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Effect of Hypothyroidism on Reproductive Hormones in Women

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Supervisor 's Certificate

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"has been prepared under my supervision at the College of Science, University of Misan; as a partial fulfillment of the requirements for the degree of Master of Biology

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Recommendation of Head of Biology Department

In view of the available recommendations; I forward this thesis to debate by the examining committee.

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(وَآخِرُ دَعْوَاهُمْ أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ)

صدق الله العلي العظيم

سورة يونس – الآية (١٠)

Dedication

To my trust and hope …. My God

To my role in life …….. father

To her pure soul……. mother

To whom supported me….. my brothers and sisters

To my friends

To everyone who helped me…. my teachers

To all who participated in the research… my patients

Eman Kamil

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Summary

Summary

The present study aimed to measure some of hormonal and biochemical parameters in women suffered from hypothyroidism in Misan province. The study included 88 women aged (20-35) years who reviewed by AL-Sader Teaching Hospital and the Diabetes and Endocrine Center for the period from 4/12/2018 – 1/6/2019. The women were divided into four groups and each group had 22 women as follows: group(1) control(healty) group, group (2) women with Infertility, group (3) women with hypothyroidism and group (4) women with hypothyroidism and Infertility. Blood samples was collected for measurement the levels of some hormonal and biochemical parameters.

The results of Thyroid Stimulating Hormone (TSH) in the third and fourth groups increased significantly($P<0.05$) compared with first and second groups , while results of Triiodothyronine (T3) and Thyroxine (T4) decreased significantly($P<0.05$) in the third and fourth groups compared with first and second groups.

The results of Follicle Stimulating Hormone (FSH) showed increased significantly($P<0.05$) in the fourth group compared with first , second and third groups, the results of progesterone and Anti-Mullerian Hormones showed decreased significantly($P<0.05$) in the third and fourth groups compare with first and second groups, the values of total testosterone decreased significantly($P<0.05$) in the third and fourth groups ,the values of Estradiol hormone(E2) showed increased significantly($P<0.05$) in the second and third groups compare with first and fourth groups . No significant differences ($P>0.05$) in the results of Luteinizing Hormone (LH) and Prolactin (PRL) among four groups.

The results of Triglyceride (TG) had increased significantly($P<0.05$) in the second and third groups compare with first and fourth groups, while the results of Low Density Lipoprotein-Cholesterol (LDL-C) significant increase($P<0.05$) in third and fourth groups compare with first and second groups , the results of Very Low Density Lipoprotein-Cholesterol (VLDL-C) showed significant increase ($P<0.05$) in the second and third groups compare with first and fourth groups . No significant differences ($P>0.05$) in Total Cholesterol(TC) , High Density Lipoprotein-Cholesterol(HDL-C) , Glucose and Total Protein among the four groups.

Table of contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Meaning
A	Absorbance
AITD	Autoimmune thyroid disease
AMH	Anti Mullerian Hormone
bv	Blood vessels
C	Capsule
DIT	Diiodo-L-Thyroxine
DUOX1	Dual Oxidase 1
DUOX2	Dual Oxidase 2
D1	Iodothyronine Deiodinase 1
D2	Iodothyronine Deiodinase 2
eGFR	Estimated glomerular filtration
ELISA	Enzyme Linked Immune Sorbent Assay
E2	Estradiol
F	Follicle
FBG	Fasting Blood Glucose
FOR	Follicles ovarian reserve
FPG	Fasting Plasma Glucose
FSH	Follicle Stimulating Hormone
GC	Granulosa Cells
GDM	Gestational Diabetes Mellitus
GLUT-4	Glucose Transporter Type 4
GnRH	Gonadotropin Releasing Hormone
GOD-PAP	Glucose Oxidase -phenol and 4 aminophenazone
HCG	Human Chorionic Gonadotropin
HDL	High Density Lipoprotein
HPT	Hypothalamus pituitary thyroid
HRP	Horseradish peroxidase

H2O2	Hydrogen Peroxidase
I	Iodine
IVF	In vitro fertilization
LDL	Low Density Lipoprotein
LH	Luteinizing Hormone
LH-RH	Luteinizing hormone-releasing hormone
LPL	Lipoprotein Lipase
MCT8	Monocarboxylate Transporter 8
MIT	Monoiodo-L-Thyroxine
NIS	Symporter of Sodium / Iodide
PRL	Prolactin
rT3	Reverse Triiodothyronine
SCH	Subclinical Hypothyroidism
SHBG	Sex Hormone Binding Globulin
TB	Trabecule
TC	Total Cholesterol
TG	Triglyceride
THs	Thyroid Hormones
Tg	Thyroglobulin
TMB	tetramethylbenzidine
TP	Total Protein
TPO	Thyroid Peroxidase
TRAb	TSH-receptor antibody
TREs	Thyroid hormone Reaction Elements
TRH	Thyroid Releasing Hormone
TRS	Thyroid Receptors
TSH	Thyroid Stimulating Hormone
TT	Total Testosterone
T2	Diiodothyronine

T3	Triiodothyronine
T4	Thyroxine
VLDL	Very Low Density Lipoprotein
VIP	Vasoactive Intestinal Peptide

Chapter One
Introduction

1: Introduction

The thyroid gland is one of the largest endocrine glands that found in the body, this gland is located in the neck infront of the thyroid cartilage (in men it's also known the Adam's apple) and at about the same level as the cricoid cartilage (Gilleron *et al*., 2006). The human thyroid gland comprises of two lobes that are lateral and below the anterior part of the larynx and are linked by an isthmus across the larynx to create a U-shaped structure with an average weight of 25 gm in adults and surrounded by a fibrous capsule (Waugh and Grant ,2014).

The thyroid gland mobilizes nutritional iodine, making it an organic compound that can accelerate the procedures of metabolism, it is essential to growth and functioning to all body cells (Wartofsky and Burman,1982).Thyroid hormones (THs) play a critical role in differentiation, development, and metabolism. In fact, THs are needed for almost all tissues to operate normally, with significant impacts on oxygen consumption and metabolic rate (Mazzaferri *et al*.,2003).

THs are released by follicular cells of thyroid gland its true structure are Thyroxine(T4) and Triiodothyronine(T3) is the overwhelming dynamic structure accessible for use(Bianco and Kim,2006).

Disorders of the thyroid is usually acquired and can occur at any time in life, thyroid autoimmunity is the most prevalent cause of thyroid disorder in the reproductive age of females (Vanderpump *et al*.,1995;Hollowell *et al*.,2002).

Hypothyroidism manifestations may be triggered by an iodine deficiency and subsequent absence of precursor moods for hormonal drugs (easy hyperplastic goiter characterized by gland compensatory enlargement), thyrotropic factor deficiency or other metabolic

irregularities, adding iodine to the diet or administering thyrotropin, respectively, can correct the first two causes. Naturally, thyroid hormone replacement treatment may be used for deficiencies of any origin (Nuovo and Wartofsky,1995).

There are two separate thyroid receptors(TRs) are TRα and TRβ found on chromosome 17 and 3 (Gurnell *et al*.,2010). The quality of each thyroid hormone receptor experiences interchange grafting to generate TRα1, TRα2, TRβ1, and TRβ2 isoforms, each with contrasting tissue appropriations (e.g. TRα1 is conveyed overwhelmingly in the focal sensory system, myocardium, colon, and skeletal muscle). TRβ1 is mostly conveyed in the liver and kidney (Huang *et al*.,2008).TRβ2 assumes a remarkable task in adverse criticism guidelines on the hypothalamic and pituitary dimensions (Cheng *et al*.,2010).

TRs were also reported to be present in human ovarian surface epithelium and follow-up on ovarian follicles and show some slight limitation in granulosa cells of ovarian follicle (Aghajanova *et al*., 2009).

Hypothyroidism has been associated with altered ovarian ability, menstrual abnormalities, subfertility, and higher (intermittent) abnormal birth cycle rates, recommending that thyroid hormone affects female reproductive axis(Krassas *et al*.,2008;Van den Boogaard *et al*.,2011).

1-1:The Aims of the Study

The current study aimed to investigate some hormonal and biochemical parameters in fertile and infertile women with hypothyroidism.

1.The hormonal parameters include :

A- Thyroid hormones (Triiodothyronine (T3) and Thyroxine (T4))

B-Thyroid stimulating hormone (TSH).

C-Reproductive hormones (Follicle stimulating hormone (FSH) , Luteinizing hormone (LH) , Prolactin (PRL) , Progesterone , Estradiol (E2) and Total testosterone(TT)

D- Anti-Mullerian hormone (AMH)).

2- Biochemical parameters include:

A-Glucose

B-Total protein

C-Lipid profile (Total cholesterol (TC) , Triglyceride (TG) , High density lipoprotein-cholesterol(HDL-C) ,Low density lipoprotein-cholesterol (LDL-C) and Very low density lipoprotein-cholesterol (VLDL-C)).

Chapter Two
Literature Review

2-Literature Review

2-1: The Endocrine System

The endocrine system is an integrated system of small organs that involve the release of extracellular signaling molecules known as hormones, it is instrumental in regulating metabolism, growth, development and puberty, tissue function, and also plays a part in determining mood (Kester *et al.*, 2004).

Endocrine system is an information signal system much like the nervous system. However, the nervous system uses nerves to conduct information, whereas the endocrine system mainly uses blood vessels as information channels , endocrine glands are groups of secretory cell surrounded by an expansive capillary network that promotes the diffusion of hormones (chemical messengers) from the secretory cells into the bloodstream ,they are also called ductless glands due to the diffusion of hormones directly into the bloodstream , which then transported through the bloodstream to target tissues and organs , where they influence cell growth and metabolism(Waugh and Grant , 2014).

Endocrine signaling is the typical mode of cell signaling in the endocrine scheme. There are also other methods of signaling, however, i.e. paracrine, autocrine, and neuroendocrine , on the other side, pure neurocrine signaling among neurons is entirel part of the nervous system (Casademont , 2005).

Thyroid gland is one of the body's largest endocrine glands, weighing 2-3 grams in neonates and 18-60 grams in adolescents, and increasing during pregnancy, this gland is found infront of the thyroid cartilage (Stagnaro-Green,2000).

2-2: Thyroid Gland

2-2-1:Histophysiology of Thyroid Gland

The thyroid gland is a highly vascularized organ located in the front of and sides of the neck , it is found foremost to the treachea only inferior to the larynx , consisting of two lobes, one on either side of the treachea associated crosswise over a thin band tissue , an isthmus which gives the organ the shape of butterfly , in a healthy adult thyroid weighting around 30 gm (Melmed *et al*.,2012).

The lobes are cone-shaped fit as a fiddle the peak of each being coordinated upwards and laterally to the extent that the intersection of the middle with the lower third of the thyroid ligament the base appears to descend and is on the fifth or sixth treacheal ring dimension. Approximately 50% of individuals have a pyramidal lobe (morgagnis or pyramidal lalouettes) that arises from either flap or the predominant bit of the isthmus and coordinated upward generally to one side (Braun *et al*.,2007).

Histologicaly, thyroid gland divided into lobules, each lobule consist of 20-40 spherical follicles that very impressively in size ,with a diameter ranging from 45-250 μm ,In the newborn , follicles are small and grow slowly, each follicle is lined by a single cuboidal layer of epithelium . Most of the thyroid follicles were lined by cubical cells; a few of them were lined by more than one layer of follicular cells. ,when stimulate become columnar with a thin basement membrane loaded with acidophilic colloid center ,thyrocyte have a distinct polarity, with there apieces directed toward the lumen of the follicles and their basis toward the basement membrane, the apical surface of the epithelial cells have various microvilli stretching out to the colloid , while the spheroid nuclei

are situated at the same level in all cells, for the most part close to their basis (Nilsson and Fagman, 2017).

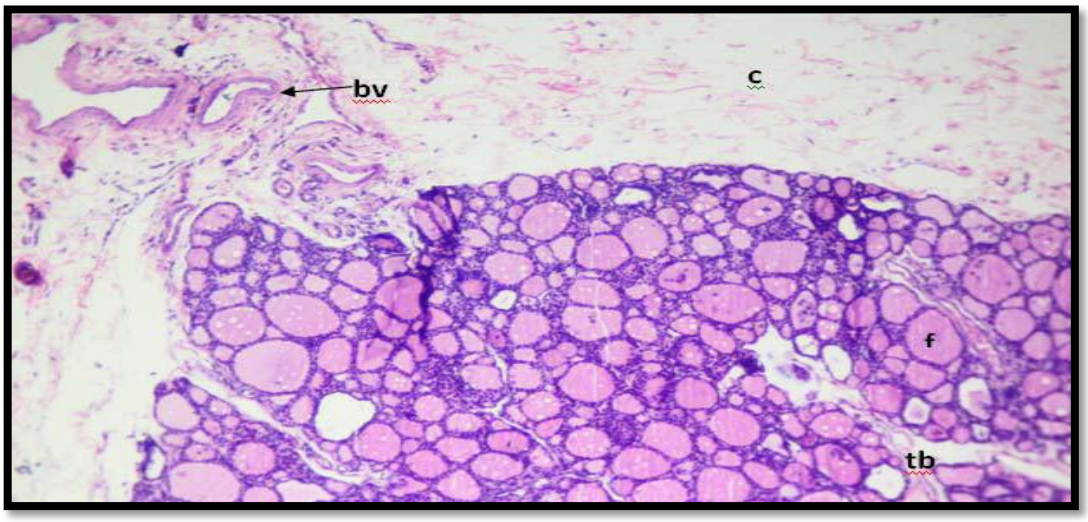
The gland consists of largely spherical epithelial follicles ,these secrete and store colloid, a dense material of sticky protein found in the lumen of the follicle cells(Figure 2-1) (Bergman *et al*.,1989; Al-Aamery and Dauod, 2017) . Parafollicular cells found individually or in small groups are found between the follicles , also called C-cells, which secrete the calcitonin hormone (Waugh and Grant , 2014).

The gland's supply of artrial blood is through the upper and lower thyroid arteries,the upper thyroid artery is an external carotid artery branch and the lower thyroid artery is a subclavian artery branch(Figure 2-2) (Waugh and Grant, 2014).Exceptionally, another artery ,the thyroidal artery(Neubauers artery) originating from either the common carotid or the mysterious troncus might be available (Mohebati and Shaha ,2012).

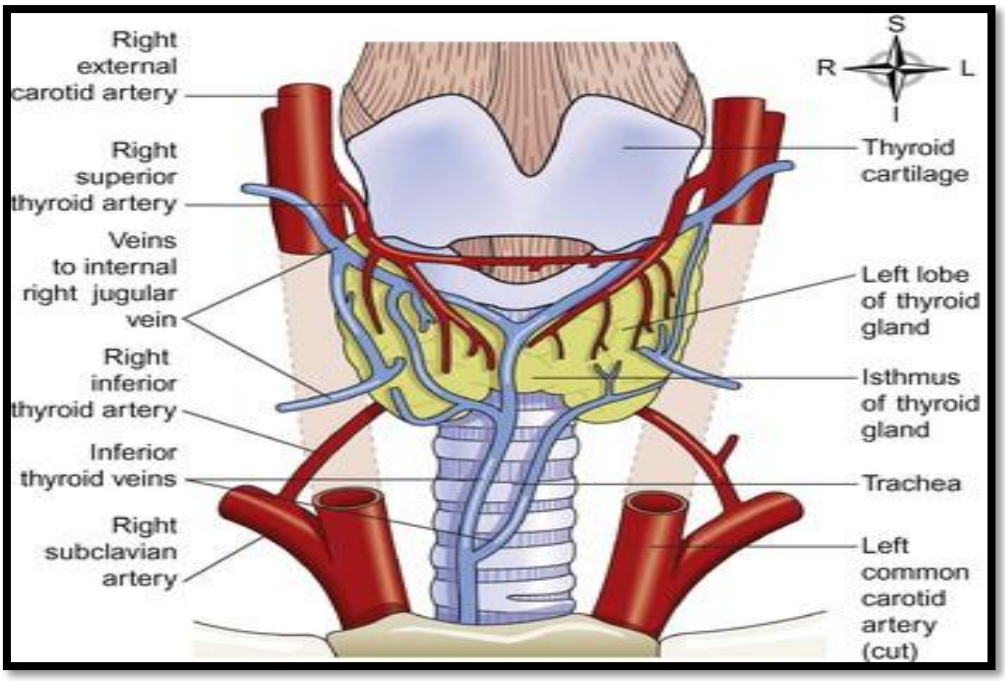
The venous blood is drained through superior thyroid veins, draining in the inner jugular vein, and through inferior thyroid veins ,flowing in the left brachiocephalic vein through the plexus thyroideus impar ,the lateral deep cervical lymph nodes and the pre-and parathracheal lymph nodes frequently pass through lymphatic drainage, the gland is provided by sympathetic nerve input from the sympathetic trunk's superior cervical ganglion and cervicothoracic ganglion, and parasympathetic nerve input from the upper laryngeal nerve and recurrent laryngeal nerve (Yalçin *et al*., 2007).The main function of thyroid gland is the production of T4, T3 and calcitonin hormones, peripheral organs such as liver, kidney, and spleen convert up to 80 percent of T4 to T3, T3 is approximately ten times more active than T4,T4 is synthesized by free tyrosine follicular cells and by a protein called thyroglobulin tyrosine residue Tg ,iodine is captured by the "iodine trap" of the hydrogen

peroxide produced by the enzyme thyroid peroxidase (TPO), and linked to the Tg and free tyrosine benzene ring sites 3' and 5'(Ekholm and Bjorkman,1997). Follicular cells reabsorb Tg when stimulated by TSH and proteolytically split iodinated tyrosines from Tg forming T4 and T3 (in T3, one iodine is absent compared to T4) and releasing them into the blood , deiodinase enzymes convert T4 to T3, thyroid hormones that are secreted from the gland is about 90% T4 and about 10% T3 (Bianco *et al*.,2002).

