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Estimation Levels of Kisspeptin , Reproductive Hormone and Biochemical Parameters in Women With Hyperprolactinemia, Obesity and Type 1 Diabetes Mellitus at Misan Province

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

(وَيَسْأَلُونَكَ عَنِ الرُّوحِ ۖ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُم مِّنَ الْعِلْمِ إِلَّا قَلِيلًا)

صدَقَ الله العلي العَظِيم

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Supervisor' s Certificate

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Dedication

To my trust and hope…… **my Lord**

To the sacrificers and supporters……**my parents**

To the lovers and the truthful…… **my sisters and brothers**

To everyone who helped me……

I dedicate this work to you

Nakaa

Acknowledgment

First, I would like to thank “God “for his care and support me throughout my life and especially through the accomplishment of this research.

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Nakaa Qassim Ghali

Summary

The current study aimed to known the health and reproductive status in women by estimation the levels of kisspeptin ,some hormonal and biochemical parameters in female with hyper prolactin ,obese and Type 1 Diabetes Mellitus (T1DM) in Maysan province, conducted in each of the Al-Sadr Teaching Hospital , Maysan for child and birth hospital ,Center for Diabetes and Endocrine and Abn Al Hitham laboratory during the period from June 2022 to February 2023. The whole sample is 92 Women (aged 20 - 40 years) , divided into four main groups (23 women / group) , as a following: Control group: healthy women with regular menstrual cycles without any hormonal disturbances , Hyperprolactinemia group : women with hyper serum prolactin ,Obesity group : women have a BMI over than 30 kg / $M^2$and Diabetic group :women with T1DM.

The present results revealed that the values of kisspeptin did not differ significantly ($P>0.05$) among all the four group .The values of gonadotropin releasing (GnRH) hormone in hyperprolactinemia, obesity and T1DM groups increased significantly ($P\leq0.05$) in comparison with control group.

The values of follicle stimulating hormone (FSH) and luteinizing hormone (LH) did not differ significantly ($P>0.05$) among the four group. The values of prolactin in the hyperprolactinemia group increased significantly ($P\leq0.05$) in comparison with T1DM, obesity and control groups.

The value of estradiol in hyperprolactinemia and obesity group increased significantly ($P< 0.05$) in comparison with T1DM and

control groups . The value of progesterone in hyperprolactinemia, obesity and T1DM groups increased significantly (P< 0.05) in comparison with T1DM, obesity and control groups .The value of testosterone in obesity and T1DM one group increased significantly (P< 0.05) in comparison with T1DM, hyperprolactinemia and control groups .

The values of 8-isoprostance in obesity and hyperprolactinemia groups increased significantly (P≤0.05) in comparison with T1DM and control groups.

The values of HBA1C and fasting glucose in the T1DM group increased significantly (P≤0.05) in comparison with obesity , hyperprolactinemia ,and control groups.

The values of cholesterol in hyperprolactinemia group decreased significantly (P≤0.05) in comparison with the T1DM and obesity groups. The values of Triglyceride (TG) in the T1DM group increased significantly (P≤0.05) in comparison with hyperprolactinemia and control groups .The values of High-density lipoprotein (HDL) in the hyperprolactinemia and obesity groups decreased significantly (P≤0.05) in comparison with control group .The values of Low-density lipoprotein (LDL) did not differ significantly (P>0.05) in control , hyperprolactinemia , obesity and the T1DM groups . The values of Very Low-density Lipoprotein (VLDL) in the T1DM group increased significantly (P≤0.05) in comparison with control group.

The physiological effect of the results be discussed according to the action of the high level of prolactin , increased concentration of

blood glucose and fat mass in hyperprolactinemia , obesity and T1DM groups

Kisspeptin wasn’t an effective agent , fertility differenced , excessive oxidative stress and Metabolic deterioration in hyperprolactinemia, obesity and T1DM.

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Abbreviations	Equivalences
4-AAP	4-aminoantipyrine
8-iso-PGF2a	8-isoprostane
ALT	Alanine aminotransferase
AMH	Anti-mullerian hormone
AMPK	Adenosine monophosphate activated protein kinase
ApoA	Lipoprotein-(Aa)
ApoB	Lipoprotein-B
BMI	Body Mass Index
CAB	Combined androgen blockade
CB	Cord blood
CPP	Central precocious puberty
CVD	Cardiovascular disease
DM	Diabetes mellitus
E2	Estradiol
EBC	Exhaled breath condensate
ECLIA	Electrochemiluminescence immunoassay
EDTA	Ethylenediaminetetracetic acid
ELISA	Enzyme-linked immunosorbent assay
ERK	extracellular signal regulated kinase
ESR2	Estrogen receptor2
eWAT	Epididymal white adipose tissue

FFAs	Free fat acids
FG	Fasting glucose
FI	Fasting insulin
FIegs	Fasting insulin equivalent
FSH	Follicle stimulating hormone
GDM	Gestational Diabetes Mellitus
GnRH	Gonadotropin releasing hormone
GnRH-R	GnRH receptor
GPCR	G-protein coupled receptor
GPR54	G-protein coupled receptor 54
HBA1C	Hemoglobin A1c
HDL	High-density lipoprotein
HFD	High-fat diet
HGNC	Human Genome Organization Gene Nomenclature Committee
HMG	Human menopausal gonadotrophin
HOMA-IR	Homeostatic model assessment index
hOT7T175,	Names kisspeptin receptor
HPE	Hyperprolactinemia
Hyper-PRL	Hyperprolactin
HPA-S	human preadipocytes-subcutaneous
ICD	International Classification of Disease
IDDM	Insulin Dependent Diabetes Mellitus
IGR	Impaired glucose regulation

IP3	inositol triphosphate
IR	Insulin resistance
ISI	Insulin sensitivity index
IsoPs	Isoprostanes
IVF	In Vitro Fertilization
KiSS1	Kisspeptin gene animal
KISS1	Kisspeptin gene human
KISS1R	Kisspeptin receptor
KNDy	Kisspeptin Neurokinin B Dynorphin
KP	Kisspeptin
Kp-10	Kisspeptin-10
LDL	Low-density Lipoprotein
LH	Luteinizing hormone
LHRH	Luteinizing hormone releasing hormone
LTGV	long-term glycemic variability
MAPK	Mitogen-activated protein kinase
MB	Mothers' blood
MRI	Magnetic resonance imaging
MS	Metabolic syndrome
NCDs	Non-communicable diseases
NIDDM	Non-Insulin Dependent Diabetes Mellitus
Pca	Prostate cancer
PİP2	Phosphatidylinositol diphosphate
PKC	Protein kinase C
PRI	Prolactin

PRLRs	Prolactin receptor
pWAT	peri-pancreatic white adipose tissue
S.E	Standard error
SHBG	Sex hormone binding globulin
SPSS	Statistical package for social science
STGV	Short-term glycemic variability
STZ	Streptozotocin
T1DM	Type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
TG	Triglyceride
TINIA	Turbidimetric inhibition immunoassay
TRH	Thyrotropic releasing hormone
TTAB	Tetradecyltrimethylammonium bromide
TyG	Triglyceride/glucose
VIP	Vasoactive intestinal peptide
WC	Waist circumference

Chapter one

Introduction and Literature Review

1 Introduction and literature review

1.1 Introduction

The hypothalamus regulates complex autonomic mechanisms that maintain the chemical constancy of the internal environment, and regulates metabolic endocrine processes to control body temperature and satiety. It synthesizes and secretes hypothalamic hormones, and these in turn stimulate or inhibit the secretion of pituitary hormones. The hypothalamus also functions with the limbic system as a unit that regulates emotional and instinctual behavior (Barrett,2019).

Kisspeptin, is a group of neuropeptides necessary for both puberty and maintenance of healthy reproductive function, increase the release of gonadotrophin-releasing hormone (GnRH) .The development of reproductive ability is necessary for the survival of all species. Mammals begin to become fertile during puberty when a limited number of neurons in the hypothalamus secrete GnRH in a pulsatile manner. Nerve terminals in the palisade layer of the median eminence of the hypothalamus release GnRH into the hypophyseal portal blood stream. Luteinizing hormone (LH) and follicle stimulating hormone (FSH), two gonadotrophic hormones, are stimulated by the GnRH's action on the anterior pituitary. The gonads are affected by the gonadotrophic hormones, which promote gametogenesis and sexual maturation (d'Anglemont and Colledge,2010).

Hyperprolactinemia (HPE) is a common endocrine disorder (Souter *et al.*,2010). HPE defined by elevated serum prolactin levels above the normal range (Davis,2004). HPE can present as a pathological condition

at any age, and this excess of prolactin may result from a variety of causes (Crosignani, 2006) .

Elevated prolactin may impact reproduction through inhibitory effects on hypothalamic GnRH neurons and/or on the pituitary gland to reduce secretion of the gonadotropins (FSH and LH), resulting in a reduction in both amplitude and frequency of LH pulses. Prolactin may act directly on GnRH neurons to suppress GnRH secretion, or the effects may be indirect, mediated through other afferent pathways, perhaps via other neurons influencing GnRH release (Kokay *et al* .,2011). Prolactin effects on the glucose and lipid profile , high circulating prolactin was associated with lower prevalence of diabetes and impaired glucose regulation (IGR) (Wang *et al* ., 2013). Also , in patients with prolactinoma, showed disorder in the levels of lipids (Erem *et al*.,2010) .

Obesity is a major worldwide problem, over 30% of adults in western populations are obese, and there is growing evidence of the associated health risks (Pender *et al.* ,2005). The prevalence of obesity has been rising steadily over the last several decades and is currently at unprecedented levels: more than 2.1 billion people are overweight or obese and more than 68% of United States adults are considered overweight, and 35% are obese (Smith and Smith,2016). This increase has occurred across every age, sex, race, and smoking status, and data indicate that segments of individuals in the highest weight categories (BMI > 40 kg/m2) have increased proportionately more than those in lower BMI categories (BMI < 35 kg/m2). The dramatic rise in obesity has also occurred in many other countries (Wright and Aronne, 2012).

The impact of obesity on fecundity is complex (Brewer and Balen,2010 ; Pantasri and Norman ., 2014) .Obesity induces a hormonal milieu consisting of insulin resistance, hyperinsulinemia, low sex hormone-binding globulin, elevated androgens, increased peripheral conversion of androgens to estrogens, increased free insulin-like growth factor 1 and high leptin (Pasquali *et al*. ,2006 : Norman ,2010) .The combined effect of these changes causes hypothalamic dysfunction, aberrant gonadotropin secretion, reduced folliculogenesis and lower luteal progesterone levels (Pasquali and Gambineri 2006 ; A. Jain *et al*.,2007).

Obesity is associated with increased basal lipolysis in adipose tissue, and elevated circulating free fatty acids (Van Hall *et al*.,2003). Dysregulation of lipid metabolism affects the body fat mass, fat-free mass, fatty acid metabolism, and various aspects of energy metabolism, such as basal metabolic ratio, adiposity, and obesity (Ko *et al* .,2020).

Diabetes mellitus (DM) is a group of metabolic disorders in which there are high blood glucose levels over a prolonged period due to insufficient amount of insulin producing from pancreas or insulin resistance (Kumar *et al*., 2017).

The gonadotropic axis and GnRH neurons may be directly targeted by insulin, which would alter their secretory activity (Tena-Sempere, 2007) .

Diabetes affects lipid profile, dyslipidemia is highly prevalent among diabetic population particularly in those with poorly controlled diabetes (Shahwan *et al*.,2019).

The aims of the study

The current study aimed to high light on the health and reproductive status of women with hyperprolactinemia, obesity and T1DM by measuring the following parameters:

A-Kisspeptin

B-The hormonal parameters include :

1-Gonadotropin Releasing Hormone (GnRH)

2- Follicles Stimulating Hormone (FSH)

3-Luteinizing Hormone (LH)

4- Prolactin

5-Estradiol

6- Progesterone

7-Testosterone

C- Biochemical parameters include:

1-HbA1C ,Glucose and 8-Isoprostane

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

1.2 Literature review

1.2.1 Kisspeptin (KP)

1.2.1.1 Identification of Kisspeptin ,gene ,structure, naming and receptor it .

KPs, a family of peptides with similar structures, functions, and origins, KISS1 is the gene that produces KPs, was first identified in 1996 as a preventer of metastases in human malignant melanoma (Lee *et al*.,1996). In honor of the famed chocolate "Kisses" made in the town of Hershey, Pennsylvania, where the gene was discovered, it was given that name. The KISS1 SS also refers to the protein's role as a "suppressor sequence." The KISS1 gene, which has four exons but only the last two are translated, is expressed upon this chromosome's longest arm 1 (q32) . It encode 145 amino acid peptide that is cut into 14 peptides of 14, 13, and 10 amino acids each after being cleaved into a 54 amino acid sequence (West *et al*., 1998).

The cleavage products all belong to the RF-amide group and have the same Argnine-Phenialane-NH2 C-terminal sequence (Kotani *et al*., 2001). The orphan G-protein coupled receptor 54 (GPR54) was first described in the rat and then in human brain (Lee *et al*.,1999; Ohtaki *et al*., 2001).

KP was then discovered to be a ligand for the adjacent GPR54, which is known as KISS1R. (Gottsch *et al* .,2009). On chromosome

19p13.3, the KISS1R gene codes for a 398 amino acid receptor through five exons (Muir *et al*., 2001).

According to the guidelines of the Human Genome Organization Gene Nomenclature Committee (HGNC), using the symbol KISS1 for the human gene and Kiss1 for the non-human KP gene in order to standardize nomenclature for the mouse, rat, and human genomes(Gottsch *et al*., 2009).

The protein's 54 amino acids were originally given the name "metastin" to honor its antimetastatic capabilities (Ohtaki *et al*., 2001).

The 54 amino acid metastin and each shorter 14, 10, and 13 amino acid peptide are collectively known as KPs , because they are all translated from the same KISS gene , as in the figure (1.1) .When indicate to the precise length of the peptide, the terms KP-54, KP-14, KP-10, and KP-13 are also used. Others have defined KP in terms of the numerical order in which the 145 amino acid precursor was cleaved; for example, KP (68-121) denotes KP-54, while KP (112-121) denotes KP-10 (Gottsch *et al*., 2009; Owens, 2019) .

Gottsch and colleagues (2009) recommend depends on the size of the KP and abbreviating these to KP for the human and kp in the non-human setting as The majority of publications describe how KP's bioactive fragment is used.

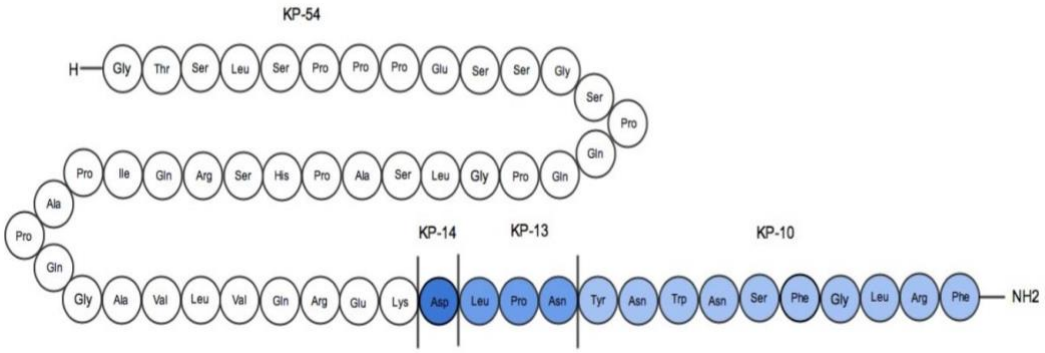


Figure (1.1) Amino acid sequences for KP. The C-terminal decapeptide sequence shared by all KP ligands has ten identical amino acids (light blue). KISS1 encodes a protein that can be further cleaved into the 54 amino acid sequences kp-10 (light blue), or kp14 (dark blue), kp13 (darker blue) (Owens, 2019) .

