Luo *et al. Thyroid Research* (2025) 18:10
https://doi.org/10.1186/s13044-025-00226-x

Thyroid Research

RESEARCH Open Access

The development of thyroid autoimmunity is potentially associated with the deficiency of vitamin D3 rather than vitamin D2 in euthyroid men

Dongdong Luo[1,2†], Chenxi Zhang[1†], Bingrui Gao[1], Deping Wang[1,4], Zhaoying Chen[1], Kan Chen[1], Bojuan Li[1], Song Leng[3*] and Jing Li[1*]

Abstract

Objective Vitamin D(VitD) deficiency has been found prevalent among patients with thyroid autoimmunity (TAI). This study aimed to investigate whether low VitD2 or VitD3 potentially contributed to the development of TAI in euthyroid male patients, which had not been reported before.

Methods A total of 2882 euthyroid male petroleum workers were recruited from those participants in the healthcare program at the second affiliated hospital of Dalian Medical University in 2021, whose serum VitD levels, thyroid functions, and autoantibody titers were all examined at the same time. Among them, 2587 (89.8%) individuals received the second health follow-up in 2022. Serum VitD including 25(OH)D2 (VitD2) and 25(OH)D3 (VitD3) levels were detected by liquid chromatography-tandem mass spectrometry. Thyroid functions and autoantibody titers were quantified using chemiluminescent immunoassays.

Results The serum levels of VitD and VitD3 were pronouncedly lower in the male euthyroid subjects with TAI ($n=195$) than those non-TAI men ($n=2687$, $P<0.05$), whereas serum VitD2 was not significantly different based on the data from the initial investigation in 2021. The prevalence of subjects with TAI among the total male euthyroid subjects with TAI population was markedly increased with the decreasing levels of serum VitD and VitD3, respectively (P for trend <0.05), but not significantly changed with that of serum VitD2. The binary logistic regression analysis revealed that either the deficiency of VitD (serum VitD <20 ng/mL, VDD) or low VitD3 level was an independent risk factor for the development of TAI, which had been further demonstrated by the follow-up observation in 2022. Among the non-TAI men in 2021, 6.52% ($n=157$) individuals became TAI patients after a one-year follow-up, and their serum VitD and VitD3 levels both exhibited significantly more reduction as compared with those of the remained non-TAI ones in 2022. More of those with VDD developed TAI than the non-VDD ones did in 2022 (8.5% vs. 5.6%, $P<0.05$). Additionally,

†Dongdong Luo and Chenxi Zhang contributed equally to this work.

*Correspondence:
Song Leng
dllengsong@126.com
Jing Li
lijingendocrine@126.com; jli23@cmu.edu.cn

Full list of author information is available at the end of the article

the change in serum VitD over the two years was more strongly correlated with serum VitD3 (rs = 0.971, $P < 0.001$) when compared with that of VitD2 (rs = 0.085, $P < 0.001$) in the whole euthyroid male population.

Conclusion Based on the cross-sectional and prospective investigations, our findings further indicate that VDD may be an independent risk factor for TAI development. Moreover, the latter is potentially associated with the deficiency of VitD3 rather than VitD2 in the euthyroid male population although the related mechanisms await in-depth exploration. Our findings also suggest that VitD3 supplementation might provide more potential benefits than VitD2 among VDD men in terms of preventing TAI development.

Study registration the Dalian Health Management Cohort (DHMC) ChiCTR2300073363.

Keywords 25(OH)D, 25(OH)D2, 25(OH)D3, Thyroid autoimmunity

Introduction

Thyroid autoimmunity (TAI) is a prevalent condition resulting from intricate interactions among immune responses, genetic factors, and environmental influences. Despite an unclear pathogenesis, TAI is characterized by the presence of antibodies, notably anti-thyroperoxidase (TPOAb) and anti-thyroglobulin (TgAb), with a particular emphasis on the former [1]. According to our recent investigation of 78,470 participants from 31 provinces in the mainland, the prevalence of TAI was 14.19% in the general population [2]. TPOAb and TgAb are involved in thyroid dysfunction and some extrathyroidal damages (e.g. nephropathy and premature labor) in TAI patients. Up to date, there is a lack of effective maneuvers to decrease the levels of serum TPOAb and TgAb.

Vitamin D (VitD) is a multifunctional steroid hormone, consisting of two main independent forms—VitD2 (ergocalciferol) and VitD3 (cholecalciferol). The former comes from ergosterol in plants after being exposed to UVB radiation, while the latter can be produced in the skin of human beings and animals under UVB radiation. Serum total 25(OH)D concentrations are reflected by the sum of circulatory 25(OH) D2 and 25(OH)D3 levels. Thus, the maintenance of circulatory total VitD levels depends on not only endogenous synthesis of VitD3 in the skin but also intake of meat, dairy, and plant food products. Vegans are susceptible to the deficiency of VitD3 even though they exhibit sufficient serum total VitD levels. Serum total 25(OH)D concentrations are well-known to indicate the nutritional status of VitD. VitD deficiency is usually considered when they are below 20 ng/ml [3]. It has been found that VitD can enhance innate immune functions and inhibit adaptive immune responses besides its regulatory actions on bone metabolism [4]. Many autoimmune diseases (e.g. multiple sclerosis, type 1 diabetes mellitus) have been found with a close association with the deficiency of VitD (VDD) [5]. Some cross-sectional investigations and meta-analyses have shown that the circulatory TPOAb and TgAb are significantly higher in VDD individuals than in non-VDD ones [6], indicating the accelerating role of VDD in the development of TAI [7, 8]. However, it has not been clarified whether VitD2 and VitD3 are equally effective at the immunomodulatory functions, on which there is a lack of investigation, and there is no report on the comparisons of their impacts on the development of TAI yet. Most of the publications have reported that they were comparatively efficacious at raising the total 25-hydroxyvitamin D (25(OH) D) concentrations [9]. However, previous studies have shown that VitD2 administration was unable to increase muscle strength whereas VitD3 supplementation was able to improve it [10]. Although the exact mechanisms are unknown, the differential binding capacities for 25-hydroxylase and VitD-binding protein and the differential degradation rates between VitD2 and VitD3 may lead to their differential effects [9]. Thus, this study aimed to investigate whether low VitD2 or VitD3 potentially contributed to the development of TAI in male individuals, which comparison analysis had not been performed before. In addition, TAI has a female predilection which has been attributed to the impacts of estrogens [11]. There were conflicting reports about the gender difference in serum VitD concentrations. Previous research reported significantly lower serum 25(OH)D levels in women compared to men [12], while the others did not be found [13–15]. To exclude the confounding role of estrogens, this study enrolled only male subjects for the investigation. Its findings would help to understand which form of VitD (VitD2 or VitD3) may be effective when administered to TAI patients in terms of reducing their serum thyroid autoantibody levels.

Methods

Study design and participants

A total of 2882 euthyroid male petroleum workers, aged 20–60 years, were recruited from those participants in the healthcare program at the Second Hospital of Dalian Medical University in 2021, whose serum VitD levels, thyroid functions, and autoantibody titers were all examined at the same time. Among them, 2587 (89.8%) individuals received the second health follow-up in 2022 (Fig. 1). All enrolled subjects in this study fulfilled the following criteria: no clinical histories of other autoimmune diseases, malignant tumors, chronic kidney/liver

