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University of Babylon
College of Medicine

Study the Thyroid and Parathyroid Hormonal Changes in Pregnant in Comparison with Non-Pregnant Women in Hilla City

A Thesis

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By

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يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ

وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ

وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴿١١﴾

صدق الله العلي العظيم

سورة المجادلة، أية (١١)

Dedication

To…

My father …

My mother …

My brothers…

My wife …

All My Friends and Teachers…

I dedicate this work

Husam

Acknowledgements

In the name of Allah, the most merciful, most compassionate, the peace and the mercy upon our Messenger Prophet Mohammad and his sanctified household. All thanks to Allah for granting me with will, strength, and patience with which this research had been accomplished.

My thanks to all patients for helping me obtain the samples and information from them. It is a pleasure to express my deep appreciation to the deanery of the College of Medicine, the headship of the Department of Biochemistry, and my supervisor Professor **Dr. Abdulsamie Hassan Alta'ee, and Asst. Prof. Dr Ban Amir Musa,** for their valuable guidance, assistance, cooperation, and motivation throughout preparing my thesis and during the progress of the present work.

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Last but not the least, I am indeed grateful to my friends, my relatives for their support and encouragement and my family for their understanding and their great help in accomplishing my study.

Husam

Summary

Background: Thyroid diseases are common during pregnancy and adequate treatment is important to prevent adverse maternal and fetal outcomes. Physiological changes in pregnancy may result in significant alterations in thyroid hormone, serum and urine electrolytes. Pregnancy specific pathophysiological processes may also affect the results of thyroid tests. Investigation of thyroid disorders in pregnancy requires knowledge of these changes and awareness of the safety of dynamic hormone testing and imaging for the mother and fetus.

Aims of the study: The current study aimed to evaluate the effect of the hormonal changes in thyroid parathyroid hormones during pregnancy and describe the best way to diagnose thyroid and parathyroid dysfunction, investigate the correlation among thyroid and parathyroid hormones in compression to healthy non-pregnant women.

Materials and Methods: A case-control study involved 40 pregnant with range aged (18-40 years). As well as 50 non pregnant women there range of age were(18-40year). The subjects were enrolled in this study between November 2022 and March 2023 at Babylon Hospital for Maternil and Children Teaching and General Shomali Hospital and all laboratory tests performed in the Clinical Chemistry and Biochemistry dept. College of Medicine, University of Babylon. All Subjects signed written informed consent forms. Body mass index was calculated as body weight (kg) divided by squared height in meters. The general data were age, and gestational age.

Results: The results show significant (p=0.0001) decreased levels of concentration of triiodothyronine (ng/mL) and thyroxine (nmol/mL) As the results show significant (p=0.0001) increased levels of concentration of thyroid-stimulating hormone (ulU/mL), parathyroid hormone (pg/mL)

and calcitonin (ng/mL) in pregnant as compared with the non-pregnant group The correlations among biochemical characteristics in the patient's group were examined There was no significant correlation among all characteristics with exception of a positive significant correlation between T3 and TSH ($r = 0.1743$; $p = 0.2259$).

Conclusion: We conclude that pregnant women experience significant hormonal changes in each of the thyroid and parathyroid hormones, which indicates the necessity of diagnosing these changes for the success of this pregnancy.

List of Contents

List of Figures

List of Tables

List of Abbreviations

Abbreviation	Key
BMI	Body mass index
CAL	Calcitonin
cAMP	Cyclic adenosine monophosphate
DIT	Diiodotyrosine
ELISA	Enzyme-linked immunosorbent assay
GD	Graves disease
HRP	Horseradish peroxidase
HT	Hashimoto thyroiditis
IUGR	Intrauterine growth restriction
MIT	Monoiodotyrosine
OD	Optical density
PTH	Parathyroid hormone
PTHrP	Parathyroid hormone -related protein
T3	Triiodothyronine
T4	Thyroxin
TBG	Thyroxine binding protein
TFTs	Thyroid Function Tests
TG	Thyroglobulin
TPO	Thyroid peroxidase
TSH	Thyroid-stimulating hormone
TSHR	Thyroid-stimulating hormone receptor

Chapter One Introduction

1 Introduction

1.1 General Introduction

Dynamic physiological processes during gestation significantly influence the maternal biochemistry that supports both the mother and fetus. Resultant changes in blood biochemistry alter the expected values of common laboratory tests(1). The thyroid gland is crucial to the regulation of metabolism, development and growth, acting via the thyroid hormones triiodothyronine (T3) and thyroxin (T4)(2). The characteristic molecular system absorbs, concentrates, oxidizes and then incorporates iodine into thyroglobulin (TG) in thyroid follicles. Tg is the protein backbone for the synthesis of thyroid hormones, which are produced by thyrocytes and secreted into the thyroid colloid. Thyroid peroxidase, also called thyroid peroxidase (TPO), contains a heme group in its ectodomain and requires iron for its activity(2).TPO catalyzes iodine oxidation, iodination of Tg, and extracellular production of monoiodotyrosine (MIT) and diiodotyrosine (DIT) near the apical membrane of thyrocytes. The combination of MIT and DIT forms T3, while T4 consists of two coupled DITs(3).

Synthesis of thyroid hormones is regulated by pituitary thyroid-stimulating hormone (TSH), which binds to its thyroid-stimulating hormone receptor (TSHR) on the surface of thyroid follicular cells All the above-mentioned thyroid proteins (Tg, TPO, TSHR) and thyroid-related proteins (TSH) are N-glycosylated; their sugar components are responsible for the proper functioning of glycoproteins(4).

Thyroid autoimmunity is more common in women than in men and affects 5–20% of the female population of childbearing age The spectrum of thyroid autoimmune disorders includes clinically heterogeneous conditions, such as Hashimoto thyroiditis (HT) characterized by primarily Tcell-mediated autoimmunity, and Graves' disease (GD) sustained by

predominately humoral autoimmunity During gestation(5), the clinical course of both HT and GD is significantly influenced either by changes occurring in maternal thyroid economy or by the condition of lowered immune responsiveness taking place throughout pregnancy(6). Even in the absence of overt maternal thyroid dysfunction(5), thyroid autoimmune diseases have been reported to be associated with an increased risk of adverse pregnancy outcomes, ranging from pregnancy loss and premature delivery to intrauterine growth restriction (IUGR) and impaired fetal neurodevelopment, among others. In addition, a large body of evidence has been provided, showing significant impacts of thyroid autoimmunity on female fecundity and infertility treatment success(7). Largely incomplete, several factors are more likely involved. These may include, either alone or in combination, general immune diathesis in the woman, possibly affecting the female reproductive system, subtle thyroid dysfunctions, predisposing genetic backgrounds, along with as yet unidentified factors(8).

1.2 Thyroid Gland

The thyroid gland (Tg) is located in front of the neck below the thyroid cartilage. The small, two-or three-in. long gland consists of two lobes connected by isthmus in the middle(9). Just beneath the skin of the neck, there is a thin platysma muscle, which covers the whole anterior neck. When the platysma muscle is dissected, the strap muscles are exposed. The strap muscles consist of the sternohyoid muscles and sternothyroid muscles. The midline is shown between right and left strap muscles. Dissection along the midline is needed to approach the thyroid gland. To perform thyroidectomy, the strap muscles should be retracted to lateral position or can be transected for better exposure in case of large goiter(10,11).

Beneath the thyroid gland, there is a trachea. The trachea is the most important landmark in performing thyroidectomy. The thyroid gland is firmly attached to the trachea from the second to the fourth tracheal ring. In normal adults, the average weight of the thyroid gland is 15–30 g(9,12). The pyramidal lobe can be seen in 20 % of the patients, which ascends from the isthmus or the adjacent part of either lobe up to the hyoid bone(9).

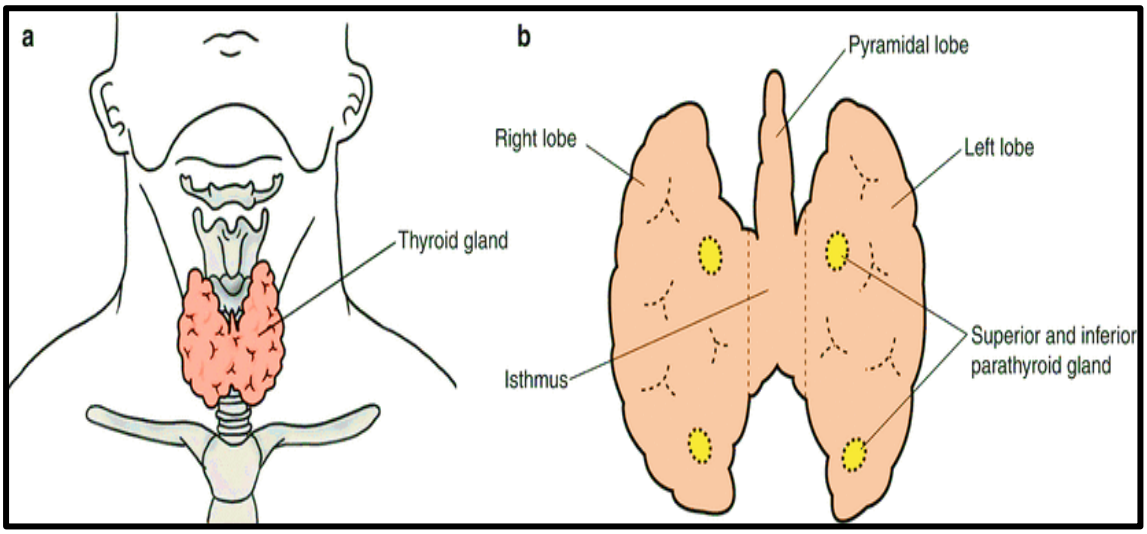

Figure 1-1: Position and anatomy of the thyroid gland. (a) Normal position of the thyroid gland, (b) anatomy of the thyroid gland (9).

The relation between the tubercle of Zuckerkandl and the distal course of the recurrent laryngeal nerve (RLN). Tubercle of Zuckerkandl is the most posterior extent of the thyroid lobe (13) as shown in Figure 1-2.

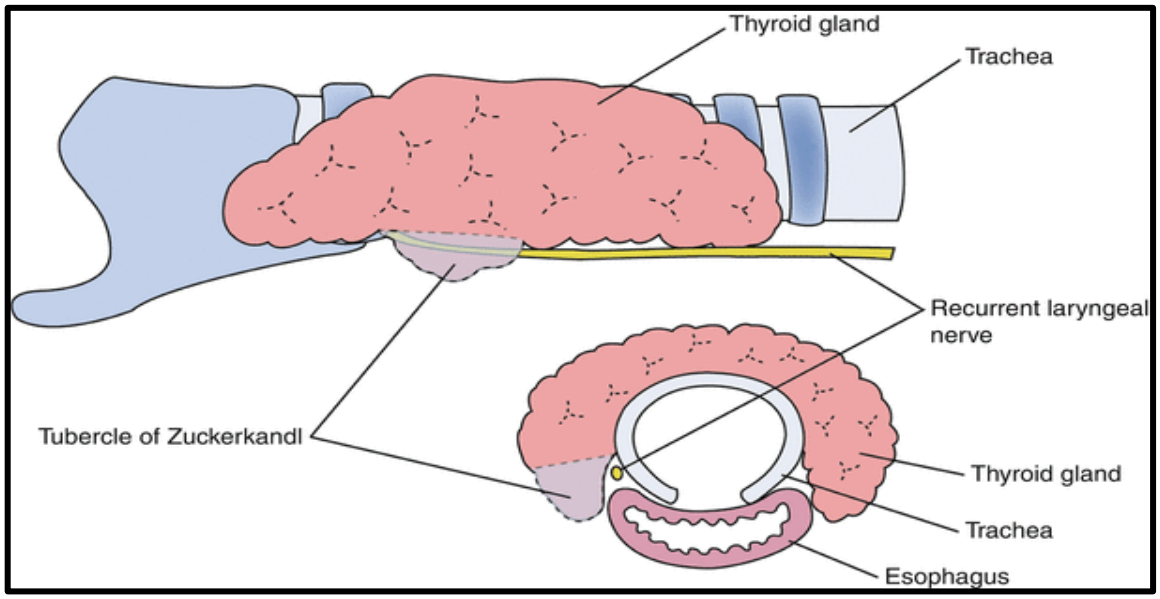

Figure 1-2: The region of the Tubercle of Zuckerkandl(13)

The thyroid is located at the front of the neck, below the Adam's apple. The thyroid gland secretes three hormones: the two thyroid hormones triiodothyronine (T3) and thyroxine (T4) and a peptide hormone, calcitonin(14). The thyroid hormones influence the metabolic rate and protein synthesis, and in children(15), growth and development. Secretion of the two thyroid hormones is regulated by thyroid-stimulating hormone (TSH), which is secreted from the anterior pituitary gland(16).

1.2.1 Vascular Anatomy of the Thyroid Gland

There are two main arterial blood supplies of the thyroid gland, the superior and inferior thyroid arteries and, to a lesser degree, the thyroid ima artery. The superior thyroid artery is the first branch of the external carotid artery and courses inferiorly to the upper pole of the thyroid gland. It enters the upper pole of the thyroid on its anterosuperior surface(17,18). The

inferior thyroid artery usually arises from the thyrocervical trunk and passes upward in front of the vertebral artery and Longus colli to the lower pole of the thyroid gland. Before entering the thyroid, the artery usually divides into 2–3 branches. One of the branches supplies the inferior parathyroid(19,20).

The thyroid ima artery can be seen in 3–10 % or less of patients. The thyroid ima ascends in front of the trachea to the lower part of the thyroid gland, which it supplies. It varies greatly in size and appears to compensate for deficiency or absence of one of the other thyroid vessels(21,22). The thyroid artery usually arises from the brachiocephalic trunk (innominate artery). It occasionally arises from the aorta, the right common carotid, the subclavian, or the internal thoracic artery (21).

Venous drainage of the thyroid gland is through a well-developed thyroid venous plexus, which usually drains through the inferior thyroid vein to the left brachiocephalic (innominate) vein. Blood from the thyroid gland also drains to the internal jugular vein via the superior and middle thyroid veins(23).

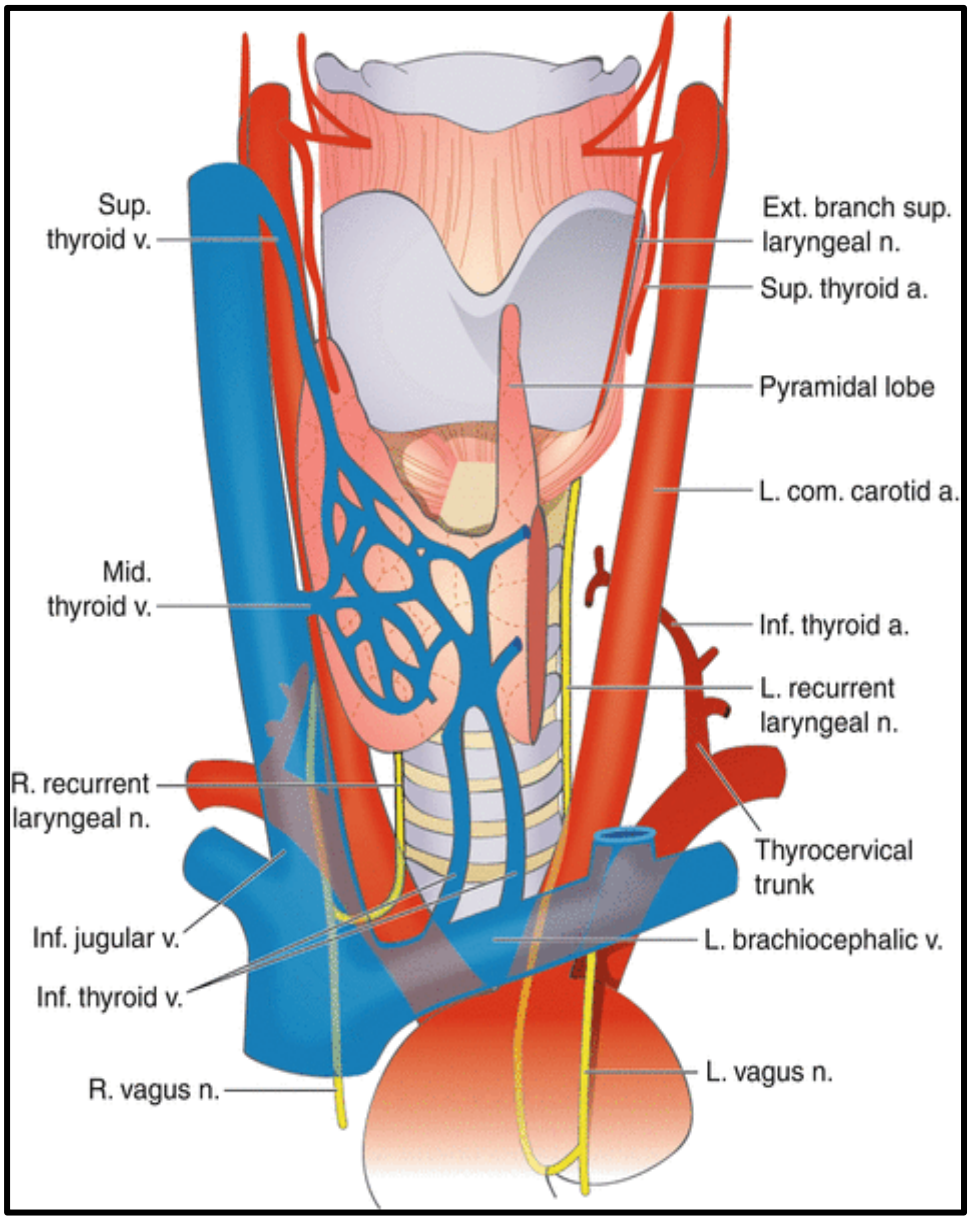

Figure 1-3: Vascular anatomy of the thyroid gland(23)**.**

1.2.2 Functions of Thyroid Gland

The thyroid hormones have a wide range of effects on the human body. These include:

1. **Metabolic Functions:** The thyroid hormones increase the basal metabolic rate and have effects on almost all body tissues. Appetite, absorption of substances, and gut motility are all influenced by the thyroid hormone (24).TH increase the absorption in the gut, generation, uptake by

cells, and breakdown of glucose, stimulate the breakdown of fats and increase the number of free fatty acid(25).

2. **Gut:** They increase the absorption in the gut, generation, uptake by cells, and breakdown of glucose, stimulate the breakdown of fats, and increase the number of free fatty acids(26). Despite increasing free fatty acids, thyroid hormones decrease cholesterol levels, perhaps by increasing the rate of secretion of cholesterol in bile (27).

3. **Developmental functions:** Thyroid hormones are important for normal development and increase the growth rate of young people. Cells of the developing brain are a major target for the thyroid hormones T3 and T4(28). Thyroid hormones play a particularly crucial role in brain maturation during fetal development and the first few years of postnatal life.TH cause increased development of type II muscle fibres. These are fast-twitch muscle fibres capable of fast and powerful contractions(29).

4. **Lungs:** Thyroid hormones stimulate the respiratory centres and lead to increased oxygenation because of increased perfusion(30).

5. **Heart:** Interpretation of Thyroid Function Tests (TFTs) In most cases, the results of TFTs are straightforward and present a familiar pattern that is easy to recognize(31). but can seem confusing, either being discordant with the clinical picture or different assay results appearing to contradict each other (30).

6. **CNS:** They influence neurogenesis, neuronal and glial cell differentiation and migration, synaptogenesis, and myelination. Neural cells express the thyroid hormone nuclear receptors THRA and THRB, which mediate most actions of T3, the active hormone(32). Brain T3 derives in part from the circulation, and part from type-2 deiodinase-mediated 5'-deiodination of T4 in glial cells (33).

1.2.3 Regulation of Thyroid Gland

There have to be some mechanisms that regulate very carefully the amount of T4 and T3 secreted by the thyroid gland so that the right - the normal - amounts are manufactured and delivered into the bloodstream. In the case of the thyroid, the 'thermostat' consists of a little gland, called the pituitary gland that lies underneath brain in skull(34).

The pituitary senses the level of thyroid hormones in bloodstream, just as the thermostat in living room senses the temperature. Under normal circumstances, if the level drops just a little below normal, the pituitary reacts by secreting a hormone called the thyroid-stimulating hormone, also known as TSH, and this hormone activates the thyroid gland to put out more T4 and T3 (35).Conversely, when the thyroid hormone levels rise above normal the 'thermostat' senses this and the pituitary stops secreting TSH so that the thyroid gland stops working so hard and the secretion of T4 and T3 is reduced(36).

1.3 Thyroid Hormones

Thyroid hormone (TH) is the hormone that's mainly responsible for controlling the speed of the body's metabolism(37). In infants, thyroid hormone is critical for brain development. Thyroid hormone s the combination of the two main hormones that the thyroid gland releases: thyroxine (T4) and triiodothyronine (T3). They're often collectively referred to as "thyroid hormone" because T4 is largely inactive, meaning it doesn't impact cells, whereas T3 is active. Once the thyroid releases T4, certain organs in the human body transform it into T3 so that it can impact cells and metabolism(38).

The thyroid also releases a hormone called calcitonin to help regulate calcium levels in the blood by decreasing it. Calcitonin

isn't grouped into the "thyroid hormone" name, and it doesn't impact body's metabolism like T3 and T4 do(39). The TH is well known for controlling metabolism, growth, and many other bodily functions. The thyroid gland, anterior pituitary gland, and hypothalamus comprise a self-regulatory circuit called the hypothalamic-pituitary-thyroid axis. The main hormones produced by the thyroid gland are thyroxine or tetraiodothyronine (T4) and triiodothyronine (T3) (40).

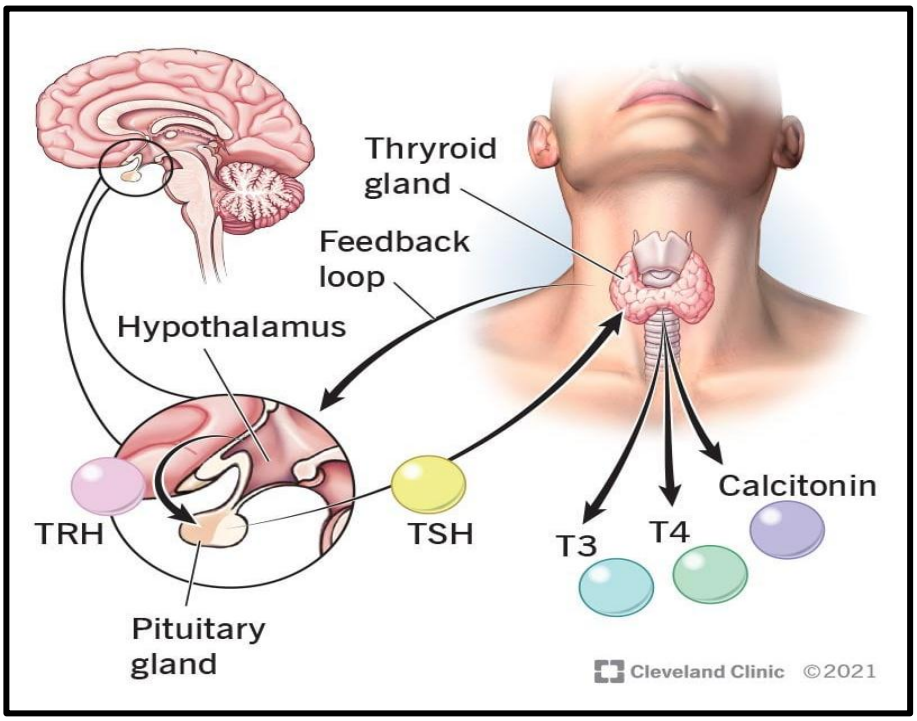

Figure 1-4: Thyroid Hormone. The body controls thyroid hormone (T3 and T4) levels through a complex feedback loop. hypothalamus releases thyrotropin-releasing hormone (TRH), which triggers pituitary gland to release thyroid-stimulating hormone (TSH), which stimulates thyroid to release T3 and T4(40).

