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Effect of low and high doses of vitamin D_3 therapy on vitamin D_3, antithyroid antibody and thyroid hormone levels in thyroid autoimmunity.

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ABSTRACT

Aims: *Vitamin D_3 deficiency is more common in autoimmune thyroid diseases (AITD). Vitamin D_3 supplementation in AITD is not questioned, but the required doses have been offered differently in several reports. This study attempts to provide a clinical answer as to what doses of vitamin D_3 may be effective in AITD.*

Methods: *Ninety-seven patients were included in the study: 44 with Hashimoto's thyroiditis (HT), 26 with Graves 'disease (GD) and 27 controls. Serum vitamin D_3, thyroid hormone and autoantibody levels against thyroid peroxidase (TPO) and TSH receptor were measured before and after treatment. Biochemical data were measured by a fully automated competitive chemiluminescence assay and presented as geometric mean(95% confidence interval).*

Results: *Baseline vitamin D_3 deficiency was detected in 22 out of 44 HT, 13 out of 26 GD and 19 out of 27 controls, and improved after vitamin D_3 therapy. Vitamin D_3 therapy significantly increased serum vitamin D_3 levels in all patient groups [46.07(16.55-128.29) vs. 71.05(40.61-124.3) nmol/l, $p<0.0001$ for HT, 44.27(8.98-218.25) vs. 64.22(38.85-106.16) nmol/l, $p<0.0207$ for GD, and 39(15.57-97.73) vs. 59.32(27.27-129.03) nmol/l, $p<0.0003$ for controls]. The increass in vitamin D_3 levels was found after low-dose vitamin D_3 therapy ($\leq$7000 NE/week) in HT ($p<0.0001$) and controls ($p<0.0002$), but not in GD. The success of vitamin D_3 therapy was dependent on high doses of vitamin D_3 (>7000 NE/week) in GD, but levels were lower in Graves' ophthalmopathy ($p<0.0302$). Anti-TPO antibody and TSH levels were decreased by low doses of vitamin D_3 in HT, but FT_4 and FT_3 decreased, and TSH levels increased only by high doses in GD.*

***Conclusion**: The efficacy of vitamin D_3 therapy was demonstrated in all patient groups. The greatest improvement in vitamin D_3 status was seen in HT. A dose-dependency of vitamin D_3 therapy was rather related to the improvement in GD.*

Keywords: vitamin D_3 status; low and high doses of vitamin D_3; autoimmune thyroid diseases; efficacy of vitamin D_3 therapy

Introduction

Autoimmune thyroid diseases (AITD) are characterized by low vitamin D_3 levels [1]. Vondra et al. suggested that proinflammatory cytokines and polymorphisms in genes encoding VDR are involved in the pathogenesis of vitamin D_3 deficiency [2]. In Hashimoto's thyroiditis (HT), vitamin D_3 status may modify serum levels of antithyroid peroxidase antibodies (anti-TPO antibodies) [3]. The results were identical to those found in patients with type 2 diabetes mellitus: patients with anti-TPO antibodies were also vitamin D_3 deficient [4]. Premenopausal women who were anti-TPO antibody positive had lower vitamin D_3 levels [5]. New-onset Graves' disease (GD) was associated with lower vitamin D_3 levels compared to controls [6]. In turn, low vitamin D_3 levels were associated with a higher incidence of GD recurrence [7]. Ke et al. showed that vitamin D_3 deficiency was more common in HT than in GD [8]. Few reports were found that compared the association with thyroid function, different doses of vitamin D_3, thyroid hormone and antithyroid antibody status.

Why is vitamin D_3 important in human diseases? Vitamin D_3 [in the form of calcidiol: 25(OH)-vitamin D_3 or calcitriol: 1,25$(OH)_2$-vitamin D_3] is involved in several systemic functions of the human organism, affecting the bone-muscle system, the endocrine system, the immunoregulatory system, the metabolic system, the cardiovascular system and the tumorigenic system. The vitamin D receptor (VDR) is expressed by most cells, including immune cells (B and T lymphocytes, antigen-presenting cells), parenchymal cells (pancreas, skin, intestine, kidney, liver, prostate, myocardium), bone, muscle and blood vessels [9-13]. Vitamin D_3-dependent endocrine processes have been demonstrated in polycystic ovary syndrome (PCOS), type 1 (T1DM) and type 2 diabetes mellitus (T2DM), thyroid disease, Addison's disease, and hyperparathyroidism [14-16]. Vitamin D_3 is synthesized from the steroid precursor 7-dehydrocholesterol in the skin by ultraviolet (UVB) radiation after hydroxylation in the liver [25 (OH)-vitamin D_3] and kidneys [1, 25$(OH)_2$-vitamin D_3]. The classification of vitamin D_3 status can be divided into 3 groups: vitamin D_3 deficiency (below 50 nmol/l), insufficiency (between 50-75 nmol/l) and sufficiency (above 75 nmol/l) [17].

