Am J Transl Res 2023;15(10):6234-6241
www.ajtr.org /ISSN:1943-8141/AJTR0151663

Original Article

Therapeutic effect of vitamin D in Hashimoto's thyroiditis: a prospective, randomized and controlled clinical trial in China

Xue Jiang[1*], Yin Huang[1*], Yanan Li[2], Yuxiao Xia[1], Lina Liu[1], Feng Lin[1], Yuhong Shi[1]

[1]Department of Nuclear Medicine, The Second Affiliated Hospital of Chengdu Medical College, China National Nuclear Corporation 416 Hospital, Chengdu 610051, Sichuan, P. R. China; [2]Department of Ophthalmology, West China Hospital of Sichuan University, Chengdu 610041, Sichuan, P. R. China. [*]Equal contributors.

Received June 9, 2023; Accepted August 11, 2023; Epub October 15, 2023; Published October 30, 2023

Abstract: Background: The study aims to explore the clinical effects of Vitamin D (VitD) supplements for Hashimoto's Thyroiditis (HT), which are unclear according to other studies. Methods: Female patients with newly diagnosed HT from January to June in 2018 were included. This study is registered in the Chinese Clinical Trials Registry with registration number ChiCTR1800014619 (URL: https://www.chictr.org.cn/). Patients were randomly assigned to the treatment group and the control group. The treated group were further randomly assigned to a VitD supplement group or VitD & Levothyroxine (L-T4) supplement group. After 6 months, we recorded and compared various indicators between different groups. Results: A total of 179 patients, aged 12 to 75, were used for statistical analysis. A significant decrease in Thyroid Peroxidase Antibody (TPOAb) level was observed (351.70±183.25 vs. 246.37±157.39, P<0.001) in the VitD-treated group compared to the control group after 6 months. Free Triiodothyronine (FT3) and Free Thyroxine (FT4) level were increased (FT3: 4.30±0.64 vs. 4.84±0.9, P<0.001; FT4: 15.15±1.93 vs. 17.38±2.97, P<0.001), and Thyroid-Stimulating Hormone (THS) level was decreased (3.58±1.78 vs. 2.25±1.22, P<0.001) in the VitD-treated group compared to the control group. Conclusion: VitD supplementation can effectively slow progression of hypothyroidism, improve thyroid function, and reduce the anti-thyroid antibody level. This suggests it is useful for HT.

Keywords: Hashimoto's thyroiditis, subclinical hypothyroidism, vitamin D, thyroid peroxidase antibody, thyroglobulin antibody

Introduction

Hashimoto's thyroiditis (HT) is an autoimmune thyroid disease characterized by an enlarged and firm thyroid gland, with or without hypothyroidism secondary to raised circulating autoantibodies (thyroid peroxidase antibody (TPOAb) or Thyroglobulin Antibodies (TGAb)). HT is typically treated with levothyroxine when hypothyroidism is present or to try to reduce the size of thyroid gland or the goiter. However, the treatment needed to reduce the antibody level and ameliorate hypothyroidism is still uncertain.

In recent years, numerous clinical and preclinical studies suggested that VitD deficiency may be associated with autoimmune thyroid diseases (AITDs) [1-3]. Furthermore, it is more frequent to observe VitD deficiency in patients with HT than in a healthy population. Bozkurt et al found that there was a correlation between VitD deficiency and duration of HT, antibody level, and thyroid volume, demonstrating a crucial role for VitD in the development of HT [3]. Simsek et al presented the first clinical study to explore the effect of VitD on thyroid antibodies in 2016 [4]. Successive studies showed a beneficial effect of VitD supplement for AITD patients [5-7]. However, the finding of a double-blinded randomized placebo-controlled clinical trial performed in Iran did not support an association between VitD treatment and improvement of thyroid autoimmune diseases [8]. Therefore, it was important to resolve controversies in this regard.