Thyrotropin-releasing hormone (TRH) from the hypothalamus, thyroid-stimulating hormone (TSH) from the anterior pituitary gland, and T4 work in synchronous harmony to maintain proper feedback mechanisms and homeostasis(40). Hypothyroidism, caused by an underactive thyroid gland, typically manifests as bradycardia, cold intolerance, constipation, fatigue, and weight gain. In contrast, hyperthyroidism caused by increased

thyroid gland function manifests as weight loss, heat intolerance, diarrhea, fine tremor, and muscle weakness(41).

Iodine is an essential trace element absorbed in the small intestine. It is an integral part of T3 and T4. Sources of iodine include iodized table salt, seafood, seaweed, and vegetables. Decreased iodine intake can cause iodine deficiency and decreased thyroid hormone synthesis. Iodine deficiency can cause cretinism, goitre, myxedema coma, and hypothyroidism(40).

1.3.1 Function of Thyroid Hormone

The thyroid gland plays a critical role in mammalian neurodevelopment, just as generally in reproduction and development. During neurodevelopment, the brain levels of the thyroid hormones thyroxine (T4) and triiodothyronine (T3) are controlled both spatially and temporally to support normal patterns of neuronal migration, neuronal differentiation and glial myelination (42).

If serum TH levels are altered beyond homeostasis (i.e. if they are either too low or too high), the effect on the structure and function of the brain depends on the magnitude, duration and timing of the thyroid perturbation(43). The foetus is fully dependent on maternal thyroid hormone until its thyroid gland starts producing thyroid hormone, which takes place around the gestational day (GD) 17 in rats. Thyroid perturbations can result in a variety of neurodevelopmental alterations. The stage of development when the foetus or newborn is exposed to low or high serum thyroid hormone levels is critical for the onset of the type of neurodevelopmental alteration(44).

The Thyroid physiology changes significantly during gestation. Advances in the assessment of thyroid function also have indicated that the interpretation of thyroid function tests depends on the stage of

pregnancy(45). Thyroid disorders are prevalent in women of childbearing age and for this reason commonly present in pregnancy and the puerperium Uncorrected thyroid dysfunction in pregnancy has adverse effects on foetal and maternal well-being. The deleterious effects of thyroid dysfunction also extend beyond pregnancy and delivery to affect neuro intellectual development in the early life of the child(46,47).

1.3.2 Synthesis of Thyroid Hormones

Synthesis of Thyroglobulin: Thyrocytes in the thyroid follicles produce a protein called thyroglobulin (TG). TG does not contain any iodine, and it is a precursor protein stored in the lumen of follicles. It is produced in the rough endoplasmic reticulum. Golgi apparatus pack it into the vesicles, and then it enters the follicular lumen through exocytosis(48).

Iodide uptake: Protein kinase A phosphorylation causes increased activity of basolateral Na^+-I- symporters, driven by Na^+-K^+-ATPase, to bring iodide from the circulation into the thyrocytes. Iodide then diffuses from the basolateral side to the apex of the cell, where it is transported into the colloid through the pendrin transporter.

Iodination of thyroglobulin: Protein kinase A also phosphorylates and activates the enzyme thyroid peroxidase (TPO). TPO has three functions: oxidation, organification, and coupling reaction (49).

Oxidation: TPO uses hydrogen peroxide to oxidize iodide (I-) to iodine (I2). NADPH-oxidase, an apical enzyme, generates hydrogen peroxide for TPO.

Organification: TPO links tyrosine residues of thyroglobulin protein with I2. It generates monoiodotyrosine (MIT) and diiodotyrosine (DIT). MIT has a single tyrosine residue with iodine, and DIT has two tyrosine residues with iodine.

Coupling reaction: TPO combines iodinated tyrosine residues to make triiodothyronine (T3) and tetraiodothyronine (T4). MIT and DIT join to form T3, and two DIT molecules form T4.

Storage: thyroid hormones are bound to thyroglobulin for stored in the follicular lumen (50).

Release: thyroid hormones are released into the fenestrated capillary network by thyrocytes in the following steps:

1. Thyrocytes uptake iodinated thyroglobulin via endocytosis
2. Lysosome fuse with the endosome containing iodinated thyroglobulin
3. Proteolytic enzymes in the endolysosome cleave thyroglobulin into MIT, DIT, T3, and T4.
4. T3 (20%) and T4 (80%) are released into the fenestrated capillaries via the MCT8 transporter
5. Deiodinase enzymes remove iodine molecules from DIT and MIT. Iodine can be salvaged and redistributed to an intracellular iodide pool(51).

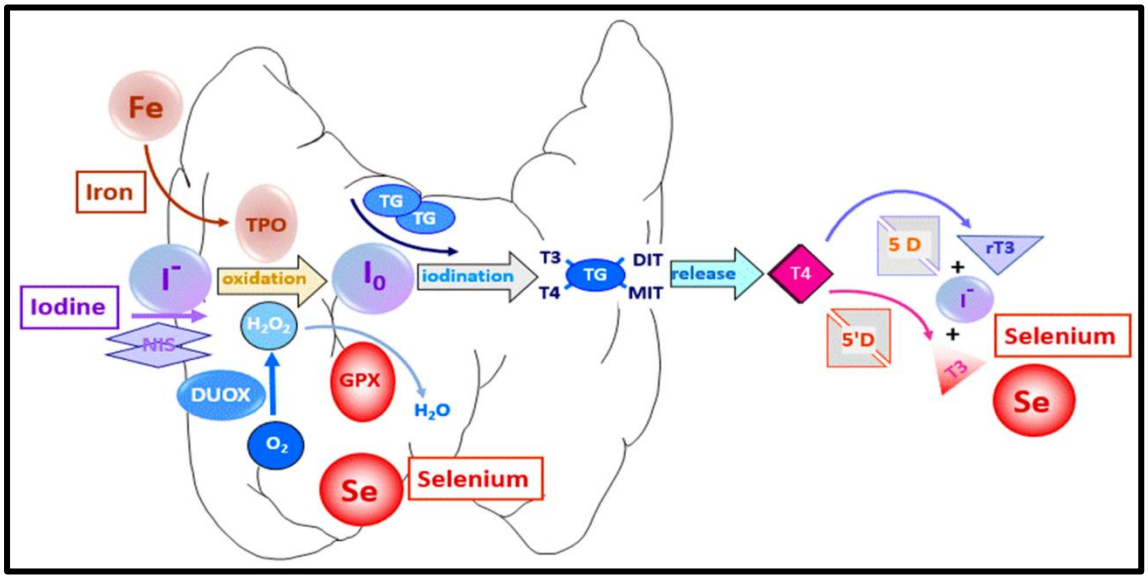

Figure 1-5: Essential role of the three trace elements iodine, selenium and iron in thyroid hormone biosynthesis and activation. Abbreviations: NIS: Na+-Iodide-Symporter (iodide uptake); DUOX: dual oxidase (H_2O_2 generation); TPO: Thyroid peroxidase (hemoprotein); TG: thyroglobulin (synthesis and storage protein); GPX: glutathione peroxidase (antioxidant defense); DIO: deiodinase (TH in-/activation) (52).

1.3.3 Triiodothyronine

Triiodothyronine (T3) is a thyroid hormone that plays vital roles in the body's metabolic rate, heart and digestive functions, muscle control, brain development and function, and the maintenance of bones(53). Triiodothyronine is the active form of the thyroid hormone, thyroxine. Approximately 20% of triiodothyronine is secreted into the bloodstream directly by the thyroid gland. The remaining 80% is produced from the conversion of thyroxine by organs such as the liver and kidneys. Thyroid hormones play vital roles in regulating the body's metabolic rate, heart and digestive functions, muscle control, brain development and function, and the maintenance of bones(16,54).

The production and release of thyroid hormones, thyroxine and triiodothyronine, is controlled by a feedback loop involving the hypothalamus, pituitary gland and thyroid gland. Activation of thyroid hormones is then controlled in body tissues such as the liver, brain and kidneys by enzymes called deiodinases which convert thyroxine into the active form triiodothyronine. Most of the body's circulating triiodothyronine (about 80%) is produced in this way(4).

The thyroid hormone production system is regulated by a feedback loop so that when the levels of the thyroid hormones thyroxine and triiodothyronine increase, they prevent the release of both thyrotropin-releasing hormone from the hypothalamus and thyroid stimulating hormone from the pituitary gland. This system allows the body to maintain a constant level of thyroid hormones in the body(40,55).

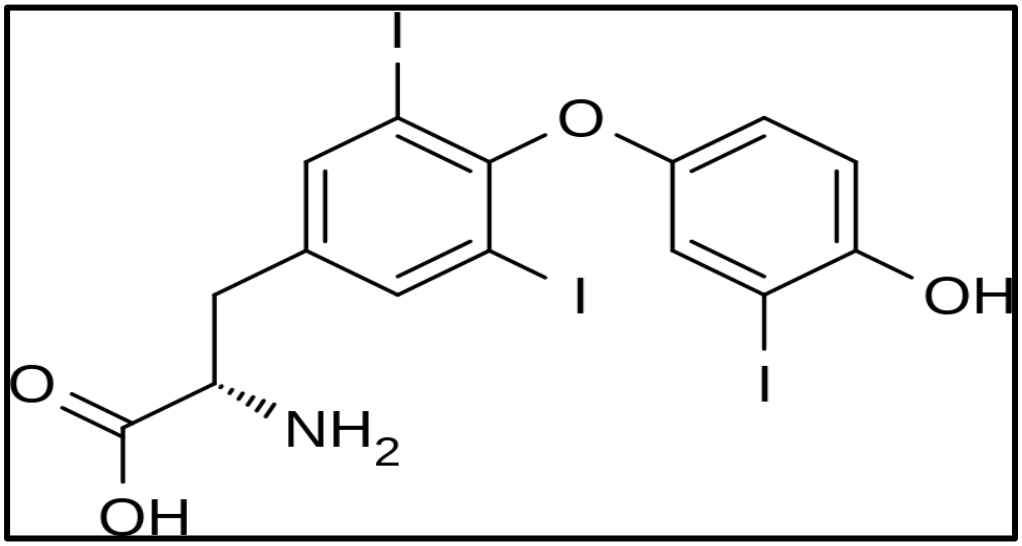

Figure 1-6: Structure of Triiodothyronine (T3)(55)

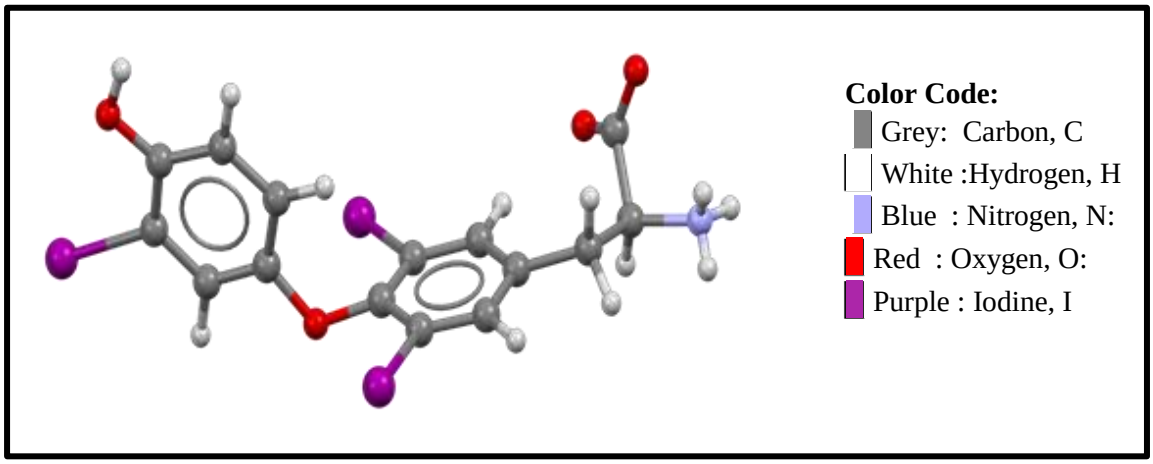

Figure 1-7: Ball-and-stick model of a triiodothyronine (T_3) molecule, $C_{15}H_{12}I_3NO_4$, based on the crystal structure of the thyroxine (T_4) *N*-diethanolamine salt, reported in *Acta Crys*(56)*t. B* (1981) **37**, 1685-1689 (CSD entry TYRXEA10). Phenol (ArOH) and phenyl (ArH that is ArI in T_4) hydrogen atom locations have been inferred(55).

1.3.4 Thyroxine

Thyroxine (T4) is the main hormone secreted into the bloodstream by the thyroid gland. It plays vital roles in digestion, heart and muscle function, brain development and maintenance of bones(16,57). Thyroxine is the main hormone secreted into the bloodstream by the thyroid gland. It is the inactive form and most of it is converted to an active form called triiodothyronine by organs such as the liver and kidneys. Thyroid

hormones play vital roles in regulating the body's metabolic rate, heart and digestive functions, muscle control, brain development and maintenance of bones(58,59).

The production and release of thyroid hormones, thyroxine and triiodothyronine, is controlled by a feedback loop system that involves the hypothalamus in the brain and the pituitary and thyroid glands. The hypothalamus secretes thyrotropin-releasing hormone which, in turn, stimulates the pituitary gland to produce thyroid stimulating hormone. This hormone stimulates the production of the thyroid hormones, thyroxine and triiodothyronine, by the thyroid gland(60).

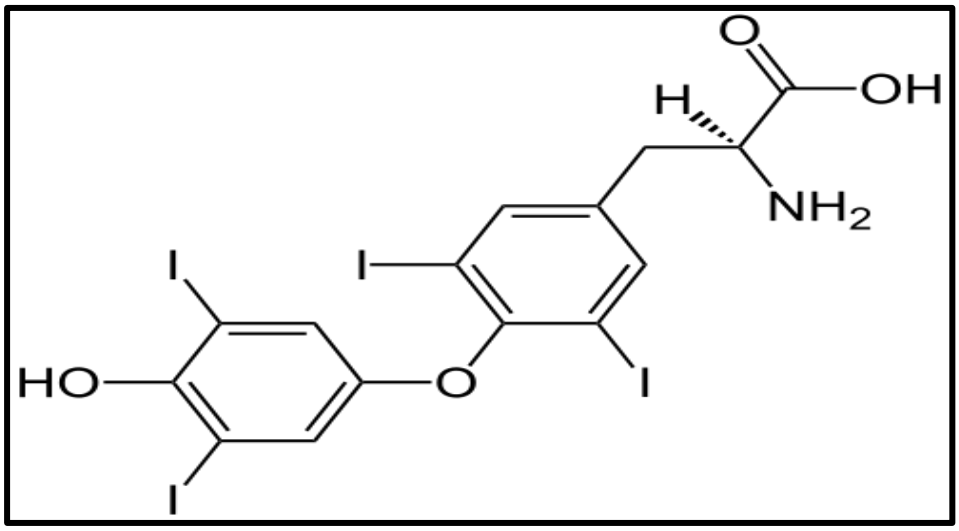

Figure 1-8: Structure of Thyroxine (T4) (60)

T4 is synthesized in the thyroid follicles, the functional units of the mature thyroid gland (Figure 1-9). The spherical follicles are covered by a monolayer of polarized epithelial cells, with the basolateral and apical surfaces facing the bloodstream and follicular lumen, respectively. The follicular lumen is filled with a colloid, mainly comprising highly cross-linked protein thyroglobulin (TG) in a concentration range of around 100–750 $mgmL^{-1}$(61).

The biosynthesis of T4 takes place on Tg in five major steps:

A. transfer of inorganic CTiodide (I) from the blood to thyroid follicles through the sodium iodide symporter (NIS)

B. generation of hydrogen peroxide (H_2O_2) by peroxidases (DUOX1 and DUOX2)

C. iodination of tyrosyl residues of Tg by thyroid peroxidase (TPO) in the presence of H_2O_2 and iodide

D. phenolic coupling of the iodotyrosyl residues on Tg by TPO to form T4

E. proteolytic liberation of THs from Tg (Figure 1-9)(62). In this process, a fraction of biologically active T3 is also produced.

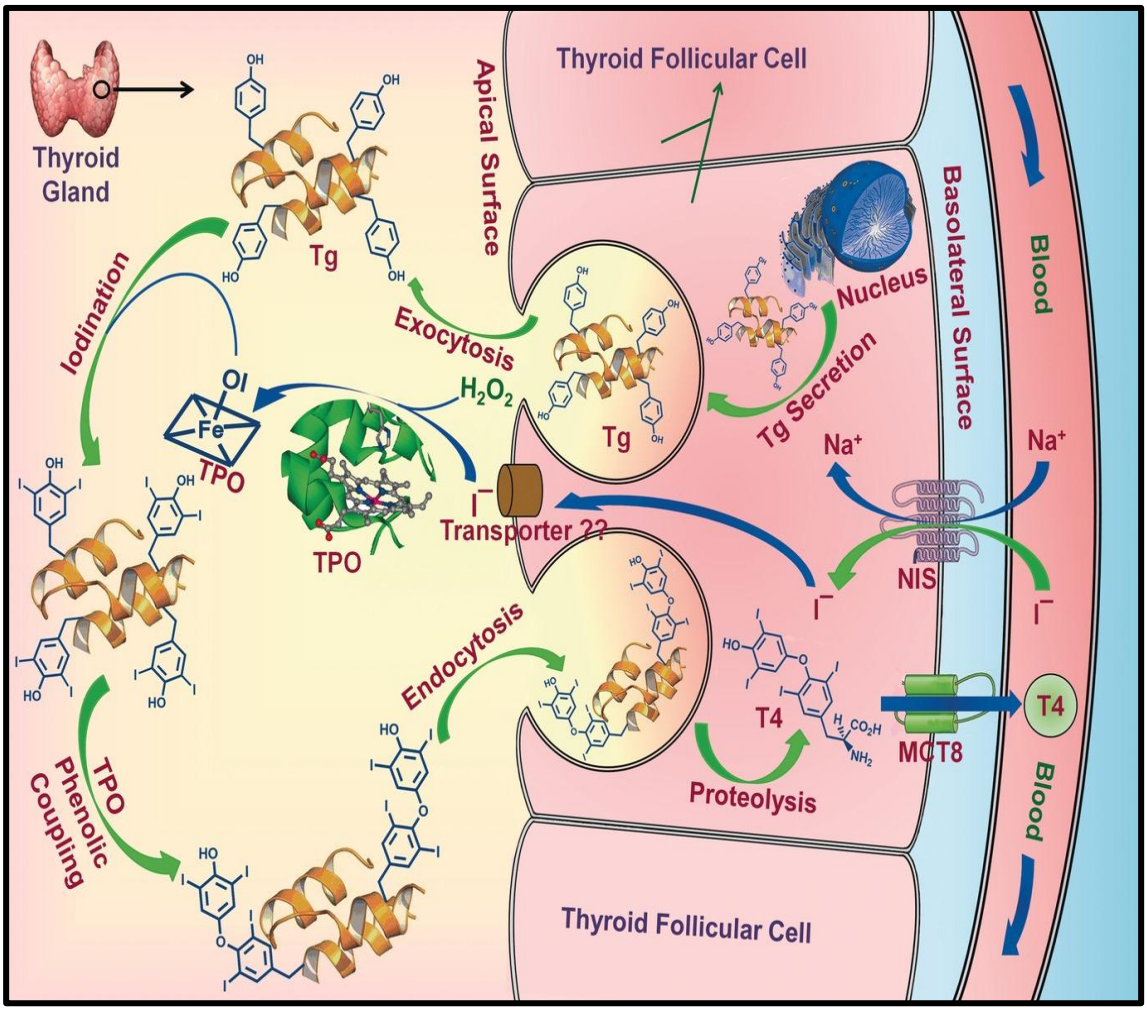

Figure 1-9: Biosynthesis of thyroxine (T4) in the thyroid follicular cells(62). A small amount of T3 is also produced in this process. Although the protein responsible for the transport of iodide through the apical membrane of the thyrocytes has been a subject of debate over the years, pendrin and anoctamin 1 are proposed to be the most probable iodide transporters(62).

1.3.5 Thyroid Stimulating Hormone

Thyroid Stimulating Hormone (TSH) is produced by the pituitary gland. Its role is to regulate (by stimulating) the production of thyroid hormones by the thyroid gland(63). Thyroid Stimulating Hormone (TSH) is produced and released into the bloodstream by the pituitary gland. It stimulates the production of the thyroid hormones, thyroxine (T4) and triiodothyronine (T3), by the thyroid gland by binding to its receptors in the thyroid gland. Thyroxine (T4) and triiodothyronine (T3) are essential for maintaining the body's metabolic rate, heart and digestive functions, muscle control, brain development and bone activity(64,65).

A gland in the brain called the hypothalamus produces a hormone called thyrotrophin-releasing hormone (TRH). Thyrotrophin is another name for Thyroid Stimulating Hormone (TSH), which is so called as 'there' refers to the thyroid gland and 'trophy' means to nourish, stimulate or grow, thus referring to TSH's action to stimulate the thyroid gland(66). Thus, the hypothalamus produces TRH which stimulates the pituitary gland to produce TSH, which in turn stimulates the thyroid gland to produce the thyroid hormones: thyroxine (T4) and triiodothyronine (T3). Thyroxine (T4) is the more abundant thyroid hormone in the bloodstream but has a weaker action than triiodothyronine (T3) T3 is the more active form and can be formed from T4 in local tissues by enzymes called 'deiodinases', enabling local tissues to regulate the local activity of thyroid hormones(67).

The hypothalamus and pituitary gland sense the amount of thyroid hormone in the body, and in response, regulate the amount of TRH released from the hypothalamus and TSH from the pituitary gland, to maintain the appropriate amount of thyroid hormones(68). For example, if too much thyroid hormone is produced, the hypothalamus will respond by making less TRH and the pituitary gland will make less TSH in order to

not stimulate the thyroid gland as much, and help bring thyroid hormone levels back down to normal. TRH is not easily measured with blood tests, whereas TSH is and can therefore indicate whether the hypothalamus and pituitary gland believe that there is the right amount of thyroid hormones in the body(69).

Conversely, if there is a problem with the thyroid gland causing it to make too little thyroid hormone, then the pituitary gland will increase the amount of TSH it produces in order to stimulate the thyroid gland to increase thyroid hormone production(64). This is called a 'negative feedback loop', whereby the controlling gland senses the amount of hormone from the gland it controls, and regulates the secretion of the controlling (often stimulating) hormone (in this case TSH) to ensure that the thyroid gland makes the appropriate amount of the end hormone (T4 and T3 in this case)(70,71).