This study demonstrated the effects of low and high doses of vitamin D_3 on serum vitamin D_3 levels and clinical signs in AITD. The different doses of vitamin D_3 help us to study their effects on vitamin D_3 levels in relation to thyroid functions, thyroid hormone and antithyroid antibody levels, and the presence or absence of ophthalmopathy. The results may provide a better understanding of the pathomechanism of GD or HT, as well as finding the appropriate vitamin D_3 doses for adjuvant treatment.

Patients and Methods

Patients

Ninety-seven patients were studied: 44 patients with HT (mean age 49±12 years, all female), 26 patients with GD (mean age 48±15 years, 4 males and 22 females; 11 cases had ophthalmopathy) and 26 controls (mean age 46±12 years, 2 males and 25 females). Patients were selected from those attending the immunoendocrine outpatient clinic run by a single physician. The selection criteria were the diagnosis of autoimmune thyroid diseases and the lack of vitamin D_3 supplementation before 6 months of the study. The control cases were healthy subjects who had not received vitamin D_3 before 6 months. All patients gave verbal consent to participate in the study after being informed in advance, and all patients accepted vitamin D_3 treatment with the clinical and biochemical controls. The presence of severe comorbidities and tumors were exclusion criteria. Therefore, 11 patients with HT out of 55, 8 patients with GD out of 34 and 8 controls out of 35 were excluded during the study. The diagnosis of Graves' ophthalmopathy (GO) was made by ophthalmologists using the American Thyroid Association (ATA) and NOSPECT classifications (18, 19). Biochemical data were measured at the start of vitamin D_3 therapy, referred to as pre-therapy data, and at the next control visit after approximately 6 months (post-therapy data), but in GD after approximately 3months, referred to as post-therapy data. Two different doses of 1,25 $(OH)_2$-vitamin D_3 medication (called vitamin D_3 therapy) were used: low vitamin D_3 doses ($\leq$ 7000 NE/week) and high vitamin D_3 doses (>7500 NE/ week). All patients with HT (receiving levothyroxine therapy, 50-175 µg/day) were euthyroid at the time of the study. In GD, 10 patients were hyperthyroid and 16 were euthyroid at baseline. Eighteen patients were treated with antithyroid drugs during the study [16 patients with methimazole (10-30 mg/day) and 2 patients with propylthiouracil (PTU) (150 mg/day)]. Twenty-seven healthy volunteers served as controls.

Methods

Determination of serum 25(OH)-vitamin D_3, thyroid hormones (TSH, FT_4, FT_3) and serum antithyroid antibodies against TPO and TSH receptor

Blood samples for analysis (25(OH)-vitamin D_3, TSH, FT_4, FT_3, anti-TPO and TSH receptor antibodies) were obtained at the outpatient clinic and sent to the laboratory within 5 hours. Samples were stored at 4 °C prior to transport. The direct competitive chemiluminescence assay (CLIA) (DiaSorin LIAISON, USA) was used for the fully automated measurement of 25(OH)-vitamin D_3 levels (called serum vitamin D_3 levels). Thyroid hormone (TSH, FT_4, FT_3) and anti-TPO antibody levels were measured by fully automated chemiluminescence immunoassay. The normal range of values was: 0.4-4 mIU/l for TSH, 11.5-12.7 pmol/l for FT_4, 3.5-6.5 pmol/l for FT_3 and 0-35 IU/ml for anti-TPO antibodies. Antibodies against TSH receptor were measured by competitive immunoassay in ELISA (Cobas anti-TSHR antibody, Roche Diagnostics, Germany). Levels greater than 1.5 IU/l were considered positive.

All procedures were performed in accordance with the 1964 Declaration of Helsinki and the Regional and Institutional Ethics Committee of the University of Debrecen.

