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ORIGINAL

25-Hydroxyvitamin D serum level in Hashimoto's thyroiditis, but not Graves' disease is relatively deficient

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Abstract. Vitamin D is a modulator of both the innate and adaptive immune system. As vitamin D deficiency was a risk factor for some autoimmune diseases, we aimed to evaluate the serum vitamin D levels in autoimmune thyroid diseases (AITD) including Graves' disease (GD) and Hashimoto's thyroiditis (HT) and investigated the association between serum vitamin D levels and AITD. 175 AITD patients including 51 GD, 61 euthyroid HT (mild HT), 63 euthyroid HT patients with hypothyroidism receiving hormone therapy (treated HT) were recruited from the outpatient department. 51 controls were from the physical checkup center of the hospital. 25-Hydroxyvitamin D levels, thyroid function, antithyroid antibodies, IL-4, IL-17, and TNF-α were determined. Compared with the controls, treated and mild HT patients had significantly lower 25(OH)D levels (45.77±3.48 *vs.* 83.49±6.24 nmol/L, $p<0.001$) and (55.25±3.88 *vs.* 83.49±6.24 nmol/L, $p<0.001$), respectively. However, GD patients had similar 25(OH)D levels (81.77±5.60 *vs.* 83.49±6.24 nmol/L, $p=0.808$). Compared to 24.1% controls with prevalent vitamin D deficiency, mild HT and treated HT patients were significantly different (55.4%, $p<0.001$) and (70.3%, $p<0.001$), respectively; no difference was seen in the GD patients (22.9%, $p=0.797$). Serum 25(OH)D levels were not associated with thyroid function, antithyroid antibodies, and serum cytokines IL-4, IL-17, and TNF-α in patients with AITD. We observed relatively low vitamin D level in mild and treated HT patients, while GD patients had similar 25(OH)D levels to those of healthy individuals. Further studies are imperative to explore the complex etiology of vitamin D deficiency in AITD.

Key words: Serum 25-Hydroxyvitamin D, Vitamin D deficiency, Graves' disease, Hashimoto's thyroiditis, Autoimmune thyroid diseases

VITAMIN D is a fat-soluble vitamin that belongs to the steroid compounds and can be ingested from the food intake. It may also be heterogeneously synthesized by 7-dehydrogenation of the skin cholesterol upon UVB irradiation (290-320 nm) and consumed for further biological activities [1]. Vitamin D is not only a major regulator of calcium, phosphorus, and bone metabolism but also regulates cell growth and differentiation [2]. Epidemiological data suggest that vitamin D deficiency may be a risk factor associated with the development of autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, and type 1 diabetes [3-5]. Recent studies indicate that 1,25-$(OH)_2D_3$, the biologically active form of vitamin D, is a modulator of both the innate and adaptive immune system. Immune cells such as monocytes, macrophages, dendritic cells, T-lymphocytes, and B-lymphocytes are targets for the active vitamin D. It has been speculated that vitamin D plays a major role in the regulation of T helper cell differentiation as well as the secretion of TNF-α, IL-4, and IL-17 [6-8]. Activated vitamin D regulates T lymphocyte functions by inhibiting the proliferation of Th1 cells and increasing the number of Th2 cells [9]. Vitamin D can suppress Th17 transcription *via* VDR [10]. 1,25$(OH)_2D_3$ analog Elocalcitol could inhibit Th17 cytokines from secreting IL-17 [11]. However, all these conclusions were obtained from *in vitro* cell experiments.

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Autoimmune thyroid diseases (AITD) including Graves' disease (GD) and Hashimoto's thyroiditis (HT) are the most common organ-specific autoimmune disorders [12], which are characterized by lymphocytic and cytokines infiltration of the thyroid parenchyma. Cytokines play a crucial role in modulating immune responses that affect the balance between maintenance of selftolerance and initiation of autoimmunity. In our very recent studies, we found IL-4 was infiltrated in the thyroid tissue of experimental autoimmune Graves' disease (EAGD) mice. IL-4 acted in an autocrine manner to activate p-STAT6 signal and stimulate unrestricted cell growth, thus aggravating GD [13]. Wang *et al*. found direct injection of TNF-α into the thyroids of mTg-primed mice can induce thyrocyte apoptosis, indicating that pro-inflammatory TNF-α may play a critical role in thyroid destruction [14]. Horie I *et al*. disclosed Th17 cells are critical for the pathogenesis of spontaneous autoimmune thyroiditis while Th17 cells were also shown to induce GD in NOD-H2(h4), but not in BALB/c mice, thus showing that the effect of IL-17 may also differ with the genetic background [15, 16].