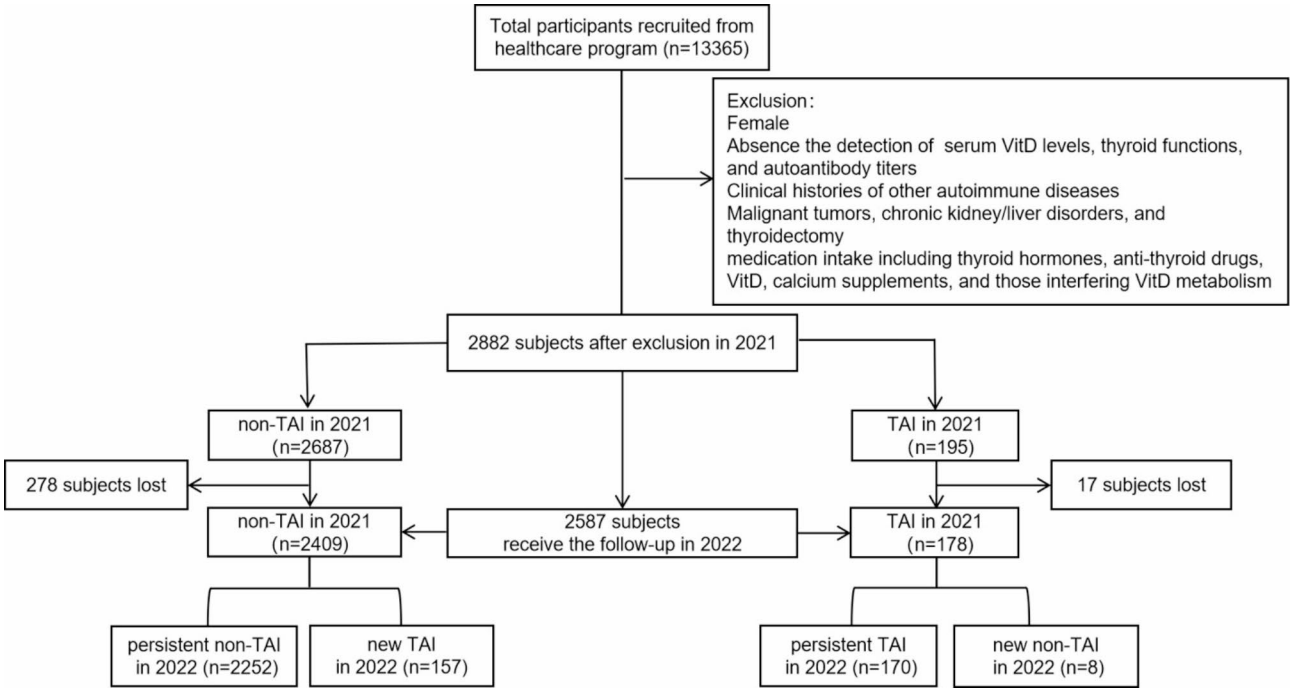

Fig. 1 Flowchart of participants recruitment in this study

disorders, and thyroidectomy, and no recent medication intake including thyroid hormones, anti-thyroid drugs, VitD, calcium supplements, and those interfering VitD metabolism (e.g. thiazides, antiepileptic drugs, and glucocorticoids) in the last 3 months. The study was approved by the ethics committee at the Second Hospital of Dalian Medical University (No. 2022090). The written informed consent was acquired from all the participants.

The medical data were simultaneously recorded and input into the His system at the Second Hospital of Dalian Medical University when the subjects were given the examinations. We collected the medical records of those subjects meeting the criteria listed above and established the final data for this study.

Laboratory analysis

In all subjects, their serum thyroid-stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3), TPOAb, and TgAb were determined using chemiluminescent enzyme immunoassays (Siemens, CENTAUR XP). Serum VitD2 [25(OH)D2] and VitD3 [25(OH)D3] levels were quantified by liquid chromatography-tandem mass spectrometry (LC/MS/MS, AB SCIEX Triple QuadTM 4500MD) after at least 8-hour fasting. Serum total VitD [25(OH)D] was calculated by serum VitD2 [25(OH)D2] plus VitD3 [25(OH)D3] levels.

TAI was defined as serum positivity of TPOAb and/or TgAb. According to the guidelines from the Endocrine Society [3], the subjects were divided into VitD sufficiency (VDS, serum total VitD ≥ 30ng/ml), insufficiency (VDI, serum total VitD 20-30ng/ml), and deficiency (VDD, serum total VitD<20ng/ml) groups. Since there were no recognized criteria for either VitD2 or VitD3 insufficiency/deficiency, the subjects were further classified based on tertiles of serum 25(OH)D2 and 25(OH)D3 levels: low VitD2 level group (D2L, bottom tercile, serum VitD2 < 0.48 ng/mL), medium VitD2 level group (D2M, middle tercile, serum VitD2 0.48–0.95 ng/mL) and high VitD2 level group (D2H, top tercile, serum VitD2 ≥ 0.95ng/mL); low VitD3 level group (D3L, bottom tercile, serum VitD3 < 19.25ng/mL), medium VitD3 level group (D3M, middle tercile, serum VitD3 19.25-26.31ng/mL) and high VitD3 level group (D3H, top tercile, serum VitD3 ≥ 26.31ng/mL).

The inter- and intra-assay coefficients of variation for serum TSH, FT4, FT3, TPOAb, TgAb, VitD2, and VitD3 measurements were (2.35%,2.85%); (4.00%,3.33%); (4.05%, 3.08%); (3.40%, 6.80%); (2.60%, 2.50%); (5.17%, 5.17%); (5.17%, 5.17%), respectively.

Statistical methods

T-tests and Mann-Whitney U tests were used to compare the normally distributed and skewed-distributed data between the two groups, respectively. One-way ANOVA and Kruskal-Wallis followed by *Bonferroni post hoc* test were used for multiple comparisons. The categorical variables were analyzed using the Chi-square test. Binary logistic regression was used to analyze the independent risk factors of TAI. Data analysis was conducted using

IBM SPSS version 26. Statistical significance was considered if $P < 0.05$.

Results

Clinical characteristics of the subjects at the baseline

This study included 2882 euthyroid male participants, with an average age of 45 years and an average body mass index (BMI) of 25.8 kg/m^2. Among them, 6.7% ($n = 195$) suffered from TAI (serum TPOAb and/or TgAb positive). The incidence of new cases in the second year is 6.5%($n = 157$)among 2409 non-TAI subjects in the first year. The baseline characteristics of the subjects with and without TAI are both shown in Table 1. The patients with TAI exhibited significantly higher age and serum TPOAb, TgAb, and TSH levels than the non-TAI subjects. Although there was a statistically but not clinically significant decrease in serum FT3 level in TAI patients in comparison to that of non-TAI participants, their serum FT4 levels were not markedly different (Table 1).

Serum VitD levels of the subjects

We compared serum 25(OH)D, 25(OH)D2, and 25(OH)D3 levels between the TAI patients (TPOAb and/or TgAb positive) and the non-TAI subjects. The levels of serum 25(OH)D and 25(OH)D3 were pronouncedly lower in the euthyroid male TAI patients than the non-TAI men. There was a significantly increased proportion of VitD deficiency in the former as compared with that of the latter ($P < 0.05$). However, there was no significant difference in serum 25(OH)D2 levels between them (Table 1). In addition, although serum total VitD levels were positively correlated with both serum 25(OH)D2 and 25(OH)D3 concentrations, a strong correlation was observed with 25(OH)D3 (rs = 0.991, $P < 0.001$) whereas weak with 25(OH)D2 (rs = 0.081, $P < 0.001$) concentrations in the euthyroid male subjects.

The associations of serum VitD levels with the development of TAI

Based on the serum total VitD levels which were the sum of serum concentrations of 25(OH)D2 and 25(OH)D3, those euthyroid male subjects were divided into VDS, VDI, and VDD groups. As shown in Table 2, the prevalence of TAI was 5.4% in the VDS euthyroid males, 6.0% in the VDI ones, and 8.7% in the VDD group, respectively. The prevalence of TAI increased with decreasing serum total VitD levels in the euthyroid male subjects (P for trend < 0.05). Subgroup analyses were further performed after being classified by serum VitD2 and VitD3 levels, respectively. The alteration trend in the prevalence of TAI was statistically significant for three groups with different serum 25(OH)D3 levels which was similar to those findings for total VitD, but not for those groups with different serum 25(OH)D2 concentrations (Table 2).

