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EFFECT OF THYROID HORMONE T3 AND T4 ON THE CENTRAL NERVOUS SYSTEM IS ASSOCIATED WITH DIABETES MELLITUS

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EFFECT OF THYROID HORMONE T3 AND T4 ON THE CENTRAL NERVOUS SYSTEM IS ASSOCIATED WITH DIABETES MELLITUS

By Mustafa Hassan Saed ALTALABANI

June 2022

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ABSTRACT

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June 2022

Thyroid hormones are critically involved in the development of the central nervous system (CNS). Fetal and/or neonatal hypothyroidism causes myelination defects, and neuronal migration and differentiation, which cause mental retardation, which can be profound, and in certain cases irreversible neurological alterations. It is accepted that most of the actions of the thyroid hormones are due to the interaction of the active thyroid hormone, triiodothyronine (T3), with nuclear receptors. Thyroid hormone regulates the expression of a series of genes that encode proteins with very diverse physiological functions: myelin proteins, proteins involved in cell adhesion and migration, signaling proteins, cytoskeleton components, mitochondrial proteins, transcription factors, etc. T3 originates partially in the thyroid gland, but for the most part is generated locally in target tissues from thyroxine (T4). The concentration of T3in the CNS is tightly regulated by type II and III 2 deiodinases. Type II deiodinase, which is expressed in tanycytes and astrocytes, produces up to 80% of the T3present in the CNS. Type III deiodinase, present in neurons, degrades T4 and T3to inactive metabolites. In the human fetus, the T3 receptor is present at least from the 10th week of gestation, indicating that thyroid hormone may have actions in the human fetal brain. Of course, in human fetal tissues, T4 can be detected in most of them, and T3 in the brain, which could be derived mostly from T4.

2022, 65 pages

Keywords: Hypothyroidism, Thyroid hormone T3 and T4, Central nervous system, Diabetes mellitus

ÖZET

TİROİD HORMONU T3 VE T4'ÜN MERKEZİ SİNİR SİSTEMİ ÜZERİNDEKİ ETKİSİ DİABETES MELLİTUS İLE İLİŞKİLİDİR

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Tiroid hormonları, merkezi sinir sisteminin (CNS) gelişiminde kritik bir rol oynar. Fetal ve/veya neonatal hipotiroidi miyelinasyon bozukluklarına ve derin olabilen mental retardasyona ve bazı durumlarda geri dönüşü olmayan nörolojik değişikliklere neden olan nöronal migrasyon ve farklılaşmaya neden olur. Tiroid hormonlarının etkilerinin çoğunun, aktif tiroid hormonu triiyodotironin (T3) ile nükleer reseptörler arasındaki etkileşime bağlı olduğu kabul edilmektedir. Tiroid hormonu, çok çeşitli fizyolojik fonksiyonlara sahip proteinleri kodlayan bir dizi genin ekspresyonunu düzenler: miyelin proteinleri, hücre yapışması ve göçünde yer alan proteinler, sinyal proteinleri, hücre iskeleti bileşenleri, mitokondriyal proteinler, transkripsiyon faktörleri, vb. T3 kısmen tiroid bezinden kaynaklanır, ancak çoğu zaman tiroksinden (T4) hedef dokularda lokal olarak üretilir. CNS'deki T3 konsantrasyonu, tip II ve III 2 deiyodinazlar tarafından sıkı bir şekilde düzenlenir. Tanisitler ve astrositlerde ifade edilen Tip II deiyodinaz, CNS'de bulunan T3'ün %80'e kadarını üretir. Nöronlarda bulunan Tip III deiyodinaz, T4 ve T3'ü inaktif metabolitlere indirger. İnsan fetüsünde, T3 reseptörü en az 10. gebelik haftasından itibaren mevcuttur, bu da tiroid hormonunun insan fetal beyninde etkileri olabileceğini gösterir. Elbette insan cenin dokularının çoğunda T4 ve beyinde, çoğunlukla T4'ten türetilebilen T3 saptanabilir.

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Anahtar Kelimeler: Hipotiroidizm, Tiroid hormonu T3 ve T4, Merkezi sinir sistemi, Diabetes mellitus

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CONTENTS

LIST OF SYMBOLS

%	Percentage
dL	Deciliter
g	Gram
mg	Miligram
mL	Milliliter
nmol	Nanomol
µg	Mikrogram

LIST OF ABBREVIATIONS

CNS	Central nervous system
CVD	Cardiovascular disease
DM1	Type1 diabetes mellitus
DM2	Type2 diabetes mellitus
FT3	Free triiodothyronine
FT4	Free thyroxin
HbA1c	Glucosylated hemoglobin
IGF	Insulin like growth factor
NGF	Nerve growth factor
rt3	Reverse triiodothyronine
T3	Triiodothyronine
T4	Thyroxin
TH	Thyroid hormon
TRH	Thyrotropin-releasing hormon

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

1.1 Thyroid Hormones (TH)

The source of TH is the thyroid gland, which predominantly secretes thyroxine (T4) from which, by deiodination, most of the circulating T3 is derived. It is from T3, a hormone that has biological activity at least 5 times greater than that of T4, which depends on the activity of practically all the tissues of the organism, since all of them potentially express TH receptors. Thus, in order to maintain the normal activity of the target tissues, adequate intracellular levels of T3 must be guaranteed, which depends not only on thyroid activity but also on the intracellular generation of this hormone, processes that depend, respectively, on, the integrity of the hypothalamic-pituitary-thyroid axis and the activity of specific enzymes, the deiodases.

In general, thyroid function is regulated by the thyrotropin-releasing hormone (TRH) produced in the hypothalamus, which, through the hypothalamic-pituitary portal system, goes to the anterior pituitary, binding to specific receptors on the thyrotroph. and inducing the synthesis and secretion of thyrotrophic hormone (TSH). This, in turn, interacts with receptors present in the thyroid follicular cell membrane, inducing the expression of proteins involved in TH biosynthesis, increasing thyroid cell activity and stimulating hormone secretion (Saifi *et al.* 2017). As for deiodases, these are the main sources of T3, both circulating and intracellular, and have specific roles due to their different characteristics and forms of regulation. So far, three isoforms of deiodines have been cloned and identified, type 1 (D1), type 2 (D2) and type 3 (D3) (Kelly, 2000). D1, expressed predominantly in liver and kidneys, has its activity increased in hyperthyroidism, decreased in hypothyroidism, being blocked by the use of propylthiouracil (PTU), an anti-thyroid compound that inhibits the activity of thyroperoxidase and, therefore, the TH biosynthesis. D2, highly expressed in the central nervous system (CNS), pituitary, brown adipose tissue and placenta, on the contrary, has its activity increased in hypothyroidism and decreased in hyperthyroidism, being insensitive to PTU, but inhibited by T4 by the reverse triiodothyronine (rT3), an inactive product of T4 deiodination (6). Both D1 and D2 are capable of generating T3 (active hormone) and 3,3' T2 (inactive iodothyronine) from the 5' deiodination of T4 and rT3, respectively (Sharma *et al.* 2021)

The third isoform of deiodinase, D3, is highly expressed in developing tissues, mainly in the central nervous system (CNS), although it is also detected in tissues such as skin, liver, placenta and CNS in adults. In the same way as D1, D3 has an increased activity in the hyperthyroid state and decreased in hypothyroidism; however, it generates only inactive products, rT3 and 3,3'T2, from T4 and T3, as it works by removing iodine from position 5 of these iodothyronines (Hulbert 2000). Furthermore, a common feature of the three deiodinase isoforms is that they can be inhibited by iopanoic acid or ipodate. The different tissue distribution and the different regulatory mechanisms of the three deiodases make them play very peculiar physiological roles. While D1 generates T3 for the extracellular fluid (ECL), thus making this hormone available to the tissues, D2 plays an important role in the generation of T3 in the very tissues in which they are expressed, which allows the its ready use (6). D3, on the other hand, is important for the degradation of TH, limiting its biological action. An additional advantage of tissues that express D2 and/or D3 is that they are, in a way, protected from thyroid functional alterations, since in the face of hypothyroidism, the activity of D2 increases, while that of D3 falls, occurring the opposite is true in situations of hyperthyroidism, in order to ensure adequate intracellular levels of T3. This role is exceptionally relevant during the embryonic and neonatal period, when thyroid hormone is essential for the establishment and completion of the differentiation process of various tissues and organs, as is the case of the central nervous system. In this, T3 is of fundamental importance for stimulating the synthesis of growth factors, such as nerve growth factor (NGF) and insulin-like growth factor (IGF), on which the activation of proliferation, synaptogenesis and neuronal myelination (Sharma *et al.* 2021).

In this way, the actions that T3 triggers in its target cells have repercussions on marked biological effects on the activity of various tissues and systems, which, in general, have a very high activity when under the action of this hormone, which increases the expression and the activity of several enzymes of oxidative metabolism, ATPases, ion transporters and proteins important for the development of several specific functions of the same and although it is very well defined that the mechanism of action of this hormone involves changes in the expression of specific genes (transcriptional action), one cannot ignore the growing number of reports in the literature of T3 actions considered non-genomic, even though the mechanism is not genomic. involved in establishing these effects is not fully understood.

1.2 Hypothyroidism

Also called myxedema, it refers to insufficient amount or activity of thyroid hormones (T3 and T4). “Hypothyroidism is the name given to the clinical situation characterized by a deficit in the secretion of thyroid hormones, produced by an organic or functional alteration of the same gland or by a lack of stimulation by TSH. (Álvarez-Sierra *et al.* 2019).

Hypothyroidism caused by primitive thyroid alterations (removal, irradiation, structural deficit) is designated as primary, while hypothyroidism that depends on insufficient TSH secretion is classified as secondary, if the failure is adenohypophyseal, or tertiary, if the alteration It comes from the hypothalamus. In the initial stage of primary hypothyroidism, there is a slight decrease in thyroxine (T4) secretion that induces an increase in TSH. This situation leads to a minimal decrease in free thyroxine (FT4), even within the reference limits, but with a progressive increase in TSH, a situation known as subclinical hypothyroidism. It should be noted that the increase in TSH preferentially produces an increase in the thyroid secretion of triiodothyronine (T3) and also an increase in the deiodination of T4 to T3. In short, there is a relative hyperproduction of T3 with respect to T4, which reduces the effect of thyroid hormone deficiency in peripheral tissues, which would explain why we sometimes find free T3 (FT3) at high limits. In later stages, there is a decrease in T4 and also in T3 with a persistent increase in TSH. In general, these phenomena occur gradually from the initial stage of subclinical hypothyroidism to severe hypothyroidism, which could culminate in myxedema coma, a more serious and rare situation of the disorder.

Primary hypothyroidism is more common in regions with iodine deficiency; It is much more common in women and increases with age. Most diagnoses occur in the sixth decade, with chronic autoimmune thyroiditis being the most common cause. In the NHANES III study, 4.6% of the subjects included had elevated TSH and the percentage increased to 15.4% in those older than 65 years.(Liappas 2009)

1.3 Thyroid Hormones and the Central Nervous System

Thyroid hormones are critically involved in the development of the central nervous system (CNS). Fetal and/or neonatal hypothyroidism causes myelination defects, and neuronal migration and differentiation, which cause mental retardation, which can be profound, and in certain cases irreversible neurological alterations. It is accepted that most of the actions of the thyroid hormones are due to the interaction of the active thyroid hormone, triiodothyronine (T3), with nuclear receptors. Thyroid hormone regulates the expression of a series of genes that encode proteins with very diverse physiological functions: myelin proteins, proteins involved in cell adhesion and migration, signaling proteins, cytoskeleton components, mitochondrial proteins, transcription factors, etc.. T3 originates partially in the thyroid gland, but for the most part is generated locally in target tissues from thyroxine (T4). The concentration of T3in the CNS is tightly regulated by type II and III 2 deiodinases. Type II deiodinase, which is expressed in tanycytes and astrocytes, produces up to 80% of the T3present in the CNS. Type III deiodinase, present in neurons, degrades T4 and T3to inactive metabolites (Di Liegro 2008).

T3 receptors are already present in the fetal rat brain, in mRNA, from day 11.5 after conception, and the protein can be detected as early as the 14-day fetus. In the human fetus, the T3 receptor is present at least from the 10th week of gestation, indicating that thyroid hormone may have actions in the human fetal brain. Of course, in human fetal tissues, T4 can be detected in most of them, and T3 in the brain, which could be derived mostly from T4. Thyroid hormones present in the fetus, especially T4They can be of maternal or fetal origin. In humans, maternally derived T4 could account for more than 50% of fetal T4 at term under normal circumstances

1.4 Objectives

1. To compare the clinical characteristics, thyroid hormone profile (TSH, total and free T4, total and free T3 and reverse T3) and systemic inflammation (CRP) between patients with DM2 and healthy individuals without DM.
2. To evaluate the presence of EDS in T2DM patients based on normal serum TSH and free T4 concentrations associated with increased T3r concentrations and/or decreased serum T4, T3 or T3L values in relation to the reference values.
3. To evaluate the association between alterations in the aforementioned thyroid hormones and clinical characteristics and serum concentrations of inflammatory markers (CRP) in patients with DM and a control group, and those with glycemic control (HbA1c) in patients with DM.
4. To evaluate clinical, laboratory and hormonal differences in the subgroups of patients with and without cardiovascular disease and in relation to the control group without DM.
5. To establish the relationship between the presence of cardiovascular autonomic neuropathy and the frequency of intraoperative cardiovascular complications in patients with Diabetes Mellitus
6. The aim of the present study was to analyze the Cardiac Autonomic Nervous System in patients diagnosed with Subclinical Hypothyroidism through the assessment of Heart Rate Variability and QT interval duration from the 24-hour Holter Electrocardiogram.