Recently, some studies have focused on the involvement of vitamin D in AITD [17-19]. However a few research dedicated to compare the Vitamin D level between GD and HT, and there is few report related to the Vitamin D level in euthyroid HT patients with hypothyroidism receiving hormone therapy. Therefore, the present study aimed to evaluate the serum vitamin D levels in GD and HT with normal thyroid function (mild HT) or hypothyroidism (treated HT) as compared to normal people. We also analyzed the correlation between vitamin D levels with thyroid function and thyroid autoantibodies in AITD patients. Consecutively, peripheral blood cytokines levels of TNF-α, IL-4, and IL-17 were also assessed. The correlation of peripheral vitamin D with these cytokines was analyzed further to investigate the involvement of vitamin D in the occurrence and development of AITD.

Materials and Methods

Study population

A total of 175 cases including 51 patients with newly diagnosed Graves' disease (GD), 61 patients with newly diagnosed Hashimoto's thyroiditis (mild HT), and 63 euthyroid HT patients with hypothyroidism that received thyroid hormone therapy (treated HT) were recruited from the Endocrinology outpatient department of the Fifth People's Hospital of Fudan University, China, between November 2015 to January 2016. GD was diagnosed by the presence of overt hyperthyroidism, diffuse goiter or normal thyroid volume on B ultrasonography, and high ^{131}I thyroid perturbation rate. Mild HT was diagnosed by the presence of diffuse goiter thyroid volume on B ultrasonography, and a high titer of TPOAb (>34 IU/mL) and/or TGAb (>115 IU/mL). On the other hand, treated HT was diagnosed by overt clinical hypothyroidism, TSH concentration above 10.0 mIU/L irrespective of the level of FT3 and FT4 (TSH > 100 mIU/mL, 4 female / 2 male; 10 mIU/mL ≤ TSH ≤ 100 mIU/mL, 31 female / 26 male), along with a high titer of TPOAb and/or TGAb, and the thyroid function returned to normal after more than six months of treatment with levothyroxine sodium. 51 control subjects normalized for parameters such as sex, age, and smoking status and with normal thyroid function, including TPOAb and TGAb, were recruited from the physical checkup center of the hospital. They were self-proclaimed good health, no family or personal history of thyroid disease. The exclusion criteria included a history of other autoimmune diseases, pregnancy during the study period, or any medication history that involved administration of immunosuppressive agents, calcium or vitamin D supplements, 3 months before blood sampling.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Helsinki declaration. The study protocols were approved by the Medical Ethics Committee of the Fifth People's Hospital of Shanghai, Fudan University (NO. 002). Informed consent was obtained from all individual participants included in the study.

Blood sampling

All the venous blood samples were collected in the morning (0700–0900 h) after an overnight fast of >8h from November 2015 to January 2016. The blood samples were centrifuged to obtain serum, which was sent to the clinical laboratory for testing or storage in a serum bank at -80°C until analysis.

Thyroid function test and antithyroid antibodies

The levels of free triiodothyronine (FT3) (reference range, 3.1-6.8 pmol/L), free thyroxine (FT4) (reference

range,12-22 pmol/L), TSH (reference range, 0.27-4.2 mIU/L), TPOAb (cut off level, 34 IU/mL), and TGAb (cut off level, 115 IU/mL) were determined in all the subjects on the same day of sampling, utilizing a commercial euglobulin clot lysis assay (ECLA) kit (ROCHE Diagnostics, cobas 601, Germany). Intra-assay coefficients of variation (CVs) for the above measurements were 1.9, 2.3, 4.2, 3.5, and 4.8%, respectively.

Cytokines level in serum

Human IL-4, IL-17, and TNF-α, using a sandwich Enzyme-linked Immunosorbent Assay (ELISA) kit (LEGEND MAX^{TM} kits, Biolegend lnc.), were quantified accurately. The kit was analytically pre-validated with ready-to-use reagents.

