NO RELATIONSHIP BETWEEN 25 OH VITAMIN D AND AUTOIMMUNITY IN WOMEN PATIENTS WITH HYPOTHYROIDISM

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ABSTRACT

Introduction: *Hypothyroidism is a common disease in the society. Based on the effects of vitamin D on immune system and endocrine system, the relationship between hypothyroidism and vitamin D gained importance. The relationship between vitamin D deficiency and thyroid was suggested for the first time by Kivity and his colleagues. In their study, they found a significant relationship between thyroid autoantibodies and vitamin D levels. In this study we want to show the relationship between autoimmunity and vitamin D deficiency in female patients with hypothyroidism.*

Materials and methods*: This study was performed at the GOP Taksim Education and Research Hospital outpatient department of internal medicine between January and June in 2015. It included 80 females patients, and 18 control females subjects*

Results*: A total of 98 participants were included in this study. The mean durations of hypothyroidism are 5,86±5,91 vs. 5,43±5,62 years. Anti-thyroid autoantibodies (Anti TPO) and anti-thyroglobulin antibodies (anti TG) levels were significantly higher in the group 1 patients compared to the group 2 patients and control subjects (p<0,01). There were no significant differences at other variables between the patients and control subjects. The serum 25-OH vitamin D levels were significantly lower in the Hashimoto thyroiditis and Non-Hashimoto thyroiditis patients compared to the healthy controls (10,88±9,19 ng/ml vs. 10,53±6,87 ng/ml, p<0.01).*

Conclusion*: It was thought that the study's being conducted during the winter months might have affected vitamin D levels. The vitamin D value of the control group cases was found to be higher as compared to the case of hypothyroidism associated with Hashimoto's thyroiditis and of hypothyroidism associated with other reasons. No significant difference was observed in vitamin D levels between the groups of hypothyroidism associated with Hashimoto's thyroiditis and hypothyroidism associated with other reasons*

Key words*: vitamin D, hypothyroidism Hashimoto's thyroiditis.*

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Introduction

Vitamin D is a fat-soluble secosteroid hormone and plays an important role in bone and mineral homeostasis[1]. In addition to its classic function of calcium homeostasis and as a result its effects on bone formation, vitamin D is a hormone known to play a role in endocrine, autocrine, paracrine, regulation of gene expression, cell differentiation and proliferation[2,3].

Deficiency of vitamin D is known to play a role in occurrence of rheumatoid arthritis, multiple sclerosis, autoimmune diseases, diabetes, inflammatory bowel disease, many cancer types and heart diseases[4-6]. Presence of vitamin D receptors (VDR) in peripheral blood cells revealed the role of vitamin D on immune system[7, 8].

Hypothyroidism is a common disease in the society.

Hashimoto's thyroiditis prevalence 8 to 15 times higher in women than men. Although it is seen in all ages ,Most commonly seen in women aged 30 to 60 years old.[9]. Based on the effects of vitamin D on immune system and endocrine sys-

tem, the relationship between hypothyroidism and vitamin D gained importance. The relationship between vitamin D deficiency and thyroid was suggested for the first time by Kivity and his colleagues. In their study, they found a significant relationship between thyroid autoantibodies and vitamin D levels[10]. In this study, we want to show the relationship between autoimmunity and vitamin D deficiency in female patients with hypothyroidism.

Materials and methods

This study was performed at the GOP Taksim Education and Research Hospital outpatient department of internal medicine between January and June in 2015. It included 80 females patients, and 18 control females subjects.

Exclusion criteria included any of the following conditions: metabolic bone disorders, primary hyperparathyroidism, liver disorders, renal disorders, diabetes mellitus, malignancy, taking oral contraceptive, anti-epileptic, vitamin D and/or calcium supplements. All participants were evaluated for thyroid function, thyroid autoimmunity, and ultrasonographic features. Hypothyroidism patients were classified into three subgroups according to their thyroid function status and thyroid autoimmunity.

Group 1 included Hashimoto's thyroiditis patients (evidenced by autoimmune features or elevated antithyroid peroxidase or antithyroglobulin antibodies), group 2 included non - Hashimoto's thyroiditis patients (no evidence of autoimmunity) and group 3 included healthy individuals In addition, we divided hypothyroidism patient groups into three subgroups: Clinic Hypothyroidic Patients(CHP), Subclinical Hypothyroidic Patients (SHP), and Euthyroid with Hypothyroidism Patients (EP). The study protocol was approved by GOP

Taksim Research and Education Hospital ethics committee, Istanbul.

