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SUMMARY OF THE PhD THESIS

BIOCHEMICAL, CLINICAL STUDIES AND CHANGES IN SARS-COV-2 INFECTION IN THYROID PATHOLOGY

PhD supervisor,

Professor CS I Natalia Roşoiu

Full Member of the Romanian Academy of Scientists

PhD student,

Drd. Timofte Anne-Lise Mihaela (Voiculescu)

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C O N T E N T

BIOCHEMICAL, CLINICAL STUDIES AND CHANGES IN SARS-COV-2 INFECTION IN THYROID PATHOLOGY

ABREVIETIONS

ACTH - Adrenocorticotropic hormone

AAP - Aminoantipyrine

ACE2 - Angiotensin converting enzyme

ADA - American Diabetes Association

ADP - Adenosine diphosphate

AG - Fatty acids

ANS - 8 anilino acid 1 naphthalene sulfonic acid

Anti-TG - Anti-thyroglobulin

Anti-TPO - Anti-thyroid peroxidase

APC - Antigen presenting cells

ARDS - Acute respiratory distress syndrome

RNA - Ribonucleic acid

ATG - Anti-thyroglobulin

ATP - Adenosine triphosphate

ATPase - Adenosine triphosphatase

ATPO - Anti-thyroid peroxidase

CD4 + - Glycoprotein that serves as a coreceptor for the T cell receptor

CD8 + - Cytotoxic T cells

CFAS U - Calibrator for automated systems

CHD - Coronary heart disease

CHER - Cholesterol esterase

CHOD - Cholesterol oxidase

CVD - Cardiovascular disease / Cardiovascular disease

DCCT - Diabetes Control and Complications Trial

DCM - Designated method of comparison

DI - Deiodinase

DIT - Diiodothyrosine

DM - Diabetes mellitus

EASD - European Association for the Study of Diabetes

ECA2 - Angiotensin converting enzyme

ECLIA - Electrochemiluminescence

EDTA - Ethylenediaminetetraacetic acid

EMSE - N ethyl N (3 methylphenyl) N succinyl ethylenediamine

FDH - Familial dysalbuminemic hyperthyroxinemia

FT3 - Free triiodothyronine

FT4 - Free thyroxine

G6PDH - Glucose-6-phosphate dehydrogenase

GK - Glucokinase

GPO - Glycerol phosphate dehydrogenase

HAMA - Human anti-murine antibodies

Hb - Hemoglobin

HBA1C - Glycosylated hemoglobin

HbAC - Hemoglobinopathy C

HbAD - Hemoglobinopathy D

HbAE - Hemoglobinopathy E

HbAS - Hemoglobinopathy S

HbCC - Type C Hemoglobin

HbF - Hemoglobin type F

HbSC - S-C type hemoglobin

HbSS - S-type hemoglobin

hCG - Human chorionic gonadotropin

HCl - Hydrochloric acid

HDL - High density lipoproteins

HK - Hexokinase

HPLC - High Performance Liquid Chromatography

I123 - Iodine isotope

I131– Iodine isotope

IDF - International Diabetes Federation

IFCC - International Federation of Clinical Chemistry

IFG - Modified basal glycemia

IFN-γ - Interferon γHemoglobin

Ig G - Immunoglobulin G

Ig M - Immunoglobulin M

IL - Interleukin

ISE - Ion Selective Electrodes

K + - Potassium / Potassium Ion

KClO4 - Potassium perchlorate

CSF - Cerebrospinal fluid

LDL - Low Density Lipoproteins

LPL - Lipoprotein lipase

MC - Quality Manager

MERS-CoV - Respiratory syndrome in the Middle East

MES - 2-morpholine-ethane-sulfonic acid

MIT - Monoiodothyrosine

Na + - Sodium Ion / Sodium

NADP + - Nicotinamide adenine dinucleotide phosphate

NADPH - Nicotinamide adenine dinucleotide phosphate reduced form

NaF - Sodium fluoride

NAPQI - N acetyl-β benzoquinone imine

NCEP - National Cholesterol Education Program

NGSP - National Glycohemoglobin Standardization Program

-NH2 - Amino group

NIH - National Institutes of Health

NIS - Sodium-iodine importer

NTI - Patients with non-thyroid disease

WHO - World Health Organization

OP - Operational procedure

PRO CELL - Washing solution

PS - Specific procedure

PS - Blood production

PTU - Propylthiouracil

QC - Quality control

RAC - Responsible for quality assurance

RCOOH - Carboxylic acid

RIC - Radio capture

RMB - Basal metabolism rate

RPM - Rotations per minute

RS - Secretion rate

rT3 - Reverse triiodothyronine

SARS - Severe acute respiratory syndrome

SARS-CoV-2 - Severe acute respiratory syndrome of Coronavirus

CNS - Central nervous system

T1 / 2 - Half time

T3 - Triidothyronine

T4 - Thyroxine

TBG - Thyroxine binding globulin

TBPA - Pre-albumin to bind thyroxine

Tc99m - Technetium isotope

Th1 - Cytokine produced by Helper T lymphocytes involved in the immune response

Th17 - Pro-inflammatory helper T cells produced by interleukin 17

Thaliu^{201} - Thallium isotope

TINIA - Inhibition immunoturbidimetric test

TMPRSS2 - Serine transmembrane protease 2

TNF - Tumor necrosis factor

TPO - Thyroperoxidase

TRAb - Anti-TSH receptor antibodies

TRH - TSH Releasing Hormone

TRIS - Tris (hydroxymethyl) -aminomethane buffer solution

TSH - Thyrotropin / Thyrotropin Hormone

TTAB - Tetradecyltrimethylammonium bromide

VLDL - Very low density lipoproteins

OBJECTIVES AND STUDY PURPOSE

I approached this topic because thyroid pathology has an increased incidence, especially following the relationship between metabolic disorders and thyroid disorders. By monitoring thyroid and biochemical markers from the first symptoms, many thyroid diseases could be prevented, or detected in stages that allow a much more effective treatment.

