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EFFECT AND PREVALENCE OF THYROID DISEASE DURING PREGNANCY

FINAL DEGREE THESIS

BIOCHEMISTRY AND MOLECULAR BIOLOGY

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1. ABSTRACT

Thyroid disorders are common during pregnancy. The prevalence of hypothyroidism, the most frequent thyroid condition during gestation, is estimated to be 0,3-0,5% for overt cases, and 2-3% for subclinical cases. Hashimoto's disease is one of the major causes of hypothyroidism. This disease is characterized by the presence of serum thyroid antibody, especially thyroid peroxidase antibody (aTPO). A 5% of euthyroid women present positive aTPO during pregnancy. This condition is treated with synthetic thyroid hormone (TH), identical to natural thyroxine (T4), to compensate for the TH deficiency. Hypothyroidism has effects in the fetus such as intrauterine death, impaired brain development, low birth weight, and preterm birth. It also presents itself as complications for the mother and the pregnancy such as premature labour and preeclampsia. In this study, data from 2021 Santa Tecla Hospital pregnant women was analysed to examine these patients based on their thyroid stimulating hormone, T4, and aTPO results. Clinical history was also considered in some cases. The study concluded with the importance of checking for thyroid disorders during pregnancy to avoid mother and fetus risks. It was also considered the link between gestational hypothyroidism and several factors such as previous thyroid conditions, gestational diabetes, and abortion, among others.

2. INTRODUCTION

2.1. THYROID HORMONES

Thyroid hormones (TH) are produced and released by the thyroid gland. TH are essential for growth and development and have a great importance in the maintenance of metabolic homeostasis. They are also vital for fetal and post-natal nervous system development and the maintenance of adult brain function. There are two forms of these hormones in mammalian: 3,3',5-triiodothyronine (T3) and 3´,5´,3,3-tetraiodo-L-thyronine (T4 or thyroxine). T4 contains four iodine atoms while T3 contains three (Stepien & Huttner, 2019).

Thyroid hormone action mechanism is mediated by several thyroid hormone receptor isoforms (TR) that are derived from two different genes. These receptors belong to a family of nuclear receptors, which have the role of regulate transcription by binding to specific thyroid hormone-responsive sequences in promoters of target genes (Zhang & Lazar, 2003). However, TH can also mediate non-genomic responses by alternative non-nuclear receptors (Stepien & Huttner, 2019).

T4 is the main product of thyroid gland secretion. Nevertheless, it has been considered a prohormone whereas T3 is the biologically active form in the classical TH pathway. This is due to the low affinity that T4 presents for nuclear TH receptors (TRs). T3 presents a much higher affinity for TRs and can be produced both in the thyroid gland or locally from T4. Only around a 20% of T3 is secreted by the thyroid itself, most circulating levels are derived from peripheral conversion of T4 (Stepien & Huttner, 2019).

Production and secretion of thyroid hormones is regulated via the hypothalamus-pituitary-thyroid (HPT)-axis (van der Spek et al., 2017). In the paraventricular nucleus of the hypothalamus is synthesized and secreted the thyrotropin-releasing-hormone (TRH). TRH stimulates the anterior pituitary gland to produce and secrete thyroid stimulating hormone (TSH), which in turn will lead to production and secretion of TH by the thyroid gland. This HPT-axis is autoregulated by a negative feedback loop **(Figure 1)**. This means that circulating levels of T3 and T4 inhibit the production of TRH

by the hypothalamus and the TSH production by the pituitary gland (Schroeder & Privalsky, 2014).

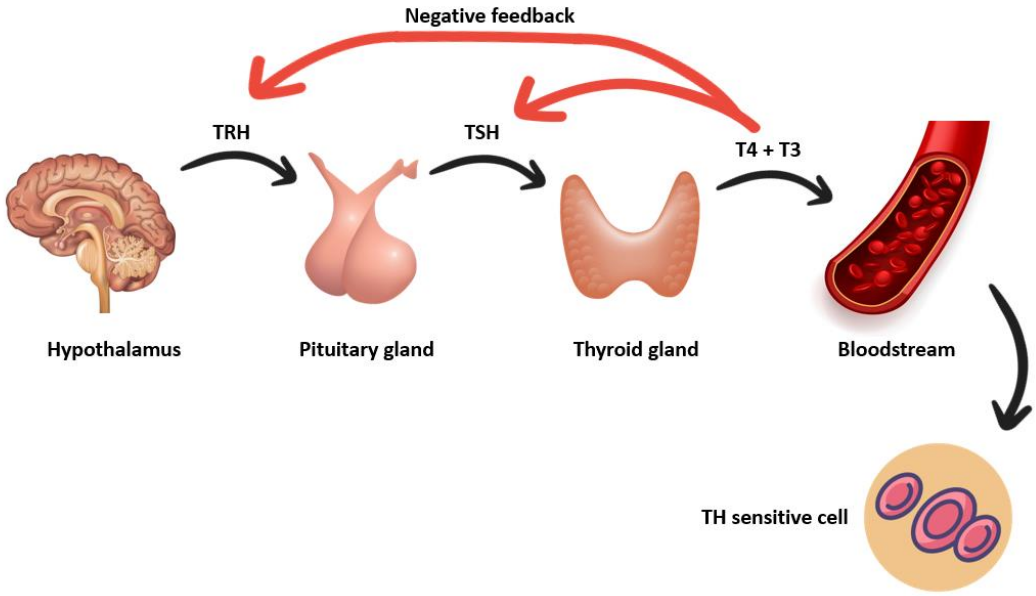

Figure 1. Hypothalamus-pituitary-thyroid axis. Hypothalamus synthesizes and secretes thyrotropin-releasing-hormone (TRH). As a response, the anterior pituitary gland produces and secretes thyroid stimulating hormone (TSH). TSH will in turn lead to the production of TH by the thyroid gland. This system is regulated by a negative feedback loop in which T4 and T3 circulating levels inhibit the production of TRH and the TSH.

Once the thyroid hormones are released into the bloodstream by the thyroid, they circulate through the body bound to serum proteins. Most T3 and T4 molecules bound to thyroxine-binding-globulin (TBG), although they can also bind to a lesser extent to other proteins such as prealbumin/transthyretin (TTR/TBPA) and albumin. Thus, only less than 1% of serum THs circulate free and available for issue uptake. Protein-bound hormones cannot enter the cells and are reservoirs of these THs (van der Spek et al., 2017).

T4 can be metabolized in different pathways that will lead to either the formation of the T3 active form or to the deactivation or excretion of T4 and T3. The main classical pathways of TH metabolism are deiodination, glucuronidation, sulfation, and ether-link

cleavage. Their predominance differs depending on the tissue (van der Spek et al., 2017). These pathways will define the amount of bioactive TH. The levels of serum TH in physiological state are relatively constant. These levels depend on the metabolism of circulating hormones. It is also important to consider that TH levels and uptake can vary strongly among tissues.

2.2. THYROID HORMONES AND PREGNANCY

Pregnancy is a natural process that involves physiological changes accompanied by metabolic and hormonic alterations. These changes are necessary and essential for fetus development. Since around the late 1960s, there has been a consensus about the importance of maternal thyroid function for the health of the new-borns (de Escobar et al., 2004). Sometimes, the changes produced during pregnancy can cause pathological processes with serious outcomes for the mother, fetus and neonate (Alemu et al., 2016). For example, several complications such as premature labour, preeclampsia, intellectual impairment, or fetal death have been associated with maternal thyroid failure at the beginning of pregnancy (Brent, 2012).

During the first trimester of pregnancy, the fetus requirement for THs is exclusively supplied by the mother. After months of gestation, fetal thyroid gland starts concentrating iodine and synthesizing THs **(Figure 2)**. Thyroid hormone receptors (TRs) can be broadly found in the fetal brain, even before the time the fetus is capable of synthesizing THs by itself. The first months are very important for brain development, but it continues considerably beyond the first trimester (de Escobar et al., 2004). On terminal phases of fetal brain development and differentiation, is when THs have most profound effects, including dendrites growth, synaptogenesis, neuronal migration, and axon myelination (de Escobar et al., 2004).