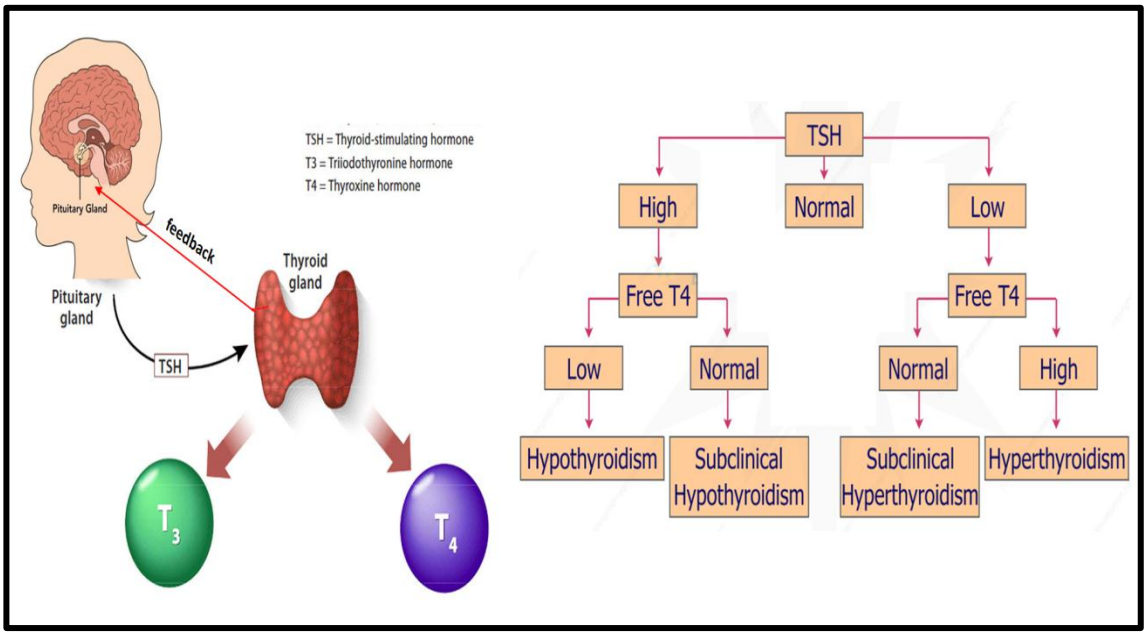

Figure 1-10: Secretion of Thyroid Stimulating Hormone(70)

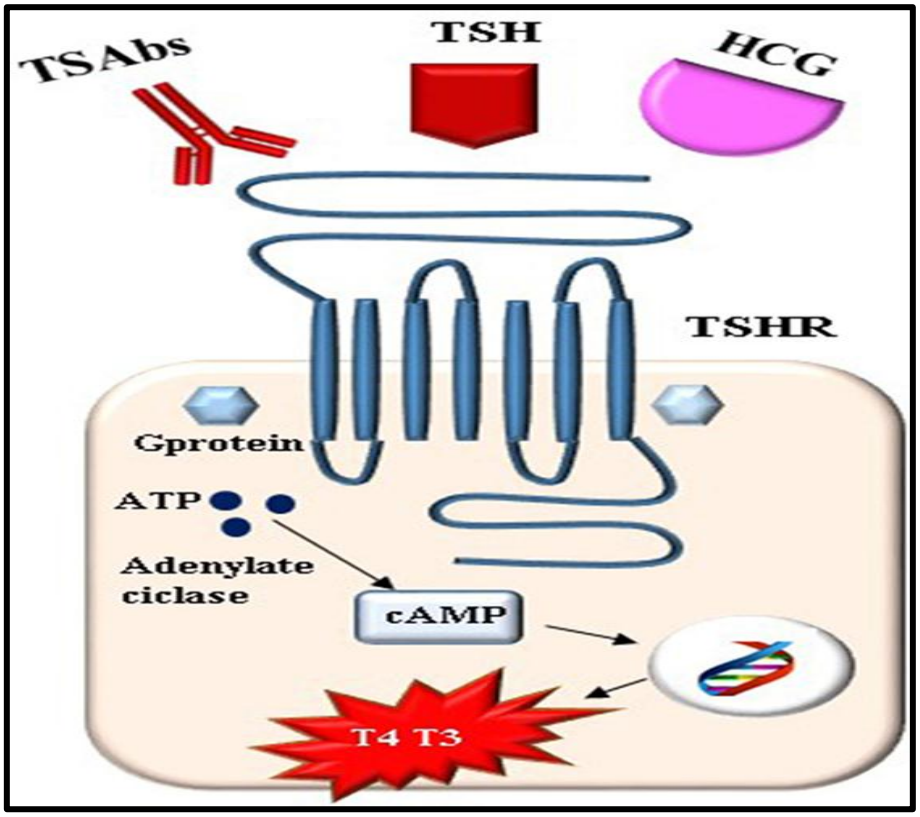

Figure 1-11: The stimulation of TSH receptor (TSHR) in pregnancy(72).

1.3.6 Calcitonin

Calcitonin is a 32 amino acid peptide hormone that is secreted by the thyroid's Para follicular cells, also referred to as C cells in humans and other chordate species(73). In contrast to the effects of parathyroid hormone (PTH), it works to lower blood calcium (Ca^{2+}). The hormone called calcitonin, which was discovered more than 50 years ago, rapidly lowers the level of calcium in the blood(74,75).

The amino acid sequences of different types of CAL have been determined for many species, and the basic structure of CT is characterized by an intramolecular disulfide bridge between the cysteine residues at positions 1 and 7 (Figure 1-12) and an amidated carboxy-terminal in which the specific amino acid residues are identical for all CTs (76–78).

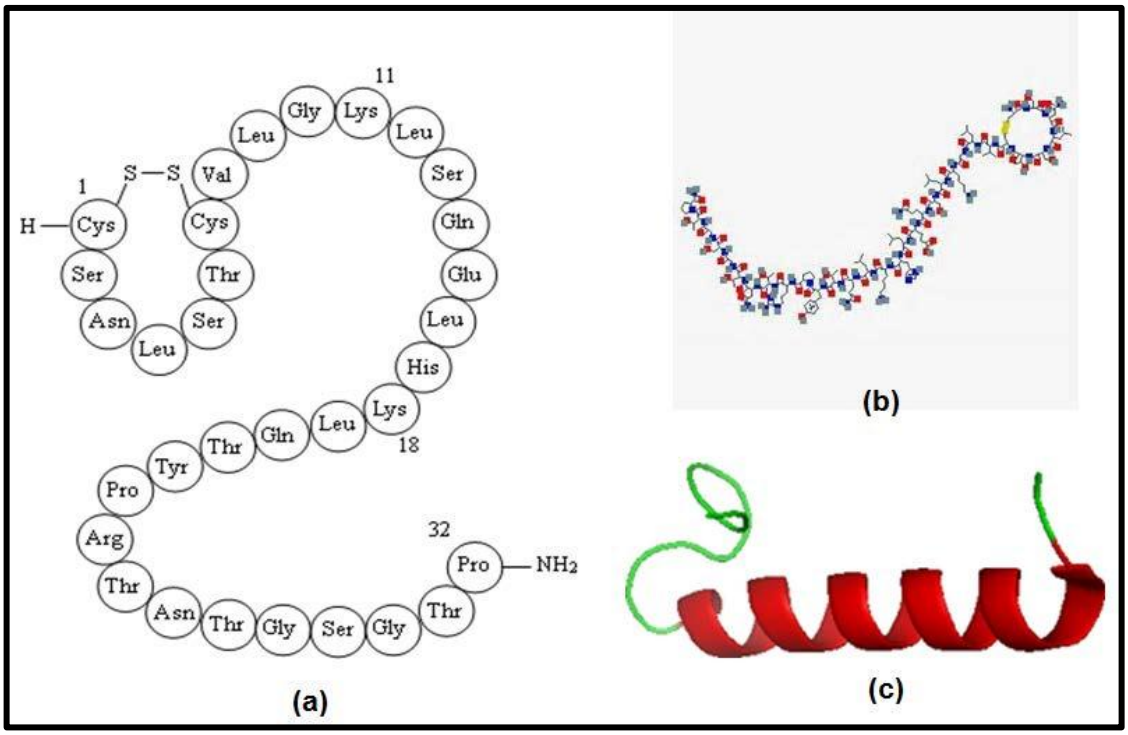

Figure 1-12: The primary (a, b) and secondary (c) structures of CAT(76)

A number of peptides that are structurally similar to calcitonin form the calcitonin family, which currently includes calcitonin gene–related peptides amylin, adrenomedullin, and intermedin. Apart from being structurally similar, the peptides signal through related receptors and have some overlapping biological activities, although other activities are peptide-specific (79).

Calcitonin is a peptide hormone produced in the thyroid gland by parafollicular cells (also called C-cells). It is produced from procalcitonin, a larger peptide, that is in turn controlled by the calcitonin gene (CAL). Calcitonin helps maintain calcium blood levels. If calcium levels are too high, then this hormone reduces them(80). This effect is caused by the inhibition of calcium efflux from bone, as calcitonin is a potent inhibitor of bone resorption. Calcitonin has been in clinical use for conditions of accelerated bone turnover, including Paget's disease and osteoporosis; although in recent years, with the development of drugs that are more potent inhibitors of bone resorption, its use has declined(80,81).

1.4 Hypothyroidism

Hypothyroidism is a clinical disorder commonly encountered by the primary care physician. Untreated hypothyroidism can contribute to hypertension, dyslipidemia, infertility, cognitive impairment, and neuromuscular dysfunction(82). Data derived from the National Health and Nutrition Examination Survey suggest that about one in 300 persons in the United States has hypothyroidism. The prevalence increases with age and is higher in females than in males(83).

Hypothyroidism may occur as a result of primary gland failure or insufficient thyroid gland stimulation by the hypothalamus or pituitary gland. Autoimmune thyroid disease is the most common aetiology of hypothyroidism in the United States(84). Clinical symptoms of hypothyroidism are nonspecific and may be subtle, especially in older persons. The best laboratory assessment of thyroid function is a serum thyroid-stimulating hormone test. No evidence screening asymptomatic adults improves outcomes. In the majority of patients, alleviation of symptoms can be accomplished through oral administration of synthetic levothyroxine, and most patients will require lifelong therapy(84,85).

Among patients with subclinical hypothyroidism, those at greater risk of progressing to clinical disease, and who may be considered for therapy, include patients with thyroid-stimulating hormone levels greater than 10 mIU/l and those who have elevated thyroid peroxidase antibody titres(86).

1.4.1 Causes of Hypothyroidism

Worldwide, too little iodine in the diet is the most common cause of hypothyroidism. Hypothyroidism is caused by inadequate function of the gland itself (primary hypothyroidism), inadequate stimulation by thyroid-stimulating hormone from the pituitary gland (secondary hypothyroidism)

or inadequate release of thyrotropin-releasing hormone from the brain's hypothalamus (tertiary hypothyroidism) (87).

In areas of the world with sufficient dietary iodine, hypothyroidism is most commonly caused by the autoimmune disease Hashimoto's thyroiditis (chronic autoimmune thyroiditis). It is characterized by infiltration of the thyroid gland with T lymphocytes and autoantibodies against specific thyroid antigens such as thyroid peroxidase, thyroglobulin and the TSH receptor(88). Autoimmune thyroiditis (Hashimoto's) is associated with other immune-mediated diseases such as diabetes mellitus type 1, pernicious anaemia, myasthenia gravis, celiac disease, rheumatoid arthritis and systemic lupus erythematosus. Iatrogenic hypothyroidism can be surgical (a result of thyroidectomy, usually for thyroid nodules or cancer) or following radioiodine ablation (usually for Graves' disease)(89).

1.5 Hyperthyroidism

When hyperthyroidism is suspected, the diagnosis should be confirmed by measurement of serum thyrotropin and total or free thyroxine, which are usually present in low and high concentrations (90). If the thyrotropin level is low but the thyroxine level is normal, serum triiodothyronine should be measured, since the patient may have triiodothyronine toxicosis(91,92). Serum total thyroxine concentrations are increased in patients with increased serum concentrations of thyroxine-binding globulin for example, pregnant women, persons taking estrogen, or persons who have an inherited increase in the production of thyroxine-binding globulin but serum concentrations of free thyroxine and thyrotropin are normal(89). The uncommon condition of familial dysalbuminemic hyperthyroxinemia, due to the presence of serum albumin with an abnormally high affinity for thyroxine, results in a spurious elevation of the free thyroxine concentration, as can thyroxine-binding

prealbumin (transthyretin) with abnormal affinity for thyroxine and the presence of thyroid hormone-binding autoantibodies. All patients with these latter conditions are clinically euthyroid and have normal serum concentrations of thyrotropin(93).

The presence of normal serum thyrotropin concentrations nearly always excludes a diagnosis of hyperthyroidism6; the exception is the rare patient with hyperthyroidism caused by excessive thyrotropin secretion(94). The converse, however, is not true, since serum thyrotropin concentrations may be low in patients with nonthyroidal illness, patients taking certain drugs (glucocorticoids or dopamine) and some healthy elderly patients (95).

1.5.1 Causes of Hyperthyroidism

Graves' disease is the most common cause of hyperthyroidism. The diagnosis is obvious if a diffuse goitre and ophthalmopathy are present. Among other causes, a multinodular goitre, toxic thyroid adenoma, and subacute thyroiditis should be evident from the history and physical examination. If the cause is not obvious, then measurement of the uptake of radioiodine by the thyroid may be indicated; low values identify patients with silent or postpartum thyroiditis or iodine-induced thyrotoxicosis (96).

1.6 Parathyroid Glands

The parathyroid gland comprises four individual pieces that are small and round, each about the size of a grain of rice. There are usually two on each side of the neck, sitting behind another gland called the thyroid gland(97,98). Parathyroid glands are derived from the epithelial lining of the third and fourth pharyngeal pouches, with the superior glands arising from the fourth pouch and the inferior glands arising from the higher third pouch. The relative position of the inferior and superior glands, which are

named according to their final location, changes because of the migration of embryological tissues(99,100).

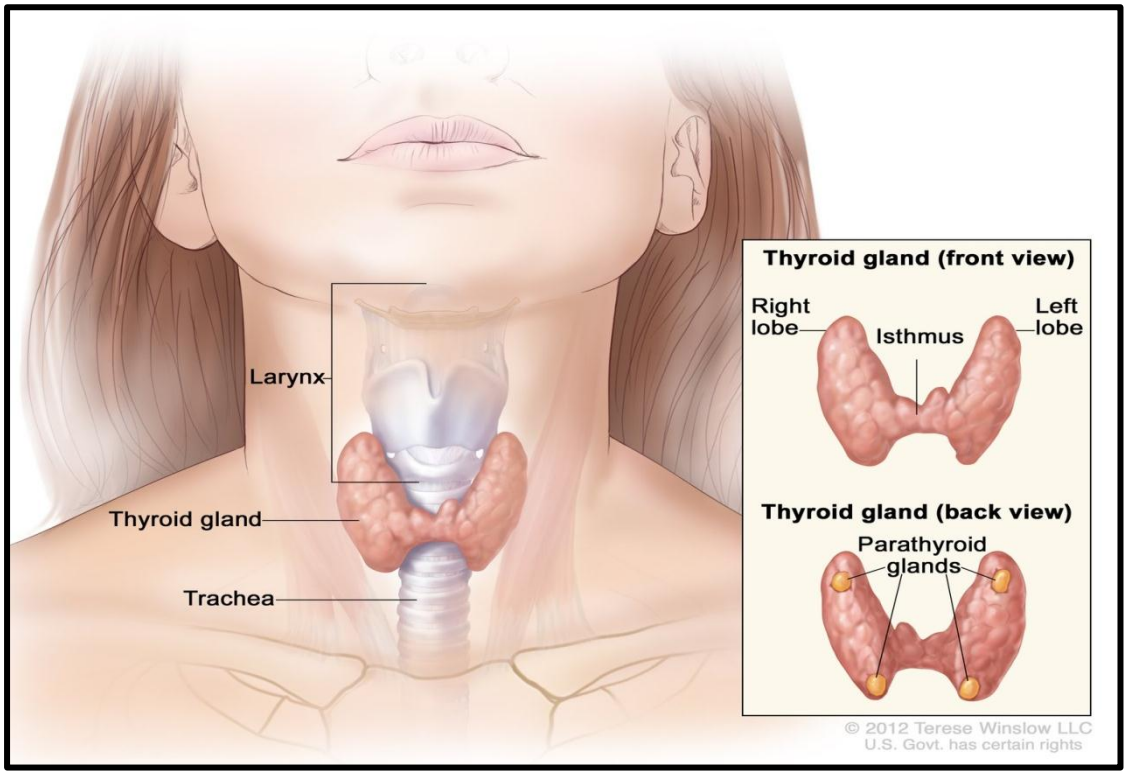

Figure 1-13: Anatomy of the Thyroid and Parathyroid Glands. The 4 parathyroid glands are located near or attached to the back side of the thyroid gland. The parathyroid glands secrete a hormone that controls blood levels of calcium(99).

These glands are a piece of the endocrine system. The endocrine system produces and directs the hormones that influence human growth, advancement, body capacity, and state of mind. When one or more of the parathyroid glands releases too much PTH, a calcium imbalance results, which can affect many of the body's functions. where the four parathyroid glands regulate calcium in the blood by releasing PTH when the body's calcium levels are low and ceasing production of PTH when the body has sufficient calcium(101,102).

Primary hyperparathyroidism is a disorder of the parathyroid glands, resulting from excess parathyroid hormone secretion "Primary" means this disorder begins in the parathyroid glands, rather than resulting from

another health problem such as kidney failure. In primary hyperparathyroidism, one or more of the parathyroid glands is overactive. As a result, the gland makes too much PTH(103–105).

In secondary hyperparathyroidism, the normal PTH effect on bone calcium release is lost. Serum PTH rises, causing generalized hyperplasia. In tertiary hyperparathyroidism, a complication of secondary hyperparathyroidism, normal feedback mechanisms governing PTH secretion are lost, parathyroid gland sensitivity to PTH decreases, and the threshold for inhibiting PTH secretion increases(106,107).

Serum parathyroid levels are slightly decreased in the second half of pregnancy. Hyperparathyroidism and hypoparathyroidism, characterized by alterations in the blood calcium levels and bone metabolism, are states of either surplus or deficient parathyroid function(108,109).

1.6.1 Function of Parathyroid Glands

The major function of the parathyroid glands is to maintain the body's calcium and phosphate levels within a very narrow range so that the nervous and muscular systems can function properly. The parathyroid glands do this by secreting parathyroid hormone(110). Parathyroid hormone (also known as parathormone) is a small protein that takes part in the control of calcium and phosphate homeostasis, as well as bone physiology. Parathyroid hormone has effects antagonistic to those of calcitonin(111) (see Figure 1-13).

The following are some of the functions that are stimulated by the parathyroid hormone:

1- Calcium is released from the bones and into the bloodstream.

2- Absorption of calcium from food via the intestinal tract.

3- Calcium storage is accomplished via the kidneys.

4- This causes the kidney cells to convert less potent forms of vitamin D into the form that is best able to absorb calcium from the intestines, and it does this by stimulating the cells.

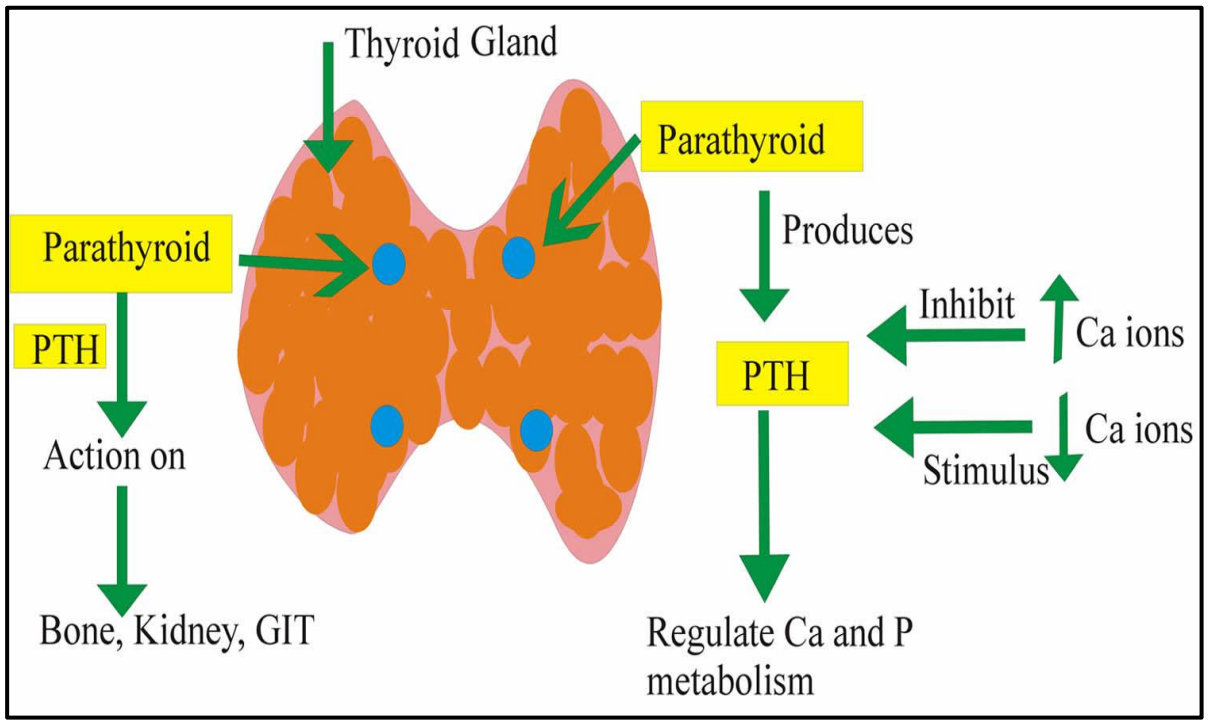

Figure 1-13: Parathyroid hormone function. Abbreviations: Ca = Calcium; P = Phosphate or Phosphorus; PTH = parathyroid hormone; GIT = gastrointestinal tract(111)

1.6.2 Parathyroid Hormone

Parathyroid hormone (PTH) or parathyrinis a polypeptide that is synthesized and secreted by the parathyroid chief cells and cleaved into an active form within the parathyroid gland(112)

Parathyroid hormone is a polypeptide composed of 84 amino acids, although the biological activity is associated with the first 20–30 amino acids(112,113) (see Figure 1-14). The hormone is synthesized as a pre-pro-parathormone containing 115 amino acids. This is cleared to produce pro-PTH containing 90 amino acids (molecular weight 12 000), which is then converted into parathormone (molecular weight 9500) and stored in granules. Subsequent to release into the circulation, the large precursor is

converted into a large inactive fragment (molecular weight 7000) and a small active fragment (molecular weight 2500)(114).

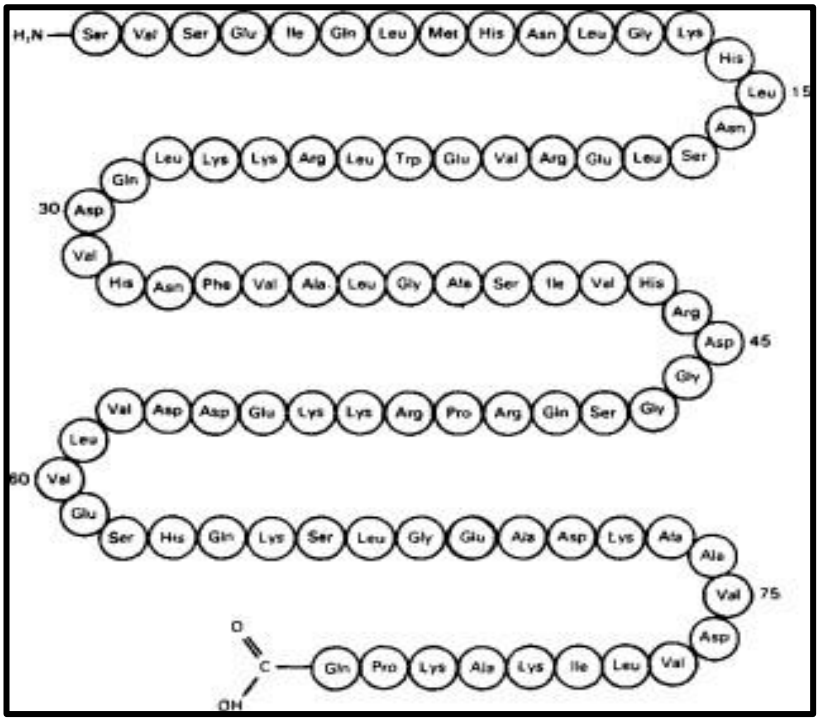

Figure 1-14: The structure of human parathyroid hormone(114).

