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Adam Maciejewski[1, A–D, F], Marlena Wójcicka[2, B], Magdalena Roszak[3, C], Jacek Losy[4, A, E], Katarzyna Łącka[5, A, F]

Assessment of Vitamin D Level in Autoimmune Thyroiditis Patients and a Control Group in the Polish Population*

[1] Student's Scientific Society, Section of Endocrinology, Poznan University of Medical Sciences, Poland
[2] Department of Clinical Neuroimmunology, Poznan University of Medical Sciences, Poland
[3] Department of Computer Science and Statistics, Poznan University of Medical Sciences, Poland
[4] Department of Clinical Neuroimmunology, Poznan University of Medical Sciences, Neuroimmunological Unit, Institute of Experimental and Clinical Medicine, Polish Academy of Sciences, Poland
[5] Department of Endocrinology, Metabolism and Internal Medicine, Poznan University of Medical Sciences, Poland

A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article

Abstract

Background. Vitamin D, known for its role in calcium-phosphorus homeostasis, is also a significant immunomodulatory factor. Vitamin D deficiency has been reported in some autoimmune disorders. Recently, vitamin D level in autoimmune thyroiditis (HT – Hashimoto's thyroiditis) has become the subject of researchers' interest.
Objectives. This study aims to assess vitamin 25-OH-D3 levels in HT patients in comparison to a control group in the Polish population. This would be the first attempt conducted in this region with such poor sunlight exposure.
Material and Methods. The group we studied consisted of 62 subjects diagnosed with HT (mean age 49.15 ± 15.51) and 32 healthy controls matched with age and sex (mean age 46.09 ± 14.32). All blood samples were collected in the first quarter of the year to minimize the impact of seasonal fluctuations of vitamin D concentrations.
Results. In the HT group the mean vitamin D level was 20.09 nmol/L (SD ± 12.66), compared to 30.31 nmol/L (SD ± 19.49) in the controls, p = 0.014. None of the patients and the controls was vitamin D sufficient (75–125 nmol/L). The deficiency (< 50 nmol/L) was significantly more common among HT patients compared to the controls (61–98.4% vs. 27– 84.4%, p = 0.029).
Conclusions. In conclusion, we found that serum vitamin D concentration is significantly lower in HT patients in comparison to the control group. This suggests vitamin D deficit as one of the risk factors for HT development. Observed vitamin D level was also low in the control group, therefore wider supplementation in general population should be recommended (**Adv Clin Exp Med 2015, 24, 5, 801–806**).

Key words: vitamin D, vitamin D deficiency, autoimmunity, Hashimoto's disease.

Autoimmune thyroiditis, also known as Hashimoto's thyroiditis, is the most prevalent type of thyroid gland inflammation, affecting about 2% of the general population, predominantly women [1]. Its etiology is autoimmune; the disease develops as a result of interactions between genetic susceptibility and further affecting environmental factors.

Among the genes predisposing to the disease, the best confirmed are Human Leukocyte Antigen region genes (*HLA*), cytotoxic T lymphocyte antigen-4 (*CTLA-4*), protein tyrosine phosphatase nonreceptor-type 22 (*PTPN22*) and thyroglobulin (*Tg*) genes. Recently, the significance of polymorphisms in cytokine gene sequences was also

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investigated [2]. According to twin studies, genetic factors are responsible for the disease's development to a degree of about 75% [3]. Environmental factors that may impact HT development are viral and bacterial infections, medications (e.g. interferon α, amiodarone), iodine excess and also an inappropriate supply of other trace elements or vitamins [4].

Lately, the relationship between vitamin D insufficiency and the diseases of autoimmune origin has become the focus of interest. Vitamin D is a secosteroid whose main source is skin synthesis in response to sunlight exposure (UVB radiation), but may also be provided by the diet or supplements. It is best known for its role in calcium-phosphorus homeostasis, but now we know that the role it plays is much bigger. Vitamin D is a significant immunomodulatory factor. Its deficiency has been reported in type 1 diabetes (TID), systemic lupus erythematosus, psoriasis, systemic scleroderma, inflammatory bowel disease or multiple sclerosis (MS) [5]. The association between inadequate vitamin D supply and autoimmunity is best confirmed in the case of TID and MS; some articles even describe the beneficial and preventive role of vitamin D supplementation in these diseases [6, 7].

The aim of this study is, therefore, to assess vitamin D (25-OH-D3) serum levels in HT patients and a control group in the Polish-Caucasian population. To the best of our knowledge (on the basis of the PubMed database), it would be the first study conducted in this region with poor sunlight exposure.