Endocrinology is the science that studies the structure and function of the endocrine system, biosynthesis, actions and metabolism of hormones, their physiological and pathological status.

Now, the assessment of a patient's health is different from previous years. Thus, it is accepted that the patient may have several diseases at the same time, and therefore it is necessary for a disease to be evaluated in the context of the presence of other pathologies.

The thyroid gland is presented in the literature as the organ that controls metabolic processes, which means that the general condition of an organism depends on the optimal functioning of this endocrine gland.

The thyroid can lead to health problems at any age, with multiple causes: nutritional factors, iodine deficiency, infectious factors, pollution, stress, exposure to various substances or the immune factor.

Decreased concentration without a known cause, recurrent melancholy, poor general condition may indicate a malfunction of the thyroid gland.

Thus, the scientific objectives of this paper were:

- Determination of biochemical parameters and thyroid markers
- Dosage of thyroid markers in patients diagnosed with Covid 19
- Data from the literature were used both nationally and internationally, through bibliographic study.
- The study aimed to analyze the relationship between carbohydrate and lipid metabolism and thyroid markers.

In the statistical analysis, recommended software applications were used to process the data obtained from the application of the research methods and to conclude the results in the most objective way possible. IBM SPSS Statistics was used to interpret the statistical results.

The study carried out and the results obtained allowed us to formulate appreciations, comments and conclusions of great topicality and of fundamental and applied scientific importance.

Thus, the data obtained may be useful to specialized medical staff in establishing the diagnosis, prognosis and application of therapy in thyroid pathology.

The aim of this thesis is to evaluate the incidence of thyroid disease and to establish correlations between thyroid and biochemical markers, as well as the influence of SARS-Cov 2 virus on the thyroid gland.

5.1 Correlations between biochemical markers in thyroid pathology and those in carbohydrate metabolism

Diabetes mellitus (DM), a common heterogeneous endocrine disorder, is on the rise globally. Long-term diabetes is associated with vascular complications that are responsible for increased morbidity and mortality in diabetic subjects. A new addition to these complications is thyroid dysfunction, which is indicated by recent studies.

Thyroid dysfunction is becoming more common in patients with diabetes. Diabetes can affect thyroid function to a variable extent, and the unknown thyroid dysfunction not only worsens metabolic control, but also prevents the management of diabetes. Studies have also suggested that patients with type 2 diabetes, with subclinical hypothyroidism, are at risk for complications such as nephropathy and cardiovascular events. Therefore, patients with diabetes should be examined for thyroid dysfunction.

Hypothyroidism is more common in areas where there is iodine deficiency. Those who have been detected with thyroid peroxidase antibodies and who have TSH values towards the upper limit of the biological reference range have an increased risk for developing hypothyroidism (**Ursu et al., 2014**).

Hypothyroidism affects carbohydrate metabolism in different ways (**Ali et al., 2020**):

1. It has been found that thyroid hormone stimulates the expression of the insulin sensitive glucose transporter (GLUT4) so that in hypothyroidism, the level of this transporter is low.

2. The activity of enzymes involved in hormone synthesis is increased in hypothyroidism because one of the most important functions of thyroid hormone is to reduce the expression of these enzymes, which leads to a decrease in the rate of insulin degradation and increased sensitivity to exogenous insulin. Therefore, when hypothyroidism is established in patients with pre-existing diabetes, there is a decrease in the need for insulin.

3. Hypothyroidism can affect the supply of glucose to the tissues. Local T3 secretion may be affected by polymorphism of the type 2-deionidase 2 (D2) gene that occurs in hypothyroidism, leading to decreased glucose uptake.

Many genes involved in glucose metabolism are regulated by the active thyroid hormone T3, which exerts its action by binding to the thyroid hormone receptor (TR). These receptors are derived from two separate genes, which encode the four major binding isomorphs of T3: TRα1, TRβ1, TRβ2, and TRβ3. TRα1 and TRβ1 are widely expressed and their relative abundance dictates whether T3 target tissues exhibit TRα1 or TRβ1

responsiveness, or no TR isomorphic specificity at all (**Yen, 2001**). TRα1 is thought to be primarily involved in the metabolic effects of thyroid hormone, while TRβ1 and TRβ2 are considered to be key regulators in balancing the hypothalamic-pituitary-thyroid axis and maintaining euthyroid status.

The aim of this study was to screen the incidence of thyroid disease in Constanta County, Romania in patients diagnosed with type II diabetes in order to assess the tendency to associate thyroid hormone dysfunction with the diabetic process by correlating glycemic parameters and thyroid profile in serum.

The study included a number of 153 patients who presented from 2018 for analysis, in compliance with GDPR rules. The following parameters were processed from these patients:

To assess thyroid function:
FT3 - free triiodothyronine

FT4 - free thyroxine

TSH - thyroid stimulating hormone, thyrotropin

Anti-TPO - thyroid peroxidase antibodies (anti-thyroid peroxidase)

To assess carbohydrate metabolism:

Serum glucose

Glycosylated hemoglobin - HbA1C

RESULTS (Voiculescu et al., 2020)

To assess thyroid function and carbohydrate metabolism, 153 patients with a mean age of 44.36 were enrolled in the study. Of these, 112 patients were female, aged 18 to 76 years, and 41 were male, aged 18 to 73 years (Table 3).

Table 3. Categories of patients analyzed according to sex and age

Sex	No. of samples	Age	
Both sexes	153	**Mean**	**44,36**
		stdev	17,2466
		Min	18
		max	76
Masculin	41	**Mean**	**43,68**
		stdev	17,4950
		Min	18
		max	73
Feminin	112	**Mean**	**44,1196**
		stdev	17,1927
		Min	18
		max	76

The average values of thyroid and carbohydrate markers are shown in Table 4.