Parathyroid hormone acts to increase the concentration of calcium (Ca^{2+}) in the blood, whereas calcitonin (a hormone produced by the parafollicular cells (C cells) of the thyroid gland) acts to decrease calcium concentration(115). PTH acts to increase the concentration of calcium in the blood by acting upon the parathyroid hormone 1 receptor and the parathyroid hormone 2 receptor(116). Parathyroid hormone regulates serum calcium, It enhances the release of calcium from the large reservoir contained in the bones. It enhances the active reabsorption of calcium and magnesium from distal tubules and the thick ascending limb. It enhances the absorption of calcium in the intestine by increasing the production of activated vitamin D(117).

Parathyroid hormone is the primary hormone that is stored, secreted, and functions in the body. The process of synthesis, cleavage, and storage

is estimated to take less than an hour. Active PTH secretion can occur as quickly as a few seconds when low serum calcium is detected(118). The mechanism of secretion is via exocytosis, a process where the hormone is released through a membrane vesicle carried to the cell membrane, releasing the hormone after the vesicle fuses with the outer membrane. The serum half-life of activated PTH is a few minutes and is removed from the serum quickly by the kidney and liver(119).

1.7 Thyroid and Pregnancy

Pregnancy is a natural physiological changes that is accompanied with hormonic and metabolic alterations caused by a variety of conditions that results in many pathophysiologic processes, some of which have potentially serious outcomes if left untreated. Thyroid diseases during pregnancy are related to maternal and fetal complications(120). Thyroid conditions, particularly hypothyroidism, are relatively common in pregnancy and important to treat. The thyroid is an organ located in the front of the neck that releases hormones that regulate metabolism (the way body uses energy), heart and nervous system, weight, body temperature, and many other processes in the body(121).

Sometimes the thyroid makes too much or too little of these hormones. Too much thyroid hormone is called hyperthyroidism and can cause many of body's functions to speed up. "Hyper" means the thyroid is overactive. Learn more about hyperthyroidism in pregnancy. Too little thyroid hormone is called hypothyroidism and can cause many of body's functions to slow down. "Hypo" means the thyroid is underactive(122).

Thyroid hormones are particularly necessary to assure healthy fetal development of the brain and nervous system throughout pregnancy. During the first trimester of pregnancy, the baby depends on a supply of thyroid hormone, which comes through the placenta. At around 12 weeks,

the baby's thyroid starts to work on its own, but it doesn't make enough thyroid hormone until 18 to 20 weeks of pregnancy, but there is still a dependence on maternal hormones(123). Two pregnancy-related hormones human chorionic gonadotropin (hCG) and estrogen cause higher measured thyroid hormone levels in blood. The thyroid enlarges slightly in healthy women during pregnancy, but usually not enough for a health care professional to feel during a physical exam(16).

The last two decades have also witnessed significant ad- advances in the diagnosis and management of hypothyroidism in pregnancy. The importance of trimester-specific reference ranges for thyroid function tests in pregnancy has been established. It has become clear that the upper reference limit of serum thyrotropin (TSH) in pregnancy is much low- er than that in the general population(124). It has also been convincingly shown that most hypothyroid women need an increased dose of levothyroxine during pregnancy. There remains uncertainty at what level of TSH should the levothyroxine replacement be considered and whether women with isolated maternal hypothyroxinaemia or isolated positive thyroid peroxidase antibodies should be treated with levothyroxine(125).

1.7.1 Physiological Changes of Thyroid Function in Mother and Fetus During Pregnancy

Thyroid hormones (TH) are very important for the growth and development of the brain for the fetus and neonate, in addition to many other aspects of pregnancy, fetal growth and development(126). The thyroid gland dysfunctions like hypothyroidism and thyrotoxicosis can affect the mother's health as well as the child's before and after delivery which can result in fetal disease; in humans, this includes a high incidence of mental retardation(126,127). The fetal thyroid gland begins concentrating iodine and synthesizing THS after first trimester of gestation.

Although the requirement for TH before this time is exclusively supplied by the mother which is most important to fetal brain development, significant fetal brain development continues considerably beyond the first trimester(128) .

Evident maternal thyroid failure during the first half of pregnancy has been associated with several pregnancy complications including preeclampsia, premature labor, fetal death and low birth weight and intellectual impairment in the offspring(129). THs have most profound effects on the terminal stages of fetal brain differentiation and development, including synaptogenesis, dendrites growth and axons myelination and neuronal migration. TH receptors are broadly dispersed in the fetal brain, and existing prior to the time the fetus is able to synthesize TH. Evidence has confirmed that it is challenging to identify the molecular targets for TH action in the developing brain, but some improvement has been made (128).

1.7.2 Mechanisms of physiological changes of thyroid function in Pregnancy

Thyroid stimulation starts as early as the first trimester by β-HCG hormone, which shares some structural homology with thyroid-stimulating hormone (TSH). There is also an estrogen-mediated increase in circulating levels of thyroid-binding globulin (TBG) during pregnancy by 2-3 times in serum TBG concentrations. TBG which is one of the numerous proteins that transports TH in the blood with a high affinity for thyroxine (T4) increases in serum a few weeks after conception and ranges to a plateau during the mid-gestational period. The mechanism for this increase in TBG involves both increased hepatic synthesis of TBG and estrogen-mediated perpetuation in the sialylation of TBG that increases the half-life from 15 min to 3 days to fully sialylated TBG(128).

Elevated levels of TBG lead to lowered bound T4 concentrations, which results in increased TSH secretion by the pituitary and, subsequently, enhanced production and TH secretion (130). The net effect of elevated TBG synthesis is to force a new equilibrium between free and bound THs and therefore a substantial increase in total T4 and triiodothyronine (T3) levels. The augmented demand for THs is reached by about 20 wk of gestation and persists until term (131).

Reflecting changes in iodine metabolism, which is an essential requirement for TH synthesis, increased demand for iodine results from a significant pregnancy-associated increase in iodide clearance by the kidney and draws off maternal iodide by the fetus. During pregnancy, there is an increased iodine excretion in the urine as a result of increased glomerular filtration and decreased renal tubular absorption. In addition, maternal iodine is actively transported to the fetoplacental unit, which contributes to a state of relative iodine deficiency(132).

The other factor is the impact of HCG secreted by the placenta of humans. Thyroid stimulation in response to the thyrotropic activity of HCG overrides the normal action of the hypothalamic-pituitary thyroid feedback system. TSH can bind and transduce signalling from the TSH receptor on thyroid epithelial cells. Closure to the end of the first trimester of pregnancy in humans, when HCG levels reach at peak, a substantial fraction of the thyroid-stimulating activity is from HCG. During this time, blood levels of TSH become suppressed. The thyroid-stimulating activity of HCG actually causes some women to cause transient hyperthyroidism(133,134).

The potential source of TH for the fetus is its own thyroid and the thyroid of the mother. Human fetuses acquire the ability to synthesize TH at approximately the first trimester of gestation. Current evidence from several species indicates that there is a significant transfer of maternal THs

across the placenta and also the placenta contains deiodinases that can convert T4 to T3(130,135). Thyroid dysfunction is one of the common complications of pregnancy and it contributes significantly to maternal and fetus morbidity and mortality. There is limited attention and information related to thyroid dysfunction and its complication during pregnancy(136).

1.8 Parathyroid hormone in Pregnancy

Physiological changes during gestation aimed at providing sufficient calcium for the growth of the fetus. Calcium homeostatic hormones, such as parathyroid hormone (PTH), are essential to increase maternal calcium absorption during pregnancy(137). PTH is involved in several processes, in particular maintenance of ionized calcium in the blood, raising the level of calcium phosphate released from bone tissue, conserving calcium, reducing tubular phosphate reabsorption, and increasing the intestinal absorption of calcium through vitamin D(138).

Through the course of pregnancy, PTH concentrations increase to reach a mid-normal range by the third trimester. The secretion of PTH is regulated primarily by extracellular calcium concentrations; lower amounts of circulating calcium trigger an increase in PTH. A rise in PTH-related protein (PTHrP) and calcitriol in the first trimester, and the flux of other hormones during pregnancy, such as estradiol, progestins, placental lactogen, and insulin-like growth factor I, may have direct or indirect effects on maternal calcium. These changes might account for the observed differences in PTH concentrations in the first and third trimesters. Additionally, nephrogenous cyclic adenosine monophosphate (cAMP) excretion, the index of parathyroid function, has been reported to decrease during the first and second trimesters of pregnancy but to be in normal range during the third trimester(139).

The transfer of calcium to the fetus increases in the case of maternal hyperparathyroidism and hypercalcemia, with consequent suppression of fetal parathyroid glands. Maternal hyperparathyroidism and hypercalcemia are known to result in a high rate of fetal complications: spontaneous abortions, intrauterine growth retardation, still births, transient neonatal tetany, and permanent hypoparathyroidism in the newborn Conversely, the placental transfer of calcium decreases in the case of maternal hypoparathyroidism and hypocalcemia, with the consequent stimulation of fetal parathyroid glands and the development of fetal hyperparathyroidism(140).

Severe cases of fetal hyperparathyroidism suffer subperiosteal bone resorption, bowing of the long bones, intrauterine rib and limb fractures, low birth weight, osteitis fibrosa cystica, spontaneous abortion, and possibly fetal death Thus, it is highly essential to understand the determinants of adequate PTH concentrations during pregnancy. Given the implications of PTH on maternal and neonatal outcomes and taking into consideration the fact that PTH concentrations are not constant but increase during pregnancy(139,141).

1.9 Thyroid Hormone Abnormalities During Pregnancy

1.9.1 Hyperthyroidism in Pregnancy

Hyperthyroidism typically is the disease process in which excessive TH is synthesized and excreted, whereas the term thyrotoxicosis refers to increased amounts of TH in the circulation(128) . It can occur in approximately 1% of the population and up to 0.4% of the pregnancies. Previous study by Wang et al found that the prevalence of thyroid dysfunction was 10.2%, hyperthyroidism, hypothyroidism and hypothyroxinemia were 1.8, 7.5 and 0.9% respectively (142).

There are 2 causes of hyperthyroidism, like classic causes which is found in the general population, and the other causes are specific during pregnancy. True hyperthyroidism is differentiated from other forms by elevated radioactive iodine uptake (RAIU). The other forms are differentiated from true hyperthyroidism by decreased RAIU like hyperthyroidism caused by factors other than thyroid gland over-activity may result from inflammatory thyroid disease, pregnancy-specific associations (like hyperemesis gravidarum and hydatid form mole) and the presence of ectopic thyroid tissue or by exogenous sources of TH(143,144).

Tissue effects of hyperthyroidism include accelerated metabolism, suppressed serum TSH, low serum cholesterol, increased bone turnover and reduced bone density with an increased risk of osteoporosis and fracture(145) . A study by Marvisi et al found that hyperthyroidism strongly is associated with lower TSH values and increased pulmonary arterial pressure which leads to severe pulmonary hypertension . In the first trimester of gestation, the normal elevation in total T4 and total T3, due to estrogen-induced increase in TBG concentration and HCG thyroid stimulation with suppression of serum TSH, may pause difficulties in the diagnosis of maternal hyperthyroidism(146).

1.9.2 Hypothyroidism in Pregnancy

The prevalence of thyroid hypofunction in pregnancy is very similar to that found in non-pregnant women of the same age: 2-3% for "mild" or subclinical hypothyroidism; 0.3-0.5% for overt hypothyroidism. The main cause is represented by autoimmune thyroiditis, which can affect 5 to 15% of the female population of childbearing age (147). It must be taken into account that pregnancy, due to the physiological increase in thyroid function demands, can accelerate the appearance of glandular hypofunction

in women affected by autoimmune thyroiditis who are still asymptomatic(148).

Hypothyroidism, even "mild" or subclinical, can be one of the reasons for early and late obstetric complications, as well as situations at risk for the fetus, all related to the severity and duration of the hypofunction. A prolonged period of maternal hypothyroidism, especially in the early stages of pregnancy, can have very serious consequences on the neuropsychic development of the fetus and therefore of the newborn(149).

Although the most recent guidelines do not recommend blanket screening of thyroid function in women planning to become pregnant or diagnosed with pregnancy in the early weeks, some authors argue that measuring thyroid hormones and TSH only in women " at risk" of hypothyroidism during pregnancy does not show about one-third of cases of subclinical and/or overt hypothyroidism Untreated hypothyroidism during pregnancy can lead to preeclampsia, anaemia, miscarriage, low birthweight stillbirth, and congestive heart failure(150). In daily practice, it is a common experience that the dosage of thyroxine in pregnancy should be increased by about 30-50% compared to the pre-conception one(151).

1.10 Aims of the study

This study aims to:

1-Investigate the hormonal Changes in thyroid and parathyroid hormones during pregnancy in comparison to healthy non-pregnant women.

2-Describe the best way to diagnose of thyroid and parathyroid dysfunction.

Chapter Two

Materials and Methods

2 Materials and Methods

2.1 Materials

2.1.1 Tools and Instruments

All the equipments and instruments that had been used in this study are listed in the table 2-1

Table 2-1: Tools and instruments that are used in this study.

No.	Equipment& instruments	Type	Country
1	1 - 50µL Micropipette	CBPP	U.K
2	10 – 100 µL Micropipette	CBPP	U.K
3	100 - 1000 µL Micropipette	GWP	U.S.A
4	5-50µL Micropipette	GWP	U.S.A
5	96-Well plate	ElabScience	China
6	Absorbance Microplate Reader	Karl Kolb	Germany
9	Centrifuge	Kokusan	Germany
10	Ependroff tubes	Eppendroff	Germany
11	Freezer (-20 °C)	Angel Antoni	Italy
14	Gel Tubes	Afco	Jordan
15	Incubator	Memmert	Germany
19	Refrigerator Centrifuge	Hettich	Germany

2.1.2 Kits

The kits that had been used in this study are shown in Table 2-2

Table 2.2: Kits (Companies and Countries of Origin) used in the present study.

No	Kit	Catalog Numbers	Company	Country
1	Human Parathyroid Hormone	MBS263675	Mybiosource	China
2	Human thyroid stimulating Hormone	MB590037	Mybiosource	China
3	Human Triiodothyronine	MBS720588	Mybiosource	China
4	Human Calcitonin	MBS169367	Mybiosource	China
5	Human Thyroxine	MBS265986	Mybiosource	China

2.2 Ethical Considerations

The study, includes blood samples collection and experimental protocols which was approved by the Ethical Committee of Women's and Children's Hospital in Hhilla, Shomali General Hospital and the College of Medicine Committee University of Babylon.

Informed consents had been obtained from the all study participants before sample collection. In addition to that all the methods and protocols were performed under the guideline and regulations of the Ethical Committee of Ceneral Directorate of Health in Babylon Province.

2.3 Study Design

This study is Disigned a case-control study which had been performed on pregnant attended Babylon Hospital for Maternil and Children Teaching and General Shomali Hospital and they were admitted as Pregnant women which were diagnosed by expert physicians.

2.3.1 Subjects

The subject involved 40 pregnant Women with range age(18-40 years). The subjects were enrolled in this study between November 2022 and March 2023 at Women's and Children's Hospital in Hilla and Shomali General Hospital , 50 healthy subjects as non-pregnant with range age (18-40 years) who visited hospital for routine check-up without any history of chronic diseases, acute illness and infection. All laboratory tests analysis was performed in at Women's and Children's Hospital in Hilla and Shomali General Hospital and the Chemistry and Biochemistry Dep,College of Medicine, University of Babylon. All Subjects signed a written informed consent forms. Body mass index (BMI) was calculated as body weight (kg) was divided by squared height in meters. The general data were age, and gestaional age.

2.3.2 Exclusion Criteria

The current study required the exclusion of a group of pregnant :

- Subjects with inflammatory diseases
- Subjects with COVID-19
- Renal disease.
- The subjects who have a history of malignancy.
- The subjects who have a chronic systemic autoimmune disease
- Thyroidectomy pregnant

2.3.3Criteria of the Control Group

The non-pregnant group samples were collected after ensuring the adequacy of the criteria which is specified in this study. The selection of the non-pregnant group was based on several criteria, including:

- Healthy non-pregnant participants should have no medical history of heart disorders
- They have not undergone surgical intervention or any illness requiring hospitalization.
- Subjective perception of good health as it is determined by a health questionnaire.

2.4 Collection of the Samples

A blood sample (5 ml) was taken from each study groups. Then serum was separated by centrifugation at (4000 rpm) for 5 min at room temperature 37°C. The separated serum was preserved using Eppendorf tubes for biochemical analysis at - 20 ˚C.

2.5 Biochemical Analysis

2.5.1 Determination of Calcitonin (CAL)

2.5.1.1 Principle

This assay is based on a sandwich ELISA format. Calcitonin present in samples or standards binds to the anti-calcitonin antibodies pre-adsorbed on the microtiter plate. Next, a biotinylated anti-calcitonin antibody is added to the plate well and binds to the captured calcitonin. A streptavidin-enzyme conjugate is then added, which binds to the biotin of the second antibody. Unbound streptavidinenzyme conjugate is removed during a wash step, and substrate solution is added to the wells. A colored product is formed in proportion to the amount of calcitonin present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from purified recombinant human calcitonin. Sample concentration is then determined by comparing to the known values of the standard curve.

2.5.1.2 Kit Components

Box 1 (shipped at room temperature)

1. Anti-Calcitonin Antibody Coated Plate(Part No. 50621B): One strip well 96-well plate.
2. Anti-Calcitonin Biotinylated Antibody (1000X)(Part No. 50622D): One 10 µL vial of anti-calcitonin antibody.
3. Streptavidin-Enzyme Conjugate(Part No. 310803): One 20 µL tube.
4. Assay Diluent(Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer(Part No. 310806): One 100 mL bottle.
6. Substrate Solution(Part No. 310807): One 12 mL amber bottle
7. Stop Solution (Part. No. 310808): One 12 mL bottle

Box 2 (shipped on blue ice packs)

Calcitonin Standard (Part No. 50623D): One 10 µL vial of 5 µg/mL human calcitonin .

2.5.1.3 Procedure

1. A volume of 100 µ L of calcitonin standards or s amples were added to the anti-calcitonin antibody coated plate.
2. Micro well plate was incubated for 1 hour at room temperature on an orbital shaker.
3. The solution was removed from the wells. Then, microwell plate was washed 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. Then, micro well plate was emptied and inverted on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. A volume of 100 µL of the diluted anti-calcitonin biotinylated antibody was added to each well.
5. Micro plate was incubated for 1 hour at room temperature on an orbital shaker.
6. The solution was removed from the wells. the strip wells was washed for 5 times according to step 3 above.
7. Avolume of 100 µl of the diluted streptavidin-enzyme conjugate was added to each well.
8. Strip well was incubated for 1 hour at room temperature on an orbital shaker.
9. The solution was removed from the wells. Then, the strip wells were washed for 5 times according to step 3 above.
10. A volume of 100 µL o f substrate solution to each well, then incubated at room temperature on an orbital shaker.

11. The enzyme reaction was stopped by adding 100 µ L of stop solution into each well.

12. Absorbances of each micro well on a spectrophotometer using 450 nm.

2.5.1.4 Calculation

Results of calcitonin concentration was calculated by use stander curve by plotting calcitonin concentration on Xaxis aganst absorbance at 450nm on Yaxis,as show figure 2-1

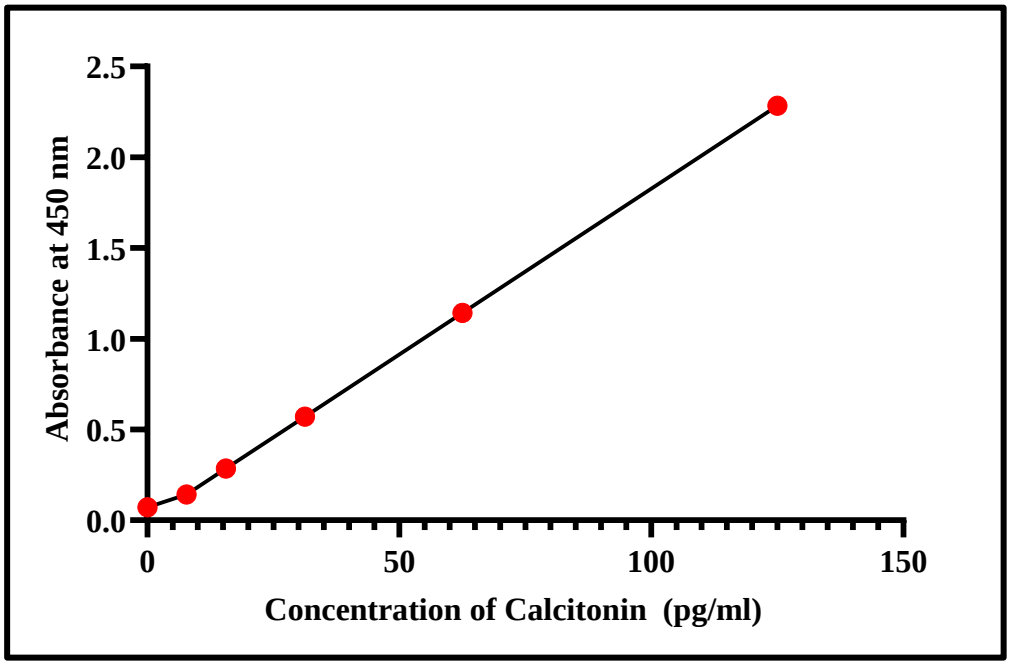

Figure 2-1: Human Calcitonin ELISA Standard Curve

2.5.2 Determination of thyroid stimulating hormone(TSH)

2.5.2.1 Principle

This ultra-sensitive TSH enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for TSH. Standards or samples are then added to the microtiter plate wells and TSH, if present, will bind to the antibody precoated on the wells. In order to quantitate the amount of TSH

present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated monoclonal antibody, specific for TSH are added to each well to "sandwich" the TSH immobilized on the plate. The microtiter plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Next, a TMB (3,3', 5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain TSH and enzymeconjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm.

In order to measure the concentration of TSH in the sample this Ultra-sensitive Human TSH ELISA Kit includes a set of calibration standards (6 standards). The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density (O.D.) versus TSH concentration (μIU/mL). The concentration of TSH in the samples is then determined by comparing the O.D. of the samples to the standard curve.

2.5.2.2 Kit Components

1. **MICROTITER PLATE** (Part EL12U-1) ***96 wells*** Pre-coated with anti-human TSH monoclonal antibody.

2. **CONJUGATE** (Part EL12U-2) ***12 mL*** Anti-human TSH monoclonal antibody conjugated to horseradish peroxidase (HRP) with preservative.

3. **STANDARD - 12 μIU/mL** (Part EL12U-3) ***1 vial*** Lyophilized human TSH in a buffered protein base with preservative that will contain 12 μIU/mL after reconstitution.

4. **STANDARD - 6 μIU/mL** (Part EL12U-4) ***1 vial*** Lyophilized human TSH in a buffered protein base with preservative that will contain 6 μIU/mL after reconstitution.

5. **STANDARD - 2 μIU/mL** (Part EL12U-5) ***1 vial*** Lyophilized human TSH in a buffered protein base with preservative that will contain 2 μIU/mL after reconstitution.

6. **STANDARD - 0.8 μIU/mL** (Part EL12U-6) ***1 vial*** Lyophilized human TSH in a buffered protein base with preservative that will contain 0.8 μIU/mL after reconstitution.

7. **STANDARD - 0.2 μIU/mL** (Part EL12U-7) ***1 vial***Lyophilized human TSH in a buffered protein base with preservative that will contain 0.2 μIU/mL after reconstitution.

8. **STANDARD - 0 μIU/mL** (Part EL12U-8) ***1 vial***

Lyophilized buffered protein base with preservative that will contain 0 μIU/mL after reconstitution.

