"OVIDIUS" UNIVERSITY OF CONSTANTA

DOCTORAL SCHOOL OF MEDICINE

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ABSTRACT OF DOCTORAL THESIS

Scientific coordinator:

University Professor Dr. Circo Eduard

PhD:

Ibadula (Musledin) Șeila

CONSTANTA 2023

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THESIS

VITAMIN D DEFICIENCY – IMPLICATIONS IN AUTOIMMUNE THYROID PATHOLOGY

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CUPRINS

The structure of the doctoral thesis:

- **General Part** - Current state of knowledge - distributed in 4 chapters
- **Special part** - Personal contribution - distributed in 4 studies
- **The thesis contains**: 154 tables and 109 figures
- **Bibliographic references**: 376 titles

Key words: thyroid, chronic autoimmune thyroiditis, Graves' disease, vitamin D, BAFF, benign breast pathology, depression, perimarine area.

Note: The content of the abstract is identical to the original content of the thesis

INTRODUCTION

Autoimmunity is incriminated in the pathogenesis of some thyroid disorders defining specific diseases: chronic autoimmune thyroiditis (Hashimoto's disease) and Graves' disease.

In the occurrence of these autoimmune thyroid diseases, a series of genetic and epidemiological risk factors are involved. Vitamin D deficiency is frequently found in patients with autoimmune thyroid pathology, being considered as one of the factors that can increase the prevalence of autoimmune diseases.

The serum level of BAFF is increased among patients with autoimmune diseases and it suspects complex autoimmunity-promoting mechanisms as well as etiopathogenic interferences. Although the literature does not provide sufficient information regarding the involvement of BAFF in the amplification of the thyroid autoimmune process, the present study emphasizes the importance of specifying much more complex immune mechanisms and their effect on clinical aspects.

The aim of this work is to verify the hypothesis of the involvement of vitamin D deficiency in the achievement of thyroid dysfunction and/or the serum level of thyroid autoantibodies, in patients with thyroid autoimmune pathology and the role of the BAFF factor in modulating this process.

Along with the analysis of autoimmunity and vitamin D, the impact of these elements on the general state of health was also monitored, a series of biological constants with a major impact on the body being determined: phospho-calcium metabolism, lipid metabolism, glycemic profile.

Benign breast lesions have the mammary epithelium or other component of the breast tissue as the lesional substrate. The coincidence of breast and thyroid lesions found in autoimmune thyroid diseases could be explained based on the existence of structures with the role of autoantigens.

Assessment of depressive syndrome among patients with autoimmune thyroid disease can be easily performed as part of the patient assessment by applying the Beck Depression Inventory.

We tried to develop a cost-effective method for determining vitamin D, through a short questionnaire to be able to anticipate the categories of patients at risk of hypovitaminosis D in order to correct the serum level.

GENERAL PART

CHAPTER 1

Vitamin D – physiology, roles and pathological implications

Vitamin D has a similar chemical structure to steroid hormones, as a result, the classification of vitamin D as a hormone is argued by the following characteristics: endogenous secretion, the presence of a specific receptor, action at the level of multiple organs and tissues, synthesis at the level of several organs and the induction of responses biological after coupling with the specific receptor. [7]

The importance of vitamin D lies mainly in the effect it exerts on phospho-calcium homeostasis and bone metabolism, but it also has extra-skeletal pleiotropic effects that are still being researched.

Vitamin D and autoimmunity

A combination of genetic and epidemiological risk factors [72,73] but also environmental factors contribute to the development of autoimmune diseases. An important factor is the availability of an adequate level of vitamin D, with epidemiological studies suggesting that vitamin D deficiency is associated with a higher incidence of autoimmune diseases [71, 74,75].

Vitamin D and implications in the functioning of the nervous system: the neuroprotective effect of vitamin D is associated with its influence on the production and release of neurotrophins, the synthesis of neurotransmitters, the regulation of intracellular calcium homeostasis and the prevention of oxidative damage to nervous tissue. [88]

Vitamin D and implications in breast pathology: the vitamin D receptor is also present in breast tissue, vitamin D having a role in the development and function of mammary glandular tissue. [90] Benign breast lesions may be associated with distinct clinical behaviors. Accurate classification of these lesions is helpful in patient management with regard to prophylaxis and surgical treatment. [91]

Implementation of a vitamin D level prediction system (questionnaire): considering the increased costs of vitamin D dosing among the population and the assumption that the majority would be vitamin D deficient led to the creation of questionnaires with the aim of identifying people with vitamin D deficiency. Studies among different population categories have concluded that these questionnaires have a beneficial, cost-effective role in predicting vitamin D deficiency. WHO encourages self-report questionnaires to assess the level of health among the population. [118,119,120]

CHAPTER 2

The thyroid and thyroid autoimmunity

The thyroid gland is the largest organ specialized in endocrine hormone production. It consists of thyroid follicles, representing the functional unit of the thyroid, having the function of secreting an adequate amount of thyroid hormones.

Autoimmune thyroid diseases

Autoimmunity is involved in the pathogenesis of many thyroid diseases, including Basedow-Graves disease, Hashimoto's thyroiditis, postpartum thyroiditis, and some forms of neonatal thyroid dysfunction. [18]

Among autoimmune diseases, autoimmune thyroid diseases have an increased prevalence, affecting between 1-5% of the population. The two most common autoimmune thyroid pathologies are Basedow-Graves disease and chronic autoimmune thyroiditis (Hashimoto's). [19]

A. Chronic autoimmune thyroiditis (Hashimoto's thyroiditis)

Autoimmune thyroiditis or Hashimoto's thyroiditis (HT), the most common autoimmune disorder of the thyroid, is an inflammatory disease of the thyroid characterized by a lymphocytic infiltration of the thyroid and production of autoantibodies against thyroid-specific antigens: thyroglobulin and thyroperoxidase, leading to a dysfunctional gland.

Hashimoto's thyroiditis is the main cause of hypothyroidism in iodine-sufficient areas of the world, as a result of fibrous transformation of the follicular cells. Approximately 20-30% of patients suffer from autoimmune thyroiditis, the cause of which is certainly multifactorial: a combination of genetic susceptibility and environmental factors that cause the loss of immunological tolerance, resulting in an autoimmune attack on the thyroid tissue and the appearance of the disease. [24,25]

B. Basedow Graves disease

Graves' disease (BG) is an autoimmune disease that primarily affects the thyroid gland. It is the most common cause of hyperthyroidism and can occur at any age with a higher incidence in women of reproductive age (20-40 years) and is responsible for 60-80% of cases of thyrotoxicosis.

Environmental factors incriminated include stress, smoking, infections and exposure to large amounts of iodine. Postpartum status, which may be associated with increased immune function, may also trigger Graves' disease in genetically susceptible women. [1]

In Graves' disease, T lymphocytes become sensitized to thyroid antigens, stimulating B cells to synthesize antibodies against these antigens. Such an antibody is directed against the TSH receptor (TRAb) in the thyrocyte membrane, stimulating thyroid growth and function. This antibody is also known as TSAb (thyroid stimulating antibody) or TSI (thyroid stimulating immunoglobulin), being produced by the B cell clones that infiltrate the thyroid.

CHAPTER 3

Vitamin D and autoimmune thyroid diseases - implications for lymphocyte-activating factor (BAFF)

Genetic, epidemiological risk factors [2,3] but also environmental factors contribute to the occurrence of autoimmune diseases. An important factor is the availability of an adequate level of vitamin D, with multiple studies suggesting that vitamin D deficiency is associated with a higher incidence of autoimmune diseases [1,4,5].

Previous research has shown that dendritic cells, macrophages, T cells, B cells, and other cells of the immune system express VDR. Thus, vitamin D has a regulatory role on the body's innate and adaptive immune mechanisms.[14]

Experimental data also suggested that these pro-inflammatory cytokines known as initiators of the autoimmune process also play a role in the prevention of autoimmune thyroiditis.[30,32]

BAFF (B lymphocyte activating factor) belonging to the tumor necrosis factor family is a vital cytokine for B cells that helps regulate both innate and adaptive immune responses. Elevated serum levels of BAFF are found in a number of different autoimmune diseases, and BAFF is found at inflammatory sites where there is lymphoid neogenesis. BAFF antagonism has been used in several autoimmune disease models, resulting in B cell depletion, decreased T cell and DC activation, and a reduction in the overall inflammatory burden. BAFF, through its interaction with its receptor (BAFF-R), is required for the survival of mature naïve B cells, all of which are depleted by blocking BAFF.[34]

BAFF is secreted both by epithelial cells and directly by B lymphocytes, which underlines the important effect of this factor in the initiation and perpetuation of lymphocyte dysfunctions [36,37].