Table 4. Average values of thyroid and carbohydrate parameters

No. of samples	Sex	Marker	TSH (µUI/ml)	FT3 (pmol/l)	FT4 (pmol/l)	Anti-TPO (UI/ml)	Glucose (mg/dl)	HbA1C (%)
153		**mean**	**3,15**	**4,96**	**15,72**	**38,51**	**93,11**	**6.50**
		stdev	3,8573	1,1177	4,3878	135,0417	24,2376	1,0177
		Min	0.005	3	8.55	7.01	64,4	4,9
		max	30.34	7.49	47.91	1185	186.8	8,6
41	M	**mean**	**2,82**	**4,93**	**15,71**	**31,17**	**92,41**	**6,21**
		stdev	2,5006	1,0983	4,5450	108,7514	24,2271	1,0363
		Min	0.005	3	10.45	10	64,4	4.9
		max	15.04	7.49	35.06	282,5	186.8	7.9
112	F	**mean**	**3,41**	**4,98**	**15,73**	**46,27**	**93,82**	**6,79**
		stdev	4,2172	0,8905	4,5769	154,5749	23,2344	1,2325
		Min	0.005	4	8.55	7.01	65.2	4.9
		max	30.34	6.99	47.91	1185	178.7	8.6

Thyroid stimulating hormone is formed in specific basophilic cells in the anterior pituitary gland and is subjected to a circadian secretion sequence. The release of the hormone TSH is the central regulatory mechanism for the biological action of thyroid hormones, having a stimulating action in all stages of formation and secretion of thyroid hormones. TSH-ul is a hormone sensitive to changes in the concentration of free thioid hormones which results in adequate positive or negative feedback of TSH. TSH is a very sensitive and specific parameter in the evaluation of thyroid function and is particularly important for detecting or excluding disorders in the central regulatory circuit between the hypothalamus, pituitary gland and thyroid (**N. Roşoiu, 2011**). In patients of both sexes studied, the mean TSH was 3.15 IU / ml, in females 3.41 µUI / ml, in males the mean TSH was 2.82 IU / ml and higher, but not above the maximum limit. Out of a total of 112 female patients evaluated, 22 (19.6%) had a value above changes in thyrotropin (TSH), but only 3 of these had increases above the reference biological range of anti-TPO antibodies. Of the male patients, only 5 had values greater than 4.2 µIU / mL of TSH. TPO (thyroid specific peroxidase) is involved in the formation of thyroid hormones T3 and T4. Dosage of anti-TPO antibodies, a marker used in the diagnosis of autoimmune thyroiditis, is recommended. 90% of patients diagnosed with Hashimoto's thyroiditis and 70% of patients with Graves' disease have an elevated anti-TPO titer (**N.Rosoiu, 2008**).

Direct measurement of T3, T4 provides a value of total circulating hormone, which does not allow the differentiation between free or bound T3 and T4 hormones. The most useful method of evaluating thyroid hormones is in response to TSH, because the value of TSH reflects the amount of free, biologically active hormone in the target tissue. The largest fraction of total triiodothyronine binds to transporter proteins (TBG, prealbumin, albumin). Free triiodothyronine (FT3) is the physiologically active form of the thyroid hormone triiodothyronine (T3). The determination of free T3 has the advantage of not depending on changes in the concentrations and binding properties of the binding proteins; thus, it is not necessary to further determine a binding parameter (T-uptake, TBG) **(Rosoiu, 2005)**. The values obtained in our study ranged from 4.98 pmol / l in female patients to 4.93 pmol / l in male patients.

Determination of free thyroxine (FT4) is an important element in routine clinical diagnosis. Free thyroxine is determined together with TSH (thyrotropin) in suspected thyroid function disorders (**Tietz, 1995**). The values obtained in our study ranged from 15.73 pmol / l in female patients and. 15.71 pmol / l in male patients.

Blood glucose ranged from an average of 92.11 mg / dl in 153 patients to 92.41 mg / dl in male patients and 93.82 mg / dl in female patients. Glycated hemoglobin (%) ranged from 6.79% in female patients to 6.21% in male patients, with a mean of 6.5%.

DISCUSSIONS

Autoimmune thyroid pathology has a significant prevalence in the general population.

In clinically manifest primary hypothyroidism, TSH is elevated from baseline, and FT4 and T3 are low below the baseline.

In subclinical hypothyroidism, TSH is elevated from the maximum baseline, and FT4 may be normal.

In autoimmune hypothyroidism, changes occur in thyroid parameters as follows: high TSH, low FT4 or normal, but with significant increases in ATPO and Anti-TG antibody titers.

In hypothyroidism, the level of thyroid hormones is high, both gluconeogenesis and glycogenolysis increase, leading to increased seric glucose level.

In hyperthyroidism, the concentration of TSH is low, with values of high thyroid hormone levels, or normal values, but towards the upper limits of the biological reference ranges.

It has been found that women and the elderly frequently have this condition. Hashimoto's thyroiditis and Graves' disease can be found in patients with type 1 diabetes (**Howson et al. 2007, Perros et al., 1995**). A number of studies recommend that patients with type I diabetes be evaluated for thyroid function by determining Anti-TPO and AntiTg (**Umpierrez et al, 2003**).

TSH, Anti-TPO and Anti-Tg screening is important for the early detection of thyroid dysfunction in patients with autoimmune diabetes, noting that autoimmune diabetes is quite commonly associated with thyroid autoimmune pathology (**Jin et al., 2004**). The incidence of thyroid dysfunction in patients with diabetes is approximately 11%. It has been found that the appearance of thyroid dysfunction increases with age, and this happens globally, and worldwide, thyroid disease is less common in men compared to women, which has been shown in our study.

Subclinical hypothyroidism has been reported to occur in approximately 4% - 8.5% and may reach 20% in women over 60 years of age, while subclinical hyperthyroidism occurs less frequently and is reported to be approximately 2%. In hypothyroidism, insulin resistance has been studied and demonstrated in various in vitro and preclinical studies (**Ali et al. 2020**).

Thyroid hormones increase the rate of hepatic glucose production, mainly by increasing hepatic gluconeogenetic activity, but also glycogenolysis. Gluconeogenesis converts nonhexose precursors into glucose molecules. This effect, in general, does not cause an increase in plasma glucose, but influences the response of pancreatic secretion by increasing insulin production (**Zimmermann, 2011**).