Body mass index (BMI) was obtained using the formula weight (kg)/height (m)2. Fasting plasma samples were obtained from the patient and the control group. For assessment of thyroid parameters, levels of free thyroxine (FT4), free triiodothyronine (FT3), thyroid-stimulating hormone (TSH), anti-thyroglobulin antibodies (TG-Ab), and antithyroid peroxidase antibody (TPO-Ab) were determined by electrochemiluminescence immunoassay using commercial kits from Roche (Cobas E411, Roche, Japan). The intraassay coefficients of variation (CVs) were 3.5% for FT4, 2.8% for FT3 and 8.7% for TSH. The intra- and inter assay variability of anti-TPO were 3,2% and 6,3% respectively and anti-TG were 7.2 % and 8.7%, respectively. Serum cholesterol, triglyceride, and high-density lipoprotein cholesterol (HDL-C), albumin, parathormone, calcium, and phosphorus were measured by enzymatic colorimetric methods with commercially available kits (COBAS 311, Roche Diagnostics GmbH, Mannheim, Germany) and low-density lipoprotein cholesterol C (LDL-C) was calculated according to the Friedewald formula. Serum 25-hydroxyvitamin D (25(OH)D) was measured with an enzyme immunoassay kit. According to guidelines, vitamin D status was estimated by measuring serum 25(OH)D. 25(OH)D level lower than 20ng/ml was defined as a vitamin D deficiency .25(OH) D level of 21-29 ng/ml was considered insufficiency of vitamin D[11]

Statistical analyses

The NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for statistical analyses. While analyzing the study data, in the comparison of quantitative data, Student's test was used in the comparison of two groups that exhibited normal distribution, and Mann Whitney U test was used in the comparison of two groups that didn't exhibited normal distribution, as well as descriptive statistical methods (mean, standard deviation, median, frequency rate, minimum, maximum).

In the comparison of three or more groups that exhibited normal distribution, one-way ANOVA test and to determine the group causing the difference Turkey HSD and Games Howel test was used; while in the comparison of three or more groups that did not exhibit normal distribution, Kruskal Wallis test and to determine the group causing the difference Mann Whitney U test was used. Fisher Freeman Halton test was used to compare qualitative data. Spearman correlation analysis was used in the evaluation of relationships between variables. Significance were assessed on $p<0.01$ and $p<0.05$ levels.

Results

A total of 98 participants were included in this study. The mean duration of hypothyroidism are 5,86±5,91 vs. 5,43±5,62 years. The clinical and biochemical characteristics of patients and controls

are shown in Table 1.

	Hashimoto thyroiditis Mean±s.d./*n*, %	**Non-Hashimoto hyroiditis** Mean±s.d./*n*, %	**Control** Mean±s.d./*n*, %
Female (n)			
Age (years)	39,79±11,27	42,63±11,09	44.53±12.62
Duration of hypothyroid (years)	5,86±5,91	5,43±5,62	0
Medicine dose	125,69±43,74	80,71±47,00	**0***
BMI (kg/m2)	28,52±7,04	27,99±4,67	27,71±5,11
Total cholesterol (mg/dl)	180,28±52,35	193,46±41,92	219,71±40,28*
Triglyceride (mg/dl)	114,45±42,9	116,6±50,28	130,12±52,53
Low-density lipoprotein (mg/dl)	119,6±32,05	125,06±25,28	132,53±39,93
High-density lipoprotein (mg/dl)	52,3±9,52	55,4±11,63	54,65±12,47
TSH (mg/l)	8,89±15,68	8,64±16,77	2,65±0,62
Free T3	2,74±0,43	2,69±0,42	**3,51±0,6****
Free T4	0,82±0,25	0,91±0,44	**1,26±0,28****
Anti-TPO	452,01±392,46	35,83±180,53	**20,47±11,57****
Anti-TG	186,94±546,61	105,76±421,78	**21,71±10,31****
Calcium (mg/dL)	9,05±0,5	8,84±0,35	**9,36±0,56****
Phosphorus (mg/dL)	3,14±0,61	3,04±0,47	**3,62±0,44****
Albumine (g/dL)	4,30±0,31	4,20±0,18	**4,37±0,23***
Parathyroid hormone (pg/mL)	67,18±50,2	58,11±30,28	46,26±18,02
25-OH D vitamin (ng/mL)	10,88±9,19	10,53±6,87	23,08±9,76 **

Table 1: Clinical and biochemical characteristics of patients and controls.
[a]Oneway Anova Test; [c]Kruskal Wallis Test; [b]Student-t Test; [c]Mann Whitney U Test; *p<0,05; **p<0,01

The group 1 patients drug dosage are significantly higher than the group 2 patients (p<0,05). The group 1 and group 2 patients had a significantly lower total cholesterol, free T3, free T4, calcium, phosphorus than the control subjects (p<0,05; p<0,01). Anti TPO and anti TG levels were significantly higher in the group 1 patients compared to the group 2 patients and control subjects (p<0,01). There were no significant differences other variables between the patients and control subjects. The serum 25-OH vitamin D levels were significantly lower in the Hashimoto and Non-Hashimoto patients compared to the healthy controls (10,88±9,19 ng/ml vs. 10,53±6,87 ng/ml, p<0.01) (Figure 1).