T4 and T3 in the blood are partly linked to globulin, transthyretin and albumin that bind thyroxin (Santoro *et al*., 2002). Only a very few fraction of the circulating hormone is unbound T4 0.03% and T3 0.3% and has hormonal activity ,like steroid hormones , thyroid hormones cross the cell membrane and bind to intracellular receptors (α1, α2, β1 and β2), which function as a transcription alone, in pairs or together with the X-receptor retinoid as transcription factors to modulate DNA transcription (Kester *et al*., 2004).



Figure(2-1):Cross section in thyroid gland showing (f)follicle,(C) capsule,(tb) trabecule (bv)blood vessels H&E 100X (Al-Aamery and Dauod,2017)



Figure(2-2): The position of the thyroid gland and its associated structure (Waugh and Grant , 2014).

2-3:Thyroid Hormones

2-3-1: Biosynthesis and Release of Thyroid Hormones

The key component engaged in thyroid hormones synthesis is iodine, which is ingested with a variety of foods including dairy products, vegetables, and meat , thyroid gland has developed to save and store iodine, which is usually produced by iodide(I^-) in the small intestine, the chemical form required for thyroid hormone biosynthesis (Pennington and Young, 1991).

Thyroid hormones synthesis is a complex process involving different activates (Figure 2-3) (Melmed *et al*.,2012) . The symporter of sodium / iodide (NIS) located in the follicular cell's basolateral membrane, is energy-dependent and saturable (Dai *et al.*, 1996). This basolateral cell membrane found in contact with the blood circulation , from there to take up the necessary elements to synthesis T3 and T4 (Melmed *et al*.,2012).

The main thyroid proteins Tg and TPO found inside the thyrocyte involved in manufacturing thyroid hormones , Tg is secreted within the follicular lumen through the apical membrane and constitutes the colloid significant element , it is a large glycosylated protein with more than 2700 amino acids and a molecular weight of 660 KD a, which represents the largest 1% protein in the vertebrate proteome and contains at least 60 residues of tyrosyl with slight differences between species (Lee *et al*.,2008;Di Jeso and Arvan,2015).

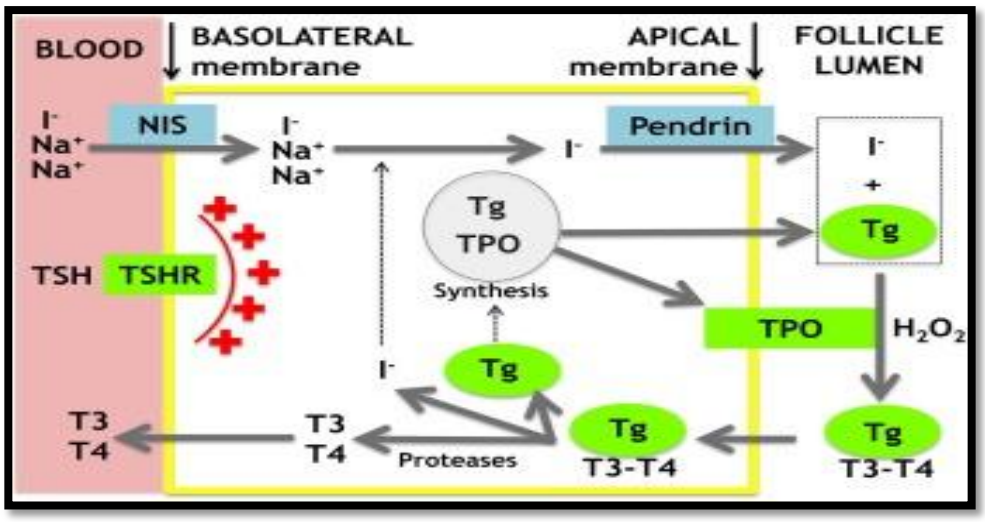
The apical boundary is the main location of synthesis T3 and T4 , I^- is transferred by a transporter called Pendrin located on the apical membrane in the follicular lumen , the particular tyrosine residues of Tg homodimers in the follicular lumen are iodinated to form 3-monoiodo-L-tyrosine (MIT) and 3,5- diiodo-L-tyrosine (DIT), the precursors of thyroid hormones, TPO acts as a donor of hydrogen peroxide (H_2O_2)and oxidized iodide besides catalyzing tyrosine iodination on Tg (also known as iodide organization) (Kopp,2013).

H_2O_2 generated by the dual oxidase 1 (DUOX1) and DUOX2 transmembrane enzymes , in order to synthesize T4 and one MIT and one DIT to create T3, TPO also catalyzes the fusion of two DIT molecules DIT, T3 and T4 are still connected to Tg protein at this point and stored in the colloid (Melmed *et al*.,2012).

Each of the thyroid hormones biosynthesis processes is stimulated by TSH secretion which is stimulated by Thyroid releasing hormone(TRH),which is generated by the paraventricular nucleus of the hypothalamus and prevents thyroid under supply (Hoermann *et al*.,2015).

Thyroid hormones release when follicular lumen endocytosis of colloid through both macropinocytosis (pseudopods) and

micropinocytosis (tiny vesicles) and on the basolateral part of thyrocytes , where T3 and T4 are secreted into the bloodstream through transmembrane protein transporters, including the thyroid hormone-specific MCT8 (Bernal *et al*.,2015).



Figure(2-3): Thyroid hormones synthesis (Melmed *et al*.,2012).

2-3-2:Chemical Structures of Thyroid Hormones

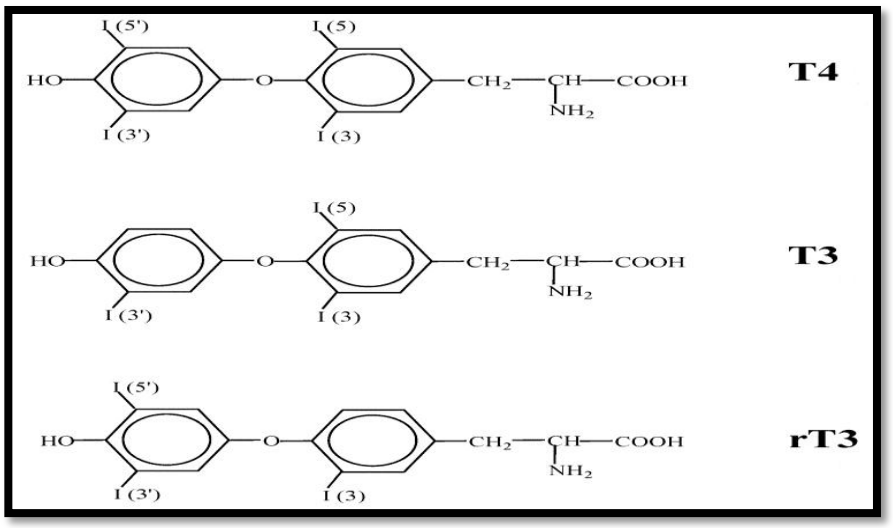
Thyroid hormones consist of a phenyl ring that is connected to tyrosine via ether and contains one to four atoms of iodine, T4 is tetraiodothyronine 3,5,3',5'- ,T3 is 3,5',3'triiodothyronine and 3,3',5' triiodothyronine (rT3; reverse T3)(Figure 2-4) (Lum *et al*.,1984). T4 is generated by follicular cells in the thyroid gland as Tg precursor , T3 is the same as T4 but it has one less iodine atoms per molecule (Ishihara *et al*.,2003).

MIT and DIT are formed when iodine combine with tyrosine in Tg, two moieties of DIT that link to produces T4 and combine one molecule of MIT and one molecule of DIT produces T3, theses two interaction are mediated by peroxidase, reduction in peroxidase associated with certain congenital and acquired thyroid disorders affects organic iodination and

increases the proportion of unbound intrathyroid iodine (Friis,1987;Roti *et al*.,1994; Ogasawara *et al.*, 2001).

- **DIT + MIT → r-T3 (biologically inactive)**
- **MIT + DIT → triiodothyronine (usually referred to as T3)**
- **DIT + DIT → thyroxin (referred to as T4)**

It is believed that T4 is a prohormone and a reservoir for the most active and primary thyroid hormone T3 , T4 is transformed through deiodinases as needed in the tissues , diodinase deficiency can mimic a defect in iodine, T3 is more active than T4 and is the hormone's final, although it is present in less than T4 (Boelaert and Franklyn, 2005).



Figure(2-4): Chemical structures of T4, T3 and rT3 (Lum *et al*.,1984)

2-3-3: Production and Metabolism of Thyroid Hormones

The total daily production rate of T4 is 80-100μg (100-130nmol), all derived from thyroidal secretion, the extrathyroidal T4 pool contains 800-1000μg (1000-1300nmol), most of which is extracellular , T4 turnover rate is 10 percent per day (serum half-life 6-7 days). Therefore, in the lack of any thyroid secretion, some T4 remain available for several weeks, approximately 80% of the T4 secreted every day is metabolized by

deiiodination, with approximately 40% being changed to T3 and 40% to rT3, the remaining 20% is used by sulfate and glucuronide conjugation , Decarboxyl and oxidative deamination to structure tetraiodothyroacetic acid , and ether connect cleavage (Leonard and Koehrle, 2000).

The change from T4 to T3, and finally diiodothyronine (T2), a metabolically inert subsidiary, is guided by three main chemicals, e.g. iodothyronine deiodinase types 1,2 and 3(D1-D2) to put it clearly, T4 is transformed by D1 and D2 into vibrant T3 while it is inactivated by D3 by transforming it into T3(rT3) , T3 and rT3 are altered by D3 and D1/D2 to T2 along these lines, separately. In what can be viewed as a standout amongst the most striking homeostatic components in the human body, D1 articulation is up regulated while D2 articulation is decreased, as a compotent of expanding T4 focuses(Bianco *et al*.,2002;Salvatore *et al*.,2011).

By comparison , the total daily production of T3 30-40 μg(45-60nmol)Indeed, 80-85% of T3 is provided by the extrathyroidal action of D1 and D2, the reminder comes from thyroid gland (Engler and Burger, 1984). Nevertheless, all things regarded, they are well understood that about 1/3 of T4 day by day produced (~130 nmol) in ordinary people is altered to T3, which is about 40 nmol and along these lines 80 percent of the total day by day assessed T3 generation of 50 nmol by exterior ring deiodination of T4 in peripheral tissue ,for early thyroid hormone digestion studies and the peruser is referred to as iodothyronine deiodinases (Bianco and Kim, 2006;Larsen and Zavacki, 2012).

Sulfation and glucuronidation are claimed reactions to detoxification in phase II, which are widely helpful in expanding substrates ' water-dissolvability and consequently, in encouraging their biliary and urinary leeway , in any event, plasma, bile and urine concentrations of

iodothyronine sulfate are frequently extremely small, given that these conjugates are rapidly debased by D1, recommending that sulfate conjugation is an essential advance prompting the irreversible inactivation of thyroid hormone (Visser,1994;Peeters *et al*.,2005).

2-3-4: Regulation of Thyroid Hormones Secretion

The hormones that make up the hypothalamic pituitary thyroid axis(HPT) regulate all cells in the body's metabolic processes and are therefore essential to the normal functioning of the organism, the hypothalamus TRH secretion activates the HPT axis (Figure 2-5)(Hiller-Sturmhofel and Bartke ,1998).The production of thyroid hormone is regulated by two mechanisms :first ,thyroidal T4 and T3 hormones are stimulated by (TSH) ,TSH production is inhibited by T3 and T4 circulation and is stimulated by the thyrotropin releasing hormone (TRH), which is produced by the hypothalamus and secreted at a higher rate in situations such as cold (in which an accelerated metabolism would generate more heat) the first mechanism offers a delicate defense in thyroid secretion against modification, Second, the production of extrathyroid T3 from T4 is controlled by a multitude of variables and the impact of these regulatory variables vary from one tissue to another , the second mechanism offers for rapid changes in tissue to non-thyroid disease, which is likely a major disease adaptation (Santini *et al*.,1996).

The hypothalamic-pituitary axis controls the release of TSH by the hypothalamus that secrete TRH , which in the anterior pituitary stimulates thyrotrophs to secrete TSH which is released by the anterior pituitary and stimulates the thyroid follicular cells to release T4 (80%) and T3 (20%), it can be transformed to T3 through the deiodination method when T4 is released into circulation ,T4 and T3 may then exert negative feedback on TSH concentrations, with high T3/T4

concentrations reducing TSH and low T3/T4 concentrations increasing anterior pituitary TSH concentrations,T3 is the predominant TSH secretion inhibitor ,because TSH is so susceptible to minor serum-free modifications in T4 through this negative feedback loop, abnormal TSH concentrations in hypothyroidism and hyperthyroidism are identified sooner than free T4 concentrations. T3/T4 and TSH have a log-linear connection with minor modifications in TH leading to significant modifications in TSH(Mariotti and Beck-Peccoz,2016).

The production of TSH is blunted by somatostatin , increased concentrations of glucocorticoids and sex hormones (estrogen and testosterone), and excessively elevated concentrations of blood iodide (De Escobar *et al*., 2004).

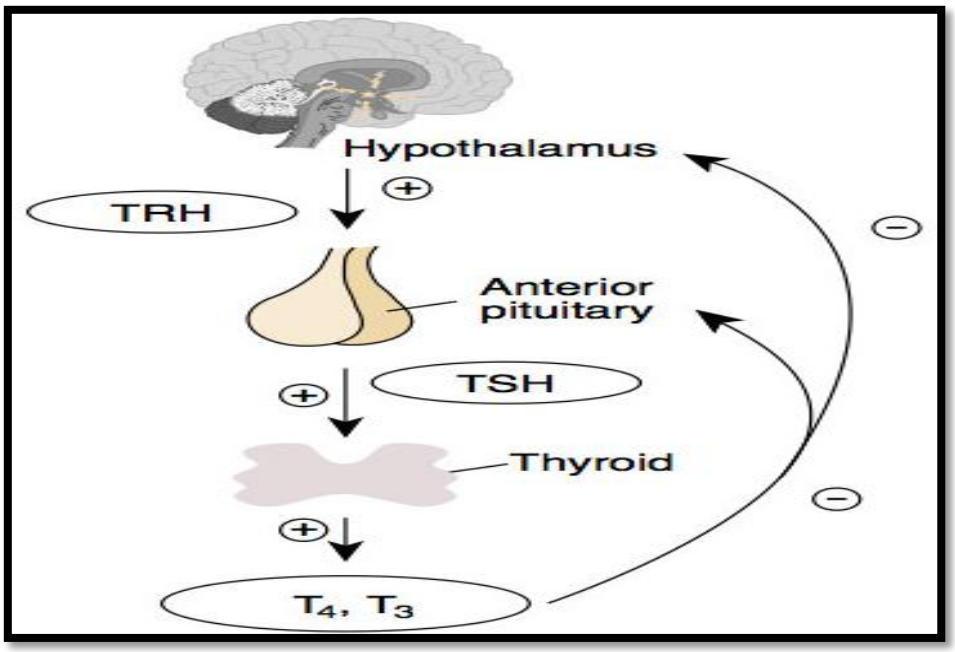


Figure (2-5): Hypothalamic – Pituitary – Thyroid Axis (Hiller-Sturmhofel and Bartke ,1998).