Statistics

The measured data were presented as GM

(geometric mean) with 95%CI (confidence interval) except for age, doses, and duration of vitamin D_3 therapy, which were presented as mean±SD. The measured biochemical data were skewed and therefore used in logarithmic form, in which they were approximately normally distributed. Student's independent t-test was used to compare different groups, and paired t-test was used to compare groups before and after vitamin D_3 therapy. One-way ANOVA was used to compare serum vitamin D_3 levels among the patient groups after therapy. Two-way repeated measures ANOVA was used to evaluate data from two time-points and two different treatments with low and high doses of vitamin D_3 within groups. The chi-squared test was used to compare categorical data. The strong correlation between two measured data was exhibited with curve estimation and 95%CI of the correlation coefficient (r) using linear regression analysis. P values less than 0.05 were considered significant. Statistical analysis was performed using Medcalc 17.9.7 and SPSS 15.0 software for Windows®.

Results

Serum vitamin D_3, thyroid hormone and antithyroid antibody levels with respect of pre- and post-therapy, and distribution of vitamin D_3 status among the patient groups

Table 1A shows the changes in vitamin D_3 levels, thyroid hormone and antithyroid antibody levels. The difference in serum vitamin D_3 levels between pre- and post-therapy was significant in all patient groups: 46.07(16.55-128.29) vs. 71.05(40.61-124.3) nmol/l, p<0.0001 for HT; 44.27(8.98-218.25) vs. 64.22(38.85-106.16) nmol/l, p<0.0207 for GD; 39(15.57-97.73) vs. 59.32(27.27-129.03) nmol/l, p<0.0003 for controls. Anti-TPO antibody levels decreased significantly after vitamin D_3 therapy compared to pre-therapy in HT: 221.57 (16.3-3012.57) vs. 125.82(6.62-2391.9) IU/ml, p<0.001. In GD, changes in FT_4 and FT_3 levels, and antibody levels against TSH receptor after vitamin D_3 therapy (patients were also receiving antithyroid drug (ATD) therapy) were compared to baseline data. The results were as follows: 20.43(9 -46.34) vs. 16.21(11.07-23.73) pmol/l, p<0.0192 for FT_4; 6.52(1.75-24.33) vs. 4.78(3.31-6.91) pmol/l, p<0.0253 for FT_3 and 2.04(0.15-27.37) vs. 1.32(0.08-21.59) IU/l, p<0.0247 for TSH receptor antibodies.

Table 1: Changes in serum vitamin D_3 levels and biochemical data after vitamin D_3 therapy (A), and age, vitamin D_3 doses with the duration of therapy (B) in the patient groups.

A

Studied parameters	Hashimoto's thyroiditis (n=44)			Graves' disease (n=26)			Controls (n=27)		
	Vitamin D_3 therapy			Vitamin D_3 therapy			Vitamin D_3 therapy		
	Pre-	Post-	p	Pre-	Post-	p	Pre-	Post-	p
Vitamin D_3 levels (nmol/l)	46.07 (16.55-128.29)	71.05 (40.61-124.3)	0.0001	44.27 (8.98-218.25)	64.22 (38.85-106.16)	0.0207	39 (15.57-97.73)	59.32 (27.27-129.03)	0.0003
TSH levels (mIU/l)	10.1 (0.06-18.35)	0.91 (0.07-12.15)		0.31 (0.002-48.75)	0.39 (0.005-29.43)		1.03 (0.27-3.93)	1.06 (0.23-4.77)	
FT_4 levels (pmol/l)	17.42 (13.02-18.35)	17.17 (12.28-24)		20.43 (9-46.34)	16.21 (11.07-23.73)	0.0192	15.42 (12.75-18.64)	15.38 (12.05-19.62)	
FT_3 levels (pmol/l)				6.52 (1.75-24.33)	4.78 (3.31-6.91)	0.0253			
Anti-TPO antibody levels (IU/ml)	221.57 (16.3-3012.57)	125.82 (6.62-2391.9)	0.001	72.54 (3.53-1488.85)	62.83 (2.86-1381.99)				
TSH receptor antibody levels (IU/l)				2.04 (0.15-27.37)	1.32 (0.08-21.59)	0.0247			

Table 1B shows the age, dose and duration of vitamin D_3 therapy. The data were age and duration matched and are as follows: 49±12 years, 1096±333 NE/day and 149±70 days for HT; 48±15 years, 1226±566 NE/day and 139±83 days for GD;

46±12 years, 966±267 NE/day and 147±62 days for controls, respectively.

