UNIVERSITY OF MEDICINE AND PHARMACY
”CAROL DAVILA”, BUCHAREST
FACULTY OF MEDICINE

POSSIBLE ASSOCIATION AS AN ENDOCRINOLOGICAL PATHOLOGY WITH AUTOIMMUNE COMPONENT BETWEEN DIABETES MELLITUS AND CHRONIC LYMPHOCYTIC THYROIDITIS

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Fundamental problem

In this paper we set out to demonstrate the association between Type 2 Diabetes Mellitus (T2DM) and Hashimoto's Autoimmune Thyroiditis (HT)based on a finding we have made in current clinical practice. We observed an increased prevalence of chronic lymphocytic thyroiditis in patients with T2DM,something not yet reported in the literature.

At the time we decided to do such research, only data on the association between Type 1 Diabetes Mellitus (T1DM) and HT were known, both being autoimmune diseases, frequently associated by the physio pathophysiological mechanism of occurrence, namely the immunological component.

Seeing repeatedly, these two pathologies occurring simultaneously, this topic stirred our interest and we wanted to see if our findings were mere coincidences or in reality there are clinical and paraclinical elements that demonstrate such an association.

This research is a pioneering, original,and has not been adressedbefore, so it represents a challenge and an opportunity to make a contribution to the specialized literature; this contribution will help us understand better the implications of this association.

In order to study this topic, we decided to design an observational, retrospective study on patients of the "Sanamed Hospital" clinic from Bucharest. The clinic's patients are predominantly patients with T1DM or T2DM patients, being a unit specialized in the treatment of metabolic and endocrinological disorders.

From the patient database, we will seek to compare whether the incidence of association between T2DM and HT is similar to the data described at the European level or it it exceeds these estimates, which will help us to prove the initial hypothesis that the two disorders are significantly and statistically associated or we are only dealing with a coincidence.

Subsequently, after proving the initial hypothesis, we want to deepen the research, to search for clinical and paraclinical elements that support the association of the two pathologies and that can give us details about the short, medium and long term implications of this association, as well as possible methods of prevention and eventually, treatment.

Purpose of research

"Possible association as an endocrinological pathology with autoimmune component between Type 2 Diabetes Mellitus and chronic lymphocytic thyroiditis".

The impact study on the possible association as endocrinological pathology with metabolic component between T2DM and chronic autoimmune lymphocytic thyroiditis is to assess the common variables that influence and lead to an association between the pathologies and the relevance to the variables to be interpreted.

Research objectives

In theresearch we pursued objectives related to the impact of clinical values, laboratory analyses specific to diabetes mellitus (DM) and HT in relation to age, gender, associated thyroid pathologies and other variables leading to a possible association between T2DM and chronic lymphocytic thyroiditis.

Research methodology

The working instrument used was the database of the clinic "Sanamed Hospital" based in Bucharest, a retrospective observational study conducted during the period: 01 January 2016 – 31 December 2018. The data collected were used to identify the possible correlations according to different clinical and paraclinical variables with focus on thyroid pathology of patients with T2DM.

The initial group/sample included a total of 5064 patients with T2DMpresenting to the centre between January 2016 and December 2018, initial assessment named Study 1 Determining the incidence of chronic autoimmune lymphocytic thyroiditis in the population of patients diagnosed with T2DM.

Subsequently we selected from this initial group a total of 150 patients who were divided into three groups: 50 patients had only T2DM, 50 patients had only HT and 50 patients had both T2DM and HT (referred to as Lot 1, Lot 2 and Lot 3), a stage reffered to as Study 2: Clinicometabolic and therapeutic features in patients with T2DM and HT.

1. GENERAL PART

1.1. Type 2 Diabetes Mellitus

Lifestyle is an important factor in the development of T2DM, including: obesity, physical activity, diet, stress and urbanization. [1] Excess body fat underlies64% of the cases of T2DM in men and 77% in women. [2] Dietary factors such as: sugar-sweetened beverages [3, 4] and the type of fat in diet also seem to play an important role [5].

A high level of physical activity, a healthy diet, avoidance of smoking and moderate alcohol consumption indicate a 82% lower rate of T2DM and in normal weight patients the rate was 89% lower. Healthy diet has been defined as one with high fibre content, high polyunsaturated fat to saturation ratio, low trans fat intake and lower mean glycemic index [6].

Obesity has been found to contribute to aproximately 55% of T2DM cases; [7] chronic obesity leads to increased insulin resistance (IR) that develops in T2DM, most likely due to the fact that adipose tissue (especially that in the abdomen around the internal organs) is a source of more chemical signals, hormones and cytokines, versus other tissues.

1.2. Hashimoto's thyroiditis

Hashimoto's thyroiditis, also known as chronic lymphocytic thyroiditis or Hashimoto's disease, is defined as a thyroid pathology of autoimmune aetiology, which will progressively produce destruction of the thyroid parenchyma [8] [9]. At first, there may be no symptoms. [8] During the course of the disease, the thyroid parenchyma may increase in volume and thus form a painless belly. [8] Some people will reach the stage of hypothyroidism where they become fat, asthenic, suffer from slow bowel movements, depressive syndrome and polyalgia. [8]. The thyroid gland will become atrophic with a long course of the disease [8]. Potential complications include thyroid lymphoma. [10]

1.3. Association between Type 2 Diabetes Mellitus and Hashimoto's thyroiditis

The relationship between thyroid disorders and DM is characterised by a complex interdependent interaction. Insulin-resistant conditions can increase nodularity of the thyroid gland and coexisting DM can increase the risk of vision loss in patients with Graves' disease. Hyperthyroidism impairs glycaemic control in diabetic subjects, while hypothyroidism may increase susceptibility to hypoglycaemia therebycomplicating diabetes management. In addition, thyroid hormones may further alter carbohydrate metabolism by interacting with leptin, adiponectin and gut hormones, namely ghrelin. This association and the resulted change in metabolic effects requires further research. Thyroid dysfunctions has been shown to be more prevalent in people with DM and in particular T1DM. Furthermore, it appears that unidentified thyroid dysfunction have a negative impact on diabetes and its complications.