CHP, SHP, and EP were similar LDL, HDL, triglyceride, albumine, parathormone (pth), and anti TPO values (Table 2).

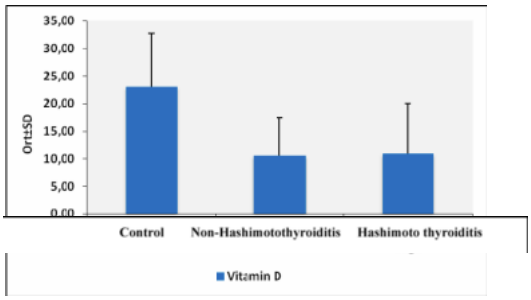

Figure 1: According to the groups, the distribution of vitamin D; *p<0,05; **p<0,01.

	Control	**Hypothyroidism**		
		Euthyroid	**Subclinic**	**Clinic**
	Mean±s.d./*n*, %	Mean±s.d./*n*, %	Mean±s.d./*n*, %	Mean±s.d./*n*, %
Total cholesterol (mg/dl)	219,71±40,28	172,69±48,37	190,21±37,71	213,71±46,83**
High-density lipoprotein (mg/dl)	54,65±12,48	54,21±10,27	55,47±12,13	50,76±9,48
Low-density lipoprotein (mg/dl)	132,53±39,93	117,08±28,53	126,32±24,65	129,12±33,83
Triglyceride (mg/dl)	130,12±52,53	113,85±51,39	107,37±37,2	128,18±42,19
TSH	2,65±0,62	1,82±1,23	6,47±1,81	26,32±26,52 **
Free T3	3,51±0,6	2,81±0,42	2,75±0,35	2,49±0,41 **
Free T4	1,26±0,28	1,01±0,41	0,75±0,15	0,65±0,22 **
Anti TPO	20,47±11,57	215,1±333,13	235,64±385,38	389,97±437,2
Anti TG	21,71±10,31	121,43±426,55	29,1±60,4	337,18±772,76 **
Calcium (mg/dL)	9,36±0,56	8,98±0,46	8,94±0,48	8,92±0,4 *
Phosphorus (mg/dL)	3,62±0,44	3,07±0,5	3,27±0,39	2,97±0,73 **
Albumine (g/dL)	4,37±0,23	4,24±0,25	4,28±0,25	4,26±0,32
Parathyroid hormone (pg/mL)	46,26±18,02	55,34±29,52	60,22±35,69	83,37±63,81
25-OH D vitamin (ng/mL)	23,08±9,76	13,03±8,77	9,48±7,86	7,10±5,23**

Table 2: Characteristics of clinic hypothyroidism patients, subclinical hypothyroidism patients, and euthyroid patients with hypothyroidism.
*[a]Oneway Anova Test; [c]Kruskal Wallis Test; *p<0,05 **p<0,01*

The serum 25-OH vitamin D levels in the euthyroid patients were significantly higher than the clinic hypothyroidism (p <0,01). The clinic hypothyroidism patients have a significantly higher TSH levels than the other groups (p<0,001; p<0,01). CHP, SHP, and EP patients have significantly lower free T3, free T4, calcium, phosphorus, and higher anti TG than the control subjects (p<0,001; p<0,01; p<0,05). The total cholesterol in the Euthyroid with Hypothyroidism patients were

significantly lower than the other groups (p <0,05). The serum 25 OH vitamin D levels showed a positive correlation with free T3 (r=0,306; p=0,004), free T4(r=0,346; p=0,001), calcium (r=0,267; p=0,009), phosphorus ((r=0,208; p=0,044), and negative correlation TSH(r=-0,353; p=0,001), parathyroid hormone levels (r=-0,415; p=0,001. There was no significant correlation between vitamin D levels and autoimmunity (anti TPO, anti TG)) in Table 3.

	25-OH D vitamin	
	r	p
TSH	-0,353	*0,001***
Free T_3	0,306	*0,004***
Free T_4	0,346	*0,001***
Anti TPO	-0,134	*0,193*
Anti TG	0,081	*0,432*
Calcium	0,267	*0,009***
Phosphorus	0,208	*0,044**
Parathyroid hormone	-0,415	*0,001***

Table 3: The relationship between the biochemical measurements and vitamin D measurement.
*r= Spearman Correlation Coefficient; *p<0,05; **p<0,01*

Discussion

Hypothyroidism is a disease resulting from deficiency or rarely ineffectiveness of thyroid hormone on tissue level. Hashimoto's thyroiditis (chronic autoimmune thyroiditis) is the most common cause of hypothyroidism in iodine sufficient regions of the world[12]. As in many autoimmune disorders, it is caused by combination of genetic and environmental factors. Studies that are conducted to show the relationship between vitamin D and Hashimoto's thyroiditis continues. In one of these studies, it was shown that development of Hashimoto's thyroiditis is more likely in those with C/C homozygote VDR FokI gen polymorphism in exon 2[13]. In another study, it was revealed that C/T polymorphism of vitamin D 1α-hydroxylase gene in intron 6 is associated with Hashimoto's thyroiditis[14].