B

Studied parameters	Hashimoto's thyroiditis (n=44)	Graves' disease (n=26)	Controls (n=27)
Age (years)	49±12	48±15	46±12
Vitamin D_3 doses (NE/day)	1096±333	1226±566	966±267
Duration of vitamin D_3 therapy (days)	149±70	139±83	147±62

The changes in vitamin D_3 status between pre- and post-therapy, - based on vitamin D_3 deficiency, insufficiency and sufficiency -, were significant only in HT (Figure 1). The cases with vitamin D_3 deficiency, insufficiency, and sufficiency were 22, 16, and 6 at pre- and 3, 23, and 18 at post-therapy for HT, $p<0.0039$; 13, 7, and 6 at pre- and 4, 14, and 8 at post-therapy for GD; 18, 6, and 2 at pre- and 6, 15, and 6 at post-therapy for controls, respectively. No changes in vitamin D_3 status were found in 5 cases of controls, 2 cases of GD patients, and 3 cases of HT patients.

Figure 1

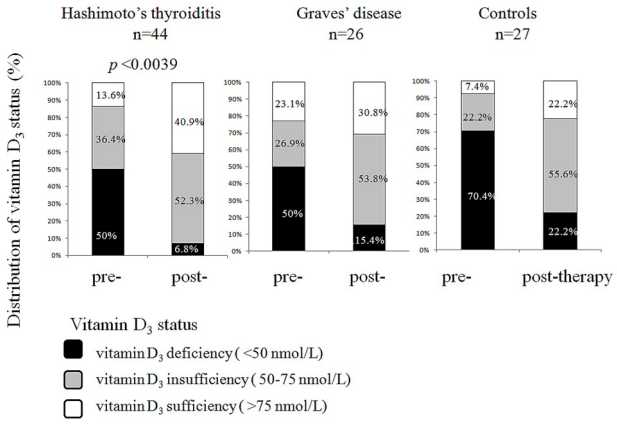

Figure 1: Changes in the distribution of vitamin D_3 status (deficiency, insufficiency and sufficiency) after vitamin D_3 therapy in the patient groups.

Changes in serum vitamin D_3 levels after treatment with low and high doses of vitamin D_3 in the patient groups

The changes in serum vitamin D_3 levels are shown in Figure 2, measured by pre- and post-therapy with low (≤7000 NE/week) and high (>7000/ week) doses of vitamin D_3 in the patient groups. The increase in vitamin D_3 levels after therapy with low doses of vitamin D_3 was significant in HT and controls [41.61(13.79-125.54) vs. 70.39 (43.66-113.48) nmol/l, $p<0.0001$ for HT and 38.17 (13.28-109.67) vs. 64.52(33.32-124.82) nmol/l, $p<0.0001$ for controls]. No relevant increase in vitamin D_3 levels could be demonstrated in GD [32.85(5.2-207.59) vs. 61.65(34.07-111.57) nmol/ l]. The increase in vitamin D_3 levels after high doses of vitamin D_3 was found only in HT [51.51 (20.86-127.22) vs. 71.78(37.52-137.33) nmol/l, $p<0.0002$]. No relevant increase in vitamin D_3 levels was found in GD and controls [57.17(17.27-189.22) vs. 66.5(43.64-101.32) nmol/l for GD and 41.06(24.84-67.89) vs. 48.58(19.09-123.62) nmol/l for controls]. Comparison of serum vitamin D_3 levels in post-therapy among patient groups was made by one-way ANOVA. No significance in serum vitamin D_3 levels could be demonstrated at low doses of vitamin D_3, while a significant difference was found at high doses of vitamin D_3 between HT and controls, $p<0.022$. Weekly doses of vitamin D_3 were approximately identical at low doses: 5842±2828 NE for controls, 5957±2828 NE for HT and 5917±3536 NE for GD, but differed at high doses: 8950±2899 NE for controls, 9557±6081 NE for HT and 10864±9546 NE for GD. The duration of therapy was almost identical at low and high doses of vitamin D_3, but differed among patient groups [177 and 191 days for controls; 487 and 449 days for HT; 234 and 282 days for GD, respectively].

Figure 2

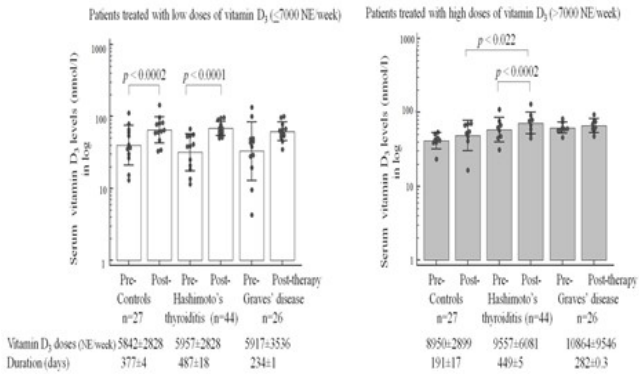

Figure 2: Increase in serum vitamin D_3 levels after treatment with low (≤7000 NE/week) and high (>7000 NE/week) doses of vitamin D_3 in the patient groups.

