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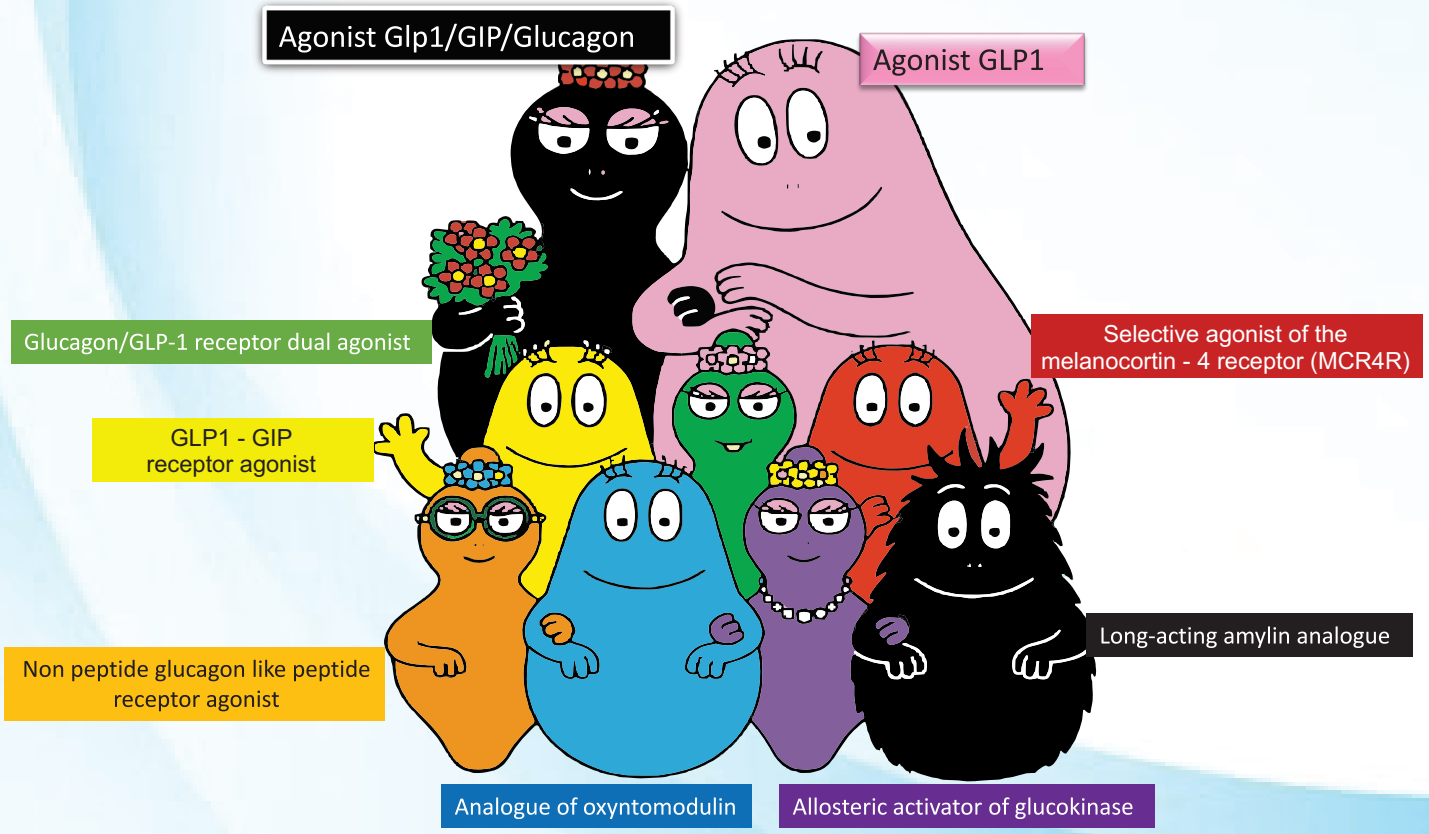
Science finally imitates life!



EDITORIAL

Obesity treatment where are we headed? Science imitating life?

Semaglutide? Tirzapatide ? Retatrutide?

Obesity is a complex and multifactorial condition, resulting from an interaction between genetic, behavioral, environmental and metabolic factors.

The treatment of obesity with drugs that act as agonists of the hormones GLP-1 (Glucagon-Like Peptide 1), GIP (Gastric Inhibitory Peptide) and glucagon is a relatively new and promising approach in the field of obesity medicine. This class of medications is known as "triple GLP-1/GIP/glucagon receptor agonists."

Preproglucagon is a precursor protein that plays a crucial role in the synthesis of several important hormones and peptides in the human body. It is primarily produced in the pancreas and the gastrointestinal tract. Preproglucagon undergoes a series of enzymatic cleavage and processing steps to yield various biologically active peptides, including glucagon, glucagon-like peptide-1 (GLP-1), and glucagon-like peptide-2 (GLP-2).

1.**Synthesis:** Preproglucagon is initially synthesized as a larger, inactive precursor protein in the pancreatic alpha cells and the L cells of the small intestine.

2.**Cleavage:** Preproglucagon is cleaved into proglucagon .

3.**Tissue-specific processing:** The subsequent processing of proglucagon differs between pancreatic alpha cells and intestinal L cells. In pancreatic alpha cells, proglucagon is cleaved by the enzyme prohormone convertase 2 (PC2) to produce glucagon, a hormone that raises blood sugar levels by stimulating the liver to release glucose. In intestinal L cells, proglucagon is processed by prohormone convertase 1/3 (PC1/3) to produce two important hormones: GLP-1 and GLP-2.

They are precursor proteins that give rise to several biologically active peptides, including glucagon, GLP-1, and GLP-2, which play important roles in regulating blood sugar levels and maintaining the gastrointestinal tract health. These peptides have significant implications for diabetes.

Glucagon is released into the bloodstream by pancreatic alpha cells and acts to raise blood sugar levels by stimulating the liver to release glucose. It has the opposite effect of insulin.

During fasting periods, glucagon stimulates glycogenolysis, ketogenesis and gluconeogenesis in the liver, lipolysis in adipose tissue and decreases glycolysis to save energy for the brain. Studies have shown that a missense mutation in the glucagon receptor was associated with type 2 diabetes, suggesting that normal functioning of this hormone is important for maintaining normoglycaemia.

Pemvidutide, analogue of oxyntomodulin, is the new drug being studied targeting both the glucagon receptor and the GLP-1 receptor which has proven useful for the treatment of obesity, type 2 diabetes and non-alcoholic fatty liver disease.

Survodutide is also a glucagon/GLP-1 receptor dual agonist that activates both the GLP-1 and glucagon receptors, which are critical to controlling metabolic functions.

GLP-1: GLP-1 (Glucagon-Like Peptide-1) is an incretin hormone secreted by intestinal L cells in response to nutrient ingestion. It plays a crucial role in regulating blood sugar levels. GLP-1 stimulates insulin release in response to a meal, inhibits glucagon release, slows gastric emptying, and promotes feelings of fullness. It is an important target for the treatment of type 2 diabetes and obesity. In cardiovascular system, GLP1reduces major adverse cardiovascular events. GLP1 protects, in animal models, against neurodegenerative disease.

GLP-2: GLP-2 (Glucagon-Like Peptide-2) is another product of proglucagon processing in intestinal L cells. It promotes the growth and maintenance of the lining of the small intestine and helps in nutrient absorption. GLP-2 is also being studied for its potential therapeutic applications in conditions that affect the gastrointestinal tract.

GIP (Glucose-Dependent Insulinotropic Peptide), it is also known as Gastric Inhibitory Polypeptide is a hormone produced by the K cells of the small intestine, particularly in the duodenum and jejunum. It plays a crucial role in regulating blood sugar levels and metabolic processes in response to food ingestion.

Insulin Release: GIP is an incretin hormone that stimulates the release of insulin from the beta cells in response to elevated blood glucose levels after a meal.

Glucagon Suppression: In addition to stimulating insulin release, GIP also inhibits the secretion of glucagon.

Lipid Metabolism: GIP is an anabolic hormone with effects on lipid metabolism. It can promote the storage of triglycerides in adipocytes and reduce release of stored fats. It regulates amino acid metabolism and inflammation of the adipose tissue by suppression of macrophage- dependent inflammation.

GIP may play a role in appetite regulation by acting on the brain's appetite centers. It can influence feelings of satiety and fullness after a meal.

Decreased: Gastric acid secretion by parietal cells.

Hypersecretion of cortisol: GIP can cause Cushing syndrome by hypersecretion of cortisol after mixed meals even with low ACTH (food induced Cushing syndrome).

Appetite Regulation: Cardiovascular action: current researches into cardiovascular action of GIP is limited.

Neuro protection: Protection against neurodegenerative disease.

That is the story

Step by step, researchers followed the path to treating obesity.

The glucagon agonist “like” peptide promised a miracle in the treatment of obesity and soon proved to be effective in the treatment of type 2 diabetes without counting its pleiotrophic effects on other organs such as the heart, kidneys, liver and brain.

The weight loss achieved with Liraglutide1.8 was not satisfactory in the treatment of obese patients. The evolution of “glutides” led us to Semaglutide, which ranged from subcutaneous to oral form (Step Study). Researches not happy with the current drugs on the market set off to study of the unknown GIP and a new drug was on the market full of promise combining GLP1-GIP – Tirzapatide (SURPASS2 Study).

Without even a real world experience with Tirzapatide, we came across with drugs glucagon/GLP-1 receptor dual agonists and finally with GIP–GLP-1–GCG receptor agonists (triagonism), imitators of human physiology!

Retatrutide and Efinopegdutide they are triple GLP-1, GIP, and glucagon receptor agonist that showed promising in individuals with obesity and mainly Efinopegdutide effectiveness in non-alcoholic fatty liver disease.

The researches believe that this potential association with peptides exhibiting activity at multiple targets, increases the effectiveness in reducing caloric intake and increasing energy expenditure.

Triple agonism! Science finally imitates life!
The imitation of life is not over yet !
Let’s wait for:
Dorzagliatin - allosteric activator of glucokinase
Orforglipron - non peptide glucagon like peptide receptor agonist
Pemvidutide - analogue of oxyntomodulin
Survodutide - glucagon/GLP-1 receptor dual agonist
Cotadutide - another GLP-1 and glucagon receptor agonist
Setmelanotide - selective agonist of the melanocortin-4-receptor (MC4R)
Cagrilintide - long-acting amylin analogue
Berberine - compound derived from the Chinese medicinal plant *Coptis chinensis,*

Mirnaluci Paulino Ribeiro Gama

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Contents

Our Cover: Barba Papas Family

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ORIGINAL ARTICLE
ANALYSIS OF AN INSTITUTIONAL SERIES OF DIFFERENTIATED THYROID CANCER IN THE PEDIATRIC AND ADOLESCENT POPULATION

ANÁLISE DE UMA SÉRIE HISTÓRICA INSTITUCIONAL DE CÂNCER DIFERENCIADO DE TIREOIDE EM UMA POPULAÇÃO INFANTOJUVENIL

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RICARDO RIBEIRO GAMA[1]

Key-words: Thyroid Cancer; Papillary; Adolescent; Head and Neck Neoplasms; Pediatrics.
Descritores: Câncer Papilífero da Tireoide; Saúde do Adolescente; Neoplasias da Glândula Tireoide; Criança

Abstract

Introduction: Differentiated thyroid cancer (DTC) is relatively rare in children and adolescents. Pediatric DTC patients present more often with an aggressive and advanced disease, although they have an excellent prognosis with lower mortality when compared to adults with DTC. **Objective:** The present study aims to analyze the population of pediatric and adolescent patients with DTC in a cancer hospital referral center. **Material and methods**: Retrospective study with analysis of medical records of 67 patients up to 21 years of age treated for DTC over a 10 year period. **Results:** The median age at diagnosis was 15 years. The mean follow-up time was 49 months, with 74.6% of disease-free survival at 12 months. Twenty patients (29.9%) presented recurrent or persistent disease. Multifocality, vascular invasion, and extrathyroidal extension were observed in 31 (46.3%), 33 (49.3%), and 32 (47.8%) patients. Extrathyroidal extension and positive neck lymph nodes were associated with residual disease or recurrence (p=0.01 and p=0.02, respectively). The presence of extrathyroidal extension, multifocality, and vascular invasion were associated with cervical lymph node metastasis (p=0.004, p<0.001 and p=0.002, respectively). Twenty-six (52%) patients presented median stimulated thyroglobulin levels greater than 14ng/mL, which was also identified as a predictor of recurrent or persistent disease (p=0.02). **Conclusion:** Thyroid cancer in children and adolescents presents initially with aggressive clinical characteristics, such as extrathyroidal extension, with a higher risk of recurrent and persistent disease, especially in cervical lymph nodes. It is important to detect cervical lymph node metastasis at diagnosis of DTC in order to minimize reoperations, especially over the first years of follow-up. **Endocrinol diabetes clin exp 2023/ 2394 - 2402.**

Resumo

Introdução: O câncer diferenciado de tireoide é raro na população pediátrica. Este possui características clínicas mais agressivas, embora apresente um excelente prognóstico com baixa mortalidade nesta faixa etária em comparação ao câncer de tireoide nos adultos. **Objetivo:** Este estudo tem o objetivo de levantar a casuística institucional dos pacientes infantojuvenis com câncer de tireoide tratados em hospital oncológico de referência. **Material e métodos:** Estudo retrospectivo com análise de prontuários médicos de 67 pacientes de até 21 anos de idade tratados por câncer de tireoide em um período de 10 anos. **Resultados:** A mediana de idade foi de 15 anos. O tempo médio de seguimento foi de 49 meses, com sobrevida livre de doença em 12 meses de 74,6%. Vinte pacientes (29,9%) recorreram ou apresentaram tumor residual. Foram observadas multifocalidade, invasão vascular e extensão extratireoidiana em 31 (46,3%), 33 (49,3%) e 32 (47,8%) pacientes, respectivamente. A presença de extensão extratireoidiana e de linfonodo cervical metastático estiveram associados à doença residual ou recorrência (p=0,01 e p=0,02, respectivamente). A presença de extensão extratireoidiana, multifocalidade e invasão vascular estiveram associadas à presença de metástase linfonodal cervical (p=0,004, p<0,001 e p=0,002 respectivamente). Vinte e seis (52%) pacientes apresentaram mediana de tireoglobulina estimulada com valor >14ng/mL, a qual foi preditora de doença residual ou recorrência (p=0,02). **Conclusão:** O câncer de tireoide em crianças e adolescentes apresenta característica clínica inicial agressiva, como extensão extratireoidiana, e com maior risco de doença residual ou recorrência, especialmente em linfonodos cervicais. É de extrema importância a detecção de metástases linfonodais cervicais já ao diagnóstico do carcinoma de tireoide, a fim de minimizar as reoperações nos primeiros anos de seguimento. **Endocrinol diabetes clin exp 2023 / 2394 - 2402.**

INTRODUCTION

Despite being the most common endocrine neoplasm in the pediatric population, thyroid cancer is relatively rare in this age group, with an annual incidence of 0.2 to 1 case per million (1,2,3). Pediatric thyroid cancer is more common in females and adolescents, and differentiated thyroid carcinoma (DTC) encompasses 90% of all types of pediatric thyroid cancer, of which papillary thyroid carcinoma is the most common subtype (3,4). DTC in children and adolescents is more aggressive at initial clinical presentation when compared to adults, although it has an excellent therapeutic response and prognosis, with low mortality rates (4).

DTC in childhood is usually associated with extrathyroidal extension and a higher risk of recurrence, when compared to DTC in adults (5). Current literature has reported that male gender, papillary thyroid tumors greater than 1cm, non-papillary histology, presence of distant metastasis, and non-surgical treatment are predictors of worse prognosis (1,5). Children under 10 years of age have a higher risk of cervical lymph node metastasis compared to children older than 10 years, with rates up to 92.5% and 71.4%, respectively (3). Distant metastasis occur most commonly to the lungs (3). Clinical and pathological features such as age, tumor size, multifocality, metastasis to the lateral cervical lymph nodes, and postoperative thyroglobulin levels, are significant predictors for disease recurrence (4).

The surgical treatment for DTC is usually curative and it

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leads to a specific disease-free survival around 99% in 10 years (6). DTC treatment usually encompasses total thyroidectomy with or without cervical lymphadenectomy, radioiodine (RAI) therapy, and thyroid-stimulating hormone (TSH) suppression. Thyroid lobectomy alone is controversial and total thyroidectomy remains the treatment of choice in pediatric patients with DTC. Having said that, aggressive management with total thyroidectomy combined with lymphadenectomy and RAI therapy seems to have better results in preventing recurrence in the pediatric population (5,7).

In Brazil, studies on the clinical and epidemiological profile of thyroid cancer in childhood and adolescence are scarce. Therefore, this study aims to report the clinical, epidemiological and treatment factors associated with DTC in pediatric and adolescent patients, treated at an institutional referral cancer center in Brazil, and to identify clinical features associated with recurrence or residual disease during the follow-up period.