A higher frequency of retinopathy and nephropathy was observed in diabetic patients with subclinical hypothyroidism, and more severe retinopathy was also observed. Therefore, treatment of subclinical hypothyroidism in diabetic patients may prove beneficial.

In conclusion, a systematic approach of thyroid testing in diabetic subjects is favourable; however, there are no definitive guidelines on screening for thyroid dysfunction in diabetic

patients. Finally, whether all patients with diabetes should be screened for thyroid function or whether patients with subclinical thyroid disease should be treated, deserves reconsideration.

In terms of treatment, the most common form of treatment is radioiodine therapy. It involves the oral administration of radioactive iodine, which slowly destroys thyroid hormone-producting cells in the thyroid, although most people who undergo radioactive iodine treatment develop hypothyroidism. Hypothyroidism is easier to manage and also has fewer long-term health complications.

If there is no significant thyroid hypertrophy and the thyroid function is normal, the mere presence of anti-thyroid antibodies does not require treatment.

Analysis of the prognostic association between diabetes and thyroiditis found that DM increases the risk of major cardiovascular events and death, but the increased risk is variable among patients, depending on age at diabetes onset, duration of diabetes, glycaemic control, blood pressure control, lipid control, tobacco control, renal function, status of microvascular complications, and other factors. When T2DM is diagnosed at the age of 40, men lose an average of 5.8 years of life and women lose an average of 6.8 years of life. Overall excess mortality in those with T2DM is about 15% higher, but ranges from ≥ 60% higher in younger adults with poor glycemic control and impaired kidney function to better than those without diabetes for those aged 65 and with good glycemic control and no kidney impairement. [11]. The cumulative prevalence of diabetic retinopathy threatens about 4.4% among adults with T2DM and appears to be higher for black non-Hispanic black people compared to non-Hispanic white people 9.3% versus 3.2%. The prevalence of end-stage renal disease is about 1% in those with T2DM (cross-sectional data), but the cumulative prevalence of nephropathy and/or chronic kidney disease is much higher. An efficient treatment requires a motivated and informed patient who actively takes responsibility for diabetes care and a team of healthcare providers willing to frequently adjust medications to support comprehensive disease management over a long period of time.[12] Symptomatic thyroid dysfunction is also the most common complication, with aproximately 5% of people with subclinical hypothyroidism and chronic autoimmune thyroiditis progressing to thyroid failure each year. Transient periods of thyrotoxicosis (thyroid overactivity) sometimes occur and, rarely, the disease can progress to full-blown Graves' hyperthyroid disorder with active orbitopathy. Rare cases of autoimmune fibrotic thyroiditis present with severe dyspnoea and dysphagia, similar to aggressive thyroid tumours, but these symptoms always improve with surgery or corticosteroid therapy. Primary B-cell thyroid lymphoma affects less than one person in a thousand and will most likely affect people with long-standing autoimmune thyroiditis. [13].

2. SPECIAL PART

2.1. Research methodology

2.1.1. Introduction/purpose

Current guidelines for the treatment of patients with T2DM are based on glycaemic standards derived from epidemiological data; however, the course of the disease, from prediabetes to end-stage complications, is not the same in all patients. Microvascular complications, including nephropathy, retinopathy and neuropathy, are strongly related to glycated haemoglobin (HbA1c). However, vascular complications may occur in patients with HbA1c <7.0% and may even occur in undiagnosed patients due totransient increases of plasma glucose concentrations.

2.1.2. Purpose of the research

To demonstrate the association between autoimmune thyroiditis and T2DM and to assess the possible risk factors involved.

2.1.3. Research objectives

In this research we pursued objectives related to impact of clinical values, specific laboratory analyses of DM and HT in relation to age, gender, associated thyroid pathologies and other variables leading to a possible association between T2DM and chronic lymphocytic thyroiditis. ***Main objectives:***

-to determine the incidence of HT in a population with T2DM and to evaluate the relationship between clinicometabolic factors in groups of patients with T2DM, HT, T2DM and HT.

- to determine the existence of independent predictors of the association between the two pathologies studied and to identify possible risk and protective factors involved in the occurrence of HT in patients with T2DM.

As secondary objectives we proposed:

- to determine the weights of associated pathologies with impact on the two pathologies in the 3 groups;
- to determine the impact of IR, assessed by surrogate indices of IR, in relation to the correlation of the presence or absence of the studied association;

- Determination of the correlation of obesity with the presence or absence of HT in the group of patients with T2DM;
- Determination of the correlation of dyslypidemia with the presence or absence of HT in the group of patients withT2DM;
- Determination of the correlation of metabolic syndrome with the presence or absence of HT in the group of patients with T2DM;
- to determine the existence of clinicometabolic features of micro and macrovascular complications of T2DM in patients with HT.

2.1.4. Materials and method

The working instrument used was the database of the clinic ”Sanamed Hospital” based in Bucharest, a retrospective observational study conducted during the period: 01 January 2016 – 31 December 2018. The data collected were used to identify the eventual correlations according to different clinical and paraclinical variables with emphasis on thyroid pathology of patients with T2DM.

The initial group/sample comprised a total of 5064 patients with T2DMpresenting to the centre between January 2016 and December 2018. Subsequently we selected from this initial group a total of 150 patients who were divided into three groups: 50 patients had only T2DM, 50 patients had only HT and 50 patients had both T2DM and HT (referred to as Group 1, Group 2 and Group 3). Group 1, 2 and 3 were selected consecutively, starting with patients presenting with the combination of T2DM and HT, and selecting patients from control, Group 1 and 2 as they presented to the clinic in the immediate aftermath.

From the initial sample we collected the data for Study 1, a study in which we followed the incidence of HT in a population with T2DM; this study was named: Study 1: Determiningthe incidence of chronic autoimmune lymphocytic thyroiditis in the population of patients diagnosed with T2DM.

The second part of this paper, presented Study 2, a study that evaluated the particularities of patients with T2DM, HT, T2DM and HT; this study was named: Study 2: Clinicometabolic and therapeutic particularities in patients with T2DM and Hashimoto’s AutoimmuneThyroiditis.

The database and processing of own data was performed in Microsoft Office Excel 2010 and SPSS.

