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"Chronic autoimmune thyroiditis and vitamin D"

Synopsis of the dissertation submitted for the academic degree of Doctor of Medicine

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Relevance of the research problem

Vitamin D was only considered as a fat-soluble vitamin, however, data from recent years have shown that it has hormone-like activity and belongs to the prohormone. Vitamin D is mainly produced in the skin under the influence of sun ultraviolet rays (UVB), although in small quantities it can be obtained from food.

Vitamin D deficiency is common worldwide problem in all age groups, even in low-latitude countries with sufficient ultraviolet radiation. Studies have shown that globally about one billion people have vitamin D deficiency.

The effects of vitamin D are mediated by its binding to the vitamin D receptor (VDR). This receptor was found not only in bone but also in kidney, intestine, immune system (T and B cells, macrophages, monocytes), muscle, endocrine system, brain, skin, reproductive organs, liver etc. This indicates that the effects of vitamin D are not limited to its influence on the musculoskeletal system and the regulation of calcium-phosphorus metabolism.

According to the data accumulated in recent years, much attention has been paid to the significant effects of vitamin D on the immune system: Vitamin D has a potential immunomodulatory effect on both the innate and adaptive immune system.

Vitamin D activation involves two enzymatic hydroxylation reaction: The first is mediated by 25-hydroxylase (CYP2R1), which converts vitamin D in the liver to 25-hydroxyvitamin D (25(OH)D). During the second reaction, 25(OH)D is converted to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) or calcitriol - active form, which is carried out in the kidneys or other organs by 1α-hydroxylase (CYP27B1). $1,25(OH)_2D3$ is an important hormone for maintaining musculoskeletal homeostasis. However, in recent years, after discovery of VDR on almost all tissues, new data have emerged providing important insights into the pleiotropic effects of vitamin D and its potential influence on immune system. Immune cells have the ability to produce $1,25(OH)_2D3$. Active vitamin D regulates T-cells: It inhibits Th1-cells, decreases production of cytokines by them and increases the level of cytokines synthesized by Th2. In particular, activated vitamin D decreases Th1 proliferation and inhibits cytokines (such as interleukin-2 (IL-2), interferon γ (IFN-γ), tumor necrosis factor (TNF)), and converts the T cells polarization to Th2, which produces cytokines: IL-4

and IL-5. In addition, Vitamin D regulates Th1 / Th2 cell ratios and inhibits tissue damage caused by the Th1 immune response. Th17 has a proinflammatory effect as it produces cytokines such as IL-17A, IL-17F, IL-21, IL-22, and IL-6. IL-17 cytokines (IL-17A - IL-17F), play an important role for the innate and adaptive immune system. IL-17A is the major cytokine produced by Th17 cells. It increases in case of various autoimmune diseases. $1,25(OH)_2D3$ inhibits the production of IL-17A in T cells. Vitamin D can inhibit the conversion of CD4 + T cells to Th17 cells. Vitamin D can also have an effect on regulatory T cells (Tregs). Vitamin D can regulate Th17 / Treg imbalance by increasing T regulatory cells and decreasing differentiation of Th17 cell.Vitamin D promotes apoptosis of dendritic cells and thereby inhibits the development of autoimmune diseases.

Chronic autoimmune thyroiditis (Hashimoto's thyroiditis) is one of the most common endocrine pathologies, characterized by increased levels of thyroid auto- antibodies in the blood (thyroperoxidase antibodies (anti-TPO) and thyroglobulin antibodies (anti-TG)), and lymphocytic infiltration of the thyroid tissue. All of the above contributes to the gradual decrease of thyroid function and its structural changes. In chronic autoimmune thyroiditis, the euthyroid condition may persist or hypothyroidism may develop, in some cases transient thyrotoxicosis may also be seen. In autoimmune thyroiditis, structural changes of the thyroid gland may present as diffuse or nodular / multinodular goiter. Progressive reduction of thyroid volume and its atrophy may also develop. The development of chronic autoimmune thyroiditis depends on environmental conditions and genetic factors. The prevalence of Hashimoto's thyroiditis depends on age (more common between the ages of 45-55), gender (women are 4-10 times more likely to get this disease than men) and race (more common in whites). Other factors such as alcohol consumption, stress, toxins, some medications may contribute to the development of chronic autoimmune thyroiditis. Autoimmune thyroid disease is caused by an imbalance between Th1 and Th2 cells. Patients with Hashimoto's thyroiditis have high levels of Th1 cells which produce cytokine IFN-y. Indeed, high levels of IFN-y and low levels of IL-4 are common in patients with chronic autoimmune thyroiditis. Excess Th1 cells in patients with Hashimoto's thyroiditis activate cytotoxic lymphocytes and macrophages, which directly affect thyroid tissue by destroying thyroid follicular cells. In addition to IL-17, Th17 cells secrete IL-22. In patients with

Hashimoto's thyroiditis, IL-22 level correlates to TPO antibody titre, that confirmes the role of this cytokine in antibody synthesis.