Another study on autoimmune thyroid diseases in which patients with TH and BG were enrolled studied the effect of BAFF and 28 other circulating factors (IFN-α, IL-4, TNF-α, eotaxin) in the evolution of these pathologies. It was reported that BAFF is the best circulating indicator to identify GD and HT among all 29 chosen

biomarkers and could be used to predict disease severity in HT and active GD. [41] The association of some autoimmune diseases in certain categories of patients suspects etiopathogenic interferences.[42]

CHAPTER 4
Current guidelines regarding vitamin D supplementation

Maintaining an adequate level of vitamin D is essential for optimal health. It is generally accepted that the serum level of 25-OH-vitamin D is the best indicator of vitamin D reserves in the body, but there is some controversy regarding the limits that define normality. In 2011, the American Institute of Medicine (IOM) proposed considering 25-OH-vitamin D levels of 20 ng/mL as the threshold of normality for organic vitamin D content [2].

An expert panel of the Food and Nutrition Board (FNB) of the National Academies of Sciences, Engineering, and Medicine (NASEM) reported that individuals with serum 25-OH-vitamin D levels less than 12 ng/mL (30 nmol /L) are at risk of vitamin D deficiency. A serum level of 20 ng/mL (50 nmol/L) or more is sufficient for most people.

The Food and Nutrition Board (FNB) also pointed out that serum concentrations greater than 125 nmol/L (50 ng/mL) may be associated with adverse effects. However, current evidence suggests that a serum level between 30-50 ng/mL (75-125 nmol/L) is beneficial for most people with higher values (close to 50 ng/mL) reducing mortality. [1]

The Endocrinology Society recommends that pregnant or breastfeeding women consume at least 600 IU of vitamin D per day. [7].

The American Academy of Pediatrics recommends that all breastfed and partially breastfed infants should receive a daily supplement of 400 IU of vitamin D. It is recommended not to exceed the cumulative dose of 1000 IU/day from supplementation and diet. The recommended daily dose for children and adolescents is 600–1000 IU/day up to 2000IU/day in the case of those with obesity. [8].

For most adults, the recommended daily allowance of vitamin D is 800–2000 IU per day, but some health organizations recommend higher doses (up to 4000 IU) for certain populations, such as the elderly, obese people, and people with exposure limited to the sun or those with dark skin. [1,10]

In conclusion, vitamin D influences the innate and adaptive immune systems to fight pathogens. Clinical studies emphasize the importance of maintaining vitamin D status in the normal range, as a low serum 25(OH)D level is correlated with various disorders related to the immune system. [21]

PERSONAL CONTRIBUTION

CHAPTER I
The motivation and objectives of the research

Currently, attention is directed to the pathological aspect, vitamin D deficiency being a widely debated topic in the specialized literature. Given that multiple prospective and epidemiological studies demonstrate the connection of this deficiency with an increased risk in the development of chronic diseases such as autoimmune diseases, type II diabetes, neoplasia, cardiovascular diseases and infectious diseases, its determination and treatment are essential in medical practice. [1]

Thyroid dysfunction is a pathology most often generated by the existence of autoimmune thyroid diseases, with a prevalence of approximately 5% [2]. The autoimmune process is triggered as a result of a combination of genetic, endogenous and environmental factors. Cytokines play an important role in the pathogenesis of autoimmune thyroid diseases. Although they often present contradictory roles in the modulation of the autoimmune process, the study of cytokines requires increased attention in the context of a specific immune response, in order to develop appropriate strategies to modulate cytokine responses to maintain or restore health. In this sense, B-cell activating factor (BAFF), belonging to the tumor necrosis factor (TNF) family, contributes to the modulation of autoimmune disorders. Although there are limited and conflicting results regarding the serum concentrations of BAFF or its functional effects, it may become a therapeutic target. [3]

As a result of the aspects mentioned above, the purpose of this paper is to provide sufficient data and arguments for the in-depth investigation of the issue and the determination of the applicability of the conclusions in current medical practice.

The main objective of this study:

Determining the correlations between vitamin D deficiency and thyroid autoimmune pathology in a group of patients from Dobrogea, the non-endemic geographical area, with an increased degree of sunshine.

In order to achieve this objective, we measured the serum BAFF level, 25-OH-vitamin D, hormonal dosage, the level of antithyroid antibodies and, additionally, the ultrasound examination of the thyroid, in all patients included in the study.

Secondary objectives of the study:

1. Identification of patients with autoimmune thyroid pathology that associates vitamin D deficiency, with the determination of the degrees of vitamin D deficiency.
2. Assessment of the thyroid functional and ecostructural status among the patients included in the study.
3. Determination of the other specific biological constants in correlation with the functional status of the thyroid.
4. Psychosocial assessment of patients with thyroid autoimmune pathology by completing a questionnaire to assess the degree of depression (Beck questionnaire)
5. Assessing the serum level of vitamin D among the patients included in the study by completing a questionnaire and comparing the results with the serum levels obtained in the patients.
6. Investigating possible implications of hypovitaminosis D and autoimmune thyroid pathology in the initiation of breast pathology assessed by ultrasonographic examination.
7. Analysis of possible factors favoring hypovitaminosis D: body mass index, age, environment of origin, personal pathological antecedents.
8. A possible involvement of hypovitaminosis D in the activation of the BAFF factor in patients with autoimmune thyroid disease.

The expected final results of the research project were as follows:

- evaluation of the incidence of vitamin D deficiency among patients with autoimmune thyropathies, in a non-endemic geographical area, with a high degree of sunshine
- determining the serum level of BAFF among patients with autoimmune thyroid pathology, comparatively assessed according to the severity of vitamin D deficiency
- comparing the serum level of BAFF depending on the type and titer of antithyroid antibodies

Prospective advantages of the study:

- identifying new aspects regarding the implications of vitamin D deficiency in thyroid autoimmune pathology
- determining the lymphocyte growth factor among autoimmune diseases, respectively in autoimmune thyroid pathology as a modulating element of the process
- communication and support of the conclusions and results within the relevant scientific conferences, their publication in national and international journals indexed and rated BDI.
- Clarifications regarding the benefits and modalities of prophylaxis and treatment in vitamin D deficiency among patients with autoimmune thyroid pathology

CHAPTER 2
Materials and method

2.1. Used materials:

- The location of the specialized Outpatient Clinic within the Circo Dentistry clinic, Constanța and the facilities in the consulting room
- The information form regarding the method of conducting and the purpose of the study and the informed consent of the patient included in the study (Extension 1)
- Patient inclusion sheets in the study and questionnaires dedicated to them (Extension 2 and Extension3, 4)
- The NPA Constanța analysis laboratory equipped with equipment corresponding to hormonal determinations but also with the special kit for determining the serum level of lymphocyte activation factor (BAFF)
- Blood samples collected for hormonal, biochemical and immunological dosage by the qualified staff of the NPA laboratory, according to the specific collection recommendations, stationery products necessary for the registration of the identification data of the biological samples by the medical staff, refrigerator for storing the collected samples.
- Vinno E10 ultrasound system equipped with linear probe F4-12L (6-12 MHz) for thyroid and breast imaging Laptop and software for using programs for statistical analysis of collected data

2.2 The methodology for carrying out the research project:

The present scientific work represents a descriptive observational clinical study of a number of 80 patients with autoimmune thyroid pathology, from the area of Dobrogea. The study was carried out between 01.2021 – 01.2022. The clinical and paraclinical data were collected by the specialist endocrinologist Ibadula (Musledin)

Șeila, under the guidance of the doctoral thesis coordinator Prof. Univ.Dr. Circus Eduard. The evaluations took place in the consulting room of the Circo Dentistry SRL clinic, Constanța. For BAFF dosing, patients were investigated at the NPA Laboratory - Constanța, str. Str. I. G. Duca, no. 44, the intersection with Str Țepeș Voda approved according to the collaboration protocol.