2.1.5. Method used

The significance thresholds has been noted with the symbol ”*” to support the ease of understandingof the values thus obtained: * $p < .05$, ** $p < .01$, *** $p < .001$.

2.1.6. Materials used

We created a retrospective observational study, Study 1, using patients from the clinic “Sanamed Hospital” based in Bucharest, in which we initially included a number of 5064 diabetic patients who presented to the center between January 2016 and December 2018. Subsequently we selected from this initial group a number of 150 patients who were divided in three groups: 50 patients had only T2DM, 50 patients had only HT and 50 patients had both T2DM and HT (referred toas Group 1, Group 2 and Group 3), this being Study 2.

For this study we obtained the approval of the Ethics Committee of the Clinic “Sanamed Hospital”, agreement number 2591/12.03.2018.

Criteria for inclusion in Study 1:

- age over 16 years,
- previous diagnosis of T2DM established according to the criteria listed below,
- previous diagnosis of HT established according to the criteria listed below for patients in group 2,
- previous diagnosis of HT established during the follow up period of the study established according to the criteria listed below for patients in group 3,
- patients who have agreed to the processing of their personal data by signing the informed consent form of the clinic in which they were investigated.

Study exclusion criteria Study 1:

- diagnosis of T1DM,
- presence of anti-GAD antibodies in patients’ serum.

Inclusion criteria Study 2:

- age over 16 years,
- previous diagnosis of T2DM established according to the criteria listed below,
- previous diagnosis of HT established according to the criteria listed below for patients in group 2,
- diagnosis established during the follow-up period of the HT study established according to the criteria listed below for patients in group 3,
- patients who agreed to the processing of their personal data by signing the informed consent form of the clinic where they were investigated.

Exclusion criteria Study 2:

- patients with other associated autoimmune pathologies (Graves-Basedow disease, rheumatoid polyarthritis, Lupus, inflammatory bowel disorders, multiple sclerosis, psoriasis, vitiligo, Guillain Barre syndrome, vasculitis, Sjogren's syndrome, celiac disease, autoimmune hepatitis, spondyloarthropathies, antiphospholipid syndrome, etc.)
- presence of anti-TPO antibodies in the serum of patients in group 1,
- pregnant or breastfeeding patients,
- patients with severe psychiatric pathologies which prevent from discerning,
- patients with physical disabilities or severe cognitive impairment that may affect their mobility and therefore their ability to move for further investigations necessary tocomplete the database in this study,
- patients who have refused to sign the clinic's informed consent to the processing of personal data,
- patients who refused to continue further investigations necessary to collect data for this study.

Patients included in study were additionally analysed for the following clinical and paraclinical parameters: total cholesterol, triglycerides, serum creatinine, estimated glomerular filtration rate (eGFR), uric acid, oxalacetic transaminase (TGO), pyruvic transaminase (TGP), height, weight, abdominal circumference, body mass index (BM)I, blood pressure. These parameters were used to identify possible correlations and identify possible risk factors.

Patients were also clinically assessed; the presence or absence of the following conditions was sought: obesity, dyslypidemia, atrial hypertension, chronic micro and/or macrovascular complications of DM, ischemic heart disease, congestive heart failure, metabolic syndrome, hepatic steatosis, hyperuricemia/goute, cataracts, glaucoma, depression and pre-existing thyroid pathology, each was defined according to international diagnostic criteria.

To determine IR we calculated surrogate markers for IR. We used the value of Triglycerides and Glucose Index (TyG Index), Visceral Adiposity Index (VAI), Lipid Accumulation Product (LAP) and Triglycerides Ratio on HDL Cholesterol (TG/HDL). We also calculated variants of TyG, more exactly TyG in relation to BMI (TyG-BMI) and in relation to abdominal circumference (TyG-WC). [14, 15]

2.1.7. Research hypotheses

In this regard, we formulated the following hypotheses:

- Patients diagnosed with T2DM have a higher risk of developing HTcompared to the general population;
- Patients with a certain degree of high risk for T2DM and HT, will have a high degree of association with medical laboratory values in relation toassociated thyroid pathologies, metabolic syndrome.

2.2. Study 1: Determination of the incidence of chronic autoimmune lymphocytic thyroiditis in a population of patients diagnosed with Type 2 Diabetes Mellitus

2.2.1. Introduction

Based on a finding from our daily practice with patients, we aimed to assess the incidence of HT in patients with T2DM. Among patients diagnosed with T2DM, we observed a large number of new cases of HT. Previously in the literature, only the association between T1DM and HT was described in terms of the underlying autoimmune pathophysiological mechanism of the two pathologies, thus our findings were either mere coincidences or there is a link between T2DM and HT.

2.2.2. Objectives Study 1

In the first phase of our research, we aimed to assess the incidence of HT in patients with T2DM, to determine what we had previously found, namely an increased proportion of thyroiditis in patients with T2DM, was confirmed or was just a chance finding without clinical significance.

2.2.3. Purpose

The main purpose of this first study was to determine the incidence of HT in the population with T2DM in the "Sanamed Hospital" clinic from Bucharest, through a retrospective observational study over 3 years, between January 1, 2016 to December 31, 2018.

2.2.4. Materials and method

A total number of 5064 patients with T2DM were evaluated using patients from the "Sanamed Hospital" clinic, which were collected over 3 years, between January 1, 2016-December 31, 2018,. Subsequently, the incidence per 100,000 inhabitants was calculated and the data were compared with those found in the European population; at the national level such data are not available, so the comparison with the Romanian population was not possible.

2.2.5. Results Study 1

Of the 5064 subjects analysed in the initial phase of our study, 2383 were men, representing 47.05% and 2681 (52.94%) were female. We can say that the initial sample was divided into almost two equal parts which did not create a research bias.

