The impacts of vitamin D supplementation on serum levels of thyroid autoantibodies in patients with autoimmune thyroid disease: a meta-analysis (#110688)

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The impacts of vitamin D supplementation on serum levels of thyroid autoantibodies in patients with autoimmune thyroid disease: a meta-analysis

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Background: Although vitamin D (VitD) deficiency had been found with close relationship with autoimmune thyroid disorders (AITD), the findings about the impacts of VitD supplementation on the production of anti-thyroid peroxidase (TPOAb) and anti-thyroglobulin antibodies (TgAb) remained inconsistent. Thus, a systemic meta-analysis was conducted to figure out the exact effects of VitD intervention on the production of TPOAb and TgAb in AITD patients.

Methods: We searched PubMed, Web of Science, Embase and The Cochrane Library databases for all clinical studies up to May 2023, which evaluated the changes in serum TPOAb and TgAb titers of AITD patients after VitD intervention.

Results: A total of 10 cohorts from 10 clinical trials with 577 patients with AITD were included. The serum titers of TPOAb and TgAb were significantly decreased after VitD supplementation as compared with their pre-treatment levels, respectively. However, a significant post-treatment reduction of serum TPOAb level was found only in the AITD patients with initial VitD insufficiency/deficiency, while an obvious decrease of serum TgAb was shown if only those studies without consideration of the pre-treatment VitD status were included. Serum TPOAb and TgAb titers were significantly decreased in the patients receiving VitD supplementation for at least 3 months, but not in the ones for less than 3 months. Serum titers of TPOAb and TgAb were both pronouncedly reduced in the patients receiving daily administration of VitD rather than weekly regimen.

Conclusions: AITD patients may benefit with the reduction of TPOAb and TgAb production after daily VitD supplementation for at least 3 months, especially with a decrease of serum TPOAb level when initially VitD insufficient/deficient, which mechanisms await further investigation.

The impacts of vitamin D supplementation on serum levels of thyroid autoantibodies in patients with autoimmune thyroid disease: a meta-analysis

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Abstract

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Systematic Review Registration

PROSPERO, identifier CRD42023424029

Introduction

Vitamin D (VitD) is currently considered as an important multifunctional hormone. The source of VitD in the body mainly relies on the endogenous production by skin epidermal cells under sunny ultraviolet radiation, and only a little comes from diet food. VitD insufficiency is very common in those people who are lack of sunshine exposure and nutrition intake. Serum 25(OH)D concentration is well-known to indicate the nutritional status of VitD with a level below 20 ng/mL as VitD deficiency and 21-29 ng/mL as VitD insufficiency[1]. The representative isomers of VitD are ergocalciferol ($VitD_2$) and cholecalciferol ($VitD_3$), and especially the latter is the main form of VitD. The outcomes caused by VitD deficiency may be improved through direct supplementation with either $VitD_2$/$VitD_3$ or their active forms [e.g. 1,25 $(OH)_2D_3$, 25$(OH)D_3$]. The active compounds can modulate the activities of many organs, such as immune system and bone. The expression of the VitD receptor (VDR) is ubiquitous across all immune cells[2, 3]. It has been found that VitD may exert some crucial influences on both innate and adaptive immune response[4]. Several autoimmune diseases (e.g. autoimmune diabetes mellitus, rheumatoid arthritis, and multiple sclerosis) have been found with close association with VitD deficiency[5, 6]. Clinical investigation and animal studies have suggested that VitD administration may inhibit the development of some autoimmune diseases[7, 8].

Autoimmune thyroid diseases (AITD) are common autoimmune diseases, mainly consisting of Hashimoto's thyroiditis (HT), postpartum thyroiditis (PPT) and Graves' disease (GD). They are usually characterized by the presence of anti-thyroid peroxidase antibody (TPOAb) and/or anti-thyroglobulin antibody (TgAb). Given the well-known immunomodulatory effects of VitD, its association with AITD has been extensively investigated in recent years[9]. Cross-sectional studies have identified a correlation between VitD deficiency and the development of AITD[10-15], which suggests the potential benefits of VitD supplementation in the AITD patients. There have been several papers published about the influence of VitD administration on the production of TPOAb and TgAb in the AITD patients. However, most of those studies were small in the sample size, and the findings were inconsistent. Therefore, the aim of this study was to clarify the alterations in the serum levels of TPOAb and TgAb in the AITD patients following VitD intervention through a meta-analysis of the related prospective studies, which may contribute to the most appropriate clinical application of VitD in the management of AITD.

Materials & Methods

Search Strategy

We conducted a systematic search of literature focusing on "Vitamin D" and "AITD" in the PubMed, EMBASE, Web of Science, and Cochrane Library databases up to May 2023. All studies on the changes of blood TPOAb and /or TgAb titers in AITD patients who were administered with VitD had been included into this analysis. Two reviewers (D.L. and B.L.) separately assessed the eligibility of the studies. The specific search strategies in the PubMed were as follows: 'Thyroiditis, Autoimmune' [Title/Abstract]) OR 'Autoimmune Thyroid*' [Title/Abstract] OR 'thyroiditis' [Title/Abstract] OR 'Hashimoto* Disease' [Title/Abstract] OR 'Hashimoto* Thyroiditi*' [Title/Abstract] OR 'Chronic Lymphocytic Thyroiditis' [Title/Abstract] OR 'Graves* Disease' [Title/Abstract] OR 'Basedows Disease' [Title/Abstract] OR 'Exophthalmic Goiters' [Title/Abstract] OR 'Postpartum Thyroiditis' [Title/Abstract] OR 'Thyroiditis, Autoimmune' [Mesh] AND 'Vitamin D' [Mesh] OR 'vitamin D*' [Title/Abstract] OR 'Ergocalciferol' [Title/Abstract] OR 'Calciferol' [Title/Abstract] OR 'Cholecalciferol' [Title/Abstract] OR 'Dihydrotachysterol' [Title/Abstract] OR 'Calcifediol' [Title/Abstract] OR 'Calcitriol' [Title/Abstract] OR '1, 25-$(OH)_2D_3$' [Title/Abstract] OR '24,25-Dihydroxyvitamin D_3' [Title/Abstract] OR '25(OH)D' [Title/Abstract] OR '25OHD' [Title/Abstract]. Detailed search strategies of other databases above are listed in the supplementary file 1.

