Original study

Vitamin D deficiency in thyroid autoimmune diseases

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Abstract

The role of vitamin D in the human body is a complex one, proven by the many studies performed related to this aspect. Data from the literature on the correlation between vitamin D deficiency and thyroid autoimmune pathology, although present and increasing in the last 10 years, have failed to establish exactly whether or not there is a link between them. The aim of the study was to assess the status of vitamin D in patients with autoimmune thyroid disease; and to determine if there is a correlation between parameters such as: thyroid stimulating hormone (TSH), free thyroxine (FT4), ATPO; and vitamin D levels. Therefore, we performed a retrospective study in which we included 60 patients, 32 with autoimmune thyroid pathology and 28 patients with negative antithyroid antibodies. The average age of those in the case group was 58 years old and 52 years old for the control group. Female sex was predominant in both groups of patients, 97% in the study group and 71% in the control group. Vitamin D values in patients with thyroid autoimmune pathology in our study were on average higher than those obtained in the control group (patients with negative thyroid antibodies), without finding a statistically significant difference between the values of the two groups of patients ($p = 0.197$). The study shows a high prevalence of vitamin D deficiency in both groups of patients (with or without autoimmune thyroid disease), the highest rate being observed among patients without autoimmune pathology, but without a statistically significant difference between values.

Keywords*: vitamin D deficiency; thyroid autoimmune diseases*

Introduction

Vitamin D is described as a steroid prohormone, being represented by two isoforms: vitamin D2 or ergocalciferol (found in plants) and vitamin D3 or cholecalciferol; the latter being synthesized in humans, on the skin, from 7-dehydrocholesterol after exposure to ultraviolet B radiation [1-3]. To become biologically active, vitamin D must go through two hydroxylation processes: the first in the liver, by activating cytochrome P450 and the enzyme 25-hydroxylase leading to the formation of 25-hydroxyvitamin D (25(OH)D), insignificant in terms of biologically aspects, but determined from serum, is the best indicator of an individual's vitamin D status; and the second renal process, by activating the enzyme 1α-hydroxylase and forming 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), its biologically active form [3, 4]. At first, the role of vitamin D in regulating phospho-calcium metabolism along with parathyroid hormone (PTH) was known, but recent research has shown the influence and importance of vitamin D in cell growth and differentiation, cell maturation and proliferation, apoptosis and angiogenesis [4].

These mechanisms occur by binding calcitriol to a specific nuclear receptor, the vitamin D receptor (VDR), forming a complex that will act primarily within the promoter of

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target genes, resulting in either activation or suppression of gene transcription [5-9].

In the immune system, the potential role of vitamin D and its active metabolite in modulating the immune response was introduced 25 years ago through three important findings: the presence of VDR in activated inflammatory cells, the ability of calcitriol to inhibit T cell proliferation, and the ability of activated macrophages to produce $1,25(OH)_2D$ [10-12].

According to the Society of Endocrinology, the classification of vitamin D status is as follows: vitamin D deficiency <20 ng/ml, vitamin D insufficiency between 20 and 29.9 ng/ml, sufficient level of vitamin D ≥ 30 ng/ml [13].

Various studies have found an association between vitamin D deficiency and certain autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, type I diabetes, multiple sclerosis, inflammatory bowel disease, Addison's disease or autoimmune thyroid disease [14]. Hashimoto's thyroiditis is the most common autoimmune thyroid pathology, found mainly in the population in areas with adequate iodine intake, being characterized by lymphocytic infiltration and progressive destruction of the thyroid gland causing primary hypothyroidism [15]. Graves' disease is another thyroid autoimmune condition, which is the main cause of hyperthyroidism, along with other specific signs and symptoms such as: diffuse goiter, exophthalmic infiltrative ophthalmopathy and infiltrative dermopathy [16].

Hashimoto's thyroiditis and Graves' disease have as main characteristic, the infiltration of the thyroid gland tissue with T lymphocytes (CD4+, CD8+) and B lymphocytes; proinflammatory cytokine secretors (T lymphocytes), but also synthesis of specific autoantibodies (B lymphocytes): antiperoxidase antibodies (ATPO), anti-thyroglobulin antibodies (ac. antiTG) and anti-TSH receptor antibodies (TRAb) [17]. The main purpose of this study is to assess the status of vitamin D in patients with autoimmune thyroid disease; and to determine if there is a correlation between parameters such as: thyroid stimulating hormone (TSH), free thyroxine (FT4), ATPO; and vitamin D levels.