The other names for the KP receptor are AXOR12, hOT7T175, KISS1, KiSS1, GPR-54 and the metastin receptor. For greater consistency, the human KP receptor gene is referred to as KISS1R by the HGNC, while Kiss1r has been allocated by mouse genome informatics to the formerly orphaned GPR-54 gene (Gottsch *et al*., 2009) .

The KP receptors are a diffuse distribution in the hypothalamus and gonads . Also, body tissues include the brain, gonads, gastrointestinal tract , and placenta express KISS1R and KISS1 (Lee *et al*., 1996; Ohtaki *et al*., 2001). As well, the infundibular and arcuate nuclei of the hypothalamus express KP neurons (Rometo *et al*., 2007 ;Oakley *et al*., 2009).

The role of a KP system ((KNDy) neurons was recognized in reproduction ,when found a connection between KISS1R mutations ,delayed puberty and idiopathic hypothalamic hypogonadism in both human and mice (Seminara *et al*., 2003). Studies in mice with targeted Kiss1r deletions also revealed similar reproductive abnormalities in these animals (Seminara *et al*., 2003 ; Funes *et al*., 2003). The same receptor can be affected by a variety of different ligands in various organs. In the human placenta, all KP ligands bind to KISS1R with a similar affinity (Kotani *et al*., 2001).

KP-54 is more effective *in vivo* than Kp-10, increasing gonadotropin release in rats and men (Jayasena *et al*., 2015). In both humans and animals, KPs increase gonadotropin secretion and the hypothalamic-pituitary axis (Gottsch *et al*., 2004; Messager *et al*., 2005). KP increase LH secretion over than FSH secretion (Dhillo *et al*., 2005; Skorupskaite *et al*., 2014). While, GnRH secretion is stopped by the administration of a KP antagonist (Roseweir *et al*., 2009).

Dynorphin and neurokinin B co-localize with KP neurons to produce the so-called KP, neurokinin B, and dynorphin (KNDy) neurons , which both effect on LH secretion (Navarro *et al*., 2009; Skorupskaite *et al*., 2014).

1.2.1.2 The mechanism of action of Kisspeptin

KP binds to the GPR54/Kiss1r receptor, activating both phospholipase C and the G-protein (Gq/11). The concentration of Ca^{+2} increase as a result of the conversion of phosphatidylinositol diphosphate (PİP2) to diacylglycerol and inositol triphosphate (IP3). When activation of this mechanism leads to the closure of potassium channels and the opening of cation channels (TRP channels), depolarization of GnRH neurons occurs. As a result, GnRH neurons begin to secrete hormones (Colledge.,2009).

Kispeptin reduce malignancy and cell proliferation ,and increase apoptosis through induction of the mitogen-activated protein kinase (MAPK) pathway via protein kinase C (PKC) and the extracellular signal regulated kinase (ERK) pathway (Figure ,1-2)(Castaño *et al*.,2009 ;d'Anglemont,2010) .

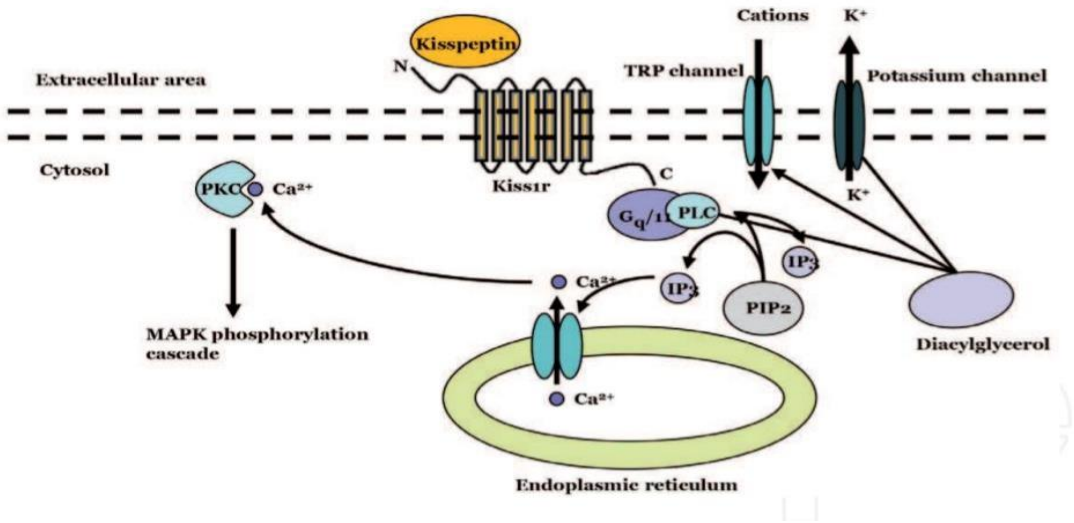


Figure (1.2): Cellular action mechanism of KPs (d'Anglemont,2010)

KP stimulates PI3K/AKT phosphorylation in a tissue-specific manner. This signaling system is crucial for primordial follicular survival, development, and transformation into primary follicles as well as granulosa cell death (Zheng *et al*., 2012; Cecconi *et al*., 2013)

According to previous studies , animals with higher follicular atresia revealed KP or KP receptor mutations (Colledge, 2009; Gaytan *et al*., 2014)

Additionally, animals (male mouse) lacking β -arrestin-1 or β -arrestin-2 have decreased KP-dependent LH secretion (Ahow *et al*., 2014). In mouse Kp-10-activated Kiss R caused calcium oscillations in GnRH neurons (Constantin *et al*., 2009).

1.2.2 Gonadotropin-releasing hormone (GnRH)

Harris' monograph from 1955 first proposed the theory that "nerve fibers from the hypothalamus liberate some humoral substance(s) into the capillaries of the primary plexus in the median eminence, and this substance is carried by the portal vessels, to excite or inhibit the cells of the pars distalis" of the anterior pituitary (Harris.,1955).When it was unknown what processes controlled anterior pituitary hormone output. It required two different teams, both led by Nobel laureates Andrew V. Shally and Roger Guilleman, to extract and sequence GnRH as a 10 amino acid peptide from porcine and ovine hypothalamic (Baba *et al*.,1971; Schally *et al*., 1971a).

Prepro-GnRH, a bigger precursor with 92 amino acids, is broken down to produce GnRH in the cytoplasm (Schally,2000), later recognized as GnRH to reflect its stimulatory action on the both LH and FSH secretion (Schally *et al*., 1971b; Conn and Crowley, 1994).

The GnRH is a deca-peptide produced in hypothalamus (Figure ,1.3). It is also named as luteinizing hormone releasing hormone (LHRH), gonadoliberin, GnRH I, gonadorelin, and luliberin . Despite the fact that GnRH is thought to have a variety of roles in mammals, investigations in biochemistry, molecular biology, neuroanatomy, pharmacology, and physiology have primarily concentrated on its function as a gonadotropin-releasing factor (Sealfon *et al*.,1997; Khazeni and Varamini,2018).

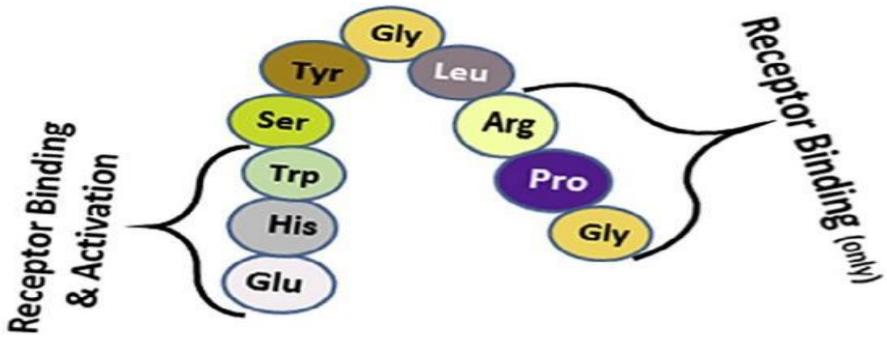


Figure (1.3): Schematic representation of mammalian GnRH peptide sequence in its folded conformation (Khazeni and Varamini, 2018).

A process that controls the reproductive system involves the hypothalamic-pituitary-gonadal (HPG) axis loop, which is regulated by hormones. The anterior pituitary gland expresses and releases follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in adult males,

and females as a result of the hypothalamic pulsatile production of the GnRH. The gonads' ability to function is controlled by the gonadotropins FSH and LH. In both sexes, FSH encourages the development of gametes, whereas LH stimulates the gonads' expression of steroid hormones. Through a negative feedback loop, increased levels of steroid hormones prevent the generation of GnRH (Schally,2000).

Over 20 isoforms of GnRH has been identified among vertebrate and protochordate species. Some of these isoforms structures have been conserved for more than 500 million years of evolution (Karten and Rivier,1986). In the majority of vertebrates, GnRH comes in at least two and frequently three different forms, including GnRH-I (also known as GnRH), GnRH-II (also known as chicken GnRH), and GnRH-III (also known as lamprey GnRH-III). The N-terminal (pGlu-His-Trp-Ser) and C-terminal (Pro-Gly-NH2) amino acid sequences of the decapeptide are conserved in these isoforms of GnRH (Millar, 2005; Okubo and Nagahama, 2008).

The gonadotropin hormones FSH and LH are released when GnRH selectively binds to the GnRH receptor (GnRH-R), which is mostly expressed on the surface of gonadotropin cells in the pituitary (Kakar *et al*., 1992). This binding stimulates the release of pituitary gonadotropins, which control the gonads' steroidogenesis and sperm and ovum maturation processes (Harrison *et al*.,2004). The GnRH decapeptide's C and N termini play a function in binding to and activating its receptor (Sealfon *et al*., 1997).

The G-protein coupled receptor (GPCR) family member GnRH-R demonstrates how GnRH-R controls cell growth and proliferation by activating mitogen-activated protein kinase (MAPK) cascades (Naor *et al*., 2000). Thus, it has been noted that pituitary adenomas express GnRH-R (Alexander and Klibanski,1994), breast, granulosa-luteal cells, testis, prostate (Kakar *et al*., 1992), and ovary (Secara *et al*., 2009).

Additionally, malignancies of gonadal steroid-dependent organs have elevated GnRH-R expression. For instance, an up regulation of medium to high-affinity binding sites for GnRH and its analogs was found in 86% of prostate malignancies, 80% of endometrial and ovarian adenocarcinomas, and 50% of breast cancer cases (Halmos *et al*., 2000; Grundker *et al*., 2001).

The GnRH derivatives are frequently given for sex-hormone dependent disorders and reproductive dysfunctions, such as hormone sensitive cancers of the breast, prostate, ovary, and endometrial. A direct growth suppression has been documented in a number of hormone-independent malignancies of the prostate, ovary, and pancreas, in addition to the GnRH-R ligands' primary effect in hormone dependent tumors. It has been demonstrated that this effect of the GnRH analogs was related to the overexpression of GnRH receptors in these tumors (Conn *et al*., 1994; Blumenfeld,2017).

1.2.3 8-isoprostane

A prostaglandin isomer called 8-isoprostane (8-iso-PGF2a) is produced without the need of enzymes during the metabolism of

arachidonic acid by free radicals (Figure,1.4); It is regarded as an accurate indicator of lipid peroxidation and is used to detect oxidative stress and a lack of antioxidants (Kaviarasan *et al*., 2009; Milne *et al* .,2015). Its finding was initially reported in 1990 named this new family of chemicals F2-isoprostanes (IsoPs) by Morrow *et al* .,(1990)

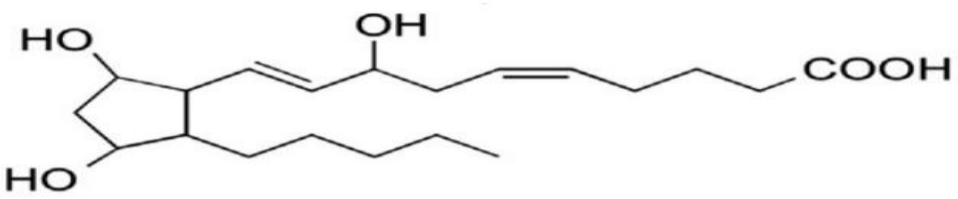


Figure (1.4):The chemical composition of 8-isoprostane (Milne et al .,2015)

In addition, one of the most prevalent endogenous in humans F2-IsoPs, 15-F2t-IsoP, has two significant urinary metabolites that have been identified: 2,3-dinor-15-F2t-IsoP and 2,3-dinor-5,6-dihydro-15-F2t-IsoP (Roberts *et al*.,1996 ; Schwedhelm *et al* .,2000 ;Nourooz-Zadeh *et al*.,2005).

Researchers from all around the world have been able to study the biological activity of these molecules depend on the chemical synthesis of certain IsoPs. The majority of investigations have concentrated on analyzing IsoPs in the form of free fatty acids. The 15-F2t-IsoP is one of the most researched IsoPs. The cardiovascular system has been the subject of most of the research on this molecule's biological activities (Bauer et al ,2014)

Congenitally obese mice had higher levels of 8-isoprostane in their blood (Frühbeck *et al*., 2017).Additionally, it has been found in rats fed a diet high in fat (Li *et al* .,2015). Similar elevated levels of 8-isoprostane

have been described in the urine of obese subjects (Devries *et al* ., 2008 ; Samocha-Bonet *et al* ., 2017) .Also, in obese Zucker rats (Ndisang *et al* .,2009) .

As a valid biomarker of lipid peroxidation, 8-isoprostane is also utilized to indicate oxidative stress and a lack of antioxidants. increased in the skeletal muscle of mice fed a high-fat diet and in the urine of obese people (Liu *et al* .,2013 ; Frühbeck *et al* .,2017)

Increased levels of 8-isoprostane are found in the serum of people with type 2 diabetes (Kaviarasan *et al* ., 2009).

In this context, decreased skeletal muscle mass is related with higher surrogate measures of systemic oxidative stress, such as urine 8-isoprostane, in older people with obesity. The development of atherosclerosis in people with sarcopenic obesity has been linked to this rise in oxidative stress, which has been theorized as a cause (Nakano *et al* .,2017) .

After three months of moderate-intensity endurance cycling exercise, obese men's 8-isoprostane concentrations were observed to be decreasing by Samjoo *et al* ., (2013).

1.2.4 Prolactin and Hyperprolactinemia

Prolactin (PRI), a protein hormone generated and secreted by the anterior pituitary gland, PRI was first identified 95 years ago (Stricker and Grueter., 1928; Riddle *et al*., 1933).