Material and Methods

Patients

The group studied consisted of 62 patients with diagnosis of HT and 32 healthy, age- and gender-matched controls. Mean age was 49.15 ± 15.51 in HT group and 46.09 ± 14.32 in controls (p = 0.33). Female to male ratio was 56/6 in HT patients, compared to 28/4 in control group (p = 0.95). HT diagnosis was made on the basis of thyroid antibody tests and thyroid ultrasound results; the duration of the disease was variable, from several months to 3 years before the study. Anti-TPO antibodies were elevated in all patients, median value was 889 IU/mL (normal range: 0–35 IU/mL) and anti-TG in 28 out of 62 patients, median value in this group was 549 IU/mL (normal range 0–115 IU/mL). In ultrasound examination, decreased echogenicity was observed, and all the patients studied have an atrophic form of autoimmune thyroiditis with decreased thyroid volume. At the time of blood collection, all patients were euthyroid, the majority due to levothyroxine (LT4) substitution therapy (4 of them stayed euthyroid without LT4) with a mean daily dose of 77 μg (ranging from 25 to 125 μg), mean TSH level was 1.2 mIU/L (ranging from 0.65 to 1.82). In the control group thyroid gland diseases were excluded by anamnesis. In both the HT group and control group, subjects with autoimmune disorders other than HT, any neoplasms, metabolic bone disorders (osteopenia, osteoporosis), renal or liver dysfunctions and with recent vitamin D supplementation were excluded. All subjects gave written informed consent. The study complied with the code of ethics of the World Medical Association (Declaration of Helsinki) and was approved by the ethics committee.

Methods

Venous blood samples were collected and 25-OH-D3 serum concentrations were measured in all subjects using a 25-OH Vitamin D EIA Kit (Immundiagnostik AG, Germany). All samples were taken in the first quarter of the year to minimize the impact of seasonal fluctuations in vitamin concentration. Inconsistency occurs between the different studies in the definition of insufficiency and deficiency states. In our publication vitamin D insufficiency or suboptimal level was defined as level ≤ 75 nmol/L and deficiency as level < 50 nmol/L (normal range – concentration higher than 75 up to 125 nmol/L), which is in accordance with the latest Polish guidelines for vitamin D supplementation [8] and most of recently published articles.

Statistical Analysis

STATISTICA v. 10 (StatSoft, Inc. (2011)) was used for statistical analysis. The normality of distribution in both groups studied was evaluated by the ShapiroWilk test. The Mann-Whitney *U* test and χ^2 test with Yates' correction were used to assess the significance of differences in vitamin D concentration and in the frequency of vitamin deficiency, respectively. A p value below 0.05 was considered statistically significant.

Results

Serum level of 25(OH)D3 was significantly lower in HT patients (mean 20.09 nmol/L, SD ± 12.66) than in the control group (mean 30.31 nmol/L, SD ± 19.49), with a p value of 0.014, concentrations ranged from 5.6 to 72.49 (Fig. 1). All patients and controls were insufficient (vitamin D