The stimulation of the thyroid starts in the first trimester by β-HCG (Human Chorionic Gonadotropin) hormone, a molecule that shares structural homology with the TSH. There is also an increase in circulating levels of thyroid-binding globulin (TBG) mediated by oestrogen **(Figure 3)**. This TH transport molecule has more affinity for T4 than T3 molecules, and begin to increase a few weeks after conception, reaching the

highest levels during mid-gestational period **(Figure 2)**. The mechanisms used to achieve this TBG increase includes both hepatic synthesis and oestrogen mediated sialylation of TBG, that produce an increase of the half-life of the protein from 15 minutes to 3 days (Alemu et al., 2016). The elevated levels of TBG will lead to a decrease in free T4 concentration due to the binding of these. This will result in increased TSH secretion and, subsequently, enhanced TH secretion and production **(Figure 3)**.

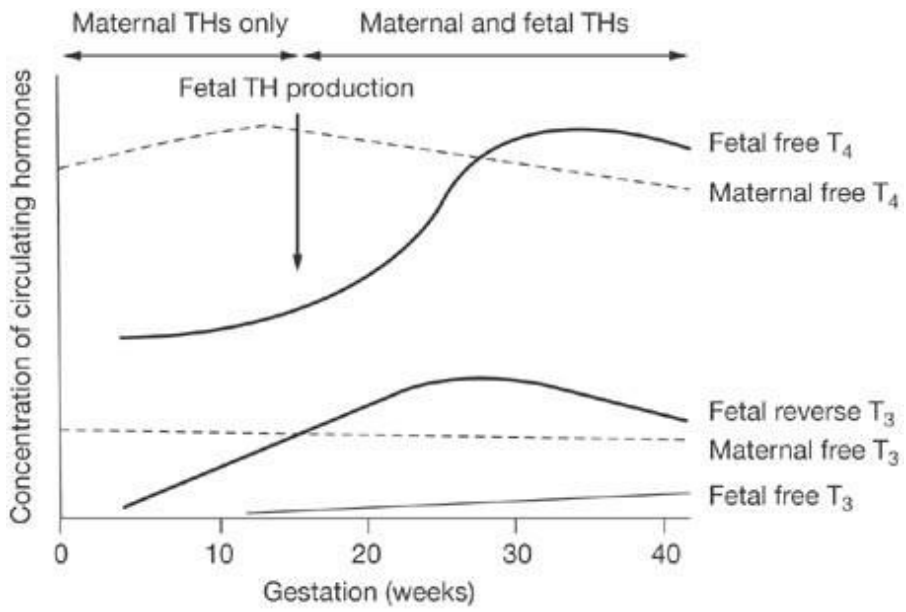

Figure 2. Thyroid hormone levels from maternal and fetal sources during gestation (S. Y. Chan et al., 2009). The fetus starts secreting thyroid hormones around 17 weeks of gestation. Prior to this time, the TH source is exclusively the mother thyroid gland. During the first 12 weeks of pregnancy, maternal TSH decreases while T4 concentration increase. With the gestation progression, TSH levels rise again and free T4 levels decrease up to 10-20%.

Focusing on iodine metabolism during pregnancy, the iodine demand increases during this time due to its necessity for TH synthesis. Besides its clearance by the kidney and urine excretion, maternal iodine is also actively transported to the feto-placental unit. Therefore, state of relative iodine deficiency can be created for the mother during pregnancy (Okosieme et al., 2008).

The impact of secreted HCG by the placenta is also important to consider. HCG stimulates thyroid gland overriding the normal action of the hypothalamic-pituitary-

thyroid feedback system. HCG reaches its peak close to the end of the first trimester in humans, and in that moment, is responsible for a great fraction of the thyroid activity. Furthermore, during this time TSH blood levels become suppressed **(Figure 3)**. Therefore, HGC action causes some women to suffer transient hyperthyroidism known as gestational thyroxicosis (Casey et al., 2006).

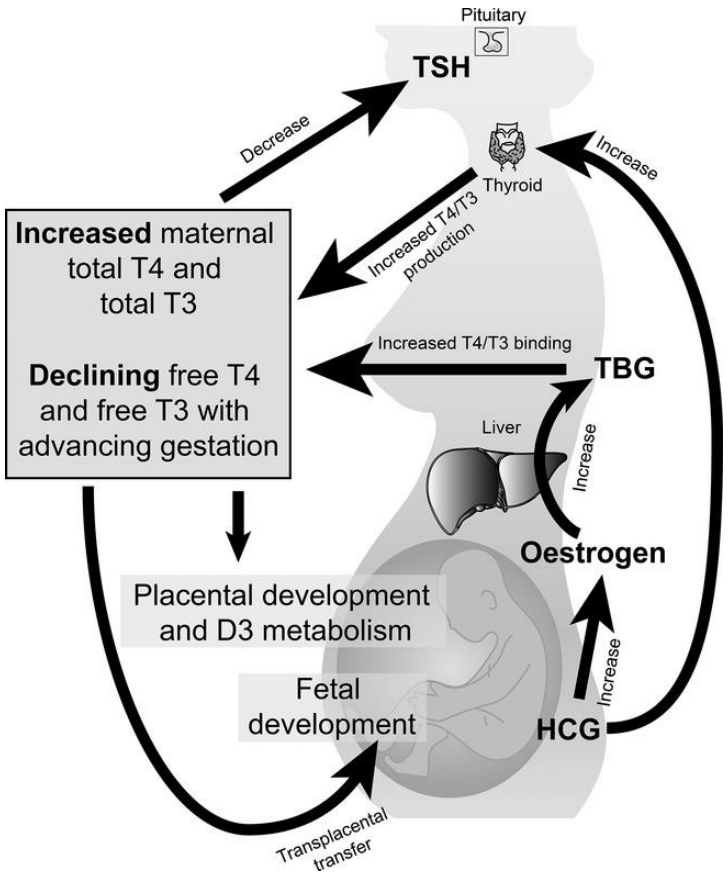

Figure 3. Thyroid hormones dynamic during pregnancy (S. Chan & Boelaert, 2015)**.** Around 10 weeks of gestation placental HCG reaches its peak and then its levels decrease. HCG produce the increasing of maternal oestrogen, which stimulate the production of TBG by the liver. TBG increase the first 20 weeks of pregnancy and then stabilize for the rest of gestation period. HCG have the ability of directly stimulate the mother's thyroid gland to produce THs. The HCG peak coincide the minimal TSH concentrations. Deiodinase type 3 enzymes (D3) metabolize maternal THs and are abundant in the placenta. Fetus receives maternal THs by transplacental supply.

In brief, the fetus source of TH is both the thyroid of the mother and its own thyroid. The ability to synthesize TH is acquired approximately from the first trimester of gestation. TH can be transferred through the placenta, moreover, the placenta

contains deiodinases able to convert T4 to T3 (Haddow et al., 1999). Thyroid dysfunction is one of the most common complications during pregnancy and can be the cause of adverse fetal and maternal outcomes. For this reason, control thyroid activity and appropriate management of thyroid disfunction in pregnancy is essential for avoiding risks.

2.3. ABNORMALITIES DURING PREGNANCY

2.3.1. HYPERTHYROIDISM

Hyperthyroidism is a condition characterized by an excessive TH synthesis and excretion. The term thyrotoxicosis refers to the increased levels of TH in the bloodstream. During pregnancy, hyperthyroidism needs to be managed to avoid risk of pre-eclampsia, premature labor, fetal loss, heart failure, and having a low birthweight baby. Approximately it occurs in 1% of the population and is present in around 0,4% of pregnancies (Michalska et al., 2008). Some tissue effects of hyperthyroidism are supressed serum TSH, accelerated metabolism, increased bone turnover and reduced bone density, and low serum cholesterol (Michalska et al., 2008).

There are different types or causes of hyperthyroidism. TSH-induced type is produced when there is a dysfunction in TSH, responsible of regulating T4 and T3 production. It can be caused by pituitary adenomas that secrete uncontrolled TSH and do not respond to normal negative feedback control. Another cause can be pituitary resistance to thyroid hormone, that can be produced for example by receptor modification.