2. LITERATURE REVIEW

Thyroid hormones are necessary for maturation of the central nervous system during development and also have actions in the adult brain. Thyroid diseases usually cause psychiatric disorders (Bernal 2002). For example, hypothyroidism presents with lethargy, hyporeflexia, and impaired motor coordination. Even subclinical hypothyroidism can be associated with symptoms such as memory loss. The most detailed review of the structural defects present in the hypothyroid rat brain was carried out by (Akhoundi *et al.* 2011). Among the defects can be found: Increases in the cellular density of the cerebral cortex, due to the reduction of the neuropil. Decreased number of cells in regions that increase their cellularity after birth, such as the olfactory bulb, and the granular layers of the dentate gyrus and cerebellum. Decrease in GABAergic interneurons in the cerebellum with accumulation of precursors. Decrease in interneurons in the cerebral cortex. Structural alteration of some neuronal types, such as cholinergic neurons, Purkinje cells and layer V pyramidal neurons. Structural changes are also seen in adult-onset hypothyroidism, such as alterations in the number and distribution of dendritic spines in pyramidal neurons, but are reversible with hormonal treatment (Wallis *et al.* 2010). All these morphological alterations are a consequence of the alteration of the following biological processes:

In studies of the effect of thyroid hormones on development, the most widely used model has been the rat and, in recent years, the mouse, with the latter having the possibility of developing genetic models. Although these models allow us to delve into pathophysiological and molecular mechanisms, they also pose a problem of extrapolation to the human body. Although the sequence of events during development is similar in vertebrates, it is important to point out that there are great differences in its relationship with the moment of birth, in the different species. Most brain growth occurs after birth in both rats and mice as well as humans, but the former are born with a less developed thyroid axis than humans. For reference, it can be said that the newborn rat can be compared to a human fetus in the second trimester of pregnancy, and that the newborn human would be equivalent to a 6-10 day old rat (Morte *et al.* 2010).

Type 2 diabetes (DM2) is one of the major public health problems worldwid and one of the greatest challenges of the 21st century. Along with obesity, it is considered one of the largest epidemics of chronic non-communicable diseases with an alarming global increase. The economic impact is enormous, in 2010 the global expenditure on health attributable to obesity which represented 12% of the total global cost of all diseases (Herman 2017).

Glycemia is an easy-to-assess laboratory parameter and plays a fundamental role in both the diagnosis and monitoring of DM. The tests that express chronic hyperglycemia must be prioritized since they constitute a tool that allows evaluating the efficacy of therapy. The determination of glycated proteins, especially hemoglobin (HbA1c) and serum proteins (fructosamine) allow quantifying their average over weeks or months, complementing glycemic monitoring. (Danese *et al.* 2015)

HbA1c allows assessment of glycemic control without fasting, at any time of the day, it has low biological variability, it is not altered by stress and it does not present sample instability. These factors, together with the documentation that it predicts the development of chronic microvascular and macrovascular complications, allow it to be considered as a variable for decision-making in the comprehensive management of patients; numerous recommendation guides suggest its measurement at regular intervals.

The increase in life expectancy in DM as a consequence of better control has generated unexpected consequences: a high incidence and prevalence of chronic disorders. Approximately 60% of deaths globally are related to chronic processes. - cos, with a projection of an additional increase of 15% by 2020 (Radin 2014) .

One of the most important chronic disorders is chronic kidney disease (CKD); its growth is favored by the aging of the population, the increase in the prevalence of DM, obesity, arterial hypertension and metabolic syndrome, among the most frequent causes. DM, as a risk factor for CKD, presents an Odds Ratio of 1.66 (1988-1994) with a progressive increase towards 2.33 (2007-2012). The global cost of DM associated with

RD (ERDM), Due to the relationship with cardiovascular diseases (CVD) and the need for substitute treatments, it implies a high economic outlay. The enormous controversy and limitations in this subject determine that it is a real challenge to carry out a review to know the advantages and limitations in the use of HbA1c as a tool in the different stages of RD and to be able to propose alternatives in the future that make possible the use of new options, for a better approach in the diagnosis, follow-up and treatment guidelines of these patients.

The only way to assess the adequacy of treatment and carry out interventions when the objectives have not been achieved is to study fasting (FA) and/or postprandial (PPG) glycemic levels and long-term glycemic control (HbA1c and/or continuous monitoring).

Given the results observed in the DCCT Study (Diabetes Control and Complications Trial) and in the UK Prospective Diabetes Study (UKPDS), there is a broad consensus of great benefit in obtaining and maintaining levels blood glucose within the established range.

Given that patients with CKD were excluded from these studies, it is our intention to share the difficulties presented by patients with CKD for the use of HbA1c as a glycemic target, highlighting the factors that are added in this context. In the first part we will review the role of HbA1c in patients with DM without RD and then show the difficulties that occur in the subpopulation with CKD, focusing especially on patients undergoing hemodialysis and peritoneal dialysis. We will address it considering that it is a controversial issue (Kuo *et al.* 2016).

2.1 Glycated Hemoglobin

Hb is a protein that is present in red blood cells, it is made up of two globin dimers, each associated with a heme group. In adults, Hb has different names according to to the type of dimer that make up the molecule. Approximately 97% corresponds to HbA (α2, β22), 1.5-3.5% corresponds to A2 (α2, δ2) and <2% to HbF (α2, η2), varying these percentages according to different haemoglobinopathies. The HbA components were identified by charge separation and named according to the order of elution: A0, A1a, A1b, and A1c. The meaning of this finding in relation to DM was contemplated by (Rahbar *et al.* 1969) who using gel electrophoresis, reported elevation of these Hb fractions in DM. Glycosylation refers to an enzymatic modification that alters protein function, half-life, or interactions with other proteins. Glycation refers to the non-enzymatic attachment of a monosaccharide (usually glucose) to an amino group of a protein. Although Hb has been the most studied and used glycated protein, others have been evaluated, such as glycated albumin, fructosamine, and advanced glycation products.

The initial step in the reaction between glucose and Hb is the condensation between the carbonyl of the former and the free primary amine of the latter, generating the formation of a Schiff base (early Maillard reaction). This base is not stable and can dissociate or undergo an Amadori rearrangement to form a stable ketoamine. The average survival of the erythrocyte is 117 days in men and 106 days in women. A blood sample contains red blood cells of different ages, with different degrees of exposure to glycemia, those with the longest survival are more exposed and the youngest are more numerous. These differences determine that the glucose levels of the 30 days closest to the determination contribute approximately 50% to the formation of HbA1c, while the period 90 to 120 days contributes only 10%. The modification of Hb is irreversible and its rate of formation reflects the minute-by-minute glycemic environment. The aforementioned allows us to understand why HbA1c reflects the glycemic status, showing the average blood glucose of the lasT3months. The first mention of the importance of HbA1c determination by the World Health Organization (WHO) and in 1988 the ADA suggested in its recommendations that it should be screened for DM monitoring. From there, a large number of methods have been developed for HbA1c, which consist of separating glycated Hb from non-glycated Hb.

In 1992, the College of American Pathologists reported discrepancies in HbA1c results, showing that values from 4% to 8.1% could result for the same sample. This evidence determined the need to introduce standardization; the National Glycohemoglobin

Standardization Program (NGSP) in the United States introduced the HPLC method used in the DCCT study, which measured HbA1c. In Sweden, another technique was proposed as standard, as well as in Japan, generating doubts regarding which would be the best method. The International Federation of Clinical Chemistry and Laboratory (IFCC), in an attempt to generate an international standardization program, set up a working group on HbA1c, with the aim of defining a simple international reference method. The HPLC method combined with mass spectrometry or with capillary electrophoresis with ultraviolet detection was postulated, (Weykamp 2013) and its implementation was accepted by the national societies of clinical chemistry and by a network of laboratories. However, the laboratories continued to carry out their own tests, but calibrated and compared with these references (Bryśkiewicz and Majkowska 2011).

IFCC HbA1c is expressed in mmol/mol Hb when expressed as a percentage, the reference range for patients without DM is approximately 1.5-2% lower than DCCT values. Both NGSP and IFCC have roles supplements in the HbA1c standardization process. Both lay a solid foundation for establishing the accuracy and reliability of the determination method in a laboratory anywhere in the world. The NGSP, like the societies of Japan and Sweden, has designed conversion equations for its own methods in relation to IFCC. The equation that allows conversion of the values obtained for HbA1c is called the "master equation ", with a linear relationship between the NGSP method and the IFCC method. In 2004, a working group (ADA/EASD/IDF Working Group of the HbA1c Assay) was set up to agree on the concepts of the definition of HbA1c. There was complete agreement on some definitions but not on which system the determination should be based on. Later, in 2007, it was agreed that HbA1c results would be reported in IFCC units (mmol glycated Hb/total mol Hb) and NGSP units (%). Those without DM would have a range of 25 to 42 (mmol/mol) and NGSP 2.5 to 4.2% (HbA1c).

Another proposal was to abandon the term HbA1c and report it as derived from an estimated mean blood glucose (eAG). This was documented based on a multinational prospective study, which showed the linear relationship between HbA1c and mean glycemia, generating controversy among the authors, some endorsing its use and others not, since there was great variability between both determinations (Weykamp 2013).

2.2 Clinical Utility

HbA1c in the clinical follow-up of patients with DM acquires relevance from the DCCT that evaluated the intensified insulin treatment compared to the conventional one, in DM1. The intensified arm presented an average glycemic lower than 155 mg/dL, with a mean HbA1c of 7.2%, compared to the conventional 231 mg/dL and HbA1c of 9.1%, with statistically significant differences. The risk of microvascular complications was reduced in the intensified group (retinopathy 76%, microalbuminuria 34%, macroalbuminuria 44%, neuropathy 64%), as well as progression (preproliferative to proliferative retinopathy 47%, microalbuminuria 43%, macroalbuminuria 56%, and neuropathy 57%). Macrovascular complications did not present significant differences between both groups. One of the conclusions to consider was that the relationship between HbA1c and the risk of chronic microvascular complications was linear, without being able to identify a level where the risk disappears (Sherwani *et al.* 2016).

The observational study EDIC continued the DCCT, evaluated the impact of intensified treatment in the long term and showed a reduction in the progression of microvascular comorbidities, as well as the incidence of cardiovascular events (42%) in intensified patients. during the DCCT. Subsequent analyzes showed that the average HbA1c was the most important predictor of progression of complications.

UKPDS (1988) the study in DM2 was published, which showed a decrease in complications in relation to the reduction in HbA1c levels. The average levels of HbA1c were 7.0% in the intensified group, and 7.9% in the intensified group. control group; this small difference showed a 25% risk reduction for microvascular disease, 16% for fatal and nonfatal acute myocardial infarction (AMI), and 12% for any DM-related endpoint, compared with the conventional treatment group. - tional. The trend in the reduction of cardiovascular events (AMI or amputations) was not significant, however it was found that for every 1% decrease, a 21% reduction in risk was observed for any endpoint related to DM, 21% for deaths related to DM, 14% for AMI, 37% for microvascular complications, and 43% for amputation. The UKPDS 35 study confirmed the relationship between HbA1c levels and complications.

Patients in the UKPDS study King *et al.* (1999) continued to be followed up with their GPs annually for the first five years and through questionnaires for the next five. The differences in HbA1c obtained were lost, despite which the reduction in the risk of micro and macrovascular events after 10 years was maintained, confirming the observations of the EDIC. This phenomenon was called "legacy effect" or "glycemic memory", allowing to demonstrate that the benefits of glycemic control at a level as close as possible to normal in the first years after diagnosis, have a prolonged effect in reducing blood glucose. risk of appearance of chronic complications and must be prioritized. In this way, it can also be affirmed in DM2 that the relationship between HbA1c and the risk of chronic complications, especially microvascular, is linear without being able to identify a level where the risk disappears.

The Kumamoto study Shichiri *et al.* (2000) presented a design similar to the DCCT but in adults with DM2. At 6 years of follow-up, a continuous relationship between the increased risk of complications and glycaemia was observed, demonstrating that an HbA1c less than 6.5%, GA <110mg/dL and GPP <180mg/dL did not present an increased risk of onset. or progression of retinopathy and nephropathy. After these studies, the impact of glycemic control on cardiovascular morbidity and mortality in DM2 continued to be the subject of debate. The ACCORD study aimed to determine whether lowering HbA1c below 6% (intensive treatment) reduced cardiovascular events compared to levels of 7 and 7.9% (standard treatment) in patients with T2DM with established CVD, or with risk factors. . The intensive group presented a higher risk of overall death, and the study was interrupted before its completion due to higher overall mortality in this arm. It was concluded that intensified treatment in those who did not have CVD at the beginning, whose HbA1c was ≤8%, presented fewer fatal or non-fatal cardiovascular events, contrary to this observation, those who had previous CVD showed an increase in overall mortality.

The Advance study Kalra *et al.* (2019) had the objective of evaluating the impact of glycemic and antihypertensive control on the reduction of the risk of cardiovascular events and chronic complications in patients with DM2. Like the previous studies, an intensive group (HbA1c ≤6.5%) and a standard treatment group (HbA1c 7.3%) were assigned, concluding that after 5 years of follow-up, the first presented a reduction in micro and macrovascular events. from 10%. There was no evidence of increased mortality in the intensive group. Another publication that evaluated the effects of intensive glycemic control on cardiovascular events in patients with DM2 is the VADT. This last group did not achieve significant reductions in the rate of major cardiovascular events or death, observing only a decrease in the progression of albuminuria and a high percentage of hypoglycemia.

In epidemiological studies, HbA1c levels >7% are strongly associated with a significant increase in the risk of developing complications. In short, the DCCT, UKPDS and the Kumamoto study(Shichiri *et al.* 2000) lay the groundwork for the design of current guidelines, confirming that complications can be avoided with defined levels of HbA1c (7% in DCCT, <6.5% in Kumamoto)especially on microvascular disease, with less evidence of its effect on macrovascular.

The ADAG studyanalyzed the relationship between mean plasma glucose and HbA1c, motivated by the scant evidence of previous studies that were mainly in DM1, with a small number of participants, with measurements for short periods of time and carried out carried out in a single moment of the day (without nocturnal or postprandial determinations (World Health Organization 2011). The results obtained showed that there is a consistent relationship between the average glucose and HbA1c (in a range of values from 5%-13%) in a large and heterogeneous group of people (population without DM, with DM1 or DM2, different ages, ethnicities, presence of smoking). An average of 2700 blood glucose tests were performed on each participant, concluding that there is a linear relationship with HbA1c (correlation coefficient 0.84) with some dispersion, probably due to errors in glucose measurement or variability in the HbA1c formation process. The ideal would be to carry out more studies with greater diversity in terms of the characteristics of each subject and with continuous and uninterrupted glucose monitoring for months.