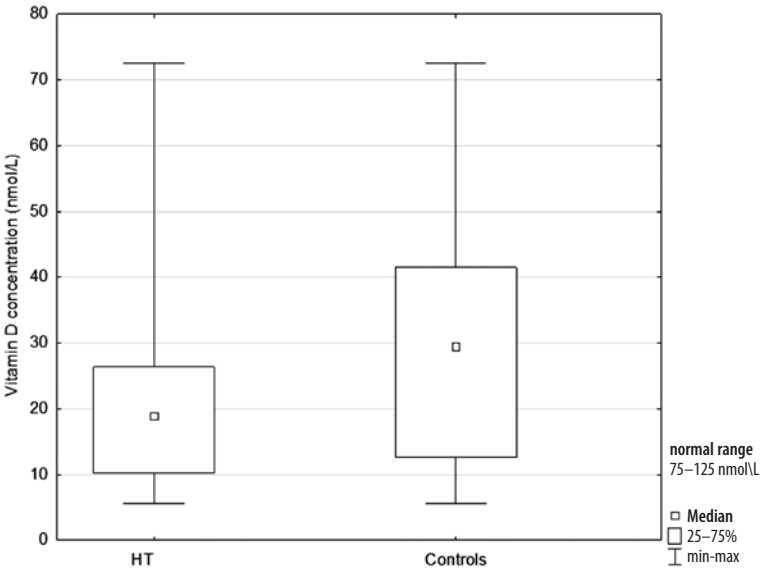

Fig. 1. Vitamin D concentration in the HT group and in the controls

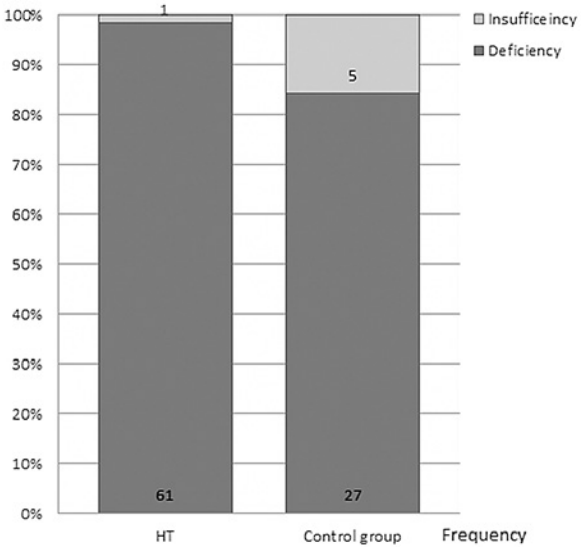

Fig. 2. Insufficiency and deficiency of vitamin D in the HT group and in the controls

serum concentration ≤ 75 nmol/L), and the prevalence of vitamin D deficiency (serum concentration < 50 nmol/L) was significantly higher in the HT group (61–98.4% vs. 27–84.4%, p = 0.029) (Fig. 2).

Discussion

While vitamin D was primarily known for its role in calcium and phosphate homeostasis, it is now clearly understood that this vitamin has many other important functions. Recent years have seen increasing evidence of the relationship between vitamin D deficiency and extraskeletal diseases, including cardiovascular and neurological diseases, neoplasms or infections [5]. Vitamin D receptors (VDRs) can be found beside the small intestine epithelium, osteoblasts and renal cells (a role in calcium and phosphorous status) also in many different tissues and organs and, what is of great importance, in immune system cells.

Vitamin D is a significant immunomodulatory factor that affects the processes of proliferation and differentiation of immune cells. VDRs are found in T lymphocytes, monocytes, dendritic cells and also in B lymphocytes [9]. One of the targets of vitamin D are dendritic cells (DCs), it affects their differentiation and maturation, decreases MHC class II and co-stimulatory molecules (CD40, CD80, CD86) expression on DCs surface and modulates cytokine production pattern [10]. Through its effect on DCs, vitamin D indirectly influences lymphocyte activation and promotes Th2 cells.

Vitamin D also inhibits T lymphocytes proliferation directly, especially Th1 cells. On the other hand, it can enhance Th2 cells development [11]. The result is a Th1–Th2 ratio shift toward anti-inflammatory Th2 lymphocytes dominance and attenuation of Th1 subpopulation (essential for inflammatory processes in the course of HT, but also T1D and MS).

Recently, another subset of proinflammatory lymphocytes – Th17 cells – has been found to be associated with HT pathogenesis, with their increased proportion in peripheral blood and thyroid gland of HT patients, when compared to the controls or Grave's disease patients [12]. Vitamin D can reduce the number of Th17 lymphocytes and the concentration of their product – Il-17A [13, 14].

It has been observed that vitamin D supplementation reduces morbidity or alleviates the manifestation of the diseases on animal models of autoimmunity, such as inflammatory bowel diseases, experimental autoimmune encephalomyelitis, rheumatoid arthritis or type I diabetes [15–18]. There are also results showing that vitamin D

supplementation may be beneficial in autoimmunity in humans – in MS, where it influenced the relapse rate [6] or TID, where researchers found that supplementation in early childhood can reduce the risk of further disease development [7].

On the basis of these *in vitro* and experimental studies, it is expected that vitamin D deficiency, accompanied by other environmental factors, can make favorable conditions for HT development in genetically predisposed people. Positive effects of 25-OH-D3 supplementation in the course of autoimmune thyroiditis, as in TID and MS, are possible and should be further studied.

These study results showed that the mean vitamin D serum concentration was significantly lower in patients with HT compared to healthy subjects and that vitamin D deficiency was more common in the HT group than in the controls. Our results stay in accordance with previously published articles (according to the PubMed database) on vitamin D concentration in HT patients [19–23] (Table 1). Effraimidis et al.'s paper contradicts these findings to a certain extent, as they found no significant difference in vitamin D status between the group at an early stage of thyroid autoimmunity (recent diagnosis of raised TPOAb concentration) and the controls [24].

