Vitamin D Level and Vitamin D Receptor Gene Polymorphisms in Iranian Azeri Turkish Patients With Autoimmune Thyroid Diseases

Amir Hossein Faghfouri[1], Morteza Bagheri[2], Alireza Mehdizadeh[3], Parvin Ayremlou[4], Rasoul Zarrin[1]

[1] *Department of Nutrition, Urmia University of Medical Sciences, Urmia, Iran*

[2] *Department of Genetics, Cellular and Molecular Research Center, Urmia University of Medical Sciences, Urmia, Iran*

[3] *Department of Endocrinology, Urmia University of Medical Sciences, Urmia, Iran*

[4] *Department of Epidemiology, Clinical Researches Development Unit, Imam Khomeini Hospital, Urmia University of Medical Sciences, Urmia, Iran*

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Abstract- The Autoimmune thyroid diseases (AITDs) are among the most common endocrine disorders. Vitamin D as an immunomodulator and Vitamin D receptor (VDR) gene polymorphisms may be effective in AITDs pathogenesis. The aim of this study was to evaluate the vitamin D level and VDR BsmI and TaqI polymorphisms in Iranian Azeri Turkish patients with AITDs. This case-control study included 121 adults with AITDs and 117 non-AITDs controls. Serum level of 25-hydroxyvitamin D was measured by electrochemiluminescence (ECL) immunoassay. BsmI and TaqI polymorphisms were assessed by polymerase chain reaction fragment length polymorphism technique. The serum level of 25-hydroxyvitamin D in AITDs patients were lower than controls (P=0.03). The frequencies of TT, TC, CC, T and C genotypes/alleles at TaqI (rs731236) marker were 52.1%, 34.7%, 13.2% , 69.4% and 30.6% in AITDs and 44.4%, 41.9%, 13.7%, 65.4% and 34.6% in controls, respectively. The frequencies of AA, AG, GG, A and G genotypes/alleles at BsmI (rs1544410) marker were 14%, 64.5%, 21.5% , 46.3% and 53.7% in AITDs and 26.5%, 58.1%, 15.4%, 55.6% and 44.4% in controls, respectively. BsmI (rs1544410) GG+AG genotypes and G allele were more frequent among patients with Hashimoto compared with control group (86.6% vs. 73.5% (OR: 2.34, 95% CI: 1.16-4.70, P = 0.014) and 54.29% vs. 44.44% (OR: 1.48, 95% CI: 1.02-2.15, P = 0.038), respectively). Vitamin D status can be related to AITDs pathogenesis. BsmI (rs1544410) GG+AG genotypes and G allele may play an important role in the predisposition to Hashimoto.

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Keywords: Hashimoto disease; Graves disease; Vitamin D receptor; Polymorphism; Vitamin D level

Introduction

Autoimmune thyroid diseases (AITDs) as part of the common thyroid gland disorders disturb thyroid functions (1). Clinically, AITDs consist of the series of diseases including Graves' disease (GD) and Hashimoto Thyroiditis (HT). Both of these are caused by producing antibodies against thyroid glands resulting in hyperthyroidism and hypothyroidism, respectively (2).

The global incidence of autoimmune hypothyroidism is more than hyperthyroidism and women are more susceptible to AITDs than men (3). Recently it has been shown that interaction between environmental and genetic factors, such as vitamin D and its nuclear receptor (vitamin D receptor) can be involved in the pathogenesis of AITDs (4).

It has been recently demonstrated that vitamin D may have an effect in the pathogenesis of AITDs (5). 1,25-dihydroxyvitamin D_3, as the active form of vitamin D, performs various functions, such as down-regulation of the immune system by binding to nuclear receptors of vitamin D (6).

Vitamin D receptor (VDR) genetic variations may have the regulatory influence on the Vitamin D metabolism. These variations in DNA sequence can be seen mainly in the population, known as VDR polymorphisms. VDR gene is located on chromosome 12 and has several genetic markers, for instance, ApaI, FokI, TaqI, BsmI, EcoRV and Tru9I (7).

VDR polymorphisms may be related to AITDs via affecting vitamin D functions and thereby with feedback mechanisms alter vitamin D status (8,9). Some previous

Corresponding Author: R. Zarrin
Department of Nutrition, Urmia University of Medical Sciences, Urmia, Iran
Tel: +98 914 8843592, Fax: +98 44 33469935, E-mail address: rasoul.zarrin@uqconnect.edu.au

studies suggested that AITDs patients have vitamin D insufficiency or deficiency compared with non-AITDs persons (10).