Given the findings of lower serum 25(OH)D and 25(OH)D3 levels in euthyroid male TAI patients, their independent associations were further evaluated using a binary logistic regression model. Odds ratios (ORs) were calculated after adjusting for age, body mass index (BMI), and serum TSH levels. VDD was an independent risk factor for the development of TAI and the positive expression of TPOAb and TgAb in euthyroid male subjects [OR 1.611 (CL 1.065–2.436); $P = 0.04$], while VDI was not. Furthermore, the month was added to the logistic regression analysis as a factor to exclude the interruption of the blooding season and the results remained consistent. Furthermore, as compared to those with high serum VitD3 level (top tercile), the euthyroid male subjects with

Table 1 Baseline characteristics and serum VitD nutritional status of all the euthyroid male subjects classified by TAI mobility

	non-TAI($n = 2687$)	TAI($n = 195$)	*P*-value
Age, median (IQR), years	47(36–52)	49(41–53)	0.023
BMI, median (IQR), kg/m^2	25.6(23.5–28.0)	25.7(24.0-28.1)	0.136
TSH, mean ± SD, mIU/L	1.55 ± 0.72	1.90 ± 0.96	< 0.001
FT4, mean ± SD, pmol/l	16.48 ± 1.86	16.50 ± 1.98	0.881
FT3, mean ± SD, pmol/l	5.37 ± 0.43	5.27 ± 0.44	0.001
TgAb, median (IQR), U/ml	15.00 (15.00–15.00)	80.98(23.56-187.86)	< 0.001
TPOAb, median (IQR), U/ml	28.00(28.00-31.67)	272.97(66.81–1300.00)	< 0.001
Total VitD, median (IQR), ng/ml	23.90(18.46–29.33)	22.13(17.15–27.33)	0.005
VDD, *n* (%)	32(860/2687)	42.1(82/195)	< 0.05
VDI, *n* (%)	45.4(1219/2687)	40(78/195)	NS
VDS, *n* (%)	22.6(608/2687)	17.9(35/195)	NS
VitD3, median (IQR), ng/ml	22.80(17.54–28.39)	20.88(16.31–26.77)	0.006
VitD2, median (IQR), ng/ml	0.68(0.4–1.17)	0.66(0.4–1.33)	0.987

The differences were compared between the patients with TAI and those without TAI by Mann-Whitney U Test, t-test, and Chi-square tests followed by Bonferroni when appropriate. TAI refers to the positive expression of either TPOAb or TgAb in the serum; non-TAI refers to those without TAI. Serum total VitD, VitD2, and VitD3 were reflected by serum total 25(OH)D, 25(OH)D2, and 25(OH)D3 concentration, respectively, which were determined by liquid chromatography-tandem mass spectrometry. Serum FT3, FT4, TSH, TgAb, and TPOAb were measured by chemiluminescent enzyme immunoassays. NS, non-significant. BMI, Body Mass Index; TSH, thyroid-stimulating; FT4, free thyroxin; FT3, free triiodothyronine; TgAb, anti-thyroglobulin autoantibody; TPOAb, anti-thyroid peroxidase autoantibody; VDD, vitamin D deficiency; VDI, vitamin D insufficiency; VDS, vitamin D sufficiency; IQR, interquartile range; SD, standard deviation

Table 2 The relationships between the nutritional status of VitD and the prevalence of TAI

	Total VitD				VitD3				VitD2			
	VDS ($n=643$)	VDI ($n=1297$)	VDD ($n=942$)	*P* for trend	D3H ($n=956$)	D3M ($n=954$)	D3L ($n=972$)	*P* for trend	D2H ($n=964$)	D2M ($n=962$)	D2L ($n=956$)	*P* for trend
non-TAI (%)	94.6	94.0	91.3[ab]	0.007	94.4	93.9[c]	91.5[cd]	0.011	92.6	94.5	92.6	0.961
TAI (%)	5.4	6.0	8.7[ab]	0.007	5.6	6.1[c]	8.5[cd]	0.011	7.4	5.5	7.4	0.961
TPOAb positivity (%)	3.6	4.6[a]	6.9[ab]	0.002	4.0	4.8[c]	6.6[cd]	0.009	5.1	4.9	5.4	0.724
TgAb positivity (%)	3.1	3.9[a]	5.7[ab]	0.009	3.3	4.0[c]	5.7[cd]	0.013	4.7	3.8	4.5	0.853

The differences were compared between the subjects with different nutritional status of VitD by Chi-square tests followed by Bonferroni when appropriate. The nutritional status of VitD was evaluated by serum total VitD, VitD2, and VitD3 levels, respectively, as described in Table 1. [a]$P<0.05$ vs. VDS; [b]$P<0.05$ vs. VDI; [c]$P<0.05$ vs. D3H; [d]$P<0.05$ vs. D3M. VDS, vitamin D sufficiency; VDI, vitamin D insufficiency; VDD, vitamin D deficiency; D3H, serum VitD3 level was in top tercile (≥26.31 ng/ml); D3M, in middle tercile (19.25–26.31 ng/ml); D3L, in bottom tercile (<19.25 ng/ml); D2H, serum VitD2 level was in top tercile (≥0.95ng/ml); D2M, in middle tercile (0.48-0.95ng/ml); D2L, in bottom tercile (<0.48ng/ml); TgAb, anti-thyroglobulin autoantibody; TPOAb, anti-thyroid peroxidase autoantibody;

low serum VitD3 level (bottom tercile) had significantly increased risks for TAI development and TPOAb positive expression, while the men with medium serum VitD3 level (middle tercile) did not. Interestingly, no independent associations were found between serum VitD2 level and TAI or between serum VitD2 and TPOAb/TgAb positivity (Table 3).

The associations between serum VitD levels and TPOAb/TgAb positive expressions during one-year follow-up

During the one year for follow-up, 89.8% ($n=2587$) of the initial 2882 participants were recruited. Among the non-TAI subjects ($n=2409$) at the baseline, 93.5% ($n=2252$) remained non-TAI, and 6.5% became TAI patients ($n=157$). We found that serum total VitD and VitD3 levels after 1 year were significantly lower in the men developing TAI in comparison to those of the subjects remaining non-TAI (both $P<0.05$) in the same period (Table 4). The percentages of subjects with D2L were higher than baseline in all four groups, and the changes showed no correlation with the development of TAI. Among those initial TAI patients ($n=178$), 95.5% ($n=170$) remained TAI, while 4.5% ($n=8$) became non-TAI individuals one year later. The patients with persistent TAI had markedly lower serum total VitD and VitD3 levels at the baseline than the subjects with persistent non-TAI (Table 4). Their serum VitD2 levels remained no difference. During the one year for follow-up, the changes in serum total VitD levels were strongly and positively associated with that of serum VitD3 concertation (rs = 0.971, $P<0.001$), whereas only weakly correlated with serum VitD2 concentration (rs = 0.085, $P<0.001$).

Among those euthyroid male subjects without TAI ($n=2409$) at the baseline, significantly higher proportions of VDD subjects (8.5%, 8.0%) developed TAI and serum TPOAb positivity during the follow-up in the next year than VDS/VDI euthyroid males (5.6%, 5.1%; both $P<0.05$) did, respectively. Nevertheless, there was no significant difference was found in the percentage of those developing TgAb positivity in the sera during the follow-up (Fig. 2.). Among all the patients ($n=335$) with TAI diagnosed either at the baseline or during the follow-up, there was an inverse correlation between the alteration magnitude of serum TPOAb level and the concentrations of both serum total VitD (rs=-0.109, $P=0.046$) and VitD3 (rs=-0.110, $P=0.044$) in the next year, while no relationship was found between serum VitD2 (rs = 0.039, $P=0.476$) and the change extent of serum TPOAb. The elevated magnitude of serum TPOAb level in those subjects with increased circulatory total VitD (from VDD to VDI\VDS)was significantly less than that of the euthyroid males remaining VDD (Fig. 3. D, E). Furthermore, serum VitD3 rather than VitD2 showed an identical trend in the alteration with circulatory total VitD levels during

Table 3 The independent association between the nutritional status of VitD and the risk for TAI development using logistic regression analyses

VitD status	TAI		TPOAb positivity		TgAb positivity	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Total VitD						
VDS	1		1		1	
VDI	1.183(0.776–1.805)	0.435	1.479(0.893–2.449)	0.129	1.298 (0.757–2.226)	0.343
VDD	1.742 (1.097–2.767)	0.019	2.338(1.351–4.047)	0.002	1.860(1.038–3.332)	0.037
VitD3						
D3H	1		1		1	
D3M	1.095 (0.737–1.626)	0.653	1.294(0.820–2.042)	0.269	1.164(0.711–1.908)	0.546
D3L	1.565(1.037–2.362)	0.033	1.826(1.130–2.949)	0.014	1.650(0.991–2.745)	0.054
VitD2						
D2H	1		1		1	
D2M	0.702(0.484–1.019)	0.063	0.909(0.599–1.379)	0.652	0.788(0.504–1.234)	0.299
D2L	1.064(0.751–1.506)	0.727	1.154(0.767–1.736)	0.492	1.002(0.649–1.545)	0.993

A binary logistic regression model was used to evaluate the odds ratios (ORs) for the development of TAI and positive expressions of TPOAb and TgAb in the euthyroid male subjects (total = 2882) under different nutritional status of VitD after adjusting for age, BMI, month and TSH. All the abbreviations were as described in Tables 1 and 2

the follow-up (Fig. 3. A-C). However, among TAI subjects, the changes in vitamin D levels show no correlation with changes in FT3 (rs = 0.00, $P = 0.98$), FT4 (rs=-0.13, $P = 0.11$), or TSH (rs=-0.01, $P = 0.88$) among individuals with elevated vitamin D levels. The changes in vitamin D levels also show no correlation with changes in FT3 (rs=-0.03, $P = 0.71$), FT4 (rs = 0.01, $P = 0.88$), or TSH (rs=-0.03, $P = 0.71$) among individuals with decreased vitamin D levels.

