Vitamin D Repletion Is Associated With Favorable Changes in the Circulating Cytokine Profile in Women With Chronic Autoimmune Thyroiditis

Karel VONDRA[1], Josef VČELÁK[1], Martina SALÁTOVÁ[2], Martin HILL[1], Michala VOSÁTKOVÁ[1]

[1]Institute of Endocrinology, Prague, Czech Republic, [2]Department of Geriatrics, Second Faculty of Medicine, Charles University, Motol and Homolka University Hospital, Prague, Czech Republic

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Summary

Vitamin D is recognized as an important immunomodulatory factor influencing both innate and adaptive immune responses. Its deficiency has been associated with dysregulation of cytokine production and an increased risk of autoimmune diseases, including chronic autoimmune thyroiditis (AIT). This study aimed to evaluate the relationship between vitamin D status and selected cytokine profiles in women with AIT before and after vitamin D supplementation. We conducted a 6-month prospective single-arm study in 29 women with chronic AIT and vitamin D deficiency (serum 25-hydroxyvitamin D<75 nmol/l). Fasting blood samples were obtained at baseline and after vitamin D supplementation. Selected cytokines (IL-1β, IL-4, IL-6, IL-10, IL-17F, IL-22, IL-25, IL-31) and biochemical markers were analyzed, and associations were assessed by Spearman correlation. Mean age was 41.4±13.0 years, and mean body mass index was 25.5±4.9 kg/m^2. Patients were clinically and biochemically euthyroid on stable levothyroxine replacement. Vitamin D supplementation increased mean serum 25(OH)D levels from 30.6±10.4 to 82.7±19.2 nmol/l, while thyroid function and other biochemical markers remained stable. During vitamin D deficiency, 25(OH)D correlated negatively with IL-1β, IL-6, IL-17F, and IL-25. After repletion, these associations disappeared, and a positive correlation with IL-31 emerged. In women with chronic AIT, vitamin D deficiency is associated with a distinct proinflammatory cytokine profile. Vitamin D repletion appears to reshape the circulating cytokine profile toward a more favorable pattern, rather than uniformly suppressing cytokine activity.

Key words

Vitamin D • Autoimmune thyroiditis • Cytokines

Corresponding author

K. Vondra, Institute of Endocrinology, Národní 8, 110 00 Prague 1, Czech Republic. E-mail: kvondra@endo.cz

Introduction

The active metabolite 1,25-dihydroxyvitamin D interacts with the vitamin D receptor (VDR), which is expressed by dendritic cells, monocytes/macrophages, B cells, and T cells. Through these pathways, vitamin D influences antigen presentation, lymphocyte differentiation, cytokine production, and immune tolerance [1-4].

Chronic autoimmune thyroiditis (AIT) is an organ-specific autoimmune disorder characterized by lymphocytic infiltration of the thyroid gland, progressive follicular damage, and eventual hypothyroidism in a subset of patients. Its pathogenesis involves loss of self-tolerance, antigen-specific immune activation, and an imbalance between effector and regulatory pathways [5,6]. Recent work has emphasized the pathogenic relevance of Th17 cells and related cytokines in AIT [7-10].

Within this framework, vitamin D is of interest because it may attenuate Th1/Th17 polarization and enhance regulatory mechanisms. Experimental and clinical data suggest that vitamin D supplementation may reduce thyroid autoimmunity markers, although results are not fully uniform and the magnitude appears to depend on baseline vitamin D status, substitution dose, study duration, disease activity and other characteristics [1,2,11-20].

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However, less is known about how vitamin D status relates to the circulating cytokine profile in clinically stable women with chronic AIT. We therefore investigated the association of vitamin D deficiency and its repletion with a panel of selected cytokines comprising both well-characterized pro-inflammatory and Th17-related cytokines, and less extensively studied cytokines whose relevance to thyroid autoimmunity remains to be fully elucidated (namely IL-1β, IL-6, IL-17F, IL-25, IL-31, IL-4, IL-10, IL-22).

Methods

Study design and participants

This 6-month prospective single-arm study included 29 women. All participants had longstanding chronic autoimmune thyroiditis that had been stable for several years with characteristic ultrasound findings and positive thyroid autoantibodies. All participants had documented vitamin D deficiency, defined as serum 25(OH)D concentration below 75 nmol/l.