The group of patients studied was formed as a result of meeting inclusion and exclusion criteria respectively. The research objectives and protocol were explained to the participants who signed the informed consent. The obligation of the investigator to ensure the protection of personal data and the use of the results obtained for personal therapeutic purposes but also as scientific data with the possibility of their communication during medical events was specified.

Criteria for inclusion in the study:

- patients with thyroid autoimmune pathology
- origin and residence in Constanța county

Exclusion criteria from the study:

- absence of informed consent
- patients with other associated autoimmune pathology
- patients with other pathologies known as causes of vitamin D deficiency

The number of patients included in the study was 87, of which 80 met the study inclusion and exclusion criteria. The study protocol was approved by the Bioethics Commission of "Ovidius" Constanța University. Evaluation of patients in the study followed the following stages:

- description of the study, obtaining agreement to participate in the study and signing the informed consent
- **History:** age, sex, environment of origin, origin and residence, studies, method of vitamin D intake, personal pathological history, hereditary-collateral history of thyropathies, current medication
- **Clinical examination** included general objective examination and endocrine objective examination
- **Paraclinical examination** included:
- hormonal dosage: TSH, FT4, ATPO, ATG, TRAb and determination of thyroid functional status: subclinical hypothyroidism, manifest clinical hypothyroidism and clinical hyperthyroidism included
- dosage: 25-OH-vitamin D and classification of patients according to the obtained value: severe deficiency, deficiency, insufficiency, optimal level
- dosage of the serum level of BAFF
- ultrasonographic evaluation of the thyroid and the breast with the help of the Vinno E10 multidisciplinary ultrasound machine, with a linear probe type F4-12L for soft parts

CHAPTER 3
Research results

3.1 The general characteristics of the two studied groups (demographic data, antecedents) and the description of the clinical-paraclinical parameters observed among the two study groups

The entire study group (N= 80) was divided into two study groups: Group 1 – including 62 patients (77.5%) with TCA and Group 2 – including 18 patients (22.5%) with BG

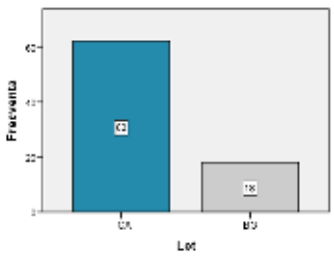

Figure no. 1- Column & pie type graphic representation for the distribution of patients by study group

In the two study groups, the mean age value for the TCA group (N = 62) is 46.47 years ± 13.37SD and for the BG group (N = 18) it is 52.33 years ± 13.81SD (Table 4).

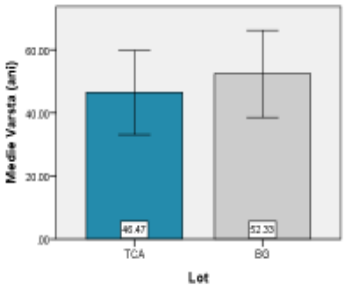

Figure no. 5 - Bar graph ± error bar of average age values for the lots analyzed

50 women (62.5%) and 12 male patients (15%) were included in the TCA study group (Table 7). For the BG study group – 17 of the patients (21.3%) were female and 1 patient (1.3%) was male.

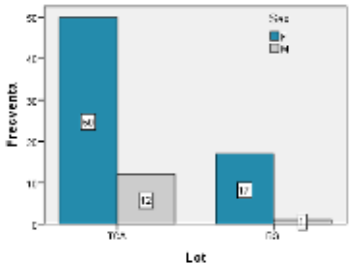

Figure no. 9 - Columnar graphic representation of the gender distribution in the two groups

The personal pathological history of the included patients were recorded and grouped as cardiovascular antecedents, metabolic disorders and oncological pathology respectively (Table 10).

Table no. 10 - Distribution of patients according to personal pathological antecedents

	Study group (n)	**Percentage (%)**
	Cardiovascular history	
Absent	51	63.75
Hypertension	29	36.25
Arrhythmia	10	12.5
Ischemic heart disease	10	12.5
	Metabolic disorders	
Absent	21	26.25
Dyslipidemia	58	72.5
Type 2 diabetes	6	7.5
Altered basal glucose	21	26.25
Obesity (IMC)	19	23.8
	Oncological pathology	
Present	9	11.25

3.2. Description of thyroid functional and ecostructural status and synological evaluation among patients in the two study groups

3.2.1. Determination of mean ATPO values in the two study groups TCA and BG

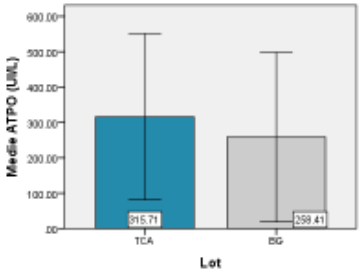

Figure no. 44 - Bar+Error bar graphical representation of the average ATPO values for the lots analyzed

3.2.2. Determination of mean values of ATG in the two study groups TCA and BG

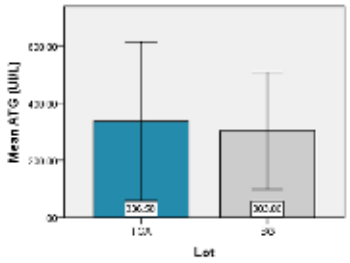

Figure no. 45 - Bar + Error bar graphical representation of the average ATG values for the lots analyzed

3.2.3. Analysis of TRAb values among the BG study group

TRAb antibodies are the marker of Graves' disease and are correlated with the severity and degree of disease activity.

Table no. 34 - Determination of mean values of TRAb among the BG study group.

TRAb value

	N	Minimum	Maximum	Average	Standard Deviation
TRAb (UI/L)	18	3.53	26.18	13.793	6.968

Table no. 36 - Distribution of patients in the two study groups according to thyroid functional status

Thyroid function in study groups

			Thyroid function				
			Euthyroidism	Subclinical hypothyroidism	Clinical hypothyroidism	Hyperthyroidism	Total
Lot	TCA	Count	30	18	14	0	62
		% of Total	37.5%	22.5%	17.5%	0.0%	77.5%
	BG	Count	0	0	0	18	18
		% of Total	0.0%	0.0%	0.0%	22.5%	22.5%
Total		Count	30	18	14	18	80
		% of Total	37.5%	22.5%	17.5%	22.5%	100.0%

3.2.4 - Determination of the ecostructural characteristics of the thyroid in the two groups

Table no. 38 - Ultrasonographic analysis of thyroid homogeneity in the two study groups

			Ultrasound thyroid echogenicity			
			Isoechogenic	Hypoechoic	Hyperechoic	Total
Lot	TCA	Count	9	52	1	62
		% of Total	11.3%	65.0%	1.3%	77.5%
	BG	Count	0	7	11	18
		% of Total	0.0%	8.8%	13.8%	22.5%
Total		Count	9	59	12	80
		% of Total	11.3%	73.8%	15.0%	100.0%

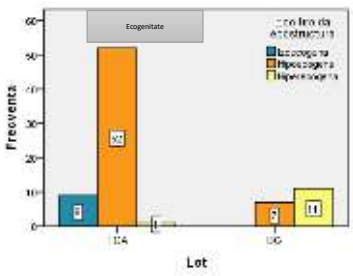

Figure no. 50 - Column-type graphic representation for the variables for the thyroid echogenicity variable in each studied batch.

In the studied groups, the changes in homogeneity show a dependency relationship (an association, a link): $\chi 2calc = 39.363$, $df = 2$, $p < 0.001 < \alpha = 0.05$ (Chi-Square Test) so that for patients with TCA, hypoechogenicity through compared with BG where hyperechogenicity is predominant.