Some studies indicate a higher incidence of vitamin D deficiency or insufficiency in patients with Hashimoto's thyroiditis compared with a healthy control group, although the results of various studies are conflicting.

So, globally, including in Georgia, autoimmune thyroiditis and vitamin D deficiency are widespread problems. Therefore, it was advisable to conduct a study to determine the possible link between chronic autoimmune thyroiditis and vitamin D deficiency / insufficiency.

The research hypothesis was the following: Patients with chronic autoimmune thyroiditis and hypothyroidism may have lower level of vitamin D, compared to healthy subjects.

The aim of our research was to compare the level of vitamin D between the patients with chronic autoimmune thyroiditis and the healthy subjects.

The primary objective of this study was to determine the association between thyroidstimulating hormone (TSH), free thyroxine (FT4), anti-TPO, anti-TG, and vitamin D, as well as structural changes of the thyroid gland and vitamin D.

Methods

For our study data of 18-70 years old patients visited the clinics “Cortex” and “National Institute of Endocrinology” in 2018 or in 2019 from mid-spring to mid-summer was retrospectively analyzed. Data were collected retrospectively, based on patients’ medical histories.

Selection criteria: Age 18–70 years and subjects who had lived in Georgia for a year or longer before visiting the clinic.

Exclusion criteria: patients with history of primary hyperparathyroidism, hypoparathyroidism, metabolic bone diseases, central hypothyroidism, history of thyroidectomy or resection of thyroid tissue, thyrotoxicosis, renal or liver failure, malabsorption syndromes, type 1 diabetes, obesity, granuloma-forming disorders, or epilepsy treated by anticonvulsants or patients with malignancy, immunodeficiency, and those on chronic medications that could interfere with thyroid hormone or vitamin D metabolism. Pregnant or lactating women and those who had received levothyroxine, vitamin D, or calcium supplementation in the last 6 months were also excluded.

In total, data of 1295 patients were collected. All patients had TSH and vitamin D measurement. From total 1295 participants, 866 subjects had FT4 data, 1263 had anti-TPO data, 295 had anti-TG data. Ultrasonographically, 262 subjects had the information about thyroid gland volume and echogenicity. In total, 85 subjects had nodular / multinodular goiter. Thyroid and vitamin D laboratory tests and thyroid ultrasound examinations were performed within one week for each patient.

Serum 25(OH)D level of ≥30 ng/ml was considered as normal level, 20 to 29 ng/ml as vitamin D insufficiency, whereas serum 25(OH)D levels of <20 ng/ml were considered as an indicative of vitamin D deficiency. Serum anti-TPO >34 IU/mL and/or anti-TG >115 IU/mL were considered as autoantibody positivity. Normal range for TSH was 0.27–4.2 μIU/ml. Normal level of FT4 was 0.93–1.7 ng/dl (according to the local laboratory reference range). The total volume of thyroid >18 cm^3 in female and >25 cm^3 in male indicated thyroid enlargement.

Elevated TSH value was used to declare primary hypothyroidism. Elevated anti-TPO and/or anti-TG levels were used to establish the diagnosis of autoimmune thyroiditis. For statistical analysis, data of study population was analyzed in the whole group and divided according to gender and age groups for women only (≤45 years and >45 y.). The statistical processing of data was performed through the Statistical Package for the Social Sciences (SPSS) version 20 program. After the data grouping (TSH and vitamin D (1295 patients), FT4 and vitamin D (866 patients), anti-TPO and vitamin D (1263 patients), anti-TG and vitamin

D (295 patients), anti-TPO-anti-TG and vitamin D (262 patients), group divided according to gender and age groups for women only (≤45 y. and >45 y.)). Their percentages were comparable to vitamin D subgroups (up to 20- deficiency, 20–29- insufficiency, and 30 and higher- normal), whose credibility was estimated by the $\chi 2$ test (chi-square test). Descriptive analysis was conducted for the same groups of vitamin D, whose credibility was assessed by the ANOVA test. A p value of <0.05 was considered as statistically significant.

The significance and novelty of the research

The study result demonstrates a possible link between chronic autoimmune thyroiditis and vitamin D insufficiency/deficiency. Research on this issue has conducted for the first time in Georgia and data of thyroid-stimulating hormone, free thyroxine, antithyroid peroxidase antibodies, antithyroglobulin antibodies and 25(OH) vitamin D deficiency / insufficiency were studied in adults of both genders, as well as in female age groups. In this study we have also analyzed thyroid structural data (total thyroid volume, heterogeneity of parenchyma, number of nodes, the size of the largest nodule) and vitamin D level in men and women, as well as in females age groups.

Main thesis of dissertation

1) There is a negative association between TSH and vitamin D levels in the total study population, in the total women population and in those female, whose age was ≤45 years.
2) The negative association between anti-TPO and vitamin D was statistically significant in the whole study group as well as in the group of women and women ≤45 years of age.
3) The negative association between anti-TG and vitamin D was only observed in the group of women.
4) The negative association between heterogeneous parenchyma of thyroid and vitamin D was revealed in women.