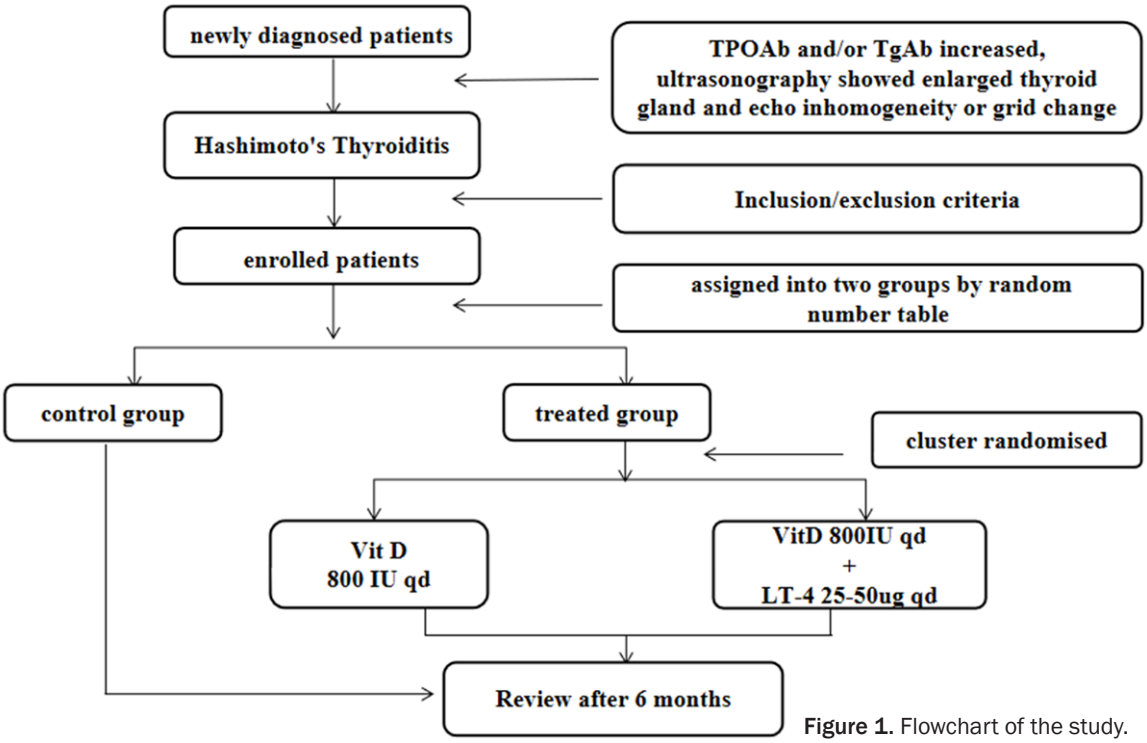

Figure 1. Flowchart of the study.

This prospective, randomized, and controlled clinical trial performed in Southwest of China aims to investigate the effects of vitamin D supplementation in patients with HT, providing support for the use of vitamin D supplements for the management of HT or in susceptible patients.

Materials and methods

Patients

A total of 193 patients were enrolled between January to June 2018, and 179 patients (aged 12-75 years) completed the study. The studies involving human participants were reviewed and approved by the Second Affiliated Hospital of Chengdu Medical College. The patients/participants provided their written informed consent to participate in this study. Examinations including serum level of thyroid stimulating hormone (TSH), free T4 (FT4), free T3 (FT3), TPOAb, TGAb, and 25-hydroxyvitamin D (25(OH)D), liver, and kidney function and ultrasound of neck were performed on all patients. Patients who had elevated serum TPOAb and/or TgAb levels, enlarged thyroid gland with echo inhomogeneity, and normal serum FT3, FT4 and TSH levels (or increased less than 10 mmol/L) were included in this study. Postmenopausal patients or patients with renal or liver disease, cancer, pregnancy, severe weight loss, and those who were on immunosuppressive medication within 6 months, addicted to alcohol or tobacco or had an abnormal Body Mass Index (BMI) (less than 18.5 or greater than 23.9) were excluded from this study.