The anterior pituitary glands lactotrophs secrete PRL, while dopamine from the hypothalamus, which enters the anterior pituitary gland through the pituitary stalk's portal circulation, inhibits PRL production. Inflammatory and viral conditions that affect the pituitary stalk, sellar and suprasellar tumors, all these conditions all increase the secretion of PRL by preventing dopamine from reaching to the lactotrophs. Additionally, a number of substances, including estrogens, serotonin, thyrotropic releasing hormone (TRH), and vasoactive intestinal peptide(VIP), release PRL (Bronstein *et al.*,2016).

The diurnal rhythm of PRL secretion is characterized by a higher concentration at night and a lower circulatory level during the day. The controlling system, apart from sleep depends on the pituitary melatonin secretion and a hypothalamic regulator (Prabhakar and Davis,2008).

Hyperprolactinemia (Hyper-PRL)is characterized by an high quantity of prolactin in the blood (Davis ,2004).Both sexes can develop hyper-PRL at any age. Men and women experience Hyper-PRL at annual incidences of 1.4 and 8.7 per 100,000 people, respectively (Soto-Pedre *et al*.,2017) .

The most prevalent pituitary tumors are prolactinomas, which are adenomas with autonomous secretion of PRL. With a prevalence of 100 cases per million, prolactinomas are ten times more common in females aged 20 to 50 than in males. However, in persons above 60, the frequency becomes comparable between sexes (Bronstein *et al*.,2016).

Hyperprolactinemia causes an aberrant lipid profile, weight gain, and cardiovascular illnesses. Additionally, elevated prolactin levels inhibit the synthesis of 17-estradiol, which lowers testosterone production, and obesity related with a prolactinoma (Ali and Mirza, 2021).

In both humans and animal models, hyperprolactinemia frequently disrupts the secretion of GnRH, FSH and LH which can result in hypogonadism and infertility (Kars *et al*.,2010; Glezer and Bronstein, 2015).

1.2.5 Causes of hyperprolactinemia

The causes of excess prolactin are outlined in table (1.1) .

Table (1.1):The causes of excess prolactin (Bahar,2016).

Dysfunction/disease	Mechanism
Idiopathic	Impaired hypothalamic dopamine secretion
Pituitary tumors: micro- or macroprolactinoma ,adenoma, hypothalamic stalk interruption	Disruption of dopamine delivery and/or secretion of prolactin
Acromegaly	Prolactin secretion from a GH adenoma
Empty Sella syndrome	Damage of the pituitary
Primary hypothyroidism	Increased hypothalamic TRH
Polycystic ovary syndrome	Raised estrogen concentration

Renal failure	Reduced PRL clearance
Drugs	Mechanism
Antidopaminergic drugs - Anti-psychotics (phenothiazines, h-aloperidol, butyrophenones, risperid-one, monoamine, oxidase inhibitors, fluoxetine, sulpiride) - Anti-emetics (metoclopramide, do-mperidone) - Tricyclic antidepressants	Inhibition of dopamine release
Opiates	Stimulation of opioid hypothalamic receptors
Oestrogens	Stimulation of lactotrophs
Verapamil	Unknown

For Hyper-PRL, dopamine agonists are recommended as the initial treatment. The PRL and gonadotropin levels can be returned to normal with dopamine agonist therapy (Wang *et al*.,2012).

One of the most common causes of female infertility is hyper-PRL. Almost all Hyper-PRL participants require magnetic resonance imaging (MRI) imaging for diagnostic procedures, yet MRI is time-consuming, difficult to access, and expensive. These participants have pituitary tumors in about 25–30% of them (Souter *et al*.,2010).

According to clinical evidence, both men and women may have infertility as a result of hyperprolactinemia, which is a common cause of reproductive failure (Newey *et al* .,2013; Glezer and Bronstein,2015).

Subjects with prolactinoma or being exposed to medications that interact with the dopamine system are the main causes of pathological hyperprolactinemia. Rarely, hyper-PRL can also result from mutations in the PRL-receptors gene that induce loss of function (Newey *et al*. ,2013).

1.2.6 Hyperprolactinemia reproductive hormone and kisspeptin

The GnRH, which in turn limits the release of both FSH and LH from the pituitary, is inhibited by hyper-PRL (Matsuzaki *et al* .,1994 ; Ordög *et al*. , 1998 ; Weintraub *et al*. ,2015).

The KP neurons are crucial afferent regulators of GnRH neurons because, they provide substantial depolarizing effects and direct synaptic input via their G-protein-coupled receptors. KP's importance in reproduction as the regulators of sexual maturity across mammalian species was demonstrated by the discovery that dysfunction mutations in receptor of KP cause GnRH insufficiency in mice and humans (de Roux *et al* .,2003 ;Seminara *et al* .,2003 ;Topaloglu *et al* .,2012).

The stimulation of GnRH secretion by KP was recoded in different mammals ,such as mice (Gottsch *et al*., 2004),rats (Matsui *et al*., 2004) ,sheep (Caraty *et al*., 2007), non-human primates (Shahab *et al*., 2005)

and humans (Dhillo *et al*., 2005). As well as, its capacity to speed up sexual maturity and a receptor antagonist's capacity to stop ovulation and reduce LH (Kinoshita *et al*.,2005; Roseweir *et al*.,2009).

Recent research reveals that the enigmatic "pulse generator," which controls the pulsatile activity of GnRH neurons, belong to the subset of KP neurons (Clarkson *et al*.,2017).

KP treatment was found to improve ovarian cyclicity and GnRH and gonadotropin output in female mice with hyperprolactinemia (Sonigo *et al*.,2012).

Generally, KP can increase GnRH induced LH production .But, measurement of individual neuroendocrine reactions to exogenous KP hasn't been thoroughly investigated, especially in the patients with reproductive problems ,In study done by Hoskova *et al* (2022) noted the exogenous KP treatment can increase the GnRH-induced LH pulses in hyperprolactinemia-affected women. So that , the claim that KP mediates the inhibitory consequences of PRL on the cascade of reproduction is supported by the fact that KP can reactive the gonadotropic axis in hyperprolactinemia women's .

The finding that the majority of Kiss1-expressing neurons also co-express the PRLRs which provides evidence that prolactin levels directly affect kiss1- expressing neurons (Kokay *et al*.,2011).

KPs may also regulate prolactin production *in vitro* ,when KP-10 administration enhanced prolactin secretion in culture media made with

anterior pituitary cells obtained from 8-month-old castrated male calves (Kadokawa *et al* .,2008).

The known dose of KP 54 subcutaneous administration in healthy women ,immediately or twice daily for a weak had no effect on the serum PRL levels in these women (Jayasena *et al*.,2014)

In recent study ,that found plasma concentration levels of KP did not differ in hyperprolactinemia from the control group(Arslan *et al*.,2022).

Elnour and his team (2021) found the level of FSH,LH and estradiol no significant difference between on women with hyperprolactinemia and women with normal prolactin level.

1.2.7 Hyperprolactinemia and blood glucose

The PRL overexpression in β-cells causes unnecessarily elevated serum insulin concentrations, an increase in islet insulin content, and persistent β -cell replication (Vasavada *et al*.,2000). The PRL has been found to encourage β-cell proliferation, insulin gene transcription, and glucose-dependent insulin secretion in both rats and humans (Petryk *et al* .,2000;Fleenor and freemark .,2001)

Chronic hyperprolactinemia has repeatedly been linked to defective insulin secretion, which is characterized in humans by postprandial hyperinsulinemia and an excess of the insulin secretory response to glucose (Kim *et al*., 1993 ; Foss *et al*., 1995)

According to the previous study, the gluco-insulinemic profile was improved when PRL levels were returned to normal, which is consistent

with PRL's negative impact on pancreatic β-cell activity(Rubí *et al* .,2005).

Fasting insulin (FI) , homeostatic model assessment index (HOMA-IR) , homeostasis model assessment of β-cell function HOMA-b, and insulin sensitivity index (ISI) have been shown to considerably improve in hyperprolactinemic male patients with concurrent hypo gonadotropic hypogonadism with long-term combined androgen blockade (CAB) therapy, which is further aided by androgen replacement and subsequent testosterone normalization . also ,testosterone levels and ISI significantly correlate. indicating that replacement therapy may have a direct, positive impact on the control of insulin sensitivity (Auriemma *et al* .,2015).

Fasting glucose (FG) was found to be significantly lower in both patients with prolactinoma after undergo a surgical procedure in pituitary or , who had dopamine agonists ,as the first line treatment for prolactinoma , this supporting the effect of PRL normalization on improving glucose metabolism (Andereggen *et al.*, 2021).

Patients with prolactinomas that are resistant to CAB conventional dose therapy have recently had the effects of various therapeutic modalities on their gluco-insulinemic profiles examined to known the effect of high-doses CAB therapy (i.e., 2 mg/week), and pituitary surgery could have an equivalent metabolic impact on these patients (Pirchio *et al* .,2021).

Plasma PRL was noted to be negatively correlated with the chance of developing diabetes in both sexes (Li *et al*., 2018). A higher risk of

developing impaired FI and diabetes after childbirth has also been linked to lower blood PRL levels (Retnakaran *et al* .,2016).

1.2.8 Hyperprolactinemia and lipid profile

Hyper-PRL caused by prolactin secretion from pituitary gland might have effects on food intake, weight gain and lipid metabolism (Posawetz *et al*.,2021). Patients with PRL overload frequently have impaired lipid profiles (Krysiak *et al* .,2022).

Patients with prolactinomas have been found to have higher rates of total cholesterol, LDL cholesterol, and TG, as well as lower HDL cholesterol, than healthy control participants (Pala *et al* .,2015; Ben-Jonathan and Hugo 2015;Medic-Stojanoska *et al* .,2015)

According to the research , it has been proposed that PRL levels and lipid fractions are directly correlated (Berinder *et al* .,2011; Medic-Stojanoska *et al*.,2015). Since, an increase in PRL receptor expression was discovered during adipocyte differentiation, indicating their role in mature adipocyte regulation, it has been hypothesized that PRL has a direct impact on lipid metabolism (McAveney *et al*.,1996; Symonds *et al*.,1998;;Hugo *et al*., 2008) .

Individuals with hyperprolactinemic disorders have higher body fat percentages than controls, lower HDL cholesterol, increased total, LDL, and TG levels, and impaired apolipoprotein production as a result of elevated PRL levels. Improvements in lipid metabolism are made possible by returning PRL levels to normal ranges. In particular, CAB

improves lipid profile regardless of changes in body weight and PRL percentage (Krysiak *et al*., 2022).

1.2.5 Obesity

Obesity is derived from the Latin term obesus, which meaning someone "who has gained weight from eating," and may have first appeared in works by Thomas Venner around 1620 (Barnett, 2005).

Both environmental and genetic variables have a role in the development of obesity. The usual environmental variables linked to this illness include a high caloric intake and a decrease in physical activity time, but not everyone exposed to these settings becomes obesity, pointing to the possibility of underlying genetic pathways at the individual level (Krentz *et al*. ,2016).

Energy balance and physiological processes related to weight are governed by a complicated system in which genes, and environment interact. By controlling food intake and energy expenditure, two groups of neurons in the hypothalamus arcuate nucleus govern the balance of energy in the body. These neurons are stimulated or inhibited by neuropeptide hormones that circulate in the body. The microbiota, pancreas, adipose tissue cells, stomach, and other organs all contribute to coordinated network of central processes and peripheral signals that regulate short- and long-term energy balance. The cognitive functions, sensory input, hedonic effects of food intake, memory, attention, and extrahypothalamic brain regions all play a role in regulating energy balance (Heymsfeld and Wadden ,2017).

Since 1980, there has been an increase in the prevalence of obesity and overweight worldwide, with about a third of the world's population now classified as overweight or obese. This increase has occurred in people of all ages and genders, regardless of location, ethnicity, or socioeconomic status; however, older adults and women are more likely to be obese. Although the absolute prevalence rates of overweight and obesity varied greatly between regions and countries (Chooi *et al*.,2019).

Body mass index (BMI) and waist circumference (WC) with waist/hip ratio are the two measurements most frequently used to describe obesity and overweight, which are conditions marked by an excess of fat mass (Fejes *et al*., 2005).

1.2.5.1 Causes of obesity

The most important causes of obesity can be summarized in the table (1.2)

Table (1.2): The causes of obesity (Bandeira and da Nobrege,2022).

Primary causes	Secondary causes
Genetic causes	Neuropsychiatric: brain injury/tumor; consequences of cranial RT; hypothalamic obesity; depression; eating disorders
Monogenetic disorders: mutation at the melanocortin-4 receptor; leptin deficiency; POMC	Endocrine: hypothyroidism"; Cushing's disease; GH deficiency; Pseudohypoparathyroidism

deficiency	
Syndromes: Prader-Willi; Bardet-Biedl; Cohen;alstrom;Froehlich	Medication: tricyclic antidepressants; STEEL; antipsychotics; anticonvulsants; Alström; Froehlich glucocorticoids; sulfonylureas; glitazones; beta blockers
"Controversial if hypothyroidism causes obesity or exacerbates obesity. RT, ACO radiotherapy, oral contraceptive	

1.2.5.2 Obesity ,reproductive hormone and Kisspeptin

Obesity has negatively impacts in both male and female reproductive systems as well as overall health (Giviziez *et al*., 2016).

In fact, PRL can directly act on adipose tissue because PRL receptors increase during adipocyte differentiation and may be involved on lipid metabolism of mature adipocytes (Symonds *et al* .,1998 ;Gualillo *et al*., 1999) .

In animals models , When given a high-fat diet (HFD), to rhesus macaques female showed decreased in LH pulse amplitude (McGee *et al*.,2014).

Estrogen play a role in the determination of regional adiposity distribution. The finding that estrogen levels are greater in non-obese premenopausal women than obese women supports the idea that

estrogens have a role in preventing adipose tissue (AT) (Freeman *et al*., 2010). Also ,Low LH levels and reduced fertility are more common in obese women (Yeung *et al*.,2013).

In study done by Al-Ttaie and his team (2021) found that PRL, LH and E2 were increased Significantly .While, Progesterone and FSH decreased significantly in the obesity women group compared with non obesity women .But , in another study by Arıkan and Sagsoz (2023) recorded the levels of FSH, LH, estradiol and AMH in obese women did not differ a significantly compared with non obesity women.

In women with obesity are more likely to have reduced fertility characterized by reduced levels of LH (Chavarro *et al*.,2012; Yeung *et al*.,2013).

According to the study done by Kołodziejski and his group (2018) found the obese women had lower KP levels in compared to non-obese women .

In study carried out in Saudi Arabia noted there are not correlation between serum KP and anthropometric indices, and the serum KP levels in the overweight and the obese young females doesn't differ significantly from normal-weight females (Rafique and latif .,2015)

In addition , KP was negatively correlated with BMI in post-menopausal women .Also, KP were independently associated with obesity, lower serum KP concentrations, are significantly associated with obesity in this women (Hestiantoro *et al*.,2019).

While, KP levels were positively correlated with BMI, WC, and weight in all children and adolescents for both gender . Obese/overweight girls had higher KP levels, and there was a positive correlation among KP ,FSH , LH and obesity-related parameters in all boys and girls (pan *et al* .,2016).