VDR polymorphisms can alter 25-hydroxy vitamin D ($25(OH)D_3$) levels in some autoimmune disorders such as Lupus and Vitiligo (11,12). However, few studies were conducted about the relationship between VDR polymorphisms and vitamin D status in HT, and there is no study about this relationship in GD (13,14).

TaqI (rs731236) and BsmI (rs1544410) are two sites of the VDR gene investigated in some previous studies, but results of these studies are conflicting (15,16). In Iran, there is no comprehensive study on the relationship between VDR polymorphisms and AITDs, and only a few reports have investigated vitamin D status in AITDs patients without considering VDR polymorphism as a risk factor (17).

The aim of this study was to assess the vitamin D status and vitamin D gene BsmI (rs1544410) and TaqI (rs731236) polymorphisms in AITDs patients in comparison with non-AITDs controls in Iranian Azeri Turkish population. Furthermore, we considered vitamin D intake from foods and supplements and sun-exposure habits to analyze the relationship between BsmI (rs1544410) and TaqI (rs731236) polymorphisms with vitamin D status.

Materials and Methods

Subjects

This case-control study was conducted in Urmia (a city in the North West of Iran), during spring and summer of 2016 and included 121 adult patients with AITDs (19 males, 102 females; 16 with GD, 105 with HT, average age: 38.7±11.8 year) referred to the Endocrinology clinic of Urmia Imam Khomeini Teaching hospital and 117 adult controls (24 males, 93 females, average: 39.5±10.5 year) in same geographic area with no family history, clinical signs and biochemical evidences of AITDs. Both groups were matched by age, sex, sun exposure habits and vitamin D intakes from foods and supplements.

All patients were diagnosed by endocrinologists based on their clinical symptoms and biochemical tests. These criteria for HT patients confirmed hypothyroidism included diffuse goiter, low radioactive iodine uptake, positive anti-thyroid peroxidase antibody (TPO), low free-T4 and high thyroid-stimulating hormone (TSH) (18). GD was diagnosed based on diffuse goiter, elevated radioactive iodine uptake, and positive anti-TPO and high thyroid hormone levels considered as hyperthyroidism (19).

Individuals with the following conditions were excluded from the study: 1) subjects who consumed any kind of medicines that alter vitamin D status including anti-seizure drugs and Bisphosphonates; 2) those who suffered from diseases that affect vitamin D status such as Cholestatic and Non-cholestatic liver diseases, Celiac disease, Crohn's disease, Ulcerative Colitis, Cystic fibrosis, Chronic kidney problems, and applying the gastrointestinal tract or hepatobiliary surgery; 3) Individuals who had Type 1 diabetes, Addison's disease, Primary biliary cirrhosis, Autoimmune hepatitis, Multiple sclerosis, Primary hyperparathyroidism, Osteoporosis, Cancers and other autoimmune diseases due to the relationship between VDR gene polymorphism and these diseases; 4) Any radiation during last 6 months (20-27).

After providing oral and written information about all aspects of the study to both patients and control groups, written informed consent was obtained from all participants. The ethics committee of Urmia University of Medical Sciences approved the research protocol.

Data collection

Amount of vitamin D intake from food sources was measured through a validated 44-item short food frequency questionnaire (FFQ) which was designed for the derivation of vitamin D, Selenium and Iodine intakes from foods. This questionnaire assessed the frequency of consumption of vitamin D sources in the past 6 months which mainly contained milk, dairy products, meat, poultry, fish, tuna, mushroom, chicken and sheep liver.

Face and content validity of the FFQ was assessed by an expert panel consisting of four nutritionists from the nutrition department of the faculty of medicine, Urmia University of Medical Sciences. For assessing the reliability of FFQ, three-day food records were collected for 20 percent of the participants in the study and Intra class Correlation Coefficient (ICC) was calculated for them. ICC for vitamin D was reported 0.83 (P<0.001; 95% CI, 0.69-0.92).

The sun-exposure questionnaire was used to assess the amount of the participant's sun-exposure in the last 6 months. The questionnaire was also used to assess the use of sunscreen creams, wearing sunglasses, clothing type against sunlight, and skin color.

Biochemical measurement

Peripheral blood samples were collected from all patients and controls. Then, serum was separated and stored at -80ºC for testing biochemical measurements.

Serum TSH was measured by ELISA (Pishtaz Teb, Tehran, Iran) and serum Anti-TPO was measured by ELISA (AccuBind ELISA Microwells, Monobind, Inc. Lake Forest, CA, USA). Serum 25-hydroxy vitamin D was determined by Electrochemiluminescence (ECL) immunoassay kit (Roche Diagnostics, Mannheim, Germany).