MATERIAL AND METHODS

Retrospective study with evaluation of paper-based and electronic medical records of pediatric and adolescent patients up to 21 years with DTC, who had undergone treatment at the Barretos Cancer Hospital, Brazil, in a ten year period (2010 to 2020), identified by the institutional hospital cancer registry. The authors found 106 patients registered and admitted at the institution in the selected period. Of these, 67 patients were selected for the study and 39 patients were excluded due to loss of follow-up or if the time of clinical follow-up was considered insufficient by the researchers.

The variables collected in the medical records were sociodemographic, clinical and pathological, besides the treatment performed and the presence of recurrence and residual tumor (follow-up data). The data were stored in the **Redcap®** platform (*Electronic Research Data Capture*) (8,9). The study was approved by the Research Ethics Committee of the Barretos Cancer Hospital.

In the statistical analysis, the qualitative variables were described through absolute frequencies and percentages. The quantitative variables were described by mean and standard deviation or median, according to their distribution. Student's t-test was used for data that followed normality and the Mann-Whitney test for non-parametric data, in order to compare groups of continuous variables. For categorical variables, the Chi-square test or Fisher's exact test were used to compare the proportions between groups. For disease-free survival curves, the Kaplan-Meier method was used. The log rank test was applied to calculate survival rates. For the univariate analysis of proportional risk, the Cox regression model was utilized. The data were analyzed using SPSS (*Statistical Package for the Social Sciences*) version 22.0, with 5% of significance level.

RESULTS

The final sample included 67 patients (**Table 1**). Of these, the majority were female - 48 (71.6%) and self-reported as white ethnicity - 45 (72.6%). The mean age of the sample was 14.7 years, with a median of 15 years. At admission, 51 (76.1%) patients presented with normal thyroid function, 12 (17.9%) with hypothyroidism and 4 (6.0%) with hyperthyroidism. The size of the punctured nodule at ultrasound, was 1 to 4 cm in 42 (79.3%) patients. Regarding the presence of lymph node metastasis documented preoperatively, 11 (50%) patients were considered N1. The location of the punctured malignant lymph node was in the lateral cervical level in 7 (63.6%) patients. More than 45% of patients had nodules classified as Bethesda VI at the preoperative cytological evaluation.

The pathological findings (**Table 2)** showed that 47 (70.1%) patients had the classic papillary histological subtype. The size of the largest malignant nodule at pathology was between 1-4cm in 43 (64.2%) patients. Multifocality, vascular invasion, and extrathyroidal extension were observed in 31 (46.3%), 33 (49.3%) and 32 (47.8%) patients, respectively.

Regarding the treatment performed (**Table 3)**, 60 (89.6%) patients underwent total thyroidectomy and 7 (10.4%) patients underwent partial thyroidectomy. In this sample, 41 (61.2%) patients underwent cervical lymphadenectomy. Of those submitted to lymphadenectomy, 26 (63.4%) patients underwent central cervical lymphadenectomy combined with lateral cervical lymphadenectomy. Of the 53 (79.1%) patients who underwent RAI therapy, 45 (84.9%) were treated with a dose between 100 to 200mCi, with stimulated thyroglobulin level median at 131I whole body scintigraphy (WBS) greater than 14ng/mL in 26 (52%) patients. The 131I whole body scintigraphy (131I WBS) showed that 24 (46.1%) patients had uptake at the surgical site exclusively, while 16 (30.8%) patients had lung radioiodine uptake.

Disease-free survival analysis

The mean follow-up time was 49 months. Disease-free survival at 12 months was 74.6% and at 60 months was 69.2%. Twenty patients (29.9%) had recurrence or residual tumor, while 47 patients (70.1%) had no events (**Figure 1**).

The stimulated thyroglobulin level (at 131I WBS after thyroidectomy) greater than 14ng/mL was statistically significant in relation to disease-free survival with p=0.01 - **Figure 2c.** However, there was no statistical significance in relation to gender and median age, with p values of 0.64 and 0.89, respectively (**Figures 2a and 2b**).

Regarding pathological variables, extrathyroidal extension and positive pathological lymph node stage were associated with lower disease-free survival (p=0.01 for both variables) - **Figures 2e, 2h**. The other variables such as multifocality, vascular invasion and thyroid tumor size at pathology were not statistically significant (p=0.12, 0.19 and 0.13, respectively) - **Figures 2d, 2f, 2g**.

Univariate disease-free survival analysis of clinical and pathological variables

The analysis of clinical and pathological variables showed that stimulated thyroglobulin level at 131I WBS greater than 14ng/mL (HR 3.83, 95% CI 1.2-11.9, p=0.02), the presence of extrathyroidal extension (HR 3.21, 95% CI 1.2-8.3, p=0.01), and the presence of neck lymph node metastasis (HR 5.49, 95% CI 1.2-23.7, p=0.02), were all predictors of recurrent or persistent thyroid cancer - **Table 4.**

Correlation of clinical and pathologic variables with cervical lymph node metastasis.

When analyzing the different demographic, clinical and pathological variables comparing patients with pathological N0 stage (negative neck for malignancy) with N1 stage (positive neck for malignancy), it was observed that the presence of extrathyroidal extension (p=0.004), multifocality (p<0.001) and vascular invasion (p=0.002) were associated with the presence of cervical lymph node metastasis - **Table 5.**

Table 1. Clinical and demographic characteristics of the study population.

Variables	Categories	Frequency - n (%)
Gender	Male	19 (28.4)
	Female	48 (71.6)
Self-reported ethnicity*	White	45 (72.6)
	Non-white	17 (27.4)
Thyroid function at admission	Hypothyroid	12 (17.9)
	Euthyroid	51 (76.1)
	Hyperthyroid	4 (6)
Nodule size punctured at ultrasound - US (cm)**	Microcarcinoma (<1cm)	6 (11.3)
	1 to 4 cm	42 (79.3)
	>4 cm	5 (9.4)
Lymph node cervical metastasis confirmed at preoperative setting***	Yes	11 (50)
	No	6 (27.3)
	Inconclusive	5 (22.7)
Location of the punctured lymph node cervical metastasis******	Central (VI level)	1 (9.1)
	Lateral (II, III or IV levels)	7 (63.6)
	More than one location	3 (27.3)
Preoperative Bethesda classification*******	I	2 (3.6)
	II	4 (7.3)
	III	4 (7.3)
	IV	4 (7.3)
	V	16 (29)
	VI	25 (45.5)

* 5 missing cases; ** 14 missing cases; *** only lymph nodes punctured at US were considered; **** only malignant punctured lymph nodes at preoperative setting were considered; ***** 12 missing cases.

Table 2. Description of the pathological variables.

Variables	Categories	Frequency - n (%)
DTC subtype	Classic papillary thyroid carcinoma	47 (70.1)
	Follicular variant of papillary thyroid carcinoma	10 (14.9)
	Non-invasive follicular thyroid neoplasm with papillary like nuclear features (NIFTP)	5 (7.5)
	Follicular carcinoma	3 (4.5)
	Other subtypes	2 (3)
Multifocality	No	36 (53.7)
	Yes	31 (46.3)
Size of the greatest malignant nodule at pathology (cm)	<1cm	12 (17.9)
	1 to 4 cm	43 (64.2)
	>4 cm	12 (17.9)
Vascular invasion	No	34 (50.7)
	Yes	33 (49.3)
Extrathyroidal extension	No	35 (52.2)
	Yes	32 (47.8)

Table 3. Description of treatment variables.

Variables	Categories	Frequency - n (%)
Thyroidectomy extension	Partial thyroidectomy	7 (10.4)
	Total thyroidectomy	60 (89.6)
Cervical lymphadenectomy#	No	26 (38.8)
	Yes	41 (61.2)
Cervical lymphadenectomy subtype#	Central lymphadenectomy	12 (29.3)
	Lateral lymphadenectomy	3 (7.3)
	Central and lateral lymphadenectomy	26 (63.4)
Radioiodine (RAI) treatment	No	14 (20.9)
	Yes	53 (79.1)

RAI dose (mCi)	<100	6 (11.3)
	100 to 200	45 (84.9)
	> 200	2 (3.8)
Stimulated thyroglobulin level at 131I WBS (ng/mL)*	≤ 14	24 (48)
	> 14	26 (52)
131I whole body scintigraphy (131I WBS) result**	Exclusive surgical site uptake	24 (46.1)
	Surgical site and neck uptake	6 (11.5)
	Mediastinal and neck uptake	5 (9.6)
	Lung uptake	16 (30.8)
	Other sites uptake	1 (2)
Presence of residual tumor or recurrence	No	47 (70.1)
	Yes	20 (29.9)

* 3 missing cases; ** 1 missing case; # the number and type of cervical lymphadenectomy can be underestimated due to difficulties in distinguishing node picking from lymphadenectomy in the pathology reports from patients operated on outside the author´s institution.

Figure 1: Disease-free survival of the study population.

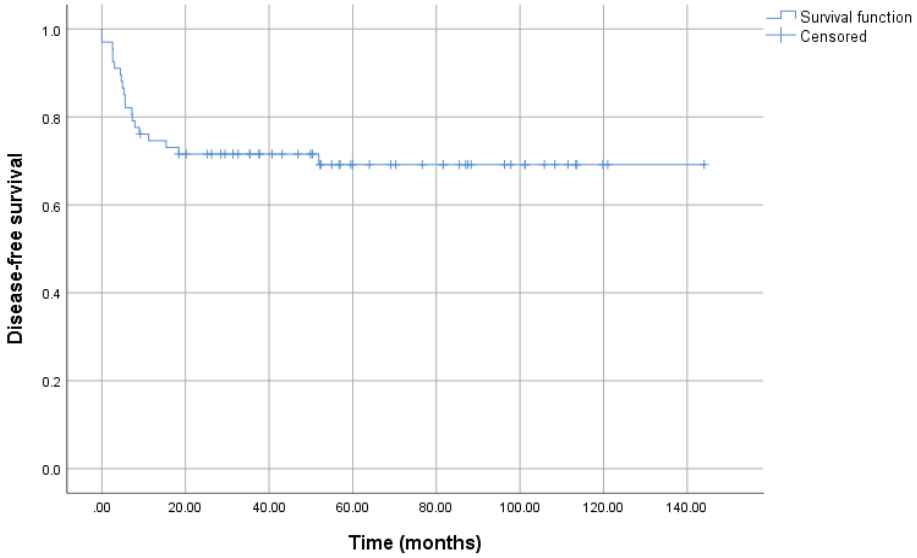

Table 4. Univariate analysis of disease-free survival predictors.

Variables	Categories	HR	CI (95%)	p value
Gender	Male	1.25	0.4 – 3.2	0.64
	Female	Reference		
Median age (years)	<= 15	Reference		
	> 15	1.06	0.4 – 2.5	0.8
Stimulated thyroglobulin level at 131I WBS (ng/mL)	<= 14	Reference		
	> 14	3.83	1.2 – 11.9	**0.02**
Tumor size (pathology) - cm	< 1	Reference	-	0.16 (*global*)
	1 a 4	1.97	0.4 – 8.8	0.3
	> 4	4.12	0.8 – 20.7	0.08
Multifocality	No	Reference		
	Yes	2.0	0.8 – 4.9	0.1
Extrathyroidal extension	No	Reference		
	Yes	3.21	1.2 – 8.3	**0.01**
Vascular invasion	No	Reference		
	Yes	1.8	0.7 – 4.4	0.1
Positive malignant cervical lymph node	No	Reference		
	Yes	5.49	1.2 - 23.7	**0.02**

Table 5. Association of cervical lymph node metastasis with demographic, clinical and pathological variables.

Variables	N0 (n/%) n=22 (32.8%)	N1 (n/%) n=45 (67.2%)	p value
Gender			
Male	7 (31.8)	12 (26.7)	0.6
Female	15 (68.2)	33 (73.3)	
Median age (years)			
≤ 15	12 (54.5)	24 (53.3)	0.9
> 15	10 (45.5)	21 (46.7)	
Nodule size at pathology (cm)			
< 1	6 (27.3)	6 (13.3)	
1 a 4	14 (63.6)	29 (64.4)	0.2
> 4	2 (9.1)	10 (22.2)	

Multifocality			
Yes	3 (13.6)	28 (62.2)	**<0.001**
No	19 (86.4)	17 (37.8)	
Extrathyroidal extension			
Yes	5 (22.7)	27 (60)	**0.004**
No	17 (77.3)	18 (40)	
Vascular invasion			
Yes	5 (22.7)	28 (62.2)	**0.002**
No	17 (77.3)	17 (37.8)	

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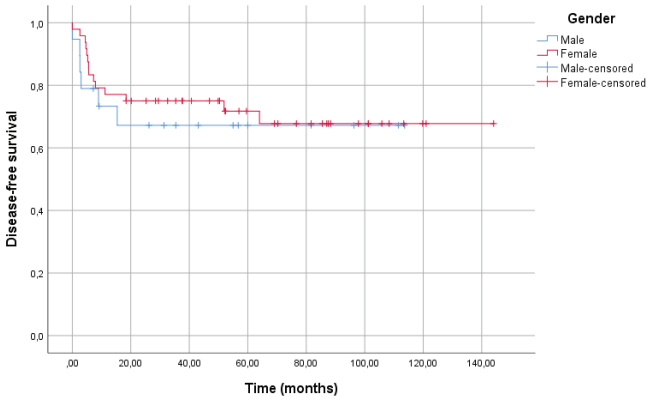

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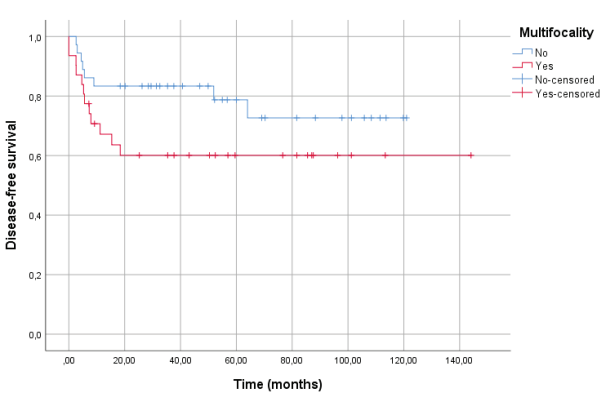

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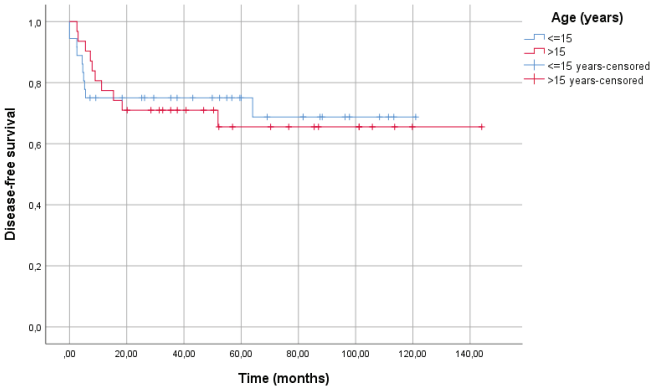

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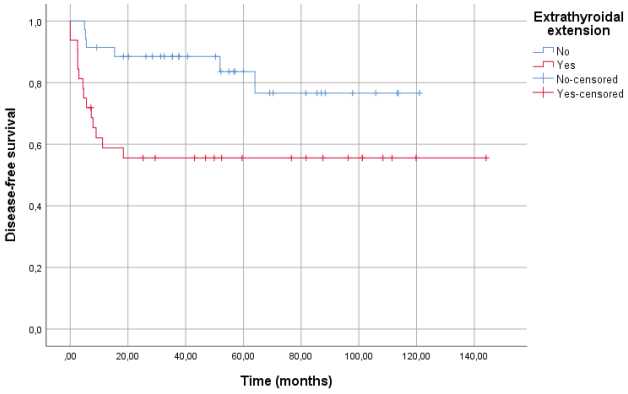

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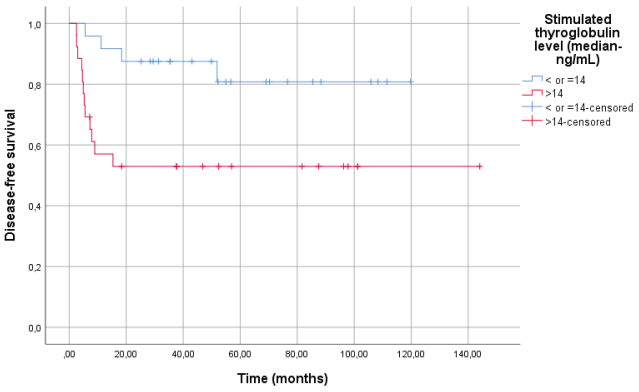

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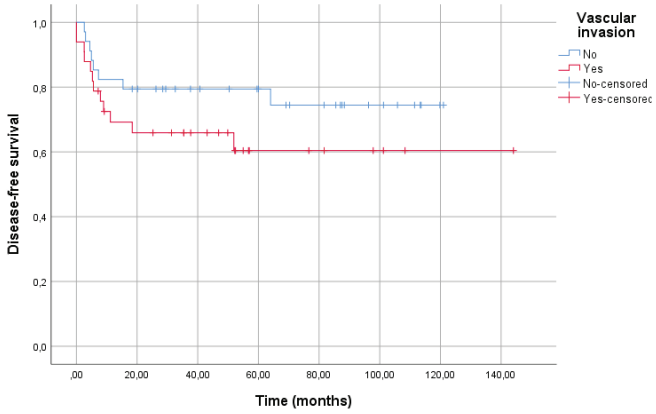