Study procedures

Patients were assigned into two groups by random number table. The first group was also randomly divided into two sub-groups. One of these was treated with Vitamin D 800 IU/day for 6 months and another group was treated with Vitamin D 800 IU/day and L-T4 25~50 ug/day for 6 months. The second group served as the control group and was treated with no drug. The levels of TSH, FT4, FT3, TPOAb, TgAb, 25(OH)D and laboratory tests of liver and kidney function were measured and recorded at the initiation of the study and after 6 month-treatment. During the study period, any patients with hypothyroidism symptoms or abnormal FT4 or FT3 levels were withdrawn from our study and instructed to supplement timely with moderate L-T4. The trial ended after 12 months. **Figure 1** shows the study clinical trial flowchart.

Table 1. Groups of the study patients

	Control group	VitD-treated group	
		VitD	VitD+LT4
Enrolled	96	49	48
Lost to follow-up	10	0	0
Excluded	0	0	4
Evaluated	86	49	44

The trial was registered in the Chinese Clinical Trials Registry with registration number ChiCTR1800014619 (URL: https://www.chictr.org.cn/).

Outcomes

The primary outcome was the change in the serum 25-hydroxyvitamin D level after treatment. The secondary outcomes were the changes in the levels of TPOAb, TgAb, TSH, FT3 and FT4 after treatment.

Sample size estimation

Sample sizes to detect differences in complication rates between sub-groups were estimated for binominal proportions assuming a Type I error of 5% (two-sided) and 80% power. These sample size determinations were made using PASS 2008 (NCSS, Kaysville, UT).

Statistical analysis

SAS 9.4 software was used for the statistical analysis. Data were presented as means ± standard error (Mean ± SE). The student t-test was used for normally distributed data and the Mann-Whitney test was used if the data were skewed. Paired t-test was used for within-group comparison and between-group comparison was performed by independent t-test. Regarding the nominal data, Chi-square and/or Fisher's exact test were used to compare the two groups. The relationships of altered 25(OH)D Level to altered thyroid function or thyroid antibody in the treated group were analyzed by a Spearman correlation test. Multivariable linear regression (as shown in the Supplementary Table 1) was used to analyze the association of decreased TPOAb level to other baseline variables, including age, FT3, FT4, TSH, TGAb, and VitD. The ΔTPOAb value (initial value minus post-treatment value) was used as the dependent variable and other variables were utilized as independent variables. A stepwise method was used to model the effect of predictor variables on adherence. A *p*-value of <0.05 was considered significant for all tests.

Results

Patients and baseline

A total of 193 patients were enrolled from January 2018 to June 2018 in the study. Finally, 179 patients with HT eligible for the study attended a baseline examination and were entered into statistical analysis. 4 patients in the VitD-treated group and 10 patients in the control group were excluded or dropped during the study period (**Table 1**). There were no significant differences in the clinical characteristics either between the control group and the treated-group, or between the VitD-treated group and the L-T4 & VitD treated group (**Tables 2**, **3**).

Serum levels of thyroid function in each group before and after trial

The levels of FT3 and FT4 (*P*=0.02, *P*=0.027, respectively) were decreased, and the levels of TPOAb and TgAb were significantly increased, while the levels of TSH (*P*=0.1764) and 25(OH)D (*P*=0.095) were similar in the control group after 6 months. There was a significant increase in FT4 level (*P*=0.0157), but the levels of TSH (*P*<0.0001), TPOAb (*P*<0.0001) and TgAb (*P*=0.001) were decreased. No changes were observed in FT3 level (*P*=0.7707) in the VitD-treated group after 6 months. The levels of FT3 (*P*=0.0152) and FT4 (*P*<0.0001) were significantly increased, accompanied by decreased TSH (*P*<0.0001), TPOAb (*P*<0.0001) and TgAb (*P*=0.004) levels in the L-T4 & VitD treated group after 6 months. As expected, the serum level of 25(OH)D was significantly increased in the VitD-treated group and the L-T4 & VitD treated group (*P*<0.0001) (**Table 4**).