EDTA whole blood was used for genomic DNA extraction with Cinnagen DNP™ (Cinnagen, Tehran, Iran) according to the kit protocol. Amplifying the TaqI (rs731236) and BsmI (rs1544410) sites with polymerase chain reaction (PCR) with specific primers was carried out by Thermocycler (Bioer, XP). Master Mix 2X (Cinnagen, Tehran, Iran) were used for this reaction that contained 3 Mm/ml $MgCl_2$, 0.4 Mm/ml of each three phosphate nucleotides and 0.08 units/ul Taq DNA polymerase. For total 25 ul reaction volume, 12.5 ul Master mix 2X, 0.5 ul forward primer, 0.5 ul reverse primer, 1 ul extracted DNA, and 10.5 ul sterile deionized water were used. The PCR condition, forward and reverse primer sequences for each site are given in table 1.

Table 1. Primers and PCR condition

	Primer sequences	PCR conditions
BsmI rs1544410	5'–GGCAACCTGAAGGGAGACGTA-3' 5'-CTCTTTGGACCTCATCACCGAC-3'	95° 5 min, 35X (93°C 45 s,66°C30 s, 72°C 45 s), 72° 10 min
TaqI rs731236	5'–CAGAGCATGGACAGGGAGCAA-3' 5'-GCAACTCCTCATGGCTGAGGTCTCA-3'	95° 5 min, 35X (93°C 45 s,66°C30 s, 72°C 45 s), 72° 10 min

PCR products for BsmI (rs1544410) and TaqI (rs731236) were in 461 bp and 740 bp band, respectively. Then, TaqI (rs731236) PCR and BsmI (rs1544410) PCR products were digested with TaqI (rs731236) and BsmI (rs1544410) restriction enzymes (Thermo Fisher Scientific, USA) and enzymes buffer for 2h in 65° C and 37° C , respectively.

After digestion with BsmI (rs1544410), 461 bp and 203, 258 bp bands represented allele "A" and allele "G," respectively (Figure 1). For the TaqI (rs731236) after enzymatic digestion, 495 and 245 bp bands indicated the allele "T" and 290, 245 and 205 bp bands indicated allele "C" (Figure 2).

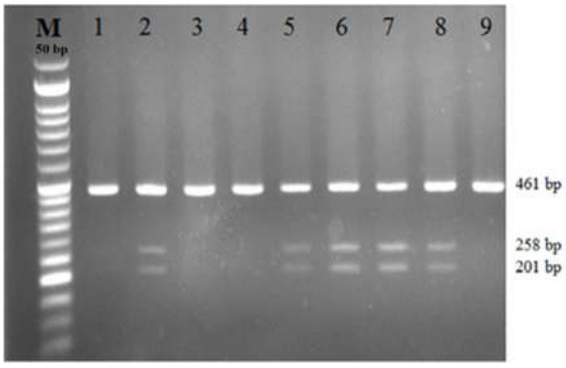

Figure 1. The PCR product analysis of BsmI genotypes after digestion. Lane 1, 3, 4, 9: BsmI AA; lane 2, 5, 6, 7, 8: BsmI AG

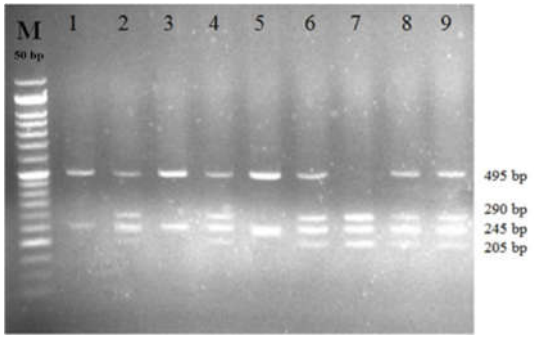

Figure 2. The PCR product analysis of TaqI genotypes after digestion. Lane 1, 3, 5: TaqI TT; lane 2, 4, 6, 8, 9: TaqI TC; lane 7: TaqI CC

Statistical analysis

A total of 116 subjects as minimum sample size per group had a statistical power of about 90% (two-tailed, α=5%). Normality tests of the data were performed using the Kolmogorov-Smirnov test. Student *t*-test was used for comparison of normal quantitative data (serum level of $25(OH)D_3$) between two groups, and Mann-Whitney U test was used if the quantitative data were not normally distributed. To compare serum levels of 25-hydroxyvitamin D in VDR genotypes analysis of variance (ANOVA) was used. The frequencies of VDR BsmI (rs1544410) and TaqI (rs731236) polymorphisms were counted directly. The data were evaluated regarding a good fitness to Hardy-Weinberg equilibrium (HWE). The chi-square (χ^2) test was done to compare genotypic/allelic frequencies between cases and

controls. χ^2 value, the odds ratio (OR), and 95% confidence interval (CI) were calculated by SPSS ver. 20.0 software (Chicago, IL) and Microsoft Office Excel 2010. Analysis of independent T-Test was performed via SPSS ver. 20.0 software. A statistical significance level α was set at 0.05.