Discussion

Thyroid autoimmunity (TAI) is a common ill condition caused by the complex interactions of immune responses, genetic, existential factors (e.g. estrogens, parity, microbiota), and environmental factors (e.g. selenium and iodine), which is defined by the positive expressions of TPOAb and/or TgAb, especially the former [1]. TAI may not only lead to thyroid dysfunction but also cause a few extrathyroidal damages. However, there are still no effective means to cure TAI. VitD is crucial for calcium regulation, bone health [16], and even autoimmune disorders prevention [17], especially in autoimmune thyroid diseases (AITDs) [18]. It is well known that VitD regulates the function of adaptive and innate immune cells [19] by binding with Vitamin D receptors (VDR) [20], weakens antigen presentation by dendritic cells, prevents T-cell activation dependent on dendritic cells (DCs), promotes monocyte differentiation, enhances phagocytosis and chemotaxis in macrophages, down-regulates HLA class II gene expression in the thyroid, shifts the balance of Th1/Th2 cell responses towards Th2, restores the Th17/Tregs ratio [21, 22]. The correlation between TAI and VitD remains controversial until now. Although researchers have not found a clear relationship between 25(OH)D levels and antithyroid antibodies [23], even a negative association between them [24], numerous studies have suggested that VitD deficiency is common in thyroid autoimmunity (TAI) [25, 26], and even increases the risk of AITD [27–29]. Previous studies have indicated that no significant difference was found between women and men [13], recent study has shown that females exhibit lower serum VitD levels compared to men [12], a phenomenon attributed to the impacts of estrogens [11]. Due to the majority of the individuals in the Health Management Center being male, this study enrolls only male subjects to investigate the association between VitD and TAI in adult males. In addition, it was still unknown which deficiency of either VitD3 or VitD2 was predominant in TAI patients when compared with that of individuals without TAI. In response to the above questions, VitD2 and VitD3 are similarly detected in our study to observe which is essential for TAI. Our study ultimately concludes that male subjects with TAI display markedly reduced serum VitD3 levels, instead of VitD2, when compared to subjects without it (Table 1). The prevalence of TAI in males increases with the decline of VitD3 levels, rather than VitD2 (Table 2). Additionally, a low VitD3 level, rather than VitD2, is also associated with an increased risk of TAI in male patients (Table 3). These findings suggest that it is VitD3, instead of VitD2, that is essential in thyroid autoimmunity modulation.

VitD3 (cholecalciferol) and VitD2 (ergocalciferol) obtained through sunlight exposure and certain foods, respectively, are two main forms of VitD [30]. The endogenous synthesis of VitD2 and VitD3 undergo the same two-step hydroxylation process, hydroxylation in the liver yields calcifediol[25(OH)D2 and 25(OH) D3] [31]. Further hydroxylation in the kidneys produces the active form, calcitriol (1,25(OH)2D) [32]. The final metabolite, 1,25-dihydroxy VitD3 (calcitriol), acts as

Table 4 The alteration of serum VitD levels in those euthyroid male subjects during the follow-up classified by TAI mobility

Serum VitD levels		Initial non-TAI				Initial TAI				
		Persistent non-TAI ($n=2252$)		becoming TAI during follow-up ($n=157$)		Persistent TAI ($n=170$)			becoming non-TAI during follow-up ($n=8$)	
Total VitD (median (IQR), ng/ml)										
	Baseline	24.06(18.73–29.49)		22.64(17.03–28.52)		22.58(17.44–27.81)*			21.74(19.37–32.32)	
	Follow-up	23.44(18.32–29.41)		21.81(16.57–27.48)*		23.51(18.13–28.96)			23.70(20.00-33.03)	
VitD3 (median (IQR), ng/ml)										
	Baseline	22.95(17.81–28.48)		21.43(15.83–27.44)		21.85(16.35–26.92)*			20.75(18.31–31.54)	
	Follow-up	22.75(17.65–28.67)		20.69(16.05–27.01)*		23.05(17.46–27.90)			23.05(17.95-32.00)	
VitD2 (%)		D2L	D2M	D2L	D2M	D2H	D2L	D2M	D2H	D2L	D2M	D2H
	Baseline	31.7	34.9	29.3	36.9	33.8	35.3	28.8	35.9	50.0	0.0	50.0
	Follow-up	77.4	3.4	19.1	79.0	3.8	17.2	80.0	3.5	16.5	62.5	0.0	37.5

Among the initial 2882 euthyroid male subjects, 2587 were followed up for 1 year. Mann-Whitney U Test test was used to analysis the alteration of serum VitD levels in the male subject. *$P<0.05$ as compared with that of persistent non-TAI male subjects. TAI refers to the presence of either TgAb or TPOAb in the serum. Non-TAI refers to those without TAI. Serum total VitD, VitD2, and VitD3 were determined as described in Table 1

a high-affinity ligand for the vitamin D receptor (VDR) [33] forming complexes that translocate to the nucleus and stimulating the transcription of target genes [34], regulating genes and functions, influencing calcium and phosphate metabolism [35]. Although their chemical structures are similar, VitD3, with an additional double bond and methyl group, is believed to be the preferred substrate in the VitD metabolic pathway. Structural differences influence the rate of VitD hydroxylation in the liver, where VitD3 is considered the favored substrate for hepatic 25-hydroxylase [36]. Although calcifediol is the predominant circulating form, it exhibits minimal hormonal activity [37]. Compared with VitD2, VitD3 has a higher binding affinity to the VitD-binding protein [38] and a lower degradation rate due to an additional step [39]. VitD2 is the preferred choice in emergencies and hepatic insufficiency, ensuring rapid elevation of serum levels. Conversely, VitD3 plays a critical role in regulating serum calcium levels, especially in hypoparathyroidism and severe renal failure [40].

Although some studies indicate that supplementation of VitD3 did not decrease [41] or even increase thyroid autoantibody titer levels [42], which may be attributed to their initially low antibody levels, most studies show a significant decrease in antithyroid antibodies after VitD supplementation [27, 28, 43–45], and down-regulation genes expression, which encoding pathways of the innate and adaptive immune systems, potentially shifting the immune system to a more tolerogenic status [46]. An observational cohort study revealed that, after matching the 78 controls based on factors that affect 25(OH) D status, 78 euthyroid female subjects with a genetic predisposition for AITD but no expression of thyroid autoantibodies, exhibited higher baseline VitD levels. Moreover, there was no significant difference in VitD levels was observed between 67 individuals who developed de novo TPOAb and those 67 who did not, both at baseline and the time of seroconversion in the longitudinal study [47], suggesting that the impact of vitamin D on AITD does not exceed the influence of genetic factors. However, our study is a large-sample observational cohort study with a one-year follow-up, and long-term follow-up will be continued, which is only focusing on males. In addition, VitD2 and VitD3 are similarly detected in our study to observe the correlation between the alteration of VitD (VitD2, VitD3) and antithyroid autoantibodies.