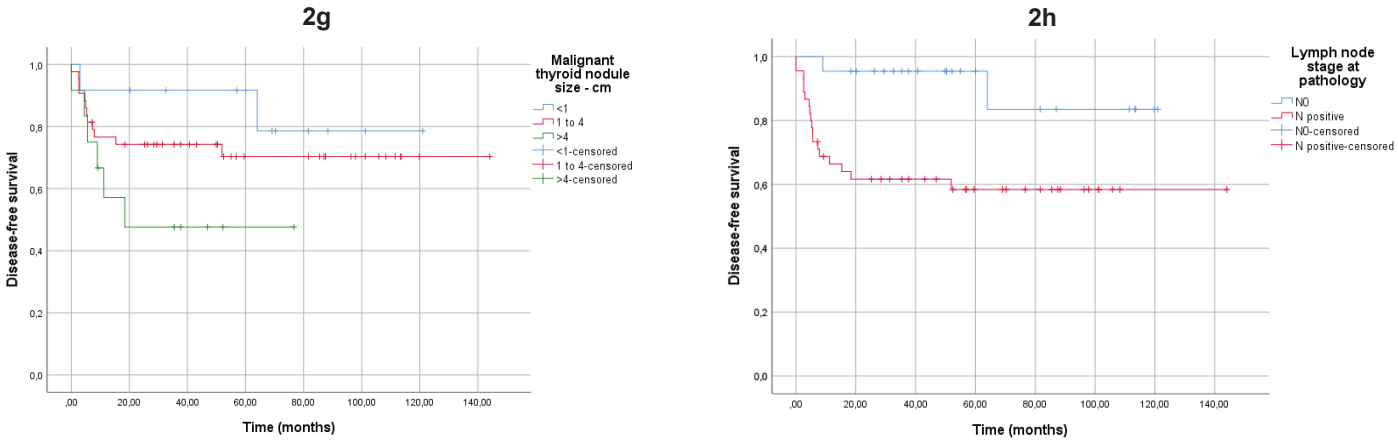

Figure 2: Disease-free survival curves according to gender – **2a**, p=0.64; age - **2b**, p=0.89; median post-treatment stimulated thyroglobulin - **2c**, p=0.01; multifocality - **2d**, p=0.12; extrathyroidal extension - **2e**, p=0.01; vascular invasion - **2f**, p=0.19; malignant thyroid nodule size at pathology - **2g**, p=0.13; and lymph node stage at patology - **2h**, p=0.01

DISCUSSION

DTC has a relatively low incidence in children and adolescents when compared to the disease in adults (1,4). In the present study, only DTC, particularly papillary thyroid carcinoma and follicular carcinoma, were retrospectively analyzed in a cancer center in Brazil, in a population up to 21 years of age.

In relation to the sample studied, the mean age was approximately 15 years, being the majority female (71.6%), with white ethnicity (72.6%), and with the classic papillary carcinoma at pathology report (70.1%). Hogan et al. (1) analyzed data published by the SEER (*Surveillance, Epidemiology, and End Results*), of the National Cancer Institute (USA), which contained 1,753 patients from 1 to 19 years who were diagnosed with thyroid carcinoma from 1973 to 2004. According to the data analyzed, thyroid carcinoma was more frequent in females when compared to males (81% and 18.8%, respectively), in white patients (86%), in patients aged between 15 and 19 years (74%), and with papillary subtype (60%). The mean age at diagnosis was 16 years and was related to previous exposure to radiation therapy for the treatment of malignant diseases in childhood in 2.4% of patients. In the present study, 3 (4.4%) patients had previous exposure to ionizing radiation. In one of the largest pediatric thyroid cancer data ever published, Hay et al. (10) obtained a sample of 218 patients. The mean age at diagnosis was 16 years, 71% were female and 17% had history of previous cervical irradiation.

Regarding preoperative cytology report, 41 patients had Bethesda V and VI categories, representing 74.5% of the sample. The authors believe that due to Barretos Cancer Hospital being also a reference in juvenile cancer treatment in Brazil, the vast majority of cases referred or accepted were Bethesda V or VI. Even so, the lower number of Bethesda III and IV cases calls attention in comparison with the adult population attended in the same institution. In the study of Byeon et al. (4) with 83 patients analyzed, 69.8% were Bethesda IV, V, and VI at the preoperative cytological evaluation.

In the present study, it was observed that most patients (64.2%) presented at pathological evaluation of the surgical specimen, tumors between 1 and 4cm. The presence of extrathyroidal extension was present in 47.8% of the patients and multifocality in 46.3%. In addition, roughly 67% of the patients had initial cervical lymph node metastasis at pathology. Byeon et al. (4) demonstrated the presence of extrathyroidal extension in 73.6% and multifocality in 22.6% of their study population. Kim et al. (5) showed that 46.8% of the sample studied had tumors larger than 2cm, 36.2% with extrathyroidal extension, and 23.4% with multifocality. Hay et al. (10) reported a median size of 2.2cm of the malignant nodule, 29% of multicentricity, 18% of extrathyroidal extension, 78% of initial lymph node involvement, and 6% of distant metastasis. In the present study, the 131I whole body scintigraphy (131I WBS) showed that 16 patients (24.2%) presented lung disease, in agreement with the literature that demonstrates a relatively high prevalence of distant metastasis, especially located in lungs, in the pediatric population with thyroid cancer (1).

Regarding the treatment performed, 89.6% of patients underwent total thyroidectomy. Patients with pediatric thyroid cancer are typically treated with total thyroidectomy and therapeutic lymph node dissection if lymph node metastasis are identified at the preoperative setting or at surgery, followed by adjuvant or therapeutic RAI and TSH suppression if the risk of recurrence is high or in the presence of distant metastasis of principle (11). The indication for total thyroidectomy is controversial, but has scientific support due to the high rates of multifocal disease (65%) and bilateral disease (30-40%), in the pediatric population with thyroid cancer (12). In the present study, most patients required cervical lymphadenectomy (61.2%) and RAI therapy (79.1%). Hay et al. (10) reported that 87% of patients underwent a bilateral lobar procedure, 19% underwent central lymphadenectomy, 13% unilateral lateral cervical lymphadenectomy, and 13% bilateral lateral cervical lymphadenectomy. Iodine treatment was performed in 35% of patients with localized disease who underwent complete tumor resection. The study of Byeon et al. (4), analyzed 18 years (2000-2018) of records of 83 pediatric patients with thyroid carcinoma, with a mean age of 16.8 years. In this study, total thyroidectomy was performed in 42% of the patients, with central cervical dissection in 48 (58%) patients and lateral cervical dissection in 19 (23%) patients. Postoperative adjuvant RAI therapy was considered in 45 (54%) patients who presented extrathyroidal extension of the tumor or presence of cervical lymph node metastasis.

The mean follow-up time of the study was 49 months. Disease-free survival at 12 months was 74.6%. The study by Hay et al. (10) showed a median follow-up time of 28 years and a specific disease-free survival of 98% in 50 years. In terms of recurrence, 32% of patients relapsed locally or at distant sites. In the present study, the recurrence rate was about 30%, corroborating with the findings of Hay et al (10). Most recurrences occured in the first 2 years after treatment, as reported by literature (13). At the end of this study, only 2 patients (3%) died from the disease, 7 (10.4%) patients still had disease under treatment, and 58 (86.6%) patients were alive without disease.

In the present study, there were predictors of recurrence or presence of residual disease such as stimulated thyroglobulin levels >14ng/mL at 131I WBS, extrathyroidal extension at pathology report and cervical lymph node metastasis. Multifocality, vascular invasion and tumor size did not reach the same significance. The study of Byeon et al. (4) showed that disease-free survival analysis was associated with significant prognostic markers such as patient age, tumor size, multifocality, lateral lymph node metastasis, and postoperative thyroglobulin levels. When analyzing clinical outcomes, Hogan et al. (1) in a multivariate analysis of the sample, showed that male gender, non-papillary histology, presence of distant metastasis, absence of surgical treatment and radiotherapy to treat medullary thyroid carcinoma were independent predictors of worse prognosis. In addition, Popvtzer et al. (7) evaluated data from 75 children with DTC treated from 1954 to 2001. The local recurrence rate was 5% and regional 9%, and all deaths occurred due to distant metastasis. The type of cervical lymphadenectomy did not affect the recurrence or distant metastasis onset and concluded that regional disease at initial presentation or at recurrence did not affect survival in the pediatric population.

A retrospective study of Kim et al. (5) analyzed 94 pediatric patients aged 5 to 19 years diagnosed with DTC who underwent surgical treatment between 1982 and 2012. Bilaterality, multifocality, and extrathyroidal extension of the disease were found in 14 (14.9%), 22 (23.4%), and 34 (36.2%) patients, respectively. Only two (2.1%) patients presented distant (lung) metastasis at diagnosis and 14 (14.9%) patients had recurrence after treatment initiation. Cervical lymph node metastasis were observed in 75.5% of the patients. Multivariate disease-free survival analysis identified tumor size greater than 2cm and positive cervical lymph nodes as significant predictors. Regarding the correlation of the amount of metastatic cervical lymph nodes on the total dissected cervical lymph nodes (LNR ratio), patients were divided in LNR <0.4 and LNR >0.4. High LNR values were significantly associated with age (p=0.044), extrathyroidal extension (p=0.022), and nodal stage (p<0.001), however, the LNR did not correlate with recurrence rate (p=0.131). In the present study, extrathyroidal extension, multifocality, and vascular invasion were associated with the presence of lymph node metastasis. Kim et al. (5) showed association between the number of metastatic cervical lymph nodes and the presence of extrathyroidal tumor extension (5). Cherella et al. (14) observed that multifocality was not necessarily related to regional lymph node involvement, since children presenting with clinically low-risk cN0 DTC, bilateral disease was present in 45% of subjects with multifocality in the primary lobe.

The main limitation of this study was the sample size and the mean follow-up time was relatively short (49 months) for a pathology that usually present late recurrences. The reduced number of patients also reflects the rarity of this type of disease in pediatric patients. The main reason for exclusion of patients in the study were when RAI therapy was the sole treatment done at our institution, without sufficient time of clinical follow-up, since many patients were followed-up in their local health units. Even so, the authors consider that the sample size of the present study, for a ten year period, is relatively adequate, since Hay et al. (10) reported a sample size of 218 patients (one of the largest series of pediatric thyroid cancer ever published), over a period of 68 years.

It is important to emphasize that the management of DTC is complex due to the rarity of the disease and due to clinical and biological differences in comparison with adult thyroid cancer. The lack of consensus for the management and treatment of pediatric thyroid cancer inspired the authors of this study to report this series. Multicenter studies of large scale and scope are important in order to define additional more robust guidelines for the management of pediatric thyroid cancer with greater level of evidence and better scientific basis.

CONCLUSION

Despite the aggressive initial clinical presentation of DTC in children and adolescents, with a high rate of positive lymph nodes and distant metastasis at the time of diagnosis, besides extrathyroidal extension, multifocality, and a considerable recurrence rate, the response to treatment is usually excellent, leading to neoplasia control and a low mortality rate. Because most relapses are seen in the first 2 years, many are residual disease detected after initial treatment, especially at 131I WBS. Therefore, it is extremely important to detect cervical lymph node metastasis already at diagnosis of thyroid carcinoma in order to minimize reoperations, especially in the first years of follow-up.

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ORIGINAL ARTICLE
BIOINFORMATICS UNRAVELS THE EPIGENETIC MECHANISMS OF HASHIMOTO'S THYROIDITIS: DECIPHERING MOLECULAR COMPLEXITY

BIOINFORMÁTICA DESVENDA OS MECANISMOS EPIGENÉTICOS DA TIREOIDITE DE HASHIMOTO: DECIFRANDO A COMPLEXIDADE MOLECULAR

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Keywords: Hashimoto's Thyroiditis; Epigenetic; Bioinformatics
Descritores: Tireoidite de Hashimoto; Epigenética; Bioinformática

Abstract

Introduction: Recent research in the field of epigenetics has shed light on the impact of epigenetic modifications in the development and progression of Hashimoto's thyroiditis (HT). However, the epigenetic roles in HT are still not fully elucidated. **Objective:** To exhibit an in silico representation of the epigenetic mechanism in HT development and explicate their function in the pathogenesis of the ailment. **Material and Methods:** Genetic data were retrieved from GEO database (NCBI) for DNA methylation assessment through bioinformatics. We evaluated 6 HT samples from GSE29315 dataset. Normalization of the data was performed to identify differentially expressed genes (DEGs). Standardization of all expression data was accomplished using the R programming language. The R package was employed for the analysis of DEGs. **Results:** The expression data from the 6 HT specimens in GSE29315 (GSM724489, GSM724490, GSM724491, GSM724492, GSM724493, GSM724494) were patterned. In total, 71 DEGs, including 63 positively regulated genes and 7 negatively regulated genes, were identified. In the *in silico* simulation of the methylated regions in gene GSE29315, we identify specific CpG sites within the analyzed regions that showed significant methylation changes: Region 1 - Promoter Region: CpG site 1: Hypomethylated (40% methylation), CpG site 2: Hypomethylated (35% methylation), and CpG site 3: Hypomethylated (38% methylation); Region 2 - Enhancer Region: CpG site 4: Hypermethylated (80% methylation). CpG site 5: Hypermethylated (75% methylation), and CpG site 6: Hypermethylated (85% methylation); Region 3 - Transcription Start Site: CpG site 7: Hypomethylated (30% methylation), CpG site 8: Hypomethylated (25% methylation), and CpG site 9: Hypomethylated (28% methylation); Region 4 - Intronic Region: CpG site 10: Hypermethylated (70% methylation), CpG site 11: Hypermethylated (65% methylation), and CpG site 12: Hypermethylated (75% methylation). **Conclusion:** Our *in silico* analysis of the GSE29315 gene revealed significant hypermethylation in specific regions, which could lead to gene silencing or altered gene expression. **Endocrinol diabetes clin exp 2023 / 2403 - 2407.**

Resumo

Introdução: Pesquisas recentes no campo da epigenética têm fornecido informações sobre o impacto das modificações epigenéticas no desenvolvimento e progressão da tireoidite de Hashimoto (TH). No entanto, os papéis epigenéticos na TH ainda não estão totalmente elucidados. **Objetivo:** Apresentar uma demonstração in silico do mecanismo epigenético no desenvolvimento da TH e explicar sua função na patogênese da doença. **Material e Métodos:** Os dados genéticos foram obtidos do banco de dados GEO (NCBI) para avaliação da metilação do DNA por meio de bioinformática. Avaliamos 6 amostras do banco de dados GEO (NCBI) de TH do conjunto de dados GSE29315. A normalização dos dados foi realizada para identificar genes diferencialmente expressos (DEGs). A padronização de todos os dados de expressão foi realizada utilizando a linguagem de programação R. O pacote R foi utilizado para a análise dos DEGs. **Resultados:** Os dados de expressão das 6 amostras de TH em GSE29315 (GSM724489, GSM724490, GSM724491, GSM724492, GSM724493, GSM724494) foram analisados. No total, foram identificados 71 DEGs, incluindo 63 genes regulados positivamente e 7 genes regulados negativamente. Na simulação *in silico* das regiões metiladas no gene GSE29315, identificamos locais específicos de CpG dentro das regiões analisadas que mostraram mudanças significativas na metilação: Região 1 - Região do Promotor: Local de CpG 1: Hipometilado (40% de metilação), Local de CpG 2: Hipometilado (35% de metilação) e Local de CpG 3: Hipometilado (38% de metilação); Região 2 - Região do Enhancer: Local de CpG 4: Hipermetilado (80% de metilação), Local de CpG 5: Hipermetilado (75% de metilação) e Local de CpG 6: Hipermetilado (85% de metilação); Região 3 - Local de Início de Transcrição: Local de CpG 7: Hipometilado (30% de metilação), Local de CpG 8: Hipometilado (25% de metilação) e Local de CpG 9: Hipometilado (28% de metilação); Região 4 - Região Intrônica: Local de CpG 10: Hipermetilado (70% de metilação), Local de CpG 11: Hipermetilado (65% de metilação) e Local de CpG 12: Hipermetilado (75% de metilação). **Conclusão:** Nossa análise *in silico* do gene GSE29315 revelou uma hipermetilação significativa em regiões específicas, o que pode levar ao silenciamento do gene ou à expressão gênica alterada. **Endocrinol diabetes clin exp 2023 / 2403- 2407.**

INTRODUCTION

Hashimoto's thyroiditis (HT) is the most prevalent autoimmune thyroid disorder, affecting millions worldwide, and the etiology of HT involves a complex interplay between genetic and environmental factors (1). Recent research in the field of

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epigenetics has shed light on the impact of epigenetic modifications in the development and progression of HT (2). Epigenetics refers to heritable changes in gene expression patterns that do not involve alterations in the DNA sequence. These modifications include DNA methylation, histone modifications, and non-coding RNA molecules, which can either activate or repress gene expression (3).