1.2.5.3 Obesity and blood glucose

Obesity is a chronic, relapsing, multifactorial disease (Bray *et al* .,2017), It is also a significant risk factor for a number of other non-communicable diseases (NCDs) such as diabetes (WHO, 2019)**.**

Human brain insulin receptors have been selectively stimulated by intranasal insulin administration. Intranasal insulin before meal reduced eating more in man than in reproductive-age women (Benedict *et al.*, 2008). Pre-meal intranasal insulin failed to prevent eating in postmenopausal women (Krug *et al*., 2010).

According to research by Aras and his associates (2021), the 5% reduction in total body weight or more can improve glycemic control, reduce the need for diabetes medication, and enhance quality of life.

Arikan and Sagsoz (2023) found that insulin and HOMA were significantly decreased, also, glucose were significantly decreased in obese women in comparison to the control.

Increased adiposity is frequently associated with IR, which is a precursor to the onset of T2DM, cardiovascular diseases, and altered metabolism (Wali *et al*., 2021).

Obesity increases levels of non-esterified fatty acids, glycerol, hormones, and pro-inflammatory cytokines generated by adipose tissue, which contribute to development of IR. Obesity is risk factor for diabetes that associated to IR (Wondmkun,2020).

1.2.5.4 Obesity and lipid profile

Abnormalities in lipid metabolism are very commonly observed in patients who are obese. Approximately 60-70% of patients with obesity are dyslipidemia (Feingold,2020).

In a study conducted on obesity students ,found the males had higher values of non-HDL-C, LDL-C, TG and VLDL-C than females, but these differences were not significant .But ,Females recorded higher TC and HDL-C than males (Hertelyova *et al* ., 2016) .

Deusdará and colleagues (2022) discovered that in early adolescence, the waist circumference was elevated for both TG/HDL and triglyceride/glucose (TyG), and the obesity-related IQs were higher than the overweight-related ones. In teenagers between the ages of 15 and 17, the FIeqs for raised WC were higher than those for obesity and overweight.

Abdominal obesity is closely associated with higher TG level and with low HDL level. Although not all obese individuals are hypertriglyceridemic, there is a significant correlation between obesity and plasma TG, with heavier individuals having higher TG level. TG correlated with cardiovascular disease (CVD) , each 1 mmol/l increase in

plasma TG concentration is associated with a 32 % increase in CVD risk in men and a 76 % increase in women. The association of triglyceridemia is greater with visceral fat deposits than subcutaneous fat deposits (Ebbert and Jensen,2013).

In obesity , there is decreased in the Lipoprotein lipase-mediated lipolysis of chylomicron-TG , and ineffective inhibition of hormone sensitive lipase mediated lipolysis in adipose tissue (Lewis *et al* .,1993).

Postprandial lipemia and elevated plasma fatty acid levels are well-recognized abnormalities in obesity. Excess fatty acid availability early in the postprandial period (when it is normally suppressed by insulin) is estimated to influence glucose uptake by as much as 50% (Yu and cooper.,2001).

Mancini and his team (2021) found the total cholesterol, LDL, HDL and TG no significant difference between overweight, obesity and normal weight.

1.2.6 Diabetes Mellitus (DM)

The Greek word "diabaino," which also means "to travel or run through," is where the word diabetes originates (Laios *et al*., 2012).

The United Nations identified diabetes as a worldwide danger in December 2006, as well as a world diabetes day was established on November 14 to honor Frederick Banting, one of the four scientists who developed insulin. This day has been observed annually since 2007 (Das *et al*., 2021).

Chronic hyperglycemia induced by disorder in insulin action, insulin production, or both characterizes the group of metabolic illnesses known as DM (Poznyak *et al*., 2020).

Diabetes be classified for main clinical types (WHO, 2019) :

• Insulin Dependent Diabetes Mellitus (IDDM) : The rate of pancreatic -cell destruction in this type of diabetes is really quite variable, being rapid in some people (mostly infants and children) and slow in others (mostly adults), and such people become dependent on insulin for survival. Type 1 diabetes mellitus (T1DM), also known as insulin-dependent diabetes or juvenile-onset diabetes, accounts for only 5–10% of all diabetes. It results from the autoimmune destruction of pancreatic cells, which is mediated by cell (ADA, 2009).

• Non-Insulin Dependent Diabetes Mellitus (NIDDM) : About 90–95 percent of all diabetes cases are type 2 diabetes mellitus (T2DM), also named as non-insulin-dependent diabetes or adult-onset diabetes. The T2DM is characterized by IR and a relative insulin deficit, and people with it do not need to take insulin to survive or frequently throughout their lives (ADA , 2009).

• Gestational Diabetes Mellitus (GDM) : Untreated mothers with GDM may result in stillbirth. The GDM is a condition in which a non-diabetic woman develops high blood sugar levels during pregnancy. It can happen during pregnancy due to IR or decreased insulin production. Babies born to mothers with GDM experience low blood sugar after

birth, jaundice, and have a higher risk of being overweight and developing T2DM (NIDDK, 2016).

• Other types of DM linked to specific illnesses.

Diabetes mellitus (DM) is characterised by hyperglycemia (an rise in blood glucose level brought on by a relative or absolute insulin shortage), abnormalities in lipoproteins, and oxidative stress (Scoppola *et al*., 2001). Long-term diabetes consequences, such as impairment of the cardiovascular system, kidneys, eyes, and nerves, are associated with chronic hyperglycemia (wild *et al*.,2004).

Hyperglycemia, ketosis, glycosuria, acidosis, and coma are all signs of DM. In addition, fatigue or illness, frequent urination, unusual thirst or hunger, unexplained weight loss, impaired vision, headache, fever, and dry, irritated skin are all probable diabetes symptoms (Young *et al*., 2000).

Today ,T1DM is understood that a single autoimmune disorder, when , the T-cell-mediated attack on insulin producing cells, T1DM is a result of complex interaction between environmental factors and the microbiome, genome, metabolism, and immune systems, which differ between individual cases (Barnett,2018; Skyler,2018).

Both the incidence and prevalence of T1DM are rising globally, with total yearly increases in incidence of roughly 2-3% each year (Maahs *et al* .,2010; Mayer-Davis *et al*.,2017).

1.2.6.1 Type 1 diabetes mellitus, reproductive hormones and kisspeptin

In GnRH neurons, prolonged hyperglycemia may result in glucotoxicity. A number of central nervous system factors, including rising dopaminergic tone, opioidergic activity, and catecholamine levels, may also play a role in the etiology of hypogonadism in T1D patients (Djursing *et al*., 1983: O'Hare *et al*., 1987; Volpi *et al*., 1998:Arrais and Dib, 2006).

Previous research found that low levels of LH, FSH, and estradiol are associated with a lack of residual insulin secretion in individuals with primary or secondary amenorrhea and insufficient metabolic regulation (Djursing *et al*., 1983; La Marca *et al*., 1999).

In comparison to healthy controls, T1D patients showed a larger total and incremental LH response to GnRH stimulation, according to South *et al*., (1993).

As the duration of diabetes increases, the response of the LH to GnRH stimuli decreases (Volpi *et al*., 1998) , an immortalized GnRH cell line subjected to 450 mg/dl of hyperglycemia, aberrant GnRH production, and elevated apoptosis have also been connected to the harmful effects of hyperglycemia on hypothalamic neurons (Pal *et al*., 2007).

Insulin raise the activity of numerous steroidogenic enzymes and promotes androgen production via theca cells (Poretsky *et al*., 1999; Codner and Escobar-Morreale, 2007).

Insulin stimulates ovarian steroidogenesis and follicular development via insulin receptors on granulosa cells (Poretsky *et al*., 1999; Sirotkin, 2011).

Studies in animal models also corroborate these findings. Female rhesus macaques feed on a high-fat diet (HFD) shown reduced LH pulse amplitude (McGee *et al* .,2014). Female mice fed an HFD exhibit longer estrous cycles (Lainez *et al* .,2018 ; Fernandez *et al*.,2017).

The research in mammals have demonstrated that interactions between Kiss1 and POMC or NPY neurons play role in integrating metabolism and gonadal axis (Luque *et al*., 2007; Backholer *et al*., 2010; Fu and van den Pol, 2010).

According to earlier research, women with T1D still experience delayed menarche and adolescence, irregular menstruation (particularly oligomenorrhoea), mild hyperandrogenism, polycystic ovarian syndrome, fewer live births, and potentially an earlier menopause .Furthermore, animal models have aided in our understanding of the fundamental causes of these disorders and have demonstrated the varying roles that aberrant insulin and KP signaling play in the mechanisms driving disturbed reproduction in type 1 diabetes (Codner *et al* .,2012).

Calcaterra and his group (2021) noted the KP levels were lower in control women when compared to the obesity women. But, did not reaching to the significance differences when compared to the women with T1DM .

Up to 40% of T1DM women will experience monthly irregularities, hyperandrogenism, or an early menopause at some point in their lives, which is a serious health issue. Different anomalies in gonadal function linked to unfavorable serum glucose levels and no physiological insulin replacement is still seen in T1D patients (Codner *et al*., 2012; de Beaufort *et al*., 2007).

According to the animal studies, injections of KP are adequate to lower testosterone levels and increase gonadotrophin synthesis in diabetic men. Similar to this, long-term administration of KP-10 to diabetic male rats greatly improves a number of long-term reproductive impairments (Castellano *et al*., 2006, 2009).

The hypogonadotropic hypogonadism condition typically seen in poorly treated T1D rat is primarily caused by a deficiency in Kiss1 tone in the hypothalamus . In long-term streptozotocin (STZ)induced diabetic rats, pharmacological investigations of central insulin or leptin infusion have looked for the metabolic signals causing altered Kiss1 expression and/or activity (Castellano *et al*., 2006).

Gonadotrophin is not necessary for the initial stage of ovarian folliculogenesis, which involves the non-cyclic recruitment of primordial follicles up to a small antral stage (2–5 mm). Gonadotrophin and other metabolic cues regulate the second stage of folliculogenesis, known as the cyclic recruitment stage, which takes place after the beginning of puberty. Insulin functions as a co-gonadotrophin by promoting the recruitment and expansion of bigger follicles, which only release a

limited quantity of anti-mullerian hormone (AMH) (Poretsky *et al*., 1999; Gougeon .,1996; Fulghesu *et al*., 1997).

Therefore, the hypothesised that whereas insulin encourages the development of tiny follicles before to puberty, it may also operate as a cogonadotrophin by promoting the maturity of big follicles that secrete less AMH when pubertal or adult concentrations of gonadotrophins are present (Codner et al., 2007, 2011).

The T1D is an uncommon reason for consultations in infertility clinics despite having diminished fecund ability , and sexual function (Healy *et al*., 1994; Hargreave and Mills, 1998).

According to the study by strotmeyer and his group (2003) recorded about 17% of T1D women suffer from involuntarily infertile, a percentage comparable to that of healthy controls (Strotmeyer *et al.*, 2003).

1.2.6.2 Type 1 diabetes mellitus and lipid profile

The circulating levels of lipid is dependent on the level normal of insulin and its effects, the connections with raised plasma lipid values were noticeably greater in patient with T1DM . Also, with the progression of diabetes . In T1DM patients develop on imbalance in their blood lipid levels(Snell-Bergen *et al*.,2010).

Patients with T1DM had notably high levels of LDL and , high levels of HDL have also been linked to T1DM (Aburawi *et al*., 2016).

Patients with T1DM had substantially increased levels of LDL, TG, TC, lipoprotein-(Aa) (ApoA), and lipoprotein-B (ApoB) compared to controls. Patients with T1DM had HbA1c levels that were considerably higher than controls. The results of the T1DM group's Spearman correlation analysis also revealed a statistically significant positive relationship between LDL and WC, TG, and HbA1c a statistically significant negative relationship between ApoA/ApoB and HbA1c. In the patient group, there was a sizable negative connection between TG levels and diabetes duration. On the other hand, among healthy controls, BMI exhibited substantial negative associations with LDL and ApoA/ApoB and positive correlations with LDL, TG, TC, ApoB, and alanine aminotransferase (ALT). People with T1DM were more likely to have higher levels of the lipid markers LDL, ApoA ,TG, TC, and ApoB in comparison to controls (Alkaabi *et al*.,2022) .

Alakkad and his associates (2020) found there was high frequency of dyslipidemia in children and adolescents with (T1DM compared to of the healthy control . Also , these changes in lipids were observed more in diabetic females than in diabetic males.The most frequent type of dyslipidemia in children and adolescents with T1DM was high TG TC LDL, while HDL level decrease the in classic diabetic dyslipidemia which was much less frequent in the form of hypertriglyceridemia.

Abdoun and his team (2022) found that hypertension and dyslipidemia were seen in 10% and 31% of children with T1DM, respectively. The elevated LDL and BMI were significantly higher in

female patients than in males. Besides, there were significant associations between high blood pressure and BMI and elevated LDL levels.

Chapter two

Materials and Methods

2 The Materials and Methods

2.1 The Materials

2.1.1 Tools and Equipment

The set of tools and equipment was used in this study, their company and origin described in the table (2-1)

Table (2-1) :The tools and equipment used with their company and origin.

No	Instrument	Company / Origin
1	Alcohol	Actisafe / France
2	Automated ELISA	HUMAN/ Germany
3	Bandge plaster	First stap / China
4	Centrifuge	Cence / China
5	Cobas c111	Roche / Switzerland
6	Cobas e411	Roche /Switzerland
7	Cold box	Halfords / Germany
8	Cotton	Life line /Iraq
9	EDTA tubes	Trust lab / China
10	Electronic scales	Beurer / Germany
11	Eppendorf tubes (2 ml)	Trust lab / China
12	Frozen deep freeze	Xsine x /Germany

13	Gel tubes	Trust lab / China
14	Gloves	Beybi / Turkey
15	Stature meter	Casa diaz /Germany
16	Syringe	Hospital & homecare / China
17	Tips(10-1000 microliter)	Trust lab / China
18	Tourniquet	Trust lab / China
19	Tubes for dilution	Trust lab / China

2.1.2 Kits

2.1.2.1 Laboratory Kits

The laboratory kits, used in this study are show in the table (2-2).

Table (2-2) : The laboratory kits was used with their company and origin.

NO.	Kits	Company /Origin
1	8-isoprostance	Shanghai yl biotech/ China
2	Cholesterol	Roche Cobas / Switzerland
3	Estradiol	Roche Cobas / Switzerland
4	FSH	Roche Cobas / Switzerland
5	Glucose	Roche Cobas / Switzerland
6	GnRH	Shanghai yl biotech / China

7	HBA1C	Roche Cobas / Switzerland
8	HDL	Roche Cobas / Switzerland
9	Kisspeptin	Shanghai yl biotech / China
10	LDL	Roche Cobas / Switzerland
11	LH	Roche Cobas / Switzerland
12	Progesterone	Roche Cobas / Switzerland
13	Prolactin	Roche Cobas / Switzerland
14	Testosterone	Roche Cobas / Switzerland
15	Triglyceride	Roche Cobas / Switzerland

2.1.2.2 ELISA Kits

The contents of kisspeptin 1 , gonadotropin-releasing hormone (GnRH) ,8-isoprostane ELISA kits are shown in the table(3-3).