3. MATERIALS AND METHODS

3.1 Methodology

3.1.1 Study area and period of the study

This research was developed at the out patient clinic of the University of Baghdad Hospital Baghdad (BTH), University of Baghdad, Iraq, during the period from January 2021 to January 2022.

3.1.2 Sample selection

The study population will consist of a total of 104 patients who were admitted with a diagnosis of Type 2 Diabetes Mellitus (DM2) in the university teaching Hospital Baghdad. Study including 104 people, being 52 patients with DM2 and 52 individuals without DM, as a control group, matched by sex, age and BMI.

3.1.2.1 Inclusion criteria

- Patients diagnosed with Type 2 Diabetes Mellitus
- Type 2 diabetics and non-diabetics aged 45 to 77 who signed an informed consent form and agreed to take part in the research were included.
- Patients admitted to out patient clinic of the University Hospital Baghdad
- The inclusion criteria used in Subclinical Hypothyroidism (SH) patients with SH and whose laboratory examination showed serum TSH levels between 4.5 and 10 μL /L or upper limit of the laboratory reference value and normal free T4 levels, of both sexes.

3.1.2.2 Exclusion criteria

- Acutely ill patients.

- Known primary thyroid dysfunction
- Presence of thyroid nodules
- Presence of serum anti-thyroid antibodies (anti-thyroperoxidase and anti-thyroglobulin).
- Cardiovascular event or clinical manifestation that evidenced atherosclerotic disease, namely: acute myocardial infarction, unstable angina, stroke or chronic arterial obstruction in the lower limbs for less than 6 months.
- Use of amiodarone or glucocorticoid
- Use of propranolol started in less than 6 months.
- Use of iodinated contrast for less than 6 months before the start of the study.
- Heart failure, severe liver disease (reduced albumin or increased INR), advanced kidney disease (creatinine clearance < 60 mL/min/m^2), hemodialysis patients.
- Patients with active neoplasia or in follow-up with remission for less than 5 years.
- Patients with inflammatory or autoimmune disease.
- Patients with chronic infections such as active tuberculosis or with cure for less than 1 year, with HIV or hepatitis B and C (even after treatment and evolution to cure).

3.1.3 Control group

Consisting of individuals without DM, according to their own report, selected among the companions of patients and employees of the BTH, Iraq, belonging to a socio-economic-cultural stratum similar to that of patients with diabetes, obeying the same inclusion criteria. and exclusion defined for patients with DM.

3.2 Study Design

Patients and individuals without DM who fit the inclusion and exclusion criteria were invited to perform anthropometric measurements of weight, height and waist and hip measurements. Other data on age, time of diagnosis of diabetes, type of therapy in use, smoking, arterial hypertension, history of cardiovascular events and chronic microvascular complications of DM were taken from the patients' medical records on the same day of the evaluation. Blood samples were collected to measure the hormonal laboratory parameters (total and free T4, total and free T3 and reverse T3), metabolic control of DM (fasting glucose and HbA1c) and inflammatory markers (CRP) with the aim of verifying relevant differences between such parameters in the groups of patients and individuals without DM. Additionally, we looked for associations between serum TH concentrations, glycemic control and systemic inflammation. We also sought to establish relationships between TH and CRP levels and clinical and anthropometric parameters, as well as their relationship with the presence of DM complications and cardiovascular events.

Finally, the TH dosages that resulted in a relevant relationship with the presence of cardiovascular events were analyzed in more detail through the construction of ROC curves. An interim analysis of statistical power was performed to evaluate the correlation between thyroid hormones and inflammatory markers, resulting in a minimum number of 47 patients.

The participants were instructed by the researchers to come to the collaborating clinic to undergo a 12-lead Micromed Wincardio electrocardiogram exam to assess possible previously unknown cardiac alterations. Then, they performed electrocardiographic assessment at home for 24 hours, with recordings in 12 channels, using the Holter DMS 300-12 recorder. The records were interpreted using the Premier XII/Suprima 12/CardioScan Digital Holter Analysis System software

The parameters of initial interest for evaluation were SDNN (Standard Deviation of all normal NN interval), SDANN (Standart Deviation of the Average NN Interval), pNN50 (Percent of normal-normal NN intervals whose difference exceeds 50 ms) and the duration of the interval. QT in milliseconds. Furthermore, the risk criteria for cardiovascular events previously established by the research were: SDNN < 70 ms, SDANN < 70 ms, pNN50 < 1.5%, QT interval duration > 490 ms and QT interval duration > 80 ms. Data were tabulated in an Excel spreadsheet and qualitatively analyzed by the researchers (Fagard 1998).

3.3 Laboratory Criteria for Metabolic-Glycemic Control

To assess glycemic control in patients with DM, glycated hemoglobin concentrations were used, measured using the high performance liquid chromatography (HPLC) method, with values lower than 7% corresponding to ideal glycemic control (reference value of method: 4%-5.7%). Fasting blood glucose was measured using the hexokinase method, with a reference value between 70 - 100mg/dL, and concentrations below 110mg/dL are considered ideal for patients with DM, as recommended by the Brazilian Society of Diabetes.

3.4 Criteria for Determining a Cardiovascular Event and Clinical Evidence of Atherosclerotic Disease

Patients with a history of acute myocardial infarction and/or stroke were considered as having a cardiovascular event on the day of data collection. The following were considered as clinical evidence of atherosclerotic disease: unstable angina, history of need for coronary artery bypass graft surgery, intermittent claudication, arterial Doppler US of the lower limbs demonstrating arterial vessel obstruction or decreased tibial/brachial index.

3.5 Clinical Parameters

The determination of microvascular complications of diabetes met the following criteria:

- **Retinopathy:** based on eye fundus examination performed routinely every year by the Ophthalmology Service of BTH, as noted in the medical record.

- **Peripheral neuropathy:** considered present in patients who had 2 or more points of insensitivity to the 10g monofilament.
- **Nephropathy:** considered present when there was a history of treatment for this condition or two or more urine samples showing concentrations of albuminuria/g creatine greater than 30mg/g.

Data collected through interviews and retrieval from medical records:

- Sex
- Age
- Time of diagnosis of diabetes
- Type of therapy in use (oral drugs only, insulin only or combination therapy)
- Presence/absence of smoking history
- Presence/absence of a history of arterial hypertension
- Presence/absence of a history of previous cardiovascular events
- Presence/absence of a history of microvascular complications of DM (retinopathy, nephropathy, neuropathy)

3.6 Anthropometric Variables

Body Mass Index (BMI):

Weight and height measurements were taken with the patient in an orthostatic position, on calibrated scales (Filizola ®) and the BMI was calculated by dividing the weight in kg by the square of the height in meters. BMI was classified accoring to the standardised examination protocols (National Center for Health Statistics 2008)

Waist/hip index (WHQ):

Waist measurement was performed with a tape measure, perpendicular to the ground and at the level of the umbilicus, and hip measurement in the same way, at the height of the greater femoral trochanters. The waist/hip index was calculated through the simple division between the values of these measures, in centimeters. The WHQ was considered normal when less than or equal to 0.95 for men and 0.8 for women.

3.7 Blood Sample Collection

20 mL of peripheral blood was collected for laboratory measurements on the same day as the clinical evaluation. Blood was collected by puncture of the antecubital vein, immediately centrifuged and stored at -80°C for further analysis.

3.8 Biochemical Parameters

The biochemical parameters evaluated and their respective dosage methods and reference values (Waithaka *et al.* 2009).

3.9 Hormone Dosages

The thyroid hormone profile was evaluated using the following methods:

⇒ **TSH** – Roche Hitachi-Elecsys Cobas E.411; electrochemiluminescence immunoassay - reference value 0.41 - 4.5 mL/L; sensitivity 0.014 mL/L; intra-assay precision: 5% coefficient of variation; inter-assay precision: 20% coefficient of variation.

⇒ **T4 Free** – Roche Hitachi-Elecsys Cobas E.411; electrochemiluminescence immunoassay - reference value 0.9 – 1.8 μ/dL; intra-assay precision: 5% coefficient of variation; inter-assay precision: 20% coefficient of variation.

⇒ **T4 Total** – Siemens Medical Solutions Diagnostics; radioimmunoassay – reference value 4.5 – 12.5 μg/dL; sensitivity 0.25 μg/dL; intra-assay precision: coefficient of variation 2.7% at mean concentration of 7.4 μg/dL; inter-assay precision: coefficient of variation of 8.1% at an average concentration of 7.2 μg/dL.

⇒ **T3 Total** – Siemens Medical Solutions Diagnostics; radioimmunoassay – reference value 86 – 187 ng/dL; sensitivity: 7ng/dL; intra-assay precision: coefficient of variation 6.5% at a mean concentration of 79 ng/dL; inter-assay precision: coefficient of variation of 9.5% at an average concentration of 95 ng/dL.

⇒ **Free T3** – Siemens Medical Solutions Diagnostics; radioimmunoassay – reference value 1.4 – 4.4 pg/mL; sensitivity: 0.2 pg/mL; intra-assay precision: coefficient of variation 6.1% at a mean concentration of 1.3 pg/mL; inter-assay precision: coefficient of variation of 8.8% at an average concentration of 2.4 pg/mL.

⇒ **Reverse T3** –Radioimmunoassay - reference value 0.090 – 0.350 ng/mL; sensitivity 0.009ng/mL; intra-assay precision: coefficient of variation 8.54% at an average concentration of 0.24 ng/mL; inter-assay precision: coefficient of variation of 8.66% at an average concentration of 0.152 ng/mL.

3.10 Dosage of Inflammatory Markers

Ultra-sensitive PCR – ELISA – Cusabio Biotech Co, Ltd (Wuhan, Hubei, China) – sensitivity (minimum detection limit): lowest protein concentration that can be differentiated from zero; no known cross-reactivity. There are no standard reference values for this marker. Results in mg/dL.

3.11 A Study on Depression and Mood Disorder Status

A sample of 40 patients diagnosed with a mild or moderate depressive episode was considered for the study, according to the International Classification of Mental and Behavioral Illnesses (ICD-10) (Evans and Roberts 2014), which considers for the diagnosis of this type of episode the following criteria:

1. Main criteria: depressed mood, loss of interest, pleasure and reduced energy, leading to increased fatigue and decreased activity.
2. Secondary criteria: reduced concentration and attention, low self-esteem and self-confidence, ideas of guilt and unworthiness (even a mild episode), self-

injurious or suicidal thoughts or acts, lack of appetite, gloomy outlook on future, sleep disorder. To determine the severity of the depressive episode, the following was taken into account:

The samples were extracted in the clinical laboratory of the facultyof BTH, by the designated technician, requiring the following protocol: they were carried out between 7:00 and 9:00 in the morning, the patients were fasting, without smoking and without medication on the day of the initial consultation, 15 mL of blood adequately to avoid hemolysis, the samples were each placed in two 10 mL plastic centrifuge tubes, with 6 mL without anticoagulant.

The biochemical determinations were all carried out together by the same team and by the same evaluator at the laboratory. TSH concentrations were determined by radioimmunoassay (RIA) technique, while T3 and T4 concentrations were determined by immunoradiometric assays (IRMA). The following values were considered normal: TSH 0.8-3.8 IU /L, T3 0.8-2.4 nm /L, T4 75-144 nm /L.

3.12 Statistical Analysis

Consisting of individuals without DM, according to their own report, selected among the companions of patients and employees of the BTH, Iraq, belonging to a socio-economic-cultural stratum similar to that of patients with diabetes, obeying the same inclusion criteria. and exclusion defined for patients with DM.

Interim analysis of statistical power to evaluate the correlation between thyroid hormones and inflammatory markers resulted in a minimum number of 47 patients. Descriptive data analysis was performed using position and dispersion measures for continuous variables and frequency tables for categorical variables. Median and interquartile range were used for continuous variables and absolute count and percentage for categorical variables. The association between two continuous variables was evaluated using the Spearman correlation test and between categorical variables using the chi-square test or Fisher's exact test, when one or more of the frequencies studied was equal to or less than 5. Differences between two groups with the Mann-Whitney test and between three or more groups with the Kruskal-Wallis test. The influence of clinical, laboratory, hormonal and inflammatory profile parameters in the presence of cardiovascular events was performed by univariate logistic regression analysis. Factors with $p \leq 0.15$ in the univariate analysis were selected for multivariate analysis, using the forward stepwise method. R2 in the multivariate logistic regression analysis. Analysis of the determining factors of T3, T4 and T3L was performed by categorizing the variables according to the reference values for subsequent logistic regression analysis, using in these cases the factors with $p \leq 0.15$ in the univariate analysis for regression multivariate.

In the regression analyses, the variables T3r and ICQ were adjusted linearly, so that the results of relative risk report variations of 0.1 unit for T3r and 0.1 unit for ICQ. An evaluation of sensitivity and specificity was carried out, as well as the determination of an ideal cut-off point by means of the creation and comparison of ROC curves and specificity of inflammatory markers and thyroid hormones that were significant in predicting the presence of cardiovascular disease. For all tests, a statistical significance value of 0.05 was adopted. The SPSS program (Statistical Package for Social Sciences, IBM, USA) version 20.0 for MacOS was used for all statistical analyses.

3.13 Bioethical Aspects

This study complied with the ethical standards established for clinical research, in coordination with the competent bodies of the BTH Baghdad, Iraq. In this virtue, the participating subjects have been respected and their desire to be part of the present study or not, has preponderated the search for the good of the participants and justice as fundamental principles.

The research responds to a valid scientific design, prepared based on the predictable risks and the possible benefits for the participants, highlighting the proportionality of the results without risks assumed by the study subjects.

Prior to participating in the proposed scientific research, an informed consent has been signed with each of the participants explaining the research methodology, the benefits that will be obtained from it and the option of choosing to participate or not in the used. strictly with an academic order, without disclosure outside the scientific community responsible for the development of this project.

4. RESULTS AND DISCUSSION

4.1 Results

4.1.1 Descriptive Analysis

Descriptive analyzes of all clinical, anthropometric, hormonal and inflammation parameters of the patient and control groups are summarized as follows.