This publication is the first attempt to assess the relationship between vitamin D level and autoimmune thyroiditis in a Polish-Caucasian population and, to the best of our knowledge; it is also the first study in a region with poor sunlight exposure, in contrast to previous articles from countries at lower latitudes. Suitable skin synthesis due to geographic location is possible in Poland only from April to September and at least 18% of the body surface must be exposed for about 15 min each day [8]. As skin synthesis is the main source of vitamin, the 25(OH)D3 serum level differs during the year. It is very important to compare samples collected at the same time of year. In our study, blood samples from both HT patients and healthy controls were drawn in the late winter and early spring, the time of the year when the lowest vitamin D levels are observed. A study by Danish authors showed that concentrations measured in winter can be nearly doubled during the summer in some groups of northern European populations [25].

As a consequence, low vitamin D level and frequent deficiency is observed in both groups in our study, what is worth emphasizing, none of the participants was vitamin D sufficient (vitamin D concentration > 75 nmol/L). There are very few publications concerning vitamin D levels among healthy Polish subjects or with different entities to compare our results, however, these available are comparable to concentrations in our study. Napiórkowska et al. measured vitamin D levels in the elderly Polish women from the general population, with

Table 1. Review of literature compared to own results

Authors		**Goswami [23]**	**Tamer [19]**	**Kivity [21]**	**Camurdan [22]**	**Bozkurt [20]**	**Own results**
Number of subjects	HT	642 randomly selected people	161	28	78	180 with long lasting disease, 180 recently diagnosed	62
	controls		162	98	74	180	32
Vitamin D concentration (mean ± SD ng/mL)	HT	7 ± 4.08	16.3 ± 10.4	–	12.48 ± 4.6	11.4 ± 5.2 and 13.1 ± 5.9	8.04 ± 5.06
	controls		29.6 ± 25.5	–	23.16 ± 7.88	15.4 ± 6.8	12.12 ± 7.80
Results		weak inverse correlation between TPOAb level and vitamin D level	$p < 0.0001$	deficiency significantly more common in HT group, inverse correlation between TPOAb level and vitamin D level	$p < 0.001$	$p < 0.001$	$p = 0.014$
Country		India	Turkey	Hungary	Turkey	Turkey	Poland

SD – standard deviation; HT – Hashimoto's thyroiditis patients; AITD – autoimmune thyroid diseases; TPOAb – thyroid peroxidase antibodies.

the mean vitamin D concentration of 34 nmol/L, samples were also collected in winter [26]. Mean vitamin D concentration in Polish obese adolescents was 29.75 nmol/L [27]. Recently published study of Kmieć et al. on a relatively large group of northern Poland population fits well as a comparison for our results (vitamin D level also measured in winter, similar age structure), although with one important difference that may explain slightly higher vitamin D concentration observed in their study (14.3 ± 6.6 ng/mL) – we excluded all participants on current or recent vitamin D supplementation while no exclusion criteria were used in their study [28].

Low vitamin D level seems to be a problem not only in Poland, but also in other countries in our region. Andersen et al. assessed vitamin D concentrations during winter in girls and women from 4 northern European countries (Denmark, Finland, Ireland and Poland), with the median results being 29.4 and 40.7 nmol/L, respectively [29]. Schilling et al. found mean vitamin D level of 25.5 nmol/L in German elderly patients [30]. All these results may be worrying as widespread functionalities of vitamin D and its role in many diseases are now described, there are also reports on inverse correlation between general, cardiovascular and cancer mortality and vitamin D concentration [31].

In conclusion, the vitamin D serum concentration is significantly lower in HT patients compared to the control group in our study. This may suggest vitamin D deficiency is one of the causative factors for HT development, although it cannot be ruled out that changes in vitamin D levels occur rather as a consequence of the disease, and further studies are needed to confirm its etiopathogenetic role. Another observation is that vitamin D concentration is low not only in HT group but also in the controls, which seems disturbing in the light of recent information of pleiotropic role of vitamin D, the need of wider supplementation in our latitude should be emphasized.

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Address for correspondence:

Adam Maciejewski
Department of Endocrinology, Metabolism and Internal Medicine
Poznan University of Medical Sciences
Przybyszewskiego 49
60-355 Poznań
E-mail: amaciejewski3@gmail.com

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