Inclusion and Exclusion Criteria

The eligibility criteria were assembled using PICOS tool as follow (supplementary file 2): (1) population: patients diagnosed with AITD; (2) interventions: treated by VitD; (3) control: placebo or no treatment; (4) outcomes: the changes of serum TPOAb and/or TgAb levels after VitD treatment; (5) study type: any related prospective studies including randomized controlled trials (RCTs) published in scientific and peer-reviewed journals; (6) language: limited to English. No ethical consent was required because this study was performed based on those previously published data.

Exclusion Criteria: those studies in which AITD patients had received any other drugs (e.g. selenium, iodine and inositol) than L-T4 and calcium were all excluded.

Data extraction

Two independent investigators (D.L. and B.L.) retrieved the studies and any discrepancies were resolved by discussion with another reviewer (J.L.). Relevant data were extracted, including the first author, publication year and country, diseases investigated, study design, initial VitD status and its measurement, VitD administration regimen, sample size, sex, age, and serum levels of TPOAb, TgAb, thyrotropin, and 25(OH)D and their changes after the intervention. The primary outcome of this meta-analysis was the effect of VitD supplementation on serum levels of thyroid autoantibodies in patients with AITD. The authors used an electronic data collection form to acquire the necessary information from each article.

Quality Assessment

Two reviewers completed a quality assessment to evaluate all included studies using the Cochrane Collaboration tool (Figure 2) and the Methodological Index for Non-Randomized Studies (MINORS) criteria scoring system (Table 3).

Statistical Analysis

Statistical analyses were conducted using Revman 5.3 software. Standardized mean difference (SMD) and 95% confidence intervals (CI) were used for continuous data. Heterogeneity was evaluated with I^2 statistics. And forest plots were created to visually reflect the heterogeneity degree among those studies included. Cochrane collaboration's tool and MINORS were used for assessing bias risk of RCT and non-RCT studies, respectively. A fixed-effects model was mainly implemented when I^2 was $\leqslant$50%, otherwise a random-effects model was adopted. Leave-one-out analyses were carried out to analyze the robustness when the heterogeneity was very high ($I^2 \geqslant 55\%$). P-value<0.05 was considered as statistically different.

Results

Characteristics of the studies included

During the screening process, 2723 potentially relevant articles were obtained through database retrieval, and only 9 studies were found eligible for the meta-analysis. Besides, another study was identified from citation searching, and then was included. Finally, 10 studies were included into the current analysis, consisting of total 416 VitD-supplemented subjects and 161 controls[16-25]. The screening process is illustrated in Fig 1.

Ten studies utilized serum TPOAb and/or TgAb levels as the primary observation outcomes (Table1). Among them, 8 studies only enrolled HT patients[16, 17, 19, 21-25], one study included both HT and GD cases[18], and one observed PPT women[20]. Active forms of VitD were administered in one study[17], while plain VitD was supplemented to AITD patients in the other 9 studies. Among them, levothyroxine (L-T4) tablet was administered to the patients besides VitD supplementation in 6 studies[17, 19-22, 24], and calcium was additionally used in one study[19].

Change in serum level of 25(OH)D after VitD intervention

Based on the data from the total 10 studies, the serum level of 25(OH)D exhibited a significant increase following VitD supplementation (SMD=3.31, 95%CI 1.95 to 4.67, P<0.00001, Fig 3D), and its average level did not reach up to 30 ng/mL only in one study (Table 2). It indicated that the nutritional VitD statuses in those AITD patients were indeed improved after VitD supplementation through the reported regimens, although not all of them finally became VitD sufficient.

Alterations in serum levels of TPOAb and TgAb after VitD intervention in all the 10 studies

In one study, the significant decrease in serum TPOAb level was not observed[21], and even an obvious increase in serum TPOAb level after VitD supplementation was shown in another

study[19]. However, the remaining 8 studies had consistently exhibited the inhibitory effects of VitD intervention on the production of thyroid autoantibodies in the patients with AITD to some extents. Based on the data from the total 10 studies, serum TPOAb and TgAb titers were significantly reduced following the administration of VitD as compared with their baseline levels before VitD treatment, respectively (TPOAb: SMD=-0.44 95%CI -0.73 to -0.15, *P*=0.003, Fig3A; TgAb: SMD=-0.21, 95%CI -0.36 to -0.05, *P*=0.009, Fig3B). No influence of VitD supplementation on TSH level was found (SMD=-0.14, 95%CI -0.43 to 0.16, *P*=0.36, Fig3C).

Alterations in serum levels of TPOAb and TgAb after VitD intervention in only 5 RCTs

When only AITD patients from 5 RCTs were included into the analysis[18, 19, 21, 22, 24], a significant increase in serum VitD concentration (SMD=3.20, 95%CI 1.59 to 4.81, *P*<0.0001, Fig4D) and reduction in serum TPOAb titer (SMD=-0.24, 95%CI -0.46 to -0.01, *P*=0.04, Fig4A) in VitD-treated subjects were found as compared with those receiving only placebo. However, no significant difference was demonstrated in circulatory TgAb although it showed a consistent decreasing trend in all the 3 studies involved (SMD=-0.20, 95%CI -0.52 to 0.12, *P*=0.22, Fig4B). No influence of VitD supplementation on serum TSH level was found (SMD=0.17, 95%CI -0.54 to 0.88, *P*=0.64, Fig4C).