Thyroid hormones increase the availability of the fundamental material for increasing gluconeogenetic activity, meaning aminoacids and glycerol, causing the expression of key enzymes in gluconeogenesis.

The general changes caused by hypothyroidism are reduced absorption of glucose from the gastrointestinal tract, accumulation of glucose in the peripheral tissues, the process of gluconeogenesis will be slowed down, glucose production in the liver will decrease and tissue resistance to insulin will increase. The relationship between diabetes and thyroid dysfunction has been shown to be very strong.

5.2. Results regarding the correlation between thyroid parameters and lipid metabolism parameters (Voiculescu et al., Study in progress)

Lipoprotein metabolism is recognized as a target for the action of thyroid hormone, which includes both lipid synthesis and degradation. Furthermore, TSH can lead to increased cholesterol synthesis and decreased hepatic excretion of bile acids. Depending on the level of thyroid hormone, the balance will be tilted towards an increased catabolism, which occurs in thyrotoxicosis, or the accumulation of lipids, due to low catabolism, in hypothyroidism. this thing. Although high levels of total cholesterol and LDL cholesterol are the main source of lipoprotein degradation in hypothyroidism, it has been observed that VLDL, which has accumulated in the blood, may also be a cause of hypothyroidism.

The aim of this study was to highlight particular changes in lipid metabolism in the context of thyroid disease.

Biochemical and hormonal markers were determined in the medical analysis laboratory of Unirea Medical Center.

The study included a number of 153 patients who presented within 3 months for analysis, in compliance with GDPR rules. The following parameters were processed from these patients:

Assessment of thyroid function:

FT3 - free triiodothyronine

FT4 - free thyroxine

T3 - triiodothyronine

T4 - thyroxine

TSH - thyrotropin

Anti-Tg - anti-thyroglobulin antibodies

Anti-TPO - thyroid peroxidase antibodies

Evaluation of lipid metabolism:

Total Serum Cholesterol (Total Chol.)

Serum triglycerides (Tg)

High Density Lipoprotein Fraction (HDL)

Low Density Lipoprotein Fraction (LDL).

Biochemical determinations were performed on serum. The laboratory equipment used for the biochemical markers was the automatic biochemistry analyzer COBAS INTERGRA 400 PLUS, by enzymatic colorimetric methods.

The COBAS E411 automated immunology analyzer by electrochemilumescence (ECLIA) immunology was used to determine thyroid markers.

The reagent kits used to determine the various biochemical and immunological markers comply with international standards and standardized methods.

Calibration and control of the equipment was performed with calibration serum and normal control serum and pathological control serum for each marker.

Results:

To assess thyroid function and lipid metabolism, 153 patients with a mean age of 44.36 were enrolled in the study. Of these, 112 patients were female, aged 18 to 76 years, and 41 were male, aged 18 to 73 years (Table 5).

Table 5. Categories of patients analyzed according to sex and age

Sex	No. of samples	Age	
Both sexes	153	**mean**	**44,36**
		stdev	17,2466
		Min	18
		max	76
Masculin	41	**mean**	**43,68**
		stdev	17,4950
		Min	18
		max	73
Feminin	112	**mean**	**44,1196**
		stdev	17,1927
		Min	18
		max	76

The average values of the thyroid markers and those of the lipid profile are presented in table 6.

Table 6. Mean values of thyroid parameters and lipid profile

No. of samples	Sex	Marker	TSH (µUI/ml)	FT3 (pmol/l)	FT4 (pmol/l)	Anti-TPO (UI/ml)	Chol total (mg/dl)	HDL Chol (mg/dl)	LDL Chol (mg/dl)	Trig (mg/dl)
41	M	**Mean**	**2,82**	**4,93**	**15,71**	**31,17**	**215,2171**	**47,4732**	**123,8171**	**169,2122**
		Stdev	2,5006	1,0983	4,5450	108,7514	54,74659	15,2984	42,46184	122,8556
		Min	0.005	3	10.45	10	127,80	25,30	55,00	78,00
		Max	15.04	7.49	35.06	282,5	367,30	118,40	250,60	860,90
112	F	**Mean**	**3,41**	**4,98**	**15,73**	**46,27**	**212,5911**	**58,0696**	**120,4384**	**139,9393**
		Stdev	4,2172	0,8905	4,5769	154,5749	55,62890	14,41508	40,41732	70,6489
		Min	0.005	4	8.55	7.01	124,00	33,80	61,70	11,40
		Max	30.34	6.99	47.91	1185	371,70	95,80	251,00	291,60

Compared to the number of patients enrolled in the study, 28 patients had changes in thyroid parameters.

Current studies indicate that thyroid pathology is more common in women than in males (**Zbranca E et al., 2008**), our study confirming this by the fact that the majority of patients presented for thyroid evaluation are women (112 patients) and also an increased percentage of 25% (28 patients) of them have thyroid pathology, and if we refer to the total number of patients it represents 18.30%.

Of the total number of patients enrolled in the study, 71.43% show values in the reference range, and 28.57% show changes in thyroid markers (Fig. 20).

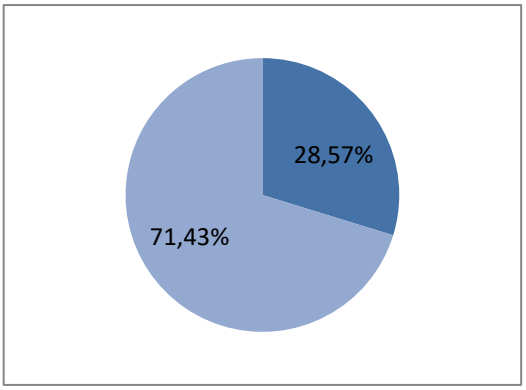

Fig. 20 Distribution of patients according to the presence of altered thyroid markers.