DNA methylation is a key epigenetic modification involved in gene silencing. Aberrant DNA methylation patterns have been observed in HT patients, contributing to altered gene expression (4). Several genes related to immune response and thyroid function have been found to be hypermethylated in HT, including those encoding thyroid peroxidase, thyroid stimulating hormone receptor, and thyroglobulin. These epigenetic changes may disrupt normal thyroid function and contribute to the autoimmune response against thyroid antigens (5).

Histone modifications play a critical role in chromatin remodeling and gene regulation. Altered patterns of histone modifications have been associated with HT development (6). Increased levels of histone acetylation and methylation have been observed in HT patients, affecting genes involved in immune response and thyroid function. Histone deacetylases and histone methyltransferases are important enzymes that regulate histone modifications. Dysregulation of these enzymes may contribute to the pathogenesis of HT (7).

Non-coding RNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), have emerged as crucial regulators of gene expression. Dysregulation of miRNAs has been implicated in various autoimmune diseases including HT. Altered expression of specific miRNAs in HT patients has been associated with immune dysfunction, thyroid hormone synthesis, and apoptosis (8). Furthermore, lncRNAs have been shown to control gene expression by interacting with chromatin and transcription factors. The role of lncRNAs in HT pathogenesis is an evolving area of research (9).

In addition to genetic factors, environmental triggers play a significant role in HT development, and these environmental factors can induce epigenetic modifications, linking the environment to altered gene expression patterns. For example, excessive iodine intake has been shown to influence DNA methylation patterns in the thyroid gland, potentially promoting autoimmune responses (10).

The purpose of this manuscript is to exhibit an *in silico* representation of the epigenetic mechanism in HT development and explicate their function in the pathogenesis of the ailment.

MATERIAL AND METODS

Study Design and Data Collection

- Genomic and epigenomic datasets were obtained from publicly available datasets established to HT.
- We selected datasets that included relevant information on epigenetic modifications such as DNA methylation, histone modifications and chromatin accessibility.
- Careful evaluation of datasets was performed to ensure inclusion of appropriate sample sizes, experimental conditions and relevant clinical information.

Data Preprocessing and Quality Control

- Data quality control checks were performed to identify and remove any discrepant samples or experimental artifacts.
- Datasets were normalized and pre-processed to minimize batch effects, technical variability, and non-biological variations.
- Established methods and tools were used for data normalization, such as quantile normalization, to ensure accurate and comparable results.

Differential Epigenetic Analysis

- Quality control checks were conducted to identify and remove any discrepant samples or experimental artifacts.
- The data sets were normalized and pre-processed to minimize batch effects, technical variability, and non-biological variations.
- Established methods and tools, such as quantile normalization, were used to ensure accurate and comparable results.

Functional annotation and pathway analysis

- Differentially methylated regions (DMRs) and differentially expressed genes (DEGs) identified with relevant genomic features, such as gene promoters, enhancers or specific genomic regions associated with autoimmunity, were annotated.
- Gene ontology (GO) enrichment analysis, pathway analysis or network analysis tools were used to identify enriched biological functions and pathways associated with epigenetic changes in HT.
- Molecular pathways and biological processes known to be involved in the pathogenesis of HT were prioritized.

Integration of epigenetic data with other omics data

- Epigenetic changes identified were integrated with other omics data, such as transcriptomic and proteomic data available.
- Integrative analyses were performed to identify possible epigenetic regulators or key regulators that could modulate gene expression and contribute to HT.

Data interpretation and visualization

- The results were interpreted in the context of current knowledge about HT and epigenetic regulations.

Bioinformatics tools used

- "R" (https://www.r-project.org/). R is a statistical programming language that offers a wide range of packages and tools for data analysis, including specific packages for epigenetic data analysis. R allows for flexible and customized analysis, enabling researchers to adjust algorithms and methods according to their specific needs and hypotheses.
- "Bioconductor" (https://www.bioconductor.org/), which is a collection of R packages specific for biological data analysis. Bioconductor offers a wide range of packages for epigenetic data analysis, such as DNA methylation analysis, histone modification analysis, and integration of epigenetic data with gene expression data.
- "PyMethylProcess" (https://pypi.org/project/pymethylprocess/) is a Python package that is also widely used for DNA methylation data analysis. It provides a variety of functions for analysis, pre-processing, visualization, and integration of DNA methylation data.

Genetic data were retrieved from GEO database (NCBI) for DNA methylation assessment through bioinformatics. We evaluated 6 HT samples from GSE29315 dataset. Normalization of the data was performed to identify DEGs. GO and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were conducted on the DEGs. Standardization of all expression data was accomplished using the R programming language. The R package was employed for the analysis of DEGs. Genes exhibiting an expression fold change greater than 4 and a P-value less than 0.05 were considered to be DEGs.

Due to its non-involvement of human subjects and the sole usage of bioinformatics tools, this study exempts the submission to the research ethics committee for evaluation.

RESULTS

Identification of HT DEGs

The expression data from the 6 HT specimens in GSE29315 (GSM724489, GSM724490, GSM724491, GSM724492,

GSM724493, GSM724494) were patterned (**Figure 1**). In total, 71 DEGs, including 63 positively regulated genes and 7 negatively regulated genes, were identified (**Figure 2**). An expression density plot was used to display the clustering of DEGs (**Figure 3**), and average log-expression was constructed to visually display all DEGs in the HT sample (**Figure 4**). Among the identified DEGs, the top 8 positively regulated genes were CD37, CD48, CD52, CXCL9, EVI2B, IGLC1, IGJ, and RGS1. Additionally, the 7 negatively regulated genes were ANXA3, HSD17B6, LMO3, LRRN3, IGSF1, MT1G, and MT1X.

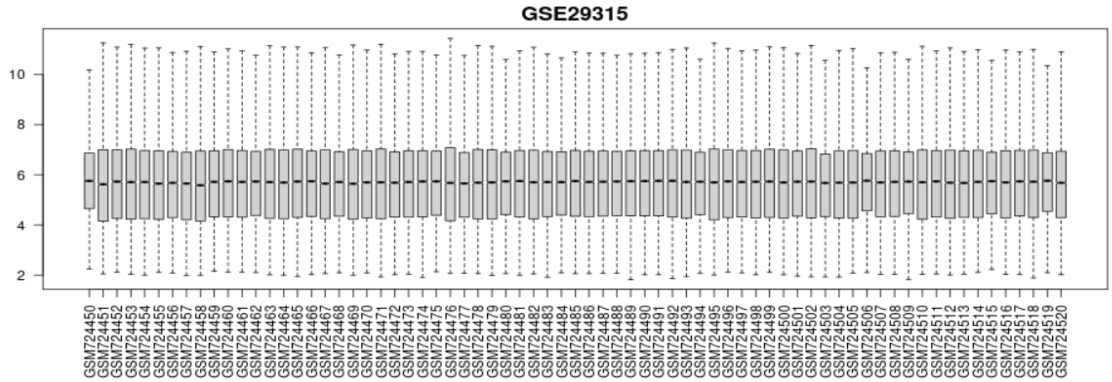
Figure 1. HT specimens in GSE29315.

Samples | Define groups

-	GSM724489	HS001	tumor	hashimoto thyroiditis
-	GSM724490	HS002	tumor	hashimoto thyroiditis
-	GSM724491	HS003	tumor	hashimoto thyroiditis
-	GSM724492	HS004	tumor	hashimoto thyroiditis
-	GSM724493	HS005	tumor	hashimoto thyroiditis
-	GSM724494	HS006	tumor	hashimoto thyroiditis

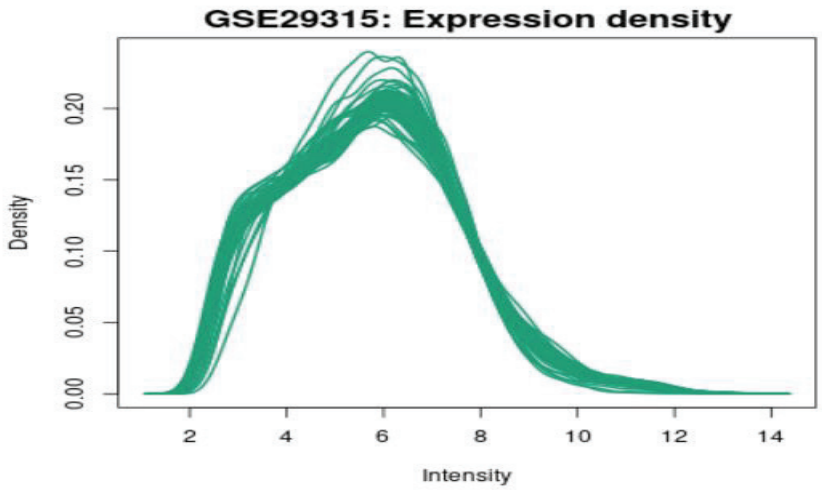
Source: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE29315

Figure 2. Standardization of gene expression data in samples.



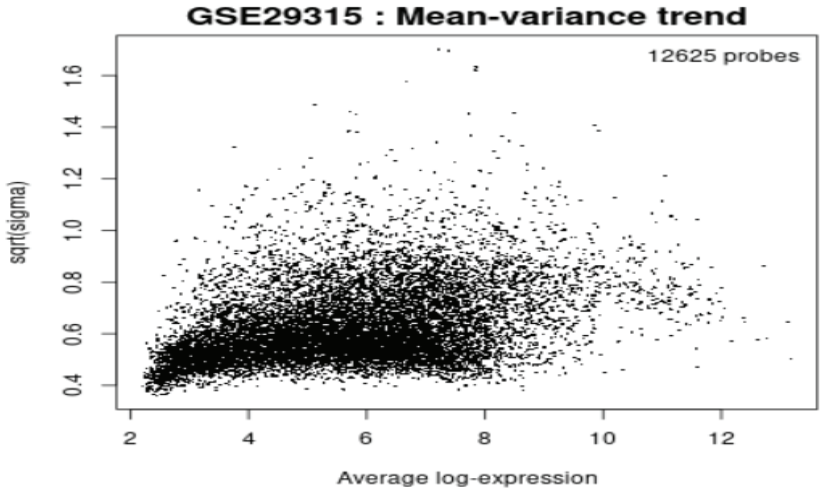
Source: https://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE29315

Figure 3. GSE29315 Expression density plot



Source: https://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE29315

Figure 4. GSE29315 Average log-expression.



Source: https://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE29315

In silico DNA Methylation in the GSE29315 Gene Regions

Using PyMethylProcess, we assessed the methylation status of CpG sites within these regions. The software provided detailed information on the percentage of methylated CpG sites, allowing us to identify patterns of hypermethylation or hypomethylation.

In our computational analysis, we observed distinct methylation patterns in the selected regions of gene GSE29315. Some regions exhibited higher levels of methylation, which may suggest gene repression or altered functionality. Conversely, other regions showed lower methylation levels, potentially indicating enhanced gene expression or regulatory activity.

In the *in silico* simulation of the methylated regions in gene GSE29315, we identify specific CpG sites within the analyzed regions that showed significant methylation changes:

Region 1 - Promoter Region:
- CpG site 1: Hypomethylated (40% methylation)
- CpG site 2: Hypomethylated (35% methylation)
- CpG site 3: Hypomethylated (38% methylation)

Region 2 - Enhancer Region:
- CpG site 4: Hypermethylated (80% methylation)
- CpG site 5: Hypermethylated (75% methylation)
- CpG site 6: Hypermethylated (85% methylation)

Region 3 - Transcription Start Site:
- CpG site 7: Hypomethylated (30% methylation)
- CpG site 8: Hypomethylated (25% methylation)
- CpG site 9: Hypomethylated (28% methylation)

Region 4 - Intronic Region:
- CpG site 10: Hypermethylated (70% methylation)
- CpG site 11: Hypermethylated (65% methylation)
- CpG site 12: Hypermethylated (75% methylation)

The identification of these specific CpG sites with significant methylation changes provides a starting point for further investigation into the functional implications of these alterations in gene expression and potential associations with HT.

DISCUSSION

We demonstrate methylation patterns of the GSE29315 gene as an epigenetic mechanism triggering HT. The observed aberrant DNA methylation patterns in the GSE29315 gene shed light on its potential involvement in the pathogenesis of HT. Gene--specific methylation changes can influence gene expression levels and alter the immune response within the thyroid gland. The identified DMRs and CpG sites may serve as potential diagnostic and prognostic biomarkers for HT.

The term epigenetics refers to heritable molecular processes that influence gene expression without causing changes to the base sequence of the DNA molecule (11). DNA methylation is the best-characterized epigenetic modification and is recognized as a mechanism for gene silencing. DNA methylation is the process by which a methyl group is added to DNA through the action of enzymes known as DNA methyltransferases. The most well characterized mechanism of DNA methylation involves the attachment of a methyl group to a cytosine base within a CpG dinucleotide, resulting in the formation of 5-methylcytosine (12). Thus, DNA methylation is a covalent modification of DNA that does not alter the DNA sequence, but has an impact on gene activity.

HT is considered a multifactorial disease, influenced by genetic, environmental, and epigenetic factors. Epigenetic modifications alter gene expression without modifying the DNA sequence, playing a pivotal role in various autoimmune diseases. The identification of specific genes and pathways associated with HT-related epigenetic alterations could provide invaluable insights into the disease mechanisms and potential therapeutic targets. HT is an autoimmune inflammatory process characterized by lymphocytic infiltration of the thyroid gland. It has been described for over 100 years and has a frequency of approximately 30%, with an increasing trend in its incidence (13,14). The genetic component is a significant factor in the development of autoimmune thyroiditis, with a prevalence rate of 33% among non-twin siblings and a staggering 79% among monozygotic twins (15). Numerous genes have been linked to autoimmune thyroiditis, with HLA-DR3 being the first gene identified as being associated with Hashimoto's thyroiditis (16). Epigenetic mechanisms have been demonstrated to play a role in the onset of Hashimoto's thyroiditis, serving as a bridge between genetic predisposition and environmental triggers (17).

Recent studies have shown that aberrant DNA methylation patterns are implicated in the pathogenesis of HT. Of particular interest is the GSE29315 gene, as it has been found to exhibit differential methylation levels in patients with HT compared to healthy controls. GSE29315 encodes for a protein involved in immune regulation, suggesting a potential role in the immune dysregulation observed in HT. Gene expression studies of thyroid disease have revealed that the GSE29315 gene is implicated in Hashimoto's thyroiditis, particularly in specimens GSM724489, GSM724490, GSM724491, GSM724492, GSM724493, and GSM724494 (18).

An epigenetic study has revealed evidence of DNA methylation in individuals with autoimmune thyroid disease, with 82 genes found to be hypermethylated and 103 genes hypomethylated (19). *In silico* simulation of the GSE29315 gene in our study showed a high percentage of hypermethylation. While

the exact mechanisms by which GSE29315 methylation affects HT onset are yet to be elucidated, it is plausible to propose that GSE29315 methylation may lead to the dysregulation of immune-related genes and subsequent activation of autoreactive lymphocytes. Thus, the high percentage of hypermethylation observed in the GSE29315 gene suggests that it may serve as a potential biomarker for the early detection and risk stratification of Hashimoto's thyroiditis.

Although there is an association between genetics and epigenetics in autoimmune thyroid diseases, the majorities of studies are limited to a small number of genes and focus primarily on Graves' disease. Most of these studies are descriptive in nature, making it difficult to establish a clear link between epigenetic factors and HT (20). By utilizing bioinformatics to evaluate the role of epigenetic mechanisms in the development of HT, specifically through the hypermethylation of the GSE29315 gene, we have gained a deeper understanding of this autoimmune disease. Through the application of computational tools and genomic analysis, we were able to identify key epigenetic modifications responsible for the dysregulation that may trigger HT.

CONCLUSION

Epigenetic mechanisms play a crucial role in the pathogenesis of HT, acting as mediators between genetic susceptibility and environmental triggers. The complex interplay of DNA methylation, histone modifications, and non-coding RNAs contributes to altered gene expression patterns and immune dysregulation in HT. The differential methylation of the GSE29315 gene in HT patients suggests its potential role in immune dysregulation observed in the disease.