4.1.1.1 Patients with DM2

In relation to the age of diabetic patients with hypothyroidism, we can observe that 53.85% are between the ages of 43 to 69 years, while 28.85% are between the ages of 70 to 77 years. More than 77 years are 17.31% only in the sample group. Where as in control group 75.00% in 43-69 years, 9.62% in 70-77 years and greater than 77 years are 13.46% (Figure 4.1). The group of DM2 carriers had a median age (interquartile range) of 69 years in patinet group and 64.5 years in control group. Where as the sex category, out of 52 patients and 52 controle subjects 18 female and 34 male in each group

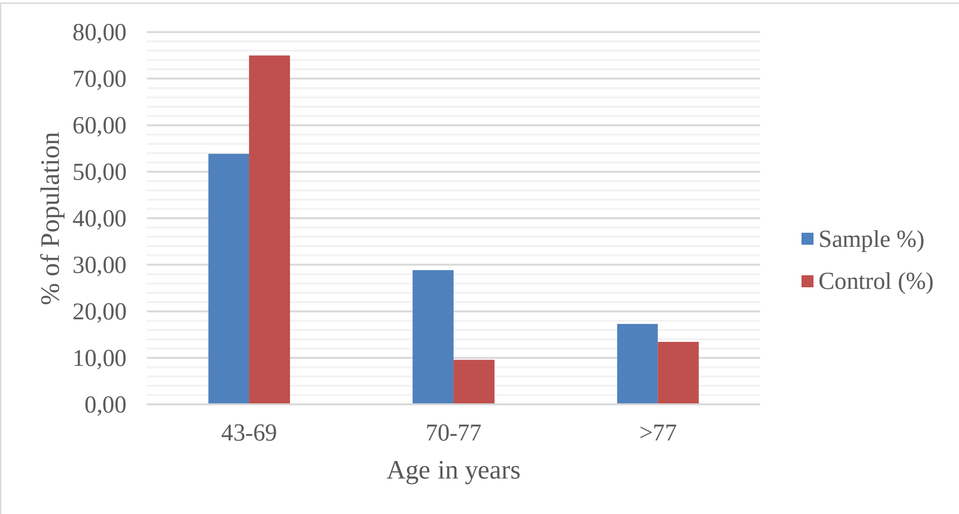

Figure 4.1 Age of diabetic patients with hypothyroidism

4.1.1.2 Frequency of hypothyroidism in patients with type 2 diabetes mellitus in patient group.

Results show thaT46.15% (n=24) of diabetics have hypothyroidism, while 53.85% (n=28)do not. The prevalence of hypothyroidism is 13.16% (n=7). No patient diagnosed with hyperthyroidism was found. 63.46% (n=33) of diabetic patients have clinical hypothyroidism, while 36.54% (n=19) is subclinical. The clinical prevalence is 7.69% (n=4), and the subclinical is 5.76% (n=3). 76% of hypothyroid patients have cardiovascular diseases, while 24% do not, the Pearson's correlation is 0.016, and that is, there is no correlation. 42.30% (n=22) of hypothyroid diabetic patients with cardiovascular disease are hypertensive, followed by hypercholesterolemia with 17.30% (n=9).

- 16% of patients have autoimmune hypothyroidism demonstrated with anti-TPO, while 84% have normal anti-TPO values.
- 10% of patients have autoimmune hypothyroidism demonstrated by thyroglobulin values, in contrast to 90% who do not.
- 33.03% of the patients included in the study have been diagnosed with type 2 diabetes for more than 15 years, and 26.78% between 11 and 15 years; In addition, it can be seen that 17.85% of diabetics over 15 years of age have hypothyroidism, while in 9.82% hypothyroidism occurs between 11 and 15 years of diagnosis of diabetes mellitus.
- Cardiovascular diseases occurred in 50% of patients who have been diagnosed with type 2 diabetes mellitus for more than 15 years, while in people with diabetes in a period of 11 to 15 years in 27.14%. Pearson's correlation was 0.350, which is a negative correlation.
- Regarding microvascular complications, nephropathy was present in 57.7% of the patients. Retinopathy was present in 67.3% and neuropathy in 44.2% of T2DM patients. The majority (80.2%) of the patients were using insulin and only 19.2% were using only oral drugs. About two-thirds (67.3%) of the patients were using a combination of oral drugs and insulin. The median HbA1c was 8.6% (8.3-11.2%).

- Among the patients with DM2, 37(71.15%) had serum concentrations of total T3 below the reference value, concomitant with normal concentrations of TSH and free T4. Of these, 48.07% (n=25)of patients with DM2 and 65.38% of those with decreased T3 had reduced serum concentrations of total T3 as the only hormonal change. Additionally, 5 of the patients (9.61% of patients and 13.46% of patients with decreased total T3) had reduced serum concentrations of total T4. 25% patients with DM and 31% of those with decreased total T3 had reduced concentrations of T3L. 10 patients, 26.3% of those with decreased total T3 had simultaneously decreased concentrations of T3 and T3L. Finally, three patients (5.76% of patients and 7.9% (n=4) of those with decreased total T3) had increased reverse T3 concentrations.
- Five (9.68%) patients had increased T3r concentrations, and 2 of them had total T3 concentrations within reference values. Two patients had decreased free T3 concentrations accompanied by normal total T3 concentrations. All patients with decreased total T4 had simultaneously decreased total T3.
- Within the group of patients with DM, there was a difference between genders only in relation to serum concentrations of T3r, with men showing higher concentrations (0.230ng/dL; $p<0.05$). There was no difference between men and women regarding CRP concentrations ($p = 0.196$).

4.1.1.3 Control group

Recent fasting blood glucose values were available for 18 of the control subjects, with a median of 86 mg/dl. The other members of this group denied a previous diagnosis of DM. Among the individuals belonging to the control group and, therefore, not having DM, 22 (42.3%) had decreased total T3 concentrations, concomitantly with normal TSH and free T4 concentrations. Of these, only one individual presented simultaneously low concentrations of total and free T3 and only one decreased concentrations of T3 and total T4. No subject had increased reverse T3 concentrations simultaneously with decreased total T3 (Figure 4.2).

No individual without DM had increased concentrations of reverse T3 or decreased free T3 simultaneously with total T3 within reference values. As already mentioned, the only patient with decreased total T4 had a low total T3.

In the control group, there was a difference between genders regarding the concentrations of T3 (101.69 to 84.50ng/dL for men to women; p=0.029) and T3L (2.689 to 2.394pg/mL for men to women; p=0.032). There was a difference in the waist/hip ratio; 0.95 to 0.87 for men to women; p=0.003.

There was no difference in serum CRP concentrations between men and women (p = 0.241). Individuals without DM and with a history of previous cardiovascular events had T3r concentrations (0.259 to 0.171ng/mL; p = 0.039) higher than those without this characteristic. There was no difference in relation to other TH or CRP concentrations.

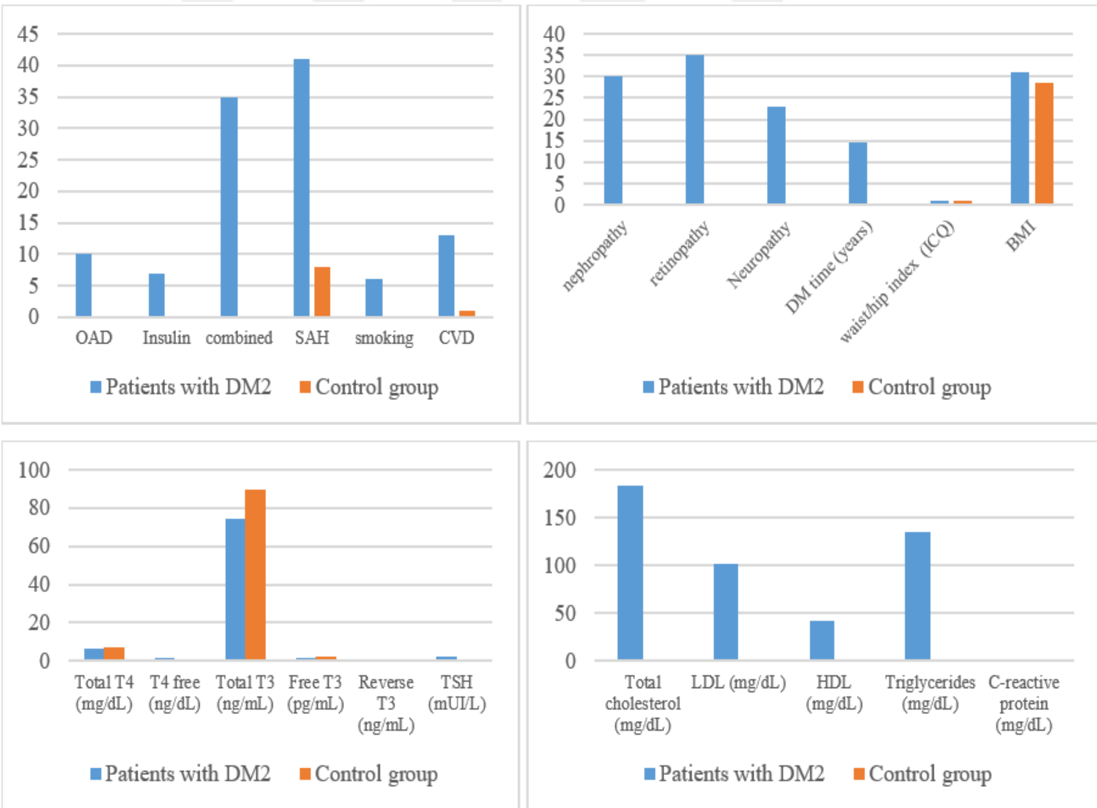

Figure 4.2 Variables studied in the groups of patients with DM2 and control and their respective comparisons. [OAD = oral antidiabetic; SAH = systemic arterial hypertension; CVD = cardiovascular disease; BMI = body mass index; CRP = C-reactive protein]

When the HbA1c levels of female and male patients over 43-70 and ≥71 years of age were compared, there was a statistically significant difference and the HbA1c values of male patients were higher ($p < 0.001$). When the HbA1c values of Type 2 diabetic women under 45 and over 65 years of age were compared, there was a statistically significant difference, and patients under 65 years of age had lower HbA1c values (Table 4.1).

Table 4.1 Difference analysis in female and male patients diagnosed with Type 2 Diabetes Mellitus

	Woman		Men			
	43 -70 years	**≥71 years**	**43 -70 years**	**≥71 years**	**Woman**	**Men**
Parameters	Mean±SD	Mean±SD	Mean±SD	Mean±SD	p value	p value
Hb A1c (%)	6.88±1.9	7.51±1.9	7.93±2.4	7.77±2.0	0.0001	0.0001
Blood Glucose (mg/dL)	138.43±72.1	156.95±73.5	171.10±88.4	165.51±74.5	0.0001	0.0001

It is known that standardized applications are needed all over the world for the use of HbA1c in the diagnosis of diabetes mellitus. Differences that may develop due to age, and gender should be carefully evaluated in diagnostic applications. Studies reported in Japanese and Chinese adults have shown that HbA1c values increase with age (1, 8). As far as we know, the number of studies on the variation between HbA1c and age groups in adult DM patients in the Turkish population is few. We think that this study will contribute to the literature in this context (Figure 4.3)

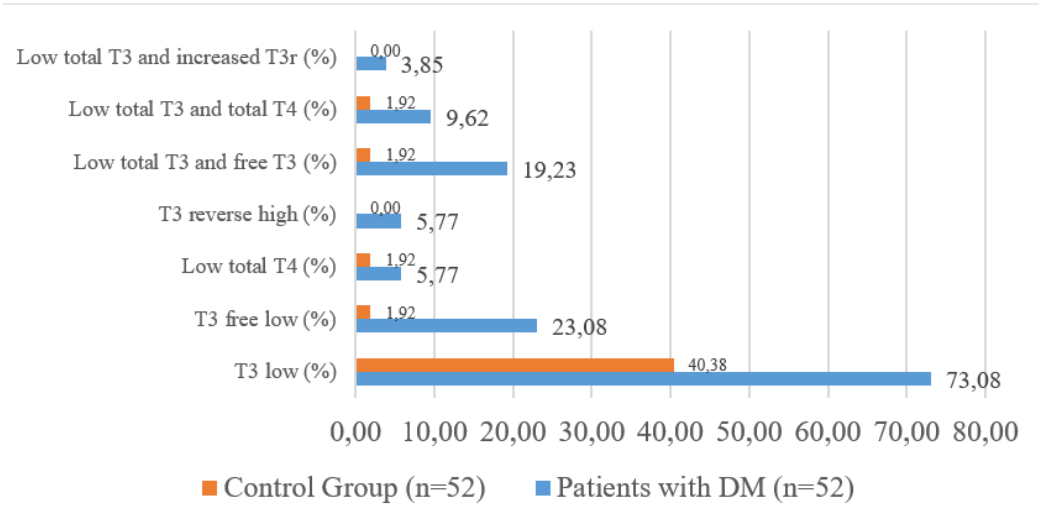

Figure 4.3 Frequency and percentage values of each thyroid hormone alteration and their combinations in the DM and control groups.

4.2 Comparative Analysis Between the Groups of Patients with DM and Control

The comparative analysis of the variables between the two groups is summarized in Figure 4.2. There was no significant difference between the groups in terms of sex (p=1.02), age (43 to 66 years; p=0.219) and BMI (31.08 to 28.96; p=0.492). However, patients with diabetes had a higher waist/hip ratio (1.00 to 0.91; p=0.0019).

Patients with DM had, compared to non-diabetics, significantly lower concentrations of T3 (74.12 to 88.81ng/dL, respectively; p=0.0021), T3L (1.74 to 2.624pg/mL; p=0.025) and T4 (6.26 to 7.23 µg/dL; p=0.059). There was also a difference between the concentrations of TSH and T4L in these groups, and individuals with DM had higher concentrations of both hormones (TSH: 2.694 to 1.580mIU/L; p=0.049 and T4L: 1.246 to 1.162ng/dL; p=0.029) – Figure 4.3

In the comparison between patients with DM and the control group, when separated by sex, differences in serum concentrations of total T3 (72.928 to 83.950ng/dL; p=0.0074), T3L (1.69 to 2.39pg/mL; p=0.026) and total T4 (6.63 to 7.24µg/dL; p=0.026). Among men, a difference was found in the concentrations of total T3 (75.62 to 101.81ng/dL; p=0.026) and T3L (1.79 to 2.81pg/mL; p=0.0061). There was no difference in T4 concentrations between men (p=0.220) or T3r concentrations between women (p=0.221) and men (p=0.53). The concentrations of T4L and TSH also showed no differences in the comparison between patients and the control group when separated by sex (Figure 4.4).