Correlations

	TSH	COL
Pearson Correlation	1	0,530**
TSH Sig.(2-tailed)		0.000
N	112	112
Pearson Correlation	0,530**	1
COL Sig.(2-tailed)	0,000	
N	112	112

**. Correlation is significant at the 0.01 level (2-tailed).

	TSH	COL
Pearson Correlation	1	0,671**
TSH Sig.(2-tailed)		0.000
N	41	41
Pearson Correlation	0,671**	1
COL Sig.(2-tailed)	0,000	
N	41	41

**.Correlation is significant at the 0.01 level (2-tailed).

Elevated TSH levels have been associated with higher total cholesterol levels for both women (Fig. 21) and men (Fig. 22).

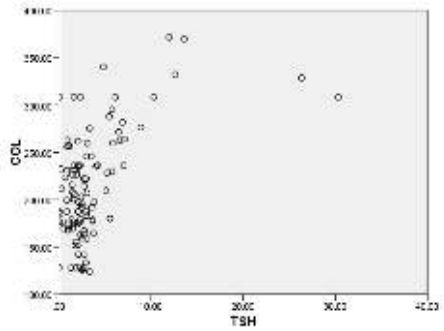

Fig. 21 Association of TSH with Cholesterol in women

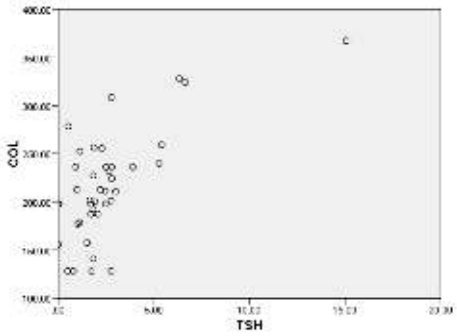

Fig. 22 Association of TSH with Cholesterol in men

Modified lipid metabolism was also present in patients with Anti-OTP, in which one patient had hypertriglyceridemia and another patient had low HDL-cholesterol. showing high and TSH values. The study also included patients whose lipid profile was not evaluated, although thyroid markers showed changes: elevated TSH values, but with normal, or slightly lower FT4 values.

Elevated anti-TPO levels have also been found in both patients with hypercholesterolemia and patients with optimal cholesterol levels. For the other thyroid parameters, FT3, FT4, T3, T4, AntiTg, no significant changes were observed that could be taken into account. What is found is that patients with changes in lipid metabolism have an increased risk of developing cardiovascular disease due to high levels of favorable factors: total cholesterol, triglycerides, HDL-cholesterol and HDL-cholesterol, low cardiovascular protective factor. The consequence of disorders in the functionality of the thyroid gland is cardiovascular diseases, to which is added diabetes due to hyperglycemia that may accompany thyroid pathology.

DISCUTIONS

Thyroid dysfunction may be associated with cardiovascular disease, although the role of thyroid function in lipid metabolism is not well known.

Elevated levels of TSH have been associated with elevated levels of total cholesterol and LDL Cholesterol emphasizing the importance of the pituitary-thyroid-cardiac axis in lipid metabolism.

Further studies are needed to show the importance of FT4 and FT3 in the involvement of lipid metabolism.

Cardiovascular disease accounts for a fairly high percentage of deaths, associated with dyslipidemia, obesity and metabolic syndrome, which are risk factors for cardiovascular disease.

The thyroid gland is an important endocrine organ, involved in the onset and development of cardiovascular disease.

Although thyroid hormones are known to have prominent effects on cholesterol metabolism, their association is still under study.

Currently, the international approach to assessing a patient's health is different from previous years. It was considered necessary to take into account the fact that a patient may have several diseases at the same time and therefore it is necessary that a disease is not evaluated as an independent entity, but in the context of the presence of other pathologies.

Thus, thyroid pathology is no exception, especially since the thyroid gland is involved in the control and regulation of glucose metabolism. The involvement of the thyroid gland in the metabolism of carbohydrates, lipids, proteins and phospho-calcium minerals, its disorders, will eventually lead to the appearance of serious pathologies associated with thyroid disease.

The manifestations of thyroid pathology vary considerably from one geographical area to another and are mainly determined by the availability of iodine in the diet. Hypothyroidism predominates in areas with a low iodine deficiency, while in areas with a low iodine content, autoimmune thyroid disease is more present (**Vanderpump, 2011**).

Studies try to highlight the manifestations of diseases such as dyslipidemia, cardiovascular disease, osteoporosis, neuromuscular disorders, neuropsychiatric disorders, which, people initially diagnosed with these diseases, found in an assessment of thyroid profile that they have markers of thyroid function.

Studies on disorders of lipid metabolism as well as phospho-calcium metabolism have shown the presence of cardiovascular disease and osteoporosis associated with changes in thyroid markers (**Wright et al., 2009, Xiang et al. 2008**).

Recent studies have shown an increase in cardiovascular risk in young people with elevated TSH (**Rodondi et al. 2010**).

Studies in patients with metabolic syndrome have shown low levels of T4 and high levels of TSH. These patients had low levels of HDL cholesterol and high levels of total cholesterol, LDL cholesterol and triglycerides (**Gutch M. et al., 2017; Chugh K. et al.,**

2012; Kumar HK et al., 2009; Agarwal P. et al., 2015). Similar results were obtained in another study performed in patients with subclinical hypothyroidism. These patients had elevated levels of TSH, total cholesterol, LDL cholesterol and triglycerides, but low levels of HDL cholesterol (**Liang LB, et al., 2013**).

The effects of thyroid hormones on lipid metabolism include: use of lipid substrates, increased synthesis and mobilization of triglycerides stored in adipose tissue, increased concentration of unsaturated fatty acids, and increased lipoprotein-lipase activity.

Severe hypothyroidism may be associated with elevated concentrations of total cholesterol and atherogenic lipoproteins.

5.3. Results on immunological parameters recorded in patients diagnosed with COVID-19 (Voiculescu et al., 2020, 2021,)

COVID-19 disease, which broke out in December 2019 and caused by SARS COV 2, continues to be a victim at this time. It is known that the main organs affected by SARS-COV-2 are the lungs and the immune system, but it is not clear whether COVID-19 has an effect on thyroid function.

Knowing that the virus enters the body through ACE2, and that it is found in large amounts in the thyroid gland, studies have begun on COVID-19 and thyroid disease.