Evaluation of vitamin D status

Serum 25(OH)D levels were used to evaluate the vitamin D status. All sera from the case-control studies were measured for 25(OH)D levels by ECLA. The intra-assay coefficient of variation of the commercial kit was <8.8%. The inter-assay coefficient of variation was <8.5%. The Endocrine Society guidelines, defined the 25(OH)D status as vitamin D deficient if <50 nmol/L, insufficient when ranging over 50–75 nmol/L, and sufficient when >75 nmol/L [20].

Statistical analysis

Statistical analysis was performed using the SPSS software (version 17.0; Chicago, IL). Data are presented as mean ± standard deviation or median with 25th and 75th percentiles. Student's *t*-test and ANOVA were used for continuous variables. Non-Gaussian parameters underwent log-transformation to the normal distribution before comparison or were directly compared with Mann-Whitney U test and Wilcoxon signed-rank test. The chi-square and Fisher's exact test were used for categorical variables. The Spearman correlation analysis was employed to establish the relationship between serum 25(OH)D and various factors, *e.g.* controlled age and BMI factor. $p<0.05$ was statistically significant.

Results

The clinical and laboratory characteristics of the 226 subjects (96 males and 130 females) are shown in Table 1. There were no significant age and sex differences between GD patients, mild HT patients, treated HT patients, and controls. GD patients demonstrated increased thyroid hormones and decreased TSH levels, whereas mild and treated HT patients displayed normal levels. The autoantibody levels were significantly high in mild HT, treated HT patients, and GD patients. Compared with the GD group, the TGAb levels were remarkable in mild and treated HT patients ($p<0.05$). On the contrary, any significant difference in the TGAb levels among mild HT and treated HT patients was not observed. The TPOAb levels were remarkably increased in treated HT patients than those in mild HT patients and in GD patients ($p<0.05$). The TPOAb levels were similar in mild HT and GD patients.

Table 1 Clinical characteristics of subjects

	Mild HT	Treated HT	GD	Control	*p* value
N (female/male)	61 (34/27)	63 (35/28)	51 (30/21)	51 (31/20)	-
Age, y *	40.88 ± 1.61	42.41 ± 1.49	39.79 ± 1.73	36.48 ± 1.68	0.190
BMI *	22.42 ± 1.78	23.01 ± 1.56	22.12 ± 1.33	22.73 ± 1.41	0.239
FT3, pmol/L *	4.15 ± 0.11	4.54 ± 0.09	25.02 ± 1.60	4.75 ± 0.13	$p<0.001$
FT4, pmol/L *	12.27 ± 0.90	12.77 ± 0.73	61.14 ± 4.01	13.37 ± 0.91	$p<0.001$
TSH, mIU/mL +	2.23 (1.55-3.30)	2.77 (1.96-4.06)	0.01 (0.00-0.01)	2.11 (1.65-2.86)	$p<0.001$
TPOAb, IU/mL +	245.65 (77.63-577.28)	486.40 (243.1-1300.00)	158.20 (26.28-317.08)	15.45 (10.17-26.72)	$p<0.001$
TGAb, IU/mL +	295.90 (150.05-461.55)	326.45 (156.28-475.58)	137.40 (29.50-406.30)	10.00 (6.18-15.55)	$p<0.001$
TNF-α, pg/mL *	9.79 ± 0.51	14.09 ± 4.26	15.43 ± 3.22	9.44 ± 0.66	0.359
IL-4, pg/mL *	0.67 ± 0.03	0.59 ± 0.03	0.72 ± 0.07	0.72 ± 0.03	0.075
IL-17, pg/mL *	2.27 ± 0.25	1.97 ± 0.17	1.78 ± 0.14	1.79 ± 0.27	0.324

GD, Graves' disease; HT, Hashimoto's thyroiditis. * Mean ± standard deviation. + Median with interquartile range.

Significant differences were observed in serum 25(OH)D levels among mild HT, treated HT, and GD patients (p<0.001). Compared with the controls, treated and mild HT patients exhibited significantly lower 25(OH)D levels (45.77±3.48 *vs.* 83.49±6.24 nmol/L, p<0.001) and (55.25±3.88 *vs.* 83.49±6.24 nmol/L, p<0.001), respectively. However, the GD patients' 25(OH)D levels did not alter significantly (81.77±5.60 *vs.* 83.49±6.24 nmol/L, p=0.808). Also, the 25(OH)D levels between mild HT patients and treated HT patients did not show any remarkable alteration (55.25±3.88 *vs.* 45.77±3.48 nmol/L) (Fig. 1).