The prevalence of hypertension (79% to 17.87%; p=0.0062), smoking (11.49% to 0%; P=0.031) and cardiovascular disease (25% to 3.8%; p=0.004) was more important in the group of patients with DM compared to the control group.

Patients with DM2 had higher serum CRP concentrations than individuals without DM (1.495 to 0.759mg/dL; p=0.0025).

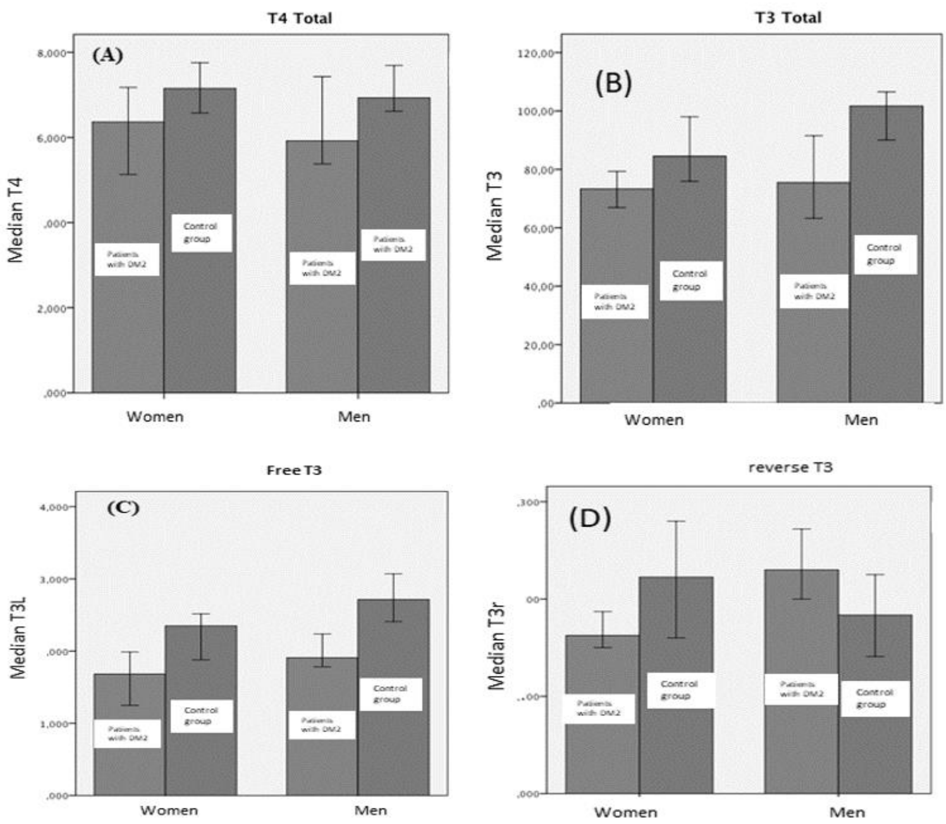

Figure 4.4 Median values and their respective 95% confidence intervals for total T4 (A), total T3 (B), free T3 (C) and reverse T3 (D) values in the groups of patients with DM and control, separated by sex. Among women, differences were significant for total T4, total T3 and free T3. Among men, differences were significant for total and free T3

4.2.1 In relation to previous cardiovascular events

Patients with DM and previous cardiovascular events had a higher waist-to-hip ratio (1.19 to 0.89; $p<0.001$), and lower serum concentrations of T3 (69.91 to 90.07 ng/dL; $p=0.016$) and T3L (1.79 to 2.39 pg/mL; $p=0.0079$), in addition to higher concentrations of T3r (0.294 to 0.201ng/dL; $p=0.036$) than subjects without diabetes. In this comparison, there was a higher proportion of men in the DM subgroup with cardiovascular disease (68.9% to 35.06%; $p=0.029$).

Comparison of individuals with diabetes without a history of cardiovascular events and the control group resulted in a higher waist-to-hip ratio (0.99 to 0.91; $p<0.001$), lower serum T4 concentrations (5.88 to 7.11 µg/dL; $p=0.0029$), T3 (73.90 to 90.08 ng/dL; $p=0.004$) and T3L (1.81 to 2.61pg/mL; $p=0.002$). There was no difference in terms of serum T3r concentrations (0.163 to 0.189 ng/dL; $p=0.152$) or in the proportion of men (23.1% to 34.6%; $p=0.256$) in the comparison between these two subgroups.

4.3 Comparative Analysis Between Patients with DM2

4.3.1 Regarding microvascular complications

Regarding the chronic microvascular complications of DM, there was a difference in hormonal parameters when analyzing the presence of nephropathy and neuropathy. Individuals with diabetic nephropathy had lower concentrations of T3 (69.81to 77.81 ng/dL; $p=0.017$). In individuals with neuropathy, serum concentrations of T3 were lower (69.15 to 79.01 ng/dL; $p=0.033$) and those of T4L were higher (1.34 to 1.29 ng/dL; $p=0.039$).

4.3.2 Regarding glycemic control and complications

There was no association between fasting glucose concentrations ($p=0.761$ for neuropathy; $p=0.74$ for retinopathy and $p=0.664$ for nephropathy) or HbA1c ($p=0.891$

for neuropathy; p=0.536 for retinopathy and p=0.296 for nephropathy) with the presence or absence of microvascular complications or cardiovascular disease (p=0.091 for fasting glucose and p=0.094 for HbA1c). Similarly, there was no difference between the parameters of glycemic control and the type of therapy being used by the patients (p=0.92 for fasting glucose and p=0.091 for HbA1c).

4.4 Association and Correlation Analyzes

4.4.1 Group of patients with DM

Among the variables of age, duration of DM, waist/hip index and BMI and TH dosages, we found a negative correlation between BMI and T4L (r= -0.29; p=0.036) and BMI and T3 (r = -0.37; p = 0.008); Figure 4.5. The correlation between TSH and BMI was not significant (r = -0.1; p = 0.95).

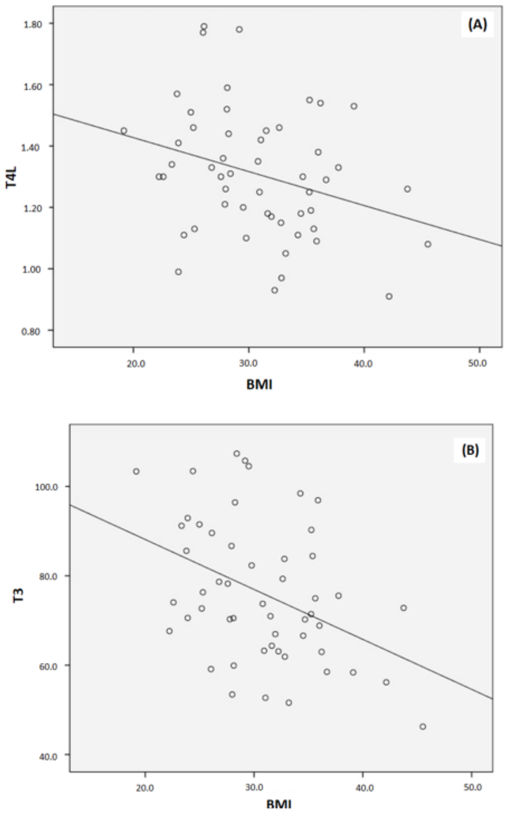

Figure 4.5 Linear correlations between free T4 and BMI (A) and total T3 and BMI (B) in the 52 patients with DM

There was a correlation between serum CRP concentrations and waist/hip ratio (r = 0.293; p = 0.035). Additionally, CRP concentrations correlated with T3r (r = 0.449; p = 0.001), but not with other hormonal variables. There was no correlation of CRP concentrations with BMI or HbA1c. Glycemic control, represented by the percentage of HbA1c, showed no significant association between clinical, anthropometric, hormonal or inflammatory variables.

Individuals with diabetes and systemic arterial hypertension had a higher prevalence of retinopathy (78.5% to 30%; p=0.06) and nephropathy (69% to 10%; p= 0.001) than patients with DM without hypertension. Smoking was not associated with the presence of microvascular complications.

4.4.2 Control group

We found a positive correlation between serum CRP concentrations and waist/hip ratio (p = 0.009) and with BMI (p = 0.003). In this group, there was no correlation between serum concentrations of CRP and T3. However, an inverse correlation was found between CRP and T3L concentrations (r = -0.354; p = 0.010).

There was no correlation between CRP concentrations and the other hormonal variables. Similarly, there was no association between clinical and anthropometric variables and hormone levels in the group of individuals without DM.

4.5 Depression and Mood Disorder Status

Regarding the failed vital event that conditioned the depressive episode (Table 4.2), there was a predominance of partner events with 16 cases (40%), followed by work and family events with 10 patients (25 %) each.

Table 4.2 Distribution of cases according to failed vital event.

Important Parameters	n	%
Labor	10	25 %
School	2	5 %
couple	16	40 %
Familiar	10	25 %
without specifying	2	5 %
Total	40	100%

It is known that in these pictures within the multifactorial etiology, vital events of an exogenous nature predominate, which is why it is called exogenous depressions by multiple authors and by different classifications as situational or adaptive.

Travers *et al.* (2013) in a study in an African population found a predominance of couple conflicts as a cause of depressive episodes. Social status, which can act as triggers or precipitants of depressive illness. When relating the evolution time of the depressive episodes with the altered recordings of thyroid hormones (Table 4.3) we found that of the 22 patients, 15 (68.2%) their depression lasted from 6 to11 months (Hazarika *et al.* 2017). reported studies of patients in outpatient endocrinology clinics after six months or more of psychiatric and psychological treatment without positive results.

Table 4.3 Time of evolution of the depressive episode in patients with altered thyroid hormones

Time of evolution	**Cases with records of altered thyroid hormones**	
Depressive episode	**n.**	**%**
15 days to 1 month	1	4.54
2 months to 3 months	2	9.09
4 months to 8 months	15	68.20
9 year to 10 months	3	13.63
More than 10 Months	1	4.54
Total	22	100

Iraq (Shaker *et al.* 2019) there is talk of a prevalence of 12% in Outpatient Psychiatry Centers in patients with manifestations that may suggest a thyroid disorder that are not studied, a situation that worsens if patients are admitted, in these conditions it is considered up to 49% prevalence. The international literature indicates that between 1% and 4% of patients with affective disorders, with poor evolution of symptoms, present clinical hypothyroidism; between 4% and 40% subclinical hypothyroidism that is diagnosed after a year of failed treatments.

The values of TSH, T3, T4 are shown in Table 4.4, Table 4.5 and Table 4.6, respectively, where we find three patients with high levels of tetraiodothyronine and three with low levels of the same hormone, each representing 7.5%. 10% presented some alteration of the T3 values, both an increase and a decrease in patients group, respectively.

TSH it behaved within the range of normal, low and high values in 55%, 37.5% and 7.5% patients, respectively.

Table 4.4 T4 values

T4	**No.**	**%**
75 - 144	34	85
< 75	3	7.5
< 144	3	7.5
Total	40	100

Table 4.5 T3 values

T3	**No**	**%**
0.8 – 2.4	36	90
< 0.8	2	5
> 2.4	2	5

Total	40	100

Table 4.6 TSH values

TSH	No	%
0.8 – 3.8	22	55
> 0.8	15	37.5
> 3.8	3	7.5
Total	40	100

Figure 4.6 shows the presence of symptoms evaluated by the psychiatric clinic and the HAM-D, which were a typical manifestation of thyroid dysfunction, sadness was found in all the patients; sleep disorders and inhibition (slow speech thinking, impaired ability to concentrate, decreased motor activity) in 91% and 63.63%, respectively.

The same number (14) of patients provided the reference to work and other activities, followed by 13 with somatic anxiety and 12 with anorexia, which we included within other symptoms and reflected 54.54%. Eleven and eight patients showed general somatic symptoms and body weight loss, respectively.

Fardella (Soon and Ting 2018) recalls that the behavior of the central nervous system with thyroid dysfunction can generate symptoms that are difficult to differentiate from those typical of illnesses primarily belonging to the psychiatric sphere.

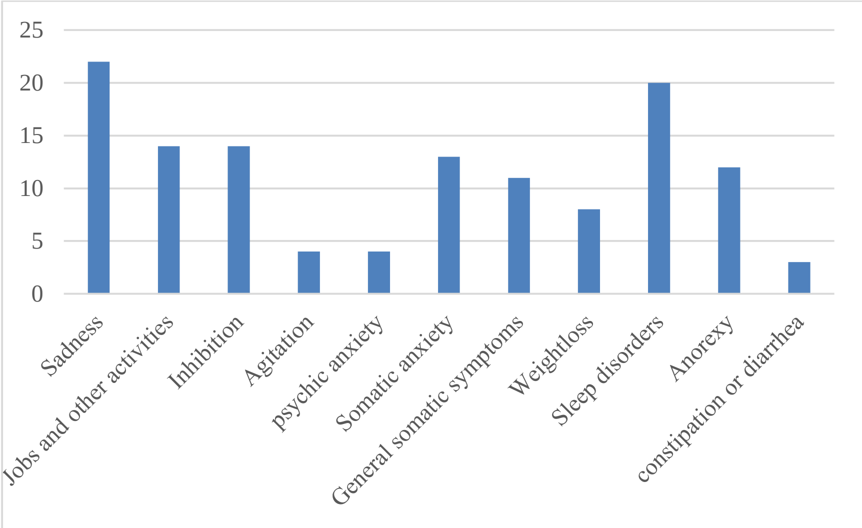

Figure 4.6 Symptoms according to the Hamilton scale

4.6 History of Cardiovascular Events in Patients with DM

Thirteen patients (25%) had a history of cardiovascular disease. In the group of patients with DM, when comparing individuals with and without a previous history of cardiovascular events, there was a significant difference in the waist/hip ratio (1.05 to 0.98; p=0.046), SAH (100% to 71.8% ; p=0.027), between T3r concentrations (0.270 to 0.170ng/dL; p=0.001) and serum CRP concentrations (2.21 to 2.36mg/dL; p=0.029).