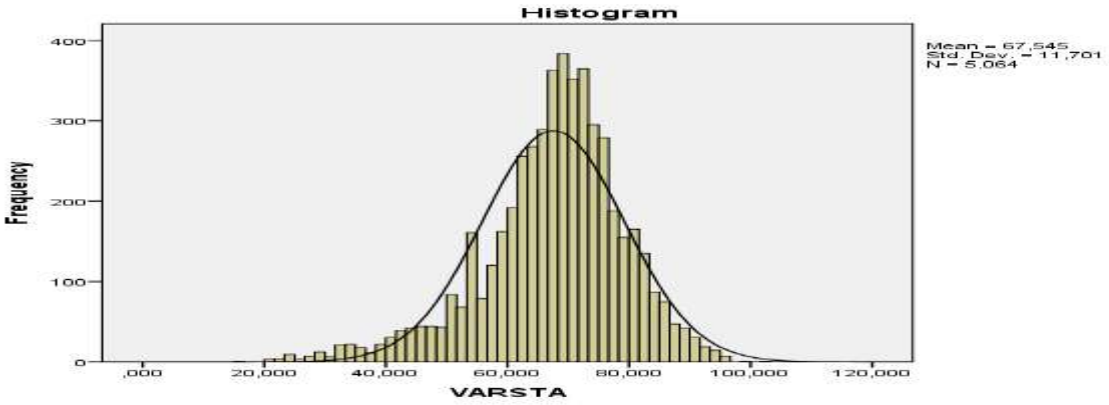

Chart 2.1 Average age in the initial lot

Regarding the mean age in the initial group, we had 67.545 ±11.701 years, with an older age for the patients evaluated in the Diabetes Mellitus section (68.508 ±10.653 years) compared to those evaluated in the Endocrinology ward (57.058 ± 16.432 years). This was natural, given that theaverage age at onset of patients with T2DM is older.

During the three-year period of the study, the incidence of HT in the group of 5064 patients diagnosed with T2DM was 789.89/10^5 per year, with an incidence of 230.80/10^5 per year among diabetic men and an incidence of 1286.83/10^5 per year among women. Compared European wide reports of HT incidence in the general European population [16] (259.12 /10^5 per year, 85.36/10^5 per year in men and 419.72/10^5 per year in women) there is a highly significant difference between the incidence in the general population and the incidence among patients diagnosed with T2DM(table 2.3.2.1). This confirms the original hypothesis of the study. Remarkably, a patient with T2DM has a 3-fold higher risk of developing chronic lymphocytic thyroiditis (HT), also female patients with T2DM have an almost 6 -fold higher prevalence of Hashimoto's thyroiditis than men diagnosed with T2DM.

	Total subjects (n=5064)	**Men (n=2383)**	**Women (n=2681)**	**Total incidence per year 10^5**	**Incidence in men per year 10^5**	**Incidence in women per year 10^5**
With HT acquired after the diagnosis of T2DM in the present study	80	11	69	789.89	230.80	1286.83

Incidence of HT in the general population of Europe*				259.12	85.36	419.72

Table 2.1. Incidence of patients diagnosed with HT after acquisition of T2DM in the study group and incidence at European level. [16]

2.2.6. Analytical discussions. Study 1

Comparing our study of incidence of HT in a population with T2DM with incidence studies of HT in the general population published in the literature, we found similarities. In a study conducted by Wiersinga et al showed a prevalence of HT of 10-12% in the general population, making it the most common autoimmune disease encountered. The higher prevalence was observed in women, increasing with age and being higher in the white versus black population worlwide.[17]

In terms of incidence worldwide, this study showed an incidence of 350 per 10^5 per year in women and 60 per 10^5 per year in men in non- iodine deficient areas and 44 per 10^5 per year in women and 12 per 10^5 per year in men in iodine deficit areas. [17] In our study the incidence was 789.89 per 10^5, which allows us to conclude that T2DM may be a risk factor involved in the occurrence of HT, since the incidence in the population with T2DM is much higher than the incidence in the general population. Also in our study the incidence was higher in women than in men, which is an agreement with the results of the study previously mentioned. The population in our study, the initial sample of 5064 patients, were mainly patients from Bucharest and from vicinity of the country's capital, so from areas without iodine deficiency, also the patients were white (Caucasian). Comparing with the results of the above study, in which the highest incidence was in white patients from areas without iodine deficiency, we can say that the results of our study are in agreement with those described in the study of Wiersinga et al. In our study the highest incidence was in females, almost 6 times higher than in males; in females it was 1286.83 per 10^5 per year, while in males it was 230.80 per 10^5 per year, these data agree with the results in the previously mentioned, where a higher incidence was shown in females versus males.

Another study led by Lee et al. showed an HT incidence in the US population of 350 per 10^5 per year in women and 80 per 10^5 per year in men, with an incidence of nearly 600 per 10^5 in the Appalachian Mountain region. In this study a global incidence of HT was estimated at

30-150 per 10^5, 10-15 times higher in women than in men, occurring most frequently in the 30-50 age group in women, with a peak incidence in men 10-15 years later. [18] The results of our study agree with those of this study, with a higher incidence among women than men 6 times higher. The age group in which ocurred most frequently in our study HT in the population with T2DM studied was in women between 60-70 years, and for men it was the same age category. These results are not in agreement with those of the study conducted by Lee et al., the peak incidence in that study being at age of 30-50 years for women and 45-60 years for men. We can say that our population being composed of patients with T2DM, had an older age than the general population studied in that study of HT incidence, thus these results are in agreement with the average age of the people studied by us. We notice, however, that the proportion in relation to the predominant gender, female, is preserved.

In another study conducted by Dong et al. in the US army, on 20.270688 soldiers, predominantly male 85.8%, an incidence of HT of 758 cases in females and 548 cases in males was described. This study showed that the highest incidence was in the white Caucasian population, and the lowest incidence was in black females (IRR, 0.33; 95% Cl, 0.21-0.51) and black males (IRR, 0.22; 95% Cl, 0.11-0.47), in Asian Pacific Islander females (IRR, 0.31; 95% Cl, 0.17-0.56) and in Asian Pacific Islander males(IRR, 0.23; 95% Cl, 0.07-0.72). [19] The results of this study are mainly determined by the preponderantly male gender of the study participants (85.8% were male), but even so we can observe that the number of HT cases in females is higher, 758, than in males, 548. Considering that the female population of the study was 14.2%, we can say that the incidence in the female population is much higher than in the male population. These results were also obtained by us in our study, a much higher incidence in women. Our study evaluated only white, Caucasian population, which by comparison to the increased incidence in the study conducted by Dong et al. in the white population, could explain a high incidence rate in our study population as well.