3.2.5. Ultrasonographic analysis of the thyroid echostructure in the two study groups

Table no. 39 - Distribution of study patients according to thyroid ecostructure

			Thyroid ultrasound - echostructure		
			Inhomogeneous	Homogeneous	Total
Lot	TCA	Count	53	9	62
		% of Total	66.3%	11.3%	77.5%
	BG	Count	10	8	18
		% of Total	12.5%	10.0%	22.5%
Total		Count	63	17	80
		% of Total	78.8%	21.3%	100.0%

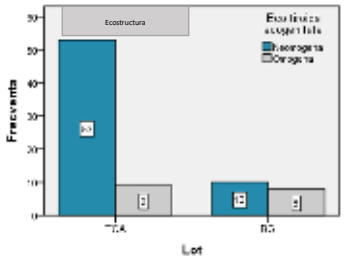

Figure no. 51 - Columnar graph for batch and thyroid ecostructure variables

Between the two categorical variables thyroid ecostructure/batch there is a dependency relationship (an association, a link): $\chi 2calc = 7.467$, $df = 1$, $p = 0.006 < \alpha = 0.05$ (Chi-Square Test). The risk of identifying an inhomogeneous thyroid in patients in the TCA group was 4.711 times higher than in patients in the BG group: OR = 4.711, 95% CI = (1.465, 15.145).

3.2.6. Analysis of the frequency of thyroid macronodules among patients

Macronodules, defined as solid entities, with dimensions > 1cm, having variable characteristics, important in the TIRADS classification, were present in 11.3% of patients with TCA and in 8.8% of patients with BG. (Table no. 41)

Table no. 41 - Distribution of patients in the two subgroups according to the presence of thyroid macronodules

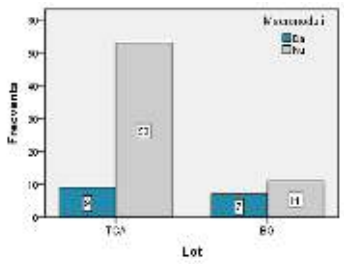

Figure no. 52 - Column type graphic representation for macronodules and lot variable

For this type of ecostructural change, between the two variables lot and macronodules there is a dependency relationship (an association, a link): $\chi 2calc = 5.179$, $df = 1$, $p = 0.023 < \alpha = 0.05$ (Chi-Square Test). The risk of identifying the presence of macronodules in patients in the TCA group was 3.745 (1/0.267) times lower than in patients in the BG group: OR = 0.267, 95% CI = (0.082, 0.870).

3.2.7. Analysis of thyroid volume in the two study groups

Table no. 47 - Distribution of study patients according to thyroid volume

	Lot	N	Average	Standard Deviation
Thyroid volume (ml)	TCA	62	7.235	3.177

	BG	18	11.714	2.980

Following this analysis, it is found that there are significant differences between the average thyroid volume values of the TCA and BG groups: t = -5.336, df = 78, p < 0.001 < α = 0.05 (Independent Samples Test)

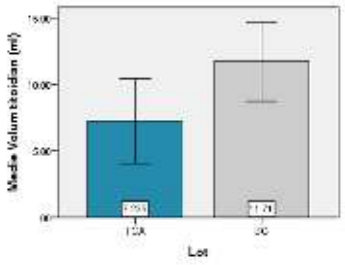

Figure no. 56 - Graphical representation type bar + error bar of the average values of the thyroid volume for the analyzed batches

Table no. 60 - TIRADS classification of patients with nodular goiter among the two study groups

			TIRADS Score				
			TIRADS 1	TIRADS 2	TIRADS 3	TIRADS 4	Total
Lot	TCA	Count	39	12	8	3	62
		% of Total	48.8%	15.0%	10.0%	3.8%	77.5%
	BG	Count	8	3	5	2	18
		% of Total	10.0%	3.8%	6.3%	2.5%	22.5%
Total		Count	47	15	13	5	80
		% of Total	58.8%	18.8%	16.3%	6.3%	100.0%

Description of breast ecostructural changes among patients with autoimmune pathology according to vitamin D level

Table no. 61 - Distribution of patients in the two study groups according to the BIRADS score and the type of antibodies present

				ATPO/ATG/ATPO+ATG			
Lot				ATPO+	ATG+	ATPO/ATG+	Total
TCA	Breast ultrasound	BIRADS 1	Count	2	1	11	14
			% of Total	4.0%	2.0%	22.0%	28.0%
		BIRADS 2	Count	5	2	15	22
			% of Total	10.0%	4.0%	30.0%	44.0%
		BIRADS 3	Count	5	1	6	12
			% of Total	10.0%	2.0%	12.0%	24.0%
		BIRADS 4	Count	1	0	1	2
			% of Total	2.0%	0.0%	2.0%	4.0%
	Total		Count	13	4	33	50
			% of Total	26.0%	8.0%	66.0%	100.0%
BG	Breast ultrasound	BIRADS 1	Count	1	0	1	2
			% of Total	5.9%	0.0%	5.9%	11.8%
		BIRADS 2	Count	2	1	4	7
			% of Total	11.8%	5.9%	23.5%	41.2%
		BIRADS 3	Count	1	0	5	6
			% of Total	5.9%	0.0%	29.4%	35.3%
		BIRADS 4	Count	0	1	1	2
			% of Total	0.0%	5.9%	5.9%	11.8%
	Total		Count	4	2	11	17
			% of Total	23.5%	11.8%	64.7%	100.0%

Determination of breast echostructural changes (BIRADS score) in the two study groups according to 25-OH-vitamin D status

Table no. 67 - Distribution of patients in the two study groups according to BIRADS score and type of 25-OH-vitamin D status

Lot				Vitamin D status				Total
				Severe deficiency	Deficiency	Insufficiency	Optimal level	
TCA	Breast ultrasound	BIRADS 1	Count	1	5	6	2	14
			% of Total	2.0%	10.0%	12.0%	4.0%	28.0%
		BIRADS 2	Count	2	10	7	3	22
			% of Total	4.0%	20.0%	14.0%	6.0%	44.0%
		BIRADS 3	Count	1	8	2	1	12
			% of Total	2.0%	16.0%	4.0%	2.0%	24.0%
		BIRADS 4	Count	1	0	1	0	2
			% of Total	2.0%	0.0%	2.0%	0.0%	4.0%
	Total		Count	5	23	16	6	50
			% of Total	10.0%	46.0%	32.0%	12.0%	100.0%
BG	Breast ultrasound	BIRADS 1	Count	0	2	0		2
			% of Total	0.0%	11.8%	0.0%		11.8%
		BIRADS 2	Count	4	3	0		7
			% of Total	23.5%	17.6%	0.0%		41.2%
		BIRADS 3	Count	1	3	2		6
			% of Total	5.9%	17.6%	11.8%		35.3%
		BIRADS 4	Count	0	2	0		2
			% of Total	0.0%	11.8%	0.0%		11.8%
	Total		Count	5	10	2		17
			% of Total	29.4%	58.8%	11.8%		100.0%

3.3. Analysis of the coexistence of depressive syndrome among patients of the two study groups

Table no. 74 - Distribution of patients in study groups according to the degree of depression and the type of antibodies present

Lot				ATPO/ATG/ATPO+ATG			Total
				ATPO+	ATG+	ATPO/ATG+	
TCA	Depression score	Normal	Count	5	5	19	29
			% of Total	8.1%	8.1%	30.6%	46.8%
		Mild depression	Count	7	1	18	26
			% of Total	11.3%	1.6%	29.0%	41.9%
		Moderate depression	Count	3	0	1	4
			% of Total	4.8%	0.0%	1.6%	6.5%
		Severe depression	Count	1	0	2	3
			% of Total	1.6%	0.0%	3.2%	4.8%
	Total		Count	16	6	40	62
			% of Total	25.8%	9.7%	64.5%	100.0%
BG	Depression score	Normal	Count	1	0	5	6
			% of Total	5.6%	0.0%	27.8%	33.3%
		Mild depression	Count	3	3	5	11
			% of Total	16.7%	16.7%	27.8%	61.1%
		Moderate depression	Count	0	0	1	1
			% of Total	0.0%	0.0%	5.6%	5.6%
	Total		Count	4	3	11	18
			% of Total	22.2%	16.7%	61.1%	100.0%

3.4. Analysis of study groups according to degree of depression and thyroid functional status

Table no. 77 - Distribution of patients in study groups according to the degree of depression and thyroid functional status