Material and methods

We conducted a retrospective, observational, case-control study, which included 60 patients (51 women, 9 men), with a mean age of 55 years; patients who benefited from endocrinological consultation at the Sibiu County Emergency Clinical Hospital, between February 2019 and December 2020. Serological samples were collected and processed, by specific laboratory methods (chemiluminescence techniques) such as: 25(OH)D, TSH, FT4, ATPO, TRAb, Total Calcium and PTH. The threshold value for which the diagnosis of Hashimoto's thyroiditis was considered was over 60 IU/ml for ATPO, and over 1.67 IU/l for TRAb in the diagnosis of Graves' disease. Based on the presence or absence of positive antibodies, two groups were subsequently organized: the study group, which included patients with positive antibodies (27 patients with ATPO +, 5 patients with TRAb +); and the control group with negative antibodies (28 patients), all patients having normal thyroid function (with or without replacement therapy) at the time of examination.

The data were initially processed with the Excel 2010 program, the statistical analysis being performed using the MiniTab 2018 program.

Results

The main parameters followed in the two groups of patients are shown in Table I.

Vitamin D deficiency (values of 25(OH)D<20 ng/dl) predominates in both groups of patients, but in a higher proportion, 72% in the control group (negative antithyroid antibodies), compared to the study group with positive antithyroid antibodies, namely 67% (p=0.464). Following the application of the non-parametric Mann-Whitney statistical test, it was found that there is no statistically significant difference between the 25(OH)D

values between the two groups of patients (p=0.197>0.05).

In the group of patients diagnosed with chronic autoimmune thyroiditis, the Pearson correlation index was calculated between 25(OH)D values and ATPO values, resulting in a weakly negative correlation (r=-0.248), statistically insignificant (p=0.213), results shown in Figure 1.

Table I. The main parameters follow in the case/control group

Parameters	Study group			Control	p value
	Thyroditis	Graves	Total		
No. patient	27	5	32	28	
Age	57±13	62±13	58±13	52±14	0.111
Sex (F:M)	26:1	5:0	31:1	20:8	0.058
25(OH)D (ng/dl)	17.29±7.12	15.27±7.35	16.98±7.07	15.06±7.34	0.197
Prevalence of 25(OH)D deficiency (<20 ng/dl)	17(63%)	3(60%)	20(67%)	20(72%)	0.464
TSH (mUI/L)	2.70±1.39	0.69±0.75	2.40±1.52	1.79±0.85	0.062
FT4 (ng/dl)	1.29±0.26	1.77±0.03	1.37±0.29	1.20±0.13	**0.006**
Total Ca (mg/dl)	9.87±0.52	9.30±0.49	9.78±0.55	9.71±0.38	0.083
PTH (µg/dl)	47.64±24.72	46.23±12.83	47.72±16.93	58.3±23.42	0.451

Data are represented as mean±standard deviation.
The p values were determined by applying the Mann-Whitney test.