Thyroid hormones modulate innate and adaptive immune responses through both genomic and nongenomic mechanisms. Concentrations of L-thyroxine (T4) and 3,3 ', 5-triiodo-L-tyronine (T3) stimulate the production and release of cytokines, which are components of the "cytokine storm" specific to systemic viral infection (**Shih et al., 2004 ; Davis et al., 2016**). Moreover, thyroid hormones are able to potentiate the antiviral action of IFN-γ.

Thus, clinicians have observed that respiratory infections could cause a thyroid storm in patients with decompensated hyperthyroidism, favoring the risk of death. It should be noted that T4, the main hormone secreted by the thyroid gland, is known to activate platelets **(Davis et al., 2018**). This may support the pathological coagulation encountered as a complication of virus infections. This observation motivates the further study of the relationship between COVID-19 and the thyroid gland.

The aim of our study was to evaluate thyroid function in patients confirmed with COVID-19 with no history of thyroid disease. The diagnosis of COVID-19 is confirmed by clinical manifestations and laboratory determinations (RT-PCR analysis).

The study involved 32 patients: 21 men and 11 women aged between 25 and 58 years, between May and July 2021. Of these, 14 patients were confirmed with COVID - 19: 4 women and 10 men. The average age for men diagnosed positively was 45 years, for those diagnosed negatively it was 46.3 years. The average age for women diagnosed positively was 39.5 years, for those diagnosed negatively it was 45.3 years. The form of their disease was moderate or severe, not reaching any critical form. Patients under study are without thyroid disease, as a medical history.

The thyroid parameters studied were T3, T4 and TSH, which were compared between patients tested positive for COVID-19 and those tested negative for COVID-19, the latter being considered a control group.

The results for the women in the study are shown in Table 7:

Table 7. Thyroid parameters in women diagnosed positively / negatively with Sars Cov-2

	N	Minimum	Maximum	Mean	Std. Deviation
T3(1,3-3,1 nmol/l) FN	7	1.48	1.91	1.7429	.17007
T4(66-181 nmol/l) FN	7	97.67	123.40	112.5386	9.60684
TSH(0,27-4,2µUI/ml) FN	7	.92	4.50	2.3466	1.33433
T3(1,3-3,1 nmol/l) FP	4	1.24	2.03	1.5825	.33310
T4(66-181 nmol/l) FP	4	62.36	97.48	84.2275	16.12489
TSH(0,27-4,2µUI/ml) FP	4	.39	2.03	1.0458	.75517

The results of the men in the study are shown in Table 8:

Table 8. Thyroid parameters in men diagnosed positively / negatively in Sars Cov-2

	N	Minimum	Maximum	Mean	Std. Deviation
T3(1,3-3,1 nmol/l) MP	10	1.55	1.99	1.7250	.15487
T4(66-181 nmol/l) MP	10	61.27	101.90	79.3800	14.02973
TSH(0,27-4,2µUI/ml) MP	10	.31	2.42	1.2183	.67812
T3(1,3-3,1 nmol/l) MN	11	1.44	2.06	1.7773	.17647
T4(66-181 nmol/l) MN	11	84.92	126.80	104.1800	11.94351
TSH(0,27-4,2µUI/ml) MN	11	1.06	4.22	2.1400	1.05905

Regarding the differences between the 2 groups, in men it is found that there are significant differences between the values of T4 and TSH, according to Table 9.

Table 9. Differences between the 2 groups: men and women tested RT-PCR for SARS-COV-2

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	T3FP - T3FN	-.09750	.49922	.24961	-.89188	.69688	-.391	3	.722
Pair 2	T4FP -T4FN	-24.04000	21.34059	10.67029	-57.99763	9.91763	-2.253	3	.110
Pair 3	TSHFP - TSHFN	.40229	1.35211	.51105	-.84821	1.65278	.787	6	.461
Pair 4	T3MN - T3MP	.06300	.24445	.07730	-.11187	.23787	.815	9	.436
Pair 5	T4MN - T4MP	25.63200	22.43782	7.09546	9.58095	41.68305	3.612	9	.006
Pair 6	TSHMN - TSHMP	1.01870	1.22307	.38677	.14377	1.89363	2.634	9	.027

DISCUSSIONS:

-TSH and T3 levels in patients with COVID-19 were lower than those in the control group.

-The more severe the COVID-19 form, the lower the TSH and T3 values. The decrease in TSH and T3 levels may be correlated with the severity of the disease. Suppressed TSH is likely to be associated with an increase in proinflammatory cytokines, such as IL 6.

-T4 does not show significant changes in any of the groups studied, although it is observed that this parameter has slightly lower values in the group of patients confirmed with COVID-19.

-Decreased TSH in patients confirmed with COVID-19 may be associated with changes in pituitary TSH secreting cells.

To determine the full impact of COVID-19 on the hypothalamic-pituitary-thyroid axis, dosing of FT4, FT3 and ATPO is recommended both during the disease and after recovery.

GENERAL CONCLUSIONS

From the analysis of the obtained results, we can formulate the following appreciations, comments and conclusions:

1. Thyroid pathology is more common in females, from young age to old age.
2. Hyperglycemia in thyroid pathology is important to evaluate because it has been found that autoimmune diabetes is associated with autoimmune thyroid pathology. TSH, Anti-TPO and Anti-Tg screening is important for the early detection of thyroid dysfunction in patients with autoimmune diabetes, noting that autoimmune diabetes is quite commonly associated with thyroid autoimmune pathology (**Jin et al., 2004**).
3. The incidence of thyroid dysfunction in patients with diabetes is about 11%. It has been found that the occurrence of thyroid dysfunction increases with age, and this is happening globally, and worldwide, thyroid disease is less common in men compared to women, which was demonstrated in our study.
4. Pathology derived from disorders of carbohydrate and lipid metabolism may be present in patients with thyroid changes, dyslipidemia may accompany thyroid pathology that complicates it.
5. Cardiovascular disorders may be a consequence of the pathology associated with dyslipidemia-thyroid pathology that may complicate the patient's life.
6. The involvement of thyroid hormones in the regulation of carbohydrate and lipid metabolism makes researchers think that thyroid hormone analogues should be used in the treatment of hypercholesterolemia and weight loss. (**Baxter JD, Webb P., 2009; Delitala AP, et al., 2017; Shoemaker T, et al., 2012**).
7. TSH and T3 levels in patients with COVID-19 were lower than those in the control group.
8. Decreased TSH but also T3 levels can be correlated with the severity of the disease. Suppressed TSH is likely to be associated with an increase in proinflammatory cytokines, such as IL.
9. T4 does not show significant changes in any of the groups studied, although it is observed that this parameter has slightly lower values in the group of patients confirmed with COVID-19.