Results

In terms of mean age, sex, amount of vitamin D intake from foods and supplements and sun-exposure habits, there were no statistically significant differences between case and control groups (Table 2).

Table 2. Basic characteristics of the study subjects

		AITDs N=121	Controls N=117	*P*
Age (years)		38.66 ± 11.78	39.5 ± 10.49	0.43*
Vitamin D intakes from foods (µg/day)		118.76 ± 77.9	129.47 ± 80.79	0.23*
Vitamin D intakes from supp. (µg/day)		1763.69 ± 1545.24	1846.78 ± 1439.33	0.75*
Women/men		102/19 (84.3/15.7)	93/24 (79.5/20.5)	0.33†
Skin color: Dark/Light		47/74 (38.8/61.2)	48/69 (41/59)	0.73†
Timing of participants sunlight exposure	**<30min/day**	73 (60.3)	68 (58.1)	0.86†
	30-60min/day	25 (20.7)	27 (23.1)	
	60-120min/day	9 (7.4)	11 (9.4)	
	>120min/day	14 (11.6)	11 (9.4)	
Sun exposure	**Face and hands**	86 (71.1)	81 (69.2)	0.9†
	Face, hands, and arms	32 (26.4)	32 (27.4)	
	More than faces, hands, and arms	3 (2.5)	4 (3.4)	
Usage of sun-screen creams	**Yes with SPF>15**	56 (46.3)	49 (41.9)	0.67†
	Yes with variable SPF	4 (3.3)	6 (5.1)	
	NO	61 (50.4)	62 (53)	
Protective Preferences against sun-light	**Moving in the shadows**	28 (23.1)	25 (21.4)	0.82†
	Usage of sun-screen creams	10 (8.3)	10 (8.5)	
	Wearing sun-glasses	1 (0.8)	3 (2.6)	
	Moving in the Shadows and usage of sun- screen	18 (14.9)	11 (9.4)	
	Moving in the Shadows and wearing sun- glasses	20 (16.5)	21 (17.9)	
	Usage of sun-screen and sun-glasses	3 (2.5)	6 (5.1)	
	All three protective actions	29 (24)	29 (24.8)	
	No Protective actions	12 (9.9)	12 (10.3)	

Quantitative data were shown as mean ± standard deviation; Qualitative data were shown as the number of cases and controls (percentage)
SPF: Sun Protection factor
* Mann-Whitney U test
† χ2 analysis.'

Serum biochemical markers of subjects with AITDs and controls are compared in table 3. TSH and Anti-TPO were two main laboratory markers for the diagnosis of HT and GD. 25(OH)D_3 levels were statistically lower in the patients with AITDs (34.55±16.01 ng/ml) than healthy control subjects (39.15±16.26 ng/ml) (*P*=0.03).

Genotypes distribution of VDR BsmI and TaqI among control subjects were in HWE (χ^2=0.65/ *P*=0.418 for TaqI, χ^2=3.66/ *P*=0.055 for BsmI). Genotypes and allelic variants distribution of TaqI and BsmI of all subjects are shown in table 4. The "G" allele frequency of BsmI (rs1544410) was significantly higher among patients with AITDs than control subjects (*P*=0.04). BsmI (rs1544410) GG+AG genotypes and G allele were more frequent among HT patients compared with control group (OR: 2.34, 95% CI: 1.16–4.70, *P* = 0.01 and OR: 1.48, 95% CI: 1.02–2.15, *P* = 0.04), respectively). There was no statistically significant difference in TaqI polymorphisms between AITDs patients and control group (*P*>0.05).

25(OH) D_3 levels in various genotypes of BsmI and TaqI are shown in figure 3. According to our analysis, vitamin D levels mean in BsmI and TaqI genotypes and

alleles did not differ among 2 groups (P>0.05).