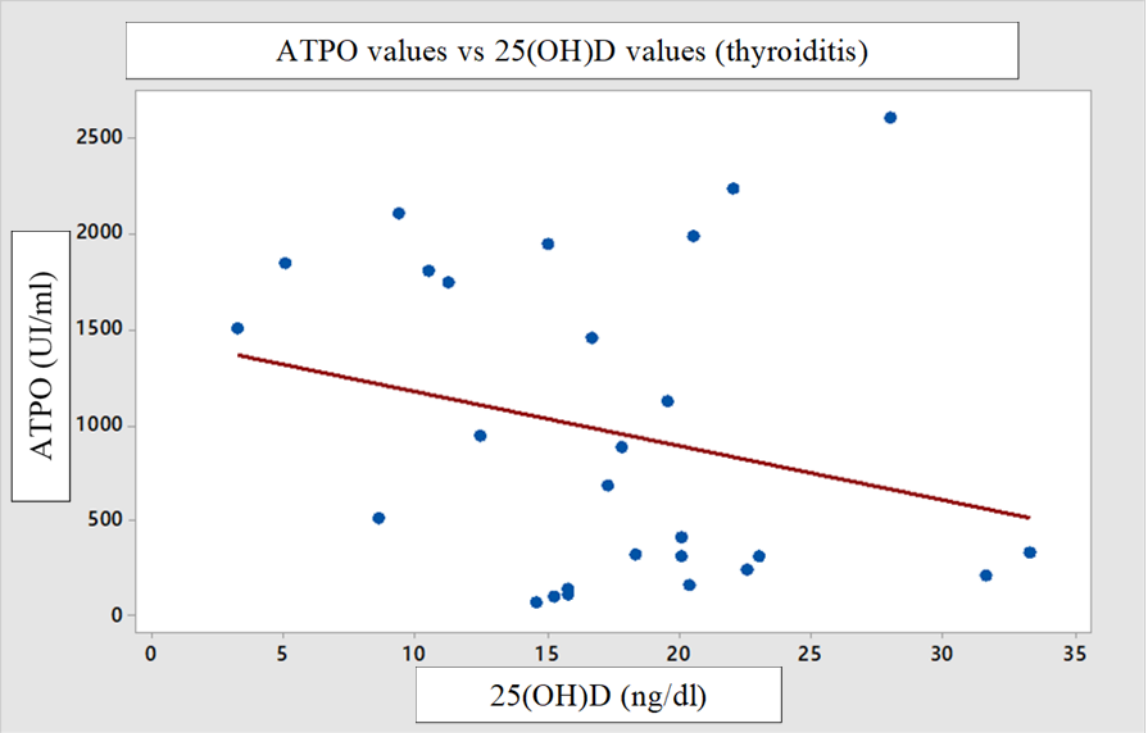

Fig. 1. Correlations between ATPO titer and 25(OH)D level in the group with Hashimoto's thyroiditis

Weakly positive correlations (r=0.221), statistically insignificant (p=0.225), were noted in the relation TSH and 25(OH)D in the case group, but also in the control group (r=0.152,

p=0.440). In patients with thyroid autoimmune pathology, a weakly negative correlation (r=-0.173), statistically insignificant (p=0.343), was found between FT4 and 25(OH)D values; the same phenomenon was observed in patients in the control group (r=-0.241, p=0.440). Next, only patients with values lower than 20 ng/dl (vitamin D deficiency) were selected from the two groups of subjects; the main values obtained being represented in the Table II. In the study group, most patients were diagnosed with chronic autoimmune thyroiditis based on increased titer of ATPO (27 patients), and the Table III shows the main results obtained in these patients according to the serum values of 25(OH)D.

Table II. Parameters of patients with vitamin D deficiency within the two groups

Parameters	Case group	Control group	p value
No. patientwith vitamin D deficiency	20	20	
Age	57±2	52±2	0.268
Sex (F:M)	20:0	14:6	
25(OH)D (ng/dl)	12.9±1.1	11.2±0.8	0.152
Measurement season (summer/autumn/winter)	1/16/3	0/14/6	
TSH (mUI/L)	2.37±1.44	1.64±0.79	0.156
FT4 (ng/dl)	1.38±0.27	1.31±0.23	0.064
Total Calcium (mg/dl)	9.83±0.46	9.68±0.42	0.126
PTH (µg/dl)	53.15±24.36	64.81±23.68	0.193

Data are represented as mean±standard deviation.
The p values were determined by applying the Mann-Whitney test.

Table III. Parameters of patients in the case group according to the values 25(OH)D

Parameters	Case group		p value
	25(OH)D<20 ng/dl	**25(OH)D>20 ng/dl**	
No. patient	20	12	
Prevalence ATPO +	17(85%)	10(83%)	0.899
ATPO (UI/ml)	934.9	350	0.884
TSH (mUI/L)	2.04	2.45	0.947
FT4 (ng/dl)	1.36	1.50	0.826

Data are represented as median.
The p values were determined by applying the Mann-Whitney test.

Discussion

Vitamin D values in patients with thyroid autoimmune pathology in our study were on average higher than those obtained in the control group (patients with negative thyroid antibodies), without finding a statistically significant difference between the values of the two groups of patients (p=0.197). Results similar to those obtained in this study were published by Effraimidis et al. [18], on two groups of 78 patients each, with and without autoimmune thyroid pathology, and who showed higher mean values of 25(OH)D in the group with autoimmune thyroiditis. Also, D'Aurizio et al. [19] obtained similar results, demonstrating that there was no significant correlation between low vitamin D levels and Hashimoto's autoimmune thyroiditis or Graves' disease, which was also highlighted in our study, where we obtained a poor negative correlation, statistically insignificant, between 25(OH)D values and ATPO values in patients in the case group.

However, there are works that contradict those found in our study, but also in other

studies that present similar conclusions; such as that published by Kivity et al. [20] demonstrating a statistically significant increased prevalence of vitamin D deficiency in a group of 50 patients with autoimmune thyroid disease, compared with 48 patients without autoimmune thyroid disease. Also, in the same study there is a statistically significant correlation between vitamin D deficiency and the presence of thyroid antibodies [20]. At the same time, a meta-analysis published by Wang et al. [21] in 2015, shows that the groups of patients with Hashimoto's thyroiditis and Graves' disease had lower levels of 25(OH)D, with patients with autoimmune thyroid disease being more likely to have vitamin D deficiency compared to those from control groups with negative antibodies.