Alterations in serum levels of TPOAb and TgAb based on the duration of VitD treatment across all 10 studies

There were 7 studies, in which total 305 AITD patients were treated with VitD for a duration of 3 months or longer[16, 20-25]. Their serum TPOAb (SMD=-0.60, 95%CI -1.01 to -0.18, *P*=0.005, Fig5A) and TgAb titers (SMD=-0.25, 95%CI -0.43 to-0.08, *P*=0.005, Fig5B) were significantly decreased after VitD treatment as compared with the pre-treatment levels, respectively. In the other 3 investigations of total 111 subjects receiving VitD treatment for less than 3 months[7, 18, 19], the post-treatment serum titer of either TPOAb (SMD=-0.18, 95%CI -0.44 to 0.07, *P*=0.16, Fig5C) or TgAb (SMD=-0.05, 95%CI -0.38 to 0.28, *P*=0.77, Fig5D) was not significantly decreased.

Differential alterations in serum TPOAb and TgAb levels after VitD treatment with respect to the initial nutritional status of VitD in all the 10 studies

In 5 studies, total 276 AITD patients with initial serum 25 (OH) D below 30ng/mL were enrolled, and supplemented with VitD[16-18, 20, 21]. Their post-treatment serum TPOAb but not TgAb titers were markedly decreased (SMD=-0.50, 95%CI -0.89 to -0.10, *P*=0.01, Fig6A), although the latter showed a consistent decreasing trend (SMD=-0.14, 95%CI -0.31 to 0.03, *P*=0.10, Fig6B). In the other 5 investigations of total 140 patients[19, 22-25], their initial nutritional status of VitD was not taken into the consideration, and even those subjects under sufficient VitD status were also enrolled and treated with VitD. Interestingly, no significant alteration was found in serum TPOAb titer (SMD=-0.38, 95%CI -0.90 to 0.13, *P*=0.15, Fig6C), whereas post-treatment serum TgAb level was markedly decreased (SMD=-0.54, 95%CI -0.92 to -0.15, *P*=0.006, Fig6D).

Alterations in serum levels of TPOAb and TgAb based on the administration frequency of VitD across all 10 studies

Among the 4 studies of total 112 AITD patients[19, 21, 24, 25], a weekly administration regimen was adopted. The post-treatment titers of serum TPOAb (SMD=-0.24, 95%CI -0.78 to 0.29, *P*=0.37, Fig7A) and TgAb (SMD=-0.35, 95%CI -0.99 to 0.30, *P*=0.29, Fig7B) were not significantly different from their pre-treatment levels, respectively. In the other 6 investigations of total 304 subjects with daily VitD supplementation[16-18, 20, 22, 23], the post-treatment serum TPOAb (SMD=-0.57, 95%CI -0.96 to -0.19, *P*=0.004, Fig7C) and TgAb titers (SMD=-0.20, 95%CI -0.36 to -0.04, *P*=0.01, Fig7D) were both markedly decreased.

Altogether, we found that continuous daily rather than weekly VitD supplementation for at least three months significantly reduced serum TPOAb level in the AITD patients with initial VitD insufficiency/deficiency, and also decreased serum TgAb in those patients potentially under VitD sufficient status. We had further done leave-one-out analyses on those results from highly heterogeneous studies ($I^2 \geqslant 55\%$), and confirmed our findings showed the relative robustness of the results except one subgroup (Fig 6A) showed insufficient robustness, where VitD were given to intervene the serum TPOAb of those patients with initial serum 25 (OH) D level below 30ng/mL (supplementary file 3).

Discussion

A series of clinical investigations and meta-analyses have demonstrated the association between VitD insufficiency/deficiency and increasing serum thyroid autoantibody levels in the patients with HT, GD and PPT, indicating the accelerating actions of VitD insufficiency/deficiency on the development of AITD[26-29]. The risks of GD, HT and PPT may be decreased by 1.55, 1.62 and 1.51 times, respectively, for every 5nmol/l increase in serum 25 (OH) D concentration as indicated by several studies[15]. Thus, the clinical application of VitD in the patients of AITD had received much attentions. However, since most of those investigations of the effects of VitD administration on the production of TPOAb and TgAb enrolled relatively small-size samples and they had not shown consistent findings. Therefore, a meta-analysis was further performed.

Indeed, there had been 2 meta-analysis publications about the effects of VitD intervention on the development of AITD yet, which included 6[30] and 7 [31] studies, respectively. In the first meta-analysis, 4 RCTs published in English and 2 in Chinese were included, and 330 HT patients and 14 GD ones were finally recruited. They found at least 6-month intervention of VitD could cause a significant decrease in serum TPOAb level, and both serum TPOAb and TgAb levels were markedly lower after VitD treatment than placebo administration. Except for the duration of VitD intervention, no other sub-group analyses were performed in the first meta-analysis[30]. In the second meta-analysis of 3 RCTs and 4 prospective studies published before 2022, the findings based on the data from 258 HT patients showed that VitD supplementation significantly decreased serum TPOAb level as compared with that of the control group with

either placebo or single use of levothyroxine, simvastatin or selenomethionine, but did not affect serum TgAb and thyroid functions[31]. They did subgroup-analyses of VitD intervention duration, pre-treatment VitD nutritional status and gender, and found that VitD intervention for at least 3months reduced serum TPOAb level more than the control maneuver in both genders who were either VitD-insufficient or sufficient[40]. In the current analysis, we included 5 RCTs and 5 prospective studies published in English, totally consisting of 552 HT, 14 GD and 11 PPT patients. Those studies in which AITD patients in the VitD intervention group had received other drugs than L-T4 and calcium were all excluded from the current meta-analysis. Our results did not only show a significant reduction in serum TPOAb and TgAb levels after VitD supplementation based on all the 10 interventional studies, but also exhibit a markedly lower serum level of TPOAb after VitD intervention as compared with that of the control group based on the 5 RCTs. Furthermore, the current meta-analysis demonstrated the effectiveness of VitD supplementation in reducing both serum TPOAb level when VitD deficient/insufficient at the baseline and serum TgAb without respect to the initial serum VitD level. Finally, we found that daily other than weekly supplementation of VitD reduced serum TPOAb and TgAb levels, which had not been investigated in the previous meta-analysis studies.