However, the most common cause of hyperthyroidism is Graves´ disease (GD). It is an autoimmune syndrome in which thyroid stimulating antibodies (TSAbs) are produced. These antibodies are capable of stimulate TSH receptors in the thyroid gland that will lead to excessive TH production, and overstimulation of gland growth (Patil-Sisodia & Mestman, 2010). Thyroid peroxidase antibodies (aTPO), the most common anti-thyroid autoantibody, are found in 10% of women at 14 weeks' gestation. TPO is a protein found in the apical membrane of the thyroid follicular cells. It catalyses the iodination of the tyrosyl groups in thyroglobulin resulting in the synthesis of thyroid hormones, T3

and T4 (Kaczur et al., 1997). GD activity is aggravated during the first trimester of pregnancy and decreased during the latter half. Afterward, a few months after delivery or late in the post-partum period it exacerbates again (Lazarus, 2005). The fetal effects of GD include intrauterine growth, short gestational age, retardation, craniosynostosis, and still birth. There are also maternal effects such as hypertension, preterm delivery, preeclampsia, thyroid crisis, heart failure, and placental abruption. The extra thyroidal manifestations of GD are due to the fact that autoantibodies that react with fibroblasts of skin and the orbital muscle of the eye are also produced (Patil-Sisodia & Mestman, 2010).

Another less common cause of hyperthyroidism during pregnancy is Trophoblastic disease. In this case, it is produced by thyroid stimulators other than TSH. This disease is probably related to the increased HCG levels secreted by the placenta, which is able to stimulate maternal thyroid through TSH receptor activation (Carranza-Lira et al., 1998). Trophoblastic disease is a general term that includes the formation of a hydatidiform mole, which is a rare growth or mass forming in the uterus, and choriocarcinomas during pregnancy (Candelier, 2016).

In general, hyperthyroidism is not a common cause of pregnancy complications. Nevertheless, if it is not controlled can promote a rise in blood pressure, congestive heart failure, thyroid stroma, premature birth, preeclampsia, miscarriage, and low birth weight. Besides, inadequately treated maternal hyperthyroidism could result in neonatal hyperthyroidism due to the transfer through the placenta of stimulatory TSAbs (Polak et al., 2004). This phenomenon occurs in about 1% of infants with mothers with Graves' Disease (KUNG & LOW, 1992).

Regarding to diagnosis of hyperthyroidism, measuring TSH level is the only initial test necessary in a patient without evidence of pituitary disease. When the TSH level is low, then free T4 (FT4) is measured to evaluate if the patient presents thyrotoxicosis. Free T3 levels (FT3) can be helpful in clinical diagnosis for thyrotoxicosis in which FT4 is unexpectedly normal (Baskin et al., 2002). Thyroid-stimulating antibody levels are also important to monitor patients with GD. Iodine uptake can be used as a parameter as well. Diseases that increase T4 synthesis such as GD imply high iodine uptake whereas

other conditions such as subacute thyroiditis and thyrotoxicosis factitia imply a decrease in iodine uptake.

During pregnancy, hyperthyroidism needs to be controlled. Mild hyperthyroidism, in which TSH is low but FT4 is normal, does not require treatment. In more severe elevated TH concentration, the patient is treated with anti-thyroid medication. This medication can cross the placenta in small amounts, therefore, to avoid hypothyroidism in the baby the lowest possible dose should be used (Alemu et al., 2016).

2.3.2. HYPOTHYROIDISM

Hypothyroidism is most common in pregnancy. The prevalence during pregnancy is estimated to be 0,3-0,5% for overt cases, and 2-3% for subclinical cases, in which patients present above normal TSH levels with normal FT4 and T3 levels (Sahay & Nagesh, 2012). In parts of the world with adequate iodine supply Hashimoto's disease is the major cause of hypothyroidism. In zones with endemic iodine deficiency, this is the cause of most hypothyroidism cases in pregnant women (Mandel, 2004).

Manifestations of hypothyroidism differ widely from asymptomatic subclinical detection to noticeable myxedema or severe hypothyroidism. Severe cases are rare due to widespread screening for thyroid disease (Kalantri et al., 2007). Hypothyroidism has effects in the fetus such as intrauterine death, impaired brain development, neonatal respiratory distress, low birth weight, preterm birth and increased fetal distress. Besides, a study reported that infants with thyroid peroxidase (TPO) positive mothers had significantly reduced brain weight, lower brain-to- body ratio and smaller head circumference than infants born to TPO antibody negative mothers. Those features are associated with a higher risk of premature delivers and miscarriage (LaFranchi et al., 2005).

Hashimoto's disease or chronic autoimmune thyroiditis is the most common cause of hypothyroidism. In this disease, the immune system attacks the thyroid, that will lead to inflammation and impairment of correct THs production. This is the cause of 90% of

hypothyroidism in pregnant women with positive test for thyroid antibodies (Klein et al., 1991). This disease is characterized by the presence of serum thyroid antibody, specially TPO antibody and thyroglobulin antibody (TGAb). A 5% of euthyroid women (with normal thyroid gland function) present positive TGAb or TPO antibodies during pregnancy. However, the prevalence of thyroid autoantibody in pregnant women has been found up to 15% (Cleary-Goldman et al., 2008).

There are other causes of hypothyroidism. For example, thyroid surgery can cause iatrogenic hypothyroidism. Moreover, iodine deficiency, thyroid enzyme defects, goitrogens and thyroid hypoplasia may also produce TH deficiency in the developing fetus. When the infant borns with congenital hypothyroidism (CH) or cretinism, there is an absence of thyroid tissue and hereditary defects in the synthesis of THs. This is one of the major preventable causes of mental retardation and it occurs in 1/3000 newborns in births with TH deficiency (Raymond & LaFranchi, 2010).

Furthermore, postpartum thyroiditis is produced during the first year after delivery and affects about 5-18% of healthy pregnant women. It is caused by a transient inflammation of the thyroid gland for 3 to 6 months. Most patients recover wholly, but a relapse is possible during a second pregnancy. Almost all women with postpartum thyroiditis have TPO antibody positivity. That is why, aTPO can be used as a marker for screening tests (Muller et al., 2001).

Similar problems caused by hyperthyroidism can occur with hypothyroidism. TSH are very important to fetal brain and nervous system development, therefore, hypothyroidism can affect those aspects of the baby, especially during the first trimester (Casey et al., 2005). Different studies reported that untreated hypothyroidism during pregnancy was related to a significant decrease of intelligence quotient of the children (Haddow et al., 1999) and deficits in mental and motor function (Kooistra et al., 2006).

Regarding management, hypothyroidism is treated with synthetic TH (LT4), identical to natural T4. Pregnant women with pre-existing TH deficiency increase their dose of LT4, and asymptomatic pregnant women are routinely screened. Has been seen that treatment with LT4 lowers the risk of adverse fetal and maternal outcomes (Velkeniers

et al., 2013). Maternal supplementation of T4 but not T3 protects from hypothyroid injury in the brain until birth. This is due to T4 being the main form transported to the central nervous system whereas the majority of T3 in the cerebral cortex comes from local tissue production (Stepien & Huttner, 2019).

It is also important to consider the role of iodine in the synthesis of THs. The baby gets iodine from the mother at the first stages of the pregnancy; therefore, dietary iodine supplements can also be helpful to prevent the progress of the deficiency (Murcia et al., 2011).

3. OBJECTIVES

The main goal of the present investigation is to analyse different thyroid parameters in pregnant women to estimate the importance and recurrency of these disorders during gestation.

The aim is to clarify if thyroid disorders are common in pregnancy and how are those conditions treated during gestation when they presented. To do that we analyse data from 2021 Sant Pau I Santa Tecla Hospital pregnant patients. These patients are studied based on their TSH, T4, and aTPO values.

As a secondary objective, we also examine clinical history of patients to assess if age is an important element when it comes to suffering these thyroid disfunctions, and we determine the frequency and possible link of several factors such as previous hypothyroid conditions, thyroiditis post-gestation, gestational diabetes, familiar history of hypothyroidism, and abortion in gestational hypothyroidism.