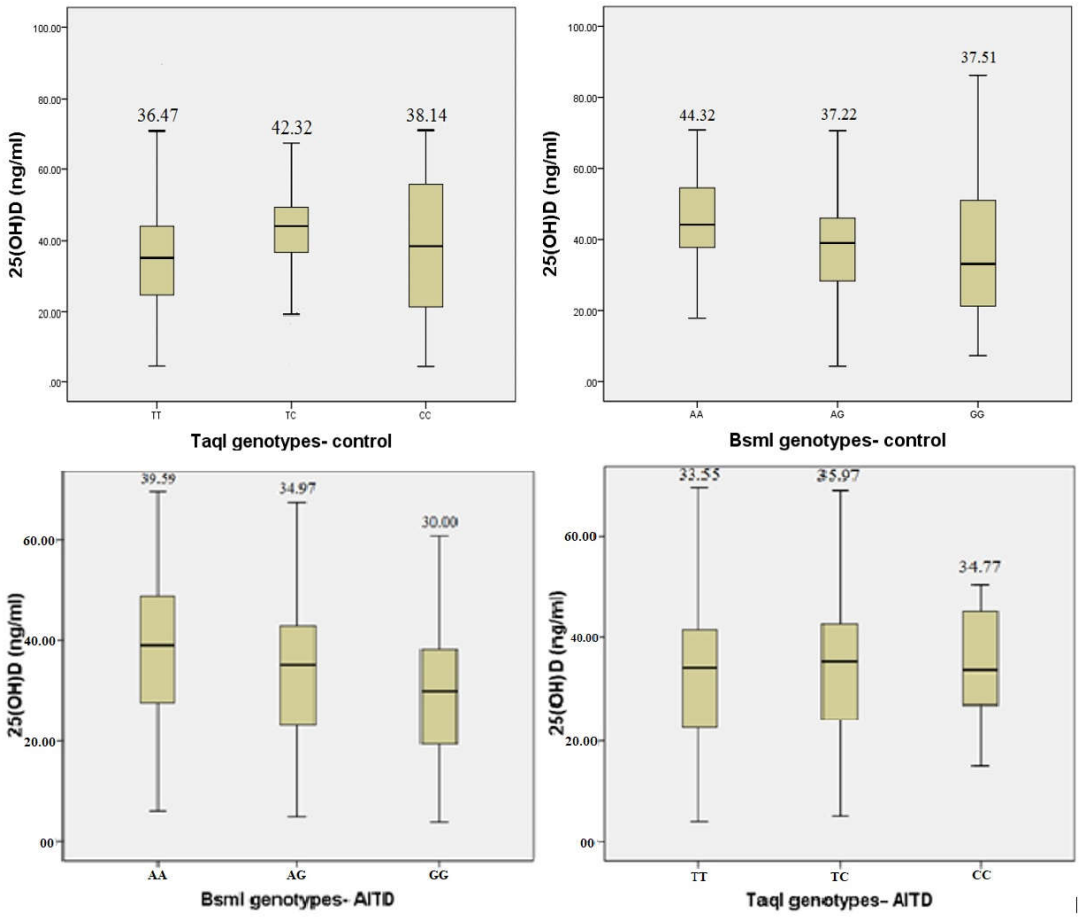

Figure 3. 25(OH)D serum levels among different genotypes of patients and controls

Table 3. Biochemical data of the study subjects

			AITDs			
		Normal range	**HashimotoN=105**	**GravesN=16**	**Controls N=117**	***P***
Anti-TPO (IU/ml)		<40	280.1 ± 270.66	219.88 ± 204.93	6.37 ± 5.73	0.0001*
TSH (mIU/L)		0.32-5.2	9.07 ± 18.2	1.48 ± 1.9	2.02 ± 1.71	0.0001*
25(OH)D (%)	**≤20 ng/ml**		24 (22.9)	2 (12.5)	14 (12)	
	20-30 ng/ml		16 (15.2)	5 (31.3)	15 (12.8)	0.06†
	≥30 ng/ml		65 (61.9)	9 (56.3)	88 (75.2)	

* Mann-Whitney U test between AITDs and Controls † $\chi 2$ analysis

Table 4. Genotype and allele distribution of TaqI (rs731236) and BsmI (rs1544410) polymorphisms among case and control groups

Marker	**Genotype/allele**	**Hashimoto N=105 (%)**	**Graves N=16 (%)**	**AITDs N=121 (%)**	**Controls N=117 (%)**
TaqI (rs731236)	TT	56(53.33)	7(43.75)	63(52.07)	52(44.44)
	TC	35(33.33)	7(43.75)	42(34.71)	49(41.88)
	CC	14(13.33)	2(12.5)	16(13.22)	16(13.68)