All patients received oral cholecalciferol (Vigantol drops, Merck Serono) for 6 months at a dose of 28 drops once weekly, corresponding to approximately 14,000 IU/week. Levothyroxine therapy was continued at a stable mean dose of approximately 105 µg/day throughout the study period, and the replacement regimen remained stable.

Laboratory measurements

Fasting morning blood samples were collected at baseline and after 6 months of supplementation. The following cytokines were analyzed: IL-1β, IL-4, IL-6, IL-10, IL-17F, IL-22, IL-25, and IL-31. Cytokines were measured by multiplex immunoassay using the Bio-Plex Pro Human Th17 Cytokine Panel on a BioPlex 200 (Bio-Rad, USA).

TSH, fT4, fT3 were measured by ECLIA (Cobas 6000, Roche, Mannheim, Germany) and 25(OH)D was measured using Elecsys Vitamin D total (Roche Diagnostics, Germany).

The study was approved by the Ethics Committee of the Institute of Endocrinology in Prague and patients signed informed consent forms in accordance with the Declaration of Helsinki (World Medical Association, 2013).

Statistical analysis

Descriptive statistics were calculated in Microsoft Excel. Associations between serum 25(OH)D and cytokine concentrations were evaluated using Spearman's rank correlation analysis. A two-sided p value <0.05 was considered statistically significant.

Results

At baseline, the cohort had a mean age of 41.4±13.0 years and a mean body mass index of 25.5±4.9 kg/m^2. Serum concentrations at study entry were 25(OH)D 30.6±10.4 nmol/l, thyroid-stimulating hormone (TSH) 2.63±1.34 mIU/l, free thyroxine (fT4) 16.2±2.25 pmol/l, free triiodothyronine (fT3) 4.56±0.51 pmol/l. These values were consistent with well-controlled thyroid function on stable replacement therapy.

After 6 months of vitamin D supplementation, mean serum 25(OH)D increased from 30.6±10.4 to 82.7±19.2 nmol/l, thus reaching the normal range. Values of TSH 2.30±1.44 mIU/l, fT4 16.74±2.61 pmol/l and fT3 4.66±0.65 pmol/l did not change significantly during the intervention (Table 1).

At baseline, during vitamin D deficiency, serum 25(OH)D showed significant negative correlations with IL-1β ($r=-0.457$; $p<0.05$), IL-6 ($r=-0.452$; $p<0.05$), IL-17F ($r=-0.496$; $p<0.01$), and IL-25 ($r=-0.370$; $p<0.05$). No significant association was observed with the remaining cytokines.

At follow-up, after repletion of vitamin D status, the baseline negative correlations between 25(OH)D and IL-1β, IL-6, IL-17F, and IL-25 were no longer present. A new significant positive correlation emerged between 25(OH)D and IL-31 ($r=0.428$; $p<0.05$) (Table 2).

Discussion

The main finding of this study is that vitamin D deficiency in women with chronic AIT was associated with a proinflammatory cytokine pattern, whereas vitamin D repletion was accompanied by disappearance of these correlations and emergence of a positive association with IL-31. Because the study design used correlation analysis, the results cannot establish causality; nevertheless, they are biologically plausible and consistent with the known immunoregulatory actions of vitamin D [1-4].

The negative associations between 25(OH)D and IL-1β and IL-6 are particularly noteworthy. Both cytokines are central amplifiers of inflammation and participate in the differentiation and maintenance of Th17 responses. IL-1β can promote inflammatory

Table 1. Thyroid hormone and vitamin D values at baseline and follow-up examination (mean ± SD).

Parameter	**Baseline**	**Follow-up**	**Significance**
TSH (mIU/l)	2.63 ± 1.34	2.30 ± 1.44	ns
fT4 (pmol/l)	16.23 ± 2.25	16.74 ± 2.61	ns
fT3 (pmol/l)	4.56 ± 0.51	4.66 ± 0.65	ns
Vitamin D (nmol/l)	30.6 ± 10.4	82.7 ± 19.2	p<0.01

ns = not significant.

Table 2. Correlation coefficients between interleukins (IL) and vitamin D at baseline and follow-up examination.