The volume and structure of dissertation:

The dissertation is presented in Georgian, 94 pages. The main part consists of 5 chapters, contains conclusions and practical recommendations. 133 references are used; 21 diagrams, 13 tables and 3 pictures are presented.

Study results and discussion:

Our study demonstrated the significant negative association between TSH and vitamin D (p=0.008). According to gender analysis, reliable negative association between TSH and vitamin D was also detected in women (p = 0.01). According to our study results, a statistically significant negative association was observed between TSH and vitamin D levels in women those whose age was ≤45 years (p = 0.036). But the association between TSH level and vitamin D was not statistically significant in women >45 years of age (p =0.232). The statistically significant association between TSH and vitamin D was not found in men (p = 0.465) (Table 1).

		Number	TSH	Number of patients	Mean of vitamin D	P
Number of patients		1295	Normal TSH	1154	17.2698	P=0.008
			High TSH	141	15.4889	
Women		1097	Normal TSH	976	17.0603	P= 0.01
			High TSH	121	15.2198	
	Women <=45 y	849	Normal TSH	768	17.2679	P=0.036
			High TSH	81	15.4319	
	Women >45 y	248	Normal TSH	208	16.2936	P=0.232
			High TSH	40	14.7905	
Men		198	Normal TSH	178	18.4185	P=0.465
			High TSH	20	17.1165	

Table 1: TSH and vitamin D

When we analysed FT4 and vitamin D, a statistically significant association was not detected neither in the total study population (p=0.325) nor in groups divided according to

gender (women- (p=0.346), men- (p=0.695)). A statistically significant association between FT4 and vitamin D was not observed in the age subgroup of women (p=0.616 <=45 y; p=0.448 >45 y) (Table 2).

		Number	FT4	Number of patients	Mean of vitamin D	P
Number of patients		866	Normal FT4	822	17.1770	P=0.325
			Low FT4	44	16.0825	
Women		749	Normal FT4	712	16.9731	P= 0.346
			Low FT4	37	15.8351	
	Women <=45 y	546	Normal FT4	524	17.1505	P=0.616
			Low FT4	22	16,3577	
	Women >45 y	203	Normal FT4	188	16.4785	P=0.448
			Low FT4	15	15.0687	
Men		117	Normal FT4	110	18.4971	P=0.695
			Low FT4	7	17.39	

Table 2: FT4 and vitamin D

The statistically significant negative association between anti-TPO and vitamin D was found in the whole study group (p = 0.011), as well as in the group of women (p = 0.021) and women <45 years of age (p = 0.044). However, no association was detected

between anti-TPO and vitamin D in women over 45 (p = 0.374) and men (p = 0.882) (Table 3).

		Number	Anti-TPO	Number of patients	Mean of vitamin D	P
Number of patients		1263	Normal anti-TPO	908	17.3127	P=0.011
			High anti-TPO	355	16.1355	
Women		1073	Normal anti-TPO	743	17.1429	P= 0.021
			High anti-TPO	330	16.0063	
	Women <=45 y	831	Normal anti-TPO	592	17.3224	P=0.044
			High anti-TPO	239	16.1721	
	Women >45 y	242	Normal anti-TPO	151	16.4389	P=0.374
			High anti-TPO	91	15.5710	
Men		190	Normal anti-TPO	165	18.0773	P=0.882
			High anti-TPO	25	17.8412	

Table 3: anti-TPO and vitamin D

The statistically significant association between anti-TG and vitamin D was not found in the whole study group (p = 0.082), as well as in the age subgroup of women ≤45 years or >45y. (p = 0.086) (p = 0.220) and in men (p=0.038). However, the statistically significant negative association between anti-TG and vitamin D was observed in the whole group of women (p =0.022) (Table 4).

		Number	Anti-TG	Number of patients	Mean of vitamin D	P
Number of patients		295	Normal anti-TG	194	19.8225	P=0.082
			High anti-TG	101	18.1015	
Women		243	Normal anti-TG	152	20.1571	P= 0.022
			High anti-TG	91	17.7321	
	Women <=45 y	183	Normal anti-TG	121	20.4066	P=0.086
			High anti-TG	62	18.3065	
	Women >45 y	60	Normal anti-TG	31	19.1832	P=0.22
			High anti-TG	29	16.5041	
Men		52	Normal anti-TG	42	18.6114	P=0.338
			მაღალი anti-TG	10	21.4630	

Table 4: anti-TG and vitamin D

The statistically significant association between both antibodies (anti-TPO, anti-TG) and vitamin D was not detected in the whole study group (p = 0.16), as well as in women (p= 0.093) or men (p=0.681). No association was found in the age subgroup of women ≤45y. or >45y. (p = 0.095) (p = 0.717).