2.2.7. Conclusions Study 1

As a conclusion of Study 1 "Determining the incidence of chronic autoimmune lymphocytic thyroiditis in a population of patients diagnosed with Type 2 Diabetes Mellitus" we can state that we have demonstrated our main objective: we have shown that T2DM is a risk factor for the development of autoimmune thyroiditis through a higher incidence of HT among patients with T2DM versus the general European population ($789.89/10^5$ per year, with an incidence of $230.80/10^5$ per year among diabetic men respectively an incidence of

1286.83/10^5 per year among women versus 259.12 /10^5 per year, 85.36/10^5 per year among men and 419.72/10^5 per year among women in the general European population.)

2.3. Study 2: Clinicometabolic and therapeutic features in patients with Type 2 Diabetes Mellitus and Hashimoto's autoimmune thyroiditis

2.3.1. Introduction

After demonstrating the main objective of this research thesis, proving the statistically significant association between T2DM and HT, by means of Study 1, Determining the incidence of chronic autoimmune lymphocytic thyroiditis in a population of patients diagnosed with Type 2 Diabetes Mellitus, we set out to evaluate the possible causes for this association: we aimed to detect the clinicometabolic and therapeutic features in patients with Type 2 Diabetes Mellitus and Hashimoto's autoimmune thyroiditis .

2.3.2. Objectives Study 2

The objectives of Study 2 are:

- to determin the weights of associated pathologies impacting the two studied pathologies in the 3 groups
- to determin the implications of IR assessed by surrogate indices of IR, i.e. whether it correlates with the presence or absence of the studied association
- determin the correlation of obesity with the presence or absence of HT in the group of patients with T2DM
- determin the the correlation of dyslypidemia with the presence or absence of HT in the T2DM group of patients
- determining whether metabolic syndrome correlates with the presence of absence of HT in the T2DM group of patients
- to determin the existence of clinicometabolic features of micro and macrovascular complications of T2DM in patients with HT

2.3.4. Purpose

We aimed to:

- evaluate the relationship between the clinicometabolic factors in the groups of patients with T2DM, HT, T2DM and HT
- determine independent predictors of the association of the two pathologies studied

- evaluate the risk factors or the protection factors involved in the occurrence of HT in patients with T2DM

After evaluating the 5064 patients diagnosed with T2DM and determing the incidence of HT in the study population, we developed a new study, Study 2- Clinicometabolic and therapeutic features in patients with Type 2 Diabetes Mellitus and Hashimoto's autoimmune thyroiditis study, to try to better understand the association between the two pathologies. We selected from the group of patients with T2DM and HT a number of 50 patients who were eligible to participate in our study. Of the initial 80 patients diagnosed with HT, 11 also suffered from other autoimmune diseases (Graves Basedow disease - 5, Rheumatoid Polyarthritis - 4, Vitiligo -1 and Anklopoietic Spondylitis – 1), 7 had advanced chronic diseases or physical disabilities that prevented them from continuing the investigations needed to complete the study database, 3 had severe mental illnesses that prevented them from making judgements and 9 refused to continue the investigations needed to complete the study database. Thus out ofthe total of 80 patients diagnosed with HT, only 50 were eligible after applying the exclusion criteria for the study; thus forming group 3. Group 1 was made up of patients with only T2DM, also from the initial group followed in Study 1, out of 5064 patients with T2DM of the clinic, this being the control group. Group 2 included 50 patients with HT from the Endocrinology department's records, also a control group. The selection of patients was consecutive, initially patients were selected from group 3, subsequently, fromthe same time period, consecutively, as they presented to the clinic, patients were selected for group 1 and 2.