2-3-5: Biological action of Thyroid Hormones

Thyroid hormones behave by binding to a particular nuclear thyroid hormone receptor (TR),T3 has a binding affinity for TRs that is 15 times

greater than T4, explaining its role as the active thyroid hormone. When bound T3 to TR generally binds to DNA in particular sequences called thyroid hormone reaction elements (TREs) as a heterodimer with a retinoid X receptor (Melmed *et al*., 2012).

Two TR genes, α and β, undergo alternative splicing forming active and inactive gene products, the active enzymes are TRα1, TRβ1, TRβ2 and TRβ3 (Lazar,2003) and the expression of the distinct TRs has tissue-specific preferences, indicating that they serve distinct functions in distinct tissues (Amma *et al*.,2001). TRα, particularly TRα2, is generally considered significant in the hypothalamus and pituitary where thyroid function regulation happens (Abel *et al*., 1999).

T3 and T4 carry out several physiological activities on various target organs and structures, including metabolic rate control, growth regulation, beneficial chronotropic and inotropic cardiac impacts, and central nervous system development (Moran and Chatterjee, 2015). Thyroid hormones are vital during organogenesis and after birth in infancy and childhood, in adult life, thyroid hormones have very essential and main impacts on target organs(Melmed *et al*., 2012 ; Moran and Chatterjee, 2015).

2-3-6: The effect of Thyroid Hormones on Proteins, Lipids and Carbohydrates metabolism

Thyroid hormones (THs) are extremely active in metabolism and are essential for most body activities such as homeostasis growth, development and maintenance ; they can boost metabolic rate by accelerating fuel oxidation in almost all tissues, THs activates lipolysis, glucose metabolism, and protein synthesis (Yen, 2001).

T3 stimulates many structural proteins, enzymes, and hormones to be synthesized (Jameson and De Groot, 1995). The effects of this intervention are most evident in the reduced neural and somatic development that accompanies infant and child thyroid deficiency, the rise in protein synthesis is mainly due to enhanced gene transcription, but the proliferation of ribosomal components involves in protein synthesis, increased translational efficiency and perhaps increased amino acid transport may also be involved , THs stimulate both lipogenesis and lipolysis, resulting in enhanced oxidation of fatty acids to produce the ATP used for thermogenesis and other energy-consuming action (Beylot *et al.*, 1991).

THs stimulate almost all aspects of carbohydrate metabolism, including enhancement of insulin-independent entry of glucose into the cells and increased gluconeogenesis and glycogenolysis to generate free glucose (Moller *et al*.,1996).

2-4:Hypothalamus Hormone

2-4-1: :Thyrotropin Releasing Hormone (TRH)

TRH is synthesized as a 26-kDa protein (proTRH) containing five copies of the proteolytic cleavage sites sequence of glutamine-histidine-proline- glucine , it is formed from ProTRH by peptidae action followed by glutamine residue cyclization to form pyroglutamyl residue (Wu *et al*., 1987).

TRH stimulates TSH secretion by binding the membrane associated with phospholipase C to receptors on thyrotrophic cells, the phosphoinositides created by this enzyme stimulate calcium release from intracellular storage locations; in turn, the excess in the concentration of cytosol calcium stimulates TSH exocytosis (Gershengorn,1986).Thyroid

hormone receptor (THR) and thyroid hormones (THs) regulate the amount of TSH receptors on the thyrotrophic neurons .TRH secretion is likely pulsatile, accounting for TSH secretion pulsatility, and TRH secretion surges are liable for the surges of TSH secretion occurring in newborn infants and in some topics during cold exposure , more importantly, TRH is needed to maintain ordinary TSH secretion and it determines the setting point for which thyroid hormones control TSH secretion (Schomberg and Bauer, 1995).

2-5:Pituitary Hormones

2-5-1:Thyroid Stimulating Hormone(TSH)

TSH , also known as thyrotropin, is a 28-kD glycoprotein hormone produced and secreted by the anterior pituitary cells called thyrotrophs. It consists of two subunits: α and β; the β subunit is peculiar to TSH and therefore determines its receptor specificity; the α subunit is the same as the luteinizing hormone, follicle-stimulating hormone, and human chorionic gonadotropin(HCG) (Melmed *et al*.,2012).

2-5-2:Follicle Stimulating Hormone(FSH)

Follicle stimulating hormone (FSH) is the oldest hormonal test of oocyte quantity. FSH is stimulated by pulsatile GnRH and inhibited through negative feedback by inhibin B and estradiol (Hoffman *et al*.,2012). As the follicular pool decreases with advanced age, the concentration of inhibin B decreases, resulting in loss of negative feedback and rise in FSH , FSH is most appropriately measured in the early follicular phase (day 3-5), when levels are generally highest before suppression by rising estradiol levels. Therefore, estradiol should be ordered with FSH to ensure that a premature elevation in estradiol has not

masked what would be an otherwise elevated FSH(Hoffman *et al*.,2012,Visser *et al*.,2012).

2-5-3:Luteinizing Hormone(LH)

LH is a hormone that is produced in the pituitary gland in both men and women, in women, LH is an important part of the menstrual cycle, it works in conjunction with follicle-stimulating hormone (FSH),the rise in estrogen tells the pituitary gland to stop producing FSH and to start making more LH, the shift to LH causes the egg to be released from the ovary, a process called ovulation .general, higher than normal levels of LH in a woman may mean the ovaries are absent or not functioning ,in a young woman, high levels may mean that puberty is early low levels of LH in the blood may indicate anorexia, an issue in the pituitary gland, stress, or damage to the hypothalamus in both men and women (Nam *et al*., 2012) .

2-5-4:Prolactin(PRL)

Prolactin plays an important role in the reproductive health of both women and men. Its main role, however, is to stimulate the production of milk in women after childbirth. In other words, prolactin triggers lactation, levels of prolactin have been found to be a measure of sexual satisfaction in both men and women (Lee *et al*., 2013).

Thyroid hormones change the sensitivity of the gonads to follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin. Besides menstrual cycle disorders, hypothyroidism can also cause an increase particularly in the release of thyrotropin releasing hormone (TRH), which in turn increases the release of TSH and prolactin, eventually causing hyperprolactinemia, which is an important factor inhibiting the development of pregnancy. So, hypothyroidism should be

taken into account in patients with hyperprolactinemia (Joshi *et al*., 1993).

2-6:Ovarian Hormones

2-6-1:Progesterone

Progesterone secreted by the corpus luteum plays an important role in the maintenance of early pregnancy ,immediately after implantation, under the influence of human chorionic gonadotropin (hCG) secreted by the trophoblast, the corpus luteum receives a signal to continue to producing 17-α-progesterone along with estradiol, estrone, and relaxin (Szlachter *et al*., 1980; Bigazzi and Nardi,1981). The corpus luteum maintains its capacity to synthesize progesterone almost throughout the pregnancy, but at approximately 7 weeks gestation, its functional ability decreases markedly at the start of the luteoplacental transition,the removal of the corpus luteum before the eighth week of gestation results in abortion, whereas after the ninth week it does not (Csapo *et al*., 1973).

2-6-2:Estradiol (E2)

The major estrogen is estradiol, which, in addition to small amounts of estrone and estriol, is produced primarily in the ovaries,other production sites of estrogens include the corpus luteum ,the placenta, and the adrenal glands(Dorgan *et al*.,1994).

2-6-3:Testosterone

androgens are synthesized in both the adrenals and the ovaries in response to adrenocorticotropic hormone and luteinizing hormone, respectively, these steroids are also derived from conversion of precursors in peripheral tissues. Until recently the major androgens in the female circulation were believed to be dehydroepiandrosterone, androstenedione,

and testosterone(Burger,2002). In premenopausal women, circulating testosterone levels fluctuate during the menstrual cycle, with a peak occurring midcycle ,there is also diurnal cyclicity, with rising levels in the early morning ,approximately 25% of circulating testosterone is derived from the ovaries, 25% from the adrenals, and the remaining from peripheral tissue(Storbeck *et al*.,2013).

2-6-4:Anti-Mullerian Hormone(AMH)

Anti-Mullerian hormone (AMH) is a dimeric glycoprotein and a member of the transforming growth factor-b superfamily (Cate *et al*., 1986). The role of AMH as an important regulator of mammalian follicular development has recently been described (Knight and Glister, 2003; Seifer and Maclaughlin, 2007). In females AMH is produced exclusively by granulosa cells (GC) of ovarian follicles (Vigier *et al*., 1984). It is normally expressed at low levels in primary follicles, increases to maximal concentrations in large preantral and small antral follicles and then declines as the follicle grows (Weenen *et al*., 2004;Fanchin *et al*., 2005; Andersen *et al*., 2010). In addition a recent study on human cumulus GC shows that AMH remains highly expressed during the final stages of folliculogenesis (Grondahl *et al*., 2011). Studies in mammals have shown that AMH is a crucial factor in folliculogenesis due to its role in inhibition of follicular recruitment and in FSH-dependent follicle growth and selection (Knight and Glister, 2003; Visser, 2006;Seifer and Maclaughlin, 2007). It has been suggested that AMH acts through inhibition of aromatase activity in GC (Grossman *et al*., 2008) or through an inhibitory effect on oocyte meiosis (Takahashi *et al*., 1986).

2-7: Disorders of Thyroid Gland

Thyroid disorders are the world's most prevalent endocrine diseases (Vanderpump, 2011). The incidence of thyroid diseases relies on age, gender, geographic variables, and consumption of iodine (Morris *et al*.,2001).

Thyroid disorders may either occur in the form of less or excessive hormone secretion, or may occur as a goiter, which is an enlargement of the body triggered by diffuse enlargement, or as a result of one or more cell nodules, other common thyroid complications are Grave's disease , Hashimoto's thyroiditis, adenoma, thyroid carcinoma, hypothyroidism, and hyperthyroidism, which are the prevalent types of thyroid disorders (Schultz *et al*.,2011; Grais and Sowers,2014).

Thyroid disorders increase with age, following exposure to radiation, and females are ten times more probable to have thyroid issues than males ,the most prevalent endocrine defects in both the Kingdom of Saudi Arabia (KSA) and the Middle East are thyroid gland disorders(Refeidi *et al*.,2010; Al Shahrani *et al*.,2016). According to the American Association of Clinical Endocrinologists (AACE), approximately three million individuals or 4.78% of the United State population have undiagnosed thyroid dysfunction and according to the American Thyroid Association (ATA), one in eight women will develop thyroid problems during her life time (Garber *et al*.,2012).

Dysfunction of the thyroid is diagnosed by measuring the concentration of serum thyrotropin (TSH), Up to now, serum TSH is the best and most reliable test for diagnosing thyroid gland disorders (Ladenson *et al*.,2000;Baskin *et al*.,2002).

Dysfunction of the thyroid is described by clinical signs, symptoms, and TSH concentrations ,hypothyroidism is diagnosed by TSH at concentrations above the reference range and hyperthyroidism is diagnosed by TSH at concentration below the reference rang(Molina *et al*.,2007). .

In Iraq , study by Abdul-Qahar *et al* .(2016) on 92 females suffering from infertility(56 with primary infertility and 36 with secondary infertility) showed that thyroid disorders in these females are closely related with subfertility and infertility in Iraq females especially autoimmune thyroid disease .

2-7-1: Hyperthyroidism(Thyrotoxicosis)

Although often used synonymously with thyrotoxicosis, the word hyperthyroidism relates to increased thyroid gland secretion of T3 and T4. Thyrotoxicosis is exclusively the clinical, physiological, and biochemical syndrome resulting from the exposure of tissues to excessive thyroid hormone concentration. Therefore, patients with hyperthyroid have thyrotoxicosis, but thyrotoxicosis has other causes than hyperthyroidism (Vanderpump *et al.*, 1995; Helfand and Redfern, 1998).

Thyrotoxicosis is generally categorized as overt and subclinical, overt thyrotoxicosis is described as elevated T4 and T3 serum and low TSH serum, most patients have symptoms and signs of thyrotoxicosis , subclinical thyrotoxicosis is characterized as ordinary levels of serum T4 and T3 and low levels of TSH, most patients do not have symptoms and signs of thyrotoxicosis (Okamura *et al*., 1987).

It is triggered by the Graves disease , toxic goiter, and thyroiditis in females of reproductive age, a greater incidence of hyperthyroidism was correlated with irregular menstrual cycles ranging from hypomenorrhea ,

polymenorrhea and oligomenorrhea to hypermenorrhea (Krassas *et al*.,1994).

2-7-2: Hypothyroidism

Hypothyroidism is a prevalent endocrine disorder caused by thyroid hormone deficiency. It is usually a primary process where the thyroid gland cannot generate enough thyroid hormone quantities. Whether this disorder should be tested and treated is still controversial (Weetman,1997).

It is generally categorized as overt and subclinical, overt hypothyroidism is characterized as low levels of serum T4 and T3 and high levels of TSH in almost all patients; most patients with overt hypothyroidism have symptoms and signs of hypothyroidism. Subclinical hypothyroidism is described as normal levels of serum T4 and T3 and high levels of serum TSH, most of these patients have no symptoms and signs of hypothyroidism (Canaris *et al.*, 2000).

The signs of thyroid disorders depend on the impacted organ , as the thyroid starts to fail, the slight enlargement of the thyroid gland (goiter) appears as a lump or swelling, the patient may start feeling tired, the thyroid gland's insufficient production of thyroid hormones is described as hypothyroidism and can trigger various symptoms, including fatigue, weakness, weight gain, and depression, with deep impacts on the cardiovascular system (Dillmann,1990), the endocrine system *(*Krassas *et al*.,1999), and the brain (Haupt and Kurz,1993;Krassas *et al*.,1999).

Hypothyroidism becomes more serious, modifications in the tissues that result under the skin may happen that lead to a characteristic swollen appearance known as myxedema , this is often particularly apparent around face and eyes (Boelaert and Franklyn,2005). It affects the

circulation and slows the heart rate ,patient may become constipated as intestinal movement slows down, there may be a few pounds of weight gain , with leg cramps, muscles can become painful (Hoogendoorn *et al*.,2004).

2-7-3: Subclinical Hypothyroidism (SCH)

The word subclinical hypothyroidism is used in patients with slightly enhanced serum TSH concentrations but ordinary T4 and T3 concentrations of the thyroid hormones (Lorini *et al*.,2003).

An rise in the concentration of serum TSH is an early and delicate indicator of reduced thyroid reserve. However, it is more hard to interpret thyroid function tests in the range of pediatric age than in adults , although standard ranges from birth to maturity have been established for all age groups, significant discrepancies between distinct laboratories still remain, that is why it is essential it is therefore important that each laboratory determines its own normal values and the results must always be interpreted cautiously (Calaciura *et al*.,2002).

It is evident that thyroxine treatment is indicated in overt hypothyroidism and there is uniform consensus that it is also indicated in patients with a permanent increase in TSH concentrations above 10 mIU / L, therapy for these milder types is controversial for TSH concentrations ranging from 5 to 10 mIU / L. Some physicians treat all these patients in clinical practice, while others reassess the thyroid function in 3-6 months to find out if the thyroid abnormality is transient,one spectrum of autoimmune thyroiditis is subclinical hypothyroidism, the clinical course is variable and spontaneous remission may happen in adolescence (Daliva *et al*., 2000).

2-7-4: Hypothyroidism and Fertility

Normal blood concentrations of the thyroid hormone are crucial for tissue growth, development and for both tissues and organs function maintenance , changes in thyroid hormone concentrations may adversely influence fertility, outcome of pregnancy and postnatal development in humans and animals; with significant impacts on the development and functioning of the offspring's growth, hearing, mental acuity and reproductive system (Jahnke *et al.*, 2004).

Hypothyroidism in females is five to eight times higher than in males (Aoki *et al*., 2007). It is connected with a wide range of reproductive illnesses ranging from abnormal sexual growth to infertility through menstrual irregularities and it was linked to modified ovarian function, menstrual irregularities, subfertility, and greater (recurring) miscarriage rates ,suggesting that hypothyroidism commonly affects reproductive axis (Krassas *et al*.,2008;Van den Boogaard *et al*.,2011). In Women have a greater incidence of anovulatory cycles leading to infertility and have a greater rate of fetal loss in the first trimester if pregnancy occurs (Roti *et al*.,1996; Montoro,1997).

The anterior pituitary gland secrets many hormones which play significant roles in a broad range of physiological mechanisms, including metabolism, growth and development, and reproduction(Karaca *et al*., 2010). Produced in the hypothalamus, Gonadotropin-releasing hormone (GnRH) regulates the release of FSH from anterior pituitary. In oogenesis, FSH plays an significant role. It causes follicle maturation (e.g., granulosa cell proliferation) and aromatase androgen-converting enzyme synthesis. Furthermore,. In recruiting the dominant follicle, it plays a key role. LH and FSH foster ovulation and boost ovarian sex hormone secretion of estradiol and progesterone from ovaries(Gaber *et*

al.,2011). The receptors of thyroid hormones are found in human oocytes, cumulus cells and granulose cells , some of pituitary hormones are impacted by hypothyroidism, including prolactin, LH, and FSH, that may result in libido defects, erectile dysfunction, and fertility(Poppe and Velkeniers,2004).