Serum levels of biochemical measurements in the control group and the treated group after trial

The levels of FT3, FT4, and 25(OH)D were significantly increased in the treated group compared to the control group (*P*<0.001). Lower TSH and TPOAb levels were observed in the treated group than in the control group (*P*< 0.001). The level of TgAb in the treated group

Table 2. Baseline characteristics in the treated group and control group

Variable	Control group (N=86)	Treated group (N=93)	T/Z	p
Age (year)	33.30±10.20	34.86±12.00	-0.93	0.356
Weight (kg)				
FT3 (pmol/L)	4.66±0.82	4.64±0.72	0.19	0.849
FT4 (pmol/L)	15.91±2.50	16.08±2.79	-0.43	0.665
TSH (mIU/L)	3.37±1.71	3.41±1.57	-0.16	0.873
TGAb (IU/ml)	689.83±818.21	685.17±745.27	0.04	0.968
TPOAb (IU/ml)	292.85±171.25	299.37±171.09	-0.25	0.799
25(OH)D (ng/ml)	15.48±4.41	15.39±4.29	0.13	0.894
Alb (g/dL)				
BUN (mg/dL)				
Cr (mg/dL)				
TSH (mU/L)				

Table 3. Baseline characteristics between sub-treatment groups

Variable	VitD Group (N=49)	L-T4 & VitD Group (N=44)	T/Z	p
Age (year)	36.23±12.49	33.39±11.42	1.13	0.260
FT3 (pmol/L)	4.65±0.84	4.63±0.56	0.03	0.975
FT4 (pmol/L)	16.07±3.42	16.09±1.88	0.88	0.376
TSH (mIU/L)	3.35±1.82	3.48±1.25	0.65	0.513
TGAb (IU/ml)	679.32±768.62	691.69±727.19	-0.08	0.937
TPOAb (IU/ml)	281.24±165.34	319.55±176.97	-1.08	0.283
25(OH)D (ng/ml)	16.06±4.52	14.64±3.92	1.61	0.110

was nonsignificantly decreased compared to the control group (*P*=0.056) (**Table 5**).

Serum levels of thyroid function in the VitD-treated group and L-T4 & VitD treated group after trial

The levels of TPOAb and TgAb were similar in the two groups after 6 months, while higher levels of FT3 and FT4 and lower levels of TSH were observed in the L-T4 & VitD-treated group compared to the VitD-treated group (**Table 6**).

Correlation analysis of the increase in 25(OH)D and thyroid function and thyroid antibody in the treated-group after trial

Spearman correlation analysis showed that the increase in 25(OH)D level was negatively correlated with the TSH, TGAb, and TPOAb levels (*r*=-0.316, -0.261, -0.403, respectively), while positively correlated with the FT4 levels (*r*=0.287). However, there was no correlation between the change in 25(OH)D level and the FT3 level (*r*=0.070, P=0.3498) (**Table 7**).

Analysis of predictors for a decrease in TPOAb

The results of multiple stepwise linear regression showed that decreased TPOAb levels were significantly associated with the initial TSH level (T=-2.63, 95% CI=3.315-23.831, P=0.010), the initial TPOAb level (T=4.530, 95% CI=0.260-0.101, P<0.0001) and the patient age (T=-3.910, 95% CI=0.308-1.590, P<0.0001). This means the level of TPOAb has a negative correlation with a lower initial TSH level, a higher initial TPOAb level, and younger patient age (**Table 8**).

Discussion

Vitamin D receptor (VDR) is widely expressed in various immune cells, including T cells, B cells, and antigen presenting cells (APCs) such as macrophages and dendritic cells (DC) [9, 10]. It is universally acknowledged that DCs are the most potent and versatile APCs. 1,25-$(OH)_2D_3$ has been reported to regulate the expression of DC-derived factor and suppress DC maturation through inhibiting Interleukin (IL)-12 and IL-23 production and elevating IL-10 release. The high affinity for 1,25-$(OH)_2D_3$ in activated lymphocytes suggests that this steroid acts as an immunoregulatory hormone. Interference with cytokine production of lymphocytes such as T cells seems to be a key mechanism by which 1,25-$(OH)_2D_3$ interacts with immune system. This inhibits the proinflammatory function of helper T (Th) cell and stimulates regulatory T cells (Treg) activity, favoring relief of autoimmunity [11, 12]. The effects of 1,25$(OH)_2D_3$ on the proliferation and apoptosis of B cells result in the prohibition of the autoimmune process directly [13], indicating that VitD may provide a beneficial effect for autoimmune diseases such as HT.