Materials and method

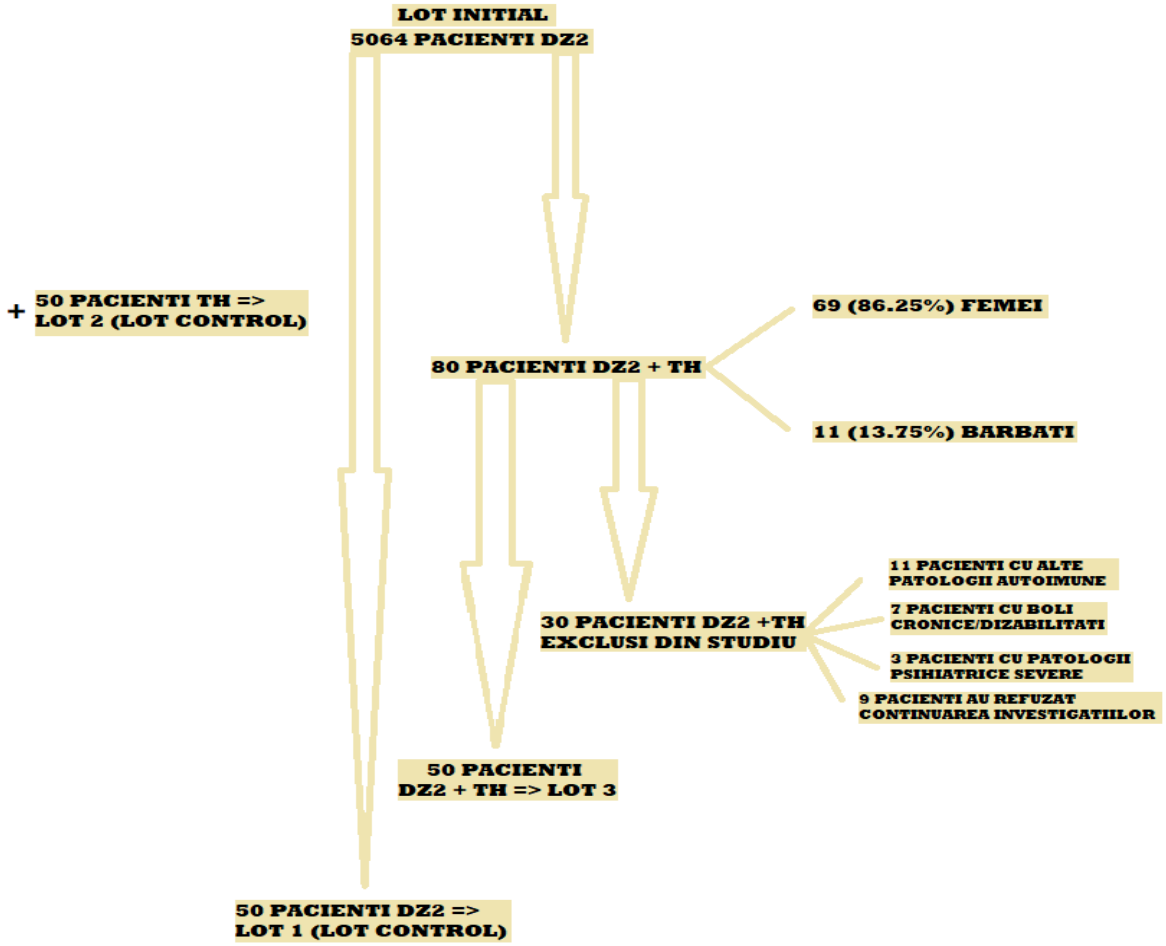

Chart 2.12 Design of Study 2

2.3.5. Results of Study 2

The mean age of the patients in the 3 groups was 58.1 years.

The study sample consisted predominantly of 122 (81.3%) female and (18.7%) male patients. [20]

We found statistically significant differences between groups 1 and 2 and between groups 2 and 3 for the parameters: abdominal circumference, weight, BMI, obesity, dyslypidemia, SM; between group 1 and 3 for TAD, cholesterol, macro and microvascular complications, depression, VAI, TyG, TyGBMI, TyGWC, LAP.

Univariate analysis revealed the following risks factors for HT in patients with T2DM: weight over 83.5 kg, BMI over 29.6 kg/m², HbA1c over 5.85%, blood glucose over 122 mg/dl, thyroid stimulating hormone (TSH) over 1.1 pmol/L, TyG over 4.7, TyG BMI over 140.8, and

TyGWC, female gender, alcohol consumption, smoking, BCI, hypothyroidism, goitre, microvascular complications of T2DM, depression and insulin treatment.

After multivariate analysis it was found that the protective factor TAD was retained and BMI, blood glucose and surrogate index of insulin resistance TyG, female gender, alcohol, smoking, hepatic steatosis, BCI, hypothyroidism and insulin treatment remained as risk factors.

RESULTS OF SUROGATE MARKERS FOR INSULIN RESISTANCE

TyG	TyGBMI	TyGWC	LAP	VAI	TG/HDL
4.8886	149.6063	449.84	57.11	1.8315	3.0914

Table 2.2 Values obtained in all 3 groups for surrogate markers Group 1, Group 2, Group 3

Group		TyG	TyGBMI	TyGWC	LAP	VAI	TG/HDL
Group 1 T2DM	Average	4.9461	157.3483	496.8141	67.3173	2.2955	3.2615
	N	50	50	50	50	50	50
	Standard Dev.	.23133	25.43355	96.40244	37.51971	1.71620	1.55994
	Median	4.9766	153.8312	480.6573	65.6570	2.1656	2.9156
Group 3 T2DM+HT	Average	5.0245	161.8517	479.7390	69.9242	1.6783	3.4651
	N	50	50	50	50	50	50
	Standard Dev.	.32335	34.00789	114.08299	52.19408	1.27814	1.99627
	Median	4.9746	158.2189	463.1977	61.6378	1.5874	3.0291
Group 2 HT	Average	4.6951	129.6190	372.9825	34.1159	1.5208	2.5476
	N	50	50	50	50	50	50
	Standard Dev.	.25271	28.41573	76.98818	28.70756	1.55027	1.56256
	Median	4.6625	124.8493	378.8840	24.5896	1.1877	2.0793
Total Groups 1,2,3	Average	4.8886	149.6063	449.8452	57.1191	1.8315	3.0914
	N	150	150	150	150	150	150
	Standard Dev.	.30473	32.60511	110.94231	43.55847	1.55201	1.75201
	Median	4.8828	149.3270	428.8168	47.8244	1.5352	2.6170

Table 2.3 Surrogate indices of IR for each group, Group 1, Group 2 and Group 3

In the three groups studied, a TyG value of more than 4.7 was observed, but the highest value was detected in the combined group 5.02 (standard deviation 0.32335).

2.3.6. Analytical discussions. Study 2

2.3.6.1.Insulin resistance in patients with Hashimoto's thyroiditis and euthyroidism

The relationship between IR and clinical and subclinical hypothyroidism is well-documented. Hashimoto's thyroiditis (HT) is the most common cause of hypothyroidism.

Hashimoto's thyroiditis is a chronic autoimmune inflammatory disease and the most common cause of hypothyroidism in adults. It is characterised by histological infiltration of T and B-cells in the thyroid gland. Autoimmune diseases detect the body's own tissue as foreign and work to destroy it. Therefore, the immune system starts producing anti-thyroid antibodies (anti-TPO) and anti-thyroglobulin antibodies (anti-Tg) to destroy the thyroid gland. It is not known why the body starts behaving this way. Perioxidase thyroid antibodies are positive in 95% of Hashimoto's thyroiditis and 85% of Graves' disease [21].

2.3.6.2.Personal discussion

In order to try to demonstrate a definitive link between IR and the association of the two pathologies studied in the present study, we used surrogate indices of IR, indices that have demonstrated their accuracy and precision in establishing the presence of IR in many other studies. [22-26]

According to the study of Kim at al., the surrogate IR marker TyG was a better predictor for T2DM than Homa-IR, VAI and LAP also had prediction for T2DM, but a more modest one than TyG. The conclusion of this study was that this TyG marker may be a useful additional tool to determine which patients are at risk of IR and T2DM. [27]

The results of this study for the surrogate markers were TyG 4.7 +/- 0.2, VAI 2.5 +/-1.9 and LAP 38.5 +/-31.9. In our study we obtained similar values TyG 4.88, VAI 1.83 and LAP 57.11, values for the whole group of 150 patients cumulated from Groups 1, 2 and 3.