Table (3-3) : The contents of kisspeptin 1 , gonadotropin-releasing hormone and 8-Isoprostane ELISA kits and it quantity.

No.	Reagents	Quantity
1	Coated ELISA Plate [3x]	12 × 8 well [3]
2	Standard dilution [3x]	3 ml [3x]
3	Chromogen solution A [3x]	6 ml [3x]
4	Chromogen solution B [3x]	6 ml [3x]

5	Streptavidin-HRP [3x]	6 ml [3x]
6	A-Kisspeptin Standard solution (1600ng/L) B-GnRH Standard solution (8ng/L) C-8-isoprostane Standard solution (1280 ng/L)	0.5 ml
7	Washing concentrate (30x) [3x]	20 ml [3x]
8	Instruction [3x]	1 [3x]
9	Seal plate membrane [3x]	2 [3x]
10	Hermetic bag [3x]	1 [3x]
11	Stop solution [3x]	6 ml [3x]
12	A-Anti KISS-1 antibodies labeled with biotin B-Anti GnRH antibodies labeled with biotin C-Anti 8-isoprostane antibodies labeled with biotin	1 ml

2.1.2.3 Cobas Automated Kits

The Instrument and contents necessary towork for FSH, LH, prolactin, testosterone, estradiol progesterone , cholesterol, triglyceride ,HDL and LDL kits by used cobas kit are :

-Materials required.

− Pipette, with disposable tips that will dispense : 3ml, 2ml and 200µl..

− Instrument of the cobas family.

2.1.3 Population of the study

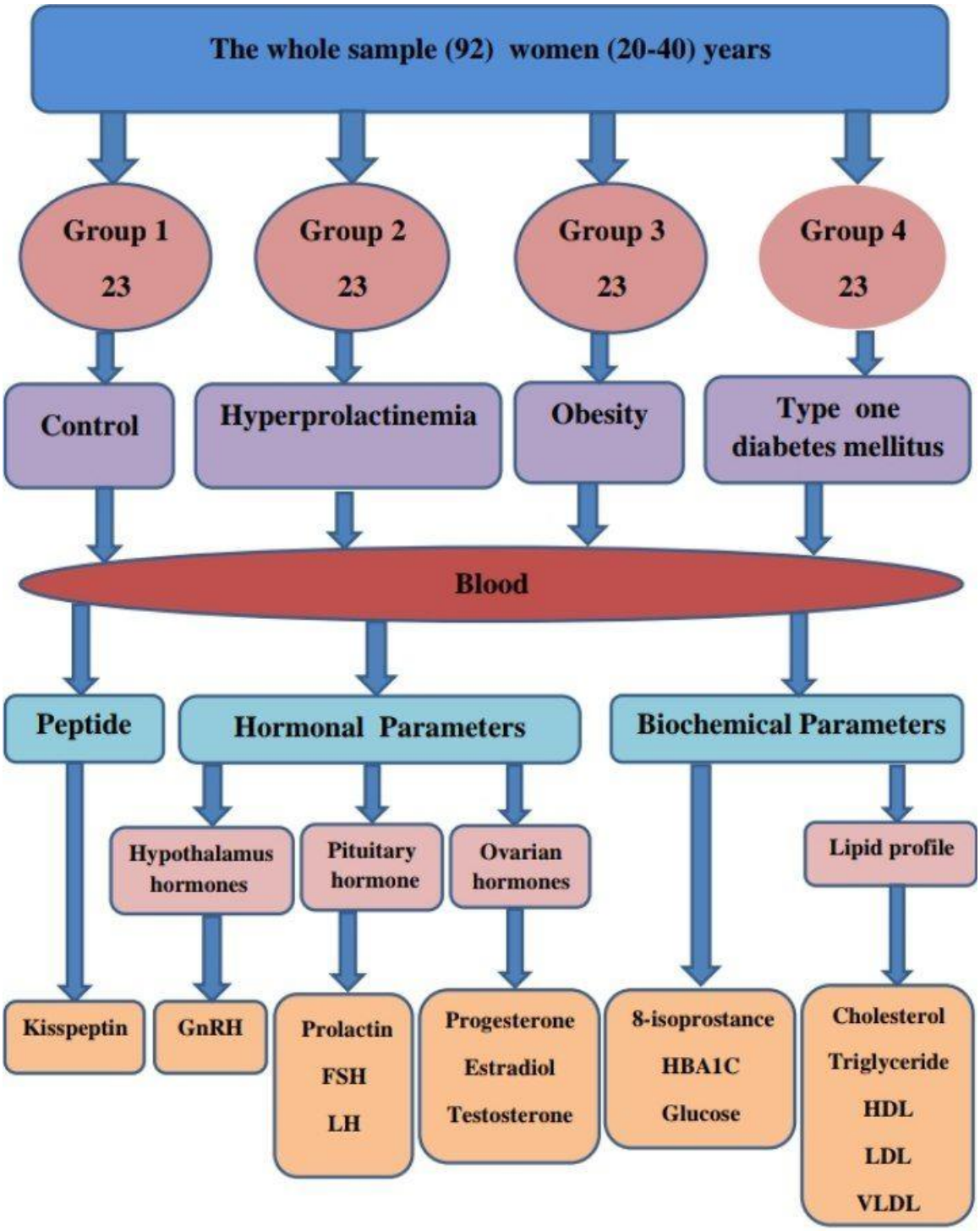
This study was conducted in Maysan governorate at the AL- Sader teaching hospital ,Maysan for child and birth hospital , the specialized center for diabetes and endocrinology ,and some private clinics and centers during the period from June 2022 to February 2023 samples were collected from 92 women , ages ranged between 20 to 40 years, divided into four groups and each include 23 women as the following :

- First group (Control group): healthy women with regular menstrual cycles without any hormonal disturbances.
- Second group (Hyperprolactineamia group) : women with hyper serum prolactin.
- Third group (Obesity group) : women have a BMI over 30 kg / M^2).
- Four group (Diabetic group) :women with T1DM.

Diabetic patients were diagnosed with T1DM had a medical history of the disease and specialized doctors . Patients with hyperprolactinemia were diagnosed by fertility doctors and gynecologists. As for obese patients, based on the body mass index was measured for them. All patient took hormonal drugs ,women with polycystic ovary syndrome, women with an irregular menstrual cycle , women with thyroid disorder, and women have BMI over than 25 kg\m in control group were excluded.

A questionnaire was conducted for all samples to obtain real information (appendix A).

2.1.4 Experimental Design



Scheme (2-1):The experimental design

2.1.5 Collection Blood samples

Samples were drawn from the vein using a single-use plastic syringe during the menstrual cycle (2-5) and after fasting (2-12 hours) at (8-10 am), where 6-10 milliliters were withdrawn of venous blood samples.

The blood was divided into fractions; 2 ml was transferred into EDTA tube for hemoglobin A1c (HbA1C) determination ,and other fraction of blood was left at room temperature for 10 minutes for coagulation at 3000 rpm ,to measure glucose and lipid profile . last serum was transferred into labeled plain tube and stored at – 20 c^{o} until used for evaluation of other parameters.

2.2 Methods

2.2.1 Body Mass Index (BMI)

The body mass index was calculated according to the World Health Organization (WHO,2020), by dividing the weight by the square of the height

$$\text{BMI}=\frac{Weight(kg)}{\left(height(m)\right)^{2}}$$

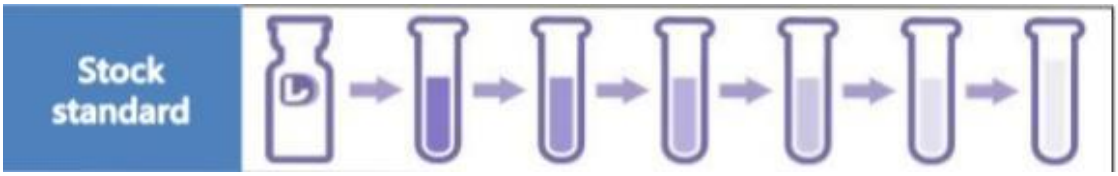
2.2.2 Determination of parameter by enzyme-linked immunosorbent assay (ELISA)

The principle of the tests of KP 1 , GnRH and 8-isoprostane according to the Wild (2013) and Risvanli *et al.*, (2020).

1. Standard solutions can be diluted by the user independently by following the instructions in small tubes. This kit comes with a standard of original concentration.

A- Kisspeptin

800ng/L	Standard No.5	120µl Original Standard +120µl Standard diluents
400ng/L	Standard No.4	120µl Standard No.5 + 120µl Standard diluents
200ng/L	Standard No.3	120µl Standard No.4 + 120µl Standard diluents
100ng/L	Standard No.2	120µl Standard No.3 + 120µl Standard diluents
50ng/L	Standard No.1	120µl Standard No.2 + 120µl Standard diluents

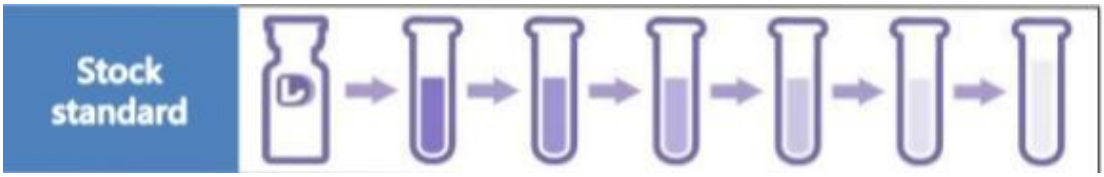


Tube	Standard	S5	S4	S3	S2	S1
ng/L	1600	800	400	200	100	50

B- gonadtropin-releasing hormone (GnRH)

4ng/mL	Standard No.5	120µl Original Standard + 120µl Standard diluents
2 ng/mL	Standard No.4	120µl Standard No.5 + 120µl Standard diluents
1 ng/mL	Standard No.3	120µl Standard No.4 + 120µl Standard diluents

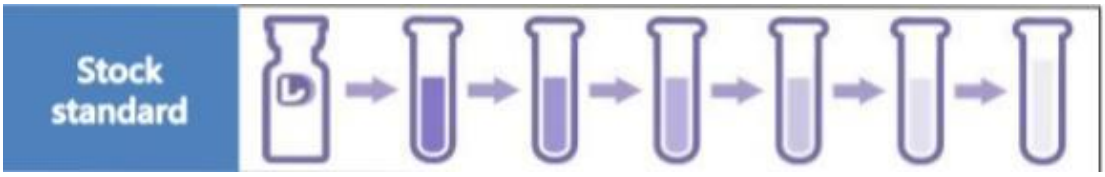
0.5 ng/mL	Standard No.2	120µl Standard No.3 + 120µl Standard diluents
0.25 ng/mL	Standard No.1	120µl Standard No.2 + 120µl Standard diluents



Tube	Standard	S5	S4	S3	S2	S1
ng/mL	8	4	2	1	0.5	0.25

C- 8-isoprostane

640 ng/L	Standard No.5	120µl Original Standard + 120µl Standard diluents
320 ng/L	Standard No.4	120µl Standard No.5 + 120µl Standard diluents
160 ng/L	Standard No.3	120µl Standard No.4 + 120µl Standard diluents
80 ng/L	Standard No.2	120µl Standard No.3 + 120µl Standard diluents
40 ng/L	Standard No.1	120µl Standard No.2 + 120µl Standard diluents



Tube	standard	S5	S4	S3	S2	S1
ng/L	1280	640	320	160	80	40

2. The required number of stripes is calculated by multiplying the number of standards by the number of test samples. It is advised that each well containing a standard solution and each well containing a blank be grouped with three or more wells whenever practical.

3. injection of a sample(a) Fill the well to the top with only Chromogen solutions A and B. (b) Prepare a standard solution by adding 50 microliter of standard and 50 µl of streptavidin-HRP. (c) 40 ml of sample, 10 ml of KISS-1 antibodies, 10 ml of GNRH antibodies, 10 ml of 8-isoprostane antibodies, and 50 ml of streptavidin-HRP should all be added to the sample well. an over it, a membrane seal plate. To blend, lightly shake the two 60 minutes of incubation at 37 °C.

4. Making the washing solution: To make the washing solution for later use, dilute the washing concentration (30X) with distilled water.

5. Cleaning: Carefully remove the seal plate membrane, drain the tank, and shake off any leftover liquid. Fill the washing solution in each well. After standing for 30 seconds, drain the liquid. After performing this step five times, blot the plate.

6.To develop color, add 50 microliter of chromogen solution A and 50 l of chromogen solution B to each well. Shake the two just enough to combine them. For color development, incubate for 10 minutes at 37 °C without light.

7. Add 50 microliter of Stop Solution to each well to halt the reaction (at this point, the blue hue turns yellow).

8. Assay: Using a blank well as the reference point, measure the absorbance (OD) of each well individually at a wavelength of 450 nm within 10 minutes of adding the stop solution.

9. Calculate the standard curve's linear regression equation using the standards' concentrations and related OD values. Next, determine the concentration of the associated sample based on the OD value of the samples. Calculations could also be performed using specialized software.(Appendix B for kisspeptin 1, C for gonadotropin-releasing hormone (GnRH) and D for 8-isoprostane)

2.2.3 Determination of hormones

The electrochemiluminescence immunoassay "ECLIA" was designed for use in the cobas e 411 immunoassay analyzers that used to measure FSH, LH, prolactin, estradiol, progesterone, and testosterone, also, used the immunoassay to quantify *in vitro* in human serum (Fung *et al*.,2017).

The cobas e 411 Elecsys assay employs two monoclonal antibodies directed specifically against human hormone. Sandwich principle is put to the test. The assay for each hormone took 18 minutes in total.

▪ First incubation: 6–40 µL of sample & a biotinylated monoclonal hormone–specific antibody (FSH, LH, prolactin, Estradiol, progesterone and Testosterone) create a first complex. The number of

immunocomplexes formed is based on the concentration of the analytic in the sample.

▪ Second incubation: The still-vacant sites of the biotinylated antibodies get occupied, leading to the development of an antibody hapten complex, after addition of streptavidin - coated microparticles and a (prolactin, FSH, LH, Estradiol, progesterone testosterone) derivate tagged with a "ruthenium complex"*. The interaction between biotin and streptavidin causes the complex to become attached to the solid phase.

$*Tris(2{,}2^1 - bipyridl)ruthenium(II) - complex(Ru(bpy)^2$

▪ The measuring cell is aspirated with the reaction mixture within, and the micro particles are then magnetically drawn to the electrode's surface. ProCell II M is then used to eliminate any remaining unbound materials. A photomultiplier measures the chemiluminescent emission that is caused when a voltage is applied to the electrode.

▪ Results are obtained by comparing a master curve given by the reagent barcode or e-barcode with a calibration curve that is instrument-specifically created via two point calibration.

2.2.4 Determination of Biochemical Parameters

Biochemical parameters were measured by a Roche Cobas c 111 (table 2-1) automates device made in Switzerland using kits from the same company

2.2.4.1 Hemoglobin A1c (HbA1C)

The anticoagulated whole blood specimen is hemolyzed automatically on the COBAS INTEGRA 400 plus analyzer. This technique used Tetradecyltrimethylammonium bromide "TTAB" as the hemolyzing reagent's detergent to get rid of leukocyte interference (TTAB does not lyse leukocytes). It is not essential to pretreat samples to get rid of labile HbA1c.