Insignificant statistical differences between the two groups ($p>0.05$), we obtained in the present study for all the parameters followed, with one exception, namely for FT4 values, values that differ statistically significantly between the two groups ($p=0.006$).

Patients with vitamin D deficiency, with autoimmune thyroid pathology, have a similar hormonal profile compared to patients with vitamin D deficiency without positive antibodies. Regarding thyroid function, we found higher average values of TSH and FT4 in patients in the case group than in the control group, who had vitamin D deficiency, a phenomenon that was highlighted in the recent study published by Botelho et al. in 2018 [22].

Weakly positive, statistically insignificant correlations between TSH and 25(OH)D were highlighted in this study, both in patients in the case group and in those in the control group; results similar to those obtained recently (in 2020) by Chao et al. [23], but showing a statistically significant positive correlation between these parameters. Contrary to these results are those published in 2020 by Aktas [24] which showed negative correlations between these parameters, as well as statistically significant negative correlations between 25(OH)D and ATPO values. Between FT4 and 25(OH)D, the correlations in our study are weakly negative, statistically insignificant, which contradicts those demonstrated by the study of Chao et al. [23], where there is a positive, statistically significant correlation between these parameters.

If we refer to the values of the other parameters followed, we noticed that, despite the existence of vitamin D deficiency, both in the case group and in patients in the control group, the mean values of calcium and PTH fall within the range of normality.

From the point of view of the seasonal variation of vitamin D deficiency, the lowest values of 25(OH)D were recorded in winter (14.15 ng/dl), and the highest were measured in autumn (16.71 ng/dl). A study published in 2017 by Niculescu et al. [25] for the Romanian population, showed a very high prevalence of vitamin D deficiency, with an average value of 18.6 ng/dl for 25(OH)D, slightly higher than the value obtained in our study in both groups of patients; the lowest values being highlighted in March, and the highest in September, similar to those obtained in our study.

Referring only to patients in the case group with autoimmune thyroid pathology, ATPO is more common in the serum of patients with vitamin D deficiency (values 25(OH)D<20 ng/dl), compared to patients without deficiency (85% versus 83%), but this difference is not statistically significant. Similar but statistically significant results were published by Muscogiuri et al. [25], which demonstrates a highly significant link between vitamin D deficiency and autoimmune thyroid disease, with the recommendation of screening for autoimmune thyroid disease in patients with vitamin D deficiency.

ATPO values are higher in patients with vitamin D deficiency, with a median of 934.9 IU/ml, compared to the median obtained in patients without vitamin D deficiency of 350 IU ml, values that are not statistically significant ($p=0.884$). The same results, but statistically significant, were published by Mazokopakis et al. [26], where 85.3% of the Greek population studied with autoimmune thyroid disease had low levels of 25(OH)D that correlated negatively with the determined serum ATPO values. Results similar to those presented in this paper, were highlighted in the paper published in 2019 by Cinpeanu et al. [26], where it has been shown that vitamin D status correlates negatively with ATPO levels.

Conclussions

In conclusion, our study shows a high prevalence of vitamin D deficiency in both groups of patients (with or without autoimmune thyroid disease), the highest rate being observed among patients without autoimmune pathology, but without a statistically significant difference between values. There was a weak negative correlation between ATPO values and 25(OH)D, without being statistically significant, with higher values of ATPO titer in patients with vitamin D<20 ng / dl.

Although the data obtained by us were not statistically significant in favor of an association between vitamin D deficiency and autoimmune thyroid disease, this topic remains controversial, with contradictory results in various studies addressing it, and given the limitations of this study (reduced number of patients, possible data selection errors) we propose to extend it to a larger scale, with the confirmation or not of those currently established in this paper.