The cut-off values for the study of Kim et al. were 4.69 for TyG, 2.54 for VAI and 36.6 for LAP. In our study we obtained cut-off values of 4.7 for Tyg, 1.4 for VAI and 27.19 for LAP.

In the study of Guerrero-Romano et al. a cut-off value for the IR determination of TyG of 4.68 was established. [28] Considering that the results of the two mentioned studies were

similar to the result of our study, we can conclude that a TyG of 4.7, obtained by us, is diagnostic for IR.

Correlating the results obtained in the two studies conducted by Kimm at al. and Guerrero-Romano et al. with the results obtained in our study, we can state that the values obtained by us were diagnostic for IR.

In the study conducted by Kim et al. a strong relationship was established between the surrogate marker of IR, TyG and the occurrence of T2DM, which was found to be a better predictor of T2DM than HOMA-IR [27].

In a meta-analysis conducted by Song et al., obesity was shown to be a risk factor for HT occurrence, with OR1.91 (Cl 95% 1.10-3.32) p=0.022. [29] In our study, after risk analysis, obesity was not retained as a risk factor, but an increased BMI at the upper limit of overweight, 29.6kg/m², close to the limit of obesity, with p =0.008 and an odds ratio of 1.230 (CL 95% 1.056-1.432), was found to be a risk factor in the occurrence of HT in patients with T2DM. Thus our data are in agreement with those stated by Song et al.

We also found similar results to our study in the publication of Du et al., where it was shown that in diabetic patients, a high blood glucose was a risk factor involved in the occurrence of thyroid pathologies, OR 2.653 (Cl95% 1.18-5.9), p=0.019 [30] our results were also in agreement with what Du et al. presented, a blood glucose value over 122mg/dL was also in our case an independent predictor of HT occurrence in diabetic patients, OR 1.023 (Cl 95% 1.007-1.040), p=0.004.

The study led by Choi et al. was the first to indicate TyG as a surrogate marker of IR in its evaluation in relation to thyroid dysfunction. It showed higher predictive values for the thyroid pathology including HOMA-IR, OR 1.81, p=0.031. [31] Our study showed that at a TyG over 4.7 the risk of developing HT among patients with T2DM is 65-foldhigher, OR 65.344 (Cl 5.067-842.607), p=0.001. We can state as a conclusion that our study is the second to demonstrate the predictive value of TyG for thyroid pathologies and the first to make this finding Hashimoto's autoimmune thyroiditis.

In the study led by Lee et al. [18], a 10-15-foldhigher incidence of HT among females was shown, similar results we also found in our study, which allows us to comment the result obtained that the female gender is a risk factor of occurrence of HT in the patients with T2DM. For female gender we obtained OR 23.176 (4.530-118.572) p= 0.0001 after multivariate analysis performed comparing the groups of patients with T2DM and with HT and T2DM. This result is not surprising in the context of the high incidence of HT among the female population.

Another risk factor for developing HT in patients with T2DM, comparing groups 1 and 3, that our study found was alcohol consumption, OR 7.048 (Cl 95% 2.187-22.720), p <0.001. From our results we can state that alcohol increases risk of developing HT, for the diabetic patients by 7 times, which is statistically significant. Comparing with the literature, we did not find another study which attesting the risk; therefore, we can state that our study is the first to certify the causal relationship between the two. Moreover, in a study conducted by Efferaimidis et al. showed that there is no association between alcohol consumption and de novo development of TPO antibodies. The results of this study are not in agreement with the results of our study. [32]

Univariate analysis between groups 1 and 3 showed that BCI is also a risk factor for developing HT in patients with T2DM, OR 5.318 (Cl 95% 2.214-12.774), p<0.001. We found no other studies in the literature that addressed this relationship, but we did find one study that addressed the inverse relationship, it described a 2-fold increasedrisk in HT patients to develop BCI, OR 2.06 (Cl 95% 1.46-2.92). [33] Interpreting the results inversely, we can say that BCI may also be a risk factor involved in the development of HT, even with a higher prediction value, the risk being in this case 5 times higher in the population with T2DM. The higher result is somewhat normal, given that the population we studied was composed of diabetics, who have a higher proportion of associated BCI. Again our study is the first to demonstrate a positive independent predictor relationship for BCI in terms of the occurrence of HT in patients with T2DM.

In the study conducted by Ogbonna et al. [34] showed a higher risk of patients with thyroid pathologies to develop diabetic nephropathy, OR 4.8, p=0.001. Considering diabetic nephropathy a part of microvascular complications of diabetes we can say that the results of our study are so similar to those of the above mentioned study. In our case, after analysis of the comparison between groups 1 and 3, it was shown that there is a 4 times higher risk of developing HT among patients with microvascular complications in among the population with T2DM, in other words the presence of microvascular complications is a risk factor for the development of the association between the two pathologies, OR 4.571 (Cl 95% 1.963-10.646), p<0.001.

Another parameter taht had statistical significance as a risk factor for the association between HT and T2DM was depression, this time in the comparison between the groups 2 and 3, OR 9.333, (Cl 1.121-77.204), p<0.001. In the study conducted by Bode et al. a positive predictive value for hypothyroidism was demonstrated for depression, OR 1.30 (Cl 95% 1.08-1.57), while for autoimmune thyroid pathologies the results were inconclusive, OR 1.24 (Cl

95% 0.89-1.74). [35] Unfortunately, this situation is also confirmed by us, the depression parameter maintain statistical significance for the other groups and not even after multivariate analysis of the data.

2.3.7. Conclusions of Study 2

The univariate analysis revealed the following risk factors for developing HT in patients with T2DM: weight over 83.5 kg, BMI over 29.6 kg/m², HbA1c over 5.85%, blood glucose over 122 mg/dl, TSH over 1.1 pmol/L, TyG over 4.7, TyG BMI over 140.8, and TyGWC, female gender, alcohol consumption, smoking, BCI, hypothyroidism, Goitre, microvascular complications of T2DM, depression and insulin treatment.

After multivariate analysis it was found that the protective factor TAD was retained and BMI, glycemia and surrogate index of insulin resistance TyG, female gender, alcohol, smoking, hepatic steatosis, BCI, hypothyroidism and insulin treatment remained as risk factors.