Several studies have indicated that VitD may be an immunomodulatory hormone and exerts significant effects on the immune system due to the expression of VDR and 1α-hydroxylase (CYP27B1) in dendritic cells, macrophages, T cells, B cells, and other immune cells[32-35]. The enzyme converts VitD into its active form of calcitriol, namely 1,25 $(OH)_2D_3$, which binds to VDR or PDIA3 on the target cells and exerts its effects through both genomic and non-genomic mechanisms[36,37]. A recent randomized controlled trial known as VITAL, involving 25,871 American participants, demonstrated that daily supplementation of 2000 IU of VitD for a continuous period of five years can lead to a significant reduction in the risk of developing any autoimmune disease including autoimmune thyroid disorders by 22%[38]. It had been found that the supplementation of 1,25 $(OH)_2D_3$ to the experimental autoimmune thyroiditis (EAT) animal model ameliorated the pathological changes of the thyroid gland, inhibited thyroid auto-antibody production and corrected the cytokine disequilibrium[39, 40].

Our study revealed significant decreases in serum TPOAb and TgAb titers following VitD supplementation. However, the subgroup analysis showed that VitD supplementation could reduce serum TPOAb level when the patients were VitD deficient/insufficient at the baseline whereas it decreased serum TgAb when those patients with potentially VitD sufficient were recruited. It suggests that higher serum VitD concentration may be needed to suppress the production of TgAb. TPOAb is well known as the most specific biomarker for the presence of thyroid autoimmunity, representing a kind of adaptive immune response. It is expressed in about 95% of HT patients with significant correlation with the degree of intrathyroidal lymphocyte infiltration[41]. TgAb is existent in only 60–80% of HT patients with less sensitivity and specificity for AITD diagnosis, reflecting an innate type of immune response[42]. It has previously reported that there is a poor but significant correlation between TPOAb and TgAb expressions[43]. VitD has been known as a threshold nutrient, and its immunomodulatory actions

may not be linearly correlated with its serum concentration[43]. An in-vitro study has reported that the regulatory actions of 1,25 $(OH)_2D_3$ on the production of IL17A, IL-6, IL-8 and MMP1/MMP3 were not linearly dependent on its concentration[45]. Besides, it has been found that VitD may have differential modulatory effects on innate immune reactions and adaptive immune functions[45]. Thus, we speculated that the different production mechanisms between TPOAb and TgAb may account for their differential changes in AITD patients after VitD intervention, and the serum threshold level for VitD to suppress the production of TgAb may be higher than that of TPOAb. In addition, the previous study of EAT has shown that VitD treatment inhibited the production of TgAb in the mice without VitD deficiency[39].

In order to figure out the appropriate regimen of VitD administration for AITD patients, we further performed the subgroup analyses on the duration and dosing frequency. Our results indicate that daily other than weekly VitD supplementation for at least three months can reduce serum levels of TPOAb and TgAb. We admitted that there were some limitations in this meta-analysis. There was still some heterogeneity in the included studies in this meta-analysis, which was attributed to the limited overall number of studies, small sample sizes in those studies, and their differential VitD administration regimens, initial VitD nutritional status, baseline thyroid functions and sex ratios of participants. Some studies combined VitD supplementation with L-T4 treatment while others did not. Although we have further done leave-one-out analyses on those results from highly heterogeneous studies, and confirmed our preliminary findings (supplementary file 3), this meta-analysis could not exclude all the heterogeneity factors among the studies. Besides, no subgroup analysis was performed based on plain and active forms of VitD supplementation due to limited data. Thus, more large-size sample RCTs are still needed to clarify the benefits of VitD supplementation and the optimal dosing regimen in AITD patients.

Conclusions

In conclusion, our current meta-analysis findings suggest that VitD supplementation for at least 3 months can lower serum TPOAb and TgAb levels in AITD patients. Mainly AITD patients with VitD deficiency/insufficiency can benefit with the decrease of serum TPOAb from VitD supplementation. But VitD may be administered to AITD patients even with VitD sufficiency so as to reduce serum TgAb. The frequency for VitD supplementation on a daily basis may be more effective than a weekly regimen. Our findings have further provided clinical evidence for the optimal administration regimen of VitD in AITD patients. However, the optimal range of 25 (OH) D level to achieve the best protection against AITD, and the optimal duration, dosing regimen, intervention form (plain vs active) of VitD to ensure the safety and maximize the benefits still await more investigations in large-size sample RCTs.

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Table 1(on next page)

Characteristics of the 10 studies included in the meta-analysis

Abbreviations: CMIA, chemiluminescent microparticle immunoassay; con, control; ELISA, enzyme-linked immunoassay; fT3, free triiodothyronine; fT4, free thyroxine; GD, Graves' disease; HT, Hashimoto thyroiditis; int, intervention; iPTH, intact parathyroid hormone; LC-MS/MS, liquid chromatography-tandem mass spectrometry; NS, not statistically significant; PPT, postpartum thyroiditis; RCT, randomized controlled trial; TgAb, anti-thyroglobulin antibodies; TPOAb, anti-thyroid peroxidase antibodies; TSH, thyroid-stimulating hormone.