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ORIGINAL ARTICLE
ACR TI-RADS® SCORE ULTRASOUND – PICTORIAL ESSAY OF ULTRASOUND / ELASTOSONOGRAPHY / ANATOMY / CYTOLOGY AND HISTOLOGY

ACR TI-RADS® CLASSIFICAÇÃO DE NÓDULOS TIREOIDIANOS DETECTADOS NA ULTRASSONOGRAFIA - ENSAIO PICTÓRICO DE ULTRASSOM / ELASTOSONOGRAFIA / ANATOMIA / CITOPATOLOGIA E HISTOLOGIA

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Keywords: TI-RADS® score; Thyroid; Ultrasound; Elastography; Histology
Descritores: Escore TI-RADS®; Tireoide; Ultrassonografia; Elastografia; Histologia

Abstract

Introduction: Thyroid Imaging Reporting and Data System (TI-RADS®) is a system for classifying thyroid nodules detected at ultrasonography, aiming at a descriptive standardization as well as to classify their risk of malignancy based on sonographic findings. **Objective:** To present a pictorial essay by composing anatomical, histological, ultrasound and elastosonography images of the TI-RADS® score based on a review of the literature. **Material and Methods:** Using software for image composition, based on the ultrasound image of the various types of thyroid nodules, we adapt to the anatomical image, the elastosonography, and histological corresponding TI-RADS® and present a pictorial essay. **Results:** The correlation between the sonographic, elastosonography, anatomical, cytology and histological images corresponding to the TI-RADS® score are demonstrated. **Conclusion:** Ultrasonographic features, elastosonography, anatomical, and histological in evaluation of thyroid nodules can correlate with features TI-RADS® score. **Endocrinol diabetes clin exp 2023 / 2408 - 2414.**

Resumo

Introdução: O Sistema de Relatórios e Dados de Imagem da Tireoide (TI-RADS®) é um sistema para classificar nódulos tireoidianos detectados na ultrassonografia, visando uma padronização descritiva, bem como classificar seu risco de malignidade com base em achados ultrassonográficos. **Objetivo**: Apresentar um ensaio pictórico compondo imagens anatômicas, histológicas, ultrassonográficas e elastossonográficas do escore TI-RADS® com base em uma revisão da literatura. **Material e Método**: Utilizando software para composição de imagem, baseado na imagem ultrassonográfica dos vários tipos de nódulos tireoidianos, adaptamos à imagem anatômica, à imagem da elastossonografia e histológica correspondente ao TI-RADS® e apresentamos um ensaio pictórico. **Resultados:** A correlação entre as imagens ultrassonográficas, elastosssonográficas, anatômicas, citológicas e histológicas correspondentes ao escore TI-RADS® são demonstradas. **Conclusão:** Características ultrassonográficas, elastosonográficas, anatômicas e histológicas na avaliação de nódulos tireoidianos podem se correlacionar com características do escore TI-RADS®.. **Endocrinol diabetes clin exp 2023 / 2408 - 2414.**

INTRODUCTION

Thyroid nodules are very prevalent in the adult population; however, about 90% are benign. Ultrasound is the imaging method usually employed in the analysis of thyroid nodules. However, ultrasound findings are in many occasions non-specific, and the conclusive diagnosis is defined by fine needle aspiration biopsy or even by histological examination after surgery. Multiple echographic studies have been proposed to characterize the risk of malignancy of the nodules (1).

Horvath et al. (2) based on the Breast Imaging Reporting and Data System (BI-RADS®) model of American College of Radiology (ACR) (3) decided to evaluate the possibility of applying the BI-RADS® in the sonographic evaluation of thyroid nodules, grouping the nodular characteristics into different categories with a percentage of malignancy analogous to the BI-RADS®, which she called Thyroid Imaging Reporting and Data System (TI-RADS®).

In order to provide physicians with evidence-based suggestions for therapeutic management based on a grouping of well-established sonographic patterns or expressions that can be adopted for each lesion, the ACR organized a committee and developed TI-RADS®, standardizing the diagnostic analysis of thyroid nodules through the elaboration of a lexicon (4).

As per the ACR TI-RADS® system (**Figure 1**), five characteristics of the nodule are included in the scores, including (1) component (choose one): cystic or almost completely cystic 0; spongiform 0; mixed cystic and solid 1; solid or almost completely solid 2; (2) echogenicity (choose one): anechoic 0; hyperechoic or isoechoic 1; hypoechoic 2; very hypoechoic 3; (3) shape (choose one): wider-than-tall, 0; taller-than-wide, 3; (4) margin (choose one): smooth 0; Ill-defined 0; lobulated or irregular 2; extrathyroid extension 3; and (5) echogenic foci (choose one): none or large comet-tail artifact 0; macrocalcification 1; peripheral (rim) calcification 2; punctate echogenic foci 3. Thus, the echographic aspects of nodules in the ACR TI-RADS® are scored according to their characteristics, and classified as benign (TI-RADS 1), not suspicious (TI-RADS 2) minimally suspicious (TI-RADS

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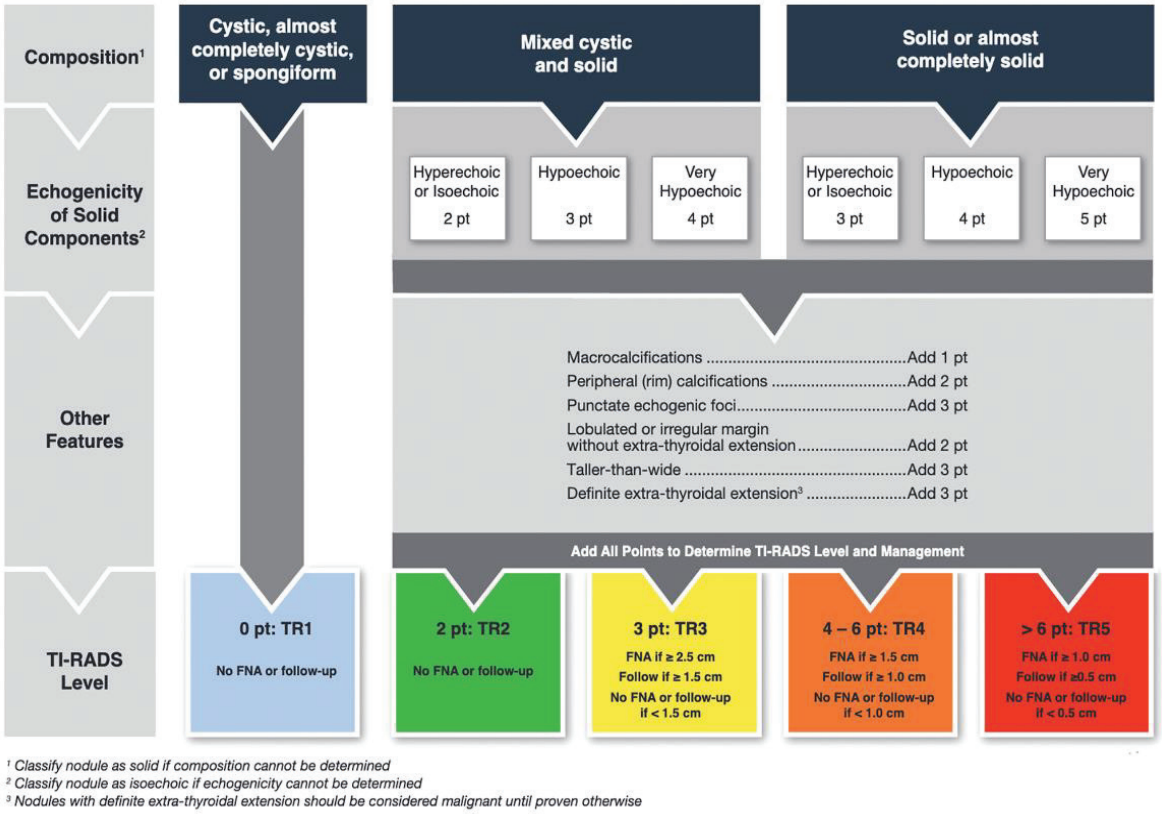
3), moderately suspicious (TI-RADS 4), highly suspicious for malignancy (TI-RADS 5) . The indications for follow-up with ultrasound or fine needle aspiration cytology are based on the ACR TI-RADS® score of the nodule as well as its maximum diameter (5). Sonoelastography because it is not yet available on many ultrasound machines, as well as autoimmune thyroiditis due to its scarcity have not yet been added categorically to the ACR TI-RADS® classification.

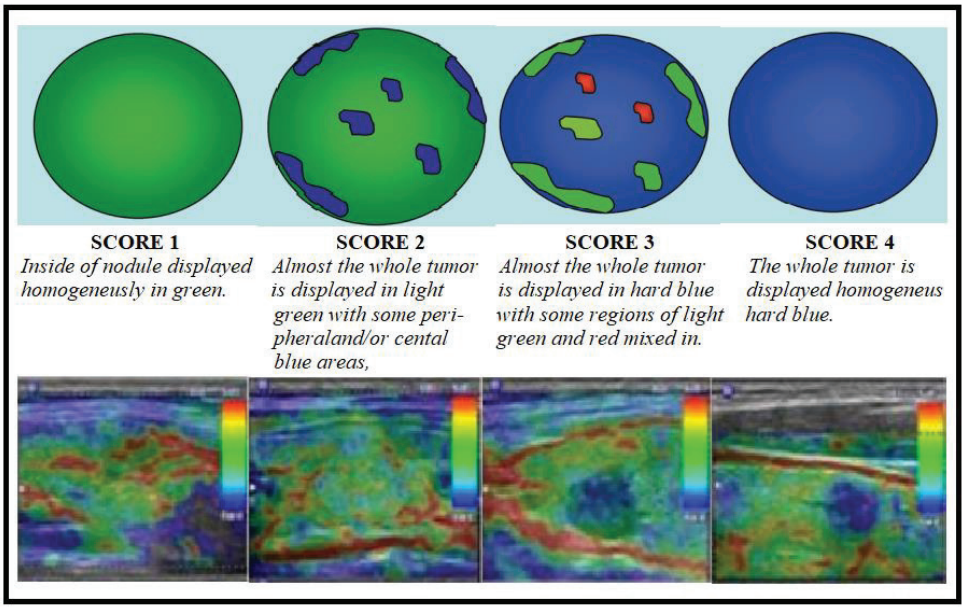
Figure 1. ACR-TIRADS® classification.



[1] *Classify nodule as solid if composition cannot be determined*
[2] *Classify nodule as isoechoic if echogenicity cannot be determined*
[3] *Nodules with definite extra-thyroidal extension should be considered malignant until proven otherwise*

Source: Hoang JK, Middleton WD, Tessler FN. Update on ACR TI-RADS: Successes, Challenges, and Future Directions, From the AJR Special Series on Radiology Reporting and Data Systems. AJR Am J Roentgenol. 2021;216(3):570-578.

Figure 2. Qualitative evaluation of elastography Strain.



Source: Asteria C, Giovanardi A, Pizzocaro A, Cozzaglio L, Morabito A, Somalvico F, et al. US-elastography in the differential diagnosis of benign and malignant thyroid nodules. Thyroid. 2008;18(5):523-31.

Elastosonography assists as a non-invasive ultrasound method that evaluates tissue strain. Lately it has been widely used in the evaluation of thyroid nodule. In such a way that both shear wave elastography as for real-time elastography show excellent results in assessing the risk of malignancy show excellent results in assessing the risk of malignancy, using nodule hardness as a criterion of suspicion for malignancy (6). In strain elastography a red-green-blue color map is displayed: red it has been agreed that characterizes soft tissue, green represents intermediate hardness (equal strain), and blue characterizes hard nodules (no strain) (7). Thus, scores have been proposed to assess tissue elasticity, such as the Asteria et al. (8) score, in

which score 1 corresponds to a totally green nodule; score 2 to a predominantly green nodule, with some blue areas; score 3 to a predominantly blue nodule, with some green areas; score 4 to a totally blue nodule (**Figure 2**).

Scores 1 and 2 are considered benign and scores 3 and 4 are suspicious for malignancy (9). In addition to the color score, tissue elasticity can be assessed using the strain ratio, a measure indicated by the device, which compares the elasticity of the thyroid nodule and the adjacent thyroid tissue (9).

Although numerous imaging examinations have been used in day to day clinical practice, incorrect analysis can occur. This manuscript aims to present a pictorial essay, composing ultrasound images with elastosonography image, anatomical image, cytological image and histological image based on ACR TI-RADS® score of thyroid nodules.

MATERIAL AND METHODS

Based on sonographic images of thyroid nodules and using the windows "paint" application, we correlate the ACR TI-RADS® classification image with the elastosonography image, the anatomy image, the cytological image and the histological image.

Correlations between the sonographic and cytological images were performed by an ultrasound specialist and a pathologist.

This study proposes to investigate the theoretical understanding of pathologies of clinical practice, and according to the Research Ethics Committee of Brazil (CEP), CNS Resolution 510/2016, there is no need for CEP evaluation.

RESULTS AND DISCUSSION

NORMAL THYROID

Anatomy

The thyroid gland consists of two lobes connected to each othThe thyroid gland consists of two lobes connected to each other by the isthmus and is located in the anterior and central part of the neck below the laryngeal cartilage (10). The thyroid gland is anatomically related to the infrahyoid muscles, namely the sternothyroid, superior belly of the omohyoid and sternohyoid anteriorly, to the larynx, pharynx, trachea, esophagus, external laryngeal and recurrent laryngeal muscles medially, and to the common carotid artery, internal jugular vein and vagus nerve laterally. The normal volume of the thyroid in the adult individual varies between 6.0cm3 and 16.0cm3, and its weight may vary in the period between 6 and 15 years from 5g to 16g in males, and 5g to 15g in females (11).

Ultrasonography

The sonographic image of normal thyroid parenchyma may vary between individuals according to the cellularity or the amount of colloid. However, the normal thyroid gland is usually homogeneous, bright, and with a slightly increased echogenicity relative to the surrounding muscles (12).

Elastosonography

Shear wave elastography (SWE) is a new technology that is able of producing complement data relative to tissue elasticity. In elastography the normal values of thyroid tissue in normal individuals are presented in kilopascals (kPa) and the shear wave velocity is expressed in meters per second (m/s) (13).

Studies demonstrated of the elastosonography values of the normal thyroid gland, and the results ranged from 1.60 ± 0.18 m/s for pSWE to 2.6 ± 1.8 m/s for 2-D SWE (14).

Cytology

Fine needle aspiration cytology (FNAC) biopsy is pointed out as gold pattern diagnostic tool in thyroid nodules (15). Benign FNAC findings avoid unnecessary procedures. The FNAC specimens of benign thyroid usually have follicular cells with variable amount of thick and thin colloid (16).

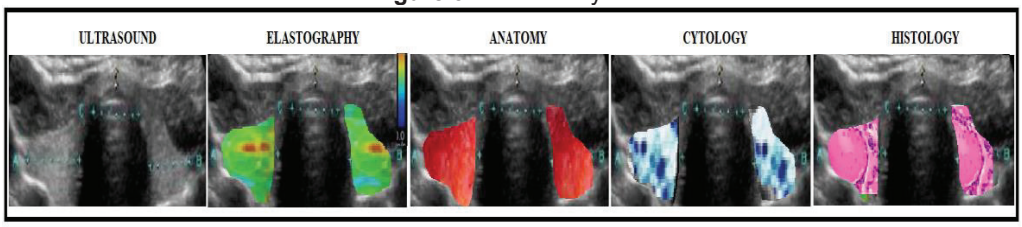
Histology

The normal thyroid gland is composed of two basics types of epithelial cells: the follicular cells, which transform iodine into thyroxine and triiodothyronine, and the parafollicular or C-cells, which contain calcitonin (17).

The thyroid gland is involved by a capsule, consisting of dense coating connective tissue and designates septa for thyroid parenchyma, distributed in multiples lobules, and each thyroid lobule includes 20 to 40 follicles. Another group of thyroid secretory cells is of C cells, which secrete calcitonin. The C cells constitutes around 0.1% of the thyroid gland (18).

Figure 3 shows the composition of images that correspond to the normal thyroid (ultrasound, elastography, anatomy, cytology and histology).

Figure 3. Normal Thyroid.



Source: Research result

ACR TI-RADS® [1]

Ultrasonography

After the large-scale use of thyroid ultrasound has been notorious that in euthyroid nodular goitre the most of the nodules is cystic or partially cystic. Composition: Cystic or almost = 0 points, completely cystic or spongiform = 0 points; Echogenicity: Anechoic = 0 points; Shape: Wider-than-tall = 0 points; Margin: Smooth = 0 points, and Echogenic foci: None or comet-tail artifacts = 0 points. Thus, nodules receiving a final score of zero are classified as ACR TI-RADS® 1 (5).

Elastosonography

Most studies with elastosonography of thyroid have been excluding cystic and/or calcified nodules or predominantly cystic nodules because real-time qualitative thyroid elastosonography only assumes malignancy risk when predominantly cystic nodules are not included (19).

Thus, elastosonography is inappropriate for predominantly cystic nodules due to artifacts artifacts that lead to unreliable measurements

Anatomy

Anatomically the thyroid cyst presents itself with homogeneous cystic component with a clear or yellowish aspect, with defined margins, capsulated and without extrathyroidal invasion and with a fibro-elastic consistency (20).

Cytology

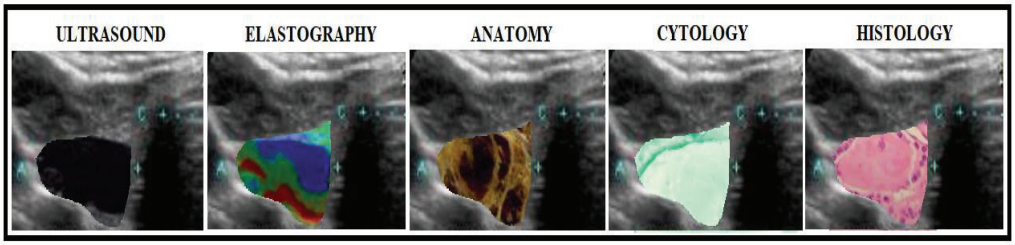
Aspirates that contain only cyst fluid are inadequate. The cystic colloid nodules contain only central colloid enclosure with a thin rim of benign follicular epithelium, which justifies the usual non-existence of follicular epithelium in aspirates, and these findings have a low risk of malignancy (21).