2.4. General conclusions

1. T2DM is a risk factor for the development of autoimmune thyroiditis through a higher incidence of HT among patients with T2DM versus general European population.
2. Female gender was found to be a risk factor involved in the occurrence of HT in patients with T2DM, probably determined by the high incidence of thyroid pathology in female population.
3. Insulin resistance seems to be the vector linking HT and T2DM, A value of TyG, a calculated insulin resistance surrogate index, above 4.7 was found to be a risk factor involved in the occurrence of HT in patients with T2DM, TyG increased the risk of occurrence of HT in the T2DM population by 65-fold..
4. Insulin resistance seems to be the vector linking HT and T2DM.
5. Overweight is a risk factor involved in the association of HT in patients with T2DM most likely through a week metabolic control, since it is known that overweight and obesity is a cause of glycemic inbalance.
6. Abdominal obesity, above 88,5cm, increases the risk of HT association with T2DM 6-fold, this is caused also by a metabolic inbalance.
7. Blood glucose above 122mg/dl was found to be an independent predictor of association of HT with T2DM, proving once more that a week metabolic control is the probable cause of the association between the tow studied pathologies.
8. Insulin treatment increased the risk of developing HT in patients with T2DM over 7 times, showing once again the involvement of the metabolic control in this association.

9. The correlation between elevated HbA1c, metabolic syndrome and elevated ATPO values was shown with increased statistical significance ($p<.001$) to be the pathophysiological link between T2DM and HT.
10. High Systolic blood pressure increases the risk of HT in patients with T2DM almost 3-fold, this is explained through the fiziopatological mechanism of cerebral insulinresistence involved in the pathyology of arterial hypertension, mechanism which was proven by high values of insulinresistence markers; this allows us to conclude that insulinresistence is the real risck factor.
11. Diastolic blood pressure below 77.5mmHg seems to have a protective effect on the occurrence of HT in patients with T2DM, because the normal or low values of diastolic blood pressure does not associate with insulinresistence, this not only does not elevates the risk, but it gives a protective effect agains the association of the two pathologies.
12. Hepatic steatosis is a risk factor for HT association in patients with T2DM, due to the high level of insulinresistence that low metabolic control patients have, as we have shown already.
13. The presence of Ischemic Heart Disease increased the risk of HT association with T2DM twice, the fiziopathological mechanism to blame for this looks to be insulinresistence, especially the cardiac one, correlated with a low metabolic control.
14. Hypothyroidism was found to be risk factor for association of HT with T2DM, an association of high values of TSH in patients with type 2 diabeteas can lead to a metabolis disorder which seems to be the real risk factor for the association of Hashimoto thyroiditis to type 2 diabetes.
15. Smoking increased T2DM patient's risk of developing HT 7-fold, it is known that smoking is a risk factor for autoimmune thyroid disorders, and in our study it was also confirmed to be a risck factor for developind HT.
16. Alcohol consumption increased T2DM patient's rick of developing HT by almost 3.5-fold; the probable cuse is the distruction of the glad due to the increased toxicity associated with alcohol intake, this being probably followed by an autoimue destruction.
17. This is the **first** study to demonstrate the predictive value of TyG for Hashimoto's autoimmune thyroiditis.
18. Our study is the **first** to certifythe causal relationship between alcohol consumption and the occurrence of HT in patients with T2DM.

19. Again our study is the **first**to demonstrate a positive independent predictive factor relationship for BCI with respect to the occurrence of HT in patients with T2DM.
20. The results of our study attest the need to introduce screening for the autoimmune thyroid pathologies among patients with T2DM.

2.5. Originality Element

The topic addressed in this paper is a topical one on which no other research has been carried out, as it is not described in national or international bibliographical references. Although this association between T2DM and HT is relatively common in the population, it has not been studied so far. The present work has carried out a pioneering study on this topic, succeedingin demonstrating some association between the two pathologies. This scientific study also revealed some possible clinical and paraclinical causes responsible for the association between T2DM and HT.

The uniqueness of this work is the large patient selection base, the initial sample of 5064 T2DM patients studied, who were evaluated demographically, clinically and paraclinically.

Through the prism of female gender, smoking, alcohol consumption, systolic hypertension, high values of blood glucose and TyG, overweight, abdominal obesity, hepatic steatosis, hypothyroidism, ischemic heart disease and insulin treatment, we demonstrated that T2DM is associated with HT.

In Romania, such research on the association of T2DM with HT, has not been conducted prior to this study.

This study is the first of its kind to demonstrate a causal relationship between alcohol consumption and the occurrence of HT, the predictive value of TyG for autoimmune thyroid pathologies and the independent predictive quality of BCI for the occurrence of HT in patients with T2DM.

The present study paves the way for further research needed to concretely understand the mechanisms involved at macro and micromolecular level in the association of the two pathologies. Future studies are needed to further explore the topic, either through the assessment of autoimmunity or the pathophysiology of IR (by testing the HOMA-IR index), aspects that this paper has not addressed. [36]

This work represents a reference point for future scientific research on this theme, both at national and international level, succeeding in demonstrating the association between T2DM and HT, which has not been demonstrated in the past.

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LIST OF PUBLICATIONS

Work number 1: Annex 2 Article of Romanian Diabetes Journal

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Authors: Parlițeanu O.A, Cheța D.M

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Electronic poster: Annex 4 Electronic Poster

"Type 2 Diabetes Mellitus and Hashimoto's thyroiditis: possible associations and clinical correlations – partial results" presented in the National Congress of the Romanian Society of Diabetes, Nutrition and Metabolic Disorders - Edition 44, Poiana Brașov, 23rd-26th May 2018 (Congress SRD 2018)

Authors: Parlițeanu O.A, Chețа D.M

Poster: Annex 5 Poster

"Type 2 Diabetes and Hashimoto's Thyroiditis-Possible Associations and Clinical Correlations-Preliminary Results" presented in the 34th International Congress of Internal Medicine, from South Africa, Cape-Town, 18th-21st October 2018 (Congress WICM 2018)

Authors: Parlițeanu O.A, Chețа D.M