This assay identifies all hemoglobin variants that are glycated at the chain N-terminus and that have antibody-recognizable areas that are the same as those of HbA1c. This assay can therefore be used to assess the metabolic status of diabetic individuals with uremia or the most common hemoglobinopathies (HbAS, HbAC, HbAD, and HbAE) (Frank *et al.* ,2000 and Jaisson *et al*.,2018).

Hemoglobin A1c

The turbidimetric inhibition immunoassay (TINIA) for hemolysis whole blood is used to determine HbA1c levels.

▪ Sample and R1 (buffer/antibody) addition:

Anti-HbA1c antibody and glycohemoglobin (HbA1c) in the sample combine to produce soluble antigen-antibody complexes. Complex formation does not occur because, there is only one instance of the particular HbA1c antibody site on the HbA1c molecule.

▪ SR (buffer/polyhapten) addition and initiation of reaction:

Excess anti-HbA1c antibodies react with the polyhaptens to create an insoluble antibody-polyhapten complex that can be measured turbidimetrically.

Hemoglobin

During the preincubation phase (sample + R1) of the aforementioned immunological reaction, liberated hemoglobin in the hemolyzed sample is transformed into a derivative with a distinctive absorption spectrum that is quantified bichromatically. As a result, a separate Hb reagent is not required.

The final result is calculated from the HbA1c/Hb ratio and is reported as mmol/mol HbA1c or % HbA1c as follows:

Protocol 1 (mmol/mol HbA1c acc. to IFCC):

HbA1c (mmol/mol) = (HbA1c/Hb) × 1000

Protocol 2 (% HbA1c acc. to DCCT/NGSP):

HbA1c (%) = (HbA1c/Hb) × 91.5 + 2.15

This method depend on Little *et al* .,(1992) and Bowling and Katayev.,(2010).

2.2.4.2 Glucose

Hexokinase is used to assess blood glucose using an enzyme reference technique (Bowling and Katayev,2010 and Freckmann *et al.*,2014).

The phosphorylation of glucose to glucose-6-phosphate by ATP is catalyzed by hexokinase.

$$GLucose + ATP \xrightarrow{HK} \text{G-6-P} + \text{NADPH} + H^+$$

In the presence of NADP, glucose-6-phosphate dehydrogenase converts glucose-6-phosphate to gluconate-6-phosphate. Nothing else in the carbohydrate is oxidized. The rate of NADPH generation during the reaction is photometrical monitored and directly proportional to the glucose concentration.

$$\text{G-6-P} + NADP^+ \xrightarrow{G-6-PDH} \text{gluconate-6-p} + \text{NADPH} + H^+$$

2.2.4.3 Lipid profile assay

2.2.4.3.1 Total Cholesterol

The principle of the total Cholesterol test is depended on the colorimetric enzymatic method (Allain *et al* .,1974 and Bowling and Katayev,2010).

The enzyme cholesterol esterase breaks down cholesterol esters to produce free cholesterol and fatty acids. The subsequent oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide is catalyzed by cholesterol oxidase. A red quinone-imine dye is created when phenol and 4-aminoantipyrine (4-AAP) undergo an oxidative coupling in the presence of peroxidase.

$$\text{Cholesterol esters} + H_2O \xrightarrow{CE} \text{Cholesterol} + \text{RCOOH}$$

Cholesterol + $O_2 \xrightarrow{CHOD}$ Cholest-4-en-one + H_2O_2

$2H_2O_2$ + 4-AAP + phenol $\xrightarrow{POD}$ quinone-imine dye + $4H_2O$

The relationship between the cholesterol concentration and the dye's color intensity is direct. It is determined by measuring the increase in absorbance.

2.2.4.3.2 Triglyceride (TG)

The principle of the test is the colorimetric enzymatic method (Siedel *et al.*,1993 and Bowling and Katayev,2010).

glycerol in addition to RCOOH are produced from the reaction of triglycerides with hydrates in the presence of LPL

Triglycerides + $3H_2O \xrightarrow{LPL}$ glycerol + 3RCOOH

Glycerol triphosphate and ADP are formed from the reaction of a triglyceride with ATP in the presence of a magnesium ion and GK.

Triglycerol +ATP $\underset{mg^{2+}}{\xrightarrow{GK}}$ glycerol-3-phosphate +ADP

Hydrogen peroxide and dihydroxyacetone phosphate are produced by the oxidation of glycerol triphosphate

glycerol-3-phosphate + $O_2 \xrightarrow{GPO}$ $dihydroxyacentone\ phosphate$ + $2H_2O_2$

$$H_2O_2 + 4-aminophenoazone + 4-Chlorophenol \xrightarrow{peroxidase} 4-(p-benzoquinone -monoimino-phenazone + 2H_2O + HCL)$$

2.2.4.3.3 High Density Lipoprotein (HDL)

The principle of the test is the homogeneous enzymatic colorimetric method **(** Katayama *et al* .,2009 and Miida *et al*.,2014).

LDL, VLDL, and chylomicrons, non-HDL lipoproteins, mix with polyanions and a detergent to produce a water-soluble complex. The enzymatic response of CHER and CHOD towards non-HDL lipoproteins is inhibited in this combination.

HDL-Cholesterol esters + $H_2O \xrightarrow{CHER}$ HDL-Cholesterol + RCOOH

Finally, CHER and CHOD can only interact with HDL particles. CHER and CHOD work together to enzymatically determine the HDL cholesterol content.

HDL-Cholesterol+$O_2 \xrightarrow{CHOD} \Delta^4 - cholestenone + H_2O_2$

CHER quantitatively converts cholesterol esters into free cholesterol and fatty acids.

Cholesterol oxidase converts cholesterol to 4-cholestenone and hydrogen peroxide in the presence of oxygen.

When peroxidase is present, the hydrogen peroxide produced reacts with EMSE and 4'-aminoantipyrine to produce a color. The level of cholesterol in the blood is evaluated based on the intensity of the color of this dye. This dye's color intensity is assessed photometrical and is directly correlated with the level of cholesterol in the blood.

$H_2O_2 + 4 - amino - antipyrine$ +EMSE+$O_2 + H_2O + H^+ \xrightarrow{peroxidase}$ colored pigment +$5H_2O$

a) N-ethyl-N-(3-methylphenyl)-N'-succinylethylenediamine

2.2.4.3.4 Low Density Lipoprotein (LDL)

The principle of the test is the homogeneous enzymatic colorimetric assay **(**Bowling and Katayev.,2010) .

Using cholesterol esterase and cholesterol oxidase in the presence of surfactants that specifically solubilize just LDL, cholesterol esters and free cholesterol in LDL are quantified using a cholesterol enzymatic technique. Surfactants and a sugar molecule block the enzyme responses to lipoproteins other than LDL. It is not known how much cholesterol is in HDL, VLDL, and chylomicron.

LDL-cholesterol + $H_2O \xrightarrow[cholesterol\ esterase]{detergent}$ cholesterol + free fatty acid

By quantitatively converting cholesterol esters into free cholesterol and fatty acids, cholesterol esterase.

LDL-cholesterol+ $O_2 \xrightarrow{cholesterol\ esterase} \Delta^4 - cholestenone + H_2O_2$

Cholesterol oxidase converts cholesterol to 4-cholestenone and hydrogen peroxide in the presence of oxygen.

a) N-ethyl-N-(3-methylphenyl)-N-succinylethylenediamine

When peroxidase is present, the hydrogen peroxide produced combines with EMSE and 4'-aminoantipyrine to produce a red-purple color. This dye's color intensity is assessed photometrical and is directly correlated with the level of cholesterol in the blood.

$$2\,H_2O_2 + 4 - aminoantipyrine + EMSE^a + H_2O + H^+ \xrightarrow{peroxidase} red\ purple\ pigment + 5H_2O$$

2.2.4.3.5 Very Low Density Lipoprotein (VLDL)

VLDL-C concentration was measured using the Equation described by Friedwald *et al*., (1972).

VLDL is calculated as VLDL=TG/5.

2.3 Statistical Analysis

Data were analyzed by one way ANOVA by general liner model procedure using statistical package for social science (SPSS) version 27 The difference were considered to be significant at $P<0.05$ using multivariate model in SPSS. The data are presented as mean ± S.E.(standard error) (SPSS,2015).

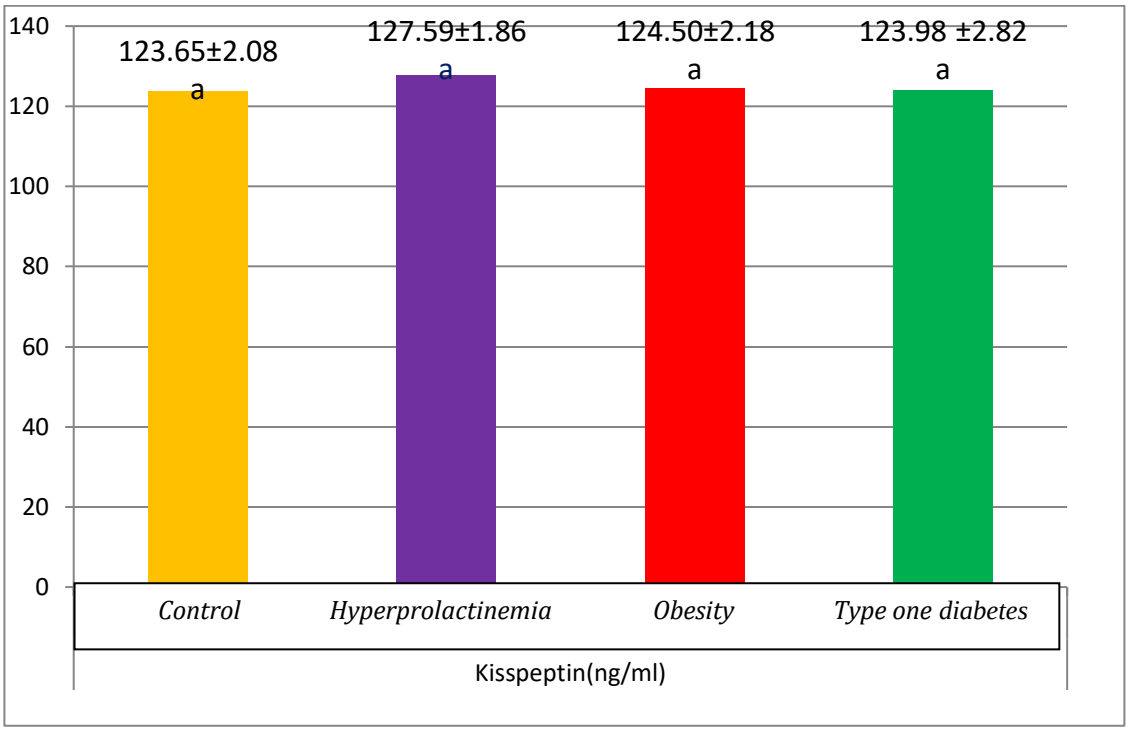
Chapter three

Results

3-Results

3.1-Kisspeptin

The levels of KP did not differ significantly (P>0.05) in the control (123.65±2.08 ng/ml), hyperprolactinemia (127.59±1.86 ng/ml), obesity (124.50±2.18 ng/ml) and T1DM groups (123.98±2.82 ng/ml) as shown in figure (3-1) Appendix E .



Figure(3-1):The kisspeptin levels in control hyperprolactinemia , obesity and T1DM women.

● levels represent mean ±SE.

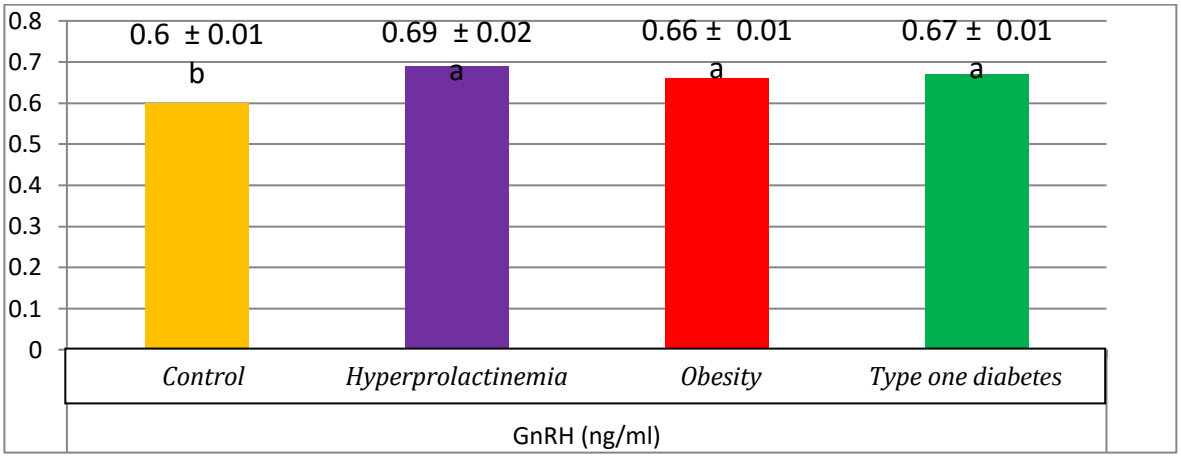
● Similar letters refer to non-significant differences among groups at level (P≤0.05).

3.2 Hypothalamus hormones

3.2.1 Gonadotropin Releasing Hormone (GnRH)

The levels of GnRH in hyperprolactinemia (0.69±0.02 ng/ml), obesity (0.66±0.01 ng/ml) and T1DM (0.67±0.01 ng/ml) increased significantly (P≤0.05) in comparison with groups control group (0.60±0.01 ng/ml)

While , non-significant (P>0.05) differences among hyperprolactinemia, obesity and T1DM groups as shown in figure (3-2) Appendix E.



Figure(3-2): The GnRH levels in control , hyperprolactinemia , obesity and T1DM women.

● levels represent mean ±SE.