Histology

The colloid nodular goiter histologically presents follicles of widely varying sizes. Some follicles are much larger than normal and contain abundant colloid. Others resemble normal follicles or are small. The follicular cells may be columnar, flattened, or cubic, indicating variation in functional activity. Beams of fibrous tissue give the gland a nodular appearance (22).

Figure 4 shows the composition of images that correspond to the colloid nodular goiter (ultrasound, elastography, anatomy, cytology and histology).

Figure 4. Colloid Nodular Goiter - Benign.



Source: Research result

ACR TI-RADS® [2]

Ultrasonography

According to the ACR TI-RADS® categorization, the TI--RADS 2 nodule presents the following sonographic characteristics smooth margins = point 0, wider than taller = point 0, hypoechoic = point 2, spongiform composition (0), and with no echogenic foci = point 0. Nodule with capsule, mixed, with solid and hyperechogenic areas hyperechogenic or anechogenic with hyperechogenic spots may show vascularization (5). Anechoic nodule, avascular and with internal echoes (colloid type 1). Isoechoic nodule with peripheral halo may show hyperechogenic spots (which are not micro calcifications). Does not demonstrate expansibility (no bulging of the thyroid lobe contours) (colloid type 2). Isoechoic nodule with cystic areas may show expansibility (colloid type 3).

Elastosonography

In elastosonography of a hyperplastic thyroid nodule the largest number of the nodules show relatively not very rigid color elasticity signal. It has been shown that in a ACR TI-RADS® 2 nodule the mean value of the shear wave are 2.56 ± 0.17 m/s (23).

Anatomy

The anatomical specimen of an ACR TI-RADS 2 nodule presents as a nodule usually with a thin fibrous capsule, some contain colloid, and others are hemorrhagic (24).

Cytology

Hyperplastic nodules may suffer cystic degeneration and remedial modifications; stromal fibrosis, hemorrhage, hyalinization, calcification, and necrosis can happen. Large colloid pools can form without or with atrophy of epithelium. The aspirates present foamy cells, macrophages filled with hemosiderin, watery colloid, granular protein debris, and scarcity of epithelium and fibroblasts. The reactive cyst enclosure cells possess characteristic cytoplasmic contours, detached to consistent granular cytoplasm, abrupt too much unipolar/ bipolar cytoplasmic processes. Nuclei can be big, rounded to oval but always with regular edges, being able to present grooves and hypochromasia with distinguished nucleoli (25).

Histology

In hyperplastic goiter the histology demonstrates a accentuated increase of follicles–a few times huge–and lowering of epithelium. Nodules present a thick viscous substance composed of thyroglobulin. It is rational that these nodules are whether it is the result of a defect of intraluminal thyroglobulin reabsorption (26).

The **figure 5** shows the composition of images that correspond to the hyperplastic nodules (ultrasound, elastography, anatomy, cytology and histology).

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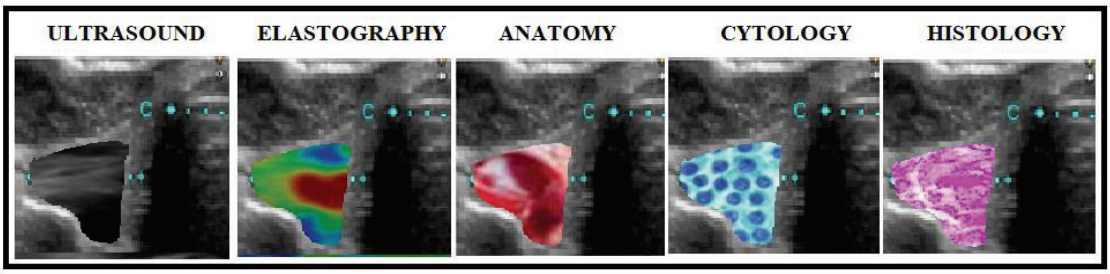
Figure 5. Hyperplastic / adenomatoid nodules thyroid - Benign.



Source: Research result

ACR TI-RADS® [3]

Ultrasonography

Mixed nodule with solid and cystic components, in the case of mixed nodules, only the solid component should be used to score the categories of echogenicity, margins and echogenic foci. Or, completely solid nodule, with echogenicity similar to the rest of the thyroid parenchyma, presenting a hypoechogenic halo that should not be considered for scoring echogenicity or margins. In total there were 3 points, being classified as ACR TI-RADS® 3 (5).

Elastosonography

The nodule is prominently in green with scarce blue areas with low kPa values and elastosonography proportions below than 1, referring that the nodule is softer than the peripheral normal thyroid tissue (27).

Anatomy

Anatomically, ACR TI-RADS® 3 nodules are solitary, encapsulated nodules with a thin capsule, usually occur in adults, and are more common in women. Evaluation of the capsule is of fundamental importance in differentiating it from a follicular carcinoma (28).

Cytology

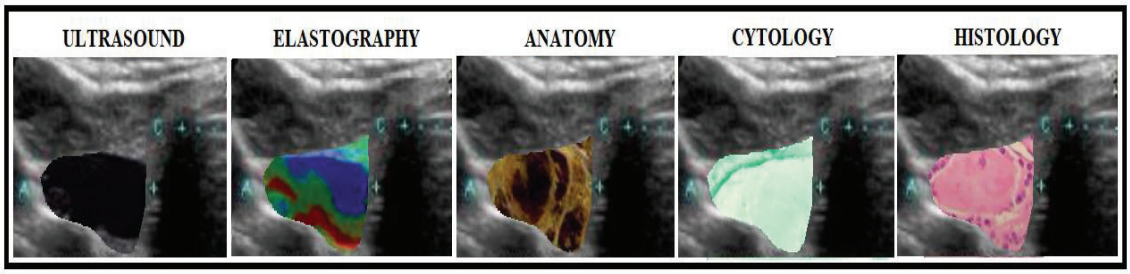
The cytological sample contains follicular cells, lymphoid or various cells with atypia or cellular matrix that do not serve the criteria to categorize it in other classification. The most usual possibilities in this category are mentioned in the following circumstances: existence of micro follicles that do not attend the patterns of the hypothesis follicular neoplasm; Hürtle cells predominance in aspirated content with low cellularity and scarce colloid; cytological atypia undermined by pre-analytical artifacts; specimen composed only by Hürtle cells; hegemony of follicular cells of benign appearance, however including focal areas that suggest papillary carcinoma; predominance of follicular cells with benign aspect but containing coating cystic that resemble atypical cells due to the presence of cracks, enlarged nuclei and nucleoli; and finally, atypical lymphoid infiltrate in which the atypia degree is insufficient to categorize it as suspicious for malignancy (29).

Histology

Histologically, Bethesda category III consists of small follicles, much smaller than normal and usually without colloid. The tumor is bounded by a fibrous capsule, and the appearance of the neoplastic tissue is regular in all areas. There are no cellular atypia, mitosis, or necrosis. There is no neoplastic infiltration of the capsule or vessels, which is the only reliable criterion for differentiating adenoma from adenocarcinoma (30).

The **figure 6** shows the composition of images that correspond to the hyperplastic nodules (ultrasound, elastography, anatomy, cytology and histology).

Figure 6. Atypia of Undetermined Significance / Follicular Lesion of Undetermined Significance.



Source: Research result

ACR TI-RADS® [4]

Ultrasonography

Solid nodule (2 points), markedly hypoechoic (3 points), wider than high (0 points), smooth margins (0 points), no echogenic foci or posterior attenuation artifacts (0 points). Or a nodule with the following sonographic characteristics, mixed solid-cystic nodule (1 point), isoechoic (1 point), wider than high (0 points), extending beyond the anterior thyroid margin (3 points), without echogenic foci or posterior attenuation artifacts (0 points). In total there were 5 points, being classified as ACR TI-RADS® 4 (5).

Elastosonography

Nodule presents on elastosonography as a low elastic nodule, without a rigid border, without internal color homogeneity, no tension in the whole lesion, with blue shading and with focal green dots (31).

Anatomy

The macroscopic characteristics of nodules classified as ACR TI-RADS® 4, the nodule in most cases, presents as a solid nodule, homogeneous in appearance and pinkish in color, without hemorrhagic or necrotic foci with well-defined margins, with a tenuous capsule, without extra thyroid invasion and of elastic consistency (32). It is not possible to distinguish it macroscopically from a follicular carcinoma. For this, a thorough microscopic study of the tumor capsule is indispensable, to look for infiltration of the capsule itself or of vessels.

Cytology

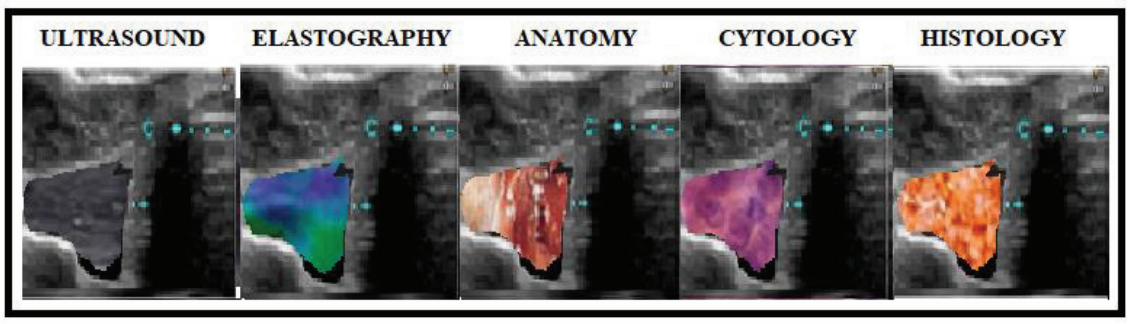
The cytology smear normally has high level cellularity with little or no colloid. It also presents modifications in cytoarchitecture where follicular cells are arranged mostly in microfollicular arrangements and syncytial plaques. Around 16% to 25% of the cases in this category are hyperplastic increases of Hürthle cell in nodular goiter or in a lymphocytic thyroiditis, while 15% to 45% of nodules are malignant (29).

Histology

Histologically, follicular adenoma of the thyroid consists of small follicles, much smaller than normal and usually without colloid. The tumor is bounded by a fibrous capsule, and the appearance of the neoplastic tissue is regular in all areas. There are no cellular atypia, mitosis, or necrosis. There is no neoplastic infiltration of the capsule or vessels, which is the only reliable criterion for differentiating adenoma from adenocarcinoma. The thyroid tissue around the tumor shows compression atrophy. Microscopically, follicular carcinoma more or less faithfully reproduces the follicular architecture of the thyroid (24).

The **figure 7** shows the composition of images that correspond to the follicular thyroid tumor (ultrasound, elastography, anatomy, cytology and histology).

Figure 7. Follicular neoplasm or Suspicious for a Follicular neoplasm.



Source: Research result

ACR TI-RADS® [5]

Ultrasonography

Solid nodule (2 points), very hypoechogenic (3 points), lobulated margin (2 points), circumscribed, not parallel to the skin (3 points), presence of peripheral calcifications (2 point) and macrocalcifications (1 point), which corresponds to the ACR TI-RADS® 5 classification. Or even, solid nodule (2 points), hypoechogenic (3 points), with irregular margins (2 points) with invasion of adjacent structures (3 points), not parallel to the skin (3 points), also classified as TI-RADS® 5. Similarly, a solid nodular image (2 point), hypoechoic (2 points), not parallel to the skin (3 points), regular and circumscribed, totaling 7 points, is classified as ACR TI-RADS® 5 (5).

Elastosonography

Elastography improves the specificity of gray-scale ultrasonography in characterization malignant lesions. The malignant nodule at elastosonography is characterized by the lack of elasticity of the tumor tissue, being a harder nodule, defined at elastosonography by the blue color, when compared to the surrounding tissue of elasticity considered normal. However, the sensitivity of elastosonography in the recognition of a malignant nodule has a wide variation among the various published studies due to the use of different score systems or different cut-off points as a predictive value of malignancy (33).

Anatomy

The macroscopic anatomy of a thyroid carcinoma is described as a solid nodule, whitish and homogeneous in appearance, most often without necrotic foci or hemorrhages, firm in consistency and without capsular invasion (32).

Cytology

The papillary carcinoma is the most prevalent cancer in cytological diagnosis among all thyroid cancers. It is the most prevalent diagnosis in thyroid cytology among thyroid cancers. It is identified due to components of thin or thick papillary tissue, presenting fibrovascular nuclei, focal grouping of juxtaposed tumor cells, regular nuclear contours, grooved nuclei and cytoplasmic inclusions within the nuclei. Metaplastic squamous cells and psammomatous bodies can also be found (29).

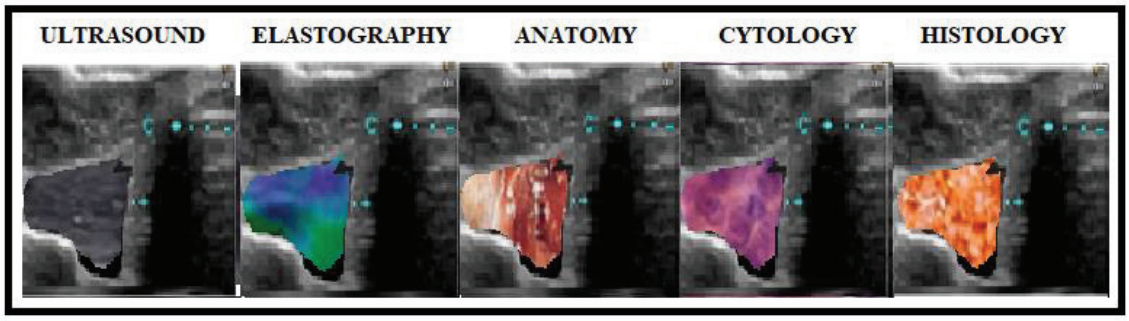
Histology

Histologically, the cells tend to be arranged along branched connective-vascular axes, forming papillae. The cells are usually cuboidal or columnar in shape, uniform and well differentiated. Currently, papillary architecture is no longer considered the decisive criterion for the diagnosis of this type of tumor, and greater importance is given to nuclear features. The chromatin of papillary carcinoma cells is thin and regularly dispersed, giving the nucleus a clear and relatively homogeneous appearance. This is called the ground-glass appearance. There is also a strengthening at the periphery of the nucleus, the chromatin being denser below the nuclear membrane. The intranuclear pseudo inclusions result from invaginations of the nuclear membrane containing cytoplasm. The image is created by superimposition and is not a true inclusion, in the sense of an intranuclear corpuscle. They are a common feature of papillary carcinoma.

Faces in nuclear membrane are folds of the membrane that, depending on the plane of observation, give the appearance of a crack in the nucleus, resembling a coffee bean. In some transverse cut nuclei the fold of the nuclear membrane is clearly visible. Psamomatic bodies are calcified corpuscles, often with a concentric lamellated structure, usually found in the connective axons of papillae. They have importance for the diagnosis of papillary carcinoma because they are much rarer in other types of thyroid carcinoma such as follicular, medullary and anaplastic. Also, the folicular component in papillary carcinomas has at least some follicles in the middle of the papillae. In some, the follicular component may greatly predominate over the papillary, or the tumor may be exclusively follicular. In this case, the diagnosis of papillary carcinoma is based on the nuclear features. These papillary carcinomas are called follicular variant and behave biologically like the usual papillary carcinomas, i.e. they are locally infiltrative and usually not encapsulated. Their prognosis is better than that of true follicular carcinoma (24).

.The **figure 8** shows the composition of images that correspond to the Thyroid Papillary Carcinoma (ultrasound, elastography, anatomy, cytology and histology).

Figure 8. Thyroid Papillary Carcinoma.



Source: Research result

CONCLUSION

The sonographic, elastosonographic, anatomical and histological features in the evaluation of thyroid nodules can correlate with the features of the TI-RADS® score, consequently avoiding unnecessary invasive procedures and helping the appropriate clinical/surgical decision making.