4. MATERIALS AND METHODS

The sample obtained for this study consists in the 1090 pregnant women registered by Sant Pau I Santa Tecla Hospital during 2021 in the area of Tarragona, Catalonia (Spain). For screening purposes, samples were taken during the first trimester of pregnancy.

Values of TSH were measured for every pregnant patient. For women that presented TSH results outside the reference range, free T4 and aTPO levels were also measured. Finally, for positive aTPO patients, clinical history was examined in this study to obtain results. The clinical history provided information about previous thyroid conditions of patients or familiar history of hypothyroidism. It also allows to check if the patients suffered from gestational diabetes, thyroiditis post-gestation, or had an abortion. Age of the mothers has been taken into consideration too **(Figure 4)**.

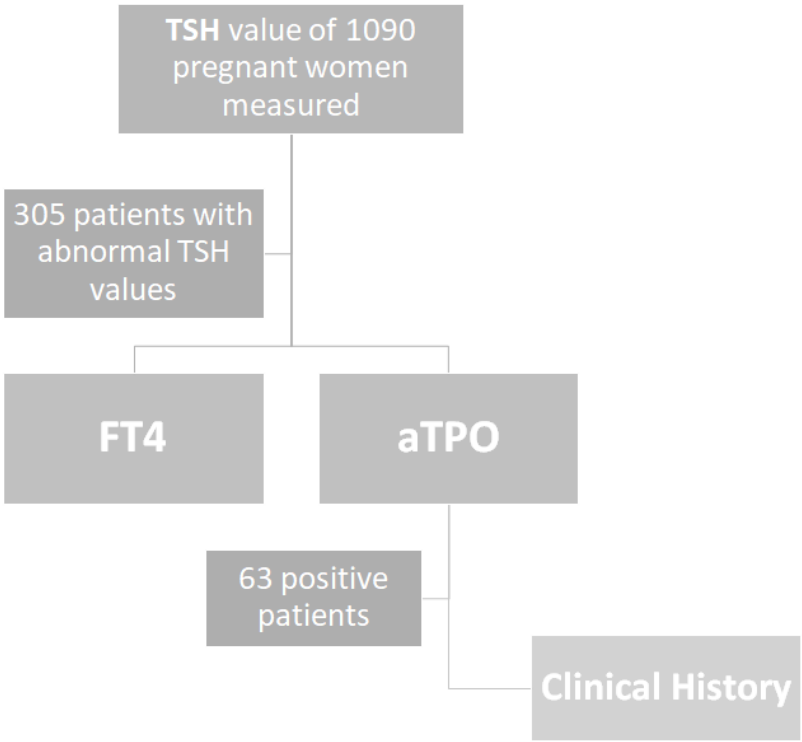

Figure 4. Study workflow. During 2021, a total of 1090 pregnant women were registered in Santa Tecla Hospital. From all this women, TSH values were measured as routine. From patients that presented abnormal values for TSH, free T4 and aTPO were measured. For positive aTPO patients, clinical history was also reviewed.

To measure the thyroid parameters, the Atellica IM analyzer from the commercial house SIEMENS was used. This clinical analyser can automatically measure specific metabolites performing different immunoassays based on chemiluminescence detection.

Before processing the patient samples, calibration and control check must be performed to create a reliable calibration curve. To do that, previously known concentrations of the metabolite to be analysed are introduced in the machine to measure the chemiluminescence (RLUs) they produce. Then, these values are related to the concentration to create the calibration curve. The correct calibrator and controls are provided by the same commercial house.

4.1. DETERMINATION OF TSH IN PATIENT SERUM

For the quantitative determination of TSH in serum, the Thyroid Stimulating Hormone 3-Ultra (TSH3-UL) assay for in vitro diagnosis was used. This assay employs an anti-FITC monoclonal antibody covalently bound to paramagnetic particles; a fluorochrome (FITC) labelled anti-TSH monoclonal mouse antibody; and a tracer that consists in a proprietary acridinium ester and an anti-TSH mouse monoclonal antibody conjugated to bovine serum albumin for chemiluminescent detection.

The assay procedure is automatically performed by the system. The steps are the following: first, it dispenses 75µL of the patient sample into a cuvette. Then, it dispenses 38µL of the reagent with the FITC conjugated to mouse monoclonal anti-TSH and 38µL of the reagent with bovine serum albumin conjugated to mouse monoclonal anti-TSH labelled with acridinium ester in HEPES buffered saline. After 5 minutes incubation at 37℃, the system dispenses 150µL of Solid Phase containing mouse monoclonal anti-fluorescein antibody covalently linked to paramagnetic particles and incubates again for 7 minutes at 37℃. Then, it separates, aspirates, and washes the cuvette with Atellica IM Wash before dispensing 300µL each of Atellica IM Acid and Atellica IM Base to initiate the chemiluminescent reaction. Finally, the system reports the results. There is a direct relationship between the amount of TSH present in the patient sample and the amount of relative light units (RLUs) detected by the system.

4.2. DETERMINATION OF FREE T4 IN PATIENT SERUM

For the quantitative determination of free T4 in serum, the Atellica IM Free Thyroxine (FT4) assay for in vitro diagnosis was used. This is a competitive immunoassay that uses direct chemiluminescent technology. The FT4 present in the patient sample competes with acridinium-ester-labelled T4 present in the Lite Reagent for a limited amount of biotinylated rabbit polyclonal anti-T4 antibody. Biotin-labelled anbti-T4 is bound to avidin that is covalently coupled to paramagnetic particles in the Solid Phase.

To complete the procedure, the system automatically performs the following steps: first, it dispenses 25µL of sample into a cuvette. Then, it dispenses 100µL of Lite Reagent and 300µL of Solid Phase before incubating for 12 minutes at 37℃. After that, the system separates, aspirates, and washes the cuvette with special reagent water. Finally, it dispenses 300µL each of Atellica IM Acid and Atellica IM Base to initiate the chemiluminescence reaction and reports the results. There is an inverse relationship between the amount of FT4 present in the patient sample and the amount of relative light units (RLU) detected by the system.

4.3. DETERMINATION OF ANTI-THYROID PEROXIDASE IN PATIENT SERUM

For the quantitative determination of anti-thyroid peroxidase (aTPO) in serum, the Atellica IM aTPO assay for in vitro diagnosis was used. This is a competitive immunoassay that uses chemiluminescent technology. In this assay, autoantibody against thyroid peroxidase in the patient sample competes with mouse monoclonal anti-TPO antibody covalently coupled to paramagnetic particles in the Solid Phase for limited amount of human TPO complexed with acridinium-ester-labelled mouse monoclonal anti-TPO antibody in the Lite Reagent. To carry out the procedure, the system automatically performs the following steps: first, it dispenses 30µL of patient sample into a cuvette. Then, it dispenses 100µL of Lite Reagent and incubates for 3 minutes at 37℃. After that, it dispenses 200µL of Solid Phase and incubates for 9 minutes at 37℃. When the incubation is done, the system separates, aspirates, and washes the cuvette with special reagent water. Finally, it dispenses 300µL each of Atellica IM Acid and Atellica IM Base to initiate the chemiluminescent reaction and

reports the results. There is an inverse relationship between the amount of anti-TPO present in the patient sample and the amount of relative light units (RLUs) detected by the system.

4.4. REFERENCE RANGES

To analyse the data of the patients, references rages are necessary to provide guidance in the results interpretation. These ranges are calculated in the way that they include 95% of statistically "normal" or "healthy" population. To set these reference ranges, factors such as gender, age, or even race must be taken into consideration. For this study, reference ranges for pregnant woman in the demographic area of Tarragona have been provided from the Sant Pau i Santa Tecla Hospital **(Table 1)**.

Table 1. Reference ranges. TSH reference range is 0.1-2.5 µIU/mL. T4 is considered in the normal range between 10.3 and 23.2 pmol/L. Finally, aTPO is considered positive above 60 U/mL.

	Reference Ranges
TSH	0.1-2.5 µIU/mL
FT4	10.3-23.2 pmol/L
aTPO	<60 U/mL

4.5. CLINICAL HISTORY OF PATIENTS

To ascertain the aetiology of the impaired thyroid function and following treatment the patients have received, the clinical history of these pregnant patients has been also reviewed for this study. Access to Sant Pau I Santa Tecla Hospital database of patients has been allowed with this purpose, always with the agreement of respecting patient confidentiality.