A granulose cell function is affected by thyroid hormones by the immediate impact of FSH, which facilitates the induction of LH/HCG, females wit hypothyroidism have lowered levels of androstenedione and estrogen metabolic clearance and show an increase in peripheral flavouring, sex hormone binding globulin (SHBG) plasma binding activity is reduced, which results in decreased plasma concentrations of both total testosterone and estradiol (E2), but their unbound fractions are increased(Ramprasad *et al*.,2012). And may have a role in enabling activation of LH receptors and progesterone production(Maruo *et al*.,1991).

Hypothyroidism can also change the pituitary feedback by altering the metabolism of estrogen and the circulation of SHBG (Krassas,2000). Other indirect impact of hypothyroidism on hyperprolactinemia owing to enhanced TRH manufacturing and modified pulsatile hormone-releasing gonadotropin secretion, resulting in delay in LH reaction and insufficient corpus luteum formation ,thyroid hormones have a direct impact on the gonads by modulating the behavior of follicles by T3 of the actions of FSH and LH on steroid biosynthesis(Cecconi *et al*.,1999).

In hypothyroidism, menstrual disturbances are prevalent, the most common being oligomenorrhoea and menorrhagia. In serious hypothyroidism, menstrual irregularities are more common compared to mild cases (Krassas *et al*., 1999).

In addation, it has been acknowledged that SCH during pregnancy has an important effect on the results of pregnancy. Complications of pregnancy such as gestational diabetes mellitus (GDM), hypertension, and pre-eclampsia have been recorded in studies by (Wilson *et al*.,2012;Gong *et al*.,2016). In comparison with topics whose thyroid function tests were normal, women with SCH were twice as probable to deliver early ,these females were also three times more probable to develop placental abruption (Casey *et al*., 2005). Other trials have noted that pregnant females with SCH were more likely to suffer miscarriage, especially during the first 20 weeks of gestation (Negro *et al*.,2010;Liu *et al*.,2014). The incidence of SCH among pregnant females in the general population is estimated to be 2% to 3% (Ertugrul *et al*.,2011).

On the other side, study by Klubo-Gwiezdzinska *et al* .(2011) recorded that the incidence of SCH in Belgium is 6.8% and as high as 13.7% in Spain .On the other hand , in the study by Knight *et al*.(2016) , on Caucasian females found the incidence of SCH was 13.9% ,While in the United State the prevalence of SCH ranged from 2% to 2.5% in pregnant women (Maraka *et al*., 2016).

Chapter Three

Materials and Methods

3- Materials and Methods

3-1 :Materials

3-1-1: Apparatus and Equipment

The apparatus and equipment used in this study are summarized in table (3-1).

Table (3-1): The apparatus and equipment that used with their producing companies and countries.

No.	Apparatus and Equipment	Company (origin)
1	Alcohol methyl	Meheco , China
2	Centrifuge	Beckman, England
3	Cobas e 411 analyzer (disk system)	Roche diagnostic, Germany
4	Cotton	Kardelen, Turkey
5	Disposable syringes 10 ml	Zhejiang INI Medical Device , China
6	ELISA reader	Bio-Tek , USA
7	ELISA washer	Bio-Tek , USA
8	Gel tube	Sun, Jordan
9	Plain tube	AFMA, Jordan
10	Refrigerator	Midea , China
11	Spectrophotometer	Aple , Japan
12	Water bath	Memmert, Germany

3-1-2: Kits

The kits used in this study are summarized in table (3-2)

Table (3-2): The kits that used with their producing companies and countries.

No	Kits	Company (origin)
1	Triiodothyronine (T3)	Roche, Germany
2	Thyroxine (T4)	Roche ,Germany
3	Thyroid Stimulating Hormone (TSH)	Roche ,Germany
4	Follicular Stimulating Hormone (FSH)	Roche ,Germany
5	Luteinizing Hormone (LH)	Roche ,Germany
6	Progesterone	Roche ,Germany
7	Prolactin (PRL)	Roche ,Germany
8	Estradiol (E2)	Roche ,Germany
9	Total Testosterone (TT)	Roche ,Germany
10	Anti-Mullerian Hormone (AMH)	Demeditec ,Germany
11	Total Cholesterol (TC)	Bio Lab, France
12	Triglyceride (TG)	Bio Lab, France
13	High Density Lipoprotein (HDL)	Bio Lab, France
14	Glucose	Bio Lab, France
15	Total Protein	Bio Lab, France

3-1-3: Diagnostic Kit

3-1-3-1: ELISA Kit

The content of ELISA kit used in the measurement AMH are listed in the table (3-3) .

Table(3-3):Anti-Mullerian Hormone (AMH) ELISA kit compotents.

Item	Specification
Micro ELISA plate	96 wells / 1 stripholder
Sample diluent	One bottle (13 mL)
Antibody-Biotin Conjugate	One bottle (13 mL)
Streptavidin-Enzyme conjugate	One bottle (13 mL)
Assay Buffer	One bottle (26 mL)
TMB chromogen solution	One bottle (11 mL)
Wash solution U (20 X)	One Vial (50 mL)
Stopping solution A	One bottle (11 mL)
Calibrator 0	One vial (0.5 mL)
Calibrator 1-6	Six vial (0.5 mL)

3-1-4: Population of the Study

This study was carried out at the AL-Sader Teaching Hospital and center for Endocrinology and Diabetes specialist in Misan province. During the period from December 2018 to June 2019.

The population of this study consists of 88 women with average age between 20-35 years , divided into four groups and each group include 22 women as the following:

- First group(A) (control group) (healthy women with regular menstrual cycles without any hormonal disturbances).
- Second group (B) (infertile women with hormonal disturbance).
- Third group (C) (fertile women with hypothyroidism).
- Fourth group (D) (infertile women with hypothyroidism).

A questionnaires were designed to obtain the actual results of women with primary hypothyroidism which diagnosis based on the measurement of thyroid hormones T3,T4 and TSH , in addition to reliance on symptoms and clinical signs such as (tiredness, weight gain, dry skin, cold intolerance, constipation, muscle weakness, puffiness around the eyes, hoarse voice, and poor memory) (Canaris *et al*.,2000).The infertile group was determined based on the doctors present in the women consultancy in the hospital. Any patients with secondary hypothyroidism, cushing syndrome, congenital adrenal hyperplasia , polycystic ovary syndrome and women who had ovarian surgery were excluded (Appendix A).

3-1-5: Experimental design

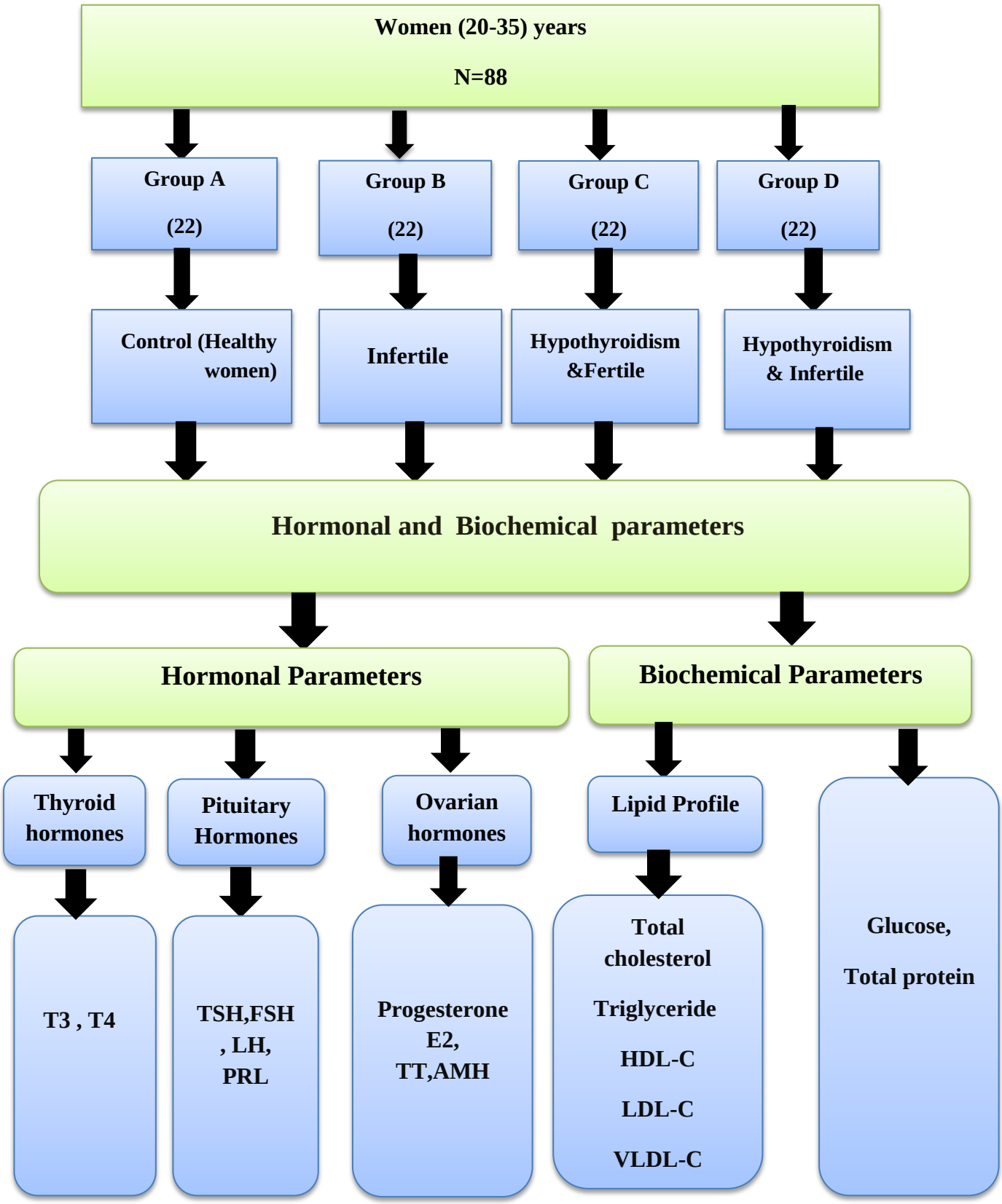


Figure (3-1): The experimental design

3-1-6: Sample Collection

3-1-6-1: Collection of Blood Sample

Three milliliters of the blood was obtained from the women of the study by a medical syringe for measurement of T3,T4 and TSH and biochemical tests including lipid profile, glucose and total protein ,another drawn two milliliters was withdrawn from these women in the follicular phase (2-3) day of the menstrual cycle at 8-10 a.m , for measurement (FSH, LH, Progesterone, PRL,E2,TT and AMH) of each subject (patients and controls woman). The blood sample puts in gel tube for 20 minutes at room temperature for clotting . Then, centrifugation was done at 3000 rpm for 10 minutes to collect the serum .

3-2: Methods

Hormonal Parameters Assesment :Concentration of hormonal levels (thyroid hormones and reproductive hormones) measured by the chemiluminescent automates immunoassay system (Cobas e 411, Roche diagnostic , Germany), while the level of AMH hormone measured by used the ELISA system .

Principle of The Hormonal levels (Thyroid Hormones and Reproductive Hormones). The Elecsys family of automated immunoassay systems , the Elecsys assay combines conventional antigen- antibody reactions on the surface of a streptavidin – coated paramagnetic microparticle with electrochemical reaction on the surface of an electrode , which generates luminescence (Wild , 2013).

Procedure of the Measurement:

1-Load assay cups and assay tips in consumables.

2-Reagent special for each parameters loading in reagent disk in the instrument.

3-Make calibration and control of each reagent.

4-Loading the sample of serum in to cuvate then put in the disk and the instrument automatically began to analysis the sample and then show the results of each sample on monitor screen.

3-2-1:Hormonal Study

3-2-1-1:Measurement of Serum (T3,T4,Progesterone,E2 and TT)

Principle of the Test: Competition principle (The Elecsys assay employs a competitive test principle with polyclonal antibodies specifically directed against) .

1-first incubation: 9-30 µl of sample and the specific antibodies anti-T3, T4,Progesterone,E2 and TT labeled with a ruthenium complex; bound T3,T4,Progesterone,E2 and TT is released of the binding proteins in the sample by ANS.

2- second incubation: After the incorporation of the coated microparticles of streptavidin and of biotinylated T3, T4,Progesterone,E2 and TT the attachment sites still free of the labeled antibody are occupied, with the formation of a antibody-hapten complex. The complex formed binds to the by the interaction of biotin and streptavidin.

3- The reaction mixture is aspirated into the reading cell, where the microparticles are magnetically attached to the surface of the electrode. Unconnected elements are then removed with ProCell. The application of an electric current to the electrode induces a chemiluminescent which is measured by a photomultiplier.

4-Result can be taken from the monitor that show the data of each patient and the number of the tests.

The measurement of the above hormones according to the (Klee , 1996 ;Nelson and Wilcox,1996; Guillaume *et al*.,1987; Bablok *et al*.,1988; Rosner *et al.*,2006).

3-2-1-2: Measurement of Serum TSH,FSH,LH and Prolactin

Principle of the Test: Sandwich principle (The Elecsys assay employs monoclonal antibodies specifically directed against human TSH,FSH,LH and Prolactin) .

1-first incubation: 10-50 µL of sample, a biotinylated monoclonal TSH‑specific antibody and a monoclonal TSH,FSH,LH and Prolactin‑specific antibody labeled with a ruthenium complex react to form a sandwich complex.

2-second incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

3-The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

4-Result can be taken from the monitor that show the data of each patient and the number of the tests.

The measurement of the above hormones according to the (Wu , 2006; Tietz and Ash, 1995; Fahie-Wilson *et al.*,2000).

3-2-1-3:Measurement of Serum Anti-Mullerian Hormone(AMH)

Principle of Method: The AMH Gen II ELISA is an enzymatically amplified two-site immunoassay. In the assay, calibrators, controls and samples are incubated in microtitration wells which have been coated with anti-AMH antibody. After incubation and washing, anti-AMH detection antibody labeled with biotin is added to each well. After a second incubation and washing step, streptavidin-horseradish peroxidase

(HRP) is added to the wells. After a third incubation and washing step, the subsubstrate tetramethylbenzidine (TMB) is added to the wells. Lastly, an acidic stopping solution is added. The degree of enzymatic turnover of the substrate is determined by dual wave length absorbance measurement at 450 nm and between 600 and 630 nm. The absorbance measured is directly proportional to the concentration of AMH in the samples. A set of AMH calibrators is used to plot a calibration curve of absorbance versus AMH concentration. The AMH concentrations in the samples can then be calculated from this calibration curve (Baarends *et al*.,1995).

Assay Procedure: Allow all samples and reagents to reach room temperature (18-25°C). Mix reagents thoroughly by gentle inversion before use. After reconstitution of reagents, mix thoroughly, avoiding foam. Calibrators, controls and samples should be assayed in duplicate.

Before adding sample to the AMH Gen II ELISA microplate, you must prepare all calibrators, controls, and samples with the AMH Gen II Assay Buffer (REF A56021). In a sample tube, prepare 1 part of each calibrator, control, or test sample respectively (including diluted pediatric male samples) with 5 parts AMH Gen II Assay Buffer (for example, 60 µL calibrator, control, or sample + 300 µL AMH Gen II Assay Buffer) Mix thoroughly.

1. Mark the microtitration strips to be used.

2. Within 1 hour, pipet 120 µL of the premixed calibrators, controls and samples to the appropriate wells using a precision pipette.

3. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for one hour at room temperature (18-25°C).

4. Prepare the wash solution as described under the "Preparation of Reagents" section of this package insert.

5. Aspirate and wash each well five times with the wash solution using an automatic microplate washer or manually using a precision pipette. Blot and dry by inverting plate on absorbent material.

6. Add 100 µL of the antibody-biotin conjugate solution to each well using a precision pipette.

7. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for one hour at room temperature (18-25°C).

8. Aspirate and wash each well five times with the wash solution using an automatic microplate washer. Blot dry by inverting plate on absorbent material.

9. Add 100 µL of the streptavidin-enzyme conjugate to each well using a precision pipette.

10. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for 30 minutes at room temperature (18-25°C).

11. Aspirate and wash each well five times with the wash solution using an automatic microplate washer. Blot dry by inverting plate on absorbent material.