A statistically significant negative association between heterogeneous parenchyma of thyroid and vitamin D was revealed in women (p =0.048) (Table 5). There was not any association between total thyroid volume and vitamin D, as well as between the number of nodules and vitamin D and between the size of the largest nodule and vitamin D in neither group.

		Number	Thyroid parenchyma	Number of patients	Mean of vitamin D	P
Number of patients		262	Normal	53	19.1728	P=0.123
			Heterogeneous	209	17.3641	
Women		210	Normal	36	19.3253	P=0.048
			Heterogeneous	174	16.7316	
	Women <=45 y	171	Normal	33	19.5245	P=0.055
			Heterogeneous	138	16.8886	
	Women	39	Normal	3	17.1333	P=0.829

	>45 y		Heterogeneous	36	16.1294	
Men		52	Normal	17	18.8500	P=0.536
			Heterogeneous	35	20.5086	

Table 5: Heterogeneous parenchyma of thyroid gland and vitamin D

From total 1295 patients, 1097 were female (84.71%) and 198 were male (15.29%) (Diagram 1).

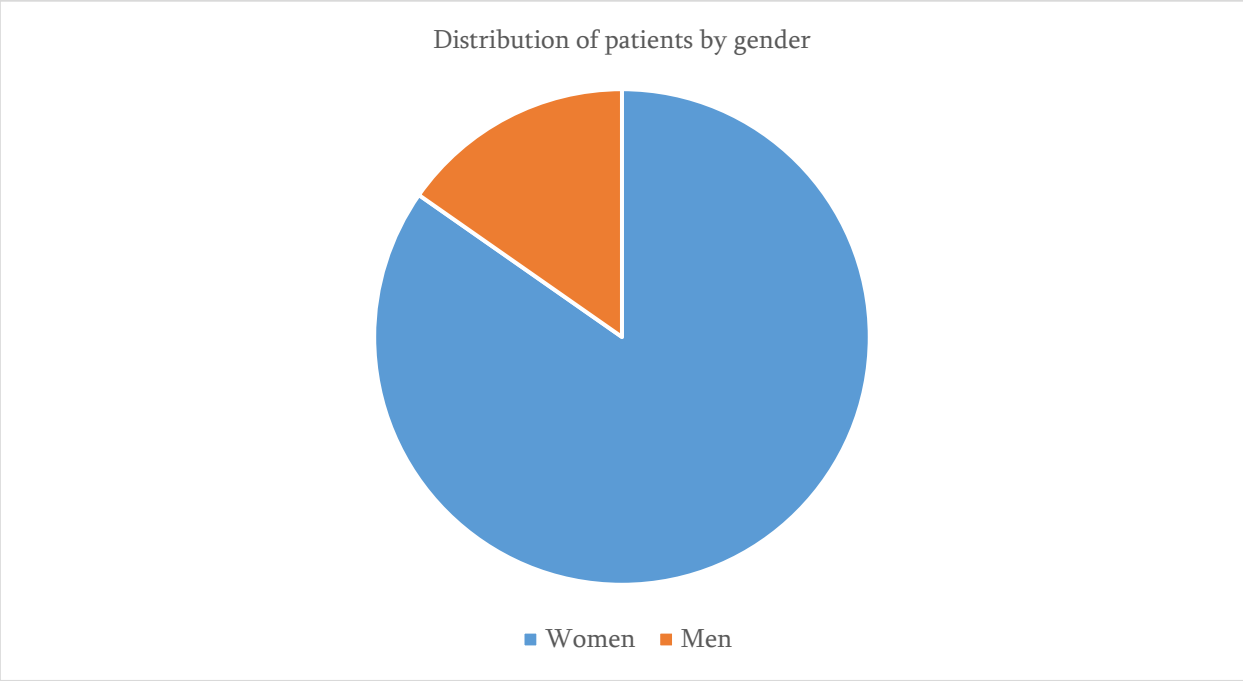

Diagram 1: Distribution of patients by gender

From female patients, 849 (77.39%) were under 45 years of age and 248 (22.61%) - > 45 y. (Diagram 2).