Although VitD2 and VitD3 are highly similar in structure, VitD2 is metabolized more rapidly. So VitD3 is primarily responsible for increasing and maintaining stable VitD levels [9]. Results from intervention studies indicate that VitD3 supplementation down-regulates the expression of genes encoding innate and adaptive immune response pathways and the proportion of Th17/Treg cells, induces immune

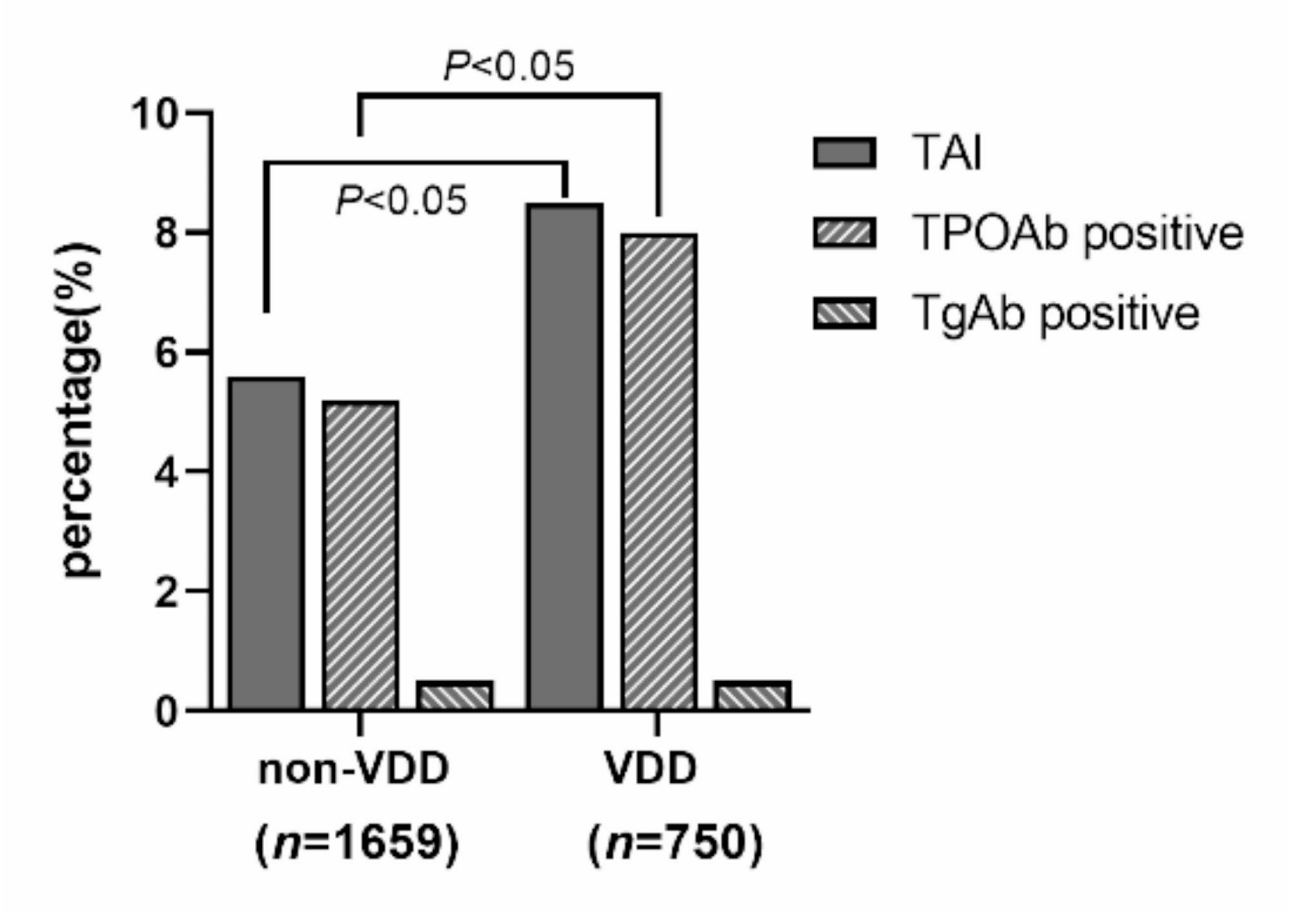

Fig. 2 The percentage of newly diagnosed TAI patients during the follow-up of those euthyroid male subjects initially without TAI. Among the initial 2882 euthyroid male subjects, 2587 were followed up for 1 year, among which 2409 were initially found without TAI at the baseline. The differences were compared between the subjects with different nutritional status of VitD at the baseline by Chi-square tests. TAI refers to the presence of either TgAb or TPOAb in the serum. VDD referred to VitD deficiency (serum total VitD < 20ng/ml) at the baseline before the follow-up; non-VDD (serum total VitD ≥ 20ng/ml) referred to VitD sufficiency and insufficiency at the baseline before the follow-up

tolerance [48], and further reduces the incidence of autoimmune diseases [49]. Considering the differences between VitD2 and VitD3, previous studies have shown that VitD3, rather than VitD2, supplementation was able to increase muscle strength [10], whereas, no study has been made to compare the difference in regulating thyroid autoimmunity between VitD2 and VitD3, which is the purpose of this study. In the prospective research, we found that the proportion of subjects with VitD deficiency at the baseline who developing TAI from non-TAI is higher than those whose VitD is non-deficiency at the baseline (Fig. 2.), indicating that individuals with VitD deficiency should supplement with vitamin D to prevent developing into TAI. Compared with subjects who had persistent non-TAI, patients who developed into TAI show lower VitD (VitD3, not VitD2) levels (Table 4). There is also shown a strong and positive correlation between the changes in serum total VitD and serum VitD3 concentrations, whereas only weakly correlated with VitD2, which further indicates that if supplementation with vitamin D is needed, D3 should be chosen over D2 because changes in VitD levels are mainly related to VitD3. Although, among patients with TAI, the increase of VitD (VitD3, not VitD2) levels in the follow-up does not appear to reverse the occurrence of TAI due to only a one-year follow-up period (Table 4), an increase in VitD from deficient to non-deficient status could delay TAI progression (Fig. 3. D). Based on the Spearman correlation analysis, which indicates an inverse correlation between the change in TPO-Ab titer and VitD3 (rather than VitD2) levels in the second year, we can readily conclude that the increase in VitD3, rather than VitD2, is responsible for slowing down the rise in TPO-Ab antibody titers. (Fig. 3. A-C), The above results suggest the significance of VitD3 in increasing VitD levels and regulating thyroid autoimmunity. It is worth noting that infectious disease can trigger AITD [50]. The outbreak of COVID-19, infecting millions of people, has also been reported in association with AITD recently [51–53]. Perhaps this is the reason why participants in our study developed into TAI within only one year.