Lot				Thyroid function				Total
				Euthyroidism	Subclinical hypothyroidism	Clinical hypothyroidism	Hyperthyroidism	
TCA	Depression score	Normal	Count	13	10	6		29
			% of Total	21.0%	16.1%	9.7%		46.8%
		Mild depression	Count	12	7	7		26
			% of Total	19.4%	11.3%	11.3%		41.9%
		Moderate depression	Count	3	0	1		4
			% of Total	4.8%	0.0%	1.6%		6.5%
		Severe depression	Count	2	1	0		3
			% of Total	3.2%	1.6%	0.0%		4.8%
	Total		Count	30	18	14		62
			% of Total	48.4%	29.0%	22.6%		100.0 %
BG	Depression score	Normal	Count				6	6
			% of Total				33.3%	33.3%
		Mild depression	Count				11	11
			% of Total				61.1%	61.1%
		Moderate depression	Count				1	1
			% of Total				5.6%	5.6%
	Total		Count				18	18
			% of Total				100.0%	100.0 %

Table no. 79 - Distribution of patients by study groups according to the degree of depression and the serum level of 25-OH-vitamin D

Lot	Depression score		N	Minimum	Maximum	Average	Standard Deviation
TCA	Normal	25-(OH)-vitamina D (ng/mL)	29	9.20	37.70	20.73	7.55
	Mild depression	25-(OH)-vitamina D (ng/mL)	26	7.00	39.00	19.53	7.65
	Moderate depression	25-(OH)-vitamina D (ng/mL)	4	14.00	38.00	23.00	11.17
	Severe depression	25-(OH)-vitamina D (ng/mL)	3	7.00	26.40	14.80	10.24
BG	Normal	25-(OH)-vitamina D (ng/mL)	6	9.00	21.00	13.58	4.63
	Mild depression	25-(OH)-vitamina D (ng/mL)	11	7.00	18.00	11.76	3.21

Moderate depression	25-(OH)-vitamina D (ng/mL)	1	28.00	28.00	28.00	.

3.4. Study of vitamin D status and BAFF among patients with autoimmune thyroid disease

Regarding the serum level of vitamin D, the values obtained were grouped into 4 categories: severe deficiency < 10 ng/ml; deficiency: 10-20 ng/dl; deficiency 21-29 ng/ml; optimal level > 30 ng/ml.

Lymphocyte-activating factor (BAFF) was analyzed for both study groups, relative to demographic factors, serum vitamin D level and factors specific to thyroid dysfunction.

3.4.1. The main statistical indicators calculated for the vitamin D variable among the two groups according to age

Table no. 81 - The main statistical indicators of the studied age variable by category of vitamin D in the TCA group

	N	Average	Standard Deviation	Minimum	Maximum
Severe deficiency	5	56.20	10.06	46.00	72.00
Deficiency	27	45.56	12.17	27.00	72.00
Insufficiency	22	47.55	14.38	22.00	75.00
Optimal level	8	40.50	14.63	22.00	61.00

Table no. 82 - The main statistical indicators of the studied age variable by vitamin D category in the BG group

	N	Average	Standard Deviation	Minimum	Maximum
Severe deficiency	5	58.80	15.83	40.00	75.00
Deficiency	11	50.09	12.56	31.00	67.00
Insufficiency	2	48.50	19.09	35.00	62.00

For the given situation, it is found that there are significant differences between the average age values (years) for at least two of the analyzed groups: $F = 9.242$, $p = 0.001 < \alpha = 0.05$ (OneWay ANOVA Test). Statistical analysis shows that there are significant differences only between the mean values corresponding to the groups Severe Deficiency and Deficiency, Severe Deficiency and Insufficiency, Severe Deficiency and Optimal Level ($p < \alpha = 0.05$).

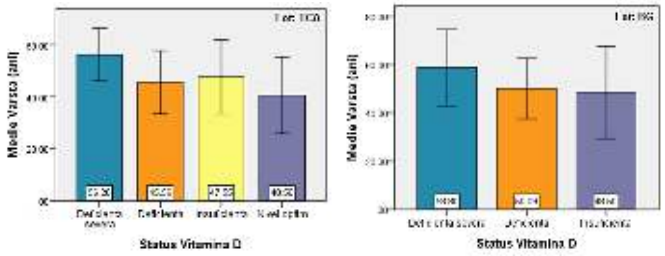

Figure no. 65 and **Figure no. 66 -** Bar + Error bar graphical representation of average age values for the analyzed groups

Table no. 83 - The age variable studied by category among TCA group patients related to vitamin D status

			Age categories (years)			
			(22-40]	(40-60]	(60-75]	Total
Vitamin D status	Severe deficiency	Count	0	4	1	5
		% of Total	0.0%	6.5%	1.6%	8.1%
	Deficiency	Count	8	15	4	27
		% of Total	12.9%	24.2%	6.5%	43.5%
	Insufficiency	Count	8	10	4	22
		% of Total	12.9%	16.1%	6.5%	35.5%
	Optimal level	Count	4	3	1	8
		% of Total	6.5%	4.8%	1.6%	12.9%
Total		Count	20	32	10	62
		% of Total	32.3%	51.6%	16.1%	100.0%

Table no. 84 - The age variable studied by category among patients of the group with BG related to vitamin D status

			Age categories (years)			
			(22-40]	(40-60]	(60-75]	Total
Vitamin D status	Severe deficiency	Count	1	2	2	5
		% of Total	5.6%	11.1%	11.1%	27.8%
	Deficiency	Count	4	5	2	11
		% of Total	22.2%	27.8%	11.1%	61.1%
	Insufficiency	Count	1	0	1	2
		% of Total	5.6%	0.0%	5.6%	11.1%
Total		Count	6	7	5	18
		% of Total	33.3%	38.9%	27.8%	100.0%

Following the statistical analysis, both for patients with TCA and for patients with BG, it was found that there is no dependency relationship (an association, a link) between the two studied variables: $\chi 2calc = 4.097$, df = 6, $p = 0.664 > \alpha = 0.05$ (Chi-Square Test); respectively $\chi 2calc = 2.354$, df = 4, $p = 0.671 > \alpha = 0.05$ (Chi-Square Test).

3.4.2. Determination of vitamin D status among the two groups according to sex

For the group of patients with TCA – a number of 54 patients had associated low level of vitamin D (severe deficiency, deficiency, insufficiency) of which 44 were female (5 patients with severe deficiency, 23 patients with deficiency and 16 patients with insufficiency) and 10 male (4 patients with vitamin D deficiency and 6 patients with insufficiency). (Table no. 85)

Table no. 85 - Gender distribution of vitamin D status among the TCA group

			Sex		
			F	M	Total
Vitamin D status	Severe deficiency	Count	5	0	5
		% of Total	8.1%	0.0%	8.1%
	Deficiency	Count	23	4	27
		% of Total	37.1%	6.5%	43.5%
	Insufficiency	Count	16	6	22
		% of Total	25.8%	9.7%	35.5%
	Optimal level	Count	6	2	8
		% of Total	9.7%	3.2%	12.9%
Total		Count	50	12	62
		% of Total	80.6%	19.4%	100.0%

From a statistical point of view, there is no relationship of dependence between the two studied variables, (an association, a link): $\chi 2calc = 2.604$, df = 3, $p = 0.457 > \alpha = 0.05$ (Chi-Square Test), although it is notable hypovitaminosis D in female patients compared to male patients where vitamin D values were slightly higher.

For the group of patients with BG – all patients presented a low level of vitamin D, of which 17 were female (5 patients with severe deficiency, 10 patients with deficiency and 2 patients with insufficiency) and 1 male patient (with deficiency). (Table no. 86)

Table no. 86 - Gender distribution of vitamin D status among the BG group

			Sex		
			F	M	Total
Vitamin D status	Severe deficiency	Count	5	0	5
		% of Total	27.8%	0.0%	27.8%
	Deficiency	Count	10	1	11
		% of Total	55.6%	5.6%	61.1%
	Insufficiency	Count	2	0	2
		% of Total	11.1%	0.0%	11.1%
Total		Count	17	1	18
		% of Total	94.4%	5.6%	100.0%

The statistical analysis applied for the two variables shows that there is no relationship of dependence (an association, a link): $\chi 2calc = 0.674$, df = 2, $p = 0.714 > \alpha = 0.05$ (Chi-Square Test), a fact explained by an inequality of the batch in terms of gender distribution.