Vitamin D deficiency was prevalent in 22.9% of GD cases, 55.4% of mild HT cases, 70.3% of treated HT cases, and 24.1% of controls. Compared with the controls, the prevalence of vitamin D deficiency was substantially different in mild HT patients (p<0.001) and treated HT patients (p<0.001). However, no differences were observed in the GD patients (p=0.797). Compared with the mild HT patients, treated HT showed a significant difference in the prevalence of vitamin D deficiency (p<0.05) (Fig. 2).

Following the amendment in the age, BMI, and gender in normal controls, the peripheral blood vitamin D was not correlated with thyroid function, TPOAb, and TGAb. The correlation analysis of vitamin D levels and thyroid hormone in GD, mild HT and treated HT groups revealed that serum 25(OH)D level was not associated with FT3, FT4, TSH, TPOAb, and TGAb (Table 2).

Compared with the controls, treated and mild HT patients had weakly lower IL-4 levels (p>0.05). On the other hand, treated HT and mild HT patients both showed only a modest increase in TNF-α and IL-17 levels (p>0.05). The TNF-α level increased slightly in the GD patients but did not achieve statistical significance (p>0.05), whereas IL-4 and IL-17 did not show any distinct changes (Table 1).

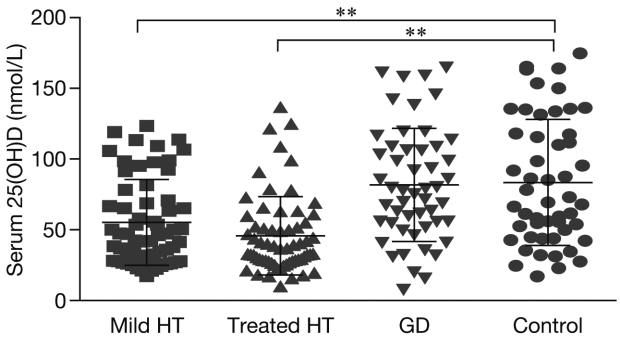

Fig. 1 Serum levels of 25(OH)D in AITD patients and controls
GD, Graves' disease; HT, Hashimoto's thyroiditis; ** p<0.001, compared with control group.

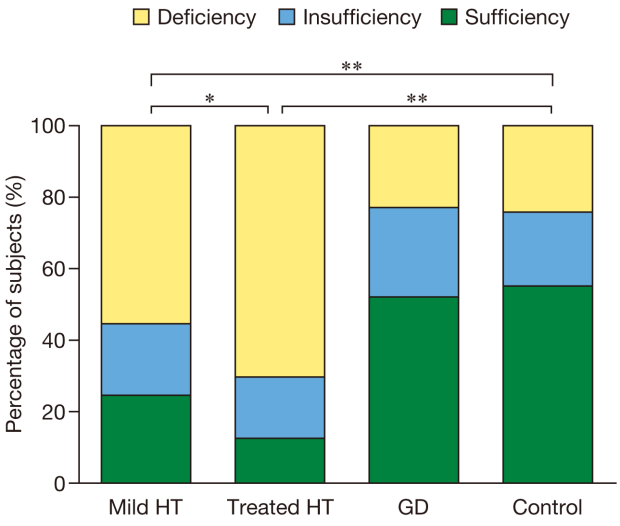

Fig. 2 Prevalence of vitamin D deficiency in AITD patients and controls
GD, Graves' disease; HT, Hashimoto's thyroiditis; Vitamin D sufficiency, 25(OH)D > 75 nmol/L; insufficiency, 50 nmol/L < 25(OH)D < 75 nmol/L; deficiency, 25(OH)D < 50 nmol/L; * p<0.05, compared with mild HT group; ** p<0.001, compared with control group.

Table 2 Correlation of serum 25(OH)D and thyroid hormone, autoantibodies, serum cytokines levels

	25(OH)D (Mild HT)		25(OH)D (Treated HT)		25(OH)D (GD)		25(OH)D (Control)	
	r	*p* value	*r*	*p* value	*r*	*p* value	*r*	*p* value
FT3	0.005	0.975	0.223	0.198	0.262	0.495	0.366	0.094
FT4	0.208	0.186	0.038	0.829	0.329	0.388	0.270	0.225
TSH	0.180	0.253	0.320	0.061	-0.302	0.429	0.201	0.369
TPOAb	-0.155	0.326	-0.029	0.870	-0.095	0.808	-0.214	0.235
TGAb	-0.099	0.531	-0.239	0.166	-0.352	0.353	-0.012	0.468
TNF-α	-0.084	0.599	-0.133	0.448	-0.344	0.365	-0.059	0.795
IL-4	0.208	0.186	0.26	0.131	0.577	0.103	0.603	0.063
IL-17	0.275	0.078	0.427	0.071	0.602	0.086	0.361	0.099

GD, Graves' disease; HT, Hashimoto's thyroiditis.