12. Add 100 µL of the TMB chromogen solution to each well using a precision pipette.

13.Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for 8–12 minutes at room temperature (18-25°C).

14. Add 100 µL of the stopping solution to each well using a precision pipette.

15. Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm(Appendix B).

3-2-2:Biochemical Study

3-2-2-1:Measurement of Serum Total Cholesterol (TC)

The employed method was based upon an enzymatic colorimetric method ,using cholesterol assay Bio lab Kit. The principle of the kit is according (Allain *et al* .,1974).

The Steps of the Procedure were Followed by the Following Table:

	Blank	Standard	Sample
Reagent	**1ml**	**1ml**	**1ml**
Standard	**-**	**10 µL**	**-**
Sample	**-**	**-**	**10 µL**

Incubated for 5 minutes at room temperature . The absorbance of the sample and the standard sample were measured against Blank reagent at 500 nm.

The color intensity is proportional to the cholesterol level in the sample that can be estimated using the spectrophotometer, color of the mixture was varying from light pink to dark red , and it was stable for 1 hour. The Calculation of Concentration Of cholesterol (mg/dl)= A. of sample /A .of standard × Concentration of standard .

Where : A(Absorbance)

3-2-2-2:Measurement of Serum Triglyceride (TG)

Procedure: The Steps of The Procedure were Followed by The Following Table:

	Blank	Standard	Sample
WR	**1ml**	**1ml**	**1ml**
Standard	**-**	**10 µL**	**-**
Sample	**-**	**-**	**10 µL**

Each tube solution was mixed and incubated for 5 minutes at 37˚C or 10 min at room temperature . The absorbance of the sample and the standard sample were measured against the reagent Blank at 505 nm. The colour is stable for at least 30 minutes(Bucolo and David,1973; Fossati and Prencipe,1982). The calculation of Concentration. of triglycerides (mg/dl) =(A) Sample –(A) Blank / (A) Standard- (A) Blank x 200 mg/dl(standard concentration).

Where :A (Absorbance)

3-2-2-3:Measurement of Serum High Density Lipoprotein – Cholesterol (HDL-C).

The Steps of The Procedure were Followed by The Following Table:

Pipette in well identified test tube	Blank	Standard	Sample
Reagent 1	**1ml**	**1ml**	**1ml**
Demineralised water	**25µl**		
Standard100 mg/dL	-	**25 µL**	-
Sample	-	-	**25 µL**

Mix and Let stand for 5 minutes at 37˚C or 10 minutes at room temperature .Record absorbances at 500 nm against reagent blank. Colour is stable for 1 hour(Tietz ,1999). The calculation of HDL-C concentration was estimated according to the following equation:

Concentration of HDL-C (mg/dL) =A. of sample /A. of standard × concentration of standard x 1.1

Where : A(Absorbance)

3-2-2-4:Measurement of Serum Low Density Lipoprotein – Cholesterol (LDL_C).

The LDL was determined according to Friedewald formula

LDL c = Total cholesterol – HDL c – (TG/5) (Friedewald *et al*.,1972).

3-2-2-5: Measurement of Serum Very Low Density Lipoprotein-Cholesterol(VLDL-C).

Concentration of VLDL-C was determined according to method by (Friedewald *et al* ., 1972 ; Tietz,1999).The calculation of VLDL is VLDL=TG/5

3-2-2-6: Measurement of Serum Glucose

The serum glucose was determined by enzymatic colorimetric (GOD-PAP) method, using kit supplied by using a kit from bio lab , France (Trinder,1969).

Assay Procedure

Solutions	Blank	Standard	Sample
WR	**1 ml**	**1 ml**	**1 ml**
Standard	**-**	**10 µl**	**-**
Sample	**-**	**-**	**10 µl**

Each tube of solution Mixed and incubated for 10 min at 37 ºC or 20 min at room temperature. Read absorbance at 500 nm against reagent blank . Coloration is stable for 15-20 min at 37 ºC, and then slowly decrease. The calculation of the concentration of glucose: (GLU) concentration(µg /dl) =(A) Sample – (A) Blank / (A) Standard –(A) Blank x 100 µg /dl

Where: A(Absorbance)

3-2-2-7: Measurement of Serum Total Protein(TP)

Total protein was determined by spectrophotometric method by using a kit from bio lab France (Gornall *et al* .,1949;Tietz and Ash,1995).

Manual Procedure

The Steps of the Procedure were Followed by The Following Table:

Pipette into well identified test tubes	Reagent Blank	Standard	Sample
Reagent R 1	**1 mL**	**1 mL**	**1 mL**
Standard		**20 µL**	
Specimen			**20 µL**
Demineralised Water	**20 µL**		

Mix and Well .Let stand for 10 minutes at room temperature . Record absorbance at 550 nm against reagent blank. The calculation of the concentration of TP :Result =(A) Sample /(A) Standard x Standard concentration

Where: A(Absorbance)

3-3:Statistical Analysis

Data were analyzed by one way ANOVA by general liner model procedure using statistical package for social science (SPSS) version 23 The comparisons between means scores were made using least significant differences (LSD) using Genstat3statistic program. The difference were considered to be significant at $P<0.05$ using multivariate model in SPSS. The data are presented as mean ± S.D.(standard deviation) (SPSS,2015).

Chapter Four

Results

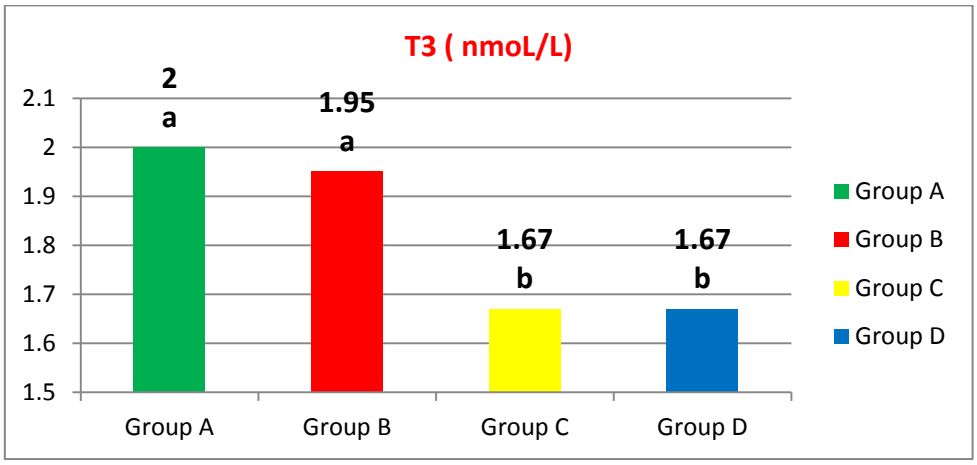
4-Results

4-1: Hormonal Study

4-1-1: Thyroid hormones

4-1-1-1: Triiodothyronine (T3)

The values of T3 decreased significantly(P≤0.05)in C (1.67± 0.41 nmoL/L) and D (1.67±0.45 nmoL/ L) groups compare with A(2±0.05 nmol/L) and B (1.95± 0.33nmoL/L) groups. A group did not differ significantly in comparison with B group. No significant differences between C and D groups (Figure 4-1 , Table 4-1).



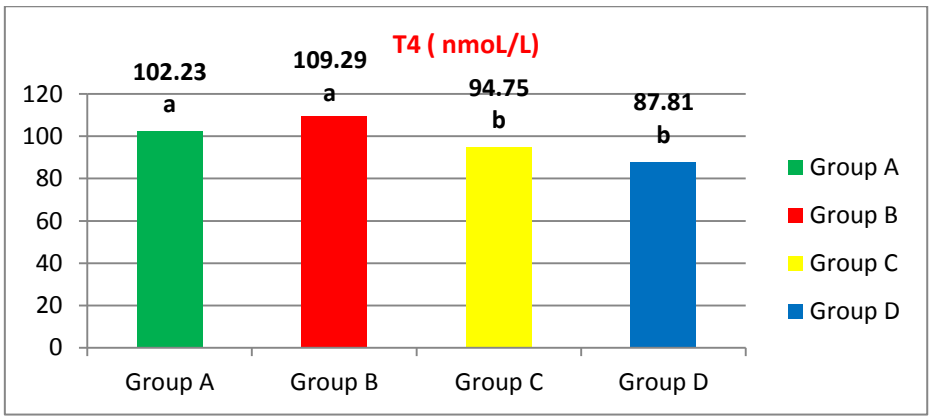
Figure(4-1): The T3 concentration in control , infertile and hypothyroidism women.

- Values represent mean ±SD.
- Different letters refer to significant differences among groups at level (P≤0.05).
- Similar letters refer to non-significant among groups.
- Group A=Control(Healthy women)
- Group B=Infertile women
- Group C=Hypothyroidism women
- Group D=Hypothyroidism and Infertile women.

4-1-1-2: Thyroxine (T4)

The values of T4 decreased significantly (P≤0.05)in C (94.75±21.62 nmoL/L) and D (87.81±22.81 nmoL/L) groups compare with A(102.23±25.85 nmol/L)and B(109.29± 16.46nmoL/L) groups. A group

did not differ significantly in comparison with B group. No significant differences between C and D groups (Figure 4-2, Table 4-1) .



Figure(4-2):The T4 concentration in control, infertile and hypothyroidism women.

- Values represent mean ±SD.
- Different letters refer to significant differences among groups at level (P≤0.05)
- Similar letters refer to non-significant difference among groups.

Table (4-1): The Values of Serum Thyroid Hormones Parameters in Control , Infertile and Hypothyroidism women.

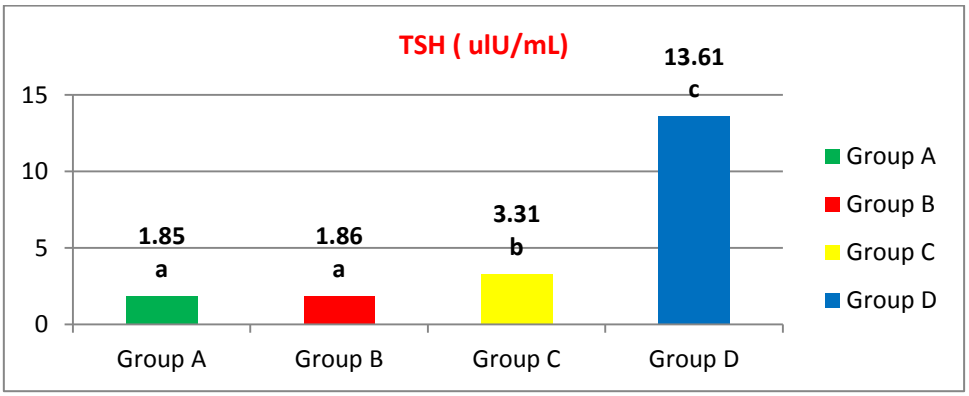
Groups	T3 nmoL/L	T4 nmoL/L
Group-A	2.009±0.50 a	102.23±25.85 a
Group-B	1.95±0.33 a	109.29±16.46 a
Group-C	1.67±0.41 b	94.75±21.62 b
Group-D	1.67±0.45 b	87.81±22.81 b
LSD	0.28	7.48

- N=22 in each group
- Values represent mean ± SD.
- Different letters refer to a significant differences among groups at level of (P≤0.05).
- Similar letters refer to non-significant differences among groups.
- GA=healthy women (control group)
- GB= infertile women.
- GC=fertile Women with hypothyroidism .
- GD=infertile Women with hypothyroidism .

4-1-2:Pituitary Hormones

4-1-2-1:Thyroid Stimulating Hormone (TSH)

The values of TSH in the D (13.61±2.37 ulU/mL) group increased significantly (P≤0.05) in comparison with A(1.85±0.50 ulU/mL), B (1.86±1.03 ulU/mL) and C (3.31±1.06 ulU/mL) groups . C group higher significantly in comparison with A and B groups. But no significant differences between A and B groups (Figure 4-3 , Table 4-2).

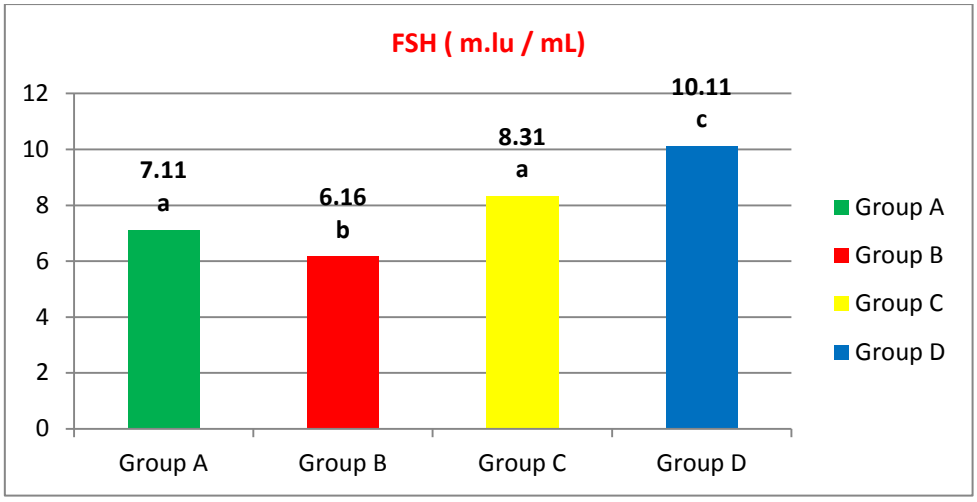


Figure(4-3): The TSH concentration in control , infertile and hypothyroidism women.

- Values represent mean ±SD
- Different letters refer to significant difference among groups at level (P≤0.05)
- Similar letters refer to non-significant difference among groups.

4-1-2-2: Follicle Stimulating Hormone (FSH)

The values of FSH in the D (10.11±1.01 m.lu/mL) group increased significantly(P≤0.05) in comparison with A (7.11± 0.82m.lu/mL),B(6.16±0.44 m.lu/mL) and C(8.31±0.74m.lu/mL) groups .B group decreased significantly in comparison with A and C groups .No significant differences between A and C groups (Figure 4-4 ,Table4-2).

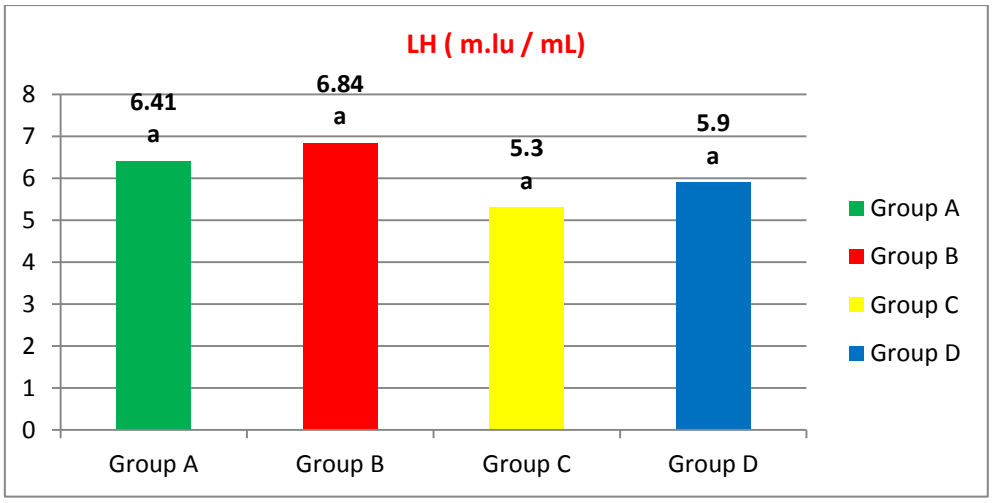


Figure(4-4) :FSH concentration in control , infertile and hypothyroidism women

- Values represent mean ±SD.
- Different letters refer to significant differences among groups at level(P≤0.05).
- Similar letters refer to non-significant differences among groups.

4-1-2-3:Luteinizing Hormone (LH)

The values of LH did not differ significantly (P>0.05) in the A (6.41 ±2.36 m.lu/mL), B (6.84±2.53 m.lu/mL) , C(5.30 ±1.45m.lu/mL) and D (5.90±1.47 m.lu/mL) groups (Figure 4-5,Table 4-2).