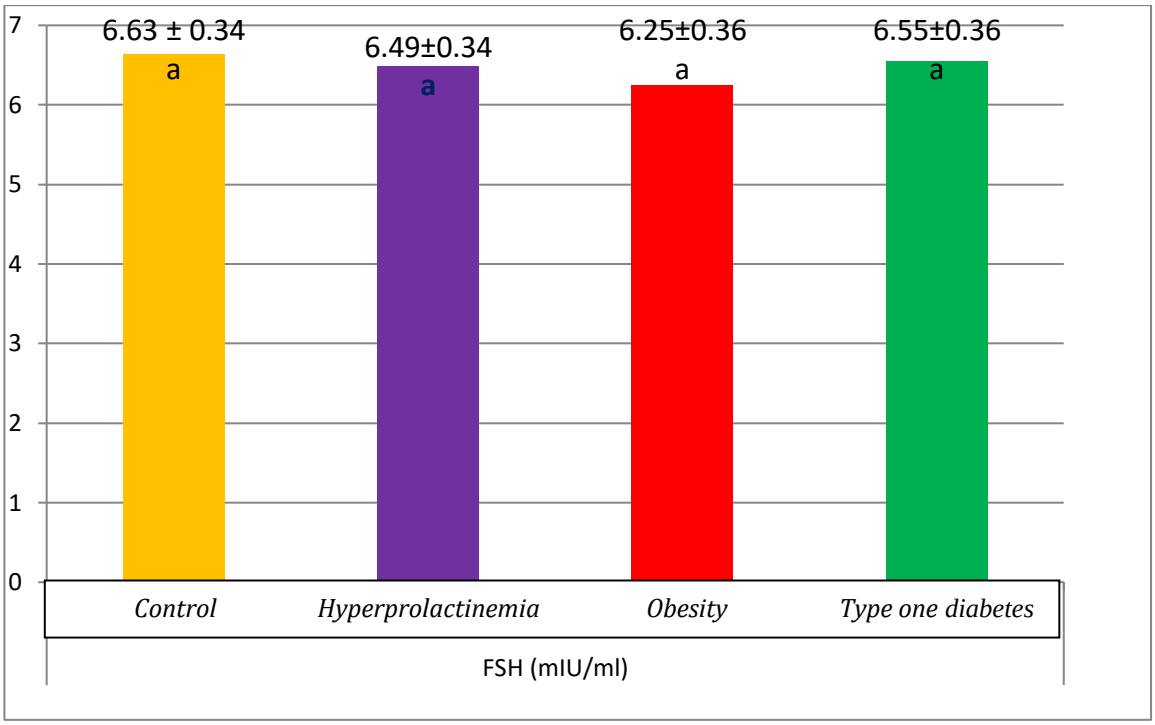
● Different letters refer to significant differences among groups at level (P≤0.05).

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

3.3 Pituitary Hormones

3.3.1 Follicle Stimulating Hormone (FSH)

The levels of FSH did not differ significantly (P>0.05) among the control (6.63±0.34 mIU/ml) , hyperprolactinemia (6.49±0.34 mIU/ml) , obesity (6.25±0.36 mIU/ml) and T1DM groups (6.55±0.36 mIU/ml) (Figure 3-3) Appendix E.



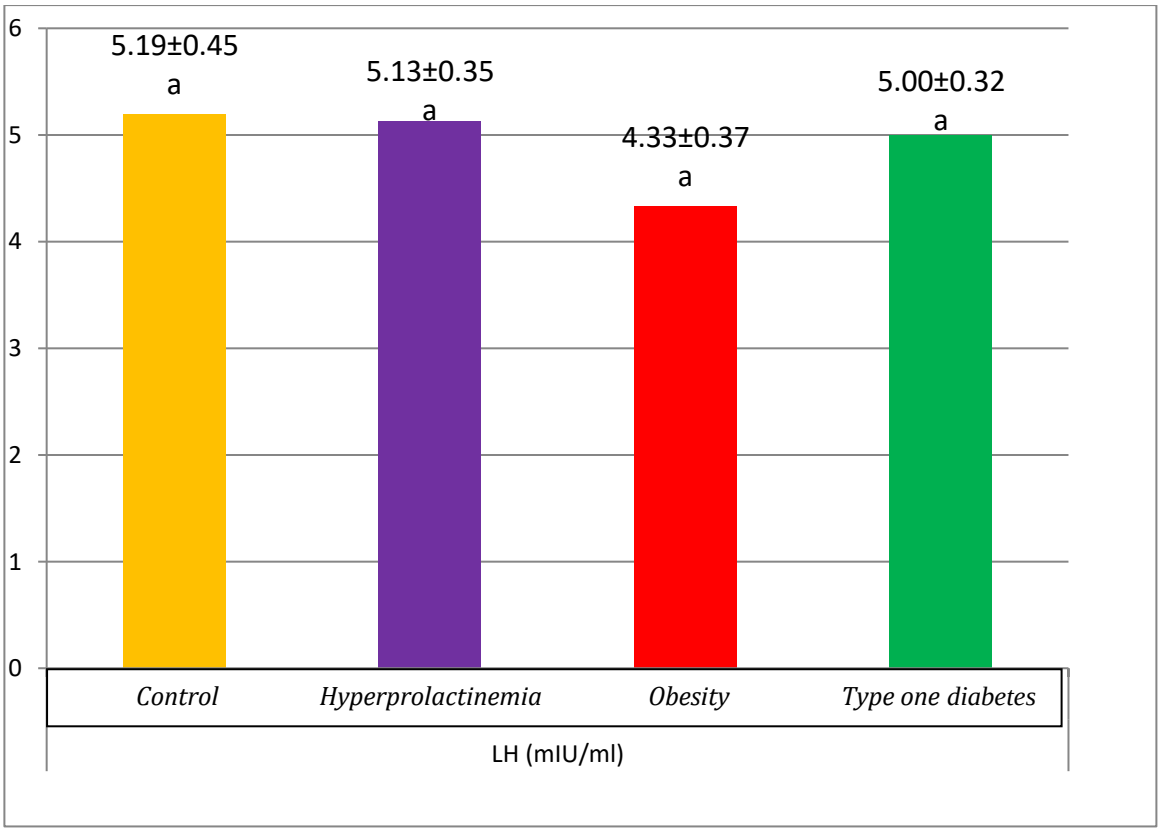
Figure(3-3): The FSH levels in control , Hyperprolactinemia , Obesity and T1DM women.

● levels represent mean ±SE.

● Similar letters refer to non-significant differences among groups at level (P < 0.05).

3.3.2 Luteinizing Hormone (LH)

The levels of LH did not differ significantly (P>0.05) in the control (5.19±0.45 mIU/ml) , hyperprolactinemia (5.13±0.35 mIU/ml), obesity (4.33±0.37 mIU/ml) and T1DM groups (5.00±0.32 mIU/ml) as shown in figure (3-4) Appendix E.



Figure(3-4):The LH levels in control , hyperprolactinemia , obesity and T1DM women.

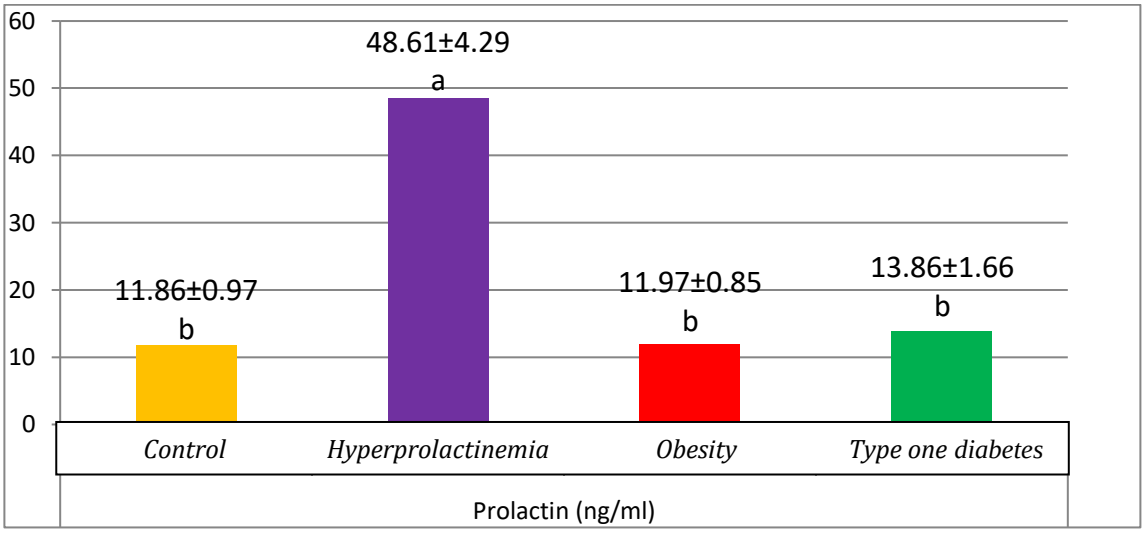
● levels represent mean ±SE.

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

3.3.3 Prolactin

The levels of Prolactin in the hyperprolactinemia group (48.61±4.29 ng/ml) increased significantly (P≤0.05) in comparison with T1DM (13.86±1.66 ng/ml), obesity (11.97±0.85 ng/ml) and control groups (11.86±0.97 ng/ml) .

While, no significant (P>0.05) differences among T1DM, obesity and control groups as shown in figure (3-5) Appendix E.



Figure(3-5): The prolactin levels in control , hyperprolactinemia, obesity and T1DM women.

● levels represent mean ±SE.

● Different letters refer to significant differences among groups at level (P≤0.05).

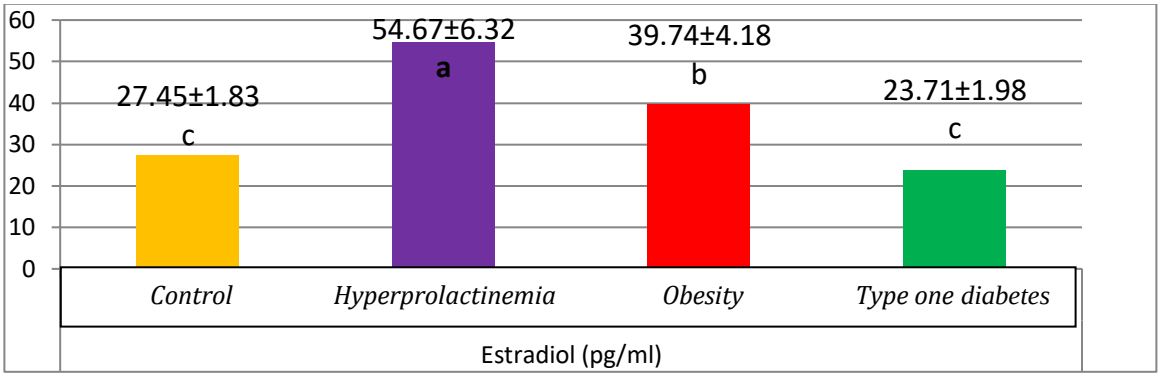
● Similar letters refer to non-significant differences among groups at level (P≤0.05).

3.4 Ovarian hormone

3.4.1 Estradiol

The levels of estradiol in hyperprolactinemia group (54.67±6.32 pg/ml) increased significantly (P< 0.05) in comparison with obesity (39.74±4.18 pg/ml) , T1DM (23.71±1.98 pg/ml) and control groups (27.45±1.83 pg/ml) .

Also, the levels of estradiol in obesity group increased significantly (P< 0.05) in comparison with control and T1DM groups .While ,no significant (P>0.05) differences in estradiol levels between control and T1DM groups as shown in figure (3-6) Appendix E.



Figure(3-6): The estradiol levels in control , hyperprolactinemia, obesity and T1DM women.

● levels represent mean ±SE.

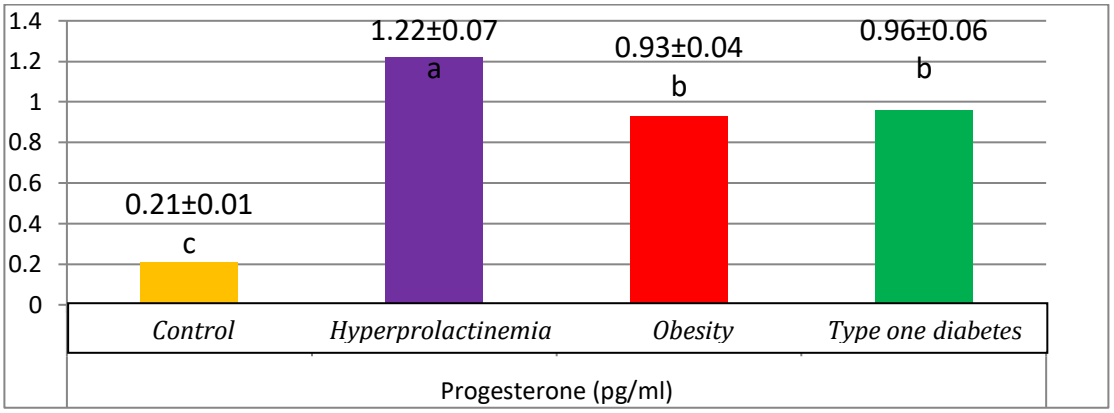
● Different letters refer to significant differences among groups at level (P≤0.05).

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

3.4.2 Progesterone

The levels of progesterone in hyperprolactinemia group (1.22±0.07 pg/ml) increased significantly (P< 0.05) in comparison with T1DM (0.96±0.06 pg/ml) , obesity (0.93±0.04 pg/ml) and control groups (0.21±0.01 pg/ml).

Also, the levels of progesterone in obesity and T1DM groups which increased significantly (P< 0.05) in comparison with control group. While, no differences significantly (P>0.05) in progesterone levels between T1DM and obesity groups as shown in figure (3-7) Appendix E.



Figure(3-7): The progesterone levels in control, hyperprolactinemia , obesity and T1DM women.

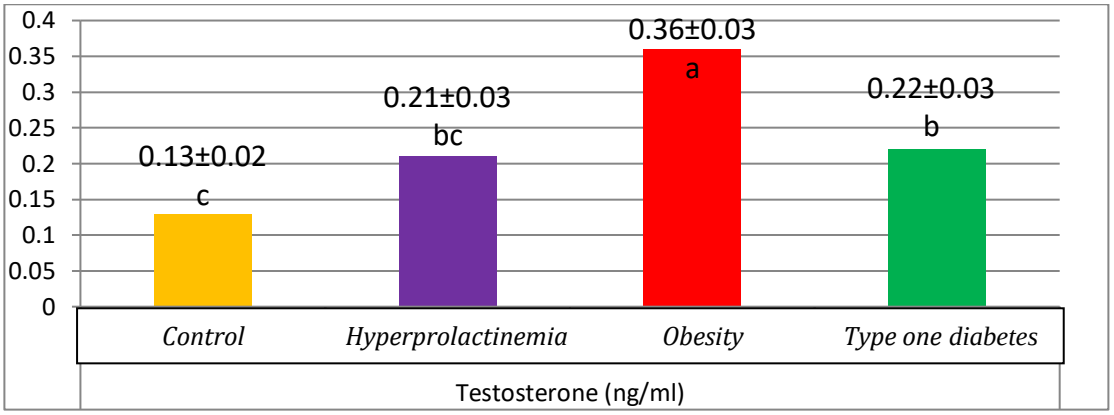
● levels represent mean ±SE.

●Different letters refer to significant differences among groups at level (P≤0.05).

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

3.4.3 Testosterone

The levels of testosterone in obesity group (0.36±0.03 ng/ml) increased significantly (P< 0.05) in comparison with T1DM (0.22±0.03 ng/ml) , hyperprolactinemia (0.21±0.03 ng/ml) and control groups (0.13 ±0.02 ng/ml) . The levels of testosterone in T1DM group increased significantly (P< 0.05) compared to the control group . The results showed non-significant (P>0.05) differences in testosterone levels between T1DM and hyperprolactinemia group .Also , non-significant (P>0.05) differences between hyperprolactinemia and control groups ,as shown in figure (3-8) Appendix E.



Figure(3-8): The testosterone levels in control , hyperprolactinemia , obesity and T1DM women.

● levels represent mean ±SE.

● Different letters refer to significant differences among groups at level (P≤0.05).