The correlation analysis between vitamin D levels and the serum cytokines levels of AITD revealed that 25(OH)D levels were neither associated with TNF-α nor with IL-4 and IL-17 levels (Table 2).

Discussion

The current state of vitamin D inadequacy around the world is discouraging, and hence, its deficiency has been recognized as a global health issue [20]. Vitamin D plays a vital role in the regulation of calcium-phosphorus metabolism, as well as in decreasing the risk of rickets, fractures, osteoporosis, and osteomalacia. In recent years, a number of studies have shown that vitamin D not only has immunoregulation function but also plays a major role in the pathogenesis of autoimmune diseases [21], affecting the interaction of vitamin D receptor on target cells.

Various studies have suggested that vitamin D level in AITD patients is lower than that in the healthy individuals, and overall the proportion of vitamin D deficient patients is higher than the normal people. Patients with vitamin D deficiency are more likely to have TPOAb and thyroid dysfunction as compared to the standard vitamin D level people [22]. Our results showed that compared to the healthy people, mild HT and treated HT groups suffered from significant vitamin D deficiency. Also, compared to the mild HT group, vitamin D level of the treated HT group was slightly decreased ($p>0.05$), and expressed a great proportion of vitamin D deficiency ($p<0.05$). Mansournia N *et al*. and Tamer *et al*. [17, 18] found that vitamin D level presented a significantly decreasing trend in hypothyroidism patients who failed to administer medication. Our study further confirmed that hypothyroidism patients continued to suffer from severe vitamin D deficiency even after the regular thyroid function was restored by drug substitution treatment. Thus, it cannot be ignored to supplement vitamin D in patients undergoing levothyroxine sodium treatment. Yasuda *et al*. [23] established that the serum vitamin D level in the group of 26 females, newly diagnosed with GD, was significantly lower than the group comprising of 46 healthy females ($p<0.05$). Ma *et al*. [24] demonstrated that vitamin D level in 70 patients with newly diagnosed GD was substantially lower than that in the healthy control group. However, in the present study, we did not find significantly decreased vitamin D level in patients newly diagnosed with GD, which was contradictory to the previous studies. Chiovato L *et al*. has proposed specifically designed studies evaluating the role of vitamin D deficiency in GD patients are needed before firm conclusions can be formulated [25]. We speculated that it might be due to the variability in the patients enrolled in different centers, which led to different vitamin D level. Zhang *et al*. [26] divided 70 patients with newly diagnosed Graves' disease into TRAb positive and negative groups. The vitamin D level of the TRAb positive group was significantly lower than that of the healthy control group, but that of the TRAb negative GD group was similar to the control group. We also performed statistical analysis of a previously collected large sample data of newly diagnosed GD patients and found a significant difference in the serum calcium levels between TPOAb>600 and ≤600 groups (2.25 *vs*. 2.31 nmol/L, $p=0.008$). Thus, we speculated that the difference in TPOAb among newly diagnosed GD patients in different research centers might be associated with variable serum calcium level and ultimately affect the vitamin D level in the patients. Previously, it was believed that GD patients required vitamin D supplements. However, we found that the proportion of GD patients with vitamin D deficiency and insufficiency was similar to that of the normal people. 52.1% of the GD patients had sufficient vitamin D, and only half of them required an appropriate amount of vitamin D supplement. However up to date the number of GD patients enrolled in recent researches focused on the vitamin D level was small, a large scale study is imperative to explore vitamin D deficiency in GD.

Kivity *et al*. [22] reported a link between vitamin D deficiency and the presence of antithyroid antibodies including TPOAb and TGAb. Zhang *et al*. [26] observed that the level of 25(OH)D in serum was inversely correlated with TRAb titer in serum of TRAb-positive GD patients. However, other studies failed to demonstrate a firm correlation. Effraimidis *et al*. [27] did not find a correlation between low vitamin D level and early AITD. Also, Chailurkit *et al*. [28] failed to find any correlation between vitamin D level with the thyroid function and thyroid autoantibodies in AITD patients. Yasuda *et al*. [23] also did not find that vitamin D level was associated with the thyroid function in GD patients. In this study, we also could not establish a correlation between vitamin D level with thyroid function and thyroid autoantibodies in both the normal controls and AITD patients.