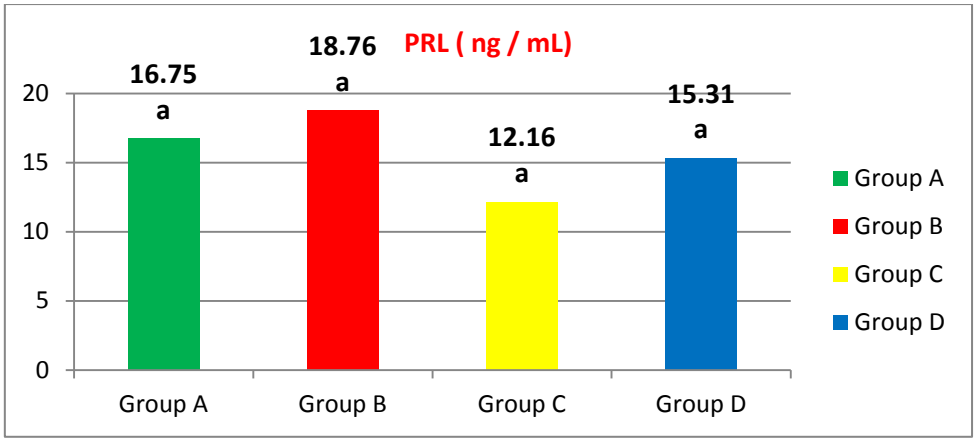


Figure(4-5):LH concentration in control , infertile and hypothyroidism women .

- Values represent mean ±SD.
- Similar letters refer to non-significant differences among groups.

4-1-2-4: Prolactin (PRL)

The values of PRL did not differ significantly (P>0.05) in the A (16.75±9.65 ng/mL) , B (18.76±9.85 ng/mL) , C (12.16± 6.58ng/mL) and D (15.31±8.37ng/mL) groups (Figure 4-6 ,Table 4-2) .



Figure(4-6): PRL concentration of control , infertile and hypothyroidism women

- Values represent mean ±SD.
- Similar letters refer to non-significant differences among groups.

Table(4-2): The Values of Serum Pituitary Hormones Concentrations in Control , Infertile and Hypothyroidism women .

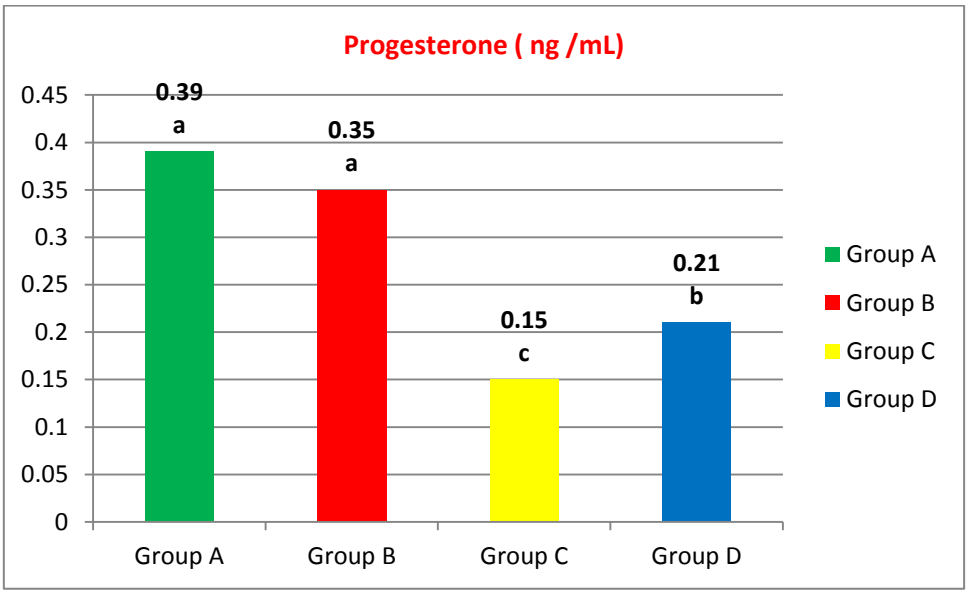
Groups	TSH ulU/mL	FSH m.lu/mL	LH m.lu/mL	PRL ng/mL
Group-A	1.85±0.50 ^{a}	7.11±0.82^{a}	6.41±2.36^{a}	16.75±9.65^{a}
Group-B	1.86±1.03 ^{a}	6.16±0.44^{b}	6.84±2.53^{a}	18.76±9.85^{a}
Group-C	3.31±1.06 ^{b}	8.31±0.74^{a}	5.30±1.45^{a}	12.16±6.58^{a}
Group-D	13.61±2.37 ^{c}	10.11±1.01^{c}	5.70±1.47^{a}	15.31±8.37^{a}
LSD	1.45	0.95	NS	NS

- N=22 in each group
- Values represent mean± SD.
- Different letters refer to a significant difference among groups at level of (P≤0.05).
- Similar letters refer to non-significant differences among groups.

4-1-3:Ovarian Hormones

4-1-3-1:Progesterone

The values of Progesterone decreased significantly(P≤0.05)in C (0.15 ±0.01ng/mL) and D (0.21±0.02 ng/mL) groups compare with A (0.39±0.08 ng/mL) and B(0.35±0.02 ng/mL)groups. A group did not differ significantly in comparison with B group. C group decreased significantly in comparison with D group (Figure 4-7, Table 4-3).

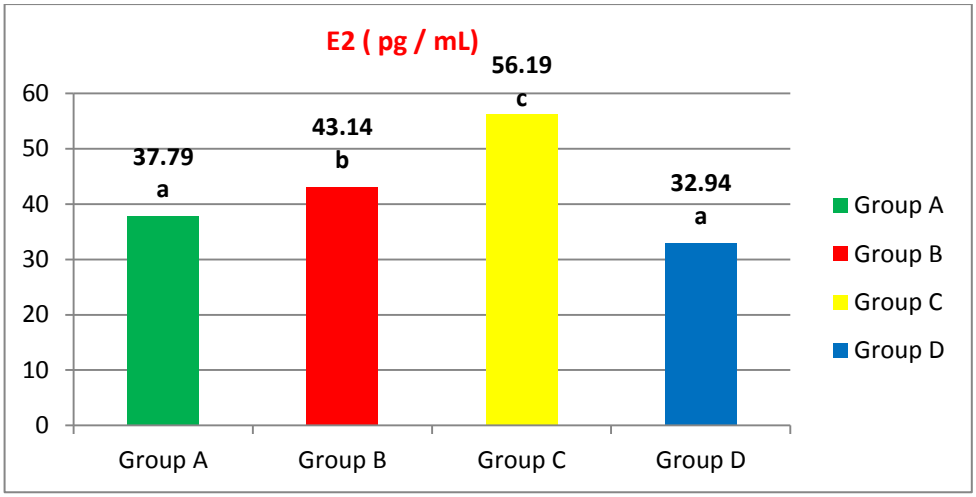


Figure(4-7):Progesterone concentration in control , infertile and hypothyroidism women.

- Values represent mean ±SD.
- Different letters refer to significant differences among groups at level (P≤0.05).
- Similar letters refer to non-significant difference among groups.

4-1-3-2: Estradiol(E2)

The values of E2 in the C (56.19±6.70 Pg/mL) group increased significantly (P≤0.05) in comparison with A(37.79±4.79 Pg/mL) , B(43.14± 4.26Pg/mL) and D(32.94±4.31 Pg/mL) groups . B group increased significantly in comparison with A and D groups. No differences significantly between A and D groups (Figure 4-8,Table4-3).

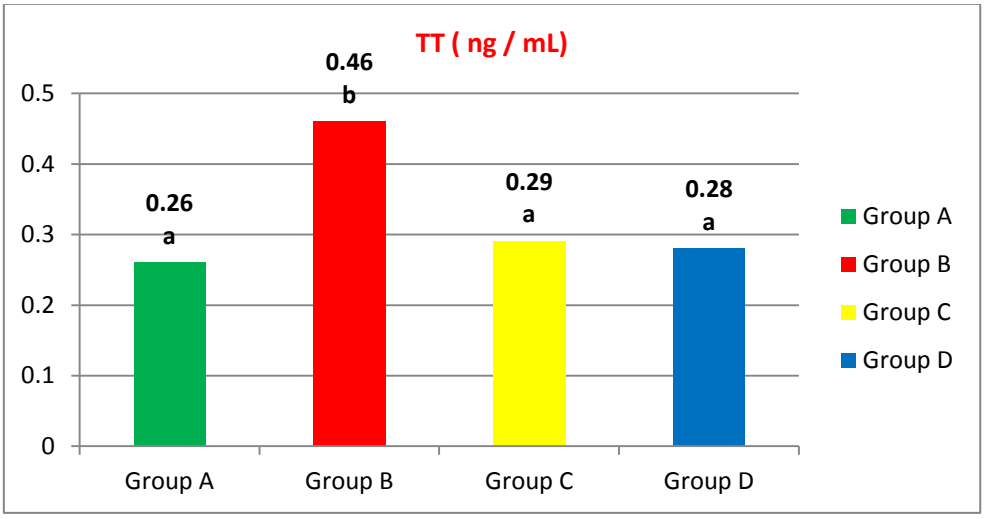


Figure(4-8): E2 concentration in control , infertile and hypothyroidism women.

- Values represent mean ±SD.
- Different letters refer to significant differences among groups at level (P≤0.05)
- Similar letters refer to non-significant differences among groups .

4-1-3-3: Total Testosterone (TT)

The values of TT in the B (0.46± 0.21ng/mL) group increased significantly (P≤0.05) in comparison with A(0.26±0.16 ng/mL) , C (0.29±0.11 ng/mL) and D (0.28± 0.17ng/mL) groups. No differences significantly among A , C , and D groups(Figure 4-9, Table 4-3).



Figure(4-9): TT concentration in control , infertile and hypothyroidism women.

- Values represent mean ±SD.

- Different letters refer to significant differences among groups at level ($P \leq 0.05$).
- Similar letters refer to non-significant differences among groups.

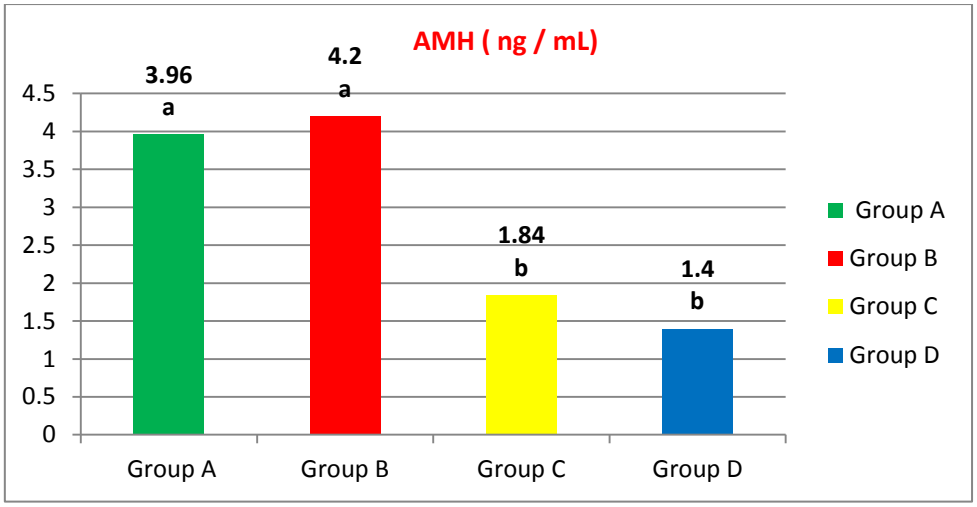
Table (4-3): The Values of Serum Ovarian Hormones Concentrations of Controls, Infertile and Hypothyroidism Women.

Groups	Progesterone ng/mL	E2 Pg/mL	TT ng/mL
Group-A	0.39±0.08[a]	37.79±4.79[a]	0.26±0.16[a]
Group-B	0.35±0.02[a]	43.14±4.26[b]	0.46±0.21[b]
Group-C	0.15±0.01[c]	56.19±6.70[c]	0.29±0.11[a]
Group-D	0.21±0.02[b]	32.94±4.31[a]	0.28±0.17[a]
LSD	0.06	5.35	0.17

- N=22 in each group
- Values represent mean± SD.
- Different letters refer to a significant difference among groups at level of ($P \leq 0.05$).
- Similar letters refer to non-significant differences among groups.

4-1-4: Anti-Mullerian Hormone (AMH)

The values of AMH decreased significantly ($P \leq 0.05$) in C (1.84±0.72 ng/mL) and D (1.4±0.19 ng/mL) groups compare with A(3.96±0.44 ng/mL) and B(4.2±0.60 ng/mL) groups . A group did not differ significantly in comparison with B group. No significant differences between C and D groups (Figure 4-10,Table 4-4).



Figure(4-10): AMH concentration in control , infertile and hypothyroidism women.

- Values represent mean ±SD.
- Different letters refer to significant differences among groups at level (P≤0.05).
- Similar letters refer to non-significant differences among groups.

Table (4-4):The Values of Serum Anti-Mullerian Hormone Concentrations in Control ,Infertile and Hypothyroidism women.

Groups	AMH ng/mL
Group-A	3.96±0.44 [a]
Group-B	4.20±0.60 [a]
Group-C	1.84±0.72 [b]
Group-D	1.40±0.19 [b]
LSD	2.12

- N=22 in each group
- Values represent mean± SD.
- Different letters refer to a significant differences among groups at level of (P≤0.05).
- Similar letters refer to non-significant differences among groups .

Table(4-5):The Researcher Summarized the Results of Hormonal Study.

Parameters		Groups				LSD
		Group A	Group B	Group C	Group D	
Thyroid Hormones	T3 nmoL/ L	2.009±0.50^{a}	1.95±0.33^{a}	1.67±0.41^{b}	1.67±0.45^{b}	0.28
	T4 nmoL/ L	102.23±25.85^{a}	109.29±16.46^{a}	94.75±21.62^{b}	87.81±22.81^{b}	7.48
Pituitary Hormones	TSH ulU/m L	1.85±0.50^{a}	1.86±1.03^{a}	3.31±1.06^{b}	13.61±2.37^{c}	1.45
	FSH (m.lu/ mL)	7.11±0.82^{a}	6.16±0.44^{b}	8.31±0.74^{a}	10.11±1.01^{c}	0.95
	LH (m.lu/ mL)	6.41±2.36^{a}	6.84±2.53^{a}	5.30±1.45^{a}	5.70±1.47^{a}	NS
	PRL (n g/mL)	16.75±9.65^{a}	18.76±9.85^{a}	12.16±6.58^{a}	15.31±8.37^{a}	NS
Ovarian Hormones	Progest erone (ng/mL)	0.39±0.08^{a}	0.35±0.02^{a}	0.15±0.01^{c}	0.21±0.02^{b}	0.06
	E2 pg/mL	37.79±4.79^{a}	43.14±4.26^{b}	56.19±6.70^{c}	32.94±4.31^{a}	5.35
	TT ng/mL	0.26±0.16^{a}	0.46±0.21^{b}	0.29±0.11^{a}	0.28±0.17^{a}	0.17
Anti-Mullerian Hormone	AMH ng/mL	3.96±0.44^{a}	4.20±0.60^{a}	1.84±0.72^{b}	1.40±0.19^{b}	2.12

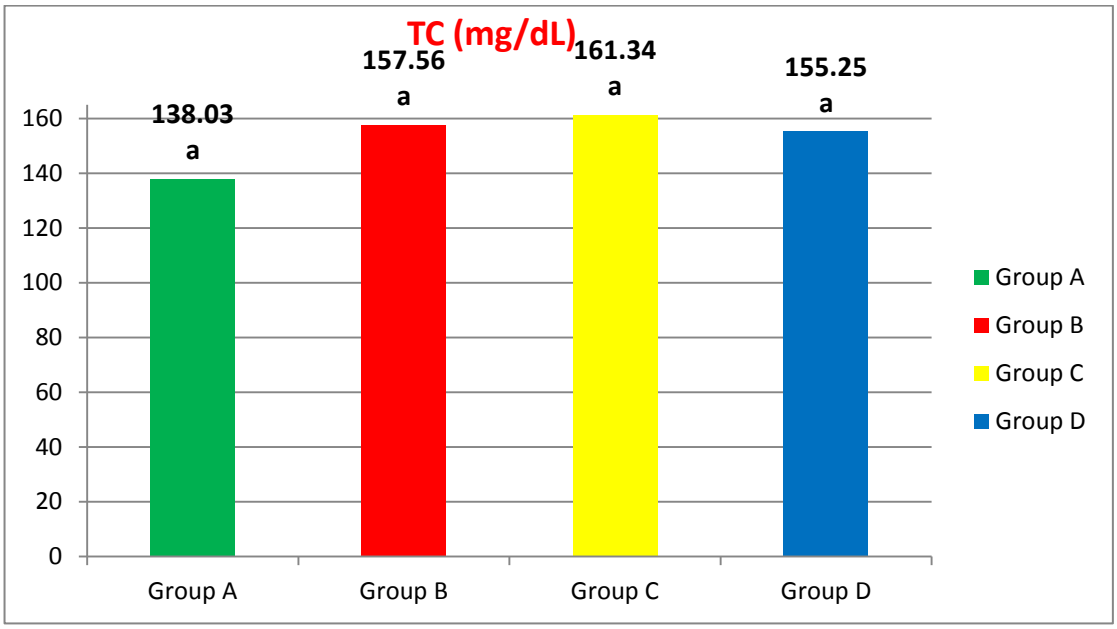
- N=22 in each group
- Values represent mean ± SD.
- Different letters refer to a significant differences among groups at level of ($P \leq 0.05$).
- Similar letters refer to non-significant differences among groups.