9. **SUBSTRATE A** (Part EL12U-9) ***10 mL*** Buffered solution with H202.

10. **SUBSTRATE B** (Part 30007) ***10 mL*** Buffered solution with TMB.

11. **STOP SOLUTION** (Part 30008) ***14 mL*** 2N Sulphuric Acid (H2SO4).

2.5.2.3 Procedure

1. All TSH Standards werer added before starting assay procedure.

2. First, the desired number of coated wells was secured in the holder, then 100 μL of Standards or samples were added to the appropriate wells of the antibody pre-coated microtiter plate.

3. Avolume of 100μL of Conjugate was added into each well. Complete mixing in this step is important. Plate was covered and incubated for 3 hours at 37°C.

4. The microtiter plate was washed 5 times using 350 mL of distilled water each time by microplate washer.

5. Avolume of 100 µL substrate solution was added to each well. Microplate was covered and incubated for 15 minutes at 37°C.

6. A volume of 100µL of stop solution was adeded to each well. Mix well.

7. The optical density (O.D.) was read at 450 nm using a microtiter plate reader within 30 minutes.

2.5.2.4 Calculation

Results of thyroid stimulating hormone concentration was calculated by use stander curve by plotting thyroid stimulating hormone concentration on Xaxis aganst absorbance at 450nm on Yaxis,as show figure 2-2

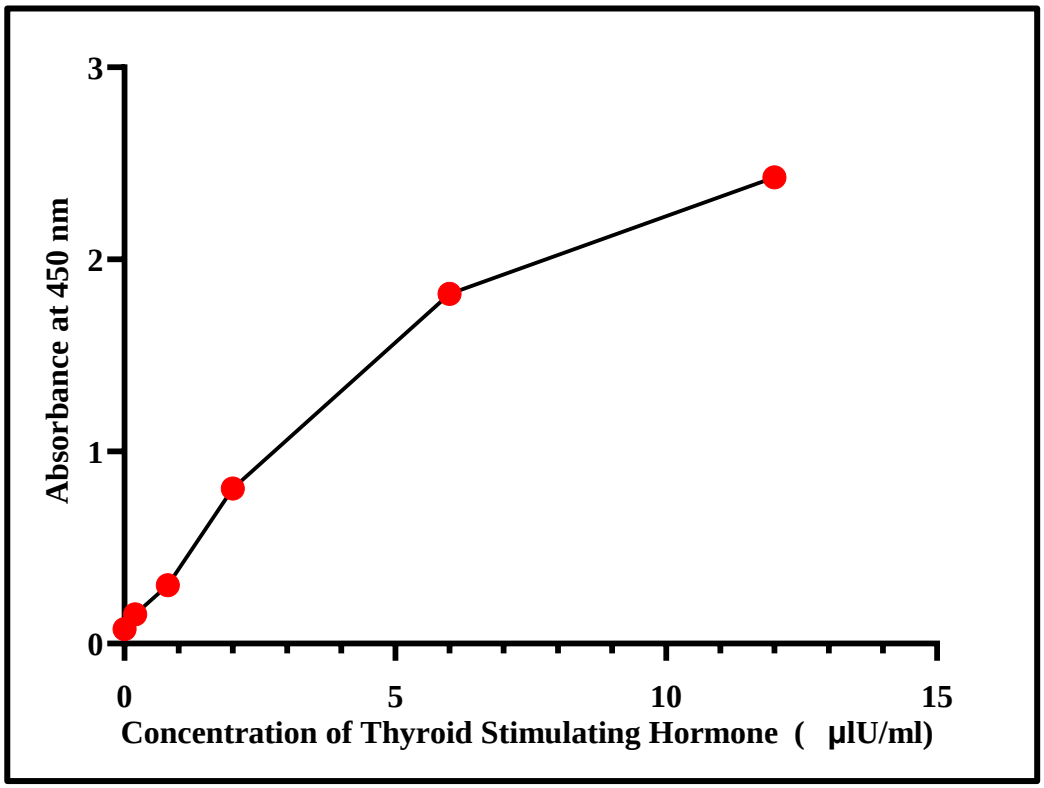

Figure 2-2: Human Thyroid Stimulating Hormone ELISA Standard Curve

2.5.3 Determination of Thyroxine (T4)

2.5.3.1 Principle

This experiment use double-sandwich ELISA technique. The pre-coated antibody is human T4 monoclonal antibody and the detecting antibody is polyclonal antibody with biotin labeled. Samples and biotin labeling antibody are added into ELISA plate wells and washed out with PBS or TBS. Then Avidin-peroxidase conjugates are added to ELISA wells in order; Use TMB substrate for coloring after reactant thoroughly washed out by PBS or TBS. TMB turns into blue in peroxidase catalytic and finally turns into yellow under the action of acid. The color depth and the testing factors in samples are positively correlated.

2.5.3.2 Kit Components

No	Name	96 Tests	48 Tests	Storage
1.	antibody precoated plate	8×12	8×6	4/-20℃
2.	Human T4 Standards	2 vial	1 vial	4/-20℃
3.	Biotinylated antibody(1:100)	1 vial	1 vial	4/-20℃
4.	Enzyme conjugate(1:100)	1vial	1 vial	4/-20℃
5.	Enzyme diluent	1vial	1vial	4/-20℃
6.	antibody diluent	1vial	1 vial	4/-20℃
7.	Standard diluent	1vial	1 vial	4/-20℃
8.	Sample diluent	1vial	1 vial	4/-20℃
9.	Washing buffer (1:25)	1 vial	1 vial	4/-20℃
10.	Colour Reagent A	1 vial	1 vial	4/-20℃
11.	Colour Reagent B	1 vial	1 vial	4/-20℃
12.	Colour Reagent C	1 vial	1 vial	4/-20℃
13.	Instruction	1 set	1 set	RT

2.5.3.3 Procedure

1. Samples or different concentration of human T4 standard samples were added to corresponding wells (100µl for each well), 0nmol/L well should be filled with standard diluent. The reaction wells were seated with adhesive tapes, hatching in incubator at 37C for 90 min.
2. Biotinylated human T4 antibody liquid was prepared 30min in advance.
3. The ELISA plate was washed for 3 times
4. The biotinylated human T4 antibody liquid was added to each well (100µl for each). Reaction wells were sealed with adhesive tapes, hatching in incubator at 37C for 60 min.
5. Enzyme-conjugate liquid was prepared 30min in advance.
6. The ELISA plate was washed for 3 times
7. Enzyme-conjugate liquid was added to each well except blank wells (100µl for each). The reaction wells were sealed with adhesive tapes, hatching in incubator at 37℃ for 30 min.
8. The ELISA plate was washed for 5 times.
9. Avolume of 100µl colour reagent liquid was added to individual well (also into blank well), hatching in dark incubator at 37C. The chromogenic reaction should be controlled within 30 min.
10. Avolume of 100µl colour reagent C was added to individual well (also into blank well). Mix well.
11. Absorbances were read at 450nm within 10 min using microplate reader.

2.5.3.4 Calculation

Results of thyroxine concentration was calculated by use stander curve by plotting Thyroxine concentration on Xaxis aganst absorbance at 450nm on Yaxis,as show figure 2-3

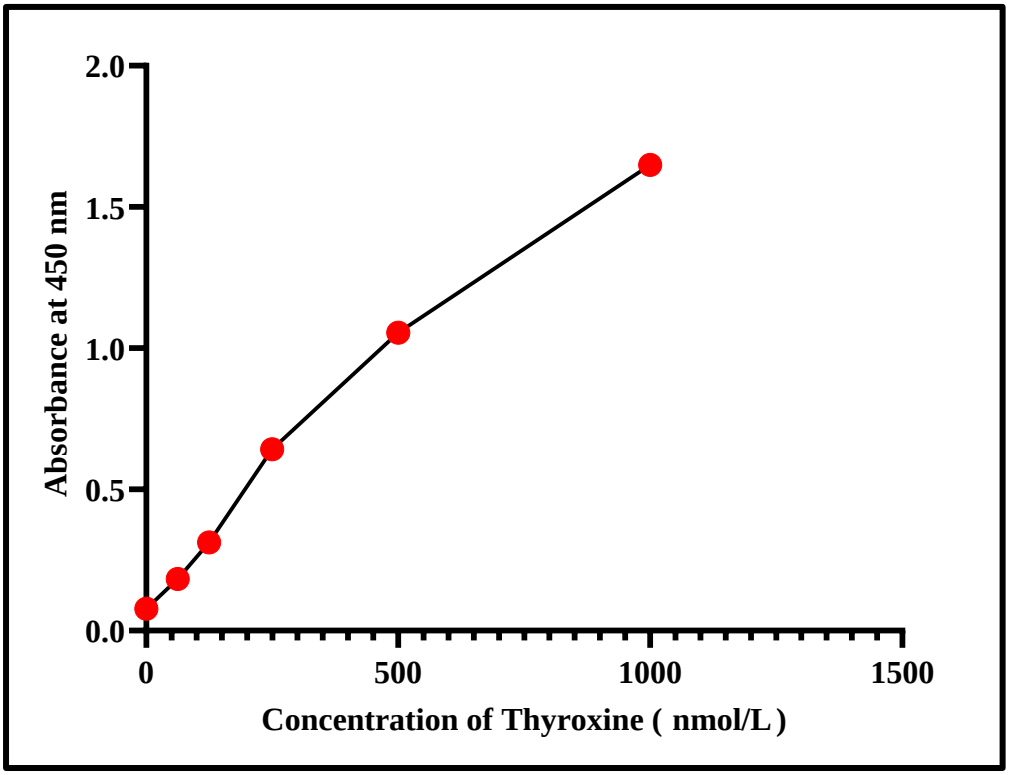

Figure 2-3: Human Thyroxine ELISA Standard Curve

2.5.4 Determination of Triiodothyron(T3)

2.5.4.1 Principle

T3 ELISA kit applies the competitive enzyme immunoassay technique utilizing a monoclonal anti-T3 antibody and an T3-HRP conjugate. The assay sample and buffer are incubated together with T3-HRP conjugate in pre-coated plate for one hour. After the incubation period, the wells are decanted and washed five times. The wells are then incubated with a substrate for HRP enzyme. The product of the enzyme-substrate reaction forms a blue colored complex. Finally, a stop solution is added to stop the reaction, which will then turn the solution yellow. The intensity of color is measured spectrophotometrically at 450nm in a microplate reader. The intensity of the color is inversely proportional to the T3 concentration since T3 from samples and T3-HRP conjugate compete for the anti-T3 antibody binding site. Since the number of sites is limited, as more sites are occupied by T3 from the sample, fewer sites are left to

bind T3-HRP conjugate. A standard curve is plotted relating the intensity of the color (O.D.) to the concentration of standards. The T3 concentration in each sample is interpolated from this standard curve.

2.5.4.2 Kit Components

No	Materials	Specification	Quantity
1.	Microtiter Plate	96 Wells	Stripwell
2.	Enzyme Conjugate	6.0 Ml	1 Vial
3.	Standard A	0 Ng/Ml 1	Vial
4.	Standard B	1.0 Ng/Ml 1	Vial
5.	Standard C	2.5 Ng/Ml 1	Vial
6.	Standard D	5.0 Ng/Ml 1	Vial
7.	Standard E	10 Ng/Ml 1	Vial
8.	Standard F	25 Ng/Ml 1	Vial
9.	Substrate A	6 Ml 1	Vial
10.	Substrate B	6 Ml 1	Vial
11.	Stop Solution	6 Ml 1	Vial
12.	Wash Solution	(100 X) 10 Ml 1	Vial
13.	Balance Solution	6 Ml 1	Vial
14.	Instruction	1	

2.5.4.3 Procedure

1) The desired numbers of coated wells was secured in the holder then add 100 uL of standards or samples were added to the appropriate well in the antibody pre-coated microtiter plate. 100 uL of PBS (pH 7.0-7.2) was added in the blank control well.

2) A volume of 50 uL of c onjugate was added to each well and mixed well. Mixing well in this step is important. the plate was covered and incubated for 1 hour at 37°C.

4) The microtiter plate was washed by using 350 mL of wash solution for 5 times by using microplate washer.

5) A volume of 50 uL substrate A and 50 uL substrate B were added to each well subsequently. The plate was covered and incubated for 10-15 minutes at 20-25°C. (Avoid sunlight).

6) A volume of 50 uL of stop solution was added to each well. Mix well.

7) Determination of the optical density (O.D.) at 450 nm using a microplate reader.

2.5.4.4 Calculation

Results of triiodothyronine concentration was calculated by use stander curve by plotting triiodothyronine concentration on Xaxis aganst absorbance at 450nm on Yaxis,as show figure 2-4

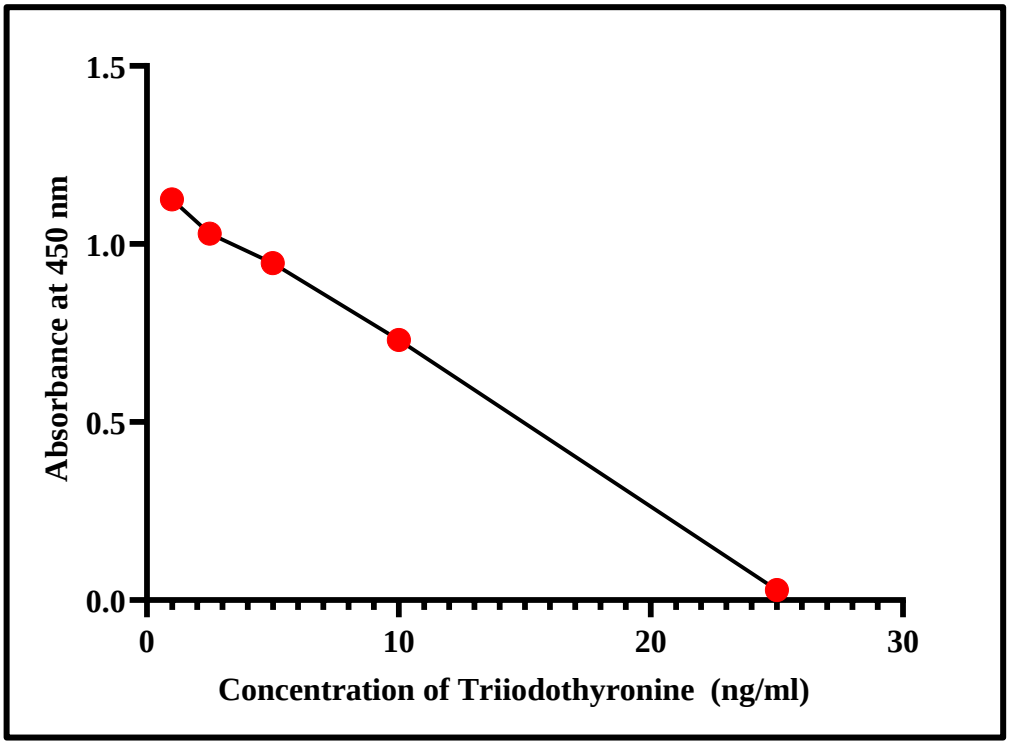

Figure 2-4: Human Triiodothyronine ELISA Standard Curve

2.5.5 Determination of Parathyroid Hormone (PTH)

2.5.5.1 Principle

This experiment use double-sandwich ELISA technique. The pre-coated antibody is human PTH monoclonal antibody and the detecting

antibody is polyclonal antibody with biotin labeled. Samples and biotin labeling antibody are added into ELISA plate wells and washed out with PBS or TBS. Then Avidin-peroxidase conjugates are added to ELISA wells in order; Use TMB substrate for colouring after reactant thoroughly washed out by PBS or TBS. TMB turns into blue in peroxidase catalytic and finally turns into yellow under the action of acid. The color depth and the testing factors in samples are positively correlated.

2.5.5.2 Kit Components

No	Name	96 Tests	48 Tests	Storage
1.	Antibody precoated plate	8×12	8×6	4/-20℃
2.	Human PTH Standards	2 vial	1 vial	4/-20℃
3.	Biotinylated antibody(1:100)	1vial	1 vial	4/-20℃
4.	Enzyme conjugate (1:100)	1vial	1 vial	4/-20℃
5.	Enzyme diluent	1vial	1 vial	4/-20℃
6.	Antibody diluent	1vial	1 vial	4/-20℃
7.	Standard diluent	1vial	1 vial	4/-20℃
8.	Sample diluent	1vial	1 vial	4/-20℃
9.	Washing buffer (1:25)	1vial	1 vial	4/-20℃
10.	Colour Reagent A	1vial	1vial	4/-20℃
11.	Colour Reagent B	1vial	1vial	4/-20℃
12.	Colour Reagent C	1vial	1 vial	4/-20℃
13.	Instruction	1set	1 set	RT

2.5.5.3 Procedure

1. Add samples or different concentrations of human PTH standard samples were added to corresponding wells (100µl for each well), 0pg/ml well should be filled with standard diluent. The reaction wells were sealed with adhesive tapes, hatching in incubator at 37C for 90 min.

2. Biotinylated human PTH antibody liquid was prepared 30min in advance.

3. The ELISA plate was washed for 2 times

4. The biotinylated human PTH antibody liquid was added to each well (100µl for each). The reaction wells were sealed with adhesive tapes, hatching in incubator at 37C for 60 min.

5. Enzyme-conjugate liquid was prepared 30min in advance.

6. The ELISA plate was washed for 3 times

7. Enzyme-conjugate liquid was added to each well except blank wells (100µl for each). The reaction wells was sealed with adhesive tapes, hatching in an incubator at 37c for 30 min.

8. The ELISA plate was washed for 5 times.

9. A volume of 100µl colour reagent liquid was added to individual well (also into the blank well), hatching in a dark incubator at 37℃. When colour for a high concentration of the standard curve becomes darker and colour gradient appears, the hatching can be stopped. The chromogenic reaction should be controlled within 30 min.

10. A volume of 100µl colour reagent C was added to individual well (also into the blank well). Mix well.

11. Absorbance was read at 450nm within 10 min.

2.5.5.4 Calculation

Results of parathyriod hormon concentration was calculated by use stander curve by plotting Parathyriod hormon concentration on Xaxis aganst absorbance at 450nm on Yaxis,as show figure 2-5

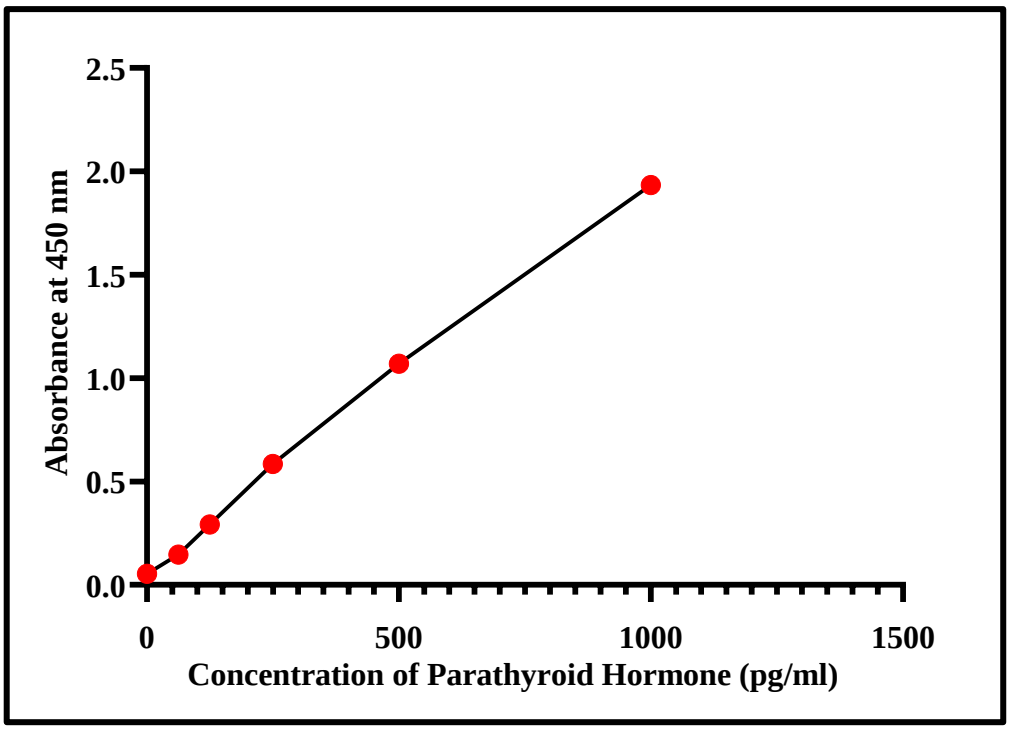

Figure 2-5: Human of Parathyroid Hormone ELISA Standard Curve

2.6 Statistical analysis

Data were summarized, analyzed, and presented using GraphPad Prism 9.2.0 and Microsoft Office Excel 2013. Numeric data were expressed as mean ± standard deviation. Unpaired t-test was used to compare the mean values among the different groups in the case of normally distributed variables. *p-value* was considered significant at *p-value* ≤ 0.05.

Chapter Three

Results and Discussion

3 Results and Discussion

Table 3.1: Comparison of mean values of thyroid function in women pregnant and non-pregnant.

Characteristic	Non-pregnant	Pregnant	P value
	50	40	
Age			
Range	**18 - 40**	**18 – 40**	**0.0186**
Mean ± SEM	**34.10 ± 1.115**	**30.07 ± 1.231**	
T3 (ng/mL)			
Range	**10.9 - 15.64**	**3.177 - 8.033**	**<0.0001**
Mean ± SEM	**13.93 ± 0.1932**	**5.469 ± 0.1799**	
T4 (nmol/L)			
Range	**231.5 - 362.5**	**130.1 - 252.8**	**<0.0001**
Mean ± SEM	**290.3 ± 4.813**	**185.9 ± 4.380**	
TSH (µlU/mL)			
Range	**0.528 - 1.808**	**1.267 - 4.196**	**<0.0001**
Mean ± SEM	**1.179 ± 0.054**	**2.886 ± 0.099**	
PTH (pg/mL)			
Range	**40.87 - 96**	**160 - 356.8**	**<0.0001**
Mean ± SEM	**72.58 ± 2.018**	**277.7 ± 6.703**	
CAL (pg/mL)			
Range	**6.335 - 14.26**	**14.37 - 29.36**	**<0.0001**
Mean ± SEM	**9.65 ± 0.303**	**21.89 ± 0.624**	

BMI: Body Mass Index**; PTH:** Parathyroid Hormone**; T3:** Triiodiothyronin**; T4:** Thyroxine**; CAL:** Calcitonin**; TSH:;** Thyroid-Stimulating Hormone

3.1 Age

The results show decreased levels of age (years)in pregnant as compared with the non-pregnant group (30.07 ± 1.231),(34.10 ± 1.115) (years) respectively; the significant difference (p-value = 0.0186). as shown in figure (3-1).