Therefore, vitamin D deficiency perhaps may not be associated with the pathogenesis of AITD. However, we observed a relatively low serum vitamin D level in mild HT, the vitamin D level in euthyroid overt HT received thyroid hormone therapy was not restored to normal levels, and GD patients had similar 25(OH)D levels to those seen in healthy individuals. Thyroid hormone levels seem to indirectly affect Vitamin D status in AITD. Although our research has not evaluated other variables known to influence vitamin D levels, such as estrogen use, month of blood sampling, sun exposure information, studies should verify the complex etiology of vitamin D deficiency in AITD.

In our study, we expected to seek the immune regulation paradigm of vitamin D in autoimmune disease patients through the detection of serum cytokines and vitamin D level in peripheral blood of the subjects. It is noteworthy serum TNF-α, IL-4, IL-17 levels did not show any distinct changes in AITD patients. Our recent observations has verified the IL-4 expression in the thyroid was significantly increased while serum IL-4 level were unaffected in EAGD mice. IL-4 derived from thyroid epithelial cells (TEC) may contribute to the pathogenesis of GD by stimulating unrestrained TEC hyperplasia [13]. Cytokines from thyroid tissue perhaps play a more critical role in the pathogenesis of GD than circulating serum cytokines. Moreover, our analysis did not find any association of vitamin D with various cytokines in AITD patients. This may be attributed to the limitations of our study which may be rectified as follows: (1) Increase in the sample size. (2) Application of levothyroxine sodium in hypothyroidism patients may affect cytokine expression. (3) The cytokine expression in the cell subsets should be analyzed more accurately with flow cytometry. In addition, we will carry out the research on the variation of cytokines from the thyroid and serum in EAGD and spontaneous autoimmune thyroiditis mice before and after vitamin D treatment.

We observed low serum vitamin D levels in HT patients, which did not alter significantly in GD patients compared with non-AITD controls. Clinical evidence suggested the administration of vitamin D and its analogs for AITD treatment, but the vitamin D supplements were not necessary for all the AITD patients. We could identify the populations requiring treatment by evaluating serum 25-(OH)D level to achieve early detection and therefore, begin early treatment.

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Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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References

1. Holick MF (2006) Resurrection of vitamin D deficiency and rickets. *J Clin Invest* 116: 2062-2072.
2. Jensen SS, Madsen MW, Lukas J, Binderup L, Bartek J (2001) Inhibitory effects of 1alpha, 25-dihydroxyvitamin D(3) on the G(1)-S phase-controlling machinery. *Mol Endocrinol* 15: 1370-1380.
3. Runia TF, Hop WC, de Rijke YB, Buljevac D, Hintzen RQ (2012) Lower serum vitamin D levels are associated with a higher relapse risk in multiple sclerosis. *Neurology* 79: 261-266.
4. Varenna M, Manara M, Cantatore FP, Del Puente A, Di Munno O, *et al*. (2012) Determinants and effects of vitamin D supplementation on serum 25-Hydroxyvitamin D levels in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 30: 714-719.
5. Greer RM, Portelli SL, Hung BS, Cleghorn GJ, McMahon SK, *et al*. (2013) Serum vitamin D levels are lower in Australian children and adolescents with type 1 diabetes than in children without diabetes. *Pediatr Diabetes* 14: 31-41.
6. Tsatsoulis A (2006) The role of stress in the clinical expression of thyroid autoimmunity. *Ann N Y Acad Sci* 1088: 382-395.
7. Peng D, Xu B, Wang Y, Guo H, Jiang Y (2013) A high frequency of circulating Th22 and Th17 cells in patients with new onset Graves' disease. *PLoS One* 8: e68446.