10. Decreased TSH in patients confirmed with COVID-19 may be associated with changes in pituitary TSH secreting cells.
11. To determine the full impact of COVID-19 on the hypothalamic-pituitary-thyroid axis, dosing of FT4, FT3 and ATPO is recommended both during the disease and after recovery.
12. Thyroid diseases occupy an important place in the case of pathologies in our country, the most under-diagnosed of these being hypothyroidism.
13. The correlation of metabolic parameters with thyroid ones is of real importance in the case of metabolic disorders due to the involvement of thyroid hormones in regulating the metabolism of lipids and carbohydrates.
14. This thesis highlighted the incidence of thyroid disease and established the correlations between thyroid and biochemical markers, as well as the influence of SARS-Cov 2 virus on the thyroid gland.
15. The study carried out and the results obtained allowed us to formulate appreciations, comments and conclusions of great topicality and of fundamental and applied scientific importance.
16. Thus, the data obtained from current events, contribute to the enrichment of specialized knowledge, presenting a relevant scientific support to specialized medical staff in establishing the diagnosis, prognosis and application of therapy in thyroid pathology.

SELECTIVE BIBLIOGRAPHY

1. Agarwal P, Singh MM, Gutch M. Thyroid function and metabolic syndrome. Thyroid Research and Practice. 2015 Sep 1;12(3):85.
2. Ali A, Allehibi K, Al-Juboori N. Glycemic status in patients with primary hypothyroidism and its relation to disease severity. Mustansiriya Med J. 2020;19:20–4.
3. Baxter JD, Webb P. Thyroid hormone mimetics: potential applications în atherosclerosis,obesity and type 2 diabetes. Nature reviews Drug discovery. 2009 Apr;8(4):308.
4. Chugh K, Goyal S, Shankar V, Chugh SN. Thyroid function tests in metabolic syndrome.Indian journal of endocrinology and metabolism. 2012 Nov;16(6):958.
5. Davis PJ, Mousa SA, Schechter GP. *New interfaces of thyroid hormone actions with blood coagulation and thrombosis. Clin Appl Thromb Hemost*. **2018**;24(7):1014–9. **https://doi.org/10.1177/1076029618774150**.
6. Delitala AP, Delitala G, Sioni P, Franciulli G. Thyroid hormone analogs for the treatment of dyslipidemia: past, present and future. Current medical research and opinion. 2017 Nov 2; 33(11):1985-93.
7. Gutch M, Rungta S, Kumar S, Agarwal A, Bhattacharya A, Razi SM. Thyroid functions and serum lipid profile in metabolic syndrome.Biomedical journal. 2017 Jun 1;40(3):147-53.
8. Howson J., Dunger D., Nutland S., Stevens H., Wicker L., Todd J. A type 1 diabetes subgroup with a female bias is sharacterised by failure in tolerance to thyroid peroxidase at an early age and a strong association with the cytotoxic T-lymphocyte-associated antigen-4 gene. Diabetologia, 2007, vol. 50, p.741-746.
9. Jin P., Zhou Z., Yang L., Yan X., WANG J., Zhang D., Huang G. Adult-onset latent autoimmune diabetes and autoimmune thyroid disease. Zhongua Nei Ke Za Zhi, 2004, vol. 43 (5), p. 363-367.
10. Kumar HK, Yadav RK, Prajapati J, Reddy CV, Raghunath M, Modi KD.Association between thyroid hormones, insulin resistance, and metabolic syndrome.Saudi Med J.2009 Mar 1;30(7):907-11.
11. Liang LB, Zhang M, Huang HJ, Wang YJ, Li SQ. Blood lipid, glucose and uric acid in people with subclinical hypothyroidism. Sichuan da xue xue bao. Yi xue ban= Journal oSichuan University. Medical science edition. 2013 Nov;44(6):954-6.
12. Perros P, McCrimmon RJ, Shaw G, Frier BM. Frequency of thyroid dysfunction in diabetic patients: value of annual screening. Diabet Med. 1995 Jul;12(7):622–7.
13. Perros P., Mccrimmon R., Frier B. Frequency of thyroid dysfunction in diabetic patients: value of annual screening. Diabet Med, 1995, vol. 12, p. 622-627.
14. Rodondi N, den Elzen WP, Bauer DC et al. Subclinical hypothyroidism and the risk of coronary heart disease and mortality. JAMA 2010;304:1365–74.
15. Rosoiu N, Serban M, Badiu G. Medical Biochemistry. Intermmediate metabolism with clinical correlations. In Constanta: Muntenia; 2005. p. 107–50.
16. Rosoiu N, Verman IG. Clinical biochemistry. In Constanta: Muntenia; 2008. p. 162–84.

17. Rosoiu N. Medical biochemistry - Course. In Constanța: Ex Pont; 2011. p. 168-170,435-438

18. Shih CH, Chen SL, Yen CC, *Thyroid hormone receptordependent transcriptional regulation of fibrinogen and coagulation proteins. Endocrinology*. **2004**; 145(6):2804–14. https://doi.org/10. 1210/en.2003-1372.

19. Shoemaker T, Kono T, Mariash C, Evans-Molina C. Thyroid hormone analogue for the treatment of metabolic disorders: new potential for unmet clinical needs?Endocrine Practice 2012 Jul 11;18(6):954-64.