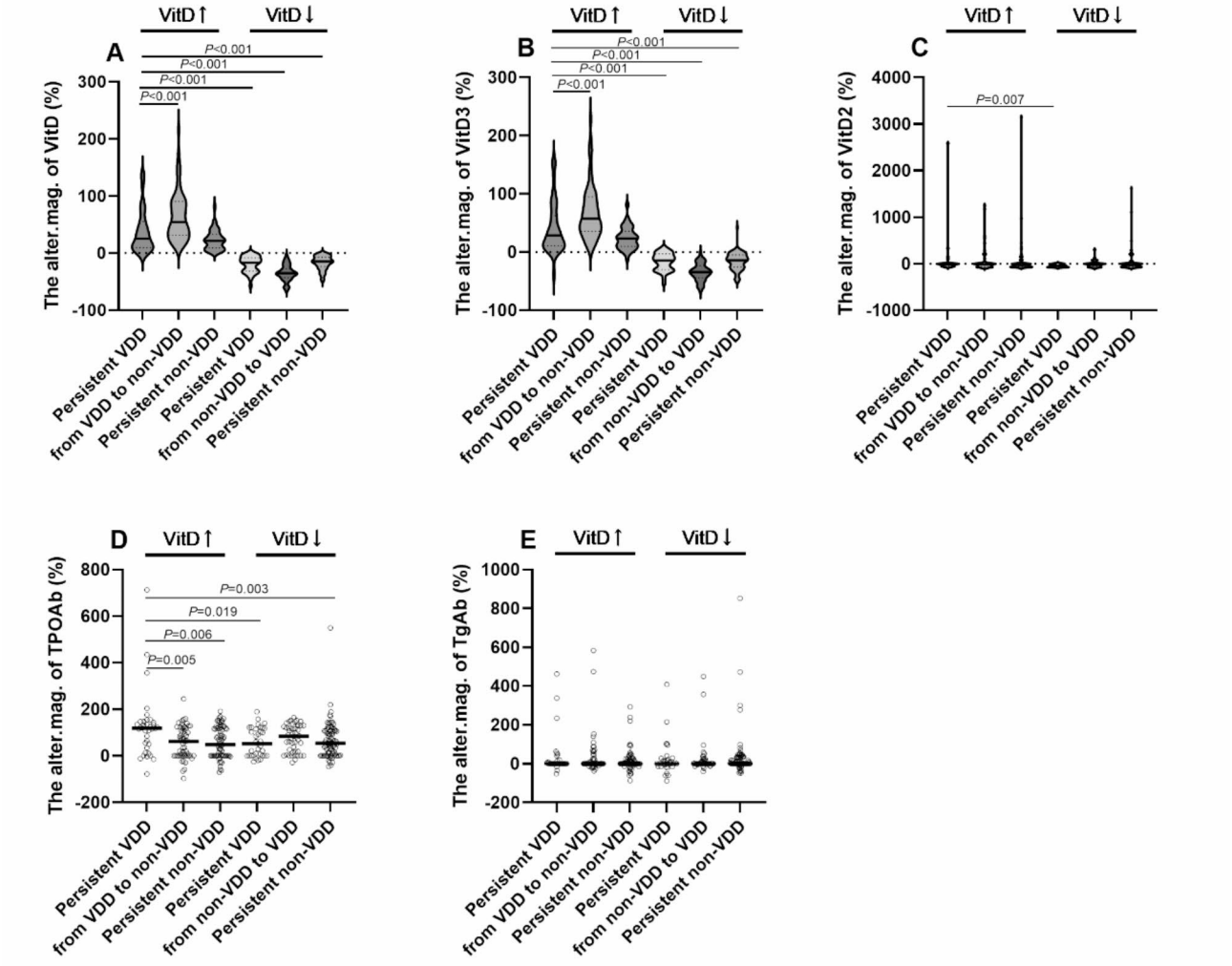

Fig. 3 Prospective observation of the relative alteration magnitudes of serum total VitD, VitD3, VitD2, TPOAb, and TgAb levels in all the male patients with TAI ($n = 335$). Among the initial 2882 euthyroid male subjects, 335 TAI male patients were followed up for 1 year. The relative alteration (alter.) magnitudes (mag.) in serum VitD levels and thyroid autoantibody titers were calculated based on the following equation: the relative alter. mag. = (the level during follow-up—the baseline)/the baseline*100%. Mann-Whitney U Test was used to compare the alteration of serum VitD levels and thyroid autoantibody titers after follow-up with those of persistent VDD. Among the 335 TAI male subjects, 36 patients had persistent VDD, 73 patients had persistent non-VDD after increasing during the follow-up, 36 patients had persistent VDD, 86 showed persistent non-VDD after decreasing during the follow-up, 57 from VDD at the baseline became non-VDD during the follow-up, 47 from non-VDD at the baseline became VDD during the follow-up. VDD referred to VitD deficiency (serum total VitD < 20ng/ml) and non-VDD referred to VitD sufficiency and insufficiency (serum total VitD ≥ 20ng/ml). Serum total VitD, VitD2, and VitD3 were determined as described in Table 1

Autoimmune thyroid disease (AITD) is characterized by an autoimmune response to thyroid antigens, necessitating a specific genetic predisposition and triggered by environmental exposures [54]. Similar to other autoimmune disorders, AITD exhibits a higher prevalence in females [55, 56]. The breakdown of self-tolerance to thyroid antigens (thyroid peroxidase (TPO), thyroglobulin (Tg), and the thyroid-stimulating hormone receptor (TSH-R)) serves as the primary driver of thyroid autoimmunity [57]. Twin studies suggest that more than 75% of the risk for developing Graves' disease (GD) and Hashimoto's thyroiditis (HT) is attributable to genetic factors [58], leaving the remaining 20% to environmental influences [59]. Moreover, AITD may be triggered by infectious disease [50]. Notably, cases of AITD, encompassing both GD and HT, were associated with SARS-CoV-2 infection.

Numerous studies indicated that the optimal concentration range for 25(OH)D is 30-50ng/mL (75-125nmol/L), which is close to the Km of 1α-hydroxylase [60]. Tissues exhibit diverse 25(OH)D concentration thresholds. The minimum effective concentration varies between tissues, and skeletal diseases with a lower minimum effective

concentration than non-skeletal diseases [61]. Until now, no gold standard exists for optimal 25(OH)D concentrations in patients with thyroid autoimmunity. Previous studies have indicated that after increasing 25(OH)D levels from <75 nmol/l to ≥75 nmol/l (30ng/ml), the anti-TPO-Ab level showed a significant decrease of 25% among patients with thyroid immunity [7]. However, based on the above results of our study, we conclude that VitD deficiency (<20ng/ml) is the risk factor of TAI, and is more likely to develop into TAI after a one-year follow-up. After elevating 25(OH)D levels from <20ng/ml to ≥20ng/ml, the progression of TAI can be delayed, suggesting that TAI patients should at least maintain VitD levels ≥20ng/ml.

Our observational study mainly focused on the relationship between serum vitamin D level and the TAI, while the VITAL trial was an intervention study, and observed the effect of vitamin D supplementation on the prevention of AITD. Although no effect was found of VitD supplementation on preventing the AITD occurrence, it does not conflict with our study. Because the effect of VitD depends on not only the serum VitD level but also the sensitivity and genetic polymorphism of VDR [62]. The previous study found that the serum PDIA3Ab level was significantly increased in euthyroid TAI women [63], which might also interfere with the effect of VitD on reducing AIT occurrence, due to PDIA3 is the membrane-associated receptor of 1,25-Dihydroxyvitamin D3 [64]. Numerous studies have confirmed that vitamin D deficiency is a risk factor for the occurrence of autoimmune thyroiditis (AIT) [27–29], which is also validated by the results of our present study. In addition, our study has also revealed the relationship between the occurrence of TAI with VitD2, and VitD3, which has not been reported previously.

There are some limitations in our study. Due to the majority of the individuals in the Health Management Center being male, this study enrolls only male subjects to investigate the association between VitD and TAI in adult males. Some studies have analyzed the seasonal variation of 25(OH)D concentrations [65, 66]. However, subjects in our study could not examine the serum VitD concentrations in the same season due to the healthcare program. Although there is an assumed association between VitD and autoimmune thyroid disease, the different function of VitD2 and VitD3 supplements remains unclear. Further studies are crucial to establish the association between VitD (VitD2 and VitD3) supplements and autoimmune thyroid disease (TAI). We acknowledged that some other reported factors (e.g. omega-3 fatty acids [67]) potentially affecting AIT development were not referred to in the present study. And their potential interactions with VitD in the occurrence of AIT had not been explored, which was a limitation of our study. Modest changes in VitD2 and VitD3 were found in our study. Among them, only the modest change of VitD3 is associated with the changes of TPOAb, but not VitD2, indicating that AITD might benefit more from VitD3. VitD2 is mainly from the diet, and it is relatively fixed in the same area, which may be one of the reasons that no great change was found in VitD2. It cannot exclude if the large changes in VitD2 could reduce thyroid autoimmune antibodies, which is another limitation of our study.

Conclusion

To conclude, our findings further demonstrate that VitD deficiency (<20ng/ml) may be an independent risk factor for TAI development. Moreover, the latter is potentially associated with the deficiency of VitD3 rather than VitD2 in the male population, which suggests the potentially beneficial effects of VitD3 than VitD2 supplementation to VDD men in terms of preventing the development of TAI. Further Prospective clinical studies with long-term follow-up and experimental studies are required to understand the mechanisms of VitD2 and VitD3 supplementation on the pathogenesis of TAI.

Author contributions

Dongdong Luo and Chenxi Zhang wrote the main manuscript text. Bingrui Gao, Deping Wang, Zhaoying Chen, Kan Chen, and Bojuan Li investigate the information of subject. Song Leng and Jing Li supervise the research. All authors reviewed the manuscript.