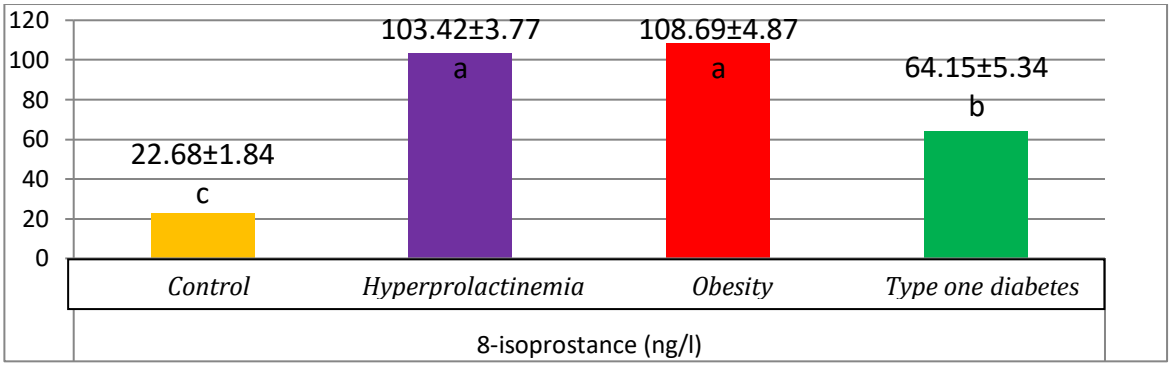
● Similar letters refer to non-significant differences among groups at level (P≤0.05).

3.5 Biochemical parameters

3.5.1 8-isoprostance

The values of 8-isoprostance in obesity (108.69±4.87 ng/l) and hyperprolactinemia groups (103.42±3.77 ng/l) increased significantly (P≤0.05) in comparison with T1DM (64.15±5.34 ng/l) and control groups (22.68±1.84 ng/l).

The values of 8-isoprostance in T1DM group increased significantly (P≤0.05) in comparison with control group .While , no differences significantly (P>0.05) in 8-isoprostance between obesity and hyperprolactinemia groups as shown in figure (3-9) Appendix E.



Figure(3-9): The 8-isoprostane values in control , hyperprolactinemia , obesity and T1DM women.

●Values represent mean ±SE.

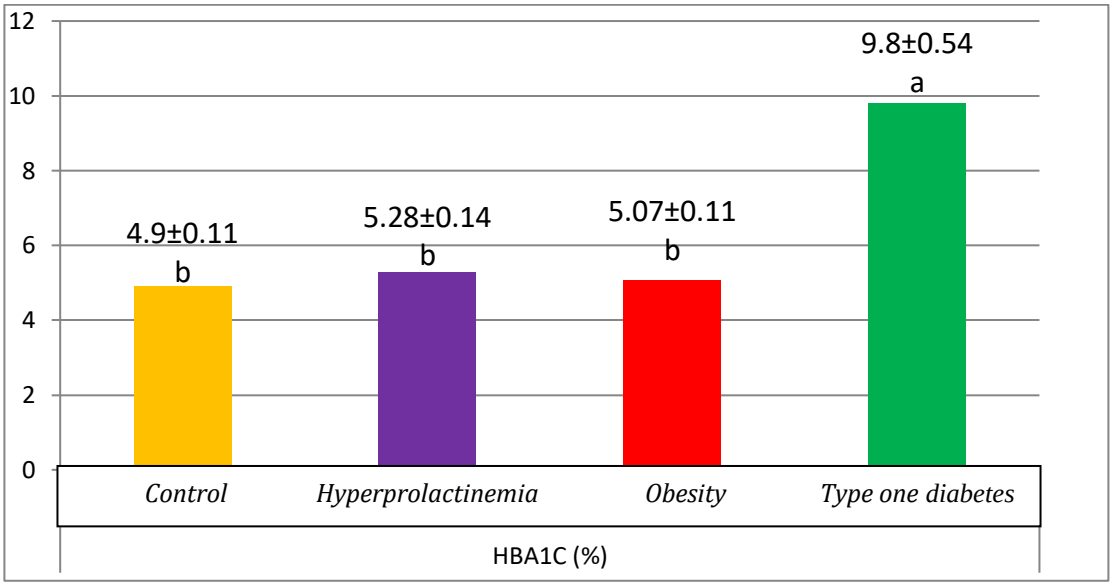
● Different letters refer to significant differences among groups at level (P≤0.05).

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

3.5.2 HbA1C

The values of HBA1C in the T1DM group (9.8±0.54 %) increased significantly (P≤0.05) in comparison with obesity (5.07±0.11 %) , hyperprolactinemia (5.28±0.14%) , and control groups (4.9±0.11 %).

There are no differences significantly (P>0.05)among obesity, hyperprolactinemia, and control groups in HBA1C values ,as shown in figure (3 -10) Appendix E.



Figure(3-10): The HBA1C values in control , hyperprolactinemia , obesity and T1DM women.

●Values represent mean ±SE.

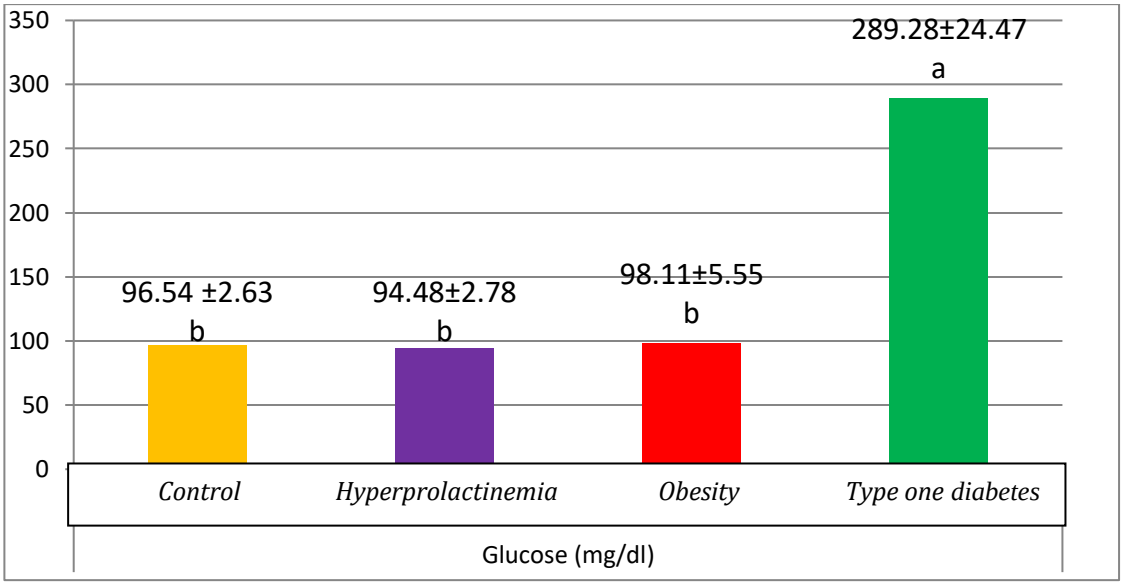
●Different letters refer to significant differences among groups at level (P≤0.05).

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

3.5.3 Glucose

The values of glucose in the T1DM group (289.28±24.47 mg/dl) increased significantly (P≤0.05) in comparison with obesity (98.11±5.55 mg/dl), hyperprolactinemia (94.48±2.78 mg/dl) and control groups (96.54 ±2.63 mg/dl)

The results showed no differences significantly (P>0.05)in glucose values among obesity, hyperprolactinemia group and control groups as shown in figure (3-11) Appendix E.



Figure(3-11): The glucose values in control , hyperprolactinemia , obesity and T1DM women.

●Values represent mean ±SE.

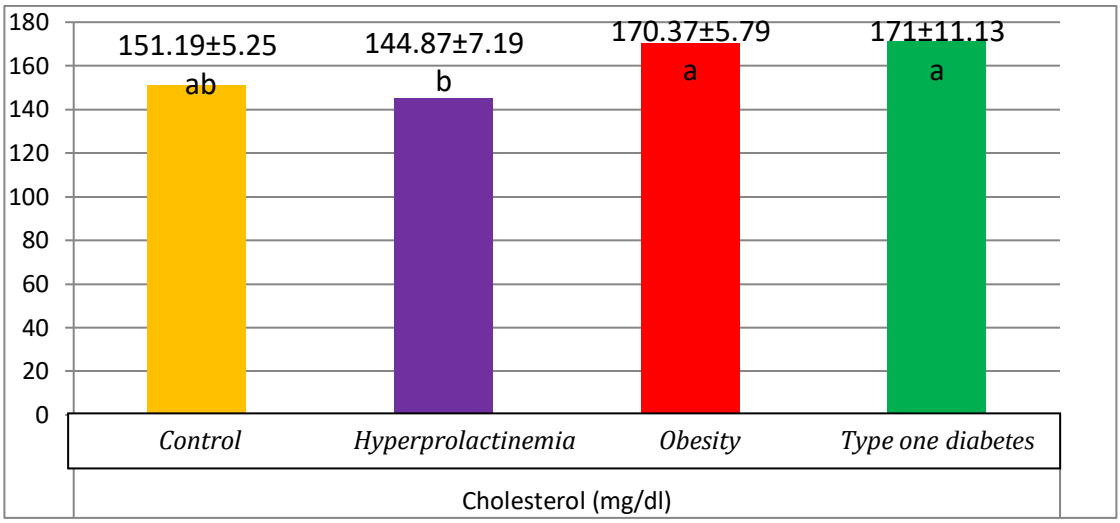
● Different letters refer to significant differences among groups at level (P≤0.05).

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

3.5.4 Lipid profile

3. 5.4 .1 Cholesterol

The values of cholesterol in hyperprolactinemia group (144.87±7.19 mg/dl) decreased significantly (P≤0.05) in comparison with the T1DM (171±11.13 mg/dl) and obesity groups (170.37±5.79 mg/dl). The levels of cholesterol in obesity and T1DM groups increased but, did not reach to significant level in comparison with control group (151.19 ±5.25 mg/dl). The results showed no differences significantly (P>0.05) in cholesterol levels among T1DM, obesity and control groups .Also, did not differ significantly (P>0.05) between control and hyperprolactinemia groups as shown in figure (3-12) Appendix E.



Figure(3-12): The cholesterol values in control , hyperprolactinemia, obesity and T1DM women.

●Values represent mean ±SE.

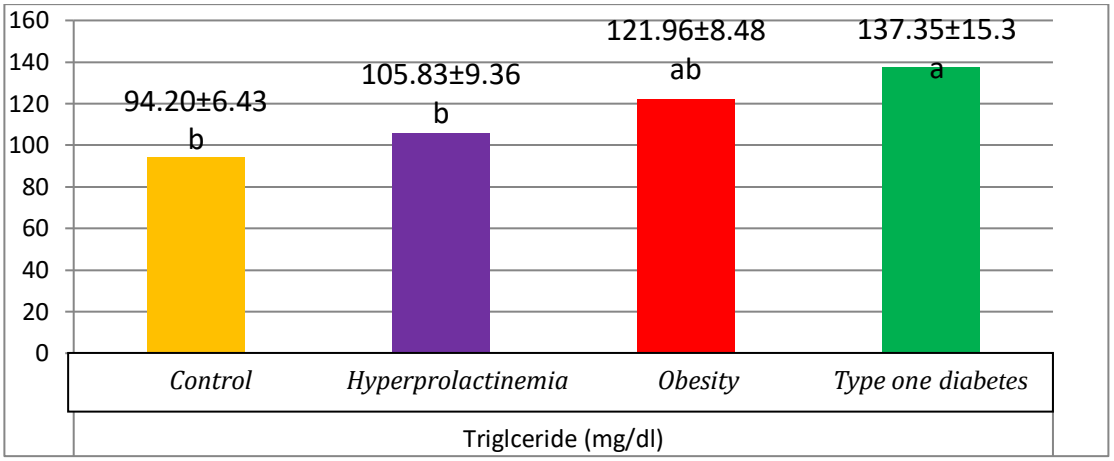
● Different letters refer to significant differences among groups at level (P≤0.05).

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

3. 5.4 .2 Triglyceride (TG)

The values of TG in the T1DM group (137.35±15.32 mg/dl) increased significantly (P≤0.05) in comparison with hyperprolactinemia (105.83±9.36 mg/dl) and control groups (94.20±6.43 mg/dl).

There are no differences significantly (P>0.05) among T1DM and obesity group (121.96±8.48 mg/dl). Also , no significant differences (P>0.05) among hyperprolactinemia and control groups as shown in figure (4-13) Appendix E.



Figure(3-13): The triglyceride values in control , hyperprolactinemia , obesity and T1DM women.

●Values represent mean ±SE.

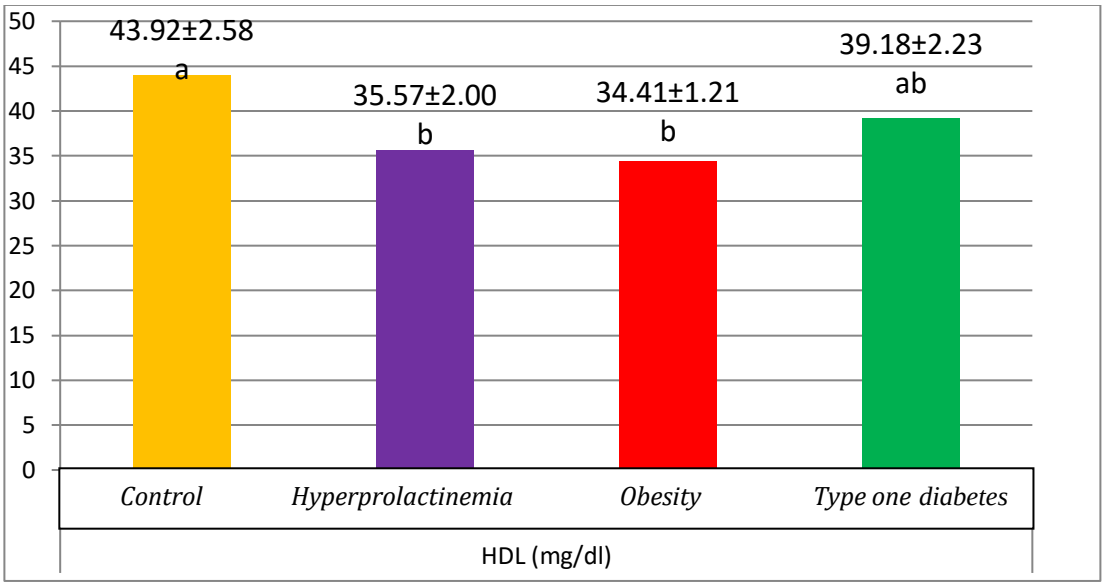
● Different letters refer to significant differences among groups at level (P≤0.05).

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

3.6.3 High-density lipoprotein (HDL)

The values of HDL in the hyperprolactinemia (35.57±2.00 mg/dl) and obesity groups (34.41±1.21 mg/dl) decreased significantly (P≤0.05) in comparison with control group (43.92±2.58 mg/dl).

There are no differences significantly (P>0.05) between control and T1DM (39.18±2.23) groups .Also, no significant (P>0.05) differences among T1DM, hyperprolactinemia and obesity groups in HDL values as shown in figure (3-16) Appendix E.



Figure(3-14): The HDL values in control , hyperprolactinemia , obesity and T1DM women.