Some research suggested that CSE (Cigarette Smoke Extract) promotes the maturation of DC

Table 4. Comparison of laboratory findings of each group before and after trial

	Variable	Initial	6 months after	t	p
Control Group (N=86)	FT3 (pmol/L)	4.66±0.82	4.30±0.64	3.93	0.02
	FT4 (pmol/L)	15.91±2.50	15.15±1.93	3.08	0.027
	TSH (mIU/L)	3.37±1.71	3.58±1.78	-1.36	0.1764
	TGAb (IU/ml)	689.83±818.21	747.62±926.17	-2.43	0.0173
	TPOAb (IU/ml)	292.85±171.25	351.70±183.25	-5.72	<0.0001
	25(OH)D (ng/ml)	15.48±4.41	16.66±5.25	-1.48	0.0950
VitD-Treated Group (N=49)	FT3 (pmol/L)	4.65±0.84	4.68±0.87	-0.29	0.7707
	FT4 (pmol/L)	16.07±3.42	16.84±3.45	-2.50	0.0157
	TSH (mIU/L)	3.35±1.82	2.49±1.22	5.07	<0.0001
	TGAb (IU/ml)	679.32±768.62	521.06±580.31	3.49	0.0010
	TPOAb (IU/ml)	281.24±165.34	231.88±147.33	5.69	<0.0001
	25(OH)D (ng/ml)	16.06±4.52	24.82±5.75	-11.70	<0.0001
L-T4 & VitD Treated Group (N=44)	FT3 (pmol/L)	4.63±0.56	5.02±0.95	-2.53	0.0152
	FT4 (pmol/L)	16.09±1.88	17.99±2.20	-4.92	<0.0001
	TSH (mIU/L)	3.48±1.25	1.99±1.18	7.94	<0.0001
	TGAb (IU/ml)	691.69±727.19	538.15±543.24	3.87	0.004
	TPOAb (IU/ml)	319.55±176.97	262.52±168.11	4.78	<0.0001
	25(OH)D (ng/ml)	14.64±3.92	23.32±4.20	-15.76	<0.0001

Table 5. Comparison of laboratory findings in the control group and treated group after trial

Variable	Control Group (N=86)	Treated Group (N=93)	T/Z	p
FT3 (pmol/L)	4.30±0.64	4.84±0.92	-4.28	0.000
FT4 (pmol/L)	15.15±1.93	17.38±2.97	-5.92	0.000
TSH (mIU/L)	3.58±1.78	2.25±1.22	5.62	0.000
TGAb (IU/ml)	747.62±926.17	529.15±560.09	1.93	0.056
TPOAb (IU/ml)	351.70±183.25	246.37±157.39	4.13	0.000
25(OH)D (ng/ml)	16.66±5.25	24.11±5.11	-9.59	0.000

Table 6. Comparison of the laboratory findings between subgroups after trial

Variable	VitD (N=49)	L-T4 & VitD (N=44)	T/Z	p
FT3 (pmol/L)	4.68±0.87	5.02±0.95	-1.78	0.079
FT4 (pmol/L)	16.84±3.45	17.99±2.20	2.90	0.004
TSH (mIU/L)	2.49±1.22	1.99±1.18	1.98	0.051
TGAb (IU/ml)	521.06±580.31	538.15±543.24	-0.15	0.884
TPOAb (IU/ml)	231.88±147.33	262.52±168.11	-0.94	0.351
25(OH)D (ng/ml)	24.82±5.75	23.32±4.20	-1.45	0.147

and initiates an acquired immune response, which is considered one of the main mechanisms leading to Hashimoto's thyroiditis [14]. Therefore, the patients who smoked were strictly excluded. The patients with abnormal BMI were also excluded because excess adipose tissue may influence the development of the autoimmune reaction [15].