Interleukin	**Baseline**	**Follow-up**
IL-1β	-0.457*	-0.032
IL-4	-0.035	0.022
IL-6	-0.452*	-0.357
IL-10	-0.003	0.024
IL-17F	-0.496**	-0.018
IL-22	-0.005	0.054
IL-25	-0.371*	-0.039
IL-31	-0.187	0.428*

* p<0.05; ** p<0.01.

activation of antigen-presenting and stromal cells, while IL-6 supports Th17 polarization and B-cell activation [7,8,21,22]. In AIT, elevated proinflammatory cytokines and increased Th17 activity have been repeatedly documented [7-10]. Our findings therefore fit well with the concept that low vitamin D is linked to a more inflammatory immune milieu in autoimmune thyroid disease.

IL-17F deserves special emphasis because it is a well-established Th17-derived cytokine implicated in tissue damage and chronic inflammation. Experimental and translational studies suggest that Th17-mediated pathways contribute to thyroid follicular injury and sustained autoimmune activity [9,10]. Recent clinical and experimental work also indicates that vitamin D can attenuate autoimmune thyroiditis by shifting the Th17/Treg balance toward a more regulatory phenotype [16,17]. The disappearance of the IL-17F correlation after vitamin D repletion in the present cohort is therefore in line with a broader anti-Th17 effect of vitamin D.

The behavior of IL-25 is more complex. Although IL-25 is a member of the IL-17 cytokine family, it is generally associated with type 2 immune responses and suppression of Th1/Th17 pathways [23,24]. At the same time, IL-25 can function as a context-dependent epithelial or tissue-derived alarmin, and its circulating levels may reflect compensatory immune signaling rather than a purely anti-inflammatory state. In our cohort, the inverse correlation between 25(OH)D and IL-25 during deficiency may indicate a disturbed immunological equilibrium. After vitamin D repletion, the association vanished, which suggests a partial restoration of cytokine profile balance rather than a simple linear rise or fall in one pathway.

The new positive correlation between 25(OH)D and IL-31 after supplementation is intriguing. IL-31 is classically linked to type 2 immune responses and pruritic inflammatory disorders, and its circulating relationship with vitamin D has mostly been explored in allergic disease [23,25-28]. In atopic dermatitis and allergic respiratory disease, studies have reported variable associations between 25(OH)D and IL-31, suggesting that this cytokine axis is highly context-dependent [23,25-28]. In our study, the emergence of the IL-31 correlation only after vitamin D repletion may reflect a new immunological equilibrium in which type 2-associated cytokine signaling becomes more tightly coupled to vitamin D status. This interpretation remains speculative but is compatible with the broader view that vitamin D does not merely suppress inflammation; rather, it appears to remodel immune homeostasis.

Our results are also consistent with recent clinical studies in AIT showing that vitamin D repletion can reduce thyroid autoantibodies or improve some immune markers, although effects on thyroid hormones and clinical relevance are less consistent [11-19]. A meta-analysis [18] reported the benefit of vitamin D on thyroid autoimmunity markers in AIT, and more recent randomized and controlled studies have continued to support an immunological effect despite mixed endocrine outcomes [11-13,17,29]. This pattern aligns with the present study, in which correction of vitamin D status did not improve thyroid function, but was associated with favorable changes in the circulating cytokine profile. The cohort studied was clinically stable and biochemically euthyroid on unchanged levothyroxine replacement, which strengthens the interpretation that the observed changes were not driven by shifts in thyroid hormone replacement.

The study has several limitations. First, the sample size was small and restricted to women, which limits the extent to which the findings can be generalized. Second, there was no placebo control group, so the findings should be interpreted as exploratory. Third, the study evaluated circulating cytokines rather than thyroid tissue immune activity. For these reasons, our data should be considered hypothesis-generating rather than definitive. Despite these limitations, the present work adds a clinically relevant signal: in chronic AIT, vitamin D deficiency is associated with a measurable shift in circulating cytokine relationships, and repletion appears to partially normalize this pattern. Future larger randomized studies should determine whether sustained vitamin D sufficiency can modify autoantibody titers, inflammatory tissue activity, or long-term thyroid outcomes in patients with autoimmune thyroiditis.

Conclusions

In women with chronic autoimmune thyroiditis, vitamin D deficiency was associated with negative correlations between serum 25(OH)D and IL-1β, IL-6, IL-17F, and IL-25. After vitamin D repletion, these associations disappeared, whereas a positive correlation with IL-31 emerged. These findings support a role for vitamin D in remodeling the cytokine profile and maintaining immune homeostasis in autoimmune thyroid disease.

Conflict of Interest

There is no conflict of interest.

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