	T	147(70)	21(65.63)	168(69.42)	153(65.38)
	C	63(30)	11(34.38)	74(30.58)	81(34.62)
	AA	14(13.33)	3(18.75)	17(14.05)	31(26.5)
	AG	68(64.76)	10(62.5)	78(64.46)	68(58.12)
BsmI (rs1544410)	GG	23(21.9)	3(18.75)	26(21.49)	18(15.38)
	A	96(45.71)	16(50)	112(46.28)	130(55.56)
	G	114(54.29)	16(50)	130(53.72)	104(44.44)

Discussion

AITDs have a complex etiology, and a series of genetic and environmental factors are involved in the pathogenesis of these diseases (4). Some evidence suggests that vitamin D insufficiency or deficiency has been linked to the development of AITDs (10). The active form of vitamin D through binding to VDR suppresses inflammatory cytokines (Interferon-γ (IFN-γ) and Interleukin-I) through Interleukin-6 production by monocytes/macrophages and T-lymphocytes (6). But, there is no instruction about prescribing vitamin D supplementation in the treatment or prevention of AITDs (28).

In this case-control study, vitamin D status was compared among patients with AITDs and non-AITDs subjects. We included 105 patients with HT, 16 patients with GD and 117 AITDs-free controls. Our results showed that serum 25(OH)D level in patients with AITDs was lower than control subjects. These results were compatible with previous studies by Kivity *et al*., and Choi et al. who showed low levels of vitamin D are related to the pathogenesis of AITDs (29,30).

VDR polymorphisms have been shown to be associated with many autoimmune diseases, for instances, Multiple Sclerosis (MS), Type 1 Diabetes, and Inflammatory Bowell Disease (IBD) (31-33). BsmI (rs1544410) and TaqI (rs731236) are two markers of the VDR gene which were investigated in the present study. Shimada et al. showed that individuals with BsmI-"A" allele have more IFN-γ production in peripheral blood mononuclear cells (34). Also Agliardi *et al*. revealed that TaqI variants were associated with VDR protein expression and therefore affect vitamin D function (35).

In the present study, we investigated the BsmI (rs1544410) and TaqI (rs731236) genotypes and alleles distribution among AITDs patients in the Iranian Azeri Turkish population. We found BsmI-"G" allele was statistically more frequent among AITDs and HT patients, while TaqI variants were not statistically different among the two groups in our study population. In the previous study, BsmI, TaqI and FokI VDR alleles were not related to AITDs in the Tunisian population (36). In contrast, the study on Caucasian outpatients which was reported by Stefanic *et al*., found that BsmI"G" allele and AG+GG genotypes frequencies were higher in AITDs patients with HT (37). Also, Feng *et al*., in a meta-analyze study revealed that TaqI polymorphisms were associated with AITDs (38). The conflicting results can be stemmed from racial diversities existing among different populations.

VDR variants may alter vitamin D status via feedback mechanism by affecting Calcium homeostasis (8). The BsmI (rs1544410) Single-nucleotide polymorphisms (SNP) is located on intron 8 without changes in VDR protein structure, TaqI (rs731236) SNP despite being located on exon 9, it is known as silent codon change and both polymorphisms are related to other polymorphisms for instance 3'-UTR that is involved in mRNA stability and thus gene expression. With these effects on VDR protein structure and function, TaqI and BsmI polymorphisms may lead to variations in vitamin D metabolite levels (39).

In the present study, we investigated the association between VDR polymorphism and vitamin D status in AITDs patients in the Iranian population with the consideration of same sun-exposure habits and vitamin D intakes from foods and supplements among all subjects. However, no association between BsmI and TaqI SNP and vitamin D status among the two groups was observed. Li *et al*., demonstrated that Vitiligo patients with ApaI-"C" and FokI-"F" alleles had lower serum 25(OH)D levels. But BsmI and TaqI were not associated with vitamin D status (11). Other studies revealed that FokI SNP was associated with 25(OH)D serum levels in Lupus and MS patients (12,40). Giovinazzo *et al*., and Guleryuz *et al.,* showed that VDR polymorphisms are not associated with vitamin D status in HT (13,14).

Other VDR polymorphisms may have more relationship with vitamin D status, and more study with other SNPs are needed to conclude about the interaction between genetic and environmental factors that are effective in vitamin D status in AITDs patients. The

limitation of our research was a low number of Graves patients and poor quality of the registration system.

In conclusion, we report that patients with HT and GD have low vitamin D status compared with free-AITDs subjects. The VDR BsmI-G allele was higher in AITDs subjects in Azeri Turkish Iranian population, and VDR polymorphisms are not associated with vitamin D status. More studies with larger samples in Iranian population especially in GD patients are needed to confirm our results.