No association was found between smoking and cardiovascular events (24.06% to 6.917%; p=0.149). There was a difference between the sexes, and the proportion of men among patients with a previous cardiovascular event was higher (68.26% to 31.09%; p = 0.0049).

There was no relationship between microvascular complications (p = 0.492 for nephropathy; p = 0.181 for retinopathy and p = 0.212 for neuropathy) or glycemic control (p = 0.318) and the prevalence of a previous cardiovascular event. The comparison between T2DM patients with and without a history of cardiovascular events is summarized in Figure 4.7.

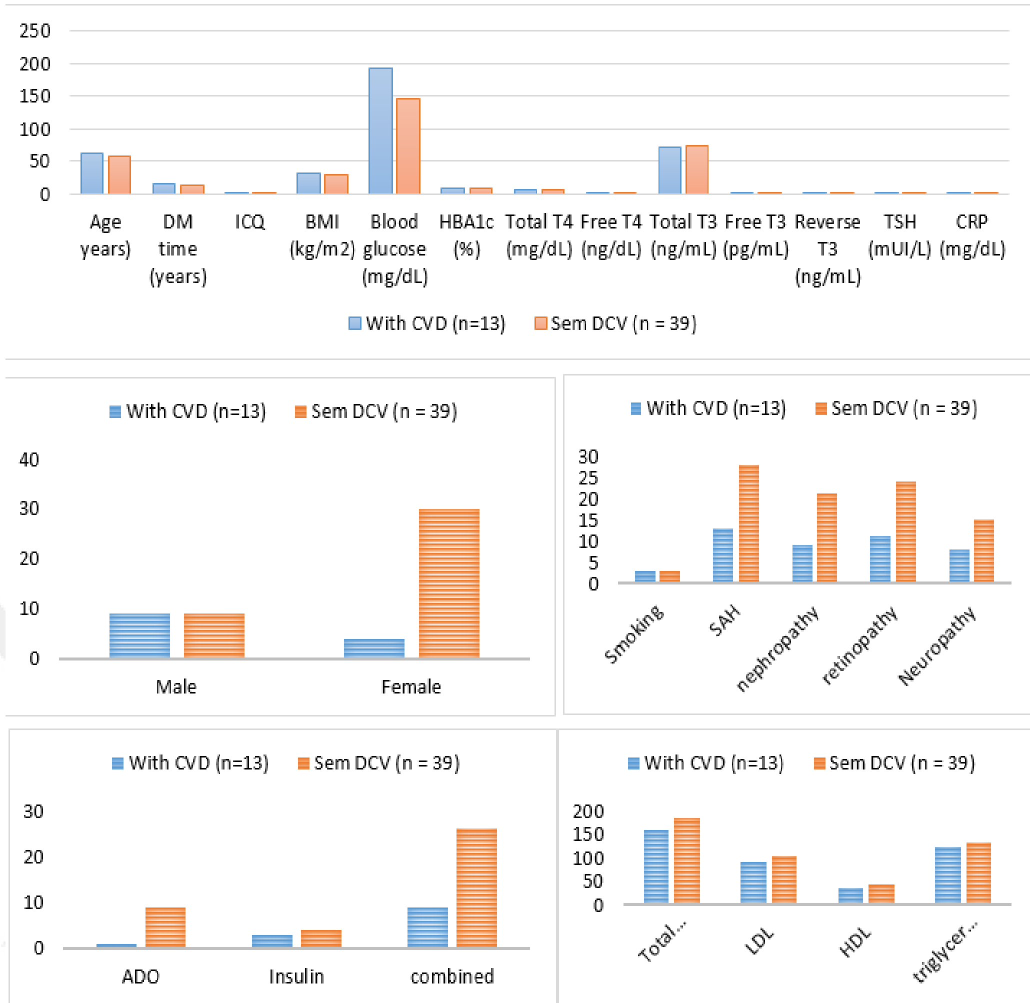

Figure 4.7 Values of the study variables in patients with DM who had or did not have a history of cardiovascular disease and the respective comparisons between the two subgroups

4.7 Logistic Regression Analysis for the Study of Factors Related to Decreased Concentrations of TH in Patients with DM

Univariate regression of factors influencing or reducing serum concentrations of T3 decreased in relation to the reference values showed that only nephropathy (p=0.049), BMI (p=0.029; RR = 1.17; 95% CI = 1.020 – 1.402) and time of DM (p=0.159) apresentaram p □ 0.149. A multivariate analysis showed that only BMI was a significant predictor for low T3 concentrations (p=0.029; RR=1.159; 95% CI=1.0129–1.3290; Nagelkerke R2=0.15).

A univariate regression of factors influencing the presence of decreased FT3 concentrations showed only male gender (p=0.056) as a predictor of decreased serum FT3 with $p \leq 0.149$. A multivariate analysis did not reveal any significant factor associated with low concentrations of FT3 (Figure 4.8). A regression analysis for decreased T4 and increased T3r concentrations was not possible due to the small number of patients (5) in each of the two categories.

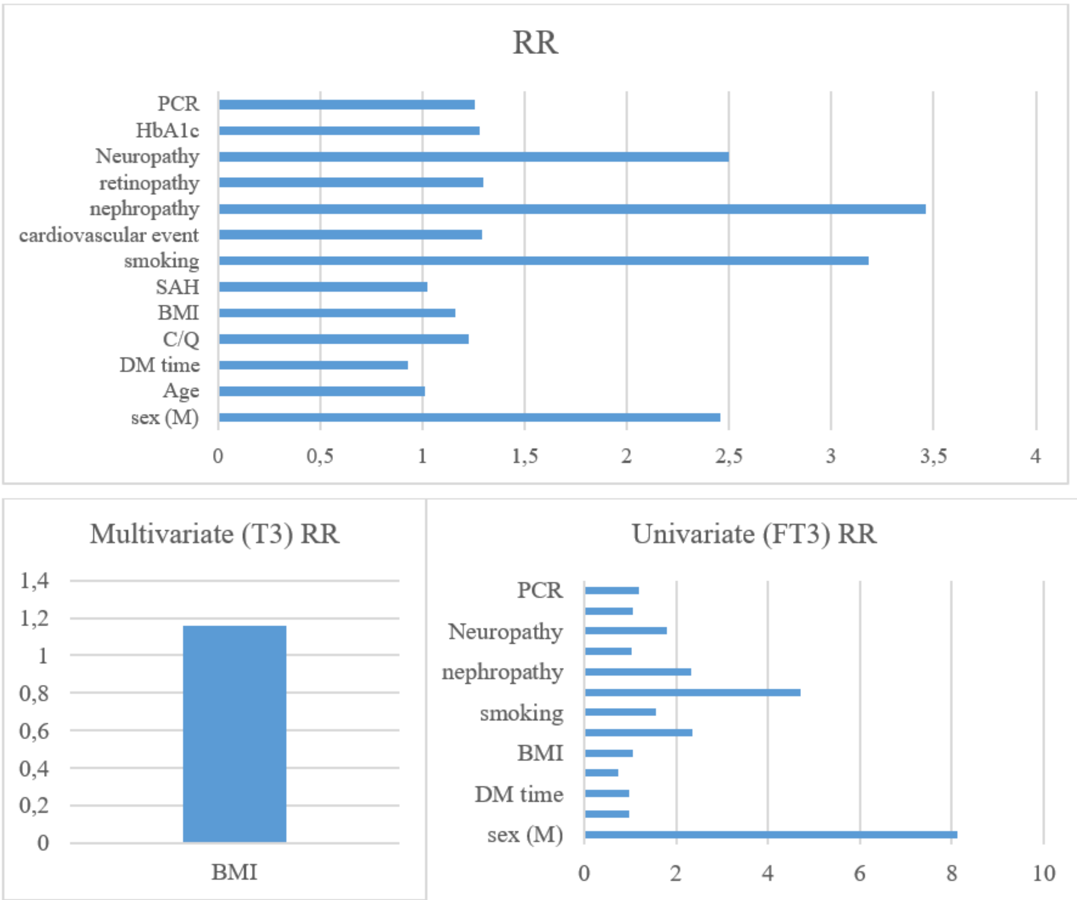

Figure 4.8 Relative risk (RR) values and their respective 95% confidence intervals, as values of statistical significance for the variables studied in the univariate and multivariate logistic regression analysis for the identification of low concentrations of of total and free T3 in the group of patients with DM

4.8 Logistic Regression Analyzes for the Study of Factors Related to Cardiovascular Disease

The regression analyzes between the clinical and laboratory factors studied and the prediction of cardiovascular disease in the group of patients with DM are presented below. The results are summarized in Figure 4.9.

4.8.1 Univariate regression

The factors studied that demonstrated the ability to predict the presence of cardiovascular disease when analyzed separately were CRP (p = 0.0179; RR = 1.4901; 95% CI = 1.059 – 2.039); the male sex (p = 0.0049; RR = 7.49; 95% CI = 1.879 – 31.209) and T3r (p = 0.019; RR = 3.629; 95% CI = 1.569 - 8.291).

The other hormonal parameters were not predictors of cardiovascular events: T4 (p=0.419), T3 (p=0.46), T3L (p=0.412), TSH (p=0.436) and T4L (p=0.655). HbA1c (p=0365), presence of nephropathy (p=0.365), neuropathy (p=0.415) or retinopathy (p=0.395), as well as smoking (p=0.26), waist/hip ratio (p=0.34), age (p=0.164), time of DM (p=0.163), BMI (p=0.481) were not good predictors of cardiovascular disease in this population. The concentrations of total cholesterol (p=0.625), LDL (p=0.416), HDL (p=0.079) and triglycerides (p=0.319) were also not associated with previous cardiovascular events.

4.8.2 Multivariate regression

The multivariate logistic regression model used took into account all factors with p ≤ 0.15 in the univariate analysis. These were: CRP, presence of retinopathy and neuropathy, male gender, waist/hip ratio, reverse T3, T4L, smoking and HDL.

In the multivariate regression including the aforementioned parameters, only males (RR=5.89; 95%CI = 1.19-25.29; p=0.019) and T3r (RR=3.0329; 95%CI = 1.208-8.031;

p=0.009) were independent risk factors for cardiovascular events in the T2DM patient population (Nagelkerke R2 = 0.361).

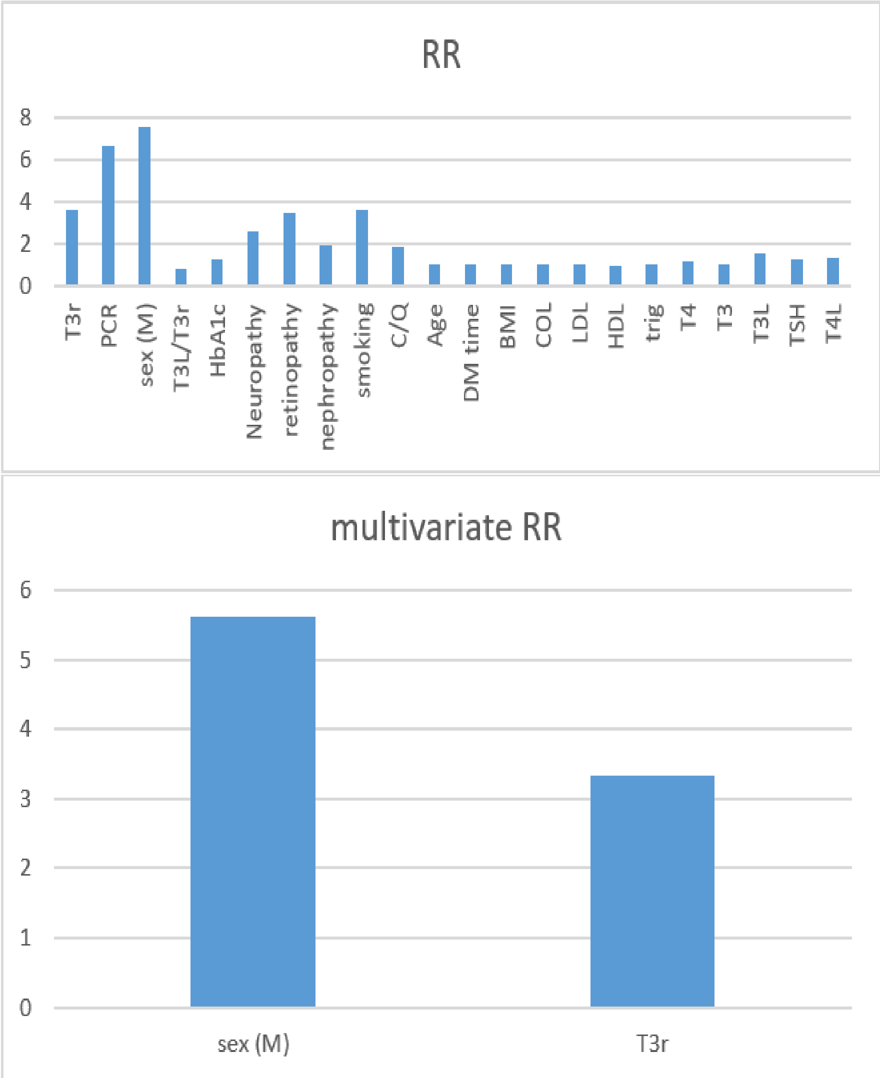

Figure 4.9 Relative risk values and their respective 95% confidence intervals, as well as statistical significance values for the variables studied in the univariate and multivariate logistic regression analysis to identify the presence of cardiovascular events in the group of patients with DM.

4.9 Analysis of ROC Curves, Sensitivity and Specificity of Values Obtained with Serum Dosages of Inflammatory and Hormonal Markers in Predicting the Risk of Cardiovascular Events

4.9.1 PCR

The ROC curve for PCR is shown in Figure 4.10. The area under the curve was 0.709 (p = 0.019; 95% CI = 0.549 – 0.869). The sensitivity and specificity values that encompass the largest area under the curve were 76.89% and 64.09%, respectively, for a CRP cut-off value of 1.649 mg/dL.