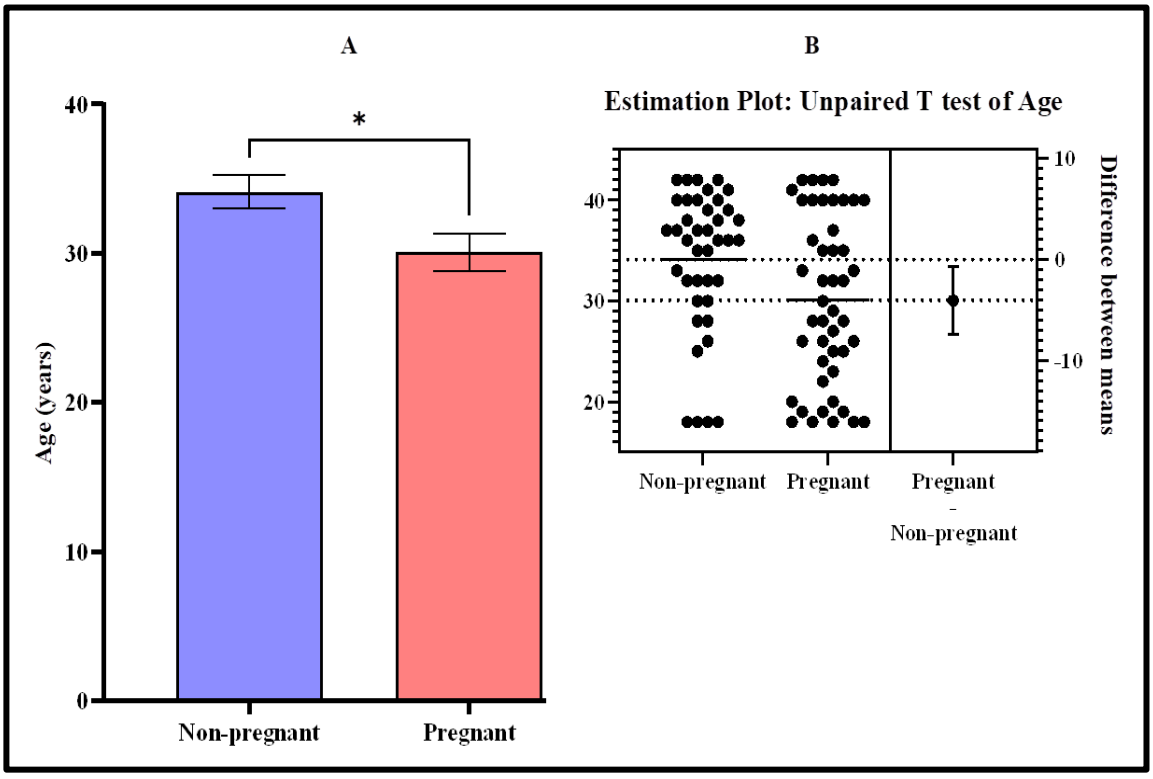

Figure 3-1: Estimation of age (years). (A) a comparison between the non-pregnant and pregnant group, (B) an estimation plot that illustrates the presence of a significant decrease in the level of Age in the pregnant group as compared to the non-pregnant, the significant difference (*p-value = 0.0186*). Data are expressed as means ± SEM. indicates *significant differences compared to the non-pregnant, P≤0.05.

3.2 Triiodothyronine

The results show decreased levels of concentration of triiodothyronine (T3) (ng/mL) in pregnant as compared with the non-pregnant group (5.469 ± 0.1799),(13.93 ± 0.1932) (ng/mL) respectively; the significant difference (p-value <0.0001). as shown in figure (3-2).

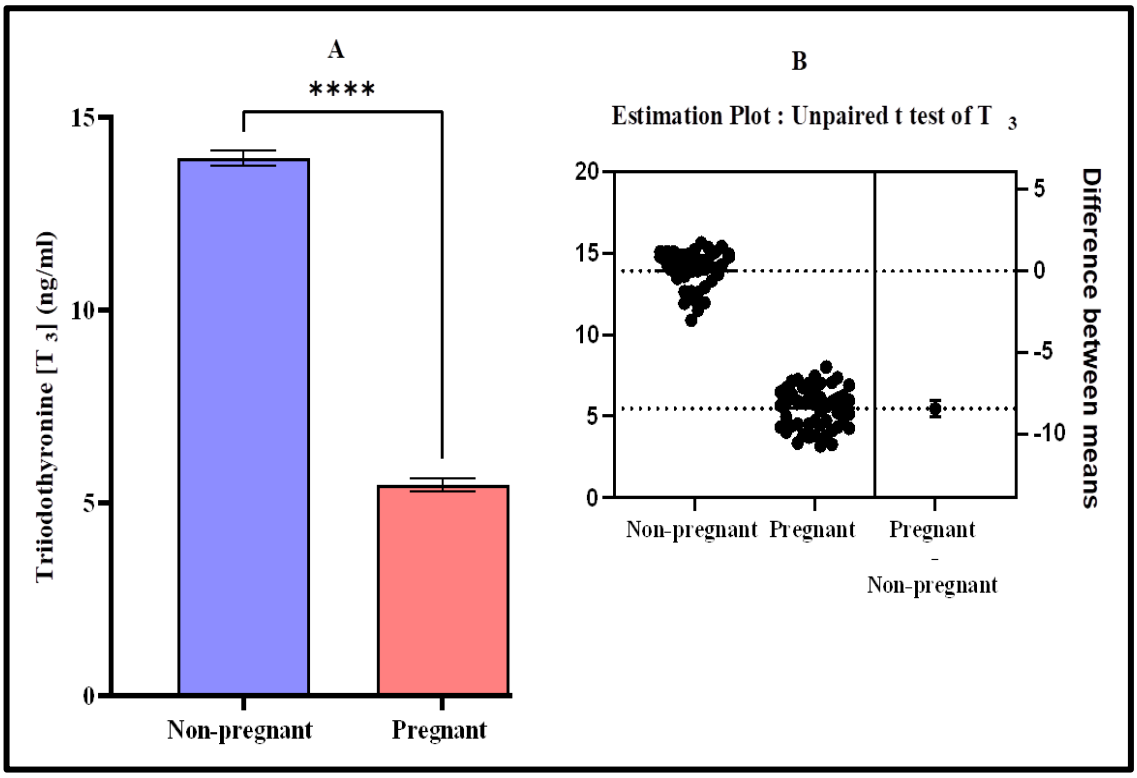

Figure 3-2: Estimation of serum triiodothyronine (T3) (ng/mL). (A) a comparison between the non-pregnant and pregnant group, (B) an estimation plot that illustrates the presence of a significant decrease in the level of T3 in the pregnant group as compared to the non-pregnant, the significant difference (*p-value <0.0001*). Data are expressed as means ± SEM. indicates *significant differences compared to the non-pregnant, P≤0.05.

The results show an decreased concentration of triiodothyronine (T3) (ng/mL) in the third trimester (4.925 ± 0.246) (ng/mL) compared with the second trimester and first trimester (5.804± 0.283), (6.346 ± 0.2962) (ng/mL) respectively; the significant difference (p-value = 0.0026) The measurement of T3 (ng/mL) showed an non-significant difference was present in mean values between the first trimester and with second trimester (p-value = 0.3634); the significant difference in mean values between the first trimester and third trimester (p-value= 0.0018); the non-significant difference in mean values between second Trimester and third Trimester (p-value = 0.0926), As shown in Figure 3-3.

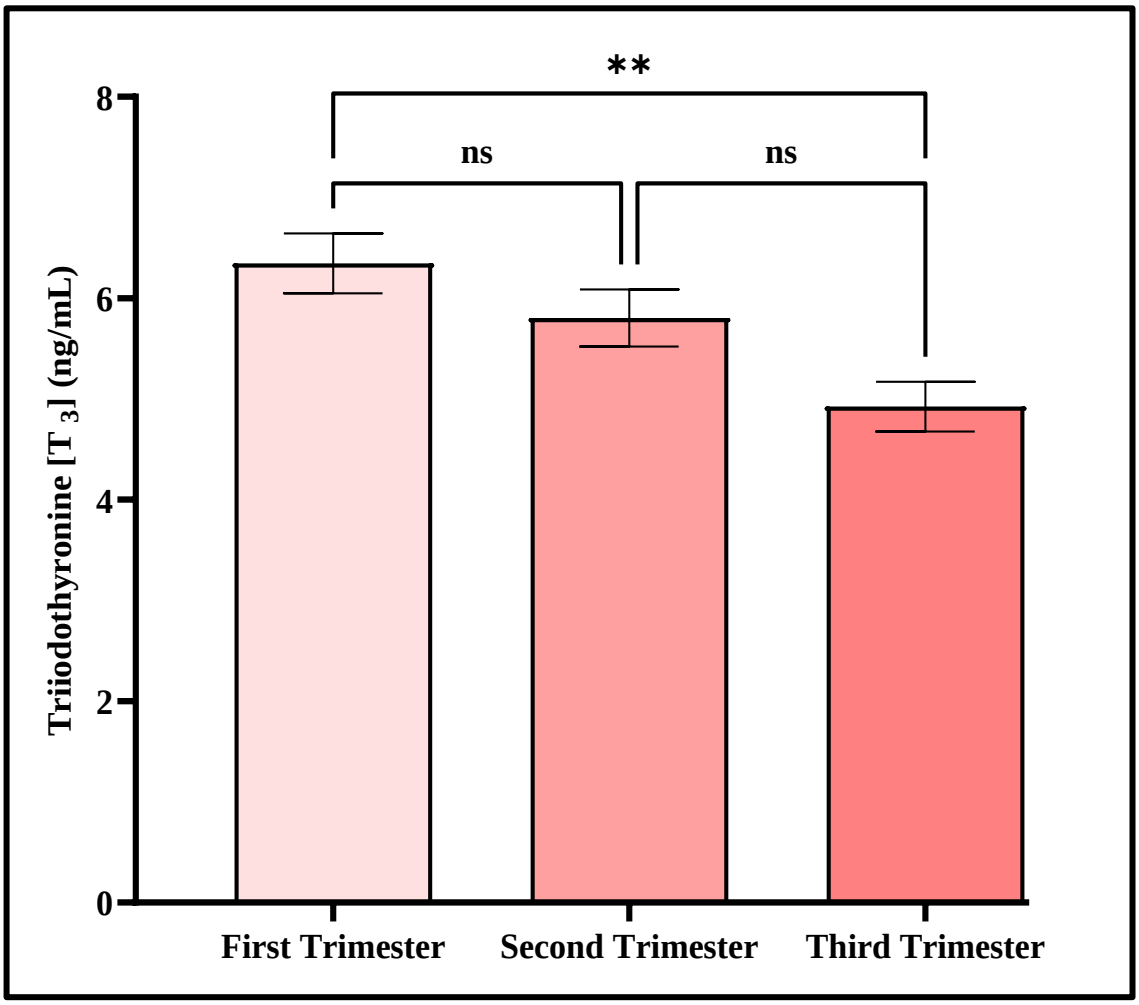

Figure 3-3: Estimation of serum triiodothyronine (T3) (ng/mL) in Stages of Pregnancy. First Trimester (0-13 Weeks), Second Trimester (14-26 Weeks) and Trimester (27-40 Weeks)

3.3 Thyroxine

The results show decreased levels of concentration of thyroxine (T4) (nmol/L) in pregnant as compared with the non-pregnant group (185.9 ± 4.380), (290.3 ± 4.813) (nmol/L) respectively; the significant difference (p-value <0.0001). as shown in figure (3-4)

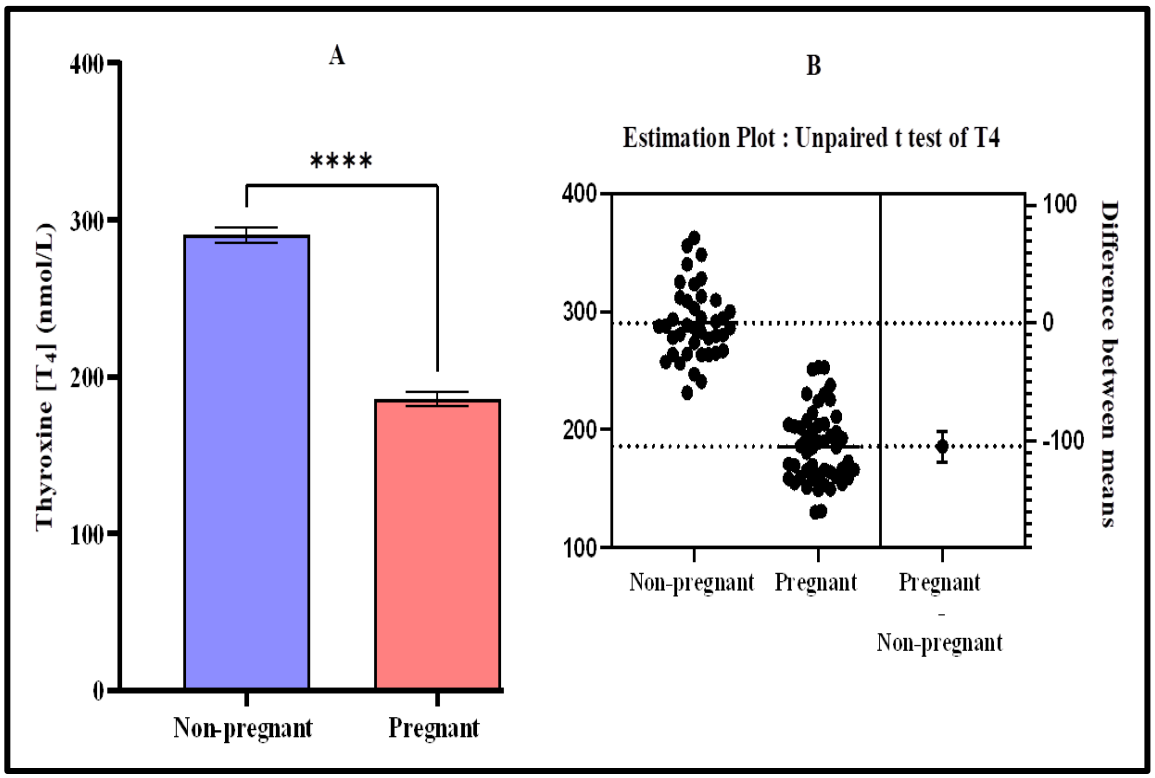

Figure 3-4: Estimation of serum thyroxine (T4) (nmol/L). (A) a comparison between the non-pregnant and pregnant group, (B) an estimation plot that illustrates the presence of a significant decrease in the level of T4 in the pregnant group as compared to the non-pregnant, the significant difference (*p-value <0.0001*). Data are expressed as means ± SEM. indicates *significant differences compared to the non-pregnant, P≤0.05.

The results show an decreased concentration of thyroxine (T4) (nmol/L) in the third trimester (164.1 ± 4.703) (nmol/L) compared with the second trimester and first trimester (190 ± 5.989), (201.5 ± 8.244) (nmol/L) respectively; the significant difference (p-value = 0.0008) The measurement of T4 (nmol/L) showed an non-significant difference was present in mean values between the first trimester and with second trimester (p-value = 0.4384); the significant difference in mean values between the first trimester and third trimester (p-value= 0.0006); the significant difference in mean values between second Trimester and third Trimester (p-value = 0.0256), As shown in Figure 3-5.

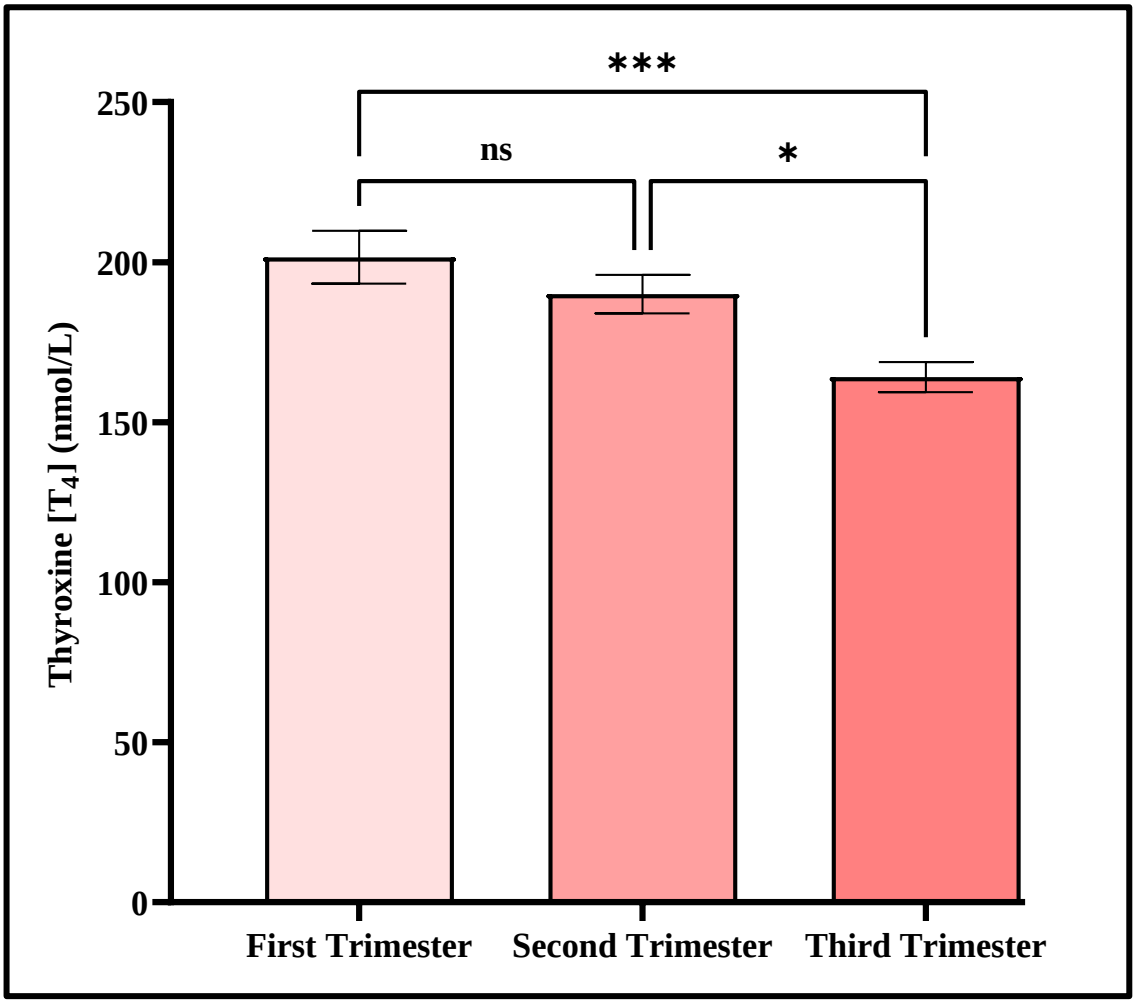

Figure 3-5: Estimation of serum thyroxine (T4) (nmol/L) in Stages of Pregnancy. First Trimester (0-13 Weeks), Second Trimester (14-26 Weeks) and Trimester (27-40 Weeks)

Proper diagnosis of thyroid dysfunction during pregnancy is essential because maternal thyroid diseases complicate pregnancy (1,2). Therefore, accurate diagnosis guidelines may provide cutoff values to correctly diagnose thyroid diseases, allowing appropriate clinical interventions. The metabolism and hormone levels of pregnant women are different from those in non-pregnant women (34,35); therefore, it is not appropriate to use generalized guidelines to detect thyroid diseases during pregnancy. During the last 20 years, it has been appreciated that thyroid physiology changes significantly during gestation (152). Thyroid disorders can have adverse reproductive and pregnancy implications. Although gestational hyperthyroidism is uncommon (0.2%), gestational

hypothyroidism occurs in higher prevalence (2.5%) and can lead to neonatal and child neurodevelopmental deficits and maternal obstetric complications (131,153).

The results of the current study revealed significantly reduced levels of both T3 and T4 in pregnant women as compared with healthy women and these consequences were compatible with the findings of other studies (154,155) which show similar results. The two major causes of this reduction are inadequate dietary iodine intake and the autoimmune condition known as Hashimoto's autoimmune thyroiditis (41). An additional factor known to contribute to thyroid dysfunction is inappropriate concentrations of selenium. Selenium deficiency is common during pregnancy leading to impair deiodinase (DIO) activity and reduce thyroid hormone concentrations (156,157). Other common causes that may result in hypothyroidism in pregnant women in our study are radio-iodine therapy, thyroidectomy, congenital hypothyroidism, drug use (i.e., rifampicin and phenytoin) and any hypothalamic-pituitary disease (158).

The outcomes of the present study conflicted with the results of other studies (159,160) which found elevated levels of T3 and T4 in comparison with healthy women. Another trial found inconsistent findings with our consequences and revealed that Thyroxine binding globulin (TBG) increases by 2–3 times compared with the pre-pregnancy level by the 20th week of gestation. This is a result of both increased production stimulated by oestrogens and the reduced clearance of the more heavily sialylated forms that are more common in pregnancy. This elevation causes an increase of total triiodothyronine (TT3) and thyroxine (TT4) by an average of 1.5 times by the 16th gestational week (161–163). in conclusion, it is important that thyroid function in pregnancy should be judged against gestational age-related reference intervals, and the results of

this study should decrease the possibility of the misinterpretation of thyroid function in pregnant women.

Serum TSH concentrations usually provide the first clinical indicator for thyroid dysfunction in the non-pregnant patient. Due to the log-linear relationship between TSH and T4, very small changes in T4 concentrations will result in very large changes in serum TSH. However, in early gestation, TSH is suppressed by 20–50% by week 10 due to the steep increase in hCG concentrations and its measurement at this time does not provide a good indicator for either the diagnosis or the control of treatment of thyroid dysfunction. Therefore, it is essential to rely on T4 and T3 (either bound or free) to assess thyroid status in early gestation, although TSH will be significantly elevated in overt hypothyroidism. Later in gestation (from about 16 weeks on) TSH is more reflective of thyroid status.

3.4 Thyroid-stimulating hormone

The results show increased levels of concentration of thyroid-stimulating hormone (TSH) (µlU/mL) in pregnant as compared with the non-pregnant group (2.886 ± 0.099),(1.179 ± 0.054) (µlU/mL) respectively; the significant difference (p-value <0.0001). as shown in figure (3-6).

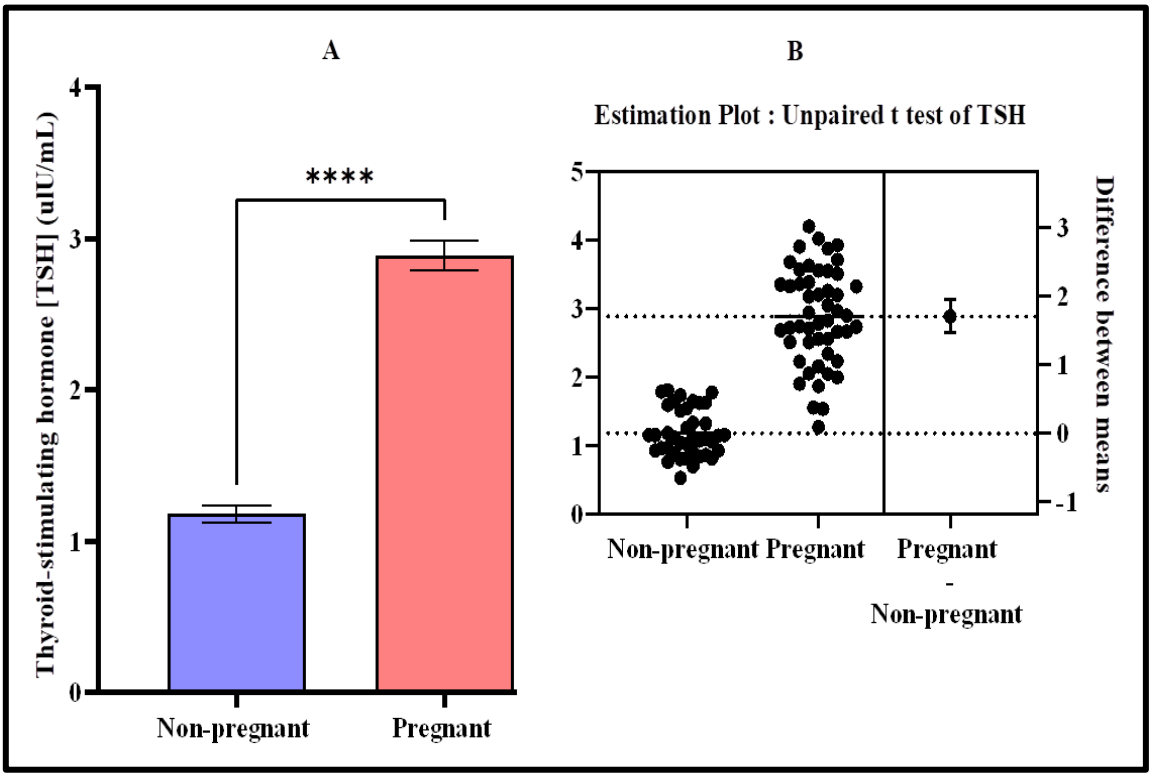

Figure 3-6: Estimation of serum thyroid-stimulating hormone (TSH) (µlU/mL). (A) a comparison between the non-pregnant and pregnant group, (B) an estimation plot that illustrates the presence of a significant increase in the level of TSH in the pregnant group as compared to the non-pregnant, the significant difference (*p-value <0.0001*). Data are expressed as means ± SEM. indicates *significant differences compared to the non-pregnant, P≤0.05.