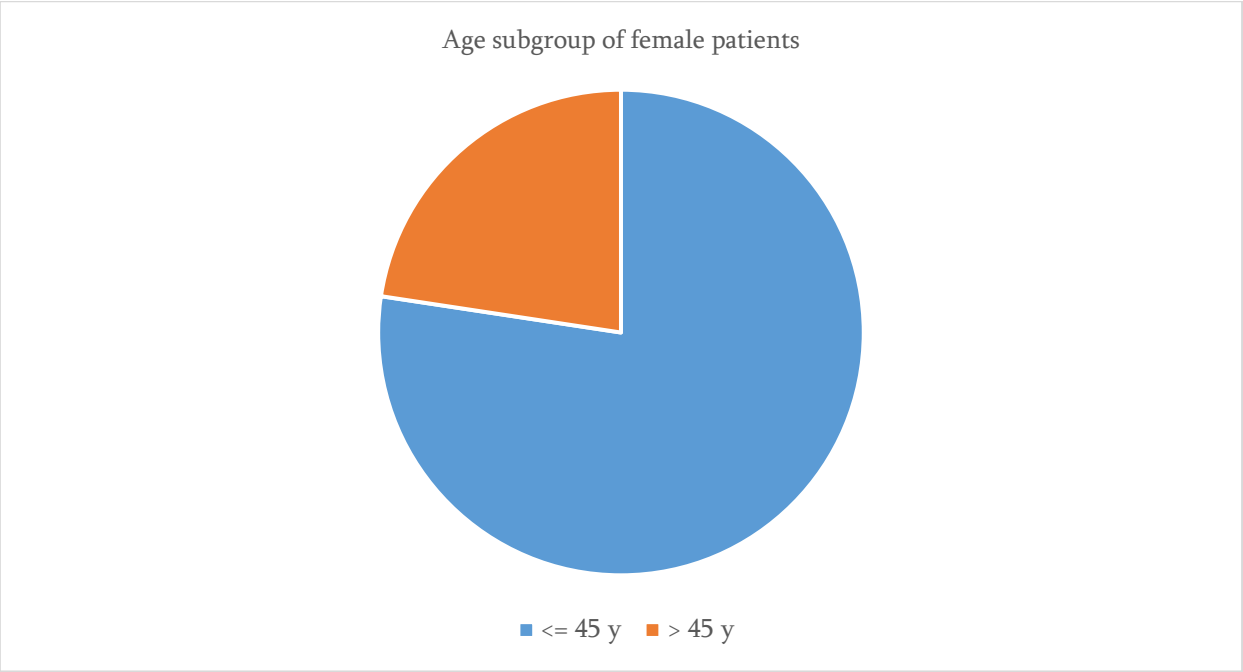

Diagram 2: Age subgroups of female patients

The study results indicate that from total 1295 patients, 70.6% had vitamin D deficiency, 24.1% had vitamin D insufficiency, and only 5.3% had normal vitamin D level. The obtained data are very remarkable and clearly indicate the widespread prevalence of vitamin D deficiency in Georgia, regardless of its geographical location (Diagram 3).

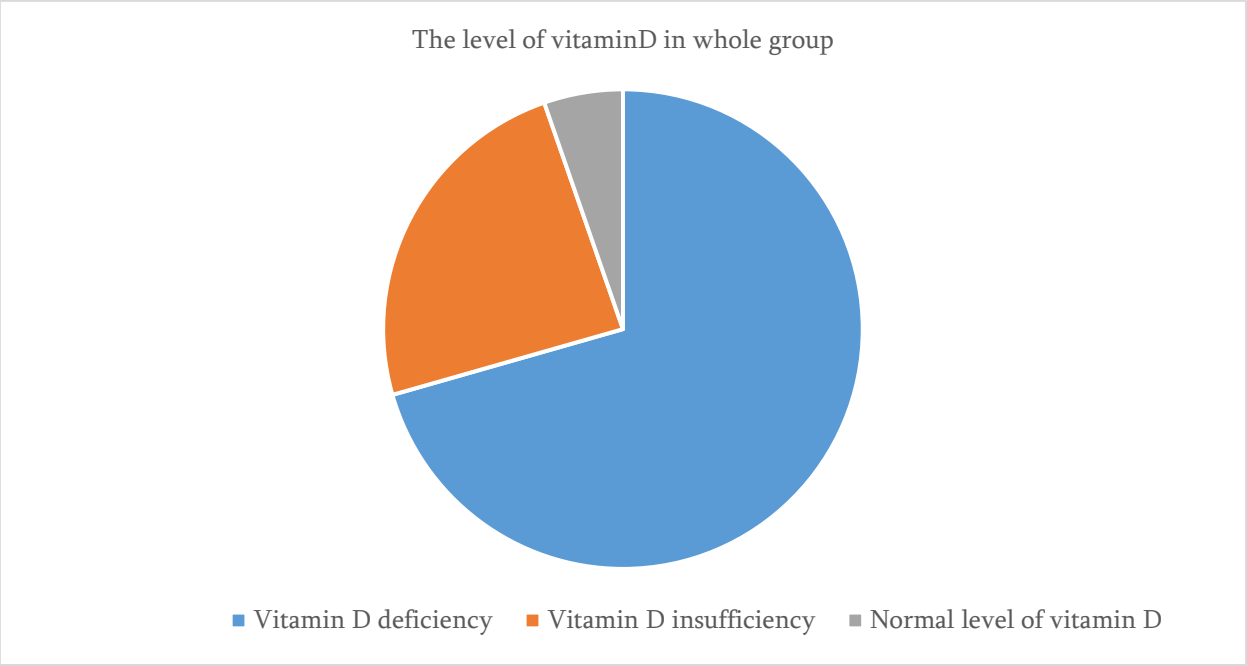

Diagram 3: The level of vitamin D in whole group.