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References

1. Vanderpump MP. The epidemiology of thyroid disease. Br Med Bull 2011;99:39-51.
2. Antonelli A, Ferrari SM, Corrado A, Di Domenicantonio A, Fallahi P. Autoimmune thyroid disorders. Autoimmun Rev 2015;14:174-80.
3. McGrogan A, Seaman HE, Wright JW, de Vries CS. The incidence of autoimmune thyroid disease: a systematic review of the literature. Clin Endocrinol (Oxf) 2008;69:687-96.
4. Guarneri F, Benvenga S. Environmental factors and genetic background that interact to cause autoimmune thyroid disease. Curr Opin Endocrinol Diabetes Obes 2007;14:398-409.
5. Chailurkit LO, Aekplakorn W, Ongphiphadhanakul B. High vitamin D status in younger individuals is associated with low circulating thyrotropin. Thyroid 2013;23:25-30.
6. Haussler MR, Whitfield GK, Haussler CA, Hsieh JC, Thompson PD, Selznick SH, et al. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. J Bone Miner Res 1998;13:325-49.
7. Valdivielso JM, Fernandez E. Vitamin D receptor polymorphisms and diseases. Clin Chim Acta 2006;371:1-12.
8. Bhanushali AA, Lajpal N, Kulkarni SS, Chavan SS, Bagadi SS, Das BR. Frequency of fokI and taqI polymorphism of vitamin D receptor gene in Indian population and its association with 25-hydroxyvitamin D levels. Indian J Hum Genet 2009;15:108-13.
9. Inoue N, Watanabe M, Ishido N, Katsumata Y, Kagawa T, Hidaka Y, et al. The functional polymorphisms of VDR, GC and CYP2R1 are involved in the pathogenesis of autoimmune thyroid diseases. Clin Exp Immunol 2014;178:262-9.
10. Ma J, Wu D, Li C, Fan C, Chao N, Liu J, et al. Lower Serum 25-Hydroxyvitamin D Level is Associated With 3 Types of Autoimmune Thyroid Diseases. Medicine (Baltimore) 2015;94:e1639.
11. Li K, Shi Q, Yang L, Li X, Liu L, Wang L, et al. The association of vitamin D receptor gene polymorphisms and serum 25-hydroxyvitamin D levels with generalized vitiligo. Br J Dermatol 2012;167:815-21.
12. Emerah AA, El-Shal AS. Role of vitamin D receptor gene polymorphisms and serum 25-hydroxyvitamin D level in Egyptian female patients with systemic lupus erythematosus. Mol Biol Rep 2013;40:6151-62.
13. Guleryuz B, Akin F, Tunc Ata M, Mergen Dalyanoglu M, Turgut S. Vitamin-D Receptor (VDR) Gene Polymorphisms (TaqI, FokI) in Turkish Patients with Hashimoto's Thyroiditis: Relationship to the levels of Vit-D and Cytokines. Endocr Metab Immune Disord Drug Targets 2016;16:131-9.
14. Giovinazzo S, Vicchio TM, Certo R, Alibrandi A, Palmieri O, Campenni A, et al. Vitamin D receptor gene polymorphisms/haplotypes and serum 25(OH)D3 levels in Hashimoto's thyroiditis. Endocrine 2017;55:599-606.
15. Ramos-Lopez E, Kurylowicz A, Bednarczuk T, Paunkovic J, Seidl C, Badenhoop K. Vitamin D receptor polymorphisms are associated with Graves' disease in German and Polish but not in Serbian patients. Thyroid 2005;15:1125-30.
16. Yazici D, Yavuz D, Tarcin O, Sancak S, Deyneli O, Akalin S. Vitamin D receptor gene ApaI, TaqI, FokI and BsmI polymorphisms in a group of Turkish patients with Hashimoto's thyroiditis. Minerva Endocrinol 2013;38:195-201.
17. Mansournia N, Mansournia MA, Saeedi S, Dehghan J. The association between serum 25OHD levels and hypothyroid Hashimoto's thyroiditis. J Endocrinol Invest 2014;37:473-6.
18. Caturegli P, De Remigis A, Rose NR. Hashimoto thyroiditis: clinical and diagnostic criteria. Autoimmun Rev 2014;13:391-7.
19. Ginsberg J. Diagnosis and management of Graves' disease. CMAJ 2003;168:575-85.
20. Menon B, Harinarayan CV. The effect of anti epileptic drug therapy on serum 25-hydroxyvitamin D and parameters of calcium and bone metabolism--a longitudinal study. Seizure 2010;19:153-8.
21. Pappa HM, Bern E, Kamin D, Grand RJ. Vitamin D status in gastrointestinal and liver disease. Curr Opin Gastroenterol 2008;24:176-83.
22. Williams S, Malatesta K, Norris K. Vitamin D and