4-2:Biochemical Study

4-2-1:Lipid Profile

4-2-1-1: Total Cholesterol (TC)

The total cholesterol values did not differ significantly (P>0.05) in the A(138.03±24.54 mg/dL) , B(157.56±36.64 mg/dL) , C (161.34±28.72mg/dL) and D (155.25±31.74 mg/dL) groups (Figure4-11, Table4-6).

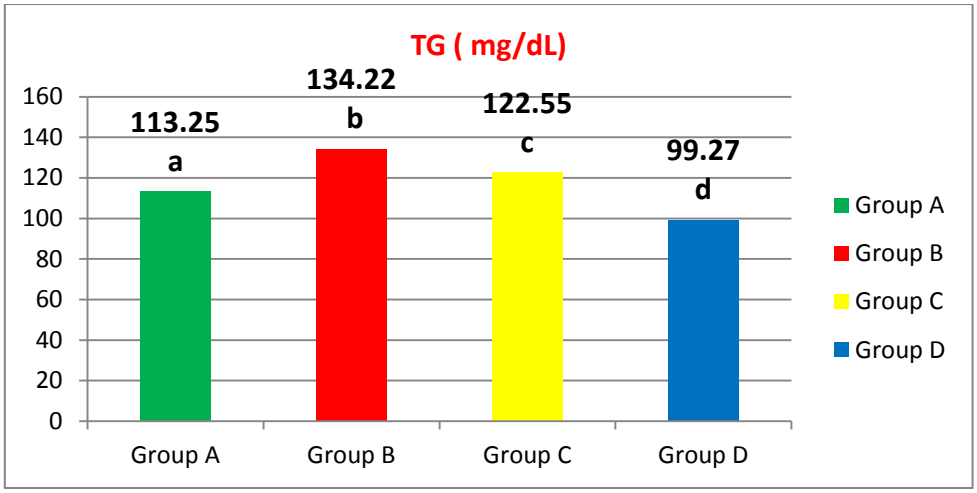


Figure(4-11):The Total cholesterol concentration in control , infertile and hypothyroidism women.

- Values represent mean ±SD.
- Similar letters refer to non-significant differences among groups.

4-2-1-2:Triglyceride (TG)

The values of TG in the B(134.22±4.39mg/dL) group increased significantly (P≤0.05) in comparison with the A(113.25± 4.77mg/dL) , C (122.55±5.45 mg/dL) and D(99.27± 4.59mg/dL) groups. C group increase significantly in comparison with A and D groups(Figure 4-12, Table 4-6).

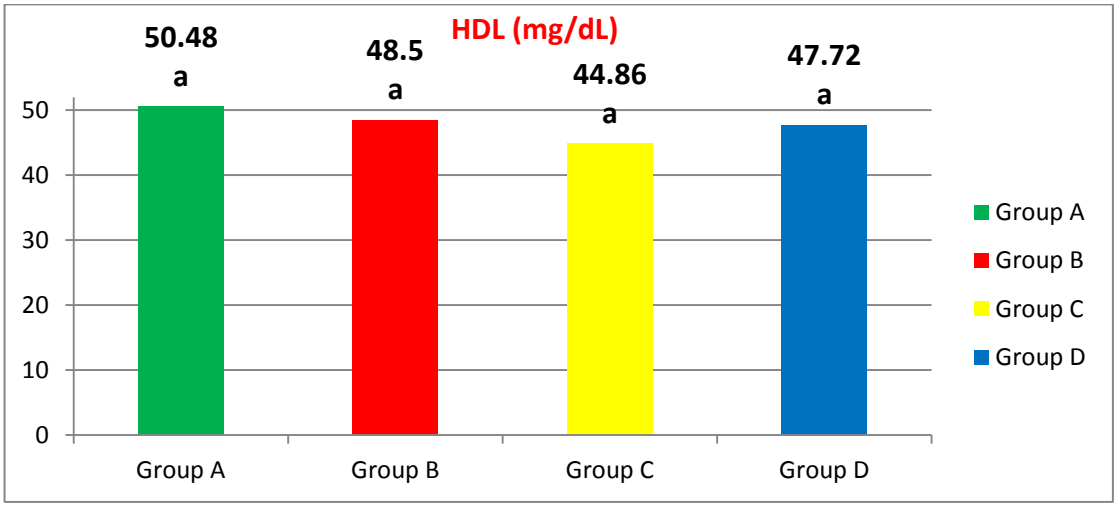


Figure(4-12):TG concentration in control , infertile and hypothyroidism women.

- Values represent mean ±SD.
- Different letters refer to a significant difference among groups at level (P≤0.05).

4-2-1-3: High Density Lipoprotein-Cholesterol (HDL-C)

The values of the HDL-C did not differ significantly (P>0.05) in the A(50.48±4.06 mg/dL), B (48.50±4.67 mg/dL), C (44.86±3.35 mg/dL) and D (47.72±3.72 mg/dL) groups (Figure 4-13, Table 4-6).

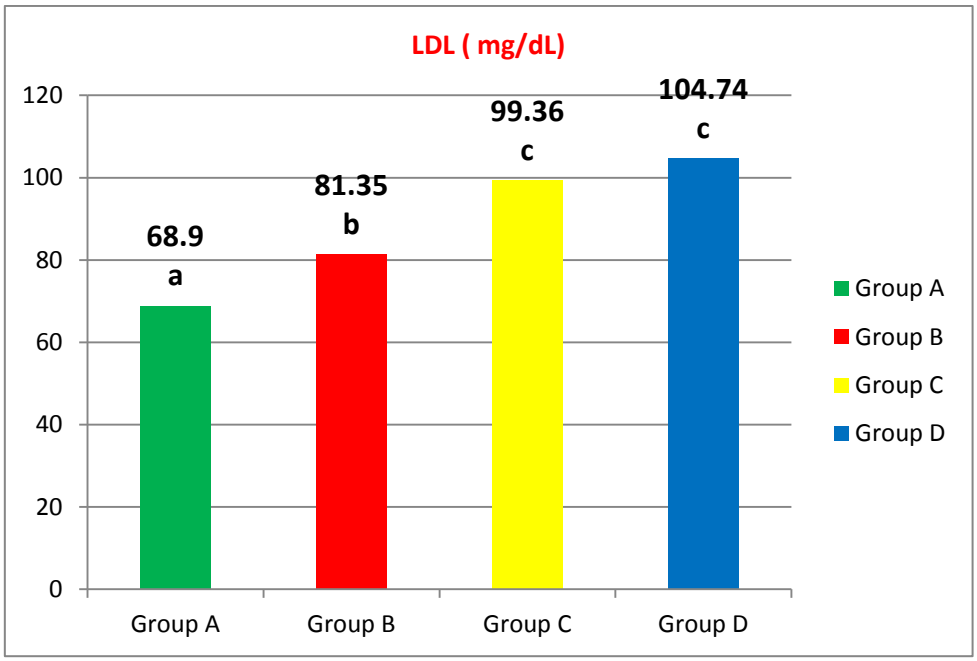


Figure(4-13):The HDL concentration in control , infertile and hypothyroidism women.

- Values represent mean ±SD.
- Similar letters refer to non-significant differences among groups.

4-2-1-4:Low Density Lipoprotein-Cholesterol (LDL-C)

The values of LDL-C in the D (104.74±4.95 mg/dL) group increased significantly(P≤0.05) in comparison with the A (68.90±5.45 mg/dL) and B (81.35±6.18 mg/dL) groups , while did not differ significantly when compared to the C(99.36± 5.81mg/dL)group (Figure 4-14,Table 4-6).

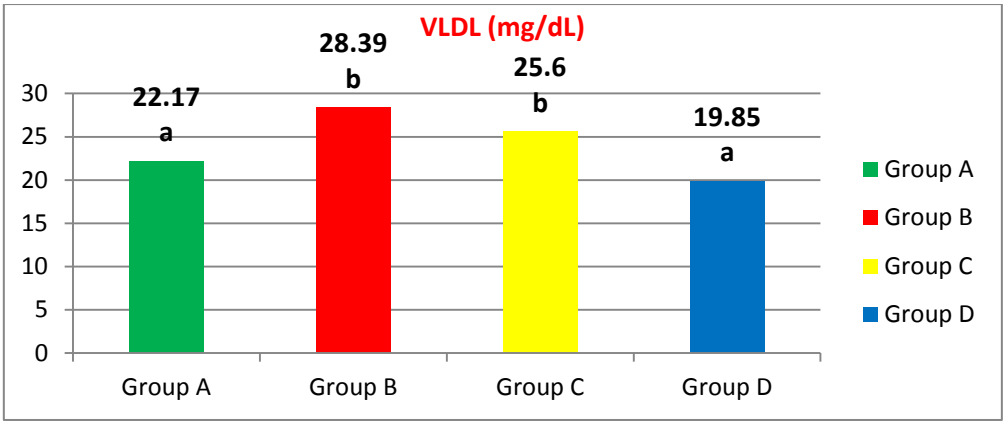


Figure(4-14) :LDL concentration in control , infertile and hypothyroidism women Values represent mean ±SD.

- Different letters refer to a significant difference among groups at level (P≤0.05)
- Similar letters refer to non-significant differences among groups.

4-2-1-5:Very Low Density Lipoprotein-Cholesterol(VLDL- C)

The values of VLDL-C in the B(28.39±2.88 mg/dL) group increased significantly (P≤0.05) in comparison with A(22.17 ±3.16mg/dL) and D (19.85 ±2.63mg/dL) groups, while did not differ significantly in comparison with C (25.60 ±3.15mg/dL) group . No significant differences between A and D groups (Figure 4-15 ,Table 4-6).



Figure(4-15): The VLDL concentration in control, infertile and hypothyroidism women.

- Values represent mean ±SD.
- Different letters refer to significant difference among groups at level (P≤0.05).
- Similar letters refer to non-significant differences among groups.

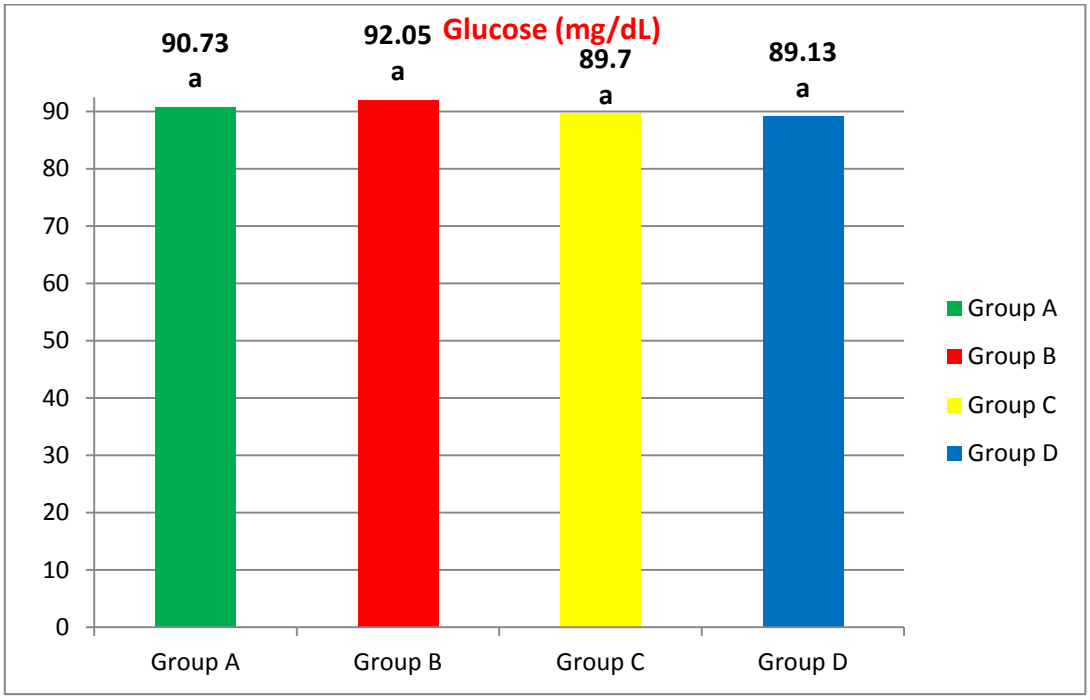
Table(4-6): The Values of Lipid Profile Concentrations in Control, Infertile and Hypothyroidism women.

Groups	TC (mg/dL)	(TG mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL (mg/dL)
Group-A	138.03±24.54^{a}	113.25±4.77^{a}	50.48±4.06^{a}	68.90±5.45^{a}	22.17±3.16^{a}
Group-B	157.56±36.64^{a}	134.22±4.39^{b}	48.50±4.67^{a}	81.35±6.18^{b}	28.39±2.88^{b}
Group-C	161.34±28.72^{a}	122.55±5.45^{c}	44.86±3.35^{a}	99.36±5.81^{c}	25.60±3.15^{b}
Group-D	155.25±31.74^{a}	99.27±4.59^{d}	47.72±3.72^{a}	104.74±4.95^{c}	19.85±2.63^{a}
LSD	NS	9.3	NS	12.45	3.43

- N=22 in each group
- Values represent mean ± SD.
- Different letters refer to a significant differences among groups at level of (P≤0.05).
- Similar letters refer to non-significant differences among groups.

4-2-2: Glucose

The results revealed that the values of glucose concentration did not differ significantly (P>0.05)in the A (90.73±30.13, mg/dL) ,B (92.05±27.36, mg/dL) , C (89.70±14.46, mg/dL) and D (89.13±19.06,mg/dL) groups (Figure 4-16,Table 4-7).

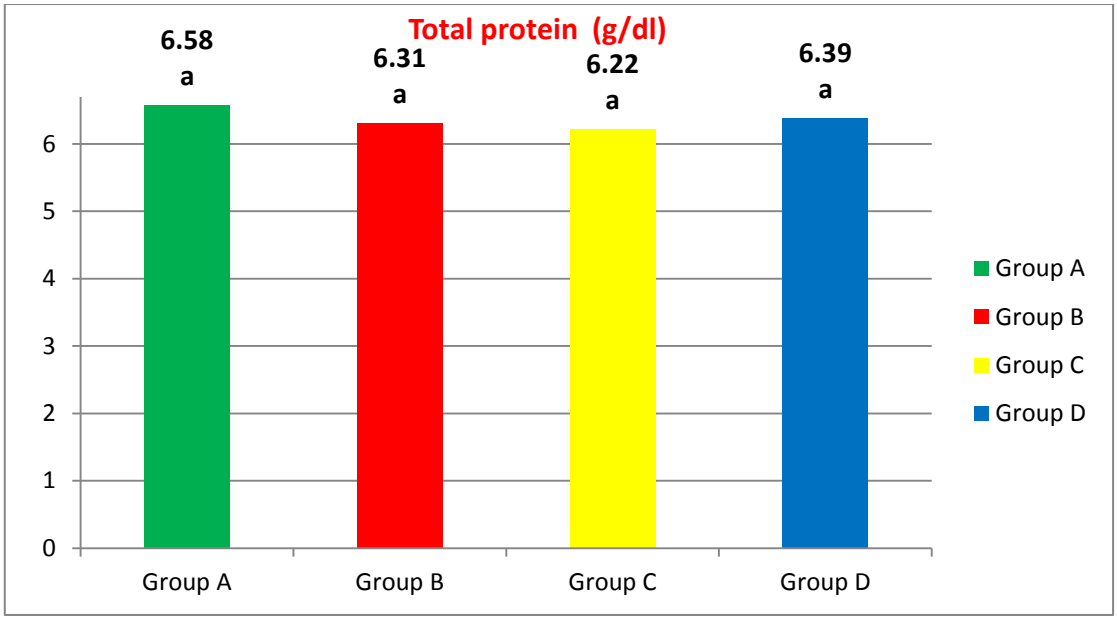


Figure(4-16):Glucose concentration in control , infertile and hypothyroidism women.

- Values represent mean± SD.
- Similar letters refer to non-significant differences among groups.