Vitamin D deficiency was higher in hypothyroidism patients (78.7%) compared to in euthyroid people (69.6%), and normal vitamin D level also was less common in hypothyroidism patients compared to healthy subjects (2.8% vs.5.6%) (Diagram 4).

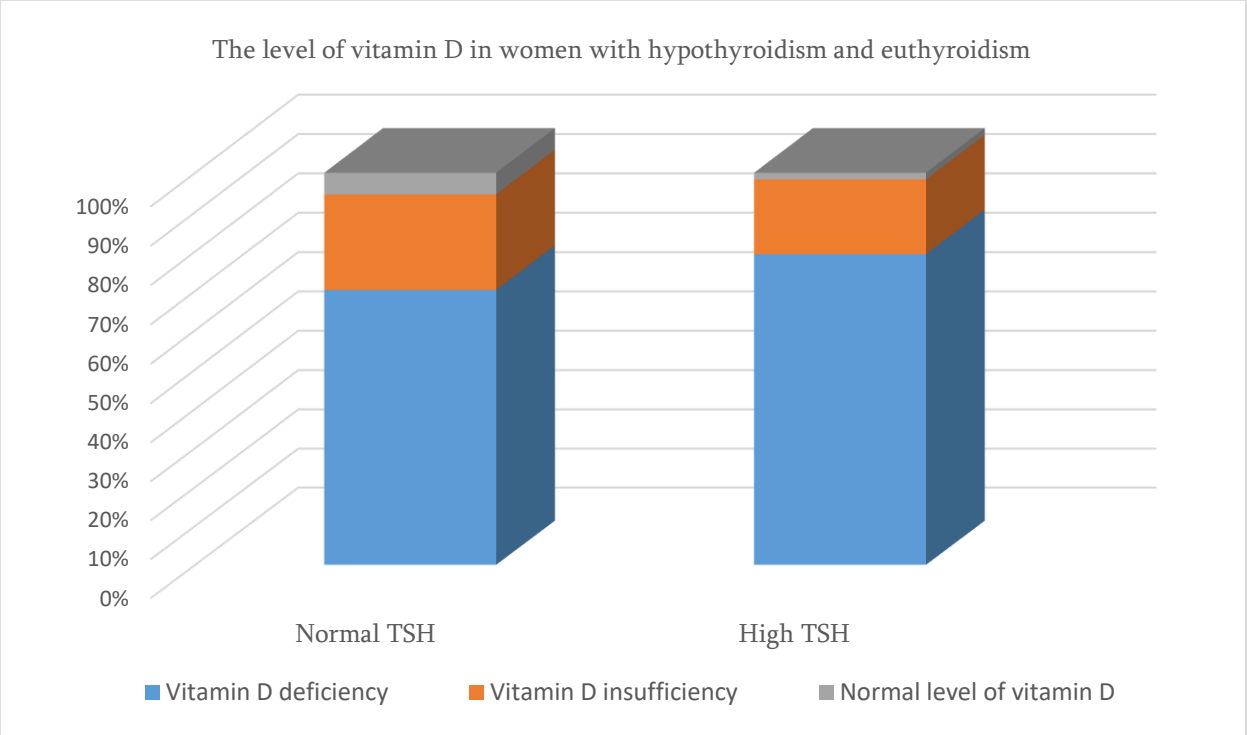

Diagram 4: The level of vitamin D in women with hypothyroidism and euthyroidism

From the collected data, 1263 patients had anti-TPO and vitamin D data. Vitamin D deficiency was more common in patients with elevated anti-TPO (75.0% and 69.7%, respectively) (Diagram 5).