Funding

This work was supported by the General Program of National Natural Science Foundation of China (grant number No.81771741) and the Distinguished Professor at Educational Department of Liaoning Province (grant number No. [2014]187).

Data availability

Data is provided within the manuscript information files.

Declarations

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author details

[1]Department of Endocrinology and Metabolism, The Institute of Endocrinology, NHC Key Laboratory of Diagnosis and Treatment of Thyroid Disease, The First Hospital of China Medical University, Shenyang, Liaoning, China
[2]Department of Endocrinology and Metabolism, The Second Hospital of Dalian Medical University, Dalian, Liaoning, China
[3]Health Management Center, The Second Hospital of Dalian Medical University, Dalian, Liaoning, China
[4]Department of Endocrinology and Metabolism, Hongqi Hospital Affiliated to Mudanjiang Medical University, Mudanjiang, Heilongjiang, China

Received: 27 June 2024 / Accepted: 22 January 2025
Published online: 18 March 2025

References

1. Caturegli P, De Remigis A, Rose NR. Hashimoto thyroiditis: clinical and diagnostic criteria. Autoimmun Rev. 2014;13(4–5):391–7.

2. Li Y, Teng D, Ba J, Chen B, Du J, He L, Lai X, Teng X, Shi X, Li Y, Chi H, Liao E, Liu C, Liu L, Qin G, Qin Y, Quan H, Shi B, Sun H, Tang X, Tong N, Wang G, Zhang J-A, Wang Y, Xue Y, Yan L, Yang J, Yang L, Yao Y, Ye Z, Zhang Q, Zhang L, Zhu J, Zhu M, Ning G, Mu Y, Zhao J, Shan Z. Teng, Efficacy and Safety of Long-Term Universal Salt iodization on thyroid disorders: epidemiological evidence from 31 provinces of Mainland China. Thyroid. 2020;30(4):568–79.
3. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911–30.
4. Mele C, Caputo M, Bisceglia A, Samà MT, Zavattaro M, Aimaretti G, Pagano L, Prodam F, Marzullo P. Immunomodulatory effects of vitamin D in thyroid diseases. Nutrients 12(5) (2020).
5. Sîrbe C, Rednic S, Grama A, Pop TL. An update on the effects of vitamin D on the Immune System and Autoimmune diseases. Int J Mol Sci 23(17) (2022).
6. Sulejmanovic M, Begić A, Mujaric-Bousbia F, Salkic S, Ramas A. The relationship between thyroid antibodies and Vitamin D Level in primary hypothyroidism. Med Arch. 2020;74(5):359–62.
7. Koehler VF, Filmann N, Mann WA. Vitamin D status and thyroid autoantibodies in Autoimmune Thyroiditis. Horm Metab Res. 2019;51(12):792–7.
8. Fang F, Chai Y, Wei H, Wang K, Tan L, Zhang W, Fan Y, Li F, Shan Z, Zhu M. Vitamin D deficiency is associated with thyroid autoimmunity: results from an epidemiological survey in Tianjin, China. Endocrine. 2021;73:447–54.
9. Wilson LR, Tripkovic L, Hart KH, Lanham-New SA. Vitamin D deficiency as a public health issue: using vitamin D2 or vitamin D3 in future fortification strategies. Proc Nutr Soc. 2017;76(3):392–9.
10. Chiang C-M, Ismaeel A, Griffis RB, Weems S. Effects of vitamin D supplementation on muscle strength in athletes: a systematic review. J Strength Cond Res. 2017;31(2):566–74.
11. Xiang Y, Jin Q, Li L, Yang Y, Zhang H, Liu M, Fan C, Li J, Shan Z, Teng W. Physiological low-dose oestrogen promotes the development of experimental autoimmune thyroiditis through the up-regulation of Th1/Th17 responses. J Reprod Immunol. 2018;126:23–31.
12. Wang X, Zynat J, Guo Y, Osiman R, Tuhuti A, Zhao H, Abdunaimu M, Wang H, Jin X, Xing S. Low serum vitamin D is Associated with Anti-thyroid-globulin antibody in female individuals. Int J Endocrinol. 2015;2015:285–90.
13. Bizzaro G, Shoenfeld Y. Vitamin D and autoimmune thyroid diseases: facts and unresolved questions. Immunol Res. 2015;61(1–2):46–52.
14. Mackawy AMH, Al-Ayed BM, Al-Rashidi BM. Vitamin d deficiency and its association with thyroid disease. Int J Health Sci (Qassim). 2013;7(3):267–75.
15. Elsammak MY, Al-Wossaibi AA, Al-Howeish A, Alsaeed J. High prevalence of vitamin D deficiency in the sunny eastern region of Saudi Arabia: a hospital-based study. East Mediterr Health J. 2011;17(4):317–22.
16. Egierska D, Pietruszka P, Burzyńska P, Chruścicka I, Buchta J. Pleiotropic effects of vitamin D3. J Educ Health Sport. 2021;11(7):143–55.
17. Lisowska KA, Bryl E. The role of vitamin D in the development of autoimmune diseases. Postepy Hig Med Dosw (Online). 2017;71(1):797–810.
18. Czarnywojtek A, Florek E, Pietrończyk K, Sawicka-Gutaj N, Ruchała M, Ronen O, Nixon IJ, Shaha AR, Rodrigo JP, Tufano RP, Zafereo M, Randolph GW, Ferlito A. The role of vitamin D in autoimmune thyroid diseases: a narrative review. J Clin Med 12(4) (2023).
19. Gallo D, Baci D, Kustrimovic N, Lanzo N, Patera B, Tanda ML, Piantanida E, Mortara L. How does vitamin D affect Immune cells Crosstalk in Autoimmune diseases? Int J Mol Sci 24(5) (2023).
20. Fletcher J, Bishop EL, Harrison SR, Swift A, Cooper SC, Dimeloe SK, Raza K, Hewison M. Autoimmune disease and interconnections with vitamin D. Endocr Connect 11(3) (2022).
21. Zhao R, Zhang W, Ma C, Zhao Y, Xiong R, Wang H, Chen W, Zheng SG. Immunomodulatory Function of Vitamin D and its role in autoimmune thyroid disease. Front Immunol. 2021;12:574967.
22. Abo-Zaid MA, Hamdi HA, Elashmawy NF. Vitamin D and immunity: a comprehensive review of its impact on autoimmunity, allergy suppression, antimicrobial defense, and cancer inhibition. Egypt J Immunol. 2023;30(4):47–66.
23. Filipova L, Lazurova Z, Fulop P, Lazurova I. Vitamin D insufficiency is not associated with thyroid autoimmunity in Slovak women with Hashimoto´s disease. Bratisl Lek Listy. 2023;124(3):182–6.
24. Aktaş HŞ. Vitamin B12 and Vitamin D Levels in patients with autoimmune hypothyroidism and their correlation with Anti-thyroid Peroxidase Antibodies. Med Princ Pract. 2020;29(4):364–70.
25. Chao G, Zhu Y, Fang L. Correlation between Hashimoto's thyroiditis-related thyroid hormone levels and 25-Hydroxyvitamin D. Front Endocrinol (Lausanne). 2020;11:4.
26. Cui A, Zhang T, Xiao P, Fan Z, Wang H, Zhuang Y. Global and regional prevalence of vitamin D deficiency in population-based studies from 2000 to 2022: a pooled analysis of 7.9 million participants. Front Nutr. 2023;10:1070808.
27. Štefanić M, Tokić S. Serum 25-hydoxyvitamin D concentrations in relation to Hashimoto's thyroiditis: a systematic review, meta-analysis and meta-regression of observational studies. Eur J Nutr. 2020;59(3):859–72.
28. Taheriniya S, Arab A, Hadi A, Fadel A, Askari G. Vitamin D and thyroid disorders: a systematic review and Meta-analysis of observational studies. BMC Endocr Disord. 2021;21(1):171.
29. Yang M, Wu H, Zhao M, Long H, Lu Q. Vitamin D status in patients with autoimmune bullous dermatoses: a meta-analysis. J Dermatological Treat. 2022;33(3):1356–67.
30. Janoušek J, Pilařová V, Macáková K, Nomura A, Veiga-Matos J, Silva DDd, Remião F, Saso L, Malá-Ládová K, Malý J, Nováková L, Mladěnka P. Vitamin D: sources, physiological role, biokinetics, deficiency, therapeutic use, toxicity, and overview of analytical methods for detection of vitamin D and its metabolites. Crit Rev Clin Lab Sci. 2022;59(8):517–54.
31. El-Sharkawy A, Malki A. Vitamin D signaling in inflammation and Cancer: Molecular mechanisms and therapeutic implications. Molecules 25(14) (2020).
32. Wierzbicka A, Oczkowicz M. Sex differences in vitamin D metabolism, serum levels and action. Br J Nutr. 2022;128(11):2115–30.
33. Carlberg C. Endocrine functions of vitamin D. Mol Cell Endocrinol. 2017;453:1–2.
34. Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G, Vitamin D. Metabolism, molecular mechanism of Action, and Pleiotropic effects. Physiol Rev. 2016;96(1):365–408.
35. Matikainen N, Pekkarinen T, Ryhänen EM, Schalin-Jäntti C. Physiology of Calcium Homeostasis: an overview. Endocrinol Metab Clin North Am. 2021;50(4):575–90.
36. Cheng JB, Levine MA, Bell NH, Mangelsdorf DJ, Russell DW. Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. Proc Natl Acad Sci U S A. 2004;101(20):7711–5.
37. Chang S-W, Lee H-C. Vitamin D and health - the missing vitamin in humans. Pediatr Neonatol. 2019;60(3):237–44.
38. Houghton LA, Vieth R. The case against ergocalciferol (vitamin D2) as a vitamin supplement. Am J Clin Nutr. 2006;84(4):694–7.
39. Horst RL, Reinhardt TA, Ramberg CF, Koszewski NJ, Napoli JL. 24-Hydroxylation of 1,25-dihydroxyergocalciferol. An unambiguous deactivation process. J Biol Chem. 1986;261(20):9250–6.
40. Wimalawansa SJ. Physiological Basis for Using Vitamin D to Improve Health. Biomedicines. 2023;11(6):1542.
41. Knutsen KV, Madar AA, Brekke M, Meyer HE, Eggemoen ÅR, Mdala I, Lagerløv P. Effect of vitamin D on thyroid autoimmunity: a Randomized, Double-Blind, controlled Trial among ethnic minorities. J Endocr Soc. 2017;1(5):470–9.
42. Behera KK, Saharia GK, Hota D, Sahoo DP, Sethy M, Srinivasan A. Effect of vitamin D supplementation on thyroid autoimmunity among subjects of autoimmune thyroid disease in a Coastal Province of India: a Randomized Open-label trial. Niger Med J. 2020;61(5):237–40.
43. Feng Y, Qiu T, Chen H, Wei Y, Jiang X, Zhang H, Chen D. Association of serum IL-21 and vitamin D concentrations in Chinese children with autoimmune thyroid disease. Clin Chim Acta. 2020;507:194–8.
44. Jiang H, Chen X, Qian X, Shao S. Effects of vitamin D treatment on thyroid function and autoimmunity markers in patients with Hashimoto's thyroiditis-A meta-analysis of randomized controlled trials. J Clin Pharm Ther. 2022;47(6):767–75.
45. Zhang J, Chen Y, Li H, Li H. Effects of vitamin D on thyroid autoimmunity markers in Hashimoto's thyroiditis: systematic review and meta-analysis. J Int Med Res. 2021;49(12):3000605211060675.
46. Durrant LR, Bucca G, Hesketh A, Möller-Levet C, Tripkovic L, Wu H, Hart KH, Mathers JC, Elliott RM, Lanham-New SA, Smith CP. Vitamins D and D have overlapping but different effects on the human Immune System revealed through analysis of the blood transcriptome. Front Immunol. 2022;13:790444.
47. Effraimidis G, Badenhoop K, Tijssen JGP, Wiersinga WM. Vitamin D deficiency is not associated with early stages of thyroid autoimmunity. Eur J Endocrinol. 2012;167(1):43–8.
48. Nodehi M, Ajami A, Izad M, Asgarian Omran H, Chahardoli R, Amouzegar A, Yekaninejad S, Hemmatabadi M, Azizi F, Esfahanian F, Mansouri F, Mazaheri Nezhad R, Fard AA, Saboor-Yaraghi. Effects of vitamin D supplements on frequency of CD4 T-cell subsets in women with Hashimoto's thyroiditis: a double-blind placebo-controlled study. Eur J Clin Nutr. 2019;73(9):1236–43.