3.4.6. Establishing the association between ATPO level and vitamin D status among the TCA cohort

The determination of the serum level of ATPO related to the vitamin D status is an element of interest for patients with TCA and respectively BG.

Table no. 89 - Establishing the main statistical indices for ATPO values related to vitamin D status in the TCA group

ATPO (IU/mL)

	N	Average	Standard Deviation	Minimum	Maximum
Severe deficiency	5	316.98	85.86	229.00	410.00
Deficiency	27	317.76	270.02	11.00	850.00
Insufficiency	22	296.22	198.98	15.00	600.00
Optimal level	8	361.61	287.53	23.00	875.00

For the given situation, it is found that there are no significant differences between the mean ATPO values for the analyzed groups: $F = 0.147$, $p = 0.931 > \alpha = 0.05$ (OneWay ANOVA Test). The variances (SD2) are considered unequal (Levene Statistic = 3.351, $p = 0.025 < \alpha = 0.05$) but, it is noted that for patients with severe vitamin D deficiency, the minimum value is significantly higher.

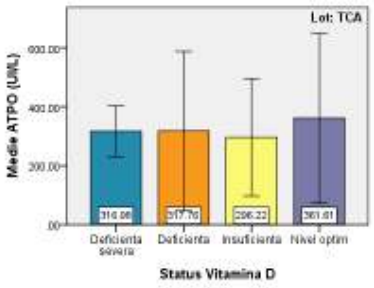

Figure no. 67 - Bar + Error bar graphical representation of the average ATPO values for the analyzed groups

3.4.5. Establishing the association between ATG level and vitamin D status among the TCA cohort

Table no. 90 - Establishing the main statistical indices for ATG values related to vitamin D status in the TCA group

ATG (IU/mL)

	N	Average	Standard deviation	Minimum	Maximum
Severe deficiency	5	357.00	347.30	20.00	790.00
Deficiency	27	295.10	224.37	17.00	898.00
Insufficiency	22	356.10	321.80	15.00	890.00
Optimal level	8	410.11	293.93	23.00	879.00

For the given situation, it is found that there are no significant differences between the average ATG values for the analyzed groups: $F = 0.424$, $p = 0.737 > \alpha = 0.05$ (OneWay ANOVA Test). The variances (SD2) are considered equal (Levene Statistic = 2.036, $p = 0.119 > \alpha = 0.05$) and compared to the level of ATPO, it is noted that there are no major differences between the minimum or maximum value of ATG.

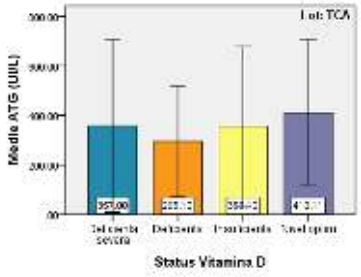

Figure no. 68 - Graphic representation Bar + Error bar of the average ATG values for the analyzed groups

Analysis of serum BAFF level among the two study groups TCA and BG relative to serum vitamin D level

Table no. 101 - Determination of the serum level of BAFF (ng/ml) among patients studied according to vitamin D status

BAFF (pg/mL)

Lot		N	Average	Standard deviation	Minimum	Maximum
TCA	Severe deficiency	5	.14	.08	.04	.20
	Deficiency	27	.13	.10	.02	.40

	Insufficiency	22	.12	.10	.01	.40
	Optimal level	8	.06	.02	.03	.10
BG	Severe deficiency	5	.42	.17	.12	.55
	Deficiency	11	.48	.15	.09	.59
	Insufficiency	2	.17	.14	.07	.27

For the given situation, it is found that there are significant differences between the average BAFF values for at least two of the analyzed groups: $F = 3.021$, $p = 0.031 < \alpha = 0.05$ (OneWay ANOVA Test). PostHoc Multiple Comparisons – Bonferroni analysis (Levene Statistic = 2.334, $p = 0.083 > \alpha = 0.05$, Variances (SD2) are considered equal) shows that there are significant differences only between the mean values corresponding to the groups: Severe Deficiency and Optimal Level, Deficiency and Optimal Level , Insufficiency and Optimal Level ($p < \alpha = 0.05$).

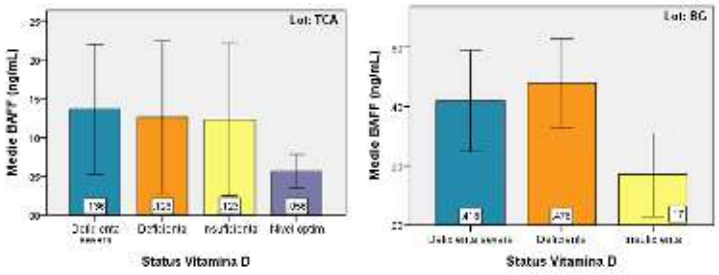

Figure no. 73 and **Figure no. 74 -** Bar + Error bar graphical representation of the average BAFF values for the analyzed groups

3.4.16. Analysis of Thyroid Autoimmune Status and Lymphocyte Activating Factor (BAFF) Among Study Patients and Study Groups

The dosage of lymphocyte activating factor (BAFF) was performed with the aim of looking for the existence of associations between it, variations of vitamin D deficiency and hormonal variations and to understand if there is a possible involvement of BAFF in the amplification of the thyroid autoimmune process.

3.4.17. Determination of BAFF serum level (pg/ml) among patients with TCA and BG

The study of the serum level of BAFF represents an important element in the work. The mean BAFF value for the TCA group (N = 62) is 0.12 pg/ml with a standard deviation (SD) of 0.10ng/ml and for the BG group (N = 18) the mean BAFF value is 0.0.43 ng/ml with a standard deviation (SD) of 0.17 pg/ml. The variances (SD2) of the two groups are considered unequal: $F = 10.444$, $p = 0.002 < \alpha = 0.05$ (Levene's Test). (Table no. 102)

Table no. 102 - Determination of the average value of BAFF among the two study groups

	Lot	N	Average	Standard deviation
BAFF (pg/mL)	TCA	62	.12	.10
	BG	18	.43	.17

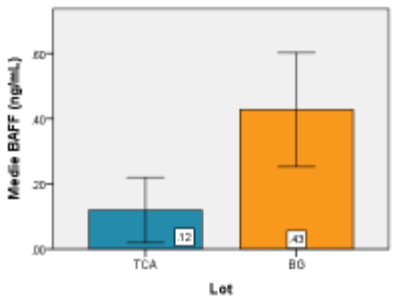

Figure no. 75 - Graphic representation Bar + Error bar of the average values of BAFF for the lots analyzed

For the given situation, it is found that there are significant differences between the average BAFF values of the TCA and BG groups: $t = -7.132$, $df = 20.346$, $p < 0.001 < \alpha = 0.05$ (Independent Samples Test).

3.4.18. Determination of the mean value of the serum level of vitamin D among the two study groups

The average value of 25-OH-vitamin D for the TCA group (N = 62) is 20.09 ng/ml ±7.87 SD and for the BG group (N = 18) the average value of 25-OH-vitamin D is 13.27ng/ml ± 5.16 DS. The variances (SD2) of the two groups are considered unequal: F = 5.871, p = 0.018 < α = 0.05 (Levene's Test). (Table no. 105)

Table no. 105 - Determination of the main statistical indicators of the serum level of vitamin D, comparatively, among the two study groups

25-(OH)-vitamin D (ng/mL)

		Lot	
		TCA	**BG**
N	Valid	62	18
Mean		20.09	13.27
Median		19.10	11.65
Mode		16.00	11.00
Std. Deviation		7.87	5.16
Minimum		7.00	7.00
Maximum		39.00	28.00
Percentiles	25	14.75	9.93
	50	19.10	11.65
	75	25.00	16.25

For the given situation, it is found that there are significant differences between the average values of 25-OH-vitamin D of the TCA and BG groups: t = 4.330, df = 42.348, p < 0.001 < α = 0.05 (Independent Samples Test).