TPOAb is not only a marker of abnormal immune activation but also acts as a cytotoxic factor in thyroid cells, to which the damage is minor based on T cell and cytokine mediated apoptosis. Furthermore, thyroid peroxidase is a poorly glycosylated membrane-bound enzyme, responsible for the oxidation of iodotyrosine and thyroid hormone production. Besides, high levels of TPOAb slow down the process of thyroid hormone synthesis by blocking the process, which directly contributes to hypothyroidism [16]. In the present study, VitD supplementation effectively improved thyroid function and decreased the level of thyroid antibodies, especially TPOAb levels, demonstrating that VitD actually does have a benefit for Hashimoto's thyroiditis.

Unlike TPOAb, TGAb can be induced by a massive release of antigens such as destruction of the thyroid gland or generation of new epitopes by a changed and more immunogenic conformation of the Tg molecule. The function of TGAb in thyroid cells is still unclear, but they have no

Table 7. Correlation analysis between changes in thyroid function, thyroid antibody, and 25(OH)D level in the treated group before and after trial

Variable	Δ25(OH)D	
	R	P
ΔFT3	0.070	0.3498
ΔFT4	0.287	<0.0001
ΔTSH	-0.316	<0.0001
ΔTGAB	-0.261	0.0004
ΔTPOAB	-0.403	<0.0001

Table 8. Multiple regression analysis of ΔTPOAb

Variable	Coefficient	SE	T	P	95% CI for Coefficient	
TSH	13.573	5.161	2.630	0.010	3.315	23.831
TGAb	0.019	0.009	2.100	0.039	0.001	0.037
TPOAb	-0.181	0.040	-4.530	<0.0001	-0.260	-0.101
Age	0.949	0.327	3.910	<0.0001	0.308	1.590

relationship to the development of thyroid cell destruction [17-19]. Thus, studies regarding the effect of the TGAb level on VitD and AITD are rare. Recently, a researcher did a multiple linear regression analysis and found that TgAb level was the most relevant indicator for the clinical symptoms of hypothyroidism such as fragile hair, face edema, edema of the eyes, and harsh voice when compared to thyroid hormones and TPOAb. This suggests the clinical significance of the TGAb level in patients with HT. In this study, the level of TGAb decreased in the VitD-treated group after 6 months. However, it showed no significant difference in the treated group and the control group, indicating that the immune responses of Tg and TPO antigens in thyroid autoimmunity were not exactly the same.

In this study, a multiple regression analysis was performed and showed that the initial TSH and TPOAb levels as well as the age of patients were predictors for a decrease in TPOAb levels, suggesting that VitD therapy might exhibit a more beneficial effect in such patients. One study in animals has confirmed that age was linearly associated with a descending mRNA level of 1α-hydroxylasein in kidneys [20], which was consistent with our results.

Previous studies showed that levothyroxine treatment reduced the degree of lymphocytic infiltration in an animal model of HT [21]. Thyroid hormones are thought to contribute to free oxygen radical cleaning by increasing nonenzymatic antioxidant molecules, and thereby decrease the oxidative stress, reduce cytokine production, and indirectly decrease the level of abnormal immune activation [22]. Some researchers found that the application of levothyroxine in euthyroid patients with HT could decrease the TPOAb and TGAb levels [23], but another study showed that those changes only occurred in patients with clinical hypothyroidism [24]. In the present study, the level of FT4 was significantly increased in the L-T4 supplement group and no obvious change in FT3, TSH, TPOAb, or TGAb level was observed. This manifests that L-T4 had an effect on upregulation of thethyroid hormone level and showed no action on thyroid reserve function or thyroid autoimmunity.

The usage of cholecalciferol substitution for VitD supplement is one of the limitations of our study. Cholecalciferol is extremely common in the clinic and no serious adverse events has been reported to date. However, the active VitD metabolites need to be hydroxylated in the liver and kidney, which is in the charge of 25-hydroxylases such as Cyp2R1 and Cyp27B1. The level of hydroxylase might influence the conclusion which we did not assessa at the beginning. Secondly, clinical symptoms of the patients were not included in this trial. In addition, a small sample size and short follow-up time may have influenced our findings.