5. RESULTS

Below are shown the results of the analysis of thyroid parameters in the 1090 pregnant women during 2021.

5. 1. TSH DISTRIBUTION OF PREGNANT WOMEN

Patients had their TSH values measured as routine. Of the 1090 patients, a total of 305 presented abnormal TSH values, which represents 27.98% of pregnant women (**Figure 5**).

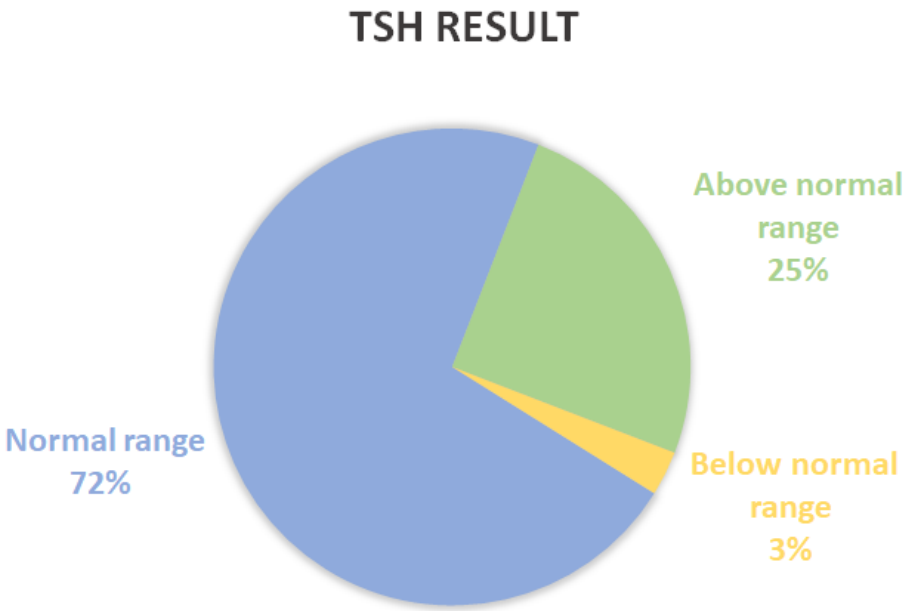

Figure 5. Classification of patients depending on their TSH value. For TSH, the reference range provided was 0.1-2.5 µIU/mL. Of the 1090 patients, 785 fit into these range, 272 presented TSH values above the reference range, and 33 presented values below them.

As is shown in **Figure 5**, TSH results of the 1090 patients have been classified in three categories: values below the normal range, above normal range, and within normal range. A total of 785 pregnant women, which represent around the 72% of patients, presented normal or non-pathological values. Therefore, most women obtained results

within the normal range. On the other hand, surrounding 28% of patients presented values outside the reference range. A total of 272 women, which represent the 25% of the sample group, obtained TSH values above the normal range. The remaining 33 patients, that represent the last 3%, presented values below the reference range. Therefore, TSH values higher than usual resulted more common than values above normal. Increased TSH value is consistent with a diagnose of hypothyroidism, which was mentioned before to be more common than hyperthyroidism during pregnancy (Michalska et al., 2008).

5.2. THE EFFECT OF AGE IN TSH VALUES

With the aim of checking if pregnant women's' age had any significance in TSH values, distribution of age in patients with normal and abnormal TSH results were represented separately **(Figure 6 and 7)**.

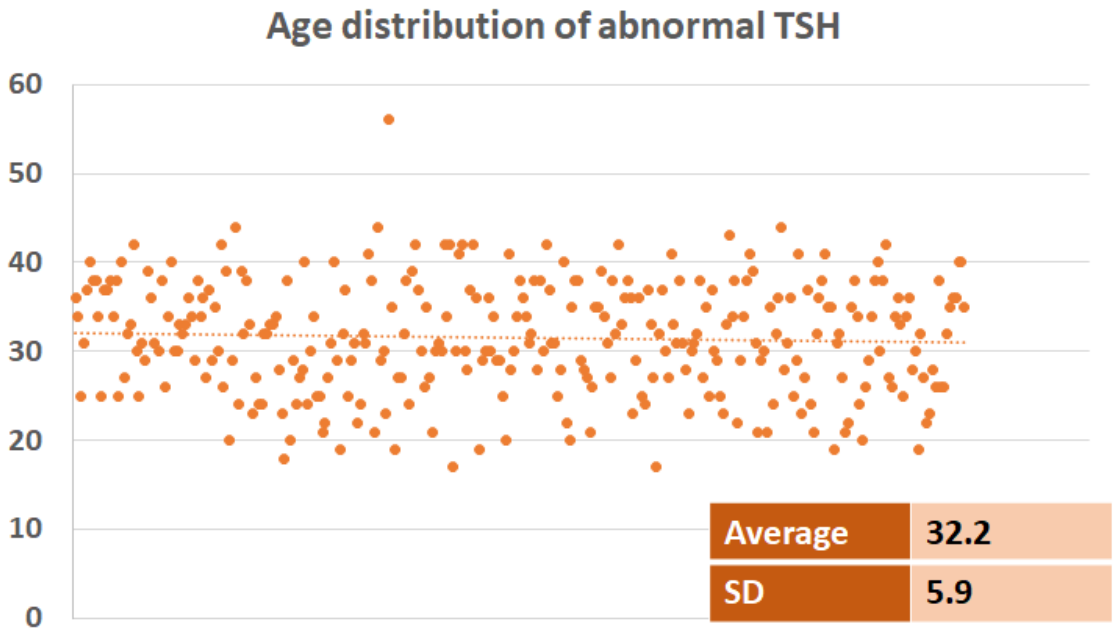

Figure 6. Distribution of patients' age with abnormal TSH values. The number of patients is n=305. The average age of these pregnant women is 32.2 years old with a standard deviation of 5.9 years.

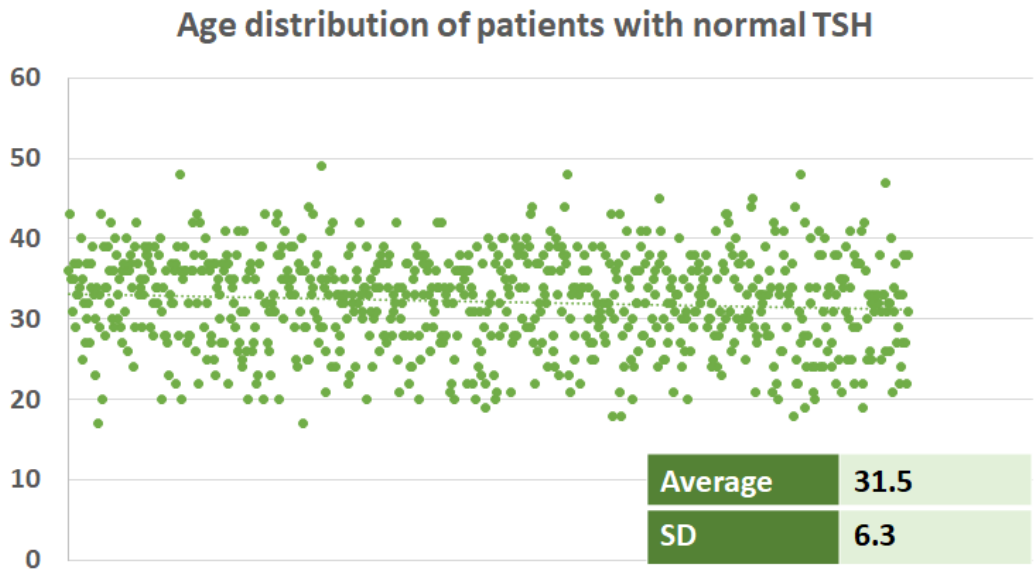

Figure 7. Distribution of patients' age with normal TSH values. The number of patients is n=785. The average age of these pregnant women is 31.5 years old with a standard deviation of 6.3 years.