Competing interests
The authors declare that they have no competing interests

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References

1. Mele C, Caputo M, Bisceglia A, et al. Immunomodulatory effects of vitamin d in thyroid diseases. *Nutrients.* 2020; 12(5):1444. https://doi.org/10.3390/nu12051444
2. Prietl B, Treiber G, Pieber TR, AmreinK. Vitamin D and immune function. *Nutrients.* 2013; 5(7):2502–2521. https://doi.org/10.3390/nu5072502
3. Bikle D. Nonclassic actions of vitamin D. *J Clin Endocrinol Metab* 2009; 94:26–34. https://doi.org/10.1210/jc.2008-1454
4. Holick MF. Vitamin D deficiency. *N Engl J Med.* 2007; 357(3):266–281. doi: 10.1056/NEJMra070553.
5. Wacker M, Holiack MF. Vitamin D-effects on skeletal and extraskeletal health and the need for supplementation. *Nutrients.* 2013; 5:111–148. doi: 10.3390/nu5010111.
6. Christakos S, Dhawan P, Liu Y, Peng X, Porta A. New insights into the mechanisms of vitamin D action. *J Cell Biochem.* 2003; 88(4):695–705. doi: 10.1002/jcb.10423.
7. DeLuca HF. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr.* 2004; 80(6Suppl):1689S–96S. doi: 10.1093/ajcn/80.6.1689S.
8. Rachez C, Freedman LP. Mechanisms of gene regulation by vitamin D (3) receptor: a network of coactivator interactions. *Gene.* 2000; 246(1-2):9–21. doi: 10.1016/s0378-1119(00)00052-4.
9. Sutton AML, MacDonald PN. Vitamin D: more than a bone-a-fide hormone. *Mol Endocrinol.* 2003; 17(5):777–791. doi: 10.1210/me.2002-0363.
10. Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC. 1,25-Dihydroxyvitamin D3 receptors in human leukocytes. *Science.* 1983; 221(4616):1181–1183. doi: 10.1126/science.6310748.
11. Rigby WF, Stacy T, Fanger MW. Inhibition of T lymphocyte mitogenesis by 1,25-dihydroxyvitamin D3 (calcitriol). *J Clin Invest.* 1984; 74(4):1451–1455. doi: 10.1172/JCI111557.
12. Adams JS, Sharma OP, Gacad MA, Singer FR. Metabolism of 25-hydroxyvitamin D3 by cultured pulmonary alveolar macrophages in sarcoidosis. *J Clin Invest.* 1983; 72(5):1856–1860. doi: 10.1172/JCI111147.
13. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: An endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2011; 96(7):1911–1930. https://doi.org/10.1210/jc.2011-0385
14. Hollenberg A, Wiersinga WM. Hyperthyroid Disorders. In: Hollenberg A WW (Ed.). Williams Textbook of Endocrinology. 14th edition. Philadelphia: Elsevier; 2019:370.
15. Pearce EN, Farwell AP, Braverman LE. Thyroiditis. *N Engl J Med.* 2003; 348(26):2646–55. doi: 10.1056/NEJMra021194.
16. Beers M, Porter R. Bolile endocrine și metabolice. In: Mușat A, Nițu G, Popescu I (Ed.). Manualul Merck de diagnostic şi tratament. XVIII. București: ALL; 2009:1194–5.
17. Antonelli A, Ferrari SM, Corrado A, Domenicantonio A, Fallahi P. Autoimmune thyroid disorders. *Autoimmun Rev.* 2015; 14(2):174–80. doi: 10.1016/j.autrev.2014.10.016.
18. Effraimidis G, Badenhoop K, Tijssen JG, Wiersinga WM. Vitamin D deficiency is not associated with early stages of thyroid

autoimmunity. *Eur J Endocrinol.* 2012; 167(1):43-8. doi: 10.1530/EJE-12-0048.

19. D'Aurizio F, Villalta D, Metus P, Doretto P, Tozzoli R. Is vitamin D a player or not in the pathophysiology of autoimmune thyroid diseases? *Autoimmun Rev.* 2015; 14(5):363-9. doi: 10.1016/j.autrev.2014.10.008.

20. Kivity S, Agmon-Levin N, Zisappl M, et al. Vitamin D and autoimmune thyroid diseases. *Cell Mol Immunol*. 2011;8(3):243-7. doi: 10.1038/cmi.2010.73.

21. Wang J, Lv S, Chen G, et al. Meta-analysis of the association between vitamin D and autoimmune thyroid disease. *Nutrients.* 2015; 7(4):2485-98. doi: 10.3390/nu7042485.

22. Botelho IMB, Moura Neto A, Silva CA, Tambascia MA, Alegre SM, Zantut-Wittmann DE. Vitamin D in Hashimoto's thyroiditis and its relationship with thyroid function and inflammatory status. *Endocr J.* 2018; 65(10):1029-1037. doi: 10.1507/endocrj. EJ18-0166.

23. 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. doi:10.3389/fendo.2020.00004

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):364-370. doi: 10.1159/000505094.

25. Muscogiuri G, Mari D, Prolo S, et al. 25 Hydroxyvitamin D deficiency and its relationship to autoimmune thyroid disease in the elderly. *Int J of Environ Res Public Health.* 2016; 13(9):850. doi: 10.3390/ijerph13090850.

26. Cinpeanu OC, Tarcea M, Voidazan S, Rogozea L. Correlations between vitamin d levels and obesity in chronic autoimmune thyroiditis. *JMB.* 2019; 1:43-48. doi: https://doi.org/10.31926/jmb.2019.1.8