Conclusion

VitD supplementation effectively improved thyroid function and decreased the level of thyroid antibodies, leading to the amelioration of hypothyroidism. There was a negative correlation between TPOAb levels and lower initial TSH level, higher TPOAb level, and younger age in patients with HT. No obvious advantages in improving thyroid reserve function and no synergistic effect in blocking the process of abnormal immune response were observed when a patient was treated with VitD and L-T4. The present findings suggest that VitD supplemen-

tation may be a useful treatment for patients diagnosed with HT who have normal thyroid function or sub-clinical hypothyroidism. However, a long-term, larger, prospective, blind-controlled trial needs to be conducted.

Disclosure of conflict of interest

None.

Address correspondence to: Feng Lin and Yuhong Shi, Department of Nuclear Medicine, The Second Affiliated Hospital of Chengdu Medical College, China National Nuclear Corporation 416 Hospital, Chengdu 610051, Sichuan, P. R. China. Tel: +86-028-84779146; Fax: +86-028-84779146; E-mail: linfeng0909@126.com (FL); shiyuhong89@hotmail.com (YHS)

References

[1] Tamer G, Arik S, Tamer I and Coksert D. Relative vitamin D insufficiency in Hashimoto's thyroiditis. Thyroid 2011; 21: 891-896.

[2] Kivity S, Agmon-Levin N, Zisappl M, Shapira Y, Nagy EV, Danko K, Szekanecz Z, Langevitz P and Shoenfeld Y. Vitamin D and autoimmune thyroid diseases. Cell Mol Immunol 2011; 8: 243-247.

[3] Bozkurt NC, Karbek B, Ucan B, Sahin M, Cakal E, Ozbek M and Delibasi T. The association between severity of vitamin D deficiency and Hashimoto's thyroiditis. Endocr Pract 2013; 19: 479-484.

[4] Simsek Y, Cakır I, Yetmis M, Dizdar OS, Baspinar O and Gokay F. Effects of vitamin D treatment on thyroid autoimmunity. J Res Med Sci 2016; 21: 85.

[5] Krysiak R, Szkrobka W and Okopien B. The effect of vitamin D on thyroid autoimmunity in levothyroxine-treated women with Hashimoto's thyroiditis and normal vitamin D status. Exp Clin Endocrinol Diabetes 2017; 125: 229-233.

[6] Ucan B, Sahin M, Sayki Arslan M, Colak Bozkurt N, Kizilgul M, Gungunes A, Cakal E and Ozbek M. Vitamin D treatment in patients with Hashimoto's thyroiditis may decrease the development of hypothyroidism. Int J Vitam Nutr Res 2016; 86: 9-17.

[7] Chaudhary S, Dutta D, Kumar M, Saha S, Mondal SA, Kumar A and Mukhopadhyay S. Vitamin D supplementation reduces thyroid peroxidase antibody levels in patients with autoimmune thyroid disease: an open-labeled randomized controlled trial. Indian J Endocrinol Metab 2016; 20: 391-398.

[8] Vahabi Anaraki P, Aminorroaya A, Amini M, Momeni F, Feizi A, Iraj B and Tabatabaei A. Effect of vitamin D deficiency treatment on thyroid function and autoimmunity markers in Hashimoto's thyroiditis: a double-blind randomized placebo-controlled clinical trial. J Res Med Sci 2017; 22: 103.

[9] Prietl B, Treiber G, Pieber TR and Amrein K. Vitamin D and immune function. Nutrients 2013; 5: 2502-2521.

[10] Baeke F, Takiishi T, Korf H, Gysemans C and Mathieu C. Vitamin D: modulator of the immune system. Curr Opin Pharmacol 2010; 10: 482-496.

[11] Dankers W, Colin EM, van Hamburg JP and Lubberts E. Vitamin D in autoimmunity: molecular mechanisms and therapeutic potential. Front Immunol 2016; 7: 697.

[12] Urry Z, Chambers ES, Xystrakis E, Dimeloe S, Richards DF, Gabrysova L, Christensen J, Gupta A, Saglani S, Bush A, O'Garra A, Brown Z and Hawrylowicz CM. The role of 1alpha,25-dihydroxyvitamin D3 and cytokines in the promotion of distinct Foxp3+ and IL-10+ CD4+ T cells. Eur J Immunol 2012; 42: 2697-2708.