3.4.22. Analysis of a possible association between the serum level of 25-OH-vitamin D and the serum BAFF level among the two studied groups

Following the investigations carried out, it was aimed to determine the existence of a possible association between the serum level of 25-OH-vitamin D and the BAFF serum level among the two studied groups (Table no. 107)

Table no. 107 - Distribution of patients by study groups according to the serum value of BAFF and 25-OH-vitamin D

Lot			**25-(OH)-vitamin D (ng/mL)**
TCA	**BAFF (pg/mL)**	Pearson Correlation	-.236
		Sig. (2-tailed)	.065
		N	62
BG	**BAFF (pg/mL)**	Pearson Correlation	-.218
		Sig. (2-tailed)	.384
		N	18

Following the correlation test both in the TCA group (r = -0.236, p = 0.065 > α = 0.05) and in the BG group: (r = -0.218, p = 0.384 > α = 0.05), the two variables are not correlated.

3.4.23. Evaluation of serum BAFF level according to vitamin D status among studied patients

Serum BAFF level related to vitamin D status indicated for the TCA group, mean BAFF values of 0.14 pg/ml for patients with severe vitamin D deficiency and values of 0.06 pg/ml among those with optimal vitamin D values.

Serum BAFF level related to vitamin D status indicated for the BG group, mean BAFF values of 0.42 pg/ml for patients with severe vitamin D deficiency and values of 0.48 pg/ml among those with vitamin D deficiency and values of 0.17 pg/ml for those with vitamin D insufficiency. (Table no. 108)

Table no. 108 - Distribution of serum BAFF values according to vitamin D status among patients in the two study groups

Lot	Vitamin D Status		N	Minimum	Maximum	Average	Standard deviation
TCA	Severe deficiency	BAFF (pg/mL)	5	.04	.20	.14	.08
	Deficiency	BAFF (pg/mL)	27	.02	.40	.13	.10
	Insufficiency	BAFF (pg/mL)	22	.01	.40	.12	.10

	Optimal level	BAFF (pg/mL)	8	.03	.10	.06	.02
BG	Severe deficiency	BAFF (pg/mL)	5	.12	.55	.42	.17
	Deficiency	BAFF (pg/mL)	11	.09	.59	.48	.15
	Insufficiency	BAFF (pg/mL)	2	.07	.27	.17	.14

3.4.27. To determine the existence of a correlation between serum BAFF and ATPO levels among the two study groups

Following the correlation test both in the TCA group ($r = -0.150$, $p = 0.246 > \alpha = 0.05$) and among the BG group ($r = 0.023$, $p = 0.929 > \alpha = 0.05$) the two variables are not correlated. (Table no. 112)

Table no. 112 – Correlations between BAFF and ATPO serum level among the two study groups

Lot			**ATPO (IU/mL)**
TCA	BAFF (pg/mL)	Pearson Correlation	-.150
		Sig. (2-tailed)	.246
		N	62
BG	BAFF (pg/mL)	Pearson Correlation	.023
		Sig. (2-tailed)	.929
		N	18

3.4.28. To determine the existence of a correlation between serum BAFF and ATG among the two study groups

Following the correlation test in the group of patients with TCA, the two variables are correlated: $r = -0.264$, $p = 0.038 < \alpha = 0.05$ (Correlation (-) weak to moderate). For the group with BG, the two variables are not correlated: $r = 0.314$, $p = 0.204 > \alpha = 0.05$. (Table no. 113)

Table no. 113 - Correlation between BAFF and ATG serum level among the two study groups

Lot			**ATG (IU/mL)**
TCA	BAFF (pg/mL)	Pearson Correlation	-.264*
		Sig. (2-tailed)	.038
		N	62
BG	BAFF (pg/mL)	Pearson Correlation	.314
		Sig. (2-tailed)	.204
		N	18

3.4.44. Analysis of the applicability of the questionnaire for the determination of vitamin D status

Questionnaires are often used with the aim of obtaining in a simple and fast way useful data for the preparation of statistical calculations and their usefulness is proven in a multitude of cases.

Comparative analysis of patients from the TCA group according to the serum level of 25-OH-vitamin D and the estimated level obtained from the questionnaire

Following the application of the questionnaire, in the TCA group an estimated value of vitamin D was described - as being low in 23 patients (37.1%) or optimal in 39 patients (62.9%), depending on the patients' answers.

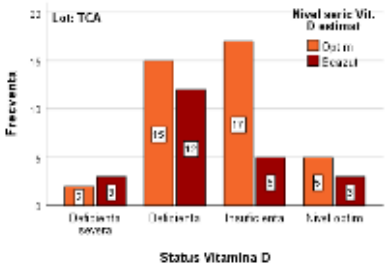

Figure no. 97 - Columnar graphic representation of vitamin D serum values compared to the estimated serum value among the TCA group

3.4.58. Comparative analysis of patients in the BG group according to the serum level of 25-OH-vitamin D and the estimated level obtained from the questionnaire

Following the application of the questionnaire, in the group with BG an estimated value of vitamin D was described - as being low in 17 patients (94.4 %) and respectively optimal in 1 patient (5.6%), depending on the patients' answers. (Table no. 154)

Table no. 154 - Distribution of patients with BG according to the determined level of vitamin D – compared to the level of vitamin D – estimated

			Estimated Vit.D serum level		
			Optimal	Low	Total
Vitamin D Status	Severe deficiency	Count	0	5	5
		% within Vitamin D Status	0.0%	100.0%	100.0%
		% within Estimated Vit.D serum level	0.0%	29.4%	27.8%
	Deficiency	Count	1	10	11
		% within Vitamin D Status	9.1%	90.9%	100.0%
		% within Estimated Vit.D serum level	100.0%	58.8%	61.1%
	Insufficiency	Count	0	2	2
		% within Vitamin D Status	0.0%	100.0%	100.0%
		% within Estimated Vit.D serum level	0.0%	11.8%	11.1%
Total		Count	1	17	18
		% within Vitamin D Status	5.6%	94.4%	100.0%
		% within Estimated Vit.D serum level	100.0%	100.0%	100.0%

CHAPTER 4
Interpretation of results and discussion

The motivation behind the conducted study was the absence of data related to vitamin D status in patients with autoimmune thyroid pathology from the perimarine area of Romania, the Dobrogea region that includes Tulcea and Constanța counties.

Although it is considered a non-endemic area and with an increased sunshine regime, vitamin D deficiency in association with thyroid pathology is frequently found in Dobrogea, a fact probably explained by the presence of a variety of factors involved in triggering the autoimmune process on the one hand and in reducing adequate vitamin D intake.

Promotion and dissemination - The frequent association of vitamin D deficiency with autoimmune thyropathies implies a screening of vitamin D status when autoimmune pathology is proven and correction of vitamin D deficiency is absolutely necessary in this situation. Informing and promoting the study among family doctors in oral presentations and scientific communications brings an important benefit in the diagnosis and follow-up of these categories of patients.

The originality of the study consists in the analysis of the BAFF factor involved in the autoimmunity cascade with exercise considered modulatory in the thyroid autoimmune process, but also the possible involvement of vitamin D deficiency, a marker that could be useful in the study of other autoimmune pathologies.

Weaknesses: the relatively small number of patients included in the study, BAFF testing – previously not applied in other laboratories in Romania for comparative results.

4.1. The general characteristics of the two groups studied (demographic data, antecedents) and the description of the determined clinical-paraclinical parameters

It is noted the existence of a greater number of patients with TCA compared to BG within the entire studied group, with the predominant age between 40-60 years in both groups, and a higher incidence of autoimmune diseases in the female sex being also identified.

For both groups, the majority of the patients in the two groups came from the urban environment.

The analysis of the pathological antecedents of the patients indicates the existence, in particular, of cardiovascular and metabolic diseases in patients with thyropathies and, to a much smaller percentage, the existence of oncological pathology.

The analysis of the pathological antecedents of the patients indicates the existence, in particular, of cardiovascular and metabolic diseases in patients with thyropathies and, to a much smaller percentage, the existence of oncological pathology.

4.2 Assessment of thyroid and breast ecostructure in correlation with thyroid functional status

Benign mammary eco-structural changes have a multifactorial determinism, the presence of thyroid autoimmunity and vitamin D deficiency being considered etiological factors in the development of benign mastopathy with a definite role but an incompletely specified mechanism.

The peculiarities of the expression of autoimmunity and the functionality of the vitamin D receptor, their quantitative fluctuations at the level of breast tissue and the specificity of the unavailability of breast tissue components provide a lesional variability with multiple individualities.