Vitamin D_3 levels in relation to thyroid function and presence of ophthalmopathy using low and high doses of vitamin D_3 in GD

The increase in serum vitamin D_3 levels in GD between pre- and post-therapy could be demonstrated in euthyroidism [40.33(10.7-152.09) vs. 62.97(37.22-106.55) nmol/l, p<0.0171] , but not in hyperthyroidism [49.22(6.38-381.08) vs. 64.91(39.22-107.42) nmol/l] (Figure 3). The presence of ophthalmopathy was associated with significantly lower serum vitamin D_3 levels in the post-therapy period compared to those without ophthalmopathy [56.67(3.12-91.45) vs. 70.38(44.7-110.81) nmol/l, p<0.0302], besides a difference in vitamin D_3 between pre- and post-therapy in GO [40.36(13.68-119.08) nmol/l vs. , p<0.0441]. Surprisingly, the significant difference in serum vitamin D_3 levels between pre- and post-therapy using both low and high doses of vitamin D_3 was only associated with low doses in GO [32.23(10.2-101.76) vs. 55.27(32.48-95.22) nmol/l, p<0.0291]. Two-way repeated measures ANOVA showed borderline significance p<0.054 for doses and p<0.061 for time-points (pre- and post-therapy data) in GD, but these data were the following in HT and controls (p<0.314 and p<0.760 for doses, and p<0.000 and p<0.039 for time-points, respectively).

Figure 3

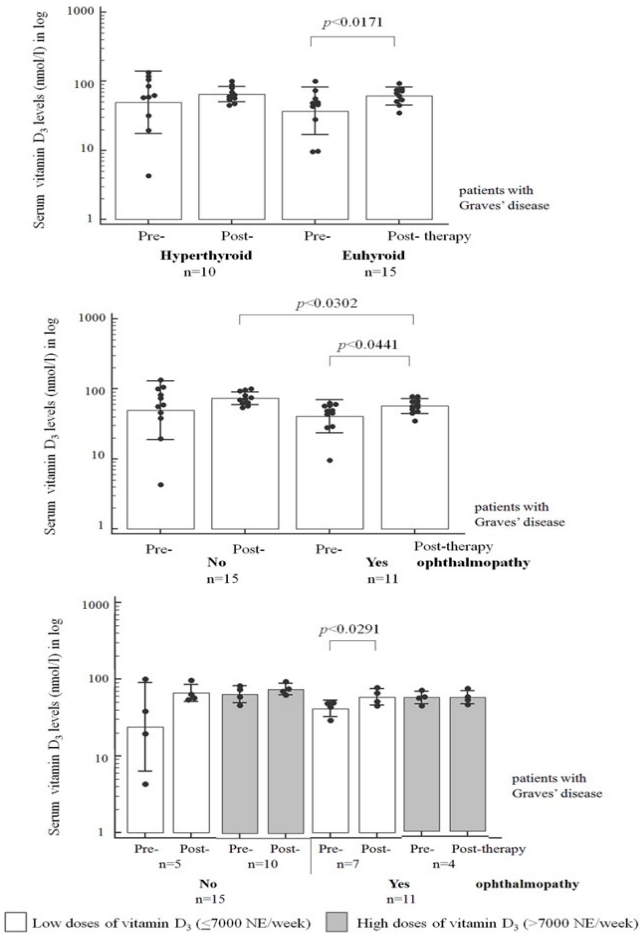

Figure 3: Changes in serum vitamin D_3 levels in relation to thyroid function and the presence of ophthalmopathy with the effect of vitamin D_3 doses [low (≤7000 NE/week) and high (>7000 NE/ week) doses] on serum vitamin D_3 levels in Graves' disease.

Changes in thyroid hormone and antithyroid antibody levels using low and high doses of vitamin D_3 in patients with HT and GD

In linear regression analysis, the relationship between thyroid hormones and anti-thyroid antibodies against TPO or TSH receptor, as depentent variables and serum vitamin D_3 levels was evaluated in all studied groups after the treatment with low and high doses of vitamin D_3.

Only two dependent variables could be found that gave a significant relationship with serum vitamin D_3 levels. A relevant inverse relationship could be demonstrated between serum TSH and vitamin D_3 levels in HT patients with low doses [log(vitamin D_3) = 2.5353 – (1.2971 * log(TSH)), r = - 0.4527 p<0.0301] (Figure 4A). In GD patients, a relevant positive relationship could be observed between anti-TPO antibody and vitamin D_3 levels with high doses [log(vitamin D_3) = 2.5353 – (1.2971 * log (anti-TPO antibody)), r = 0.5171 p<0.0068] (Figure 4B).