In the studies, it was observed that 1.25(OH)2 D vitamin prevents development of autoimmune thyroiditis in animal models in an effective way and inhibits HLA class II expression in endocrine cells[15,16]. The average 25-OHD concentration of hypothyroidism cases associated with Hashimoto's thyroiditis is 10.88 ng/dl, while it is 10.53 ng/dl in hypothyroidism cases associated with other reasons. The 25-OHD concentrations in hypothyroidisms are 7.10 ng/dl in clinical hypothyroidism 9.48 ng/dl in subclinical hypothyroidism and 13.03 ng/dl in euthyroid cases. An advanced, significant difference was observed between vitamin D measurements of the cases according to the groups (p=0.001; p<0.01).

However, it is seen that even in the control group, the average vitamin D is lower than 30 ng/dl. Deficiency of vitamin D is a common health problem. In the study conducted by Erkal and his colleagues, 25OHD level was found to be lower than 25 ng / ml in more than 78% of the patients[17]. In another study, Ergür and his colleagues reported a serious vitamin D deficiency in 27% of the women in reproductive period, an average deficiency in 54.3% of them. Only in 18.6% of the women in reproductive period, normal vitamin D levels were observed[18]. In the study conducted by Bozkurt and his colleague, 25OHD level was found to be lower than 25 ng/ml in 94.4% of the patients. Again in the same study, a serious deficiency was found in 42.8% of the women[19].

In the study conducted by Tamer and his colleague, serum vitamin D level was found to be 16.3± 10 ng / ml in the Hashimoto's thyroiditis cases, while it was 29.6 ±25.5 ng / ml in the healthy control group[19]. In the study conducted by Kivity and colleagues, vitamin D deficiency was found to be 63%. This rate was 72% in those with autoimmune thyroid disease and 30% in the healthy control group. Vitamin D deficiency was determined in 79% of the cases of Hashimoto's thyroiditis. Vitamin D deficiency was 64% in gravis patients. Again in the same study, the vitamin D level was found to be 10 ng/ml and below in 52% of the patients with non-autoimmune thyroid disease[21]. Likewise, we also found a significant difference between Hashimoto's thyroiditis and control group in our study.

However, as opposed to other studies in our study, the relationship between Hashimoto's thyroiditis and hypothyroidism associated with other reasons were compared but no difference was observed; similar vitamin D levels were determined. When we look at the literature, in the study conducted by Effraimidis and his colleagues, no relation was determined between vitamin D deficiency and thyroid autoimmunity[22].

When hypothyroid cases were divided into three groups; an advance significant difference was

found between vitamin D measurements of the cases according to the groups ($p<0.01$). In the study by Tamer and his colleagues, no significant difference was found between the three groups[20].

In our study, presence of significant difference between hyperthyroidism, subclinical hyperthyroidism, euthyroid groups demonstrated that vitamin D deficiency might be effective in the course of hypothyroidism. In conclusion, in our study it was seen that there might be a relationship between hypothyroidism and vitamin D, and Hashimoto's thyroiditis and vitamin D. However, the fact that vitamin D levels were low in hypothyroidism cases both due to Hashimoto and non-Hashimoto reasons can be interpreted that there is no relationship between vitamin D levels and autoimmunity. However, it was thought that the prevalence of vitamin D sufficiency in our country can be limiting while comparing the groups. This study may provide guidance for future studies to be conducted to show relationship between vitamin D, hypothyroidism and autoimmunity.

Conclusion

It was thought that the study's being conducted during the winter months might have affected vitamin D levels. The vitamin D value of the control group cases was found to be higher as compared to the case of hypothyroidism associated with Hashimoto's thyroiditis and of hypothyroidism associated with other reasons. No significant difference was observed in vitamin D levels between the groups of hypothyroidism associated with Hashimoto's thyroiditis and hypothyroidism associated with other reasons. The dose of medication used by the patients with hypothyroidism associated with Hashimoto's thyroiditis was detected to be higher than that of medication used by the patients with hypothyroidism associated with other reasons. Total cholesterol value of euthyroid patients was lower than that of clinical hypothyroidism patients.

We suggest that in future studies, in order to show a relationship between autoimmunity and vitamin D, including a larger population into the studies and avoiding populations with vitamin D deficiency would make them more valuable.

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