49. Lubberts E. [Vitamin D like one of few prevention possibilities for autoimmune diseases?]. Ned Tijdschr Geneeskd 166 (2022).
50. Prummel MF, Laurberg P. Interferon-alpha and autoimmune thyroid disease. Thyroid. 2003;13(6):547–51.
51. Feghali K, Atallah J, Norman C. Manifestations of thyroid disease post COVID-19 illness: report of Hashimoto Thyroiditis, Graves' disease, and subacute thyroiditis. J Clin Transl Endocrinol Case Rep. 2021;22:100094.
52. Tee LY, Harjanto S, Rosario BH. COVID-19 complicated by Hashimoto's thyroiditis. Singap Med J. 2021;62(5):265.
53. Knack RS, Hanada T, Knack RS, Mayr K. Hashimoto's thyroiditis following SARS-CoV-2 infection. BMJ Case Rep 14(8) (2021).
54. Ajjan RA, Weetman AP. The pathogenesis of Hashimoto's Thyroiditis: further developments in our understanding. Horm Metab Res. 2015;47(10):702–10.
55. Bülow Pedersen I, Laurberg P, Knudsen N, Jørgensen T, Perrild H, Ovesen L, Rasmussen LB. A population study of the association between thyroid autoantibodies in serum and abnormalities in thyroid function and structure. Clin Endocrinol (Oxf). 2005;62(6):713–20.
56. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, Braverman LE, Serum TSH. T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab. 2002;87(2):489–99.
57. Weetman AP. Autoimmune thyroid disease: propagation and progression. Eur J Endocrinol. 2003;148(1):1–9.
58. Brix TH, Kyvik KO, Hegedüs L. What is the evidence of genetic factors in the etiology of Graves' disease? A brief review. Thyroid. 1998;8(7):627–34.
59. Wiersinga WM. Clinical relevance of environmental factors in the pathogenesis of autoimmune thyroid disease. Endocrinol Metab (Seoul). 2016;31(2):213–22.
60. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. Am J Clin Nutr. 2008;88(2):S491–9.
61. Spedding S, Vanlint S, Morris H, Scragg R. Does vitamin D sufficiency equate to a single serum 25-hydroxyvitamin D level or are different levels required for non-skeletal diseases? Nutrients. 2013;5(12):5127–39.
62. Inoue N, Watanabe M, Ishido N, Katsumata Y, Kagawa T, Hidaka Y, Iwatani Y. The functional polymorphisms of VDR, GC and CYP2R1 are involved in the pathogenesis of autoimmune thyroid diseases. Clin Exp Immunol. 2014;178(2):262–9.
63. Yang Z, Wang H, Liu Y, Feng Y, Xiang Y, Li J, Shan Z, Teng W. The expression of anti-protein disulfide isomerase A3 autoantibody is associated with the increased risk of miscarriage in euthyroid women with thyroid autoimmunity. Int Immunopharmacol. 2022;104:108507.
64. Zmijewski MA, Carlberg C. Vitamin D receptor(s): in the nucleus but also at membranes? Exp Dermatol. 2020;29(9):876–84.
65. Hyppönen E, Power C. Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. Am J Clin Nutr. 2007;85(3):860–8.
66. Darling AL, Hart KH, Macdonald HM, Horton K, Kang'ombe AR, Berry JL, Lanham-New SA. Vitamin D deficiency in UK South Asian women of childbearing age: a comparative longitudinal investigation with UK caucasian women. Osteoporos Int. 2013;24(2):477–88.
67. Hahn J, Cook NR, Alexander EK, Friedman S, Walter J, Bubes V, Kotler G, Lee IM, Manson JE, Costenbader KH. Vitamin D and marine omega 3 fatty acid supplementation and incident autoimmune disease: VITAL randomized controlled trial. BMJ. 2022;376:e066452.

Publisher's note