Figure 4

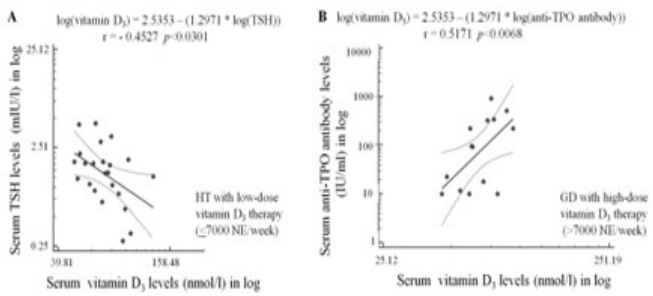

Figure 4: Relationship between TSH and vitamin D_3 levels in Hashimoto' thyroiditis with low doses (≤7000 NE/week) (A); and between anti-TPO antibody and vitamin D_3 levels in Graves' disease with high doses (>7000 NE/week) of vitamin D_3 therapy (B) using linear regression analysis.

In constrast, anti-TPO antibody levels decreased significantly after treatment with both low and high doses of vitamin D_3 in HT [265.02 (24.49-2867.29) vs. 137.68(6.19-3060.01) IU/ml, p<0.0006 for low and 182.11(10.56-3141.05) vs. 114.04(6.75-1926.24) IU/ml, p<0.0006 for high doses of vitamin D_3] (Figure 5A). Serum TSH levels in post-therapy period were significantly decreased with high doses of vitamin D_3 compared to those found with low doses in HT [1.38(0.35-5.41) vs. 0.58(0.02-15.44) mIU/l, p<0.0278]. None of the differences in serum FT_4 levels could be demonstrated between pre- and post-therapy with both low and high doses of vitamin D_3 in HT. In turn, no differences in serum anti-TPO antibody and TSH levels could be found between pre- and post-therapy with both low and high doses of vitamin D_3 in GD (Figure 5B). Serum TSH receptor antibody levels showed a significant decrease between pre-and post-therapy periods with low doses of vitamin D_3 [2.66(0.27-25.96) vs. 1.12(0.06-19.31) IU/l, p<0.0178]. The decrease in serum FT_3 and FT_4 levels was significant only with high doses of vitamin D_3 between pre- and post-therapy in GD [23.47(9.69-56.81) vs. 15.35(10.83-21.76) pmol/l, p<0.0068 for FT_4 and 8.25(1.84-36.95) vs. 4.4(3.03-6.4) pmol/l, p<0.0031 for FT_3]. The decrease in FT_3 levels was dose-dependent, with a greater decrease at high doses compared to low doses [5.27(4.05-6.86) pmol/l, p<0.0114].

Figure 5

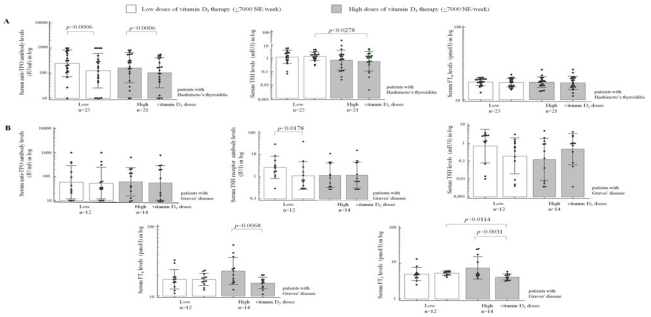

Figure 5: Decrease in serum anti-thyroid antibody and thyroid hormone levels in Hashi-moto's thyroiditis (A) and changes in antithyroid antibody and thyroid hormone levels in Graves' disease (B) with low (≤7000 NE/week) and high (>7000 NE/ week) doses of vitamin D_3 therapy.

Treatment with high doses of vitamin D_3 resulted in relevant differences in anti-TPO antibody levels in GO and thyroid hormone levels in GD without ophthalmopathy (Figure 6). Serum anti-TPO

antibody levels were significantly decreased in GO with high doses of vitamin D_3 [65.59(2.28-1884.81) vs. 30.92(1.23-778.92) IU/ml, p<0.0308], but not in GD without ophthalmopathy. No relevant differences in serum TSH receptor antibody levels were observed. GD without ophthalmopathy had higher TSH and lower FT_4 or FT_3 levels in the post-therapy period compared to the pre-therapy period [0.08(0-21.85) vs. 0.79(0.01-54.37) mIU/l, p<0.0248 for TSH, 25.89(10.34-64.83) vs. 14.72(11.1-19.51) pmol/l, p<0.0062 for FT_4 and 10.24(2.09-50.13) vs. 4.65(3.33-6.5) pmol/l, p<0.0062 for FT_3].