The correlation of the presence of antithyroid autoantibodies and hypovitaminosis D with malignant breast pathology is a motivation for an in-depth study of the issue.

Standardization of breast changes by imaging means is a useful and perfectable exploratory method.

The results of our study support the association between benign breast diseases and thyroid pathology. An important implication of this finding may be to demonstrate the need for screening for potential breast pathology in women with autoimmune thyroid disease in clinical practice.

4.3 Incidence of depressive syndrome among patients of the two study groups

The existence of depression must be evaluated among patients with autoimmune thyropathies, its presence being frequent in this category of patients.

The use of a rapid assessment test and even self-assessment can bring additional benefits in the evolution of the general condition of the patient with thyroid conditions and improve the quality of life.

Hypovitaminosis D is present among these patients but is not directly proportional to the severity of depression.

Further studies are needed to assign a specific therapeutic role for vitamin D in patients with depression associated with autoimmune thyroid pathology.

4.4 – Determination of serum BAFF level correlated with vitamin D status in patients with autoimmune thyroid pathology

Among patients with autoimmune thyroid pathology, the serum BAFF level in our study group was significantly higher in patients with BG compared to those with TCA. The quantitatively different presence of BAFF serum level among patients with BG could be correlated with the significantly lower serum level of 25-OH-vitamin D in them compared to the serum level of the vitamin recorded in patients with chronic autoimmune thyroiditis.

The immunomodulatory role of vitamin D could be related to the enhanced effect of autoimmunity differently for patients with autoimmune thyroid diseases.

The lack of statistical correlation between the average serum level of BAFF and the level of thyroid autoantibodies ATPO and TRAb in the two groups, but the existence of a statistically significant correlation with the level of ATG in the case of the group with TCA, attests to the complexity of the mechanisms of the autoimmune process.

The role of vitamin D in deficiency conditions in achieving the action variables of thyroid autoimmunity involves extensive population studies, motivated by the significant increase in the incidence of autoimmune thyroid diseases and vitamin D deficiency at the population level.

There is a wide range of factors influencing serum vitamin D levels. Given that screening for vitamin D deficiency in the general, asymptomatic population is not recommended, and most professional societies and international guidelines recommend assessing vitamin D status only in groups at increased risk, the development of a standardized questionnaire may be useful in predicting vitamin D deficiency and recommending vitamin D supplementation in at-risk categories.

In Dobrogea, including the perimarine area of Romania, there is an increased incidence of hypovitaminosis D of 76.16%, compared to the population of the other geographical regions. Studies carried out in Romania regarding the incidence and pathological implications of hypovitaminosis D find geographical, seasonal and age variations in the studied population.

Another important aspect of using a questionnaire in the evaluation of vitamin D status is the financial aspect, given the costs of analyzing the serum level in the population, especially for patients with chronic diseases that require periodic monitoring of biological constants.

Although it is an easy-to-apply questionnaire, the results may be incomplete due to individual factors involved. The questionnaire can identify the individuals and the target population for the risk of hypovitaminosis D, determining a prophylactic medical attitude.

CHAPTER 5
CONCLUSIONS

1. In the studied group of patients with autoimmune thyroid disease, it is noted the existence of a greater number of patients with TCA compared to BG within the entire studied group, predominating in both groups aged between 40-60 years - 48.8% (N= 39), being also identified a higher share of autoimmune thyroid diseases in the female sex - 83.3% (N= 67) compared to the male sex - 16.3% (N = 13).

2. The analysis of the pathological antecedents of the patients indicates the existence in particular of cardiovascular and metabolic diseases - 36.5% (N= 29) and respectively 73.5% (N = 59) in patients with thyropathies and, in a much smaller percentage, the existence of oncological pathology (11.25%).
3. The determinations of biological constants demonstrate the impact of autoimmune thyroid pathology on lipid metabolism, the increased incidence of dyslipidemia being evident in the studied patients.
4. Ultrasonographic examination of the thyroid revealed a predominantly hypoechoic appearance in patients with TCA. The determination of the thyroid volume shows significantly higher values of the thyroid volume in patients with BG (11.71ml ± 2.98 SD/7.23ml ± 3.17DS) associated with an intensified vascularization compared to patients with TCA.
5. The presence of nodular formations detected by ultrasound indicates a tendency for the formation of macronodules in patients with BG, and with regard to the appearance of micronodules, it has been observed that they can be equally present in both pathologies.
6. Following the ultrasound evaluation of the nodules in the two groups by calculating the TIRADS score, it was found that a small number of patients with autoimmune thyroid pathology had a TIRADS score of 4 (3.8%), which required additional investigations such as biopsy.
7. Benign mammary ecostructural changes were frequently encountered among patients with TCA and BG, being predominantly identified in the 40-60 age category, the presence of thyroid autoimmunity and vitamin D deficiency being incriminated as possible etiological factors associated with the development of mastopathy benign.
8. Analyzing breast changes classified according to the BIRADS score in relation to vitamin D values noted slightly lower mean serum values in patients with breast lesions with a BIRADS score of 4 compared to BIRADS 1,2 or 3, especially in patients with TCA, thus suggesting the probable role of hypovitaminosis D in the development of benign mammary pathology.
9. The existence of depression evaluated among patients with autoimmune thyropathies by using a rapid test, found its frequent presence in this category of patients - 53.2% of patients with TCA and 66.7% of patients with BG studied. The identification of the severity of the condition can bring additional benefits in the evolution of the general condition of the patient with thyroid conditions, the control of the associated symptomatic disorders and the improvement of the quality of life.
10. Hypovitaminosis D was present among patients with autoimmune thyroid diseases studied, associating, according to the Beck test, different degrees of depression, but it was not proportional to the severity of depression.
11. The evaluation of vitamin D status on a group of patients from the perimarine area of Romania, associating autoimmune thyroid pathology indicated an increased incidence of vitamin D deficiency among this category of patients - 90% (N= 72) of the studied patients, contrary to expectations , related to the increased level of sunshine in this geographical area.
12. The identification of hypovitaminosis D was found both in TCA and BG patients, especially for the age group of 40-60 years, but not in a dependent relationship with the level of thyroid antibodies or with the thyroid functional status, and without an influence of their environment of origin.
13. Screening for vitamin D deficiency in the general population is not currently recommended, but assessing vitamin D status only in high-risk groups by developing a standardized questionnaire may be useful in predicting vitamin D deficiency and recommending vitamin D supplementation in these at-risk groups.
14. Although it is an easy-to-apply questionnaire, the results may be incomplete due to some individual factors involved: age, sex, nutritional status, associated pathologies. The questionnaire can identify the individuals and the target population for the risk of hypovitaminosis D, determining a prophylactic medical attitude.
15. Among patients with autoimmune thyroid pathology, the BAFF serum level for the study group was significantly higher in patients with BG (0.43 ng/ml ± 0.17 SD) compared to those with TCA (0.12 ng/ml ± 0.10 SD) although, in the group in TCA patients, significantly higher BAFF values are observed in relation to the degree of hypovitaminosis.
16. Patients with BG showed higher values of BAFF compared to TCA but also a significantly lower mean value of the serum level of 25-OH-vitamin D, the role of vitamin D in deficiency conditions in achieving the action variables of thyroid autoimmunity being obvious but requiring extensive population studies.
17. The lack of statistical correlation between the average serum level of BAFF and the level of thyroid autoantibodies ATPO and TRAb in the two groups, but the existence of a statistically significant correlation with the level of ATG in the case of the group with TCA, attests to the complexity of the mechanisms of the autoimmune process.
18. BAFF measurement in patients with BG obtained higher concentrations in those with ocular symptoms, which can be used as an indicator for Graves' disease activity with extrathyroidal complications on an autoimmune basis such as Graves-Basedow ophthalmopathy.
19. The results obtained in the study group argue for a possible correlation between vitamin D deficiency and the mechanism by which it is involved in the modulation of the autoimmune process, the limitation in the present case

being the small number of patients enrolled in the study. However, these results motivate the continuation of research on extended study groups.
20. The benefits of relatively easy diagnosis and treatment of vitamin D deficiency in autoimmune thyroid diseases, diseases with a constantly increasing incidence, justify the continuation of research in this segment of pathology.

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