8. Joshi S, Pantalena LC, Liu XK, Gaffen SL, Liu H, *et al*. (2011) 1,25-Dihydroxyvitamin D3 ameliorates Th17 autoimmunity via transcriptional modulation of interleukin-17A. *Mol Cell Biol* 31: 3653-3669.
9. Kongsbak M, von Essen MR, Levring TB, Schjerling P, Woetmann A, *et al*. (2014) Vitamin D-binding protein controls T cell responses to vitamin D. *BMC Immunol* 15: 35.
10. Chang SH, Chung Y, Dong C (2010) Vitamin D suppresses Th17 cytokine production by inducing C/EBP homologous protein (CHOP) expression. *J Biol Chem* 285: 38751-38755.
11. Borgogni E, Sarehielli E, Sottili M, Santarlasci V, Cosmi L, *et al*. (2008) Elocalcitol inhibits inflammatory responses in human thyroid cells and T cells. *Endocrinology* 149: 3626-3634.
12. Antonelli A, Ferrari SM, Corrado A, Di Domenicantonio A, Fallahi P (2015) Autoimmune thyroid disorders. *Autoimmun Rev* 14: 174-180.
13. Jiang X, Zha B, Liu X, Liu R, Liu J, *et al*. (2016) STAT6 deficiency ameliorates Graves' disease severity by suppressing thyroid epithelial cell hyperplasia. *Cell Death Dis* 7: e2506.
14. Wang SH, Bretz JD, Phelps E, Mezosi E, Arscott PL, *et al*. (2002) A unique combination of inflammatory cytokines enhances apoptosis of thyroid follicular cells and transforms nondestructive to destructive thyroiditis in experimental autoimmune thyroiditis. *J Immunol* 168: 2470-2474.
15. Horie I, Abiru N, Nagayama Y, Kuriya G, Saitoh O, *et al*. (2009) T helper type 17 immune response plays an indispensable role for development of iodine-induced autoimmune thyroiditis in nonobese diabetic-H2h4 mice. *Endocrinology* 150: 5135-5142.
16. Horie I, Abiru N, Saitoh O, Ichikawa T, Iwakura Y, *et al*. (2011) Distinct role of T helper Type 17 immune response for Graves' hyperthyroidism in mice with different genetic backgrounds. *Autoimmunity* 44: 159-165.
17. Mansournia N, Mansournia MA, Saeedi S, Dehghan J (2014) The association between serum 25OHD levels and hypothyroid Hashimoto's thyroiditis. *J Endocrinol Invest* 37: 473-476.
18. Tamer G, Arik S, Tamer I, Coksert D (2011) Relative Vitamin D insufficiency in Hashimoto's thyroiditis. *Thyroid* 21: 891-896.
19. Wang J, Lv S, Chen G, Gao C, He J, *et al*. (2015) Meta-analysis of the association between vitamin D and autoimmune thyroid disease. *Nutrients* 7: 2485-2498.
20. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, *et al*. (2011) Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 96: 1911-1930.
21. Bikle D (2009) Nonclassic actions of Vitamin D. *J Clin Endocrinol Metab* 94: 26-34.
22. Kivity S, Agmon-Levin N, Zisappl M, Shapira Y, Nagy EV, *et al*. (2011) Vitamin D and autoimmune thyroid diseases. *Cell Mol Immunol* 8: 243-247.
23. Yasuda T, Okamoto Y, Hamada N, Miyashita K, Takahara M, *et al*. (2012) Serum vitamin D levels are decreased and associated with thyroid volume in female patients with newly onset Graves' disease. *Endocrine* 42: 739-741.
24. Ma J, Wu D, Li C, Fan C, Chao N, *et al*. (2015) Lower Serum 25-Hydroxyvitamin D Level is Associated With 3 Types of Autoimmune Thyroid Diseases. *Medicine (Baltimore)* 94: e1639.
25. Rotondi M, Chiovato L (2013) Vitamin D deficiency in patients with Graves' disease: probably something more than a casual association. *Endocrine* 43: 3-5.
26. Zhang H, Liang L, Xie Z (2015) Low Vitamin D Status is Associated with Increased Thyrotropin-Receptor Antibody Titer in Graves Disease. *Endocr Pract* 21: 258-263.
27. Effraimidis G, Badenhoop K, Tijssen JG, Wiersinga WM (2012) Vitamin D deficiency is not associated with early stages of thyroid autoimmunity. *Eur J Endocrinol* 167: 43-48.
28. Chailurkit LO, Aekplakorn W, Ongphiphadhanakul B (2013) High Vitamin D Status in Younger Individuals Is Associated with Low Circulating Thyrotropin. *Thyroid* 23: 25-30.