1

First author, year	Country	Disease	Study design	VitD status	VitD measurement	VitD dose	Duration	Sample size (int./con.)	TPOAb in int. group (before vs. after)	TGAb in int. group (before vs. after)	Sex	Age (int./con.), years	Main outcome
Elias, 2015[16]	Greece	HT	Prospective	<30 ng/mL	CMIA	$VitD_3$ 1200-4000 IU/day	4 months	n=186	Significant reduction	Reduction but NS	Both	35.3±8.5	TPOAb, TGAb, BMI, TSH
Ucan, 2016[17]	Turkey	HT	Prospective	<20 ng/mL	RIA	$25(OH)D_3$ 50,000 IU/week	8 weeks	n=25	Significant reduction	Significant reduction	Both	35.9±11.2	fT3, fT4, TSH, TPOAb, TgAb
Simsek, 2016[18]	Turkey	GD+HT	RCT	<20 ng/mL	LC–MS/MS	VitD 1000 IU/day	1 month	n=82 (46/36)	Significant reduction	Significant reduction	Both	35.8±12/39.7±12.6	TSH, fT4, fT3, TPOAb, TgAb
Chaudhary, 2016[19]	India	AITD	RCT	No limited	RIA	$VitD_3$ 60,000 IU/week	8 weeks	n=100 (50/50)	Significant reduction	/	Both	28.48±6.57/ 27.86±7.29	fT4, TSH, Calcium, phosphate, iPTH
Krysiak, 2016[20]	Poland	PPT	Prospective	<20 ng/mL	/	VitD 4000 IU/day	3 months	n=11	Significant reduction	Reduction but NS	Female	32±4	fT3, fT4, TSH, Ca, phosphate, PTH
Vahabi, 2017[21]	Iran	HT	RCT	<20 ng/mL	ELISA	VitD 50,000 IU/week	12 weeks	n=56 (30/26)	NS	/	Both	43.55±1.56/ 44.12±1.56	TPOAb, PTH, TSH
Krysiak, 2017[22]	Poland	HT	RCT	⩾30 ng/mL	ELISA	VitD 2000 IU/day	6 months	n=34 (16/18)	Significant reduction	Reduction but NS	Female	34±7/35±6	fT3, fT4, TSH, TPOAb, TgAb
Krysiak, 2019[23]	Poland	HT	Prospective	Not limited	Competitive immunoassay	$VitD_3$ 400 IU/day	6 months	n=20	Significant reduction	Significant reduction	Male	35±8	fT3, fT4, TSH, TPOAb, TgAb
Chahardoli, 2019[24]	Iran	HT	RCT	Not limited	ELISA	$VitD_3$ 50,000 IU/week	3 months	n=40 (19/21)	Reduction but NS	Significant reduction	Female	36.4±5.2/35.9±7.8	T3, T4, TSH, TPOAb, TgAb, Ca
Kumar, 2020[25]	India	HT	Prospective	Not limited	CMIA	$VitD_3$ 60,000 IU/week	6 months	n=23	Significant increase	/	Both	35.5±11.03	fT4, TSH, TPOAb

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Table 2(on next page)

The levels of serum thyroid autoantibodies, 25(OH)D and thyrotropin in the 10 studies

Abbreviations: con, control; int, intervention; 25(OH)D, 25-hydroxyvitamin D; TgAb, anti-thyroglobulin antibodies; TPOAb, anti-thyroid peroxidase antibodies; TSH, thyroid-stimulating hormone. ND, not detected.

First author, year		TPOAb (IU/mL)		TGAb (IU/mL)		25(OH)D (ng/mL)		TSH (μIU/mL)	
		Before	After	Before	After	Before	After	Before	After
Elias, 2015[16]	Int.	364±181	290±116	16.8±7.3	15.9±5.4	14.6±7.2	45.7±4.3	2.5±1.7	2.4±1.5
Ucan, 2016[17]	Int.	448±443	434±446	365±812	329±831	9.56±5.01	52.9±39.1	3.20±1.03	3.48±1.49
Simsek, 2016[18]	Int.	223±591	210±592	312±1612	244±915	11.5±5.9	21.4±9.9	4.1±4.0	3.5±2.5
	Con.	201±593	166±592	273±566	237±592	8.6±4.2	10.9±6.0	4.0±2.5	3.5±2.2
Chaudhary, 2016[19]	Int.	739.1±343.2	387±1146	ND	ND	33.25±93.77	98.52±124	6.88±138.98	3.16±2.07
	Con.	687.8±255.1	553.5±1002	ND	ND	39.61±116.31	41.61±100.06	6.80±149.36	3.39±2.19
Krysiak, 2016[20]	Int.	1395±451	705±314	1410±623	918±456	14±4	39±7	2.5±1.0	2.6±1.2
Krysiak, 2017[21]	Int.	1405±402	955±358	1210±465	934±415	52±12	64±10	4.3±1.4	4.1±1.5
	Con.	1455±390	1410±425	1265±420	1212±385	50±10	47±12	4.2±1.3	4.5±1.0
Vahabi, 2017[22]	Int.	820.25±92	734±102.93	ND	ND	12.76±0.74	45.53±1.84	3.3±0.5	3.88±0.82
	Con.	838.07±99.37	750.03±108.71	ND	ND	13.28±0.86	14.92±1.06	3.45±0.43	2.66±0.38
Krysiak,2019[23]	Int.	836±245	638±211	756±302	562±267	25±10	42±8	2.9±0.7	2.7 ±0.7
Chahardoli, 2019[24]	Int.	131.4±108	118.1±97.9	192.6±161.8	140.2±134.3	25.38±11.02	50.16±14.98	3±2.09	1.83±1.4
	Con.	174.1±141.8	181.6±122.5	182.5±153.9	176.7±167.1	19.80±8.81	22.03±9.45	2.56±1.36	2.77±1.9
Kumar, 2020[25]	Int.	746.84±332.24	954.09±459.76	ND	ND	15.33±5.71	41.22±12.24	7.23±3.16	3.04±2.62

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Table 3(on next page)

Bias risk assessment of non-randomized trials included in this study by the MINORS scale

①Clearly stated aim; ②Inclusion of consecutive patients; ③Prospective collection of data; ④Endpoints appropriate to the aim of the study; ⑤Unbiased assessment of the study endpoint; ⑥Follow-up period appropriate to the aim of the study; ⑦Loss to follow up <5%; ⑧Prospective calculation of the study size; ⑨Adequate control group; ⑩Contemporary groups; Baseline equivalence of groups; Adequate statistical analyses. Abbreviation: MINORS, Methodological Index for Non-Randomized Studies