4-2-3:Total Protein

The total protein values did not differ significantly (P>0.05) among the A (6.58±0.64, g/dl) , B (6.31±0.95, g/dl) , C (6.22± 0.57,g/dl) and D (6.39±0.80, g/dl) groups (Figure 4-17,Table 4-7).



Figure(4-17):The Total protein concentration in control , infertile and hypothyroidism women.

- Values represent mean ±SD.
- Similar letters refer to non-significant differences among groups.

Table(4-7):The Values of Glucose and Total Protein concentrations in control, infertile and hypothyroidism women.

Groups	Glucose (mg/dL)	Total protein (g/dl)
Group-A	90.73±30.13[a]	6.58±0.64[a]
Group-B	92.05 ±27.36[a]	6.31±0.95[a]
Group-C	89.70 ±14.46[a]	6.22±0.57[a]
Group-D	89.13± 19.06[a]	6.39±0.80[a]
LSD	NS	NS

- N=22 in each group
- Values represent mean ± SD.
- Similar letters refer to non-significant differences among groups.

Table (4-8):The Researcher Summarized The Results of Biochemical Study.

Biochemical Parameters	Groups				LSD
	Group A	Group B	Group C	Group D	
TC (mg/dL)	138.03±24.54^{a}	157.56±36.64^{a}	161.34±28.72^{a}	155.25±31.74^{a}	NS
TG(mg/dL)	113.25±4.77^{a}	134.22±4.39^{b}	122.55±5.45^{c}	99.27±4.59^{d}	9.3
HDL-C(mg/dL)	50.48±4.06^{a}	48.50±4.67^{a}	44.86±3.35^{a}	47.72±3.72^{a}	NS
LDL-C(mg/dL)	68.90±5.45^{a}	81.35±6.18^{b}	99.36±5.81^{c}	104.74±4.95^{c}	12.45
VLDL-C(mg/dL)	22.17±3.16^{a}	28.39±2.88^{b}	25.60±3.15^{b}	19.85±2.63^{a}	3.43
Glucose (mg/dL)	90.73±30.13^{a}	92.05±27.36^{a}	89.70±14.46^{a}	89.13±19.06^{a}	NS
Total Protein(g/dl)	6.58±0.64^{a}	6.31±0.95^{a}	6.22±0.57^{a}	6.39±0.80^{a}	NS

- N=22 in each group
- Values represent mean ± SD.
- Different letters refer to a significant differences among groups at level of ($P \leq 0.05$).
- Similar letters refer to non-significant differences among groups.

Chapter Five

Discussion

5-Discussion

5-1:Hormonal Study

5-1-1:Thyroid Hormones

The present study showed significant decrease in the T3 and T4 levels in the C (hypothyroidism women) and D(hypothyroidism and infertile women) groups, while the TSH levels significant increase in the C and D groups . The results of the present study are accedes with the study that done by Sridevi and Sandhya Rani, (2015) which found that T3 and T4 levels are significantly decreased and TSH levels was significantly increased in clinical hypothyroid females compared to controls. These decreases in T4 and T3 lead to hyper-secretion of TSH from pituitary gland and amplified rises of its concentrations in serum (Erem *et al*., 1999).

The results of the present study are accedes with the study carried out by Elzobir *et al* .,(2014) who found that the serum TSH concentration highly increased in primary hypothyroidism group as compared to the control group , also the results of the current study are agreed with the study carried out by Hussein *et al* .,(2017) they found higher significant rise in serum TSH levels in the infertility women group compared to the control group.

This study agrees with the study that done by Kaushik and Kalal ,(2018) who found that serum TSH level was higher in infertile women than fertile . Generally, serum TSH measurement in infertile females is used for hypothyroidism detection, TSH is a major thyroid function hormone (Baloch *et al*.,2003).

Hypothyroidism was diagnosed on the basis of history, physical examination findings (symptoms and signs of hypothyroidism) and

thyroid function tests showing high serum levels of TSH and low serum T3 and T4 (Sibia *et al*.,2018). Women with elevated serum TSH concentrations above 2.5 mIU / L had more menstrual disturbances and an ovulatory cycles, reduced oocyte fertilization and pregnancy rates, increased risk of in-vitro fertilization failure and greater recurrent miscarriage rates(Karaca and Akpak , 2015; Jefferys *et al*.,2015). In a research conducted on 299 infertile females in Finland by Arojoki *et al*.,(2000) which found that TSH was found to be rises in 4% and overt hypothyroidism was identified in 3.3% ,in these cases, ovulatory dysfunction was found to be dominant compared to other cases of infertility.

The prevalence of hypothyroidism differs in different studies , in the infertile females, especially subclinical hypothyroidism , was revealed to range from 0.7% to 43%, this broad prevalence range was attribute to the variations in serum TSH measurement sensitivity (Poppe and Velkeniers,2003; Poppe *et al*.,2008; Krassas *et al*.,2010;Cho,2015;). In other study done by Orazulike and odum, (2018) that found 0.9% of infertile women had overt hypothyroidism but none had subclinical hypothyroidism.

5-1-2:Reproductive Hormones

The results of the present study showed that FSH values significant increase in the D(infertile women with hypothyroidism) group , LH and PRL values did not differ significantly among four groups, while progesterone values showed significant decrease in the C(fertile women with hypothyroidism) and D(infertile women with hypothyroidism) groups in comparison with A(control) group ,and TT values showed significant decrease in the C(fertile women with hypothyroidism) and D(infertile women with hypothyroidism) groups and showed significant

increase in the B(infertile women) group, E2 values significant increase in the B (infertile women) and C(fertile women with hypothyroidism) groups and decrease in the D (infertile women with hypothyroidism) group.

The results of the present study are agrees with the study that done by Ramadhan and Abdala,(2012) which found there are significant decrease in the levels of testosterone and progesterone in hypothyroid females , and also agrees with the study that done by Saran *et al* .,(2016) that found hypothyroidism is associated with decreases levels of serum testosterone.

About 66% of testosterone is bound to SHBG and 33% is bound to albumin, leaving only 1-2% of testosterone unbound, this unbound or bioavailable testosterone that enters target tissues (Strauss and Barbieri, 2009). Hypothyroid women demonstrate a reduced androgen metabolite proportion of 5 α/β and an increase in the excretion of 2-oxygenated estrogens(Krassas and Pontikides ,2013).

In hypothyroidism, plasma binding activity of SHBG is reduced, resulting in reduced plasma levels of both total testosterone and E2, but their free fractions are increased (Hampl *et al*.,2003). Also, reduction in the metabolic clearance of androstenedione and estron may increase the aromatization of peripheral estrogens (Longcope *et al*.,1990).

Progesterone stimulation thyroid hormones assimilation into the cells, allowing the thyroid gland to work properly and provide energy, Sathi *et al*.,(2013) ,has discovered that progesterone therapy increases free thyroxine (T4) .

In mild hypothyroidism pregnancy may be happen and are linked with greater pregnancy loss rates and maternal complications (Stray-Pedersen and Tray-Pedersen, 1984; Davis *et al*., 1988). Although there is

an association between low thyroid function and loss of pregnancy, there is no direct proof of a causal role (Clifford *et al*., 1994). One hypothesis for this correlation is that luteal phase defect has been linked to thyroid hypofunction. In consideration that production of progesterone is a pivotal element of a successful pregnancy, it is possible that pregnancy loss could be related to a deficient corpus luteum action (Daya *et al*., 1988).

The results of the current study did not agree with another recent study by Lal *et al* .,(2016) that found 40.7% of infertile women with hypothyroidism exhibiting hyperprolactinemia ,the results also, did not agree with the same study that found serum LH and FSH decreased in infertile women with hypothyroidism in compare with control group , LH and FSH negatively correlated with prolactin.

The increasing of FSH level in this study may tell us that ovarian reserve is poor ,while the decrease in the level of LH and FSH in hypothyroidism women was recorded in study of Kalsum and Jalali, (2002) they found significant decrease in serum LH in follicular, ovulatory and luteal phase in hyperprolactinemic women having primary and secondary infertility and significantly low serum FSH value observed in ovulatory phase in women with primary infertility and in luteal phase in women with secondary infertility, and may be because these hormones measured in hypothyroidism and infertility women with hyperprolactinaemia.

The normal value of the PRL and LH levels in our study may be because treatment with thyroxine in accordance of the result observed by Bachimanchi *et al* .,(2019) which found PRL elevation in 36% of patients with overt hypothyroidism and 22% of patients with subclinical hypothyroidism, the PRL level decreased to normal in all patients after

thyroid functions were normalized with L-thyroxine treatment and E2 levels increased significantly, L-thyroxine treatment also reverse menstrual abnormalities and increases spontaneous fertility (Atis *et al*.,2010).

In contrast to the results of the present study, other study showed that the increasing of the PRL levels in hypothyroidism women , these increase may be due to TRH stimulation where the increase in PRL level under hypothyroidism is related to TRH stimulation, as lactotropic which resemble thyrotropic cells, express membrane receptors to releasing hormone. In addition, the increase in PRL may be due to an increase in pituitary vasoactive intestinal peptide(VIP), which acting as a paracrine or autocrine to affects PRL secretion regulator in rats according to study by Tohei *et al*.,(2000).

THs interact with both estrogens and progesterone to maintain a normally functioning uterus and are necessary for the normal maturation of the oocytes, the impact of thyroid hormones has been reported to be both direct through the presence of thyroid hormone receptors on the ovaries and indirect through an impact on the secretion of sex hormone binding globulin SHBG, prolactin and luteinizing hormone releasing hormone (LH-RH) (Poppe *et al.*, 2008).

5-1-3:Anti-Mullerian Hormone

The present study showed significant decrease in AMH concentration in the C(fertile women with hypothyroidism) and D(infertile women with hypothyroidism) groups. This results are in agreement with study done by Kuroda *et al* .,(2015) which found the concentration of AMH was inversely correlated with concentration of TSH in women at reproductive age suffering from infertility . Also, the agreement with the study is done by Kucukler *et al* .,(2016) which investigated the association of AMH

values and ovarian reserve in women with autoimmune thyroid disease(AITD). Although this study demonstrated low serum AMH concentration in women with AITD, the difference was not statistically significant, Since ovarian reserve decreases with increasing age, women with hypothyroidism are at increased risk for development of ovarian insufficiency.

Our study results agrees with the study that is done by Al-Jaff ,(2018) which found that the AMH level decreases in the hypothyroid group compared with control this result is incoming with Kuroda *et al*.(2015) and Weghofer *et al*.,(2016). The inversely association between TSH and AMH in hypothyroidism women may be belonging to, the negatively effects of TSH on Follicles ovarian Reserve (FOR) which leading to significant decrease in AMH (Hansen *et al*.,2011; Meng *et al*.,2017).

Because subclinical females with hypothyroid are at enhanced danger of infertility, high concentrations of TSH may have deleterious impacts on ovarian function (Kuroda *et al*.,2015). In infertile patients with elevated serum TSH concentrations also revealed high prevalence clinical information of decreased ovarian reserve (Michalakis *et al*.,2011).

The ovarian responsiveness of thyroid hormone receptors could be explained by the existence of thyroid hormone receptors in human oocytes(Wakim *et al*.,1993). THs also synergize with the FSH-mediated LH / hCG receptor to directly stimulate cell function (progesterone production) (Cecconi *et al*.,1999).

Cramer *et al*.,(2003) showed that serum TSH concentrations were an important predictor of in vitro fertilization (IVF) failure, as TSH

concentrations were considerably greater among females who manufactured non-fertilized oocytes.

One hypothesis which found that TSH in patients with subclinical hypothyroidism could directly suppress the growth of follicles. In addition, depleted thyroid hormones secretion in patients with overt hypothyroidism may have an additional adverse effect on follicle recruitment(Kuroda *et al* .,2018).

5-2: Biochemical Study

5-2-1:Lipid Profile

The results of the present study showed that the TC and HDL-C levels did not differ significantly among four groups , while the levels of LDL-C which significant increase in the C (fertile women with hypothyroidism) and D(infertile women with hypothyroidism) groups, and results of TG and VLDL-C levels significant increase in the B(infertile women) and C(fertile women with hypothyroidism) groups in comparison with A(control) group.

The results of the current study agrees with the study that is done by Al salmi *et al*., (2018) ,they found a significant increase in LDL-C, VLDL-C, and TG values in hypothyroidism patients compared to normal thyroid group. And the present study did not agreed with the above study in regarding TC and HDL-C.

The results of the present study agrees with the study that was done by Hiregoudar *et al* .,(2019) which found that the HDL-C value was normal in hypothyroid patients also this value did not differ significantly between overt and subclinical hypothyroidism patients . While, the LDL value was elevated in overt hypothyroidism patients significantly. But the

present study did not agreed with the above study in regarding to TC and TG values.

The present study did not agree with the study that is done by Satyajit and Arindam ,(2017) that found high prevalence of hypercholesterolemia in hypothyroid females with significant positive correlation with TSH in compare to control group.

The increase in LDL-C, VLDL-C and TG because of decreasing in TG-rich lipoprotein clearance, the high concentrations of TG in overt hypothyroidism patients may associated with increasing concentrations of VLDL and sometimes fasting chylomicronemia (Al-Tonsi *et al*.,2004).

5-2-2:Glucose and Total protein

The results of the present study showed that there are no significant differences in glucose level among four groups. This study agrees with the study that carried out by Satyajit and Arindam,(2017) which found no significant differences between fasting plasma glucose(FPG) and hypothyroidism in females.

The present study did not agree with the study that performed by Dogantekin *et al* .,(2015) that found Fasting blood glucose(FBG) level was decreased in the hypothyroidism group in comparision with hyperthyroidism group and found a negative correlation between FBG and TSH. Also, another study performed by Mushtaq *et al* ., (2017) which found a positive correlation between FBG and TSH in hypothyroidism patients .

The glucose level in hypothyroidism patients differ according to the results of researchers .When it's decreased this may be because lower of insulin level. Maratou *et al* ., (2009) which found decreased level of insulin in patients with overt and subclinical hypothyroidism due to

disrupted translocation of the glucose transporter type 4(GLUT- 4) in the plasma membrane lead to decreased level of insulin –stimulated glucose transporter in monocytes. While the increased of glucose level may be due to TSH level increased in hypothyroidism patients because many studies showed that the interaction between hypothyroidism and diabetic patients ,and may be because the increase in TSH level lead to decreased the metabolism and turnover of glycated serum proteins, lead to increase HbA 1c and also, accumulate free radicals in the tissues and inhibit proteolysis leading to further aggravating in hyperglycaemia in these patients (Kim *et al*.,2010;Anantarapu *et al*., 2015).

The normal values of glucose in the present study may be because all the women in the present study did not suffer from diabetes mellitus or have problems in insulin level.

The results of total protein concentration in this study revealed that there is not significant difference among four groups. The present study agrees with the study that presented by Deepika *et al* .,(2015) which found that there are no significant differences in the level of total protein in patient with hypothyroidism , but this study did not agrees with the study carried out by Kalita *et al* .,(2016) which found the Serum total protein significant increase in hypothyroid patients in comparison with control group. Also, the study done by Chang *et al*.,(2018) which found a significant increase in proportion of subclinical, and overt hypothyroidism with decreasing estimated glomerular filtration (eGFR) level and an increased severity of proteinuria .The causal relationship between hypothyroidism and proteinuria is also uncertain, the increase urinary loss of thyroid hormones binding protein in patient with nephrotic syndrome results in a decrease in total T4(Feinstein *et al*.,1982) .

Thyroxine therapy has been shown to decrease edema, capillary permeability of albumin, and plasma colloid osmotic pressure even in patients with subclinical hypothyroidism, indicating thyroid hormone deficiency may increase protein vascular permeability (Wheatley and Edwards,1983).

The normal values of total protein in the present study may be because that all the women did not suffer from any other disease such as proteinuria or nephritic syndrome.

Chapter Six

Conclusions and Recommendations

6:Conclusions and Recommendations

6-1:Conclusions

Results of the present study showed that the following conclusions.

1. Hypothyroidism in women can affected fertility by decreased levels of AMH, and Progesterone .

2.There are reverse relationship between AMH and TSH , when TSH level high the level of AMH low and vice versa.

3.The results of PRL showed the normal range in the healthy ,infertile and hypothyroidism women groups.

4.The level of Total Testosterone increased in infertile women.

5.The level of LDL increased in the hypothyroidism women .

6-2: Recommendations

1. Comparative study can be made between women and men affected with hypothyroidism.

2.Comparative study can be made between overt , clinical and subclinical hypothyroidism.

3.Study can be made between of hypothyroidism and other metabolic disorders such as Diabetes mellitus and obesity in women.

4.Studying the thyroid receptors gene and metabolic regulation.

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