20. Tietz N.W. Clinical Guid to Laboratory Tests. 3rd ed. Philadelphia, Pa: WB Saunders Co 1995:596.

21. Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia: WB Saunders, 1995;124-127(chloride), 840-841 (lithium), 502-507(potassium), 562-565 (sodium).

22. Tietz NW, ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders Company 1995;610-611.

23. Umpierrez G.E., Latif K. A., Murphy M. B., Lambeth H. C., Stentz F., BUSH A., Kitabchi A. E. Thyroid dysfunction in patients with type 1 diabetes: a longitudinal study. Diabetes Care, 2003, vol. 26, p. 1181-1185.

24. Vanderpump M. P. J. The epidemiology of thyroid disease. British Medical Bulletin 2011; 99: 39–51.DOI:10.1093/bmb/ldr030.

25. **Voiculescu A-L M**, Anghel A, Gurgas L, Roșoiu N, Significance of Hematological Parameters and Biochemical Markers in Severe Forms of Covid-19, Academy of Romanian Scientists, Annals Series on Biological Sciences, 9, 2, 88-99, (2020).

26. **Voiculescu A-L M**, Anghel A, Gurgas L, Petcu L,Roșoiu N, Correlations between biochemical markers in thyroid pathology with those in carbohydrates metabolism, ARS Medica Tomitana. 26, 2,100 – 104, (2020).

27. **Voiculescu A-L M**, Anghel A, Roșoiu N , Effects of COVID-19 on the Thyroid Gland, The Publising House Medicine of the Romanian Academy, Proc. Rom. Acad., Series B, 23(1), 132-135 (2021).

28. **Voiculescu A-L M**, Anghel A, Roșoiu N , Immunity from SARS-COV- 2 The Publising House Medicine of the Romanian Academy, Proc. Rom. Acad., Series B, 23(1), 90-93 (2021).

29. Wright HL, McCarthy HS, Middleton J, Marshall MJ. RANK, RANKL and osteoprotegerin in bone biology and disease. Curr Rev Musculoskelet Med 2009;2(1):56-64.

30. Xiang G.D, Sun H.L, Hou J. Changes in endothelial function and its association with plasma osteoprotegerin in hypothyroidism with exercise-induced silent myocardial ischemia. Clin Endocrinol (Oxf) 2008;69(5):799-803.

31. Zbranca E. și col. Endocrinologie. Ghid de diagnostic și tratament în bolile endocrine. Ediția a-III-a. Ed. Polirom, 2008, 103-166.

32. Zimmermann MB. Iodine deficiency. Endocr Rev. 2009 Jun;30(4):376–408.

PUBLICATIONS

I. SCIENTIFIC PUBLICATIONS PUBLISHED IN BDI AND ISI RATED JOURNALS

1). VOICULESCU A-L M., ANGHEL A., GURGAS L., ROŞOIU N., Significance of Hematological Parameters and Biochemical Markers in Severe Forms of Covid-19**, Academy of Romanian Scientists, Annals Series on Biological Sciences, 9**, 2, 88-99, **(2020).**

2). VOICULESCU A-L M., ANGHEL A., GURGAS L.,PETCU L. ROŞOIU **N.,**Correlations between biochemical markers in thyroid pathology with those in carbohydrates metabolism, **ARS Medica Tomitana. 26,** 2,100 – 104, **(2020).**

3). VOICULESCU A-L, ANGHEL A., ROŞOIU N., Effects of COVID-19 on the Thyroid Gland, **The Publising House Medicine of the Romanian Academy, Proc. Rom. Acad., Series B**, **23**(1), 132-135 **(2021).**

4). VOICULESCU A-L, ANGHEL A., ROŞOIU N., Immunity from SARS-COV- 2 **The Publising House Medicine of the Romanian Academy, Proc. Rom. Acad., Series B**, **23**(1), 90-93 **(2021).**

II. PAPERS PRESENTED AT VARIOUS NATIONAL AND INTERNATIONAL SCIENTIFIC EVENTS AND PUBLISHED IN ABSTRACT BOOKS)

1). GHIDUS D., VERMAN G.I., MIHAILOV I.C., **TIMOFTE A.M.,** ROSOIU N., The iron in associated pathology of rheumatoid arthritis and chronic hepatitis C, **Academia Oamenilor de Stiinta din Romania, Sesiunea stiintifica de toamna,** 24-26 septembrie, Iasi, 51-52, **(2015).**

2). VERMAN G.I., GHIDUS D., MIHAILOV C.I., **VOICULESCU A.M.,** ROSOIU N, Fibrinogen, iron and antioxidants in chronic hepatitis C, **Academia Oamenilor de Stiinta din Romania, Sesiunea stiintifica de toamna,** 24-26 septembrie, Iasi, 66-67, **(2015).**

3). GHIDUS D., VERMAN I.G., **VOICULESCU A-L.,** MIHAILOV C., ROSOIU N, Rheumatoid Arthritis and Associated Comormobidities, **Academia Oamenilor de Stiinta din Romania, Sesiunea stiintifica de toamna,** 22-24 septembrie Durau - Neamt, 73, **(2016).**

4). VOICULESCU A-L.M., SAMARGIU D.M., ROSOIU N., Thyroid pathology in diabetus mellitus, **Academia Oamenilor de Stiinta din Romania, Sesiunea stiintifica de primavara**, 24 martie Bucuresti, 36, **(2017).**

5). VOICULESCU A.L. SAMARGIU D.M., ROSOIU N., Variation of some Biochemical markers of Thyroid function, **Archives of the Balkan Medical Union, Vol.52,** October Sofia, **P47 (2017).**

6). VOICULESCU A-L., ANGHEL A., GURGAS L., ROSOIU N., The Significance of Hematological Parameters and Biochemical Markers in Severe Forms of COVID-19, **Academia Oamenilor de Stiinta din Romania, Conferinta Stiintifica de Toamna,** 59-61**, (2020).**

7). VOICULESCU A-L., ANGHEL A., GURGAS L., ROSOIU N., Efficiency of vaccines against COVID 19**, Academia Oamenilor de Stiinta din Romania, Conferinta Stiintifica de Primavara,** 43-44**, (2021).**