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TOPICS IN MEDICAL CLINIC
ORIGINAL ARTICLE
CLINICAL PROFILE OF SYSTEMIC LUPUS ERYTHEMATOSUS ACCORDING TO THE PRESENCE OR ABSENCE OF HYPOTHYROIDISM

ESTUDO DO PERFIL CLÍNICO E SOROLÓGICO DO LÚPUS ERITEMATOSO SISTÊMICO DE ACORDO COM A PRESENÇA OU NÃO DE HIPOTIREOIDISMO

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Key words: Systemic lupus erythematosus; Hypothyroidism; Serology; Clinical Epidemiology
Descritores: Lúpus eritematoso sistêmico; Hipotireoidismo; Sorologia; Epidemiologia clínica

Abstract

Background: Systemic lupus erythematosus (SLE) is a disease with various clinical manifestations that may be associated with hypothyroidism. In this study, the clinical and serological profile of lupus patients with and without hypothyroidism was studied. **Material and Methods:** Retrospective study for the presence of hypothyroidism, SLE clinical profile and antibody profile. **Results:** We studied 534 patients with SLE; in 120/534 (22.4%) there was hypothyroidism. Patients with hypothyroidism were more women (p=0.001) and older (p=0.05). From the clinical point of view, hypothyroidism was associated with discoid lesion (p=0.01), malar rash (p=0.04) and joint complaints (p=0.04) Regarding the serological data, it was observed that the presence of anti-Ro (p=0.04) increased the chance of hypothyroidism by 1.5 times. **Conclusion:** The profile of SLE with associated hypothyroidism is that of a milder disease with more cutaneous and articular characteristics. Regarding the serological profile, no significant differences were found beyond the presence of the anti-Ro/SS-A antibody. **Endocrinol diabetes clin exp 2023 / 2415 - 2419.**

Resumo

Justificativa: O lúpus eritematoso sistêmico (LES) é uma doença com variadas manifestações clínicas e que pode estar associado ao hipotireoidismo. Neste estudo estudou-se o perfil clinico e sorológico de paciente lupicos com e sem hipotireoidismo. **Material e Métodos:** Estudo retrospectivo para presença de hipotireoidismo, perfil clínico e perfil de anticorpos do LES. **Resultados:** Estudaram-se 534 pacientes com LES. Em 120/534 (22,4%) existia hipotireoidismo. Pacientes com hipotireoidismo eram mais mulheres (p=0,001) e mais velhos (p=0,05). Do ponto de vista clínico, o hipotireoidismo se associou com lesão discoide (p=0.01), rash malar (p=0.04) e queixas articulares (p=0.04). Em relação aos dados sorológicos, observou-se que a presença de Anti-Ro (p=0.04) aumentava a chance de hipotireoidismo em 1,5 vezes. **Conclusão:** O perfil do LES com hipotireoidismo associado é o de uma doença mais branda e com mais características cutâneas e articulares. Em relação ao perfil sorológico, não foram encontradas diferenças significativas além da presença do anticorpo Anti-Ro/SS-A. **Endocrinol diabetes clin exp 2023 / 2415 - 2419.**

INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that affects multiple organs such as kidney, liver, joints, blood, skin, vessels, nervous system, lungs and heart. Its prevalence varies between 19-59 per 100,000 people (1-5). Due to the great progress in the diagnosis and treatment of SLE, its mortality rate has gradually decreased; however, it remains three times higher than that of the general population (6).

Hypothyroidism may also be autoimmune and characterized as an organ-specific disease. Autoimmune thyroiditis is quite common. In this case it is characterized by the infiltration of immune cells into the thyroid tissue (7).

Both diseases can occur simultaneously and previous studies have shown that thyroid disease is more common in patients with SLE, when compared to the general population (8) which corroborates studies that speculate that SLE may have an impact on thyroid disease (9). The relationship between hypothyroidism and lupus activity has also been pointed out (10). However, it is not known whether the presence of hypothyroidism impacts the clinical and serological profile of SLE.

It is in this context that the present study is situated, which aims to compare individuals with SLE with and without hypothyroidism regarding the clinical and serological profile.

MATERIAL AND METHODS

This is a retrospective study, in which data were collected from the medical records of patients at the rheumatology outpatient clinic of the Hospital Universitário Evangélico Mackenzie (HUEM) for the period from January 1, 2013 to January 1, 2023, in a total of 656 patients. This study was duly approved by the Research Ethics Committee of FEMPAR under protocol n° 6.054.344.

To be included, patients had to meet 10 points in the classification criteria for lupus of the ACR/EULAR (11) and have a medical record with sufficient data for the analysis. Individuals with the juvenile form of the disease and pure discoid form were excluded.

The following data were collected: (a) - epidemiological data: such as age, sex, race, age at diagnosis of the disease, use of tobacco; (b) - clinical: presence of acute, subacute or chronic skin lesions, Raynaud's, oral ulcers, alopecia, serositis, arthritis, glomerulonephritis (and its classification), convulsions,

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psychoses, hemolytic anemia, leukopenia, lymphopenia, thrombocytopenia; c) - serological: presence of anti-ds DNA, anti-Ro, anti-La, anti-RNP, anti-Sm, direct Coombs, anticardiolipins IgG, IgM and anticoagulant lupus antibodies in addition to TSH and T4 data and the presence of thyroid autoantibodies (anti--thyroperoxidase).

Next, patients with hypothyroidism were compared with those without hypothyroidism regarding clinical and serological characteristics.

Data were described as percentages when nominal and as mean with standard deviation if parametric numeric and median with interquartile ranges if nonparametric and numeric. The comparison of nominal data was made by the chi-square and Fisher tests and of numerical ones by the unpaired t or Mann Whitney according to the distribution of the sample. The significance adopted was 5%. The tests were calculated with the aid of the Graphpad Prism software, version 9.1.

RESULTS

Of the 656 medical records analyzed, there were data on thyroid function in 534. Of these 534, about 120 had hypothyroidism (22.4%). Of the 120 patients with hypothyroidism, 91 were investigated for Hashimoto's disease and this was found in 47 (51.6%).

Sample description is on **Table 1**.

Table 1: Studied sample - 534 systemic lupus erythematosus' patients.

Females (n)	494/534 - 92.5%
Ethnic background (n)	Euro descendants = 289/476 - 60.7%
	Afro descendants = 185/476 - 38.8%
	Asian 2/476- 0.4%
Age (years)	Median of 42.2 (30-54)
Tobacco exposure(n)	167/517 - 31.7%
Age at disease onset (years)	Median of 29.5 (21-39)
Clinical profile (n)	
Discoid lesion	63/465 - 13.5%
Alar rash	286/518 - 55.2%
Photosensitivity	385/526 - 73.1%
Oral ulcers	222/519 - 42.8 %
Arthritis	415/531 - 78%
Convulsions	48/525 - 9.1%
Psychosis	29/523 - 5.5%
Serositis	111/527 - 21.1%
Hemolytic anemia	54/526 - 10.3%
Leukopenia	150/526 - 28.5%
Thrombocytopenia	123/521 - 23.6%
Glomerulonephritis	221/526 - 42.1%
Antiphospholipid antibody syndrome	28/468 - 6.0%
Dry eye	138/488 - 28.3%
Dry mouth	193/493 - 39.1%
Autoantibodies (n)	
Anti Ro	203/517 - 39.2%
Anti La	95/516 - 18.4%
Anti ds DNA	216/521 - 41.5%
Anti Sm	134/509 - 26.3%
Anti RNP	155/481 - 32.2%
Anti cardiolipin IgG	74/554 - 13.4%
Anti-cardiolipin IgM	67/512 - 13.1%
Lupus anticoagulant	51/482 - 10.6%
Coombs	65/465 - 14.0%

N=number.

The comparison between patients with and without hypothyroidism regarding epidemiological data is on **Table 2**, which shows that patients with hypothyroidism are preferentially female, older and with later-onset disease.

Table 2: Comparison of epidemiological data of SLE patients with and without hypothyroidism.

Variable		With hypothyroidism	Without hypothyroidism	p
Females/ males (n)		119/1	375/38	0.001
Median age (Years)		48 (41-59)	40 (28,2-51,9)	<0,0001
Median age at disease onset (years)		36 (25-43,2)	28 (20-38)	<0.0001
Ethnic background (n)				0.64
	Caucasians	68	212	
	Afro descendants	41	144	
	Asian	0	2	
Smokers (n)	Yes	26	104	0.49
	No	81	269	
	Ex	6	32	

N= number.

The comparison of clinical data is shown in **Table 3.** It is possible to verify that patients with hypothyroidism have more cutaneous-articular complaints and dry mouth.

Table 3: Comparison of clinical data in SLE patients with and without hypothyroidism.

Variable (n)	With hypothyroidism	Without hypothyroidism	p
Discoid lesions	1460	49/405	**0.01***
Malar rash	56/119	230/399	**0.04 ****
Photosensitivity	83/118	302/408	0.47
Oral ulcers	51/117	171/402	0.91
Alopecia	57/110	202/376	0.72
Arthritis	102/120	313/411	**0.04#**
Convulsions	6/118	42/407	0.08
Psychosis	5/118	25/405	0.64
Serositis	31/119	80/408	0.12
Hemolytic anemia	8/118	46/408	0.12
Leukopenia	32/118	118/408	0.17
Thrombocytopenia	28/115	95/406	0.83
Glomerulonephtitis	45/119	176/407	0.29
AAF	8/100	20/368	0.33
Dry eye	39/112	99/376	0.07
Dry mouth	53/112	140/381	**0.04##**

**OR=2.2 (95%IC=1.1-4.3); ** OR=1.5 ((95%IC=1.01-2.30); # OR=1.7 (95%IC=1.7-3.0); ## OR=1.5 (95%IC=1.01-2.3).*

n= number; AAF= antiphospholipid antibody syndrome.

Table 4 shows the comparison of serological profile data between patients with and without hypothyroidism. The only difference found was in the anti-Ro antibody, which is more common in patients with hypothyroidism.

Table 4: Comparison of serological profile of SLE patients with and without hypothyroidism.

	With hypothyroidism	Without hypothyroidism	p
Anti Ro	54/114	149/403	**0.04***
Anti La	24/114	71/402	0.40
Anti ds DNA	48/118	168/403	0.91
Anti Sm	27/116	107/393	0.47
Anti RNP	38/108	117/373	0.45
Anti cardiolipin IgG	15/115	60/399	0.59
Anti cardiolipin IgM	19/115	48/397	0.21
Lupus anticoagulante	13/113	48/369	0.67
Coombs	12/112	53/353	0.40

**OR=1.5 (95%CI=1.008-2.3)*

DISCUSSION

The association of hypothyroidism and lupus was recognized by other authors such as Viggiano et al. (12) who evaluated a total of 208 patients, 106 patients with SLE and 102 controls, obtaining a prevalence of 24% in the group with SLE and 7% in the control group. In the sample currently studied, the prevalence was very similar, and was around 22%.

Although Hashimoto's thyroiditis is the classically recognized form of hypothyroidism in patients with SLE (13), other causes of hypothyroidism are also common although much less studied and rarely identified. In this sample, in the subgroup of patients with data to judge the presence of Hashimoto's thyroiditis, it was responsible for hypothyroidism in half of the cases. Nevertheless this was judged only by the presence of anti-thyroid peroxidase antibody; most of the studied patients did not have thyroid ultrasound. Autoimmunity diseases tend to coexist in the same individual either because they have a common predisposing genetic background or because of possible exposures to environmental triggers in common (13).

Hypothyroidism was found to be more common in women and in older individuals, which reflects the epidemiology of hypothyroidism itself within a sample of individuals with lupus. The findings of Lin et al. (14) corroborate the present ones, demonstrating a higher prevalence of this association in women. Moreover, Tsai et al. (15) and Domingues et al. (16) noticed that the mean duration of SLE was longer in patients with thyroid diseases, while Kumar et al. (17) did not find this association. Although in this study this data was not studied, it was observed that the age of onset of the disease was higher in those with hypothyroidism than in those without.

Clinical differences marked the patients with SLE and hypothyroidism in the studied sample, showing that cutaneous--articular manifestations (which are milder) but not severe manifestations such as glomerulonephritis and central nervous system were more common on these individuals. This may point to the fact that the association with hypothyroidism reveals a lupus with a less severe prognosis. These data differ from those found by Gao et al. (18) who observed that patients with lupus glomerulonephritis tend to present subclinical and persistent hypothyroidism. It should not be forgotten that lupus is a disease whose clinical profile suffers genetic influences and that these differ according to the population studied.

Regarding autoantibodies, the only difference observed was a greater presence of anti-Ro antibody in the hypothyroidism group. The anti-Ro antibody classically has an association with sicca symptoms and Sjögren's syndrome (19). This fact is in agreement with the results of association of hypothyroidism with dry mouth clinic and with a tendency to associate with dry eye observed in the present study. These findings differ from those of Domingues et al. (16) who associated hypothyroidism with anti-Sm antibodies.

The findings of this study have at least two important practical implications. The first one is to demarcate – in our geographical region – a lupus with a better prognosis, guiding the clinician who accompanies these patients on this direction. The second is that patients with hypothyroidism of autoimmune etiology have a higher prevalence of thyroid cancer. Indeed, individuals with SLE have a higher rate of papillary thyroid cancer (13). Thus, the attending physician should remain alert to this possibility.

This study is limited by the retrospective nature of its design and the fact that not all patients have thyroid ultrasound, which would help in the diagnosis of Hashimoto's thyroiditis. On the other hand, it has the advantage of demonstrating the high prevalence of hypothyroidism in patients with SLE and that this finding seems to delimit a specific niche of the disease.

CONCLUSION

In the studied SLE population, a high prevalence of hypothyroidism was found, and in half of the cases, it can be attributed to Hashimoto's thyroiditis. Women and older individuals have more hypothyroidism as well as those with cutaneous--articular manifestations, sicca syndrome and presence of anti-Ro antibody.

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TOPICS IN MEDICAL CLINIC
ORIGINAL ARTICLE
ANTI-ENDOMYSIAL ANTIBODIES AND CELIAC DISEASE ASSOCIATED TO SJÖGREN'S SYNDROME

ANTICORPOS ANTI-ENDOMÍSIO E DOENÇA CELÍACA ASSOCIADOS COM SÍNDROME DE SJÖGREN

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Descritores: Doença celíaca; Síndrome de Sjögren; Autoimunidade

Abstract

Background: SAutoimmune disease may co-occur in the same patient probably due to a shared genetic background or to a common environmental exposure. **Aim:** To study the prevalence of Celiac Disease (CD) autoantibodies in a sample of Sjogren's syndrome (SS) patients and to review the literature. **Material and Methods:** The search for EmA (anti-endomysium antibody)-IgA in 39 SS patients was done by indirect immunofluorescence technique and the results were compared with the prevalence in the general population. A literature review was done searching Pubmed/MEDLINE, Embase and Scielo for the terms: "Sjögren's syndrome", "sicca syndrome" and "celiac disease" from the last 30 years. **Results:** In SS sample there was one patient with known CD diagnosis; other 2 had a positive IgA EmA. All positive patients had some gastrointestinal complaints. Compared with the prevalence in general population (1/286), SS patients had an OR=23.7;95%CI= 3.4-234.0; p=0.005 for positive EmA-IgA. The literature review showed 8 articles in the search for CD in SS with prevalence of autoantibodies for CD and for CD itself ranging from 1% to 14.7%. In the search for SS in CD, 5 articles were found with the prevalence of SS varying from 1.2% to 5%. **Conclusion:** The present work and literature review show a high prevalence of coexistence of these two diseases. **Endocrinol diabetes clin exp 2023 / 2420 - 2425.**

Resumo

Introdução: Doenças autoimunes podem coincidir em um mesmo paciente provavelmente por causa de uma mesma predisposição genética ou exposição ambiental em comum. **Objetivos:** Estudar a prevalência de anticorpos da doença celíaca (DC) em uma amostra de pacientes com síndrome de Sjögren (SS) e rever a literatura. **Material e Métodos:** A pesquisa de EmA (anticorpo anti-endomísio) IgA foi realizada em 39 pacientes com SS por imunofluorescência indireta e comparada com prevalência da população em geral. A revisão da literatura foi feita através das bases de dados: Pubmed/MEDLINE, Embase e Scielo usando os descritores "Sjögren's syndrome", "sicca syndrome" e "celiac disease" dos últimos 30 anos. **Resultados:** Na amostra de SS já existia um paciente com diagnóstico de DC; outros dois apresentaram EmA IgA positivos. Todos tinham queixas gastrintestinais. Comparados com prevalência da população em geral (1/286), pacientes com SS tinham OR=23.7;95% CI= 3.4-234.0; p=0.005 deste anticorpo. A revisão de literatura mostrou 8 artigos em procura de DC em SS com prevalência de autoanticorpos para DC e DC de 1% a 14,7%. A busca de SS em DC mostrou 5 artigos com prevalência de SS de 1,2 a 5%. **Conclusão:** Esta pesquisa e a revisão de literatura mostram alta prevalência de associação destas duas doenças. **Endocrinol diabetes clin exp 2023 / 2420 - 2425.**

INTRODUCTION

Association of autoimmune diseases is unusual; a shared genetic background and exposure to a common triggering environmental factor are some of the proposed explanations (1).

Sjögren's syndrome (SS) is a systemic autoimmune disease characterized by exocrine glandular damage leading to dry eye and dry mouth; it also has extra glandular manifestations in 20-60% of patients with articular involvement, vasculitis, cryoglobulinemia, etc.(2). High mortality is seen in SS patients with extra glandular features mainly due to vasculitis and malignancies (3,4). Moreover SS patients have higher risk of non-Hodgkin's lymphoma than general population, which is credited to the existence of a chronic inflammatory process secondary to persistent antigenic stimulation of B lymphocytes (4-8). The presence of anti-Ro/SS-A autoantibodies, salivary gland biopsy and objective tests for documentation of dry eye and dry mouth help to establish the diagnosis (2). SS may be primary or secondary to other diseases, mainly connective tissue diseases such as rheumatoid arthritis and systemic lupus erythematosus (9).

