 0.7	0.005* [§]
Anti-TPO (IU/ml)	215 (108-420)	1.2 (0.2-4.6)	0.9 (0.2-4.2)	0.0001*
25(OH)D (ng/ml)	14.2 ± 5.8	18.3 ± 6.1	20.4 ± 8.2	0.012**
Calcium (mg/dl)	8.9 ± 0.7	9.2 ± 0.4	9.3 ± 0.5	0.126
Phosphorus (mg/dl)	3.9 ± 0.5	3.8 ± 0.3	3.8 ± 0.2	0.324

SCH: Subclinical hypothyroidism; AIT: Chronic autoimmune thyroiditis; ANSCH: Antibody negative subclinical hypothyroidism; Anti-TPO: Anti-Thyroid Peroxidase; 25(OH)D: 25-hydroxy vitamin D, *One-Way ANOVA results of 3 groups, $p<0.05$ is statistically significant. + Statistical analysis was performed by ANOVA followed by Tukey's multiple comparison test, $p=0.096$ control group Vs ANSCH group; $p=0.009$ control group Vs AIT group; $p=0.01$ AIT group Vs ANSCH group & Statistical analysis was performed by ANOVA followed by Tukey's multiple comparison test, $p=0.08$ AIT group Vs ANSCH group; $p=0.001$ control group Vs AIT group; $p=0.006$ control group Vs ANSCH group.

studies suggest a relationship between AITD and vitD deficiency, considering how vitD regulates inflammatory response and autoimmunity (12-16). Previous research has found that vitD deficiency is one of the features of AITDs, particularly Hashimoto's thyroiditis (HT), and may cause the autoimmune process that leads to HT and hypothyroidism (16). In the study by Tamer et al. (13), 92% of patients with HT had vitD deficiency, and vitD deficiency was significantly higher in patients with HT than in healthy controls. In this study, vitD levels were similar in euthyroid and hypothyroid HT patients. Shin et al. (14) observed lower vitD levels in patients with AITD compared with patients without AITD in their study of 304 patients. They did not show a difference in vit D levels related to thyroid function. Mazokopakis et al. (15) showed inversely relation between anti-TPO and 25(OH)D levels in HT. Bozkurt et al. demonstrated that the severity of 25(OH)D deficiency correlated with the duration of HT, thyroid volume, and antibody levels, suggesting a possible role of 25(OH)D in the development of HT and progression of hypothyroidism (16). Our study also confirms the literature and shows that AIT and VitD deficiency are related independently of thyroid hormone levels. The relationship between vitD and autoimmunity is not clearly established but is likely related to its anti-inflammatory and immunomodulatory functions (15,19). Expression of the nuclear vitD receptor (VDR) and the vitD-activating enzyme 1α -hydroxylase (CYP27B1) has been detected in most immune cells, leading to the idea that vitD may play a role in the pathogenesis of the immune system and autoimmune diseases (20-23). $1-25(\text{OH})\text{D}$ suppresses B cell proliferation, immunoglobulin production, and differentiation into plasma cells and promotes the apoptosis of B cells (20). In the $\text{CD4} + \text{T-cell}$ response, vitD directly inhibits the production of Th1 cytokines

(IL2 and IFN- γ) and increases the production of Th2 cytokines (IL-4) (24). In light of these data, recent studies have suggested that vitD supplementation may improve autoimmunity. These studies reported a decrease in anti-TPO levels after cholecalciferol supplementation in HT patients with vitD deficiency (15,25). Mazokopakis et al, reported a significant decrease in anti-TPO levels after 4 months of oral vitD supplementation in patients with vitD deficiency (15). Another study from our country showed that vitD treatment can reduce the development of hypothyroidism in patients with HT (26). Some literature studies have shown that hypothyroidism is associated with vitD deficiency in addition to autoimmunity (4-6). Kim D found that patients with overt hypothyroidism had lower 25(OH)D levels than euthyroid individuals or patients with SCH with or without HT. In this study, researchers found that lower serum 25(OH)D levels were associated with higher TSH levels (6). A few studies have also found an inverse association between 25(OH)D and TSH levels or disease severity in HT, suggesting a link between poor vitD status and progressive thyroid destruction (6,16,27,28). We did not include patients with overt hypothyroidism in this study, so we can not definitively state whether there is a relationship between TSH and vitD. However, we found that vitD levels in SCH patients without AIT were similar to those in euthyroid healthy controls, and SCH patients with positive antibody had lower vitD levels than SCH patients with negative antibody.

The limitations of the study are the small number of patients and its retrospective nature. There may be missing data because the medications taken by the patients and concomitant diseases are recorded retrospectively via the computer system. Patients not currently taking vitD were included in the study, but if they had recently taken vitD and then stopped, this may have affected patients' vitD levels. Seasonal variation in blood collection may have affected the results because patient samples were not collected in a single season. We did not include euthyroid and overt hypothyroid patients with AIT in the study. If we included this group of patients, the association between vitD and hypothyroidism and autoimmunity could have been more clearly demonstrated.

CONCLUSION

Our results show that patients with SCH are more likely to have lower vitD levels than the control population, and this finding was statistically significant in anti-TPO(+) patients. VitD levels can be measured in patients with SCH and anti-TPO positivity. New studies are needed to investigate the effects of vitamin D deficiency on bone and other systems in this patient population.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval of the University of Health Sciences Antalya Training and Research Hospital Clinical Researches Ethics Committee (Date: 27.05.2021, Decision No: 7/2).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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