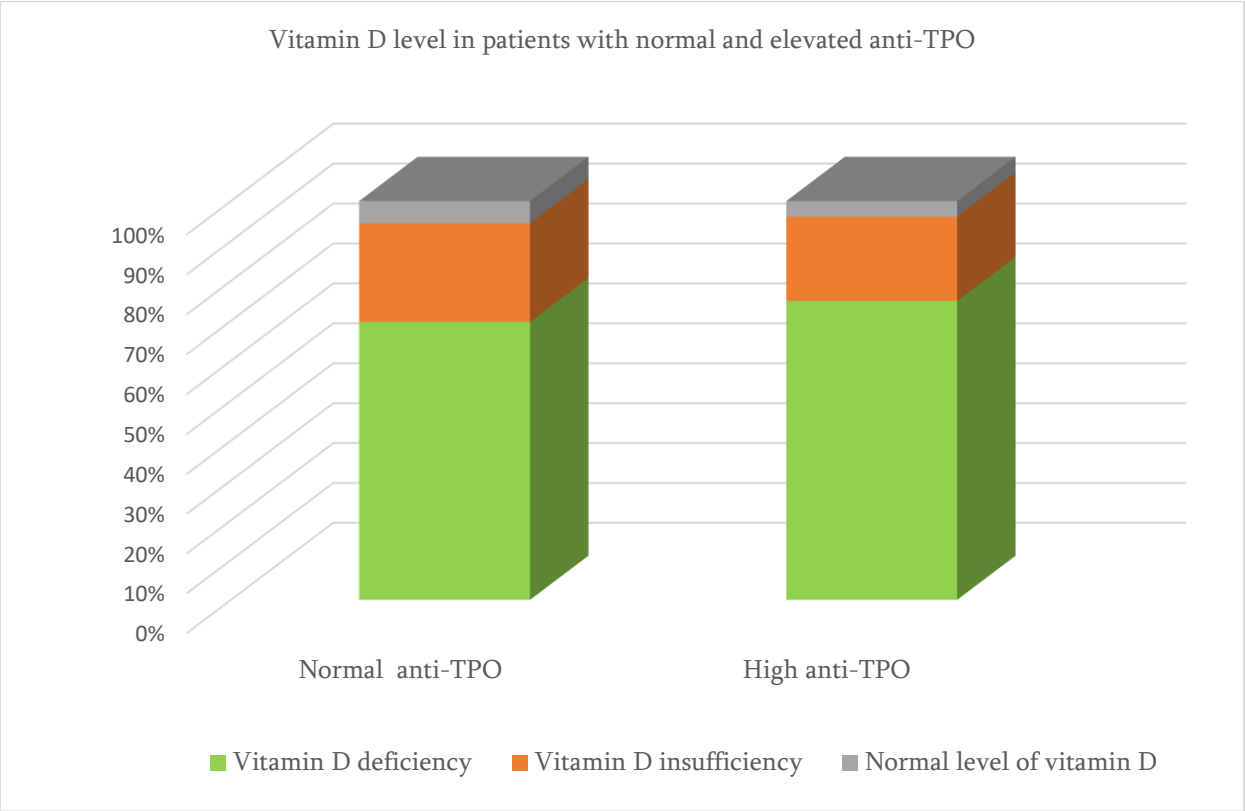

Diagram 5: Vitamin D level in patients with normal and elevated anti-TPO

The negative association between anti-TPO and vitamin D was found in the whole group of women and women ≤45 years of age.

The negative association between anti-TG and vitamin D was observed in the whole group of women (Diagram 6).