Figure 6

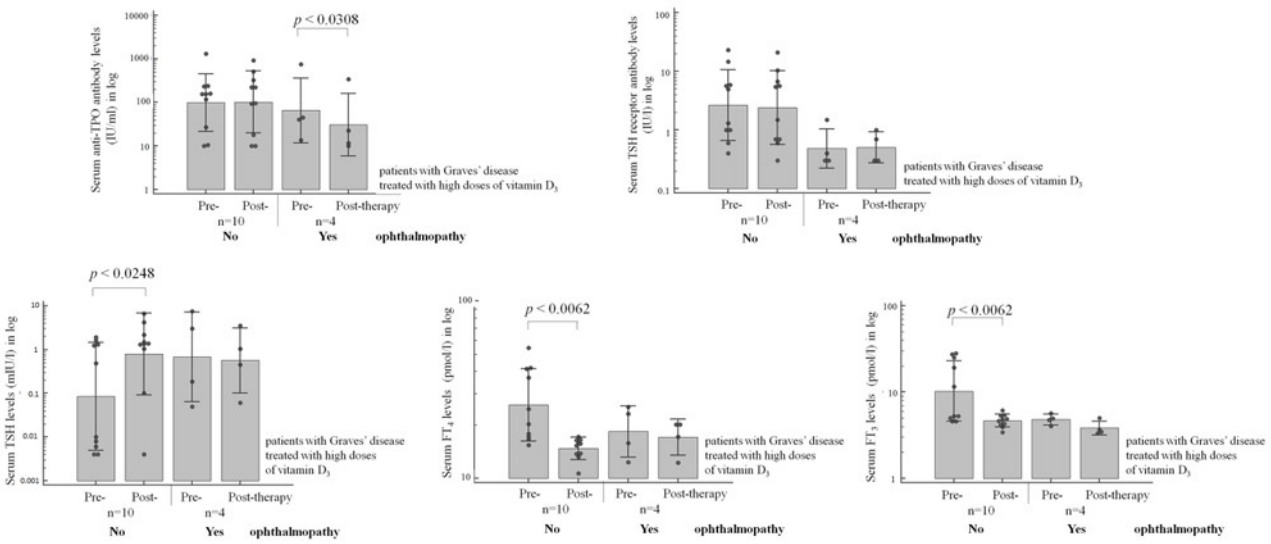

Figure 6: Effect of high doses of vitamin D_3 therapy (>7000 NE/week) on serum antithyroid antibody and thyroid hormone levels in Graves' disease with and without ophthalmopathy.

Discussion

Several reports have shown that the occurrence of vitamin D_3 deficiency is more common in AITD than in other autoimmune diseases [20-22]. Recently, data have published that vitamin D_3 supplementation is beneficial for repigmentation in vitiligo patients [23]. The greater importance of vitamin D_3 supplementation has been emphasized in postmenopausal HT patients [24]. Higher anti-TPO antibody levels have been found in vitamin D_3 deficient HT patients [21, 25]. Studies in healthy subjects receiving vitamin D_3 supplementation showed a high variability of thyroid hormone levels, except for TSH levels [26]. Few reports could be found, in which the changes in vitamin D_3 levels together with antithyroid autoantibody and thyroid hormone levels were investigated in thyroid autoimmunity using low and high doses of vitamin D_3 in follow-up. In our study, both low-dose (≤7000 NE/week) and high-dose (>7000 NE/week) vitamin D_3 therapy increased vitamin D_3 levels in all patient groups. Vitamin D_3 deficiency was more frequent in about half of all patients by baseline vitamin D_3 levels, which improved more better after vitamin D_3 therapy in patients with HT and GD, and less in controls (6 cases out of 27 remained in vitamin D_3 deficiency). The vitamin D_3 receptor gene polymorphism may be one of the factors causing the lack of increase in vitamin D_3 levels after vitamin D_3 therapy, which represents therapy resistance [27]. In this study, 5 control cases, 2 GD cases and 3 HT cases may be suffering from

vitamin D_3 receptor gene polymorphism because none of these cases showed any changes in their vitamin D_3 status after therapy.