The results show an increased concentration of thyroid-stimulating hormone (TSH) (µlU/mL) in the third trimester (3.436 ± 0.096) (µlU/mL) compared with the second trimester and first trimester (3.08 ± 0.1493), (2.252 ± 0.112) (µlU/mL) respectively; the significant difference (p-value <0.0001) The measurement of TSH (µlU/mL) showed an significant difference was present in mean values between the first trimester and with second trimester (p-value <0.0001); the significant difference in mean values between the first trimester and third trimester (p-value<0.0001); the non-significant difference in mean values between second trimester and third trimester (p-value = 0.1119), As shown in Figure 3-7.

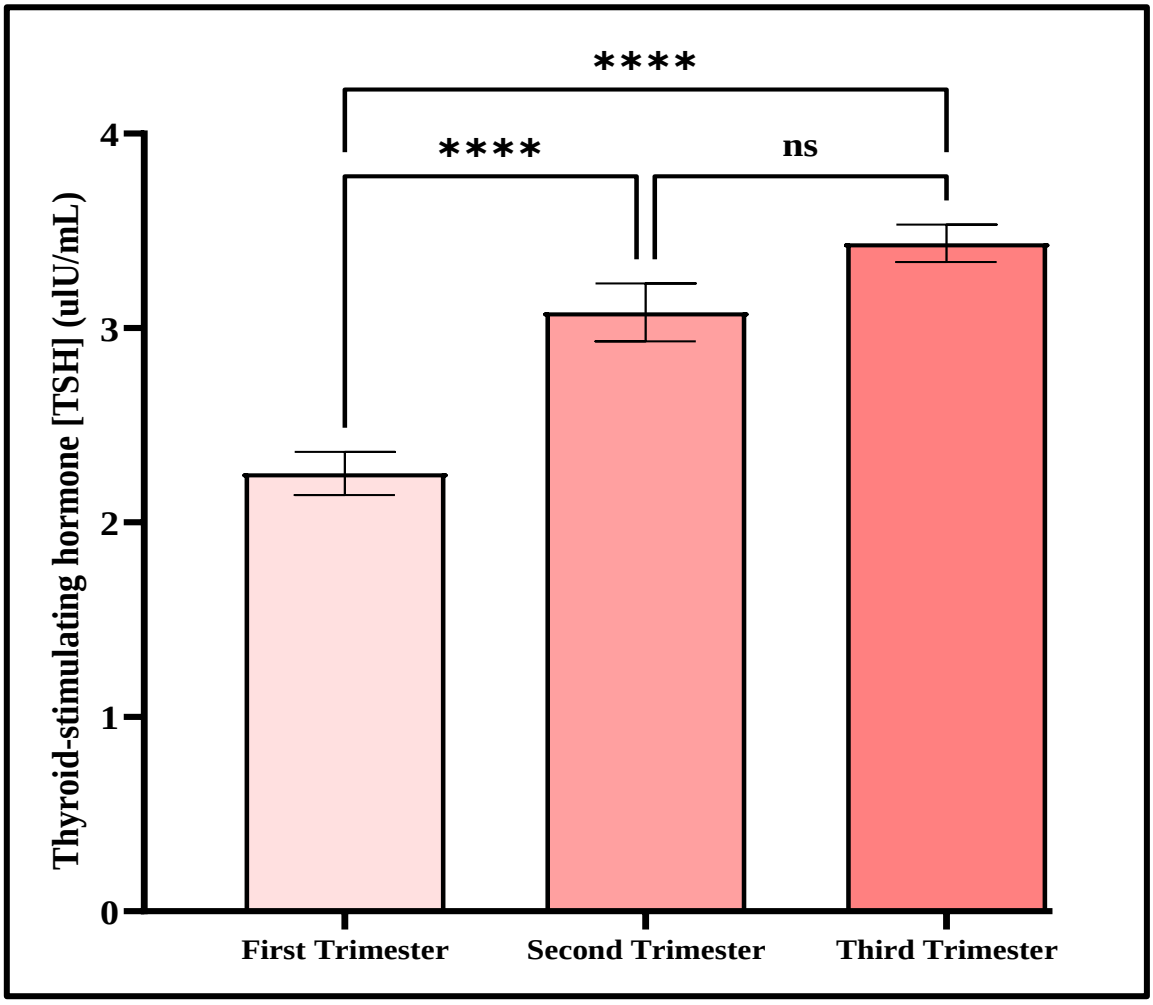

Figure 3-7: Estimation of serum thyroid-stimulating hormone (TSH) (µlU/mL) in Stages of Pregnancy. First Trimester (0-13 Weeks), Second Trimester (14-26 Weeks) and Trimester (27-40 Weeks)

Thyroid stimulating hormone is a glycoprotein hormone produced by the anterior pituitary. It is the primary stimulus for thyroid hormone production by the thyroid gland. It also exerts growth effects on thyroid follicular cells leading to enlargement of the thyroid. TSH has been widely accepted as the universal screening tool for thyroid dysfunction in patients, including pregnant women. The physiological log/linear reverse correlation found between serum TSH and thyroxine (T4) concentrations and the excellent sensitivity of the pituitary to detect abnormal T4 values corresponding to the genetically adjusted T4 setpoint contribute to the superiority of TSH in providing reliable detection of abnormal values within the individual. There are strong arguments implying that commonly

available T4 immunoassays may not work well during pregnancy. The outcomes of our present study offered higher levels of TSH in comparison with non-pregnant groups. This elevation may be due to the decline in HCG levels as pregnancy progresses and this decline induces an increase in TSH (164).

Our current study results were consistent with the results of another study (165) which showed that among pregnant women who had their TSH measured in the first trimester, 62.8% had a TSH level greater than 2.5 mU/L, with 7.4% greater than 10 mU/L. Women with TSH greater than 2.5 mU/L in the first trimester had an increased risk of miscarriage. While another study exhibited that the largest decrease in serum TSH is observed during the first trimester because of elevated levels of serum hCG directly stimulating the TSH receptor and thereby increasing thyroid hormone production. Human chorionic gonadotropin (HCG) levels increase following fertilization and peak at 10~to 12 weeks of gestation, leading to a rise in the total serum T4 and T3 concentrations and subsequently reduction of thyrotropin-releasing hormone (TRH) and TSH levels as a result of negative feedback (166).

Therefore, we have focused our attention, particularly on the TSH reference interval. When comparing the TSH reference interval between pregnancy and the manufacturer's proposed reference limits, upper TSH reference limits showed a noticeable high during the first trimester. Even though a decrease was observed as the pregnancy progressed, cut-off values had not returned to the non-pregnant levels by the following trimesters. The upper and the lower TSH reference limits decreased compared with the non-pregnant in the first trimester. The high TSH reference limits also decreased throughout pregnancy and reached times the non-pregnant level at the second and third trimesters.

3.5 Parathyroid hormone

The results show increased levels of concentration of parathyroid hormone (PTH) (pg/mL) in pregnant as compared with the non-pregnant group (277.7 ± 6.703),(72.58 ± 2.018) (pg/mL) respectively; the significant difference (p-value <0.0001). as shown in figure (3-8).

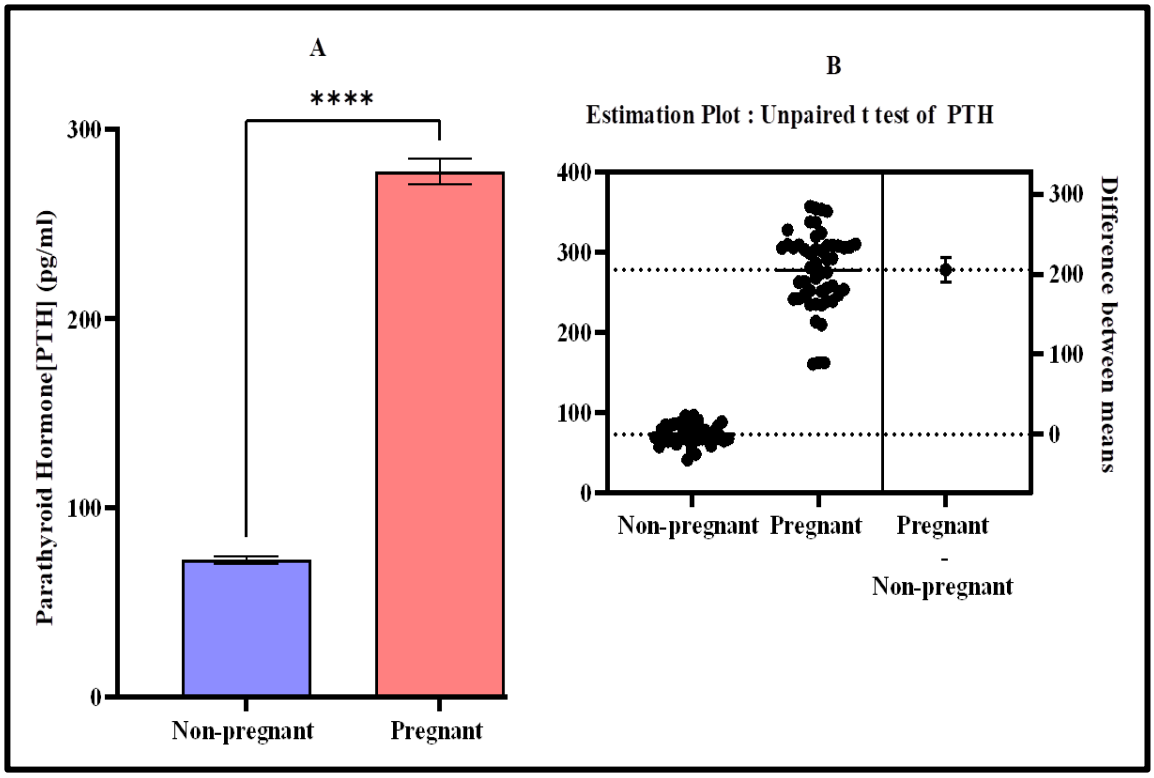

Figure 3-8: Estimation of serum parathyroid hormone (PTH) (pg/mL). (A) a comparison between the non-pregnant and pregnant group, (B) an estimation plot that illustrates the presence of a significant increase in the level of PTH in the pregnant group as compared to the non-pregnant, the significant difference (*p-value <0.0001*). Data are expressed as means ± SEM. indicates *significant differences compared to the non-pregnant, P≤0.05.

The results show an increased concentration of parathyroid hormone (PTH) (pg/mL) in the third trimester (290.9± 11.19) (pg/mL) compared with the second trimester and first trimester (285.8± 7.642), (253.5 ± 13.2) (pg/mL) respectively; the significant difference (p-value = 0.0431) The measurement of PTH (pg/mL) showed an insignificant difference was present in mean values between the first trimester and with second trimester (p-value = 0.1242); the significant difference in mean values

between the first trimester and third trimester (p-value= 0.0497); the non-significant difference in mean values between second trimester and third trimester (p-value = 0.9451), As shown in Figure 3-9.

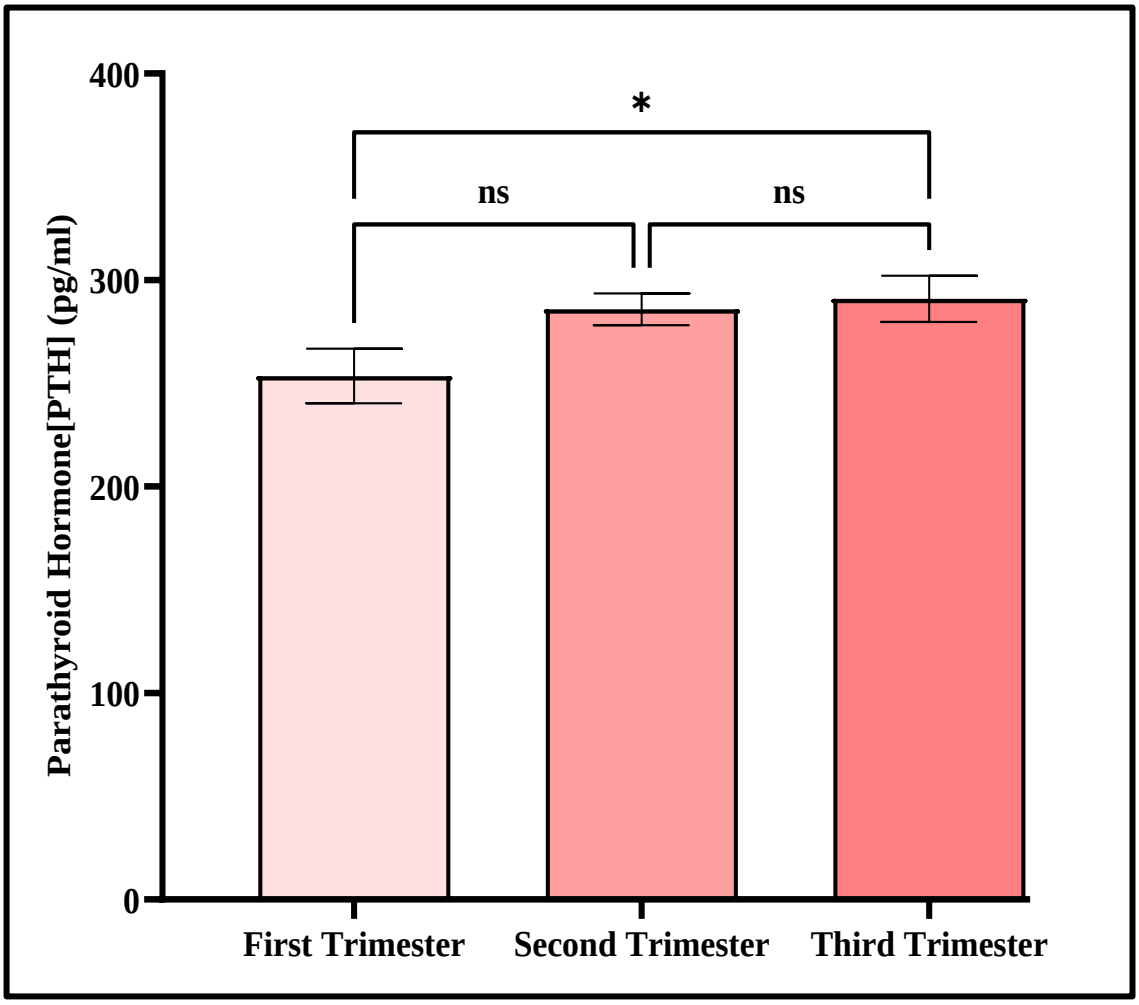

Figure 3-9: Estimation of serum parathyroid hormone (PTH) (pg/mL)in Stages of Pregnancy. First Trimester (0-13 Weeks), Second Trimester (14-26 Weeks) and Trimester (27-40 Weeks)

Parathyroid hormone (PTH) is a very essential hormone in calcium homeostasis. It has a very short half-life of 5 min and is influenced by subtle changes in serum calcium levels. Calcium requirement increases during pregnancy. Maternal PTH levels are positively associated with birth weight, fetal upper arm, and calf circumferences(167,168). Parathyroid hormone regulates fetoplacental mineral homeostasis and skeletal development and stimulates placental calcium transfer(169).

Parathyroid hormone is involved in several processes, in particular maintenance of ionized calcium in the blood, raising the level of calcium phosphate released from bone tissue, conserving calcium, reducing tubular phosphate reabsorption, and increasing the intestinal absorption of calcium through vitamin D (170). Our estimate showed a highly significant elevation of parathyroid hormone in pregnant women as compared with non-pregnant women. In addition, our estimate is similar to estimates obtained in populations in other studies performed during pregnancy (111,171,172).

This increase in PTH in our study results and findings of other studies may be interpreted as follows: Half of the serum calcium circulates bound to plasma proteins, primarily albumin. During pregnancy, due to hypoalbuminemia, increased renal clearance, and placental transfer to the fetus, total serum calcium is slightly decreased. As a result, the parathyroid glands secrete a parathyroid hormone to maintain calcium homeostasis (173,174). Previous reports found incompatible outcomes with our findings and these reports revealed that PTH concentrations are suppressed into the low normal range and may even decline below the normal range (175). During pregnancy, a remarkable series of physiologic adaptations aimed at preserving maternal calcium homeostasis and at providing the requirements for growth and skeletal mineralization of the fetus occurs. The increase in serum 1,25(OH) 2D levels during pregnancy may be the primary factor responsible for the maintenance of maternal calcium homeostasis(176–179). The stimulus for enhanced renal and/or placental 1-alpha-hydroxylase activity is unclear. Some investigators have found a rise in PTH levels during pregnancy and suggested that a PTH increase could stimulate 1,25(OH) 2D synthesis(177,180–182).

Conflicting information regarding PTH concentrations in pregnancy may reflect the use of antibodies with different specificities to PTH and the

heterogeneity of inactive fragments of the hormone that result from peripheral metabolism and from increased glomerular filtration that occurs in pregnancy(177,183–186). Naylor et al. reported a longitudinal study with 16 subjects. PTH levels were found to decrease by 47% during the first trimester of pregnancy and subsequently increased but remained below baseline(178,187,188).

A study by Ardawiet et al. found that intact-PTH concentrations increased from 1.31 pmol/l in the first trimester to 2.26 pmol/l in the second trimester, but then declined to values of the first trimester and increased significantly postpartum. While pregnancy-induced, increase in calcitriol concentration was postulated to be the primary mediator of changes in maternal calcium metabolism(189). Similarly, Rasmussen et al. in their study on 20 healthy pregnant women concluded that pregnancy is not associated with a state of physiological hyperparathyroidism(190). It is known that parathyroid hormone (PTH) levels are increased in women during pregnancy and lactation(191). Pitkin et al(192) suggested that physiologic hyperparathyroidism exists during pregnancy(193). In this regard, Heaney and Skillman(194) found that intestinal absorption of calcium doubled in pregnancy, a fact they attributed, in part, to increased PTH. Perhaps the osteolytic activity of PTH is countered by the hypersecretion of calcitonin. This action may permit the calciumretaining actions of PTH to be exerted on the gut and kidney while the calcium needs of the fetus are met, thus, sparing the maternal skeleton.

3.6 Calcitonin

The results show increased levels of Concentration of calcitonin (CAL) (pg/mL) in pregnant as compared with the non-pregnant group (21.89 ± 0.624),(9.65 ± 0.303) (pg/mL) respectively; the significant difference (p-value <0.0001). as shown in figure (3-10).

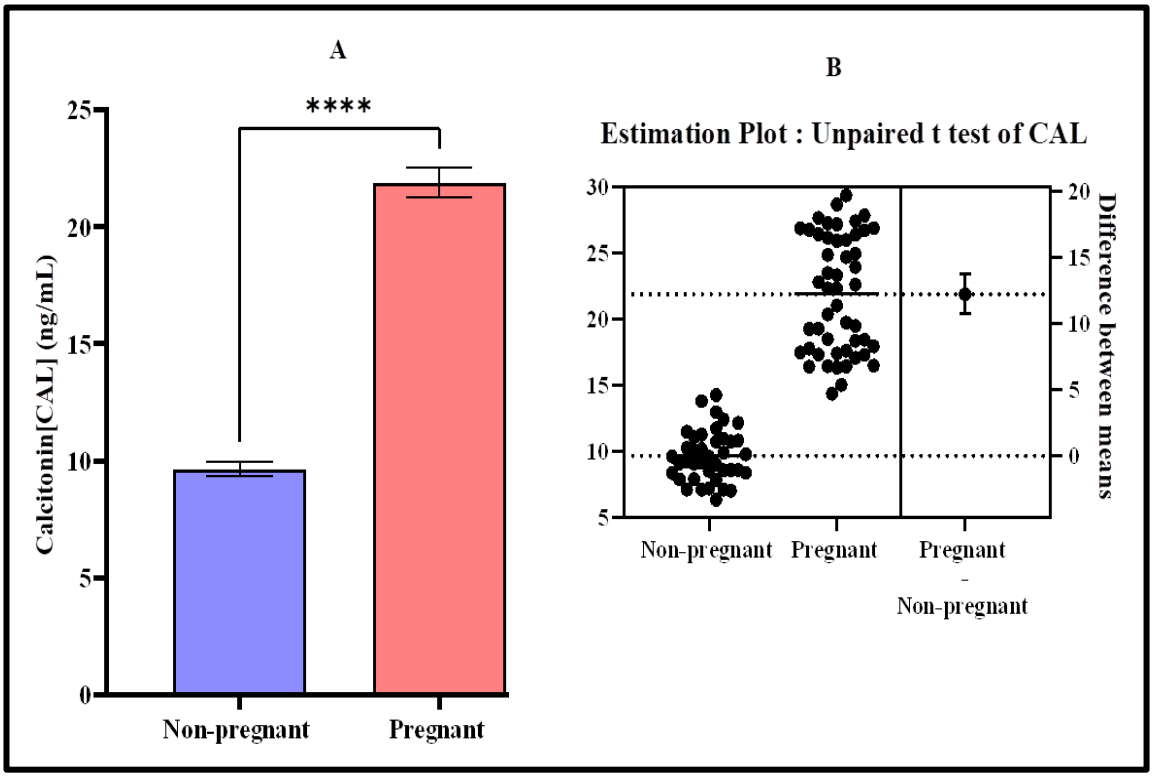

Figure 3-10: Estimation of serum calcitonin (CAL) (pg/mL). (A) a comparison between the non-pregnant and pregnant group, (B) an estimation plot that illustrates the presence of a significant increase in the level of CAL in the pregnant group as compared to the non-pregnant, the significant difference (*p-value <0.0001*). Data are expressed as means ± SEM. indicates *significant differences compared to the non-pregnant, P≤0.05.

The results show an increased concentration of calcitonin (CAL) (pg/mL) in the third trimester (25.55± 0.8794) (pg/mL) compared with the second trimester and first trimester (21.21± 0.9156), (19.65 ± 0.9495) (pg/mL) respectively; the significant difference (p-value = 0.0003). The measurement of CAL (pg/mL) showed an insignificant difference was present in mean values between the first trimester and with second trimester (p-value = 0.4368); the significant difference in mean values between the first trimester and third trimester (p-value= 0.0002); the significant difference in mean values between second trimester and third trimester (p-value = 0.0059), As shown in Figure 3-11.