Using this cut-off point of 1.649 mg/dL, patients with higher CRP concentrations had a relative risk of cardiovascular event 5.949 (95% CI = 1.409 – 25.249; p=0.019) times greater than patients with CRP below this level.

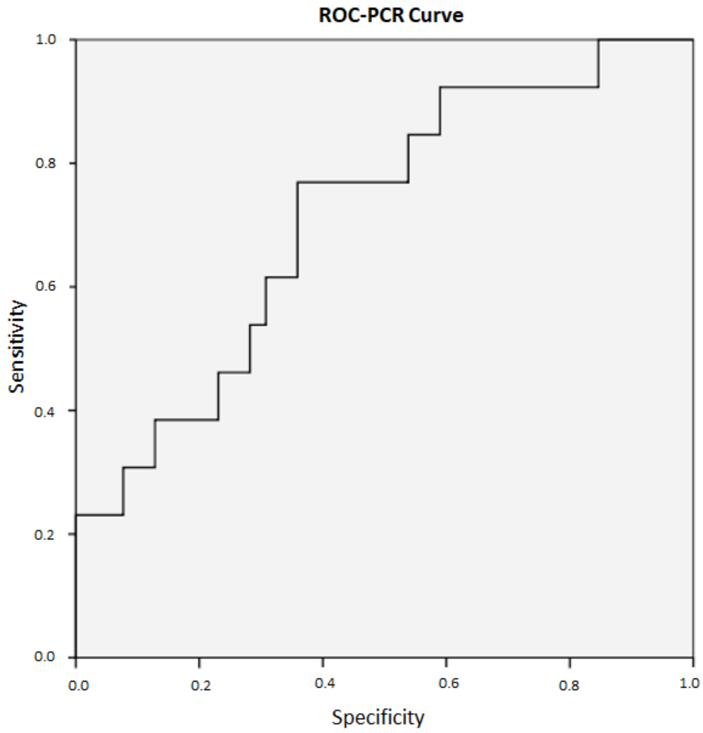

Figure 4.10 ROC curve for CRP performance as a predictor of cardiovascular events. Area under the curve = 0.714 (p = 0.019); sensitivity = 78.7%; specificity = 63.91%

4.9.2 Reverse T3

Regarding the T3r values, the ROC curve is shown in Figure 4.11. The area under the curve was 0.789 (p = 0.0019; 95% CI = 0.649 – 0.929. The best sensitivity and specificity values were 84.6% and 71.8%, respectively, with a cut-off point of 0.206 ng/mL.

Patients with T3r concentrations above the cut-off point of 0.206 ng/mL had a relative risk of cardiovascular disease 13.91 (95% CI = 2.659 – 73.681; p=0.0018) times greater than patients with T3r below this level.

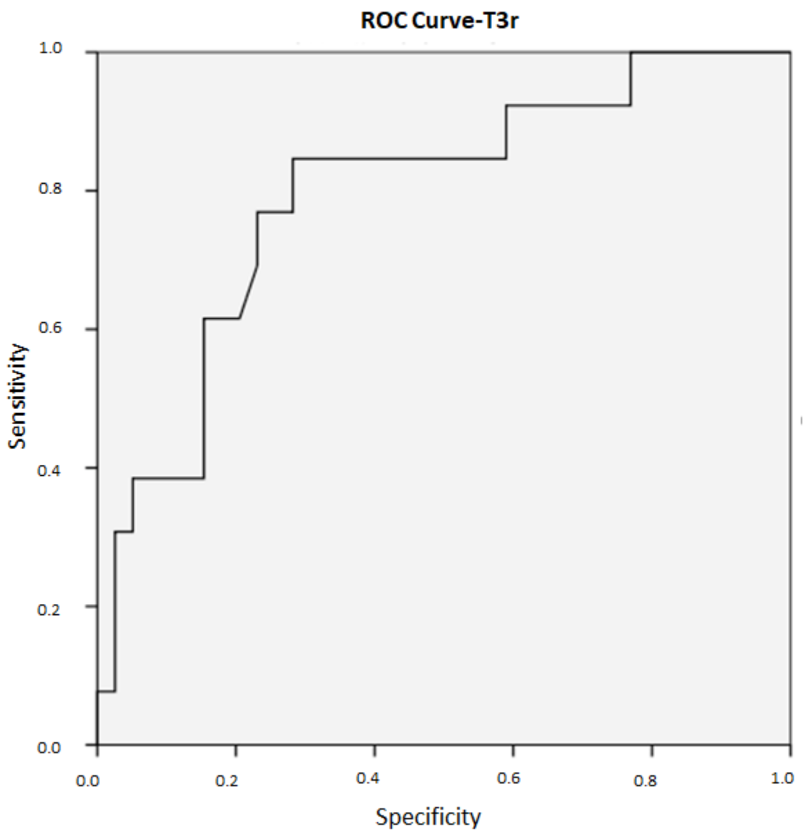

Figure 4.11 ROC curve for reverse T3 performance as a predictor of cardiovascular events. Area under the curve = 0.794 (p = 0.0019); sensitivity = 83.8%; specificity = 72.01%

4.10 Discussion

The present study was carried out in a total of 52 patients diagnosed with type 2 diabetes mellitus admitted to the clinical service of the BTH and who met the criteria to be taken into account in our research. In my study it was shown that the prevalence of hypothyroidism associated with type 2 diabetes mellitus was 7 members, lower than previous studies at the local level such as the one carried out BTH, where it was observed that 36.54% of diabetic patients have hypothyroidism, in which it was possible to determine that 24/52 of the population presented hypothyroidism; however, within the 52 patients included in the study, the frequency of diabetic patients associated with hypothyroidism is 46.15%. This could be explained due to the high percentage of patients who debut with a diagnosis of hypothyroidism, which is 36%, and who also presented subclinical hypothyroidism, while in 63.46% their hypothyroidism was clinical, 16% being autoimmune due to anti-TPO present and 10% by thyroglobulin. The clinical prevalence is 8.42%, while the subclinical prevalence is 4.73%. No studies on the prevalence of clinical, subclinical and autoimmune hypothyroidism were found, so the information cannot be contrasted. Patients with type 2 diabetes mellitus associated with hyperthyroidism were not observed in the present study, in contrast to the aforementioned local and national studies, where a percentage of 13 and 5%, respectively, was found. The percentage of hypothyroid diabetic patients associated with cardiovascular diseases was high (76%), of which 39.47% presented arterial hypertension, followed by hypercholesterolemia with 18.42%; The Pearson's correlation is 0.016, which means that there is no correlation between hypothyroidism and cardiovascular disease. The presence of cardiovascular disease and hypothyroidism are directly proportional to the time of diagnosis of diabetes mellitus and occur in 50% and 17.85%, respectively, of patients over 15 years of age, while in people with diabetes in a period of 11 to 15 years in 27.14% and 9.82%; No studies of cardiovascular disease in hypothyroid diabetic patients were found, so it is not possible to verify my information.

This work demonstrated that the prevalence of low total T3 status in an outpatient population of patients with type 2 diabetes was greater than 70% and about 25% of patients had low T3L concentrations. It was also possible to demonstrate that about 10% of the patients had decreased serum concentrations of T4, suggesting a change of greater relevance in the economy of TH. These results become more important considering that the concentrations of these TH were lower when compared to individuals without DM. Additionally, patients with DM had higher concentrations of T4L and TSH, but without differences in T3r concentrations. However, we emphasize that the differences in reverse T3 concentrations between diabetic patients and the control group were evident when patients with a previous cardiovascular event were analyzed separately.

In relation to the control group, we similarly found a high prevalence, around 40%, of low total T3 status. This high prevalence of low total T3 in the control group may be due to other factors unrelated to DM, such as age, insulinemia and its influence on the hepatic production of thyroid hormone-binding proteins (Brenta *et al.* 2007).

On the other hand, free T3 concentrations were shown to be reduced by only about 2%. This finding indicates, in our opinion, that the free T3 fraction would be a more reliable parameter to discriminate low T3 states, given the high prevalence of low total T3 in both groups. Thus, the diagnosis of EDS based only on total T3 concentrations could be overestimated. The free fraction of T3 would more accurately reflect the serum concentration of the biologically active hormone. In agreement with this hypothesis, a study by (Iervasi *et al.* 2003) showed that T3L was the best predictor of death in patients hospitalized for heart failure.

Other authors have demonstrated decreased serum concentrations of total and free T3 in patients with DM. Most of these studies were performed in patients with type 1 diabetes in the presence of poor glycemic control, unlike our study where the median HbA1c was 8.5%, and showed decreased serum concentrations of T3, increased T3r and inappropriately normal levels of TSH. These changes are similar to those seen in patients with a mild form of sick euthyroid syndrome. Regarding DM2, the finding of decreased total T3 is reported in several previous studies. However, there is disagreement regarding the concentrations of total T4, which were reduced in some studies and normal in others (Welsh and Soldin 2016). Additionally, in previous publications, serum concentrations of T3r were shown to be increased in patients with DM and inversely correlated with concentrations of HbA1c, differing from the results of this study.

In one study, Sherwani *et al.* (2016) HbA1c levels were found to be higher in men than in women, and it was observed that this difference increased with increasing age. HbA1c levels were found to be significantly higher in men in the 43-59 age group than in women in the same age group. One of the factors that may be effective in this has been shown to be lower hemoglobin levels in menstruating women who have faster erythrocyte turnover. In our study, it was seen that the rate of female cases was higher in each age group when looked at by gender. When the HbA1c levels of men and women over 43 to >70 years of age were compared, there was a statistically significant difference and the HbA1c values were higher in men (p=0.00). When the HbA1c values of Type 2 diabetic women under 45 and over 70 years of age were compared, there was a statistically significant difference, and the cases under 43 years of age had lower HbA1c values. It has been shown that women in the peri- and post-menopausal age groups have a more significant increase in HbA1c than men. Many changes occur in humans with age, for example: pancreatic islet function declines with age, tissue sensitivity to insulin and insulin receptor activity gradually decreases, muscle tissue gradually decreases, and glucose consumption generally decreases. Under the combined effect of these factors, blood glucose may increase and HbA1c levels increase, especially with advancing age.

Tighter glycemic control is targeted in young people, while older people seem to be more flexible. In general, higher HbA1c values are expected due to polypharmacy, comorbidities, and low use of glucometer due to socio-cultural reasons with age. In our study, no statistically significant difference was found in HbA1c values between the five, six, seven, eight and ninth age groups. The reason for this can be shown as the possibility of regular follow-up by giving appointment priority to elderly patients, and the provision of diabetes education and nutrition education to all patients. All these factors may have led to tighter controls on HbA1c targets and lower HbA1c values.

In our study, patients who had suffered a previous cardiovascular event had higher serum concentrations of reverse T3, as well as CRP. Interestingly, T3r values were higher in patients with higher CRP concentrations, even when they remained within reference values.

When looking for factors that would influence the presence of reduced serum concentrations of TH in patients with DM, only BMI proved to be a predictive factor of low concentrations of total T3. However, this variable explained less than 15% of the variation in T3 concentrations in this population. As for T3L, we did not find factors associated with reduced serum concentrations of this hormone. Probably, the determining factors of changes in the concentrations of these TH are different from those analyzed in this work. Possibly, factors such as inflammatory cytokines not evaluated in the present study may have a greater influence on the changes observed in TH in patients with DM2.

In critically ill patients, the pathophysiology of EDS is multifactorial. Changes in thyroid hormone metabolism, transport, and peripheral conversion are implicated together with alterations in the hypothalamic-pituitary-thyroid axis. Measurements of thyroid hormones in their free form are considered problematic due to variations in TBG concentrations as well as in the affinity of TH for the carrier proteins present in the underlying disease. T4L concentrations, for example, have previously been reported as normal, decreased, or even increased, depending on the method used (Segni 2017).

Abnormalities in the expression and function of deiodases, responsible for converting T4 to T3 and T3 to T3r, are important causes of changes in TH. Studies conducted in critically ill patients showed decreased activity of type 1 deiodinases and 2 and increased expression of type 3 deiodinase.(Luongo 2013) The expected result in the physiology of TH, given these changes, would be a decreased conversion of T4 to T3 and an increase in deiodination from T4 to T3r and from T3 to T2, with a consequent increase in serum T3r concentrations and a decrease in T3. The induction of D2 activity in the hypothalamus and pituitary reported in some studies would partly explain the normal or decreased concentrations of TSH in these situations. The increased expression of D2 would lead to normal concentrations of T3 in the pituitary tissue, making the pituitary gland euthyroid, while the rest of the body would be in a hypothyroid state.

In the present study, the higher concentrations of T4L and TSH, together with the decrease in total and free T3 demonstrated in patients with DM in relation to individuals in the control group, could raise as hypotheses the development of alterations in the activity of deiodases, leading to lower conversion from T4 to T3, causing an increase in T4L and a slight increase in TSH, although remaining within the normal range, in response to the drop in T3. Failure to induce higher magnitude elevations in TSH concentrations, as would be expected in cases where T3 concentrations fall below reference values, may be due to suppression of the hypothalamic-pituitary axis in patients with diabetes, as a result of the action of pro-inflammatory cytokines, as well as the presence of greater oxidative stress in these patients. These results are in agreement with previously published studies, which found increased TSH and T4L concentrations in patients with DM compared to individuals without DM. In some of the studies (Shimon *et al.* 2002), a flattened TSH response to exogenous TRH administration was also observed, thus confirming that there is a hypothalamic-pituitary suppression component in the genesis of these alterations.

Oxidative stress has been described as one of the factors associated with the presence of EDS in critically ill patients, as it decreases glutathione concentrations and, consequently, the activity of deiodases, which have glutathione as a necessary cofactor for their activity.