Our results reflected a high incidence of vitamin D_3 deficiency in AITD who did not take vitamin D_3 supplementation, which has been reported in numerous publications [28]. On the other hand, our controls had significantly lower serum vitamin D_3 levels after vitamin D_3 therapy compared to those found in HT and GD, especially at high doses. Both low and high doses of vitamin D_3 therapy resulted in significantly lower serum anti-TPO antibody and TSH levels, with a dose-dependent decrease in HT patients. The changes in anti-TPO antibody and TSH levels were very often associated with vitamin D_3 supplementation used in HT with variable results [29, 30]. Our results confirmed that adequate vitamin D_3 supplementation could be effective to increase vitamin D_3 levels or achieve better vitamin D_3 status, which was associated with the decrease in anti-TPO antibody and TSH levels in HT [31-33]. These results suggested that vitamin D_3 status may have an important influence on the patho-mechanism of HT, and the reduction of autoantibodies may be associated with the improvement of hypothyroidism [34]. Our results confirmed the beneficial effect of vitamin D_3 therapy on anti-TPO antibody and TSH levels, but not on FT_4 levels in HT with low and high doses of vitamin D_3. Chao et al. found a positive correlation between serum FT_4 or FT_3, and vitamin D_3 levels in HT [35].

Both low and high doses of vitamin D_3 did not significantly increase serum vitamin D_3 levels between pre- and post-therapy in GD. A relevant difference could be demonstrated between GD with and without ophthalmopathy with low-dose vitamin D_3 therapy. However, our results did not support the modifying effect of hyperthyroidism on serum vitamin D_3 levels. In contrast, vitamin D_3 levels were significantly more reduced in patients with ophthalmopathy compared to patients without ophthalmopathy highlighting the role of vitamin D_3 supplementation in immune modulation [36]. The small number of patients did not allow a detailed investigation of the role of vitamin D_3 levels and doses according to the classification of ophthalmopathy. The majority of publications found no relationship between vitamin D_3 levels and thyroid function in GD, but Sheriba et al. demonstrated a strong correlation between vitamin D_3 levels and thyroid volume, and degree of proptosis [37, 38]. In our study, no relevant differences in anti-TPO antibody and TSH levels could be demonstrated with low and high doses of vitamin D_3. The significant decrease in TSH receptor levels with low doses of vitamin D_3 seems to be insufficient to accept any relationships between vitamin D_3 and TSH receptor antibody levels. Our results in GD showed a relationship between vitamin D_3 doses and FT_4 or FT_3 levels. Only high doses of vitamin D_3 resulted in significant differences in anti-TPO antibody, TSH, FT_4, and FT_3 levels measured by patients with and without ophthalmopathy, highlighting the importance of vitamin D_3 dose dependence in GD. GD without ophthalmopathy showed convincing improvement of hyperthyroidism at high doses of vitamin D_3. This fact may explain that vitamin D_3 acts directly on the expression of type 2 deiodinase and moderates T_3 and T_4 hormone levels [39]. Adequate vitamin D_3 supplementation may improve immune tolerance by suppressing dendritic cell differentiation and maturation, which is associated with suppressed T lymphocyte

proliferation and decreased proinflammatory cytokine production [40]. Our results confirmed that low vitamin D_3 levels may be a risk factor for new-onset or recurrent hyperthyroidism in GD [6, 7].

The limitation of the study may be the small number of patients who met the main study criteria: did not receive vitamin D_3 supplementation before 6 months of the study. The number of specialists also plays as a limiting factor in the selection of patients. Nevertheless, the results provided convincing data based on the changes in serum vitamin D_3, antithyroid autoantibody and thyroid hormone levels with low and high doses of vitamin D_3 therapy for vitamin D_3 supplementation in AITD.

Conclusion

Our results confirmed that vitamin D_3 deficiency is frequent in patients with AITD who have not yet received vitamin D_3 therapy. However, vitamin D_3 deficiency was even more frequent in our controls. The efficacy of vitamin D_3 therapy was demonstrated in all patient groups. The success of vitamin D_3 therapy depended on the dose of vitamin D_3 in GD. Hyperthyroidism required higher doses of vitamin D_3 therapy (at least more than 1500 NE/day). The most favorable changes in vitamin D_3 status were seen in HT, associated with a gradual and significant decrease in anti-TPO antibody and TSH levels. In turn, more decreased serum vitamin D_3 levels could be found in GO compared to GD without ophthalmopathy, that patients demonstrated modifying effect of high doses of vitamin D_3 on FT_4 and FT_3 levels.

Competing interests

Authors declare that no competing interests exist.

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