As shown in **Figure 6**, the age distribution of patients that obtained abnormal TSH results fluctuates between 17 and 56 years old. Nevertheless, this '56 years old' point is isolated in the chart, and most women find themselves in the age between 26 and 38. For these patients, the average age calculated was 31.5. The standard deviation obtained was 5.9.

On the other hand, in **Figure 7** is represented the age distribution of patients that obtained normal TSH results values. In this case, the age of pregnant women fluctuates between 17 and 49 years old. However, most patients find themselves in the age between 25 and 38, with an average of 31.5 years old. The standard deviation obtained was 5.

In brief, both pregnant patients with and without abnormal TSH values presented very similar average ages. Patients with normal results presented a slightly younger age in general. Nevertheless, this difference does not seem significant, and the results obtained suggest that age is not a determinant factor that dictates the probability of suffering thyroid disfunction during pregnancy.

5.3. THYROID PARAMETERS RESULT OF aTPO POSITIVE PATIENTS

For every patient with abnormal TSH result, the presence of TPO autoantibodies was tested. The table below **(Table 2)** includes the thyroid parameters measured for every patient that obtained a positive aTPO result.

Table 2. TSH and FT4 result of aTPO positive patients. List with patient number, age, TSH (µIU/mL.) and FT4 value (pmol/L) of women with positive aTPO results (n=63). TSH reference range is 0.1-2.5 µIU/mL. T4 is considered in the normal between 10.3 and 23.2 pmol/L. Every patient presented aTPO positive results above 60 U/mL. Abnormal values are shown in red.

PATIENT	AGE	TSH	FT4
1	40	0.01	23.37
2	31	0.06	16.7
3	29	2.57	14.38
4	26	2.6	13.44
5	32	2.68	15.5
6	38	2.74	12.18
7	30	2.78	15.11
8	31	2.81	14.85
9	32	2.83	12.51
10	25	2.84	16.6
11	39	2.96	15.24
12	30	3.02	14.78
13	31	3.02	12.51
14	17	3.09	14.82
15	42	3.1	13.26
16	28	3.1	16.43
17	37	3.11	16.95
18	19	3.14	14.33
19	36	3.16	13.43
20	29	3.18	15.17
21	29	3.19	14.87
22	41	3.21	13.87
23	34	3.22	17.55
24	31	3.28	12.25
25	22	3.42	14.96
26	21	3.51	16.13
27	35	3.52	16.21
28	39	3.54	18.91
29	38	3.64	14.38
30	32	3.64	15.68
31	38	3.68	13.67

PATIENT	AGE	TSH	FT4
32	36	3.68	13.41
33	23	3.71	11.97
34	30	3.86	14.42
35	41	3.9	17.23
36	38	3.95	13.86
37	31	3.97	12.53
38	33	4.16	15.11
39	29	4.37	13.49
40	44	4.41	13.68
41	28	4.44	16.45
42	31	4.51	15.46
43	38	4.83	11.29
44	41	4.89	10.83
45	35	4.93	15.15
46	32	5.01	12.81
47	27	5.04	15.41
48	38	5.39	13.69
49	34	5.66	12
50	38	5.67	9.41
51	40	5.72	11.85
52	38	5.82	14.84
53	34	6.64	12.97
54	36	6.74	15.19
55	33	6.8	14.1
56	36	7.06	14.52
57	28	7.13	11.54
58	30	7.32	14.08
59	26	9.4	13.86
60	38	10.54	12.55
61	26	10.73	12.66
62	26	16.14	12.33
63	35	37.45	8.12

There was a total of 63 aTPO positive patients. **Table 2** shows age, TSH, and FT4 results for each one of these women. Regarding age, mothers with positive TPO autoantibodies presented mixed ages between 17 and 44 years old. The average age calculated was 32.6 years old, which is very similar to the averege age of the whole group of patients with abnormal TSH values (32.2 years old), and to the average of patients with normal TSH values (31.5 years old) showed above. Therefore, once again it can be seen that age distribution is very similar no matter the group that is analysed, and does not seem to have an impact on test result.

Table 2 also shows TSH and FT4 results. TSH values are sorted in ascending order. It can be seen that most patients presented TSH values above the normal range (>2.5), whereas only two patients presented values below normal range (<0.1). On the other hand, only three patients presented abnormal FT4 results. Therefore, the most common combination was patients with abnormally high TSH levels acompanied by a normal TF4 result. As mentioned before, these are cases of subclinical hypothyroidism, the most common thyroid condition during pregnancy (Sahay & Nagesh, 2012).

5.4. PREVALENCE OF TPO ANTIBODIES IN PREGNANT WOMAN

To analyse if the prevalence of TPO autoantiboides positivity during pregnacy is significant, the percentatge of patients that presented TPO autoantibodies was represented in both the whole sample group **(Figure 8A)** and patients with abnormal TSH values **(Figure 8B)**.

TPO autoantibodies can be present in both hyperthyroidism (Graves' disease) and hypothyroidism (Hashimoto's disease). In this study, each one of the 63 pregnant women that obtained a positive aTPO test result presented gestational hypothyroidism.

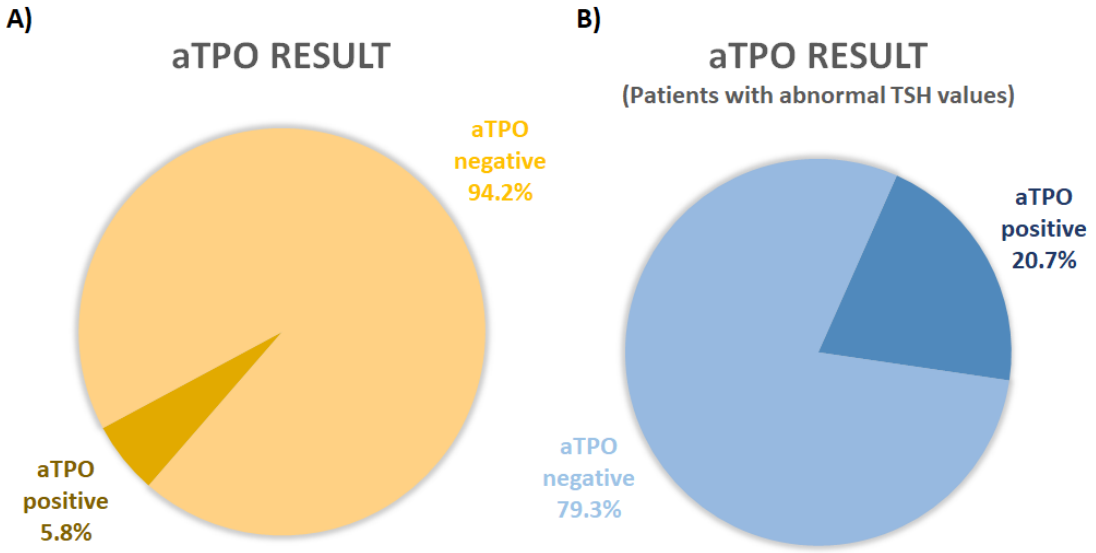

Figure 8. Results of aTPO screening. Chart A represents the number of positive aTPO results in comparation with the whole group of pregnant women (n=1090). Chart B represents the number of positive aTPO results in comparation with patients with abnormal TSH values (n=305). A total of 63 patients were positive for anti-thyroid-peroxidase antibody, which represents the 5.8% of the whole pregnant woman and the 20.7% of the women with abnormal TSH values.

As shown in **Figure 8**, aTPO positivity represents a great percentage in patients with hypothyroidism. In **Figure 8A**, the percentage of patients with positive aTPO result is represented taking into consideration the whole 1090 women group. It shows that 5.8% of the studied women had TPO autoantibodies. Even if this does not seem a great percentage, we have to consider that in this figure healthy pregnant women with normal TSH values are also contemplated in the statistics.

In contrast, in **Figure 8B**, the percentage is calculated taking into consideration the group of patients with abnormal TSH values, and therefore, with impaired thyroid function. In this case, women with aTPO positivity represented the 20.7% of patients. This evidence that autoantibodies was one of the main causes of thyroid disfunction in Sant Pau I Santa Tecla pregnant women.