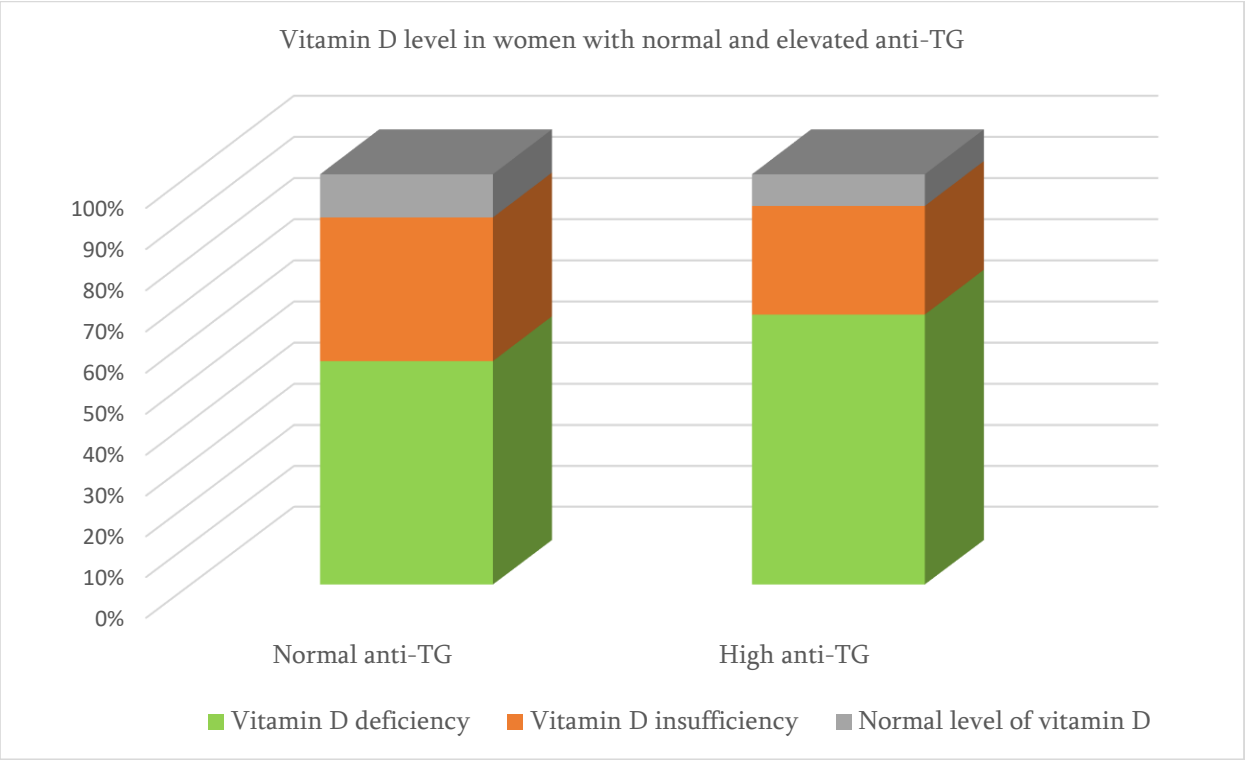

Diagram 6: Vitamin D level in women with normal and elevated anti-TG

High prevalence of vitamin D deficiency in patients with chronic autoimmune thyroiditis and primary hypothyroidism may be explained by vitamin D's immunomodulatory ability: Vitamin D deficiency promotes the production of various cytokines, which causes the development of autoimmune thyroiditis and often leads to decreased thyroid function. In autoimmune thyroiditis, an imbalance between Th1 and Th2 cells is common: There is an excess level of Th1 cells and the level of cytokine-IFN-γ produced by them also is increased. The production of cytokines from Th17 cells is also increased. Whereas normal level of vitamin D promotes to maintain balance between Th1, Th2, Th17 cells and, consequently, regulates their cytokine production. T and B lymphocytes as well as dendritic cells and macrophages have VDR. After connection with VDR, 1,25 (OH) $_2$D inhibits the maturation and differentiation of dendritic cells, regulates the production of major cytokines from Th1 (IL-12, IL-23), from Th17 (IL-17), and promotes production of IL-10. Calcitriol promotes polarization of T cells -from Th1, Th17 to Th2. It also promotes the production of Th2 anti-inflammatory cytokines (IL-3, IL-4, IL-5, and IL-10) and the development of T regulatory cells. 1,25 (OH) $_2$D inhibits proliferation of B lymphocyte and

their differentiation into plasma cells, as well as the production of IgG and IgM, and promotes B cell apoptosis. This may explain why low levels of vitamin D is a risk factor and causes the development of autoimmune thyroiditis.

According to analysis of our study results by gender, vitamin D deficiency was detected in both women and men. However a statistically significant negative association between TSH and vitamin D was detected in whole women group and in women ≤45 years. The negative association between anti-TPO and vitamin D was found in whole women group and in women ≤45 years. The negative association between anti-TG and vitamin D was observed in the whole group of women. These results mainly in premenopausal women may explain by the influence of vitamin D and estrogens in the development of autoimmune thyroiditis.

According to our study, significant negative association between heterogeneous parenchyma of thyroid and vitamin D was revealed in women. Heterogeneity of parenchyma is a one of the common characteristic sign for chronic autoimmune thyroiditis: Lymphocytic infiltration of the thyroid as a result of autoimmune process, leads to gradual destruction and fibrous replacement of the thyroid tissue.

The results of the study have great scientific and practical value: The results of the study confirm the widespread prevalence of vitamin D deficiency in the population of Georgia and its negative association with chronic autoimmune thyroiditis and primary hypothyroidism, mainly in women.

Conclusions and recommendations

TSH and vitamin D levels was observed predominantly in women ≤45 years.

2) There is a negative association between anti-TPO and vitamin D: Vitamin D is lower in patients with elevated anti-TPO. The statistically significant negative association was found mostly in women ≤45 years.

3) There is a negative association between anti-TG and vitamin D in women. Vitamin D deficiency is more common in women with elevated anti-TG.

4) A statistically significant negative association between heterogeneous parenchyma of thyroid gland and vitamin D was revealed in women.

5) There was not any association between TSH, anti-TPO, anti-TG, structural changes of thyroid and vitamin D in men.

6) The association between FT4 and vitamin D was not detected.

7) Vitamin D deficiency, chronic autoimmune thyroiditis and primary hypothyroidism is widespread disease in Georgia.

8) Low levels of vitamin D may contribute to development of chronic autoimmune thyroiditis and primary hypothyroidism, predominantly in women.

9) For patients with Hashimoto's thyroiditis and primary hypothyroidism vitamin D screening is required, and in case of insufficient levels, its normalization is advisable, especially in women.

10) Further studies are needed to evaluate the influence of vitamin D supplementation on thyroid autoantibody positivity and primary hypothyroidism.

List of published papers

1) Turashvili, N., Javashvili, L., & Giorgadze, E. (2021). Vitamin D Deficiency Is More Common in Women with Autoimmune Thyroiditis: A Retrospective Study. *International journal of endocrinology*, *2021*.

2) Turashvili, N., Javashvili, L., & Giorgadze, E. (2021). Association between vitamin D and thyroid structural abnormalities in male patients. *Translational and Clinical Medicine-Georgian Medical Journal*, *6*(2), 9-11.

3) Turashvili, N., Javashvili, L., & Giorgadze, E. (2022). The Role of Vitamin D on Thyroid Antibodies in Patients with Chronic Autoimmune Thyroiditis. *Journal of Endocrinology Research, 2022*