In the last two decades, obesity and DM 2 have been recognized as subclinical inflammatory response states. In the present study, the differences observed in patients regarding the concentrations of T4, T3 and free T3 in relation to the control group, as well as the failure to raise TSH above the reference values, could be related to the centripetal obesity observed in patients with DM2. Although there was no difference in BMI between patients with DM and individuals without DM, the former had a higher waist/hip ratio. The role of visceral fat in the genesis of the inflammatory response and type 2 DM is well established. An increased waist-to-hip ratio classically relates to risk factors for cardiovascular disease such as high blood pressure, DM, and a lipid profile with low HDL and high triglycerides. Thus, individuals with a higher waist/hip ratio, and consequently, a greater amount of visceral fat, would present an exacerbated inflammatory response, leading to the appearance of DM and explaining, at least in part, the changes in TH. This association between inflammation and centripetal obesity was corroborated by the positive correlation between serum CRP concentrations and waist/hip ratio verified in the present study.

Previous work (Tsalamandris 2019) has linked changes in serum HT concentrations to the metabolic control of DM. Our study showed that at least part of these changes may be due to inflammation resulting from centripetal obesity. A study by (Mahendran *et al.* 2013) showed that lower serum concentrations of total T3 and higher serum T3r were associated with the presence of increased concentrations of free fatty acids, which could indicate an adaptive state to avoid hypercatabolism in patients with poorer control. glycemic. Similar changes in the pituitary-thyroid axis have been reported in patients with type 2 diabetes, especially in patients with very poor metabolic control, translated by HbA1c concentrations above 12% . We also demonstrated that T3r and CRP concentrations were good predictors of the occurrence of cardiovascular events when analyzed separately. However, when we studied all risk factors simultaneously, only T3r, but not CRP, remained a significant risk factor, together with male gender. In fact, T3r proved to be the parameter most significantly associated with the presence of cardiovascular disease, unlike the other TH.

Additionally, there was an association between higher serum T3r concentrations and the presence of a history of cardiovascular events in the group of patients with DM, whether compared to the other patients with DM or to the control group. In the group of patients with DM, the serum concentration of T3r provided greater specificity and sensitivity for differentiating between the presence or absence of cardiovascular disease than did CRP.

(Iervasi *et al.* 2003), studying patients with heart failure found similar results. The authors demonstrated that L3T at concentrations below the reference values was the best predictor of mortality, more significant than classically known risk factors such as dyslipidemia, age or left ventricular ejection fraction. (Chaudhary *et al.* 2018) evaluated the thyroid hormone profile of 83 patients with chronic obstructive pulmonary disease and observed a 25% decrease in T3L with no differences between T4L and TSH compared to healthy individuals. Decreased T3L concentrations were associated with increased serum concentrations of IL-6 and TNF-α. In turn, acute exacerbations of the disease were associated with an even greater drop in T3L. In both studies, T3r assessment was not performed.

In the mildest form of EDS, there may be an increase in T3r as a result of changes in peripheral TH conversion. The assessment of T3r makes it possible to complete the thyroid hormone profile, being able to detect cases in which T3 concentrations remain within the normal range. However, the decrease in serum T3 concentrations and the T3L/T3r (or T3/T3r) ratio are considered the most sensitive markers for EDS, and are even correlated with prognosis in some studies (DeGroot 2015).

In DM, as already mentioned, similar changes in the TH profile have been reported and associated with the degree of glycemic control. As DM 2 has a strong inflammatory component in its pathophysiology, we postulate that the abnormalities in TH metabolism found in patients with EDS would be better explained by the degree of inflammation and cardiovascular risk than by glycemic control alone. In fact, we found a low T3 state in these patients, characterized by a decrease in total and free T3. CRP concentrations correlated with the waist/hip ratio, indicating that possibly centripetal obesity would lead to an inflammatory state capable of altering the economy of thyroid hormones in patients with T2DM, since higher concentrations of reverse T3 were associated with greater serum concentrations of CRP.

Another relevant difference between the present work and the others was the absence of an inverse correlation between T3 and HbA1c concentrations, frequently reported in other studies (Dubey *et al.* 2020) The median glycated hemoglobin in our patients (8.5%) was lower than in most previous studies, as we did not select only patients with poor metabolic control. Additionally, we work with an elderly population (median age of 60 years) under care at a tertiary center outpatient clinic, with a high prevalence of microvascular complications (44%-67%) and a long duration of disease (median of 14.5 years). Thus, we found no correlation of HbA1c with total T3, free T3 or T3r. However, in the population studied, composed of clinically stable patients with DM2, T3r and CRP were correlated and among those with established cardiovascular disease, we found higher concentrations of T3r and CRP. Studies that reported a correlation between HbA1c and T3r were conducted in patients with metabolically decompensated DM 1 or in cases of diabetic ketoacidosis, clinical situations of greater severity. (Kabadi 2017) showed that newly diagnosed DM 2 patients and HbA1c > 10.8% had higher T3r and lower T3. These changes were reversible with improved metabolic control. Furthermore, patients with poor glycemic control showed a flattened TSH response to the administration of exogenous TRH, an alteration that was also reversible with the normalization of glycemia after adequate treatment of DM.

Inclusion only of patients with poor glycemic control, especially in cases of DM1, which progresses with absolute insulin deficiency, can further complicate data analysis, causing a bias due to weight loss and catabolic state present in these patients, a known cause decrease in T3 concentrations and increase in T3r.

4.10.1 HRV results and QT interval duration

Regarding the parameters used to assess HRV and the duration of the corrected QT interval, the following results were identified in Table 4.7

Table 4.7 Parameters evaluated in HRV

Characteristics	**Group -1 (± S.D)**	**Controle Group (95% CI) (± S.D)**
SDNN (ms)	183± 17	89± 12
SDANN (ms)	179± 16.7	94± 11.61
pNN50 (%)	22± 21	26±13.25
rMSSD (ms)	58± 22	33 ± 18
QTc (ms)	491± 6	449±4

pNN50: Percent of normal-normal NN intervals whose difference exceeds 50 ms; QTc: Corrected QT interval duration in milliseconds.

QT duration corrected for heart rate (QTc) emerged as an important predictor of cardiac death. According to the data analyzed, we observed that ⅔ of the patients had an increase in the duration of the QTc interval (VR: > 490 ms), with values of 449 ms in control and 534 ms in patient 3. (Bakiner *et al.* 2008) demonstrated that the mean QTcmin and QTcmax intervals of their study group were significantly longer than those of the control group ($p = 0.001$, $p = 0.000$, respectively). These findings also corroborate those described by (Kalra *et al.* 2016). The other HRV parameters did not show changes according to the risk criteria for cardiovascular events established in the study methodology. These findings contrast with those found in the literature, in which there is a decrease in SDNN, SDANN and pNN50%, as reported by Hoshi. et. al. and Galetta. et. al. Therefore, it was not possible to correlate the presence of SH with ANS changes through the parameters of Heart Rate Variability identified in these other studies.

It was also observed that presenting an asymptomatic clinic, the participants had considerable cardiac dysfunctions, with risks of relevant cardiovascular events. Sinus pauses longer than 4.0 seconds are cited, suggesting obstructive sleep apnea, periods of ST depression (-1.5 MM), indicating silent ischemia and intermittent left bundle-branch block, which cannot exclude Wounds. Thus, it is not possible to affirm that the alterations found are correlated with SH or with other possible previous diseases unknown to the research patients.

In summary, we demonstrated that individuals with DM2 had lower serum concentrations of total and free T3 and total T4 than individuals without DM matched by sex, age and BMI. Additionally, about three quarters of patients had reduced serum concentrations of T3, as did 40% of individuals without DM. We consider that total T3 alone could overestimate the diagnosis of EDS and that perhaps the assessment of free

T3 more reliably reflects the changes observed in the economy of TH present in patients with T2DM.

We also found that higher T3r concentrations in T2DM patients correlated with CRP concentrations and were associated with cardiovascular disease, but not with glycemic control. Our analysis suggests that T3r may be a reliable marker to predict cardiovascular events in this population, given its similar performance to CRP and its correlation with this inflammatory marker. Changes in serum thyroid hormone concentrations in patients with T2DM and insulin resistance states followed in an outpatient setting should be investigated in prospective studies with larger numbers of patients before T3r can be recommended as a laboratory test marker of cardiovascular events in clinical practice.

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

The TH alterations observed in critically ill patients or in some diseases under outpatient care are of complex and multifactorial etiology. There is considerable variation in the laboratory presentation, depending on the underlying disease and the duration of the disease. In critically ill patients and in some outpatient settings, particularly heart disease, TH concentrations have been shown to be good predictors of disease progression.

Changes in TH metabolism follow a biphasic time course. Initially, there is a drop in T3, which would represent an evolutionarily programmed response to the state of acute physical stress, aiming at reducing the basal metabolic rate and conserving the protein and energy balance, especially when associated with situations of caloric deprivation.

If, however, the adaptive response is not sufficient for the organism to overcome the moment of acute aggression, or if the disease has a prolonged character, as is the case with patients on prolonged artificial life support, a second phase of alterations begins. This phase of chronicity has no evolutionary parallel, since advanced support and maintenance of life under extreme clinical conditions is a very recent phenomenon. Thus, there is a greater reduction in serum T3 concentrations, accompanied by a drop in T4 without the expected increase in TSH, or even its paradoxical decrease occurring in parallel with TH, which for most authors represents a maladaptive response. Physiologically, there is suppression of other pituitary hormones, insulin resistance, negative protein balance and positive fat balance if there is sufficient caloric intake. Thus, these authors consider that the administration of TH, glucocorticoids, growth hormone and androgens would be beneficial in these situations.

The information that is currently available is decisive in the sense of the potential role of thyroid receptors in the development of various regions of the nervous system from the earliest stages. Their participation is not limited to their action as thyroid signal transducers, but there are several examples where wild receptors that are not bound to thyroid hormone naturally (such as TR α 2 or some truncated isoforms), or transiently (such as TR α 1, TR β 1 and TR β 2 apororeceptors) can exert regulatory actions on transcriptional activity, and even generate by themselves harmful effects in the absence of thyroid hormone.

On the other hand, mutations in thyroid receptor genes produce defects in thyroid hormone binding or DNA binding, with which thyroid signal transduction also suffers alterations that lead to various pathologies in both function and formation. of structures that are under the control of thyroid hormones in neural development. There is growing evidence regarding the very function of thyroid hormones as regulators of their own receptors, and in this sense their regulation during neuraxis development should be a particular focus of attention in prenatal monitoring of thyroid hormone levels. in the maternal circulation.

Women suffered more from depression than men, by a ratio of six to one. The age groups of both sexes between 30-49 years were where more patients were found. Mild depressive episodes were more common in women, but not in men where, out of six patients, five were moderate. 55% of the patients presented some type of alteration in the thyroid hormones and it was in them, where the evolution of the episode lasted more than six months. Couples conflicts were the most common as possible triggers of the depressive episode, which were reflected with somatic and physical symptoms of various kinds that were caused or aggravated by thyroid dysfunction.

- This study showed that T3 concentrations are decreased in about 70% of patients with DM2 and in 40% of individuals without DM. T3L concentrations are reduced in 25% of patients with DM and in only 2% of individuals without DM. Additionally, T3r concentrations are increased in about 10% of patients with type 2 DM in outpatient follow-up.

- It was possible to assume that the assessment of total T3 as the only parameter for diagnosing EDS in patients with DM may overestimate the prevalence of this condition. Free T3 would more reliably reflect changes in TH economy.

- It was possible to observe that patients with DM2 had lower serum concentrations of Total T4, Total T3 and Free T3 compared to non-diabetic individuals. There was no difference in terms of serum T3r concentrations between the two groups. Differences regarding T3r concentrations were revealed only when comparing patients with cardiovascular events and the control group.
- The increase in T3r correlated with the degree of systemic inflammation and not with glycemic control, and there was no correlation of the other thyroid hormones with glycemic control.
- This study showed that individuals with type 2 diabetes mellitus and established cardiovascular disease had higher serum concentrations of CRP and T3r than those patients without this characteristic and that both were predictors for the presence of cardiovascular events in the studied population, conferring risk 5, 9 and 14 times higher for this characteristic, respectively, when using the cut-off points of the ROC curves.
- This study showed that, among all the factors involved, T3r and male gender were the independent risk factors for cardiovascular disease in patients with DM2, conferring 3.3 and 5.6 times greater risk for this characteristic, respectively.
- The relationship between TH and mental disorders is multidirectional and involves controversial aspects in all areas, from preclinical findings to diagnosis and treatment. The relevant pathophysiological mechanisms in this scenario are numerous and diverse. The relative relevance of each of these phenomena must be evaluated in an inclusive manner in the future. This would allow a greater focus on the development of new approaches in the clinical context.

Likewise, further research is needed in the future with robust methodological designs aimed at exploring three specific problems: the true prevalence of clinical and subclinical thyroid pathology in each of these mental disorders, their impact on long-term comprehensive health and the refinement of the therapeutic approach to these diseases in patients with mental disorders. Indeed, despite the fact that the link between the thyroid and psychiatric pathology is a historically old clinical observation, the understanding of this situation still requires extensive research. The results allow encouraging a more in-depth evaluation of the thyroid hormone profile in patients with DM 2 as well as presenting a new possibility of laboratory evaluation for the detection of cardiovascular disease in these patients, to be confirmed in larger and prospective studies. The clinical impact of reduced TH concentrations in DM patients still needs further investigation.

5.2 Recommendations

Once this research work is finished, I allow myself to make the following recommendations that are aimed at improving patient care and reducing morbidity and mortality.

- Encourage the study of diabetic patients with thyroid disorders that encompass a greater number of participants and a longer period of time.
- Encourage the study of thyroid diseases, due to the high percentage of them that occur in our population.
- Establish a protocol for requesting laboratory tests that include a thyroid profile beyond those normally requested in subsequent check-ups of hypothyroid diabetic patients in order to verify the control of said pathologies.
- Promote physical exercise in diabetic patients and the importance of quitting tobacco consumption in order to reduce cardiovascular risk.

The study was unable to achieve the desired results, due to the inexpressive sample. However, it was observed that, although asymptomatic, the participants presented considerable cardiological dysfunctions, and it was not possible to conclude that the alterations found are related to SH and/or other previously unknown diseases.

Therefore, more national and international studies are needed that address this issue and emphasize the need to assess the cardiac function of these patients.

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