[13] Chen S, Sims GP, Chen XX, Gu YY, Chen S and Lipsky PE. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. J Immunol 2007; 179: 1634-1647.

[14] Ueno A, Jijon H, Traves S, Chan R, Ford K, Beck PL, Iacucci M, Fort Gasia M, Barkema HW, Panaccione R, Kaplan GG, Proud D and Ghosh S. Opposing effects of smoking in ulcerative colitis and Crohn's disease may be explained by differential effects on dendritic cells. Inflamm Bowel Dis 2014; 20: 800-810.

[15] Harpsoe MC, Basit S, Andersson M, Nielsen NM, Frisch M, Wohlfahrt J, Nohr EA, Linneberg A and Jess T. Body mass index and risk of autoimmune diseases: a study within the Danish National Birth Cohort. Int J Epidemiol 2014; 43: 843-855.

[16] Lisi S, Segnani C, Mattii L, Botta R, Marcocci C, Dolfi A, McCluskey RT, Pinchera A, Bernardini N and Marino M. Thyroid dysfunction in megalin deficient mice. Mol Cell Endocrinol 2005; 236: 43-47.

[17] Zaletel K and Gaberscek S. Hashimoto's thyroiditis: from genes to the disease. Curr Genomics 2011; 12: 576-588.

[18] Benvenga S and Trimarchi F. Thyroid hormone autoantibodies in Hashimoto's thyroiditis: often transient but also increasingly frequent. Thyroid 2003; 13: 995-996.

[19] Baric A, Brcic L, Gracan S, Skrabic V, Brekalo M, Simunac M, Lovric VT, Anic I, Barbalic M, Zemunik T, Punda A and Boraska Perica V. Thyroglobulin antibodies are associated with symptom burden in patients with Hashimoto's thyroiditis: a cross-sectional study. Immunol Invest 2019; 48: 198-209.

[20] Han JC, Zhang JL, Zhang N, Yang X, Qu HX, Guo Y, Shi CX and Yan YF. Age, phosphorus, and 25-hydroxycholecalciferol regulate mRNA expression of vitamin D receptor and sodium-phosphate cotransporter in the small intestine of broiler chickens. Poult Sci 2018; 97: 1199-1208.
[21] Padberg S, Heller K, Usadel KH and Schumm-Draeger PM. One-year prophylactic treatment of euthyroid Hashimoto's thyroiditis patients with levothyroxine: is there a benefit? Thyroid 2001; 11: 249-255.
[22] Ates I, Altay M, Yilmaz FM, Topcuoglu C, Yilmaz N, Berker D and Guler S. The impact of levothyroxine sodium treatment on oxidative stress in Hashimoto's thyroiditis. Eur J Endocrinol 2016; 174: 727-734.
[23] Aksoy DY, Kerimoglu U, Okur H, Canpinar H, Karaagaoglu E, Yetgin S, Kansu E and Gedik O. Effects of prophylactic thyroid hormone replacement in euthyroid Hashimoto's thyroiditis. Endocr J 2005; 52: 337-343.
[24] Romaldini JH, Biancalana MM, Figueiredo DI, Farah CS and Mathias PC. Effect of L-thyroxine administration on antithyroid antibody levels, lipid profile, and thyroid volume in patients with Hashimoto's thyroiditis. Thyroid 1996; 6: 183-188.

Supplementary Table 1. The conversion of continuous variables (age, FT3, FT4, TSH, TGAb and VitD) to categorical variables

Variables	Control group (N=86)	Treated Group (N=93)	p
age (year)			>0.05
20-30	14	22	
30-40	25	32	
40-50	47	39	
FT3 (pmol/L)			>0.05
3-4	33	37	
4-5	18	21	
5-6	35	35	
FT4 (pmol/L)			>0.05
12-15	42	45	
15-18	12	27	
18-21	32	21	
TSH (mIU/L)			>0.05
1-3	24	21	
3-5	33	37	
5-7	29	35	
TGAb (IU/mL)			>0.05
0-600	22	18	
600-1200	37	42	
1200-1800	27	33	
25(OH)D (ng/mL)			>0.05
10-14	31	32	
14-18	38	33	
18-22	17	28	