5.5. ANALYSIS OF GESTATIONAL HYPOTHYROIDISM USING THE CLINICAL HISTORY OF PATIENTS

As mentioned above, for aTPO positive patients, clinical history was also reviewed. This allowed us to represent the number of cases of patients that presented or exhibit different factors that have been linked to thyroid disfunction during pregnancy in comparison with the entire group of positive aTPO patients **(Figure 9)**. The factors that have been taken into consideration are previous hypothyroid conditions, thyroiditis post-gestation, gestational diabetes, familiar history of hypothyroidism and abortion.

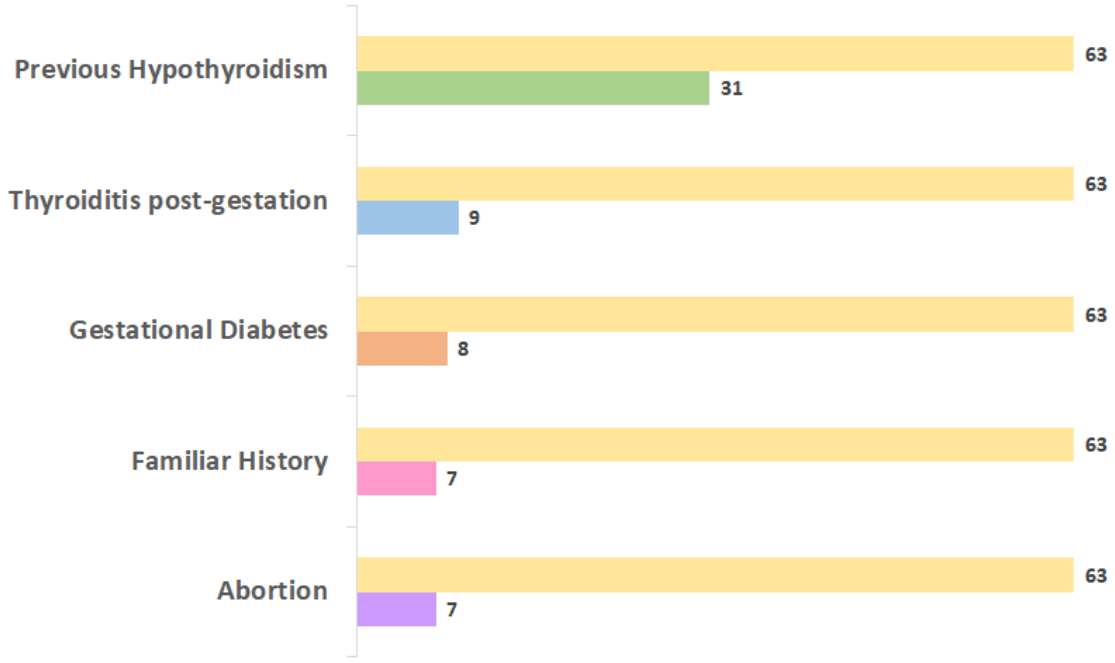

Figure 9. Link between gestational hypothyroidism and previous conditions, thyroiditis post-gestation, gestational diabetes, familiar history of hypothyroidism and abortion. The first bar represents the total of positive aTPO patients (n=63), and the second bar represents how many of these patients also presented those factors.

As we can see in **Figure 9**, these factors seem to have a link with gestational hypothyroidism. By order of prevalence, previous hypothyroid condition was present in almost half of aTPO positive patients (49.2%). After delivery, a total of 9 patients of the 63 presented thyroiditis post-gestation, which represents around the 14% of

women. Gestational hypothyroidism is sometimes presented accompanied by gestational diabetes. In this case, 8 patients (almost 13% of total) also exhibit gestational diabetes. On another hand, 7 pregnant patients (11%) had a family history of hypothyroidism. Lastly, abortion has also been taken into consideration. In this study, 7 hypothyroid mothers, which represents the 11% of the aTPO positive patients, suffered a miscarriage.

Therefore, **Figure 9** shows that each one of the factors hypothesized to have a link with gestational hypothyroidism had representation in the sample group of pregnant women.

Lastly, it must be taken into consideration for factors such as thyroiditis post gestation and abortion that data was collected as of April 29 (2022). The sample group is formed by 2021 pregnant women, therefore, depending on the month gestation began, some of those women were still expecting by the time the data was collected. Thus, some women could have suffered from thyroiditis post gestation or an abortion after that date, that could not be considered in the present study.

6. DISCUSSION

Thyroid stimulating hormone is the first thyroid function test usually made during pregnancy. This simple hormone determination enables to detect thyroid function abnormalities, that need to be treated to ensure normal pregnancy outcomes (Baskin et al., 2002). Gestational hypothyroidism may be difficult to diagnose, considering that its signs can be attributed to pregnancy itself. Therefore, it is important to perform this kind of tests as a routine during the first trimester to detect abnormalities, which will lead to the conduction of more specific tests such as TPO antibody detection (Tudosa et al., 2010).

Considering the relevance of this, the aim of the present study was to clarify if thyroid disorders are common in pregnancy and how are those conditions treated during gestation when they presented, using the information collected from Sant Pau I Santa Tecla Hospital and from previous literature. In order to do that, data from 2021 pregnant women was analysed.

Regarding the prevalence of thyroid disfunction, in this study, the patients that showed abnormal TSH values represented the 27.98% of pregnancies **(Figure 5)**. Thus, for this group of women, almost 28 of each 100 mothers presented thyroid impairment. These results confirm that these disorders are not uncommon among pregnant patients and highlight the importance of monitoring thyroid parameters such as TSH to enable adequate treatment at the first stages of gestation.

Only 3% of women presented very low serum TSH (with values below the reference range). On the other hand, 25% of pregnant women presented abnormally high TSH levels in serum **(Figure 5)**, mainly accompanied by normal T4 concentrations **(Table 2)**. Those are cases of subclinical hypothyroidism, in which the patient presents TSH levels above normal with normal FT4 and T3 levels (Kalantri et al., 2007). This was expected, due to subclinical Hypothyroidism being the most common thyroid disorder during pregnancy. Besides, pregnant patients with subclinical hypothyroidism are more likely to have positive TPO antibody results than euthyroid women (Sahay & Nagesh, 2012).

Furthermore, the age of the mothers seemed to not have impact on these TSH values. The distribution of age for these women was similar for both mothers with abnormal TSH results **(Figure 6)** and for mothers with normal values **(Figure 7)**. In this sense, women with abnormal TSH results presented an average age of 32.2 years old whereas those with apparent normal thyroid function obtained an average age of 31.5. Therefore, age does not seem a crucial factor to determine if women are more susceptible to suffer from thyroid disorders during pregnancy. Besides, age did not seem to have any impact on aTPO positive mothers either **(Table 2)**. This finding is consistent with other studies in which it was also reported that prevalence of autoimmune thyroid disease did not increase with age in pregnant women (Potlukova et al., 2012).

As mentioned above, hypothyroidism is much more common during pregnancy than hyperthyroidism (Michalska et al., 2008). This was also noticeable in Sant Pau I Santa Tecla patients, where every aTPO positive mother presented gestational hypothyroidism. It was also previously mentioned that hypothyroidism can originate due to different causes. Nevertheless, the most common cause, together with iodine deficiency, is autoimmune thyroid disease, in which autoantibodies are produced against cells in the thyroid gland (Mandel, 2004). The most common autoantibodies present in this condition are anti-TPO autoantibodies, followed by thyroglobulin autoantibodies (Cleary-Goldman et al., 2008). That is why mothers with abnormal or outside the reference range TSH values have been screened for it. Many studies have link aTPO positivity to adverse fetal and maternal outcomes in pregnancy such as pre-term birth and miscarriage (Rajput et al., 2017). In this study there was a total of 5.8% of positive aTPO patients **(Figure 8A)**. However, aTPO positive women represented 20.7% of pregnant women with abnormal TSH values **(Figure 8B)**. Therefore, as expected, the presence of autoantibodies represented one of the main causes of hypothyroidism in these patients.