chronic kidney disease. Ethn Dis 2009;19:S5-8-11.
23. Kennel KA, Drake MT. Adverse effects of bisphosphonates: implications for osteoporosis management. Mayo Clin Proc 2009;84:632-8.
24. Abd El Gawad SS, Abdul Samee ER, Metwali AA, Abd El Gawad MS. Vitamin D receptor gene polymorphism and its association with 1,25-dihydroxyvitamin D(3) in patients with Graves disease in an Egyptian population: a pilot study. Endocr Pract 2012;18:132-9.
25. Gandini S, Gnagnarella P, Serrano D, Pasquali E, Raimondi S. Vitamin D receptor polymorphisms and cancer. Adv Exp Med Biol 2014;810:69-105.
26. Jia F, Sun RF, Li QH, Wang DX, Zhao F, Li JM, et al. Vitamin D receptor BsmI polymorphism and osteoporosis risk: a meta-analysis from 26 studies. Genet Test Mol Biomarkers 2013;17:30-4.
27. Wei S, Baloch ZW, LiVolsi VA. Parathyroid adenoma in patients with Graves' disease: a report of 21 cases. Endocr Pathol 2015;26:71-4.
28. Muscogiuri G, Tirabassi G, Bizzaro G, Orio F, Paschou SA, Vryonidou A, et al. Vitamin D and thyroid disease: to D or not to D? Eur J Clin Nutr 2015;69:291-6.
29. Kivity S, Agmon-Levin N, Zisappl M, Shapira Y, Nagy EV, Danko K, et al. Vitamin D and autoimmune thyroid diseases. Cell Mol Immunol 2011;8:243-7.
30. Choi YM, Kim WG, Kim TY, Bae SJ, Kim HK, Jang EK, et al. Low levels of serum vitamin D3 are associated with autoimmune thyroid disease in pre-menopausal women. Thyroid 2014;24:655-61.
31. Huang J, Xie ZF. Polymorphisms in the vitamin D receptor gene and multiple sclerosis risk: a meta-analysis of case-control studies. J Neurol Sci 2012;313:79-85.
32. Kamel MM, Fouad SA, Salaheldin O, El-Razek Ael R, El-Fatah AI. Impact of vitamin D receptor gene polymorphisms in pathogenesis of Type-1 diabetes mellitus. Int J Clin Exp Med 2014;7:5505-10.
33. Wang L, Wang ZT, Hu JJ, Fan R, Zhou J, Zhong J. Polymorphisms of the vitamin D receptor gene and the risk of inflammatory bowel disease: a meta-analysis. Genet Mol Res 2014;13:2598-610.
34. Shimada A, Kanazawa Y, Motohashi Y, Yamada S, Maruyama T, Ikegami H, et al. Evidence for association between vitamin D receptor BsmI polymorphism and type 1 diabetes in Japanese. J Autoimmun 2008;30:207-11.
35. Agliardi C, Guerini FR, Saresella M, Caputo D, Leone MA, Zanzottera M, et al. Vitamin D receptor (VDR) gene SNPs influence VDR expression and modulate protection from multiple sclerosis in HLA-DRB1*15-positive individuals. Brain Behav Immun 2011;25:1460-7.
36. Maalej A, Petit-Teixeira E, Chabchoub G, Hamad MB, Rebai A, Farid NR, et al. Lack of association of VDR gene polymorphisms with thyroid autoimmune disorders: familial and case/control studies. J Clin Immunol 2008;28:21-5.
37. Stefanic M, Papic S, Suver M, Glavas-Obrovac L, Karner I. Association of vitamin D receptor gene 3'-variants with Hashimoto's thyroiditis in the Croatian population. Int J Immunogenet 2008;35:125-31.
38. Feng M, Li H, Chen SF, Li WF, Zhang FB. Polymorphisms in the vitamin D receptor gene and risk of autoimmune thyroid diseases: a meta-analysis. Endocrine 2013;43:318-26.
39. Smolders J, Peelen E, Thewissen M, Menheere P, Tervaert JW, Hupperts R, et al. The relevance of vitamin D receptor gene polymorphisms for vitamin D research in multiple sclerosis. Autoimmun Rev 2009;8:621-6.
40. Smolders J, Damoiseaux J, Menheere P, Tervaert JW, Hupperts R. Fok-I vitamin D receptor gene polymorphism (rs10735810) and vitamin D metabolism in multiple sclerosis. J Neuroimmunol 2009;207:117-21.