Included studies	①	②	③	④	⑤	⑥	⑦	⑧	Additional criteria for comparative studies				Total scores
									⑨	⑩	⑪	⑫	
Elias, 2015	2	2	1	2	0	2	2	0					11
Ucan, 2016	2	2	1	2	0	2	2	0	1	1	2	2	17
Krysiak, 2016	2	2	1	2	0	2	2	0	2	2	2	2	19
Krysiak, 2019	2	2	2	2	2	2	2	0					14
Kumar, 2020	2	1	2	2	0	2	2	1					12

①Clearly stated aim; ②Inclusion of consecutive patients; ③Prospective collection of data; ④Endpoints appropriate to the aim of the study; ⑤Unbiased assessment of the study endpoint; ⑥Follow-up period appropriate to the aim of the study; ⑦Loss to follow up <5%; ⑧Prospective calculation of the study size; ⑨Adequate control group; ⑩Contemporary groups; ⑪Baseline equivalence of groups; ⑫Adequate statistical analyses.

Abbreviation: MINORS, Methodological Index for Non-Randomized Studies

Figure 1

Search flow diagram according to PRISMA guideline.

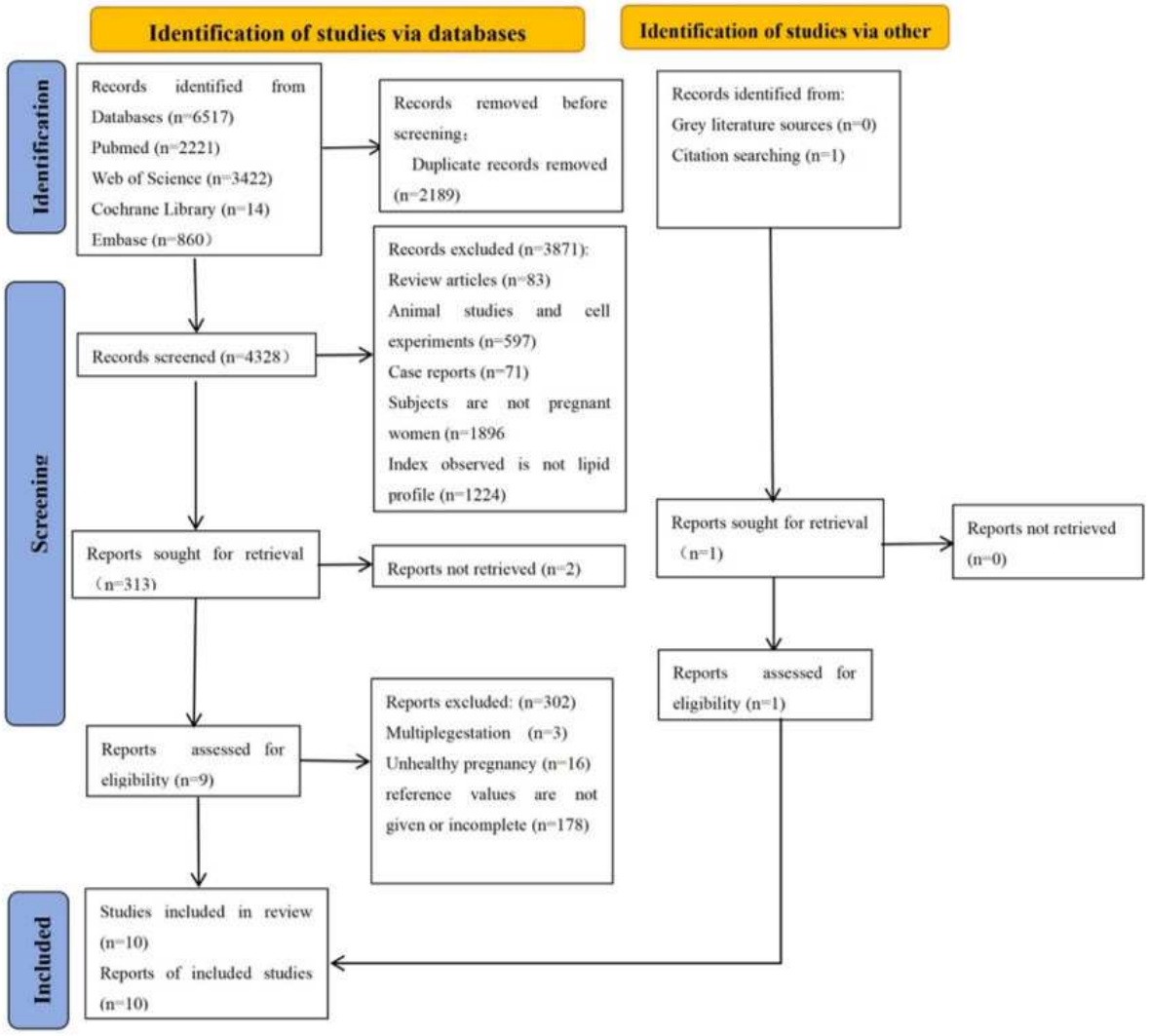

Figure 2

Risk of bias assessments

(A) Upper panel presents risk of bias for each inolude study; (B) Lower panel presents overall risk of bias of included studies; green indicates low rish, red indicates high risk, yellow indicates some concerns.