Finally, for patients that presented gestational hypothyroidism with positive autoantibody result, different factors from their clinical history were checked to observe if there was usually a link between their condition and that factor. Factors taken into consideration were previous hypothyroid conditions, thyroiditis post-

gestation, gestational diabetes, familiar history of hypothyroidism and abortion **(Figure 9)**. These factors will be discussed by order of prevalence.

In gestational hypothyroidism, thyroid pathology can begin during gestation period or can be pre-existent. Most patients that suffer from hypothyroidism during pregnancy have a previous clinical history of thyroid disease (Sahay & Nagesh, 2012). In the case of 2021 Sant Pau I Santa Tecla patients with positive TPO autoantibodies, almost half of women presented previous history of thyroid disorder. A total of 63 patients were positive for aTPO. Of these patients, 31 had pre-existent hypothyroidism according to their clinical history **(Figure 9)**. 6 of these 31 with pre-existing history had subclinical hypothyroidism before pregnancy that did not have required treatment. Therefore, most women had already presented THs problems prior to gestation.

As mentioned above, the first year after delivery woman can suffer from postpartum thyroiditis. This transient inflammation of the thyroid gland affects about 5-18% of healthy pregnant women, and the patients usually present positive TPO antibody results (Muller et al., 2001). In this study, 9 of the 63 aTPO positive patients presented postpartum thyroiditis, which represents around the 14% of studied women **(Figure 9)**. Thus, screening of patients after delivery with high risk of developing postpartum thyroiditis such as previous aTPO positive women is highly recommended. It is important to clarify that by the time the data was collected (April 29), 18 women were still expecting. Therefore, it is a possibility that these mothers and women with recent deliveries to that date experience postpartum thyroiditis in the future. Those cases could not be considered in this study.

Another factor that was taken into consideration was the link between gestational diabetes and gestational hypothyroidism. It was observed that 8 of the 63 aTPO positive patients also presented gestational diabetes, which represents almost the 13% of studied women **(Figure 9)**. The reason behind this relationship is that hypothyroid women present increased insulin resistance, and therefore higher risk of developing gestational diabetes (Gong et al., 2016). Thyroid hormones have an important effect in the regulation of glucose homeostasis and insulin secretion, that might be the reason why both conditions sometimes appear simultaneously during pregnancy. Besides, it

was also observed in previous studies that the risk of developing gestational diabetes increases with TSH levels (Tudela et al., 2012).

In the case of familiar history of hypothyroidism, 7 of the 63 studied women presented family with hypothyroid background. Therefore, around 11% of aTPO positive patients had close family with these characteristics **(Figure 9)**. Nevertheless, not every mother with family history presented hypothyroidism before pregnancy.

In **Figure 9**, the number of abortions suffered by these mothers was also considered. Once more, since some of the mothers that got pregnant in 2021 were still expecting, gestation problems produced after the collection of data could not be considered. The abortion information was collected as of April 29. To that date, there are still 18 expecting women that did not yet experienced an abortion. Also to that date, 7 patients had suffered an abortion. That represents around 11% of the pregnant women with TPO autoantibodies. As mentioned before, women with hypothyroidism present an increased risk of miscarriage. The presence of aTPO has also been associated with increased risk of abortion (Dhillon-Smith & Coomarasamy, 2020). Most abortions occur during the first trimester and are strongly associated with higher TSH levels (Gahlawat et al., 2017).

In brief, those features or complications were present in 14-11% of pregnant women with autoimmune disease. Even if it might not seem a significant percentage, it must be taken into consideration that the sample number in which those factors were studied is relatively small. Therefore, just the fact that every factor had representation in the sample group gives an idea of their relevance in relationship with gestational hypothyroidism.

Finally, the clinical history of patients also showed the treatment that aTPO positive patients had to follow after being diagnosed with gestational hypothyroidism. Each one of the patients were prescribed with Eutirox. Eutirox is a medicine that contains the active ingredient levothyroxine. This is a synthetic form of L-thyroxine or synthetic T4 that is used to treat the lack of sufficient thyroid hormones in hypothyroidism. The goal of this treatment is to return TSH to the normal range (Velkeniers et al., 2013).

For Sant Pau I Santa Tecla patients with previous hypothyroid condition, the dose of levothyroxine was increased during pregnancy. Synthetic T4 has been tested to be safe and is necessary for the well-being of the mother and the fetus if hypothyroidism is presented (Alemu et al., 2016). For patients with subclinical hypothyroidism, there is not an obvious consensus about if they should be treated or not. Recommendations differ due to the existence of inconsistent data regarding the benefits for the child and the mother (Teng et al., 2013). Treatment of pregnant women with overt hypothyroidism has shown to improve the complications and negative outcomes of pregnancy. Nevertheless, there is less evidence for women with subclinical hypothyroidism. More studies of the offspring of these mothers are necessary to assess if treatment could improve the neurodevelopmental outcome of these infants (Casey, 2006). Furthermore, evidence seems to indicate that treating these mothers does not entail any downside for the new-borns. Therefore, LT4 supplementation seems to be recommended in every case to improve pregnancy outcome (Velkeniers et al., 2013). However, further studies will help to define which subgroups of pregnant women require or will benefit from treatment, in order to determine the risks and benefits of both strategies.

Regarding treatment, many Sant Pau I Santa Tecla pregnant patients were also prescribed with yodocefol. This medicine is used to treat iodine deficiencies, especially during pregnancy. As mentioned before, iodine is essential for the synthesis and composition of thyroid hormones. During pregnancy, iodine demand increases due to its necessity for TH synthesis to supply the fetus (Murcia et al., 2011). Besides its clearance by the kidney and urine excretion, maternal iodine is also actively transported to the feto-placental unit (Okosieme et al., 2008). Therefore, state of relative iodine deficiency can be created for the mother during pregnancy. This iodine deficiency contributes to the emergence of hypothyroidism and supplementation of patient with iodine can help detaining the disease progression (Zimmermann, 2012).

7. LIMITATIONS OF THE STUDY

The current study arises from the necessity to clarify the effect and prevalence of thyroid disease during pregnancy. However, it faces some limitations.

Firstly, the sample group considered was relatively small. Some aspects have been able to be studied in the whole 1090 pregnant group, but some others could have only been examined in the 63 patients with aTPO positive result. This was the case of the information taken from the patients' clinical history, which includes previous hypothyroid conditions, thyroiditis post-gestation, gestational diabetes, familiar history of hypothyroidism and abortion.

Secondly, as mentioned before, some women were still expecting at the time of data collection. Therefore, some of them could have suffered thyroiditis post-gestation or an abortion that could not be considered in this study.

On another note, thyroid autoantibodies and iodine deficiency are the main causes of hypothyroidism (Mandel, 2004), but in this study only the first one was examined.

Finally, checking for thyroid disorders in pregnancy is important in part because of the negative effects that can cause in the fetus. For this reason, monitoring the infant's health after delivery could have been interesting. However, as currently all pregnant women with hypothyroidism are treated, no impact should be observed in the offspring (Martínez et al., 2018).

8. CONCLUSIONS

This study confirms that hypothyroidism is common during pregnancy, especially compared to hyperthyroidism. Around of 28% of pregnant patients presented abnormal thyroid function during the first trimester of gestation. It also showed that autoimmune disease is usually one of the main reasons behind these abnormal values. Sant Pau I Santa Tecla patients were aTPO positive in 20.7% of the cases with abnormal thyroid function. On another hand, no link between this thyroid disfunction and the age of the patients was found. Furthermore, this study also shows the importance of factors such as thyroiditis post-gestation, gestational diabetes, familiar history of hypothyroidism, and abortion. Each one of these factors were present in 14-11% of pregnant women with autoimmune disease. In the case of previous hypothyroid conditions, almost half of patients with autoimmune hypothyroidism presented history prior to gestation. Therefore, is also noticeable that a great number of pregnant women did not develop the hypothyroidism from pregnancy itself.

Lastly, the study also reveals the importance of future research on the field. Despite the progress made in the treatment of thyroid function deficiency disorders, the entire role of THs in fetal brain formation remains unknown. A better understanding of thyroid function in child development would be useful to improve treatment and secure the successful progress of pregnancy. Besides, further studies should also focus on defining which subgroups of pregnant women require or will benefit from treatment.

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