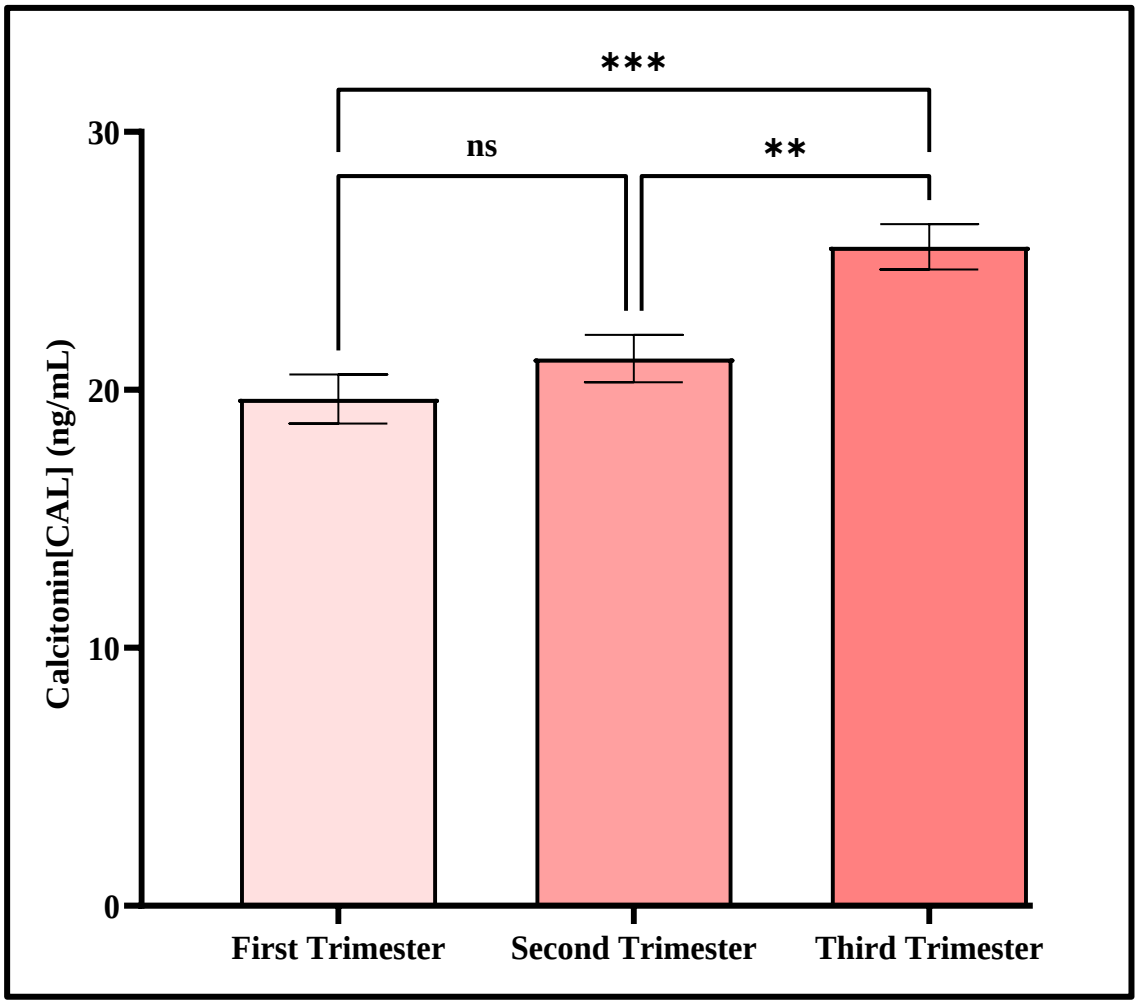

Figure 3-11: Estimation of serum calcitonin (CAL) (pg/mL) in Stages of Pregnancy. First Trimester (0-13 Weeks), Second Trimester (14-26 Weeks) and Trimester (27-40 Weeks)

Calcitonin is a hormone that is produced and released by the C-cells of the thyroid gland. Its biological function in humans is to have a relatively minor role in calcium balance(195,196). Circulating levels of calcitonin are high during pregnancy. The most likely sources are hypertrophied C cells of the thyroid and possibly from the breast and placenta. Though postulated to affect the maternal bone, human studies have not convincingly shown any significant effect of calcitonin on calcium metabolism during pregnancy(197,198).

Pregnancy has a profound impact on thyroid homeostasis which results in a change in thyroid function and thyroid volume (TV). Moreover,

calcitonin (CT), and its gene-related peptide have been demonstrated to play an important role in the implantation process. Our findings found elevated levels of serum calcitonin in pregnant women in comparison with non-pregnant women. The consequences were agreed with the findings of another study which exhibited that the levels of this hormone were determined in 56 pregnant women in all trimesters and found to be above normal at 72 %.

The high serum calcitonin levels seen during pregnancy gradually fall to normal a few weeks postpartum. Its role in bone physiology in human pregnancy is unclear(197,198). Konopka et al(34) measured the serum calcitonin of no pregnant and pregnant women by bioassay and found that 57.4 % of pregnant women had increased values(34). The increase during gestation was statistically significant in the second and third trimester. Values in the second and third trimesters were similar, but values postpartum were slightly higher. These authors proposed that hypercalcitonemia may serve to protect the skeleton against demineralization during pregnancy. Samaan *et al* first reported that CT levels were increased in women at delivery(168).

It would appear that the hypercalcitonemia of pregnancy is not explicable exclusively based on maternal to fetal calcium transfer or hyperestrogenism. If the high CT levels measured were the result exclusively of increased mobilization and transport of calcium from the mother to fetus, this hormone would be expected to be at its highest during the third trimester, when the fetus accumulates the bulk of its calcium(199), which supports the findings of the current study. However, there was no significant increase in calcitonin from the second to the third trimester. Similarly, estrogen production by the placenta is not likely to be the sole stimulus to calcitonin secretion since placental estrogen increases progressively until delivery(200,201).

Pregnancy and perinatal periods are hallmarked by alterations in calcium homeostasis. The regulation of calcium homeostasis involves PTH, 1,25 dihydroxy vitamin D (1,25 (0H)*D) and calcitonin (CT) but the exact role of each in pregnancy and on the 1st day of life is not well understood. Some authors found high amino-terminal PTH levels and increased biological activity of PTH in the third trimester of pregnancy whereas others showed normal values of the carboxyl-terminal, amino-terminal, and intact hormone(108,202,203). The increased levels of CT during gestation propose that this hormone plays a role in the defence of the maternal skeleton.

3.7 Correlations Biochemical Characteristics

The correlations in biochemical characteristics in thyroid function in women the pregnant are shown in Table 3.3. There was no significant correlation among all characteristics except for a significant correlation between T4 and PTH (r = 0.3136; p = 0.0266; 95% CI= 0.03865 to 0.5444; R squared=0.09836).; T3 and CAL (r = 0.3381; p = 0.0163; 95% CI= 0.06597 to 0.5634; r squared=0.1143)

The correlations in biochemical characteristics in thyroid function in women the non-pregnant are shown in Table 3.4. There was a significant correlation among all characteristics except for a negative significant correlation between; (PTH and CAL); (PTH and TSH); (T3 and CAL); (T3 and TSH) and (T4and CAL).

Table 3.2: Correlations of biochemical characteristics in pregnant

	Pearson r	Age (years)	BMI (kg/cm^2)	PTH (pg/mL)	T3 (ng/mL)	T4 (nmol/L)	CAL (pg/mL)	TSH (µlUg/mL)
Age	r		0.1905	-0.06983	-0.1722	0.009668	-0.1761	-0.1061
	95% CI		-0.1056 to 0.4556	-0.3530 to 0.2250	-0.4405 to 0.1243	-0.2814 to 0.2991	-0.4437 to 0.1203	-0.3846 to 0.1900
	R squared		0.0363	0.004877	0.02966	0.00009346	0.03101	0.01127
	P value		0.2047	0.6447	0.2524	0.9492	0.2417	0.4826
PTH	r				-0.2598	0.3136	-0.1776	-0.08229
	95% CI				-0.5018 to 0.02002	0.03865 to 0.5444	-0.4345 to 0.1060	-0.3526 to 0.2007
	R squared				0.06748	0.09836	0.03154	0.006771
	P value				0.0685	0.0266	0.2172	0.57
T3	r					-0.159	0.3381	0.1743
	95% CI					-0.4188 to 0.1249	0.06597 to 0.5634	-0.1093 to 0.4317
	R squared					0.02527	0.1143	0.0304
	P value					0.2702	0.0163	0.2259
T4	r						0.0008825	-0.1254
	95% CI						-0.2775 to 0.2792	-0.3901 to 0.1585
	R squared						7.788E-07	0.01572
	P value						0.9951	0.3857
CAL	r							0.193
	95% CI							-0.09017 to 0.4473
	R squared							0.03726
	P value							0.1793

r: correlation coefficient**;CI:** Confidence Interval**; BMI:** Body Mass Index**;PTH:** Parathyroid Hormone**;T3:** Triiodiothyronin**;T4:** Thyroxine**;CAL:** Calcitonin**;TSH:;** Thyroid-Stimulating Hormone

Table 3.3: Correlations of biochemical characteristics in non-pregnant

	Pearson r	Age (years)	BMI (kg/cm^2)	PTH (pg/mL)	T3 (ng/mL)	T4 (nmol/L)	CAL (pg/mL)	TSH (µlUg/mL)
Age	r	1	-0.8655	0.8991	-0.8644	0.8147	-0.3742	0.3334
	95% CI		-0.9223 to -0.7723	0.8279 to 0.9417	-0.9211 to -0.7717	0.6937 to 0.8910	-0.6141 to -0.07097	0.02447 to 0.5843
	R squared		0.7491	0.8083	0.7471	0.6638	0.14	0.1112
	P value		<0.0001	<0.0001	<0.0001	<0.0001	0.0174	0.0355
PTH	r			1	-0.9045	0.8922	-0.1781	0.2195
	95% CI				-0.9449 to -0.8368	0.8167 to 0.9377	-0.4639 to 0.1412	-0.09875 to 0.4970
	R squared				0.8181	0.7961	0.03173	0.04819
	P value				<0.0001	<0.0001	0.2715	0.1735
T3	r				1	-0.9507	-0.1617	0.3092
	95% CI					-0.9719 to -0.9144	-0.4505 to 0.1577	-0.002521 to 0.5662
	R squared					0.9039	0.02616	0.09562
	P value					<0.0001	0.3188	0.0522
T4	r					1	-0.1032	-0.2766
	95% CI						-0.4018 to 0.2153	-0.5415 to 0.03817
	R squared						0.01065	0.07652
	P value						0.5264	0.084
CAL	r						1	-0.3132
	95% CI							-0.5692 to -0.001852
	R squared							0.09808
	P value							0.0491

r: correlation coefficient**;CI:** Confidence Interval**; BMI:** Body Mass Index**;PTH:** Parathyroid Hormone**;T3:** Triiodiothyronin**;T4:** Thyroxine**;CAL:** Calcitonin**;TSH:;** Thyroid-Stimulating Hormone

3.8 The diagnostic performance of biomarkers in thyroid function in women with pregnant

To evaluate the diagnostic performance of thyroid function in pregnant, receiver operator characteristic (ROC) curve analysis was

performed and the results are shown in Figures 3.13 through 3.17 and Table 3.4.

Regarding T3, the cutoff value was ≤8.033 (ng/mL) with 100 % sensitivity, and 100 % specificity, as shown in Figure 3.13 and Table 3.4. Regarding T4, the cutoff value was ≤252.824(nmol/L)with 100 % sensitivity, and 92.5% specificity, as shown in Figure 3.14 and Table 3.4. Regarding TSH, the cutoff value was >1.808 (µlU/mL)with 94 % sensitivity, and 100 % specificity, as shown in Figure 3.15 and Table 3.4.

Regarding CAL, the cutoff value was >14.26 (pg/mL)with 100 % sensitivity, and 100 % specificity, as shown in Figure 3.16 and Table 3.4. Regarding PTH, the cutoff value was >95.997 (pg/mL)with 100 % sensitivity, and 100 % specificity, as shown in Figure 3.17 and Table 3.4.

Table 3.4: Characteristics of ROC curves of T3, T4, TSH, CAL and PTH as predictors of pregnant diagnosis

Characteristic	T3 (ng/mL)	T4 (nmol/L)	TSH (µlU/mL)	CAL (pg/mL)	PTH (pg/mL)
Cutoff	**≤8.033**	**≤252.824**	**>1.808**	**>14.26**	**>95.997**
AUC	**1**	**0.995**	**0.984**	**1**	**1**
Standard Error a	**0**	**0.00384**	**0.01**	**0**	**0**
95% CI	**0.960 to 1.000**	**0.950 to 1.000**	**0.932 to 0.999**	**0.960 to 1.000**	**0.960 to 1.000**
P value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Sensitivity %	**100**	**100**	**94**	**100**	**100**
Specificity %	**100**	**92.5**	**100**	**100**	**100**

AUC: Area under the ROC curve**;CI:** Confidence Interval**; BMI:** Body Mass Index**;PTH:** Parathyroid Hormone**;T3:** Triiodiothyronin**;T4:** Thyroxine**; CAL:** Calcitonin**;TSH:** Thyroid-Stimulating Hormone

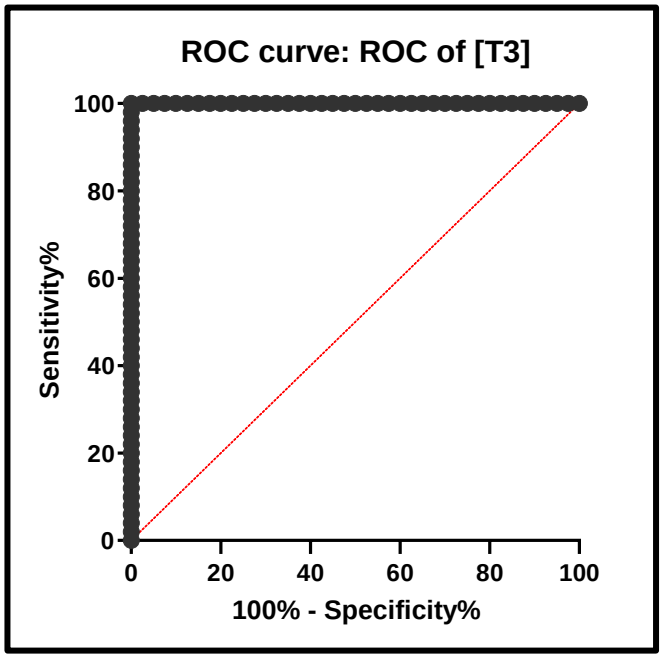

Figure 3-13: Receiver operator characteristic (ROC) curve analysis to find the best serum T3 cutoff value that can predict a diagnosis of pregnant

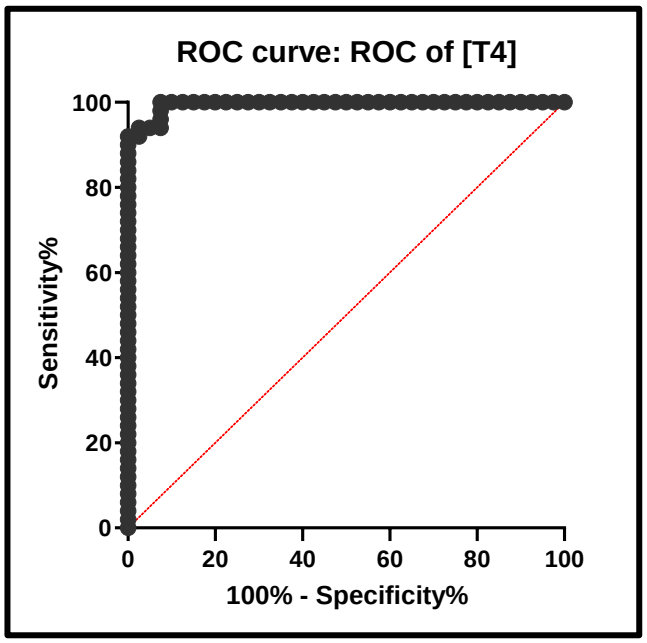

Figure 3-14: Receiver operator characteristic (ROC) curve analysis to find the best serum T4 cutoff value that can predict a diagnosis of pregnant

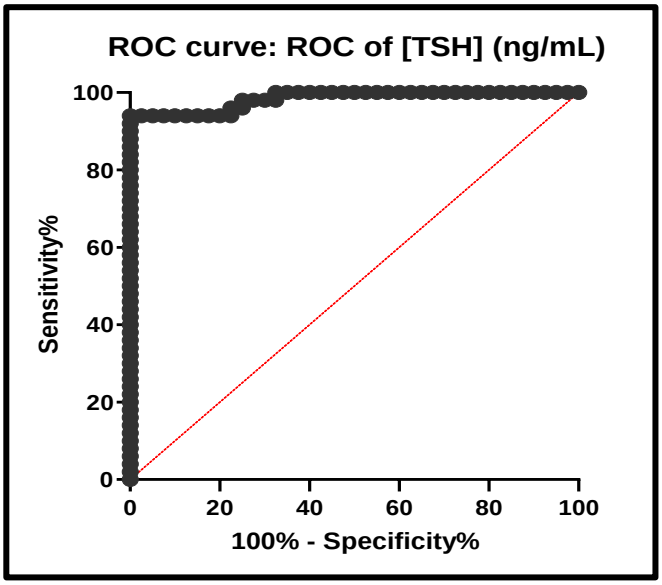

Figure 3-15: Receiver operator characteristic (ROC) curve analysis to find the best serum TSH cutoff value that can predict a diagnosis of pregnant

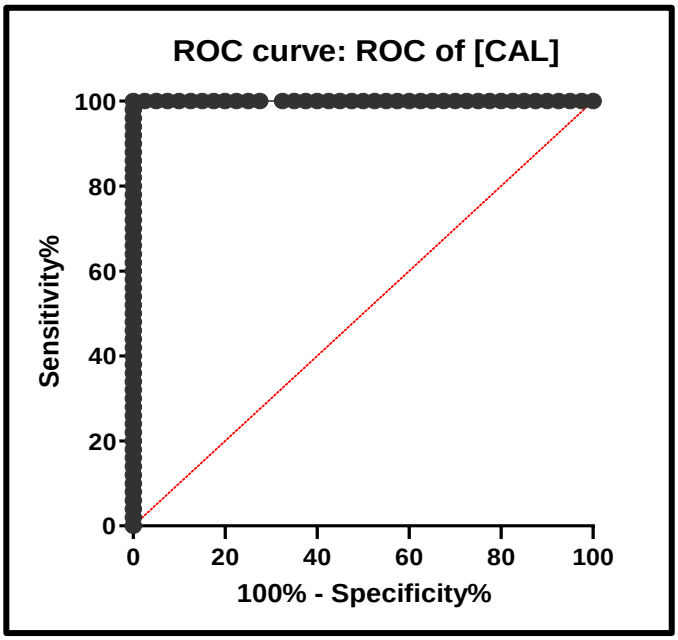

Figure 3-16: Receiver operator characteristic (ROC) curve analysis to find the best serum CAL cutoff value that can predict a diagnosis of pregnant

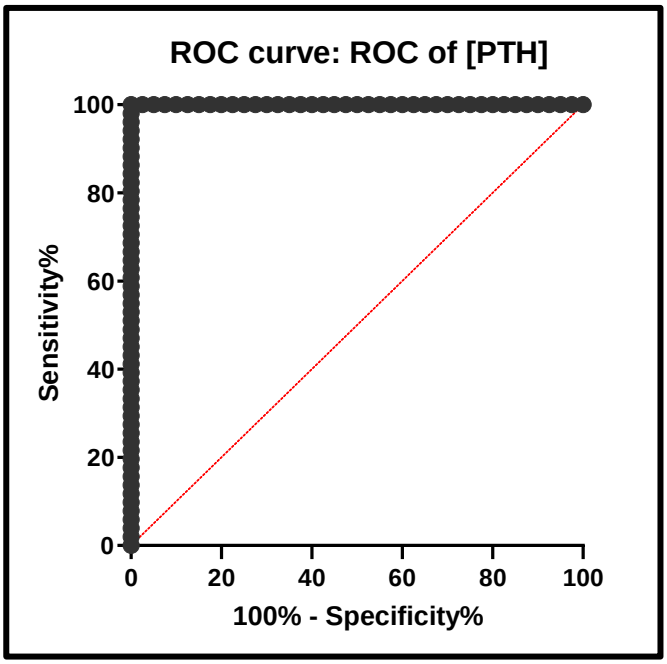

Figure 3-17: Receiver operator characteristic (ROC) curve analysis to find the best serum PTH cutoff value that can predict a diagnosis of pregnant

Conclusions and Recommendations

Conclusions

We conclude that pregnant women experience significant hormonal changes in each of the thyroid and parathyroid hormones, which indicates the necessity of diagnosing these changes for the success of this pregnancy.

Recommendations

The following recommendations are presented as a starting point for future studies and research:

1. Calcium and phosphorous levels are affected by vitamin D insufficiency, thus they should be monitored on a regular basis.
2. Measurement of thyroid hormones at the beginning of pregnancy to avoid the disorders of thyroid hormones disorders and the dangers to the fetus.
3. Providing vitamin supplements in health centers that are related to the factors of delayed childbearing is a point that must be looked at with attention.

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الخلاصة

الخلفية العلمية : ان أمراض الغدة الدرقية شائعة أثناء الحمل والعلاج المناسب مهم لمنع النتائج السلبية للأم والجنين. قد تؤدي التغيرات الفسيولوجية في الحمل إلى تغيرات كبيرة في هرمون الغدة الدرقية ، وشوارد المصل والبول. قد تؤثر العمليات الفيزيولوجية المرضية الخاصة بالحمل على نتائج اختبارات الغدة الدرقية. يتطلب التحقيق في اضطرابات الغدة الدرقية أثناء الحمل معرفة هذه التغييرات والوعي بسلامة اختبار الهرمونات الديناميكية والتصوير للأم والجنين.

المواد والطرق: شملت دراسة الحالات والشواهد 40 حامل تتراوح أعمارهم (18-40 سنة). وكذلك المرأة التي تبدو بصحة جيدة(غير الحامل) هناك مجموعة من الأعمار (18-40 سنة). تم تسجيل المشاركين في هذه الدراسة بين نوفمبر 2022 ومارس 2023 في مستشفى النساء والأطفال في بابل ومستشفى الشوملي العام ، وتم إجراء جميع التحاليل المخبرية في مستشفى النساء والأطفال في بابل ومستشفى الشوملي العام وقسم الكيمياء السريرية والكيمياء الحيوية. كلية الطب جامعة بابل. وقعت جميع المواد على نماذج موافقة خطية مستنيرة. كانت البيانات العامة هي العمر والجنس وفترة الحمل.

النتائج: أظهرت النتائج انخفاضًا معنويًا ($p = 0.0001$) في مستويات تركيز ثلاثي يودوثيرونين (T3)نانوغرام / مل) وهرمون الغدة الدرقية) (T4) نانومول / مل) حيث أظهرت النتائج زيادة معنوية ($p = 0.0001$) مستويات تركيز مرتفعة. الهرمون المنبه للغدة الدرقية / ulU) (TSH) (mL، وهرمون الغدة الجار درقية (pg / mL) (PTH) و الكالسيتونين(CAL) (نانوغرام / مل) في الحمل مقارنة بالمجموعة غير الحوامل. تم فحص مجموعة المريض لم يكن هناك ارتباط معنوي بين جميع الخصائص باستثناء وجود علاقة سلبية معنوية بين ($r = 0.314; p = 0.027$).

الاستنتاجات : نستنتج أن المرأة الحامل تعاني من تغيرات هرمونية كبيرة في كل من هرمونات الغدة الدرقية والغدة الدرقية مما يدل على ضرورة تشخيص هذه التغيرات وعلاجها لإنجاح هذا الحمل.

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ
وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ
وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴿١١﴾

صدق الله العلي العظيم

سورة المجادلة، أية (١١)

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية الطب

دراسة التغيرات الهرمونية للغدة الدرقية والجارات الدرقية عند الحوامل مقارنة بالنساء غير الحوامل في مدينة الحلة

رسالة

مقدمة الى مجلس كلية الطب / جامعة بابل
كجزء من متطلبات نيل شهادة الماجستير
في علم الكيمياء الحياتية السريرية

تقدم بها

حسام شلمان حمادي
بكالوريوس علوم كيمياء جامعة القادسية (2018)

بأشراف

أ.د عبد السميع حسن الطائي
أ.م.د بان عامر موسى

تموز 2023 م
محرم هـ 1444