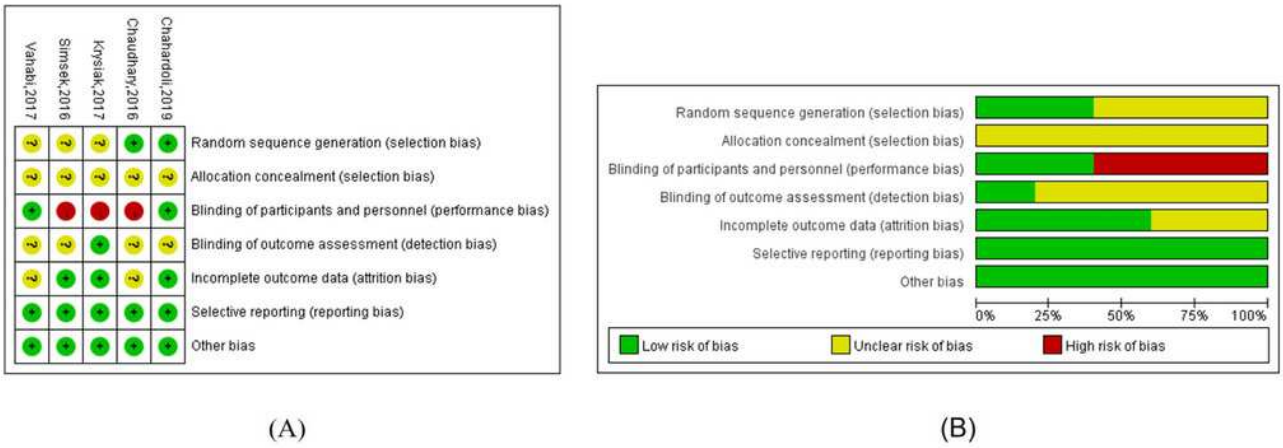

Figure 3

Forest plots of serum thyroid autoantibodies, 25(OH)D and thyroid-stimulating hormone levels based on the whole 10 prospective studies.

(A) Anti-thyroid peroxidase antibody; (B) Anti-thyroglobulin antibody; (C) Thyroid-stimulating honmone; (D) 25-Hydroxyvitamin D.

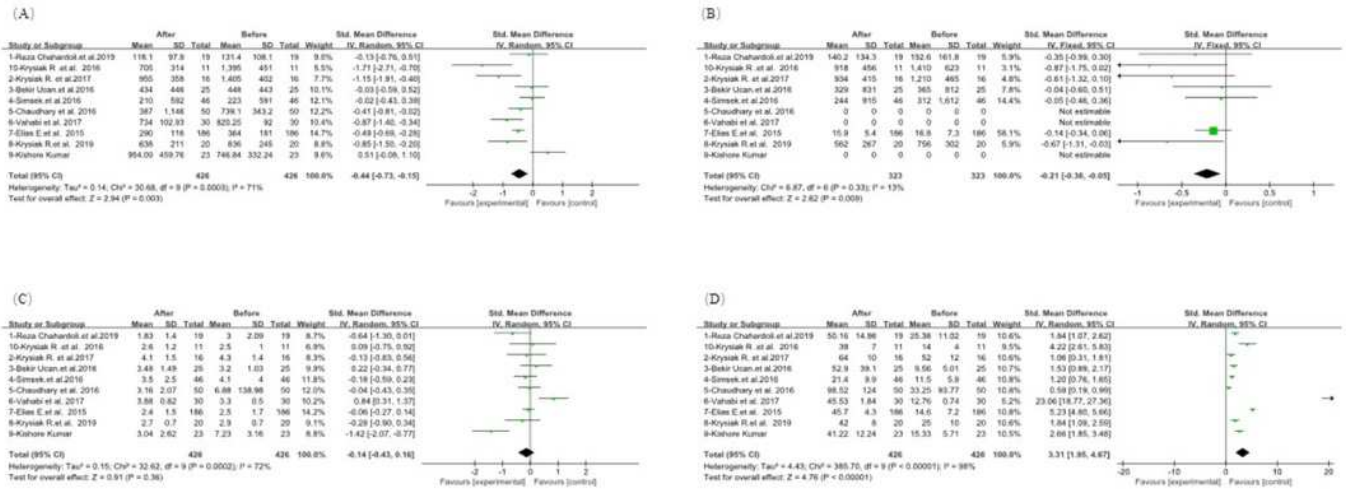

Figure 4

Forest plots of serum thyroid autoantibodies, 25(OH)D and thyroid-stimulating hormone levels based on the 5 randomized controlled trials.

(A) Anti-thyroid peroxidase antibody; (B) Anti-thyroglobulin antibody; (C) Thyroid-stimulating honmone; (D) 25-Hydroxyvitamin D.

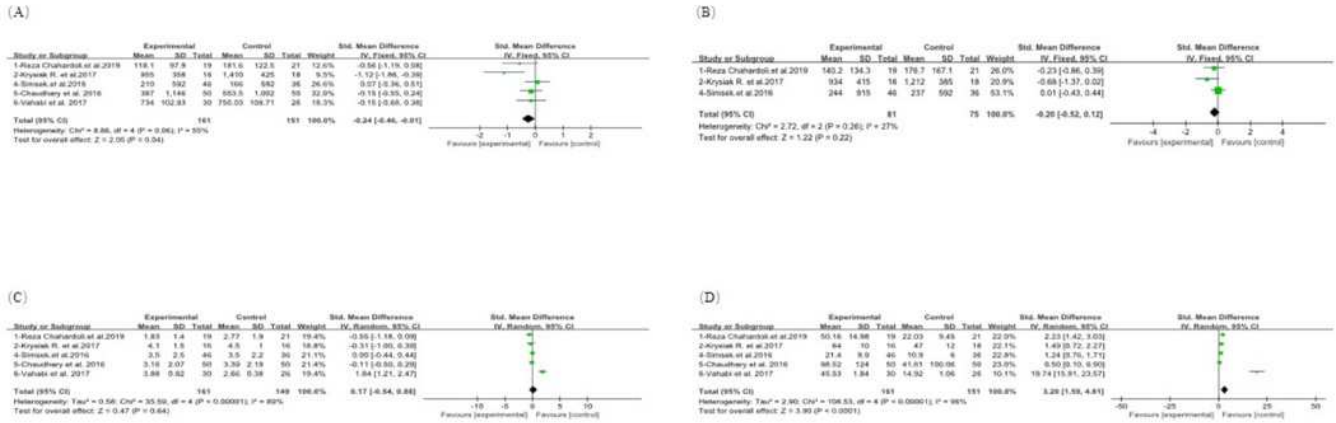

Figure 5

Forest plots of serum thyroid autoantibodies in the 10 prospective studies classified by the duration time of VitD supplementation.

(A) Anti-thyroid peroxidase antibody in AITD patients treated for at least 3 months; (B) Anti-thyroglobulin antibody in AITD patients treated for at least 3 months; (C) Anti-thyroid peroxidase antibody in AITD patients treated for less than 3months; (D) Anti-thyroglobulin antibody in AITD patients treated for less than 3 months.

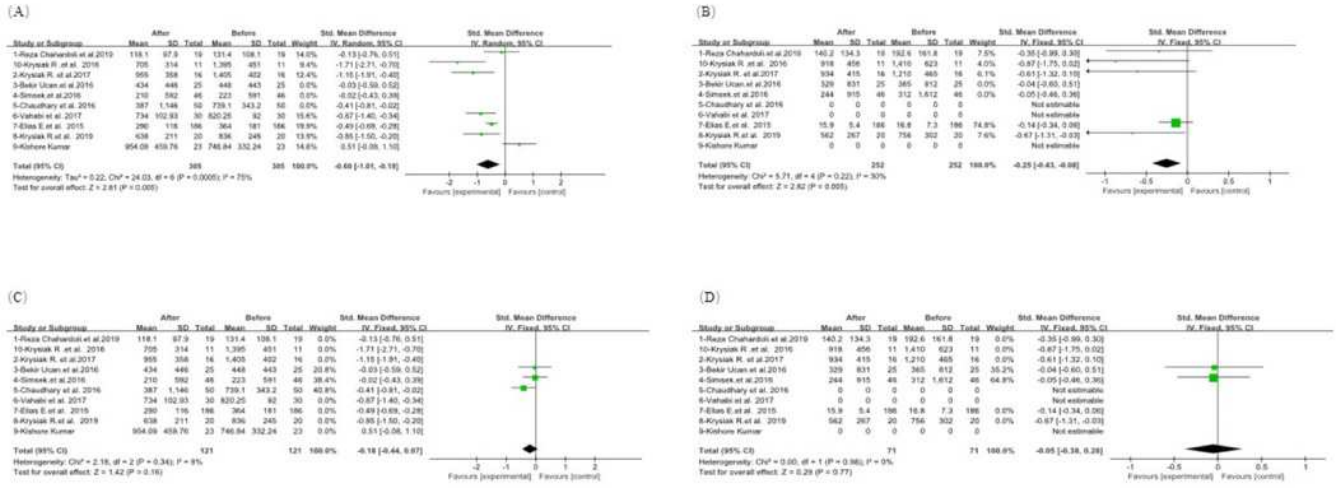

Figure 6

Forest plots of serum thyroid autoantibodies in the 10 prospective studies classified by initial VitD nutritional statuses of the AITD patients.

(A) Anti-thyroid peroxidase antibody in treated AITD patients with initial serum 25(OH)D below 30ng/ml; (B) Anti-thyroglobulin antibody in the treated patients with initial serum 25(OH)D below 30ng/ml; (C) Anti-thyroid peroxidase antibody in treated AITD patients without initial VitD status considered; (D) Anti-thyroglobulin antibody in treated AITD patients without initial VitD status considered.

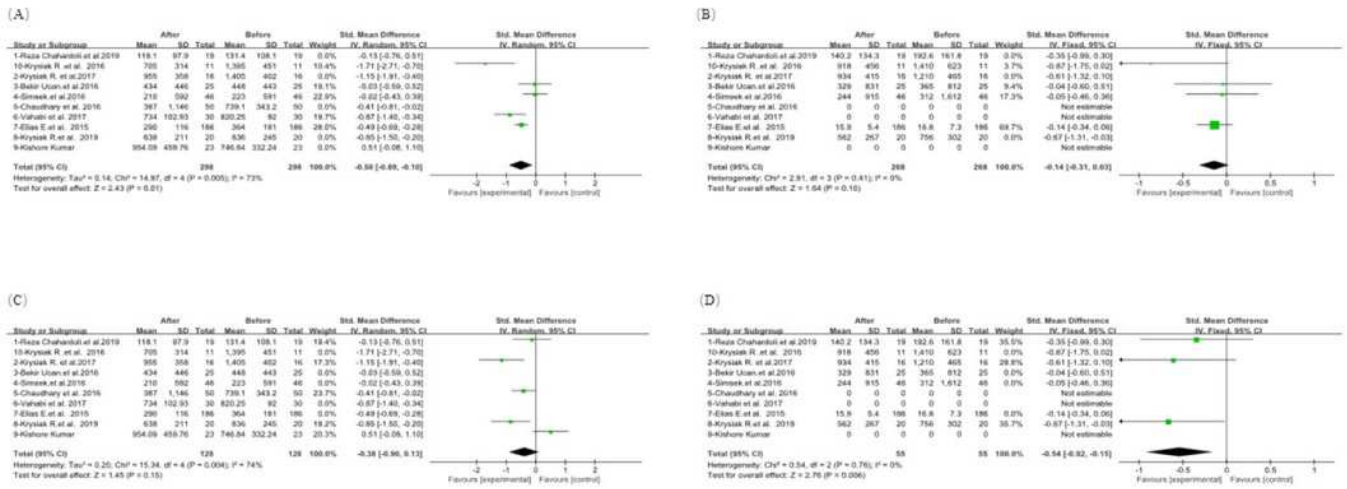

Figure 7

Forest plots of serum thyroid autoantibodies in the 10 prospective studies classified by VitD administration regimen.

(A) Anti-thyroid peroxidase antibody in AITD patients with weekly VitD supplementation; (B) Anti-thyroglobulin antibody in AITD patients with weekly VitD supplememation; (C) Anti-thyroid peroxidase antibody in AITD patients with daily VitD supplementation; (D) Anti-thyroglobulin antibody in AITD patients with daily VitD supplementation.