Celiac disease (CD) is an autoimmune enteropathy triggered by the ingestion of gluten in genetically susceptible individuals causing disabsortion (10). In the classical form, intestinal symptoms are prominent. Atypical forms with extra intestinal manifestations such as hematological, dermatological, endocrinological and skeletal involvement as well as silent forms with no evidence of clinical symptoms are also found (11,12). CD is considered one of the most common autoimmune diseases, with a prevalence estimated in 0.5–1% of the general population (13). CD diagnosis is done by a combination of mucosal alterations detected by intestinal biopsy and positivity of serological tests. Anti-endomysial (EmA) and anti-tissue transglutaminase (tTG) antibodies are considered highly specific serological markers; biopsies of the proximal small bowel confirm the diagnosis (11-13). Occurrence of intestinal lymphoma might complicate the clinical course of CD. Non-Hodgkin T cell lymphoma are the commonest (six to nine times higher than in general population) and appear particularly in those with refractory or untreated disease (10).

An association between CD and SS has been reported. The prevalence of CD among patients with SS ranges from 1% to

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14.7% (10,14-16). Nevertheless, the knowledge of this link by the medical community remains unsatisfactory (10); such information could allow an early diagnosis of either disease avoiding their long-term consequences.

Both SS and CD are diseases with frequencies that oscillate according to the ethnic background. SS is associated with the presence of HLA-DR3 (17) while CD is linked to HLA-DQ2/8 among other predisposing genes (18). So, the frequency of this association may also change according to the studied population.

Herein we aimed to study the frequency of IgA anti-EmA antibodies in patients with SS and to review the literature on the prevalence of CD and its autoantibodies in SS.

AIM

To evaluate the association between primary and secondary Sjogren's syndrome, and the presence of the anti-endomysial (anti-EmA) amidst the population.

MATERIAL AND METHODS

1- Study of anti-EmA prevalence in the local SS sample

This was a cross sectional study approved by the local committee of ethics in research. A convenience sample of primary and secondary SS patients that fulfilled the ACR (American College of Rheumatology)/EULAR (European League Against Rheumatism) classification criteria (19,20) and older than 18 years were invited to participate. To be included patients must have Ig A serum measured to exclude IgA deficiency (normal values ≥50 mg/dL) (21). All patients were recruited from a single Rheumatology Unit and came for regular consultation during the year of 2020. Epidemiological and clinical data were obtained from chart review or upon direct questioning.

Serum samples were screened for EmA-IgA by indirect immunofluorescence technique using a commercial kit (Euroimmun®, product code FA1911). Briefly, testing was performed using sections of monkey esophagus as substrate. Sections were incubated first with patient sera diluted 1:10, followed by incubation using fluorescein conjugated anti-IgA. Slides were read using a fluorescent microscope (Olympus, Japan). Positive and negative controls of commercial kit were used as reference for positivity analysis.

All EmA-IgA positive patients were invited to perform an upper gastrointestinal endoscopy with duodenal biopsy for histological analysis and answered the Gastrointestinal Symptom Rating Scale (GSRS) questionnaire for gastrointestinal symptoms (22). GSRS is a 15-item scale on several gastrointestinal symptoms rated using the following scale: 0 = totally irrelevant;1 = relevant but not important; 2 = moderately important; 3 = very important. GSRS ranges from 0-45 with higher scores indicating greater severity.

Data was collected in frequency tables. Numerical data had central tendency expressed in median values and interquartile range (IQR). To compare EmA- IgA in SS sample with general population, Fisher test was applied using data from a previous work done in blood donors from the same geographical region that showed a prevalence of positive antibodies in 1/286 in 4000 tested individuals (23).

2-Literature review

Articles published in Pubmed/ MEDLINE, Embase and Scielo for the last 30 years (from July 1990 to July 2021) using the following MeSH entry terms: "Sjögren's syndrome", "sicca syndrome" and "celiac disease" in different combinations were systematically searched without language restriction. The reference lists of the selected articles were analyzed to identify other publications.

A standardized form to extract information from relevant articles was designed to include authors and year of publication, number of studied patients, geographical distribution of the studied population, methods used to access the association CD and SS and prevalence of this association. The included studies were all cross-sectional; case descriptions were excluded. The articles found from the search strategy were submitted to the reading of the titles and abstracts, and the articles included in the review were read in full by the authors. The results were made available in a flow chart and tables.

RESULTS

EmA IgA em SS patients

Thirty-nine patients were included; 28 (71.7%) with primary SS (pSS) and 11 (28.3%) with the secondary SS (sSS) form (9 associated to systemic lupus erythematosus and 2 associated to rheumatoid arthritis). In this sample, 37/39 or 94.8% were females with median age of 49 years (IQR=42-57) and 22/39 (66.6%) were auto- declared as Caucasians, 9/39 (27.2%) as Afro-descendants and 2/39 (6.1%) as with Asiatic background. The main clinical data of studied sample is on **Table 1**.

Table 1- Main clinical and laboratory findings of studied sample (39 patients with Sjögren's syndrome)

	N	
Subjective dry eye	32/38	84.2%
Objective dry eye (Schirmer's test)	29/39	74.3%
Dry mouth	33/38	86.8%
Parotid swelling	8/38	21.0%
Musculosketal symptoms	30/38	78.9%
Skin rash	8/37	21.6%
Vasculitis	4/39	10.2%
Positive anti-Ro	33/39	84.6%
Positive salivary gland biopsy	27/39	69.2%

In the obtained sample there was already one patient with known CD diagnosis proved by duodenal biopsy. Other 2 patients had a positive IgA EmA. All positive patients had the primary form of Sjögren's disease and all had some gastrointestinal complaint: 66% complained of loose stools, 66% of passing gas or flatus; 33% of urgency to evacuate and 33% of the sensation of not completely emptying bowel with GSSG rating from 9 to 23. One positive patient had also vitiligo and Hashimoto thyroiditis. Endoscopy with biopsy was offered to these patients, but this exam was postponed by the patient's choice due to COVID-19 pandemic.

When the prevalence of positive IgA EmA found in the present work was compared with literature results (1/286), p=0.005 (OR=23.7; 95%CI=3.4-234.0) was obtained.

Results of literature review

The search for the articles to review the literature followed the flowchart on **Figure 1**.

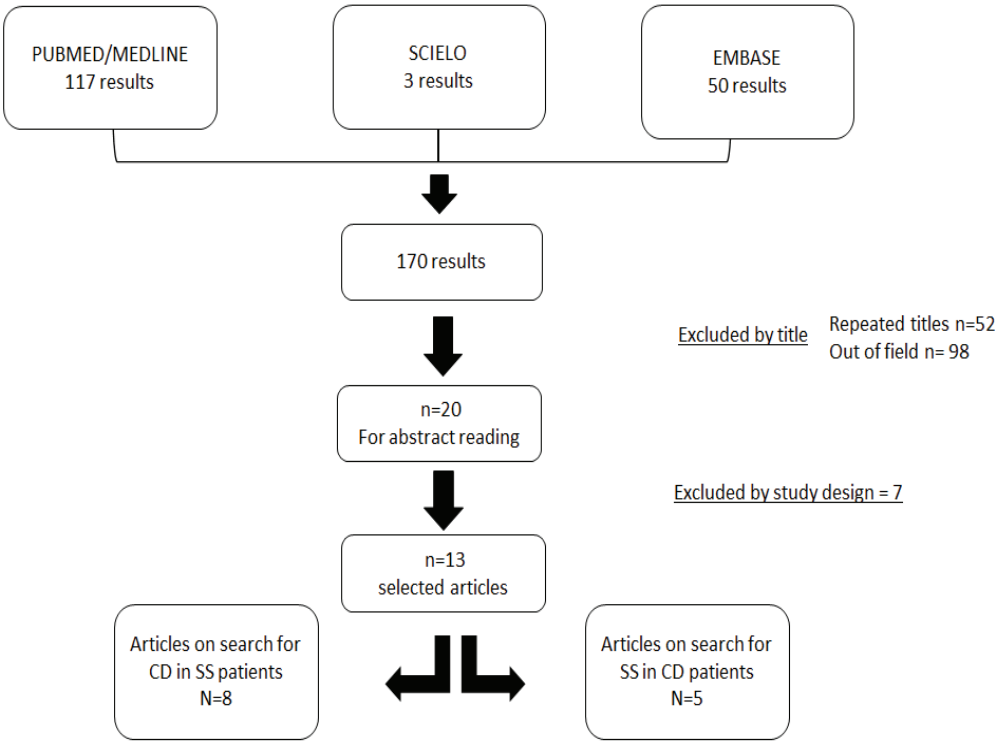

Figure 1 - Flowchart on article's selection.

The result of the studies done in SS sample to identify CD autoantibodies and/or CD diagnosis are on Table 2. The prevalence of autoantibodies for CD and for CD itself ranged from 1% in the work of Bizzaro et al. (24) (that is considered like the prevalence in the general population) to 14.7% in a study done in Finland (14).

Table 2 - Data on cross-sectional studies in Sjogren's syndrome (SS) patients for possible link with celiac disease (CD).

Author/year	Background	SS patients (n)	Females/Males (n)	Searched Autoantibodies	Duodenal biopsy
Bartoloni et al., 2019. [16]	Italy	354	343/11	IgA tTG IgA and IgG EmA	25/354 (7.06%)
Caio et al., 2018. [25]	Italian	52	45/7	3/52 (5.7%) + for anti-tTG IgA; 3/52 (5.7%) + for EmA IgA 4/52 (7.6%) + for anti-DGP IgG	Not done
Koszarny et al.,2015. [26]	Poland	30	30/0	2/30 (6.7%) IgG AGA	Not done
Szodoray et al.,2004. [15]	Hungary	111	NA	6/111 (5.4%) with + serology - not specified (IgA anti- tTG and/or IgA EMA and/or IgG/IgA anti-AGA)	+5/111 (4.5%)
Luft et al., 2003. [27]	USA	50	NA	5/50 (10%) anti-tTG + and EmA + 1/50 (2%) anti-tTG+ and EmA –	+ 5/50 (10%)
Bizzaro et al.,2003. [24]	Italy/Israel	100	NA	1/100 (1%) anti-tTG+	Not done
Fasano et al., 2003. [28]	USA	98	NA	IgA anti-tTG and IgA EmA	2 (2%)
Iltanen et al.,1999. [14]	Finland	34	28/6	3/34 (8.8%) +for EmA IgA 13/34 (38.22%) +for anti-gliadin IgA	+ 5/34 (14.7%)

N= number; SS=Sjögren's syndrome; tTG= tissue transglutaminase; EmA=anti-endomysial antibody; DGP= deamidated gliadin protein; AGA= anti-gliadin antibodies.

In five studies from **table 1**, a duodenal biopsy was done (14,15,16,27,28) and the positivity for CD ranged from 2% in a sample from American population 28 to 14.7% in the study of Iltanen et al. (14).

The work by Koszarny et al. (26) describes that SS patients with positive anti-gliadin antibodies (AGA) had more autoimmune thyroid diseases and higher prevalence of antimitochondrial antibodies than those AGA negative, stressing the association among autoimmune diseases.

Caio et al. (25) studying the frequency of anti-tTG IgA in several connective tissue diseases (rheumatoid arthritis, systemic lupus, polymyositis, dermatomyositis), observed that the highest prevalence of this association was detected in SS patients.

The multicentric study of Bortoloni et al. (16) - that has the largest sample of this review - found CD in 6.7% of their 354 SS patients, reporting that those with associated disease were younger than those with SS without CD.

It is important to note that some authors did not refer if the serum tested for antibodies was IgA sufficient (25,26) and this may have caused false negative results.

Table 3 shows the studies done in patients with celiac disease diagnosis looking for associated SS. This prevalence went from 1.2% found in a Turkish sample (29) to 5% in another study also done in Turkey (30). The comparison of the studies in this context is difficult due to different criteria adopted for SS diagnosis with one study (31) using only the specialist report for SS diagnosis.

Table 3 - Studies of Celiac Disease patients for possible link with Sjogren's syndrome

Author/year	Background	CD patients (n)	Females / Males (n)	Eye/mouth sicca symptoms (n)	Objective dry eye	Anti-Ro/SS-A	Salivary gland biopsy	SS diagnosis
Ayar et al., 2020.[30]	Turkey	80	63/17	18/21	17 (*)	0 (*)	5 (*)	3.8% -ACR criteria,2012 5.0% -AECG criteria,2002
Erbasan et al., 2017.[29]	Turkey	82	60/22	20/ 24	10	0	1	1.2%- ACR criteria, 2012
Bibbò et al., 2017.[31]	Italy	255	206/49	NA	NA	NA	NA	2.3% Written report by specialist.
Caglar et al., 2009.[32]	Turkey	31	NA	NA	NA	2	NA	NA
Collin et al., 1994.[33]	Finland	335	NA	NA	NA	NA	NA	3.3% (Californian Criteria)

(*) The authors adopted a stepwise evaluation not performing all tests in all patients.
CD= celiac disease, SS= Sjogren's syndrome, n= number; NA= not available; ACR= American College of Rheumatology; AECG= American-European Consensus Group classification criteria.

In the study of Caglar et al. (32) only SS autoantibodies were searched: they detected anti-Ro positivity in 2 of their 31 patients with CD. Although anti-Ro positivity is one of classification criteria in the three most used SS classification criteria (the 2012 ACR criteria (34), the criteria from American-European Consensus Group (AECG) (35) and the 2016 ACR/EULAR criteria (20)), this autoantibody can be found in several other diseases. In fact, in this work one of the patients had SS but the other had LES.

Bibbó et al. (31) studied the prevalence of several autoimmune diseases (including SS) in CD patients and found that SS was the fourth autoimmunity more frequent in this context, being less common than thyroid autoimmune diseases, type 1 diabetes mellitus and psoriasis. The results of Collin et al. (33) that studied several autoimmune disorders in CD, showed a prevalence of 7.2 % of connective tissue diseases being SS the most common of them (in 3.3%).

In the analysis of Table 3, it is worthwhile to note that anti--Ro was not searched in two of the studies, (31,33) and biopsy of salivary gland was not performed in 3 of them (31-33). Both, anti-Ro and biopsy of minor salivary gland, are part of the three classification criteria for SS already mentioned.

DISCUSSION

The results of this work show a prevalence of IgA anti-EmA in this sample of SS patients of 7.6%, much higher than in general population (OR=23.7). This prevalence is within the range of results found in other studies as seen on Table 2. This table shows the prevalence of anti-tTG IgA, and/or anti-EmA IgA and/or of celiac disease proved by intestinal biopsy in 6 other cross-sectional studies. Auto-antibodies most used for initial CD screening are anti-EmA and anti-tTG. The sensitivity and specificity of IgA anti-EmA - used presently as the screening tool, was reported to be >95%, although a recent review reported a lower sensitivity (73%) but with specificity still around 99% (36). Gastrointestinal complaints not properly valued previously were present in all positive patients of this sample.

There are no current guidelines on routine investigation of SS in CD patients and vice and versa (37,39). Nevertheless, it is important that the clinicians that follow these patients have a high level of suspicion and initiate this investigation in cases of minor gastrointestinal symptoms, presence of unexplained anemia or even suspicion of secondary osteoporosis. The CD investigation follows well-defined steps. According to the American Society of Gastroenterology, patients should undergo serological screening followed by gastrointestinal biopsies that is the gold standard for this diagnosis (37). However, the diagnosis of Sjogren may take a more complicated course as the disease may have heterogenous clinical manifestations and there is no single diagnostic test to prove it. So, the diagnosis of SS is made by a combination of clinical and laboratory features, after

the exclusion of other causes of ocular and/or oral dryness (40). In order to help data interpretation in SS, classification criteria for this disease have been built but the existence of several heterogenous classification sets turn this interpretation difficult.

Both diseases may complicate with a lymphoma's appearance. In SS the most common lymphoid neoplasia is a low-grade marginal-zone lymphoma related to mucosa-associated lymphoid tissue with primary extra nodal involvement of the major salivary glands (mainly parotid) (41). In this context, lymphomas seem to result from chronic and excessive stimulation of B cells that play a central role in the pathophysiology of this disease, producing excessive amounts of immunoglobulins and various autoantibodies (41). In celiac disease, the enteropathy associated T cell lymphoma is found mainly in those with refractory disease. A deregulation of cytokine signaling due to mutations in members of the JAK/STAT pathway seems to be an early event in lymphomagenesis in this context (42).

According to Caio et al. (25), CD treatment with gluten free diet does not have any efficacy in the natural history of SS, but its recognition is important to improve intestinal absorption of drugs used for SS treatment, to prevent osteoporosis and other diseases resulting from nutrients malabsorption, and to exercise vigilance in the appearance of complications such as lymphomas. On the other hand, there is a curious case description of SS treatment with rituximab that dramatically improved DC symptoms (43).

The present study has some limitations: the sample is small and the duodenal biopsy was not done in positive individuals. Furthermore, it compared the prevalence of autoantibodies with literature data. The study from Alencar et al. (23) used for comparison had a different demographic composition, including males and younger individuals. Consequently, the presented comparison should be interpreted with care. However, it provided data in IgA EmA prevalence in a sample of SS Brazilian patients.

CONCLUSION

Concluding, adult patients presenting with Sjögren's syndrome may be at risk for CD. The prevalence of EmA IgA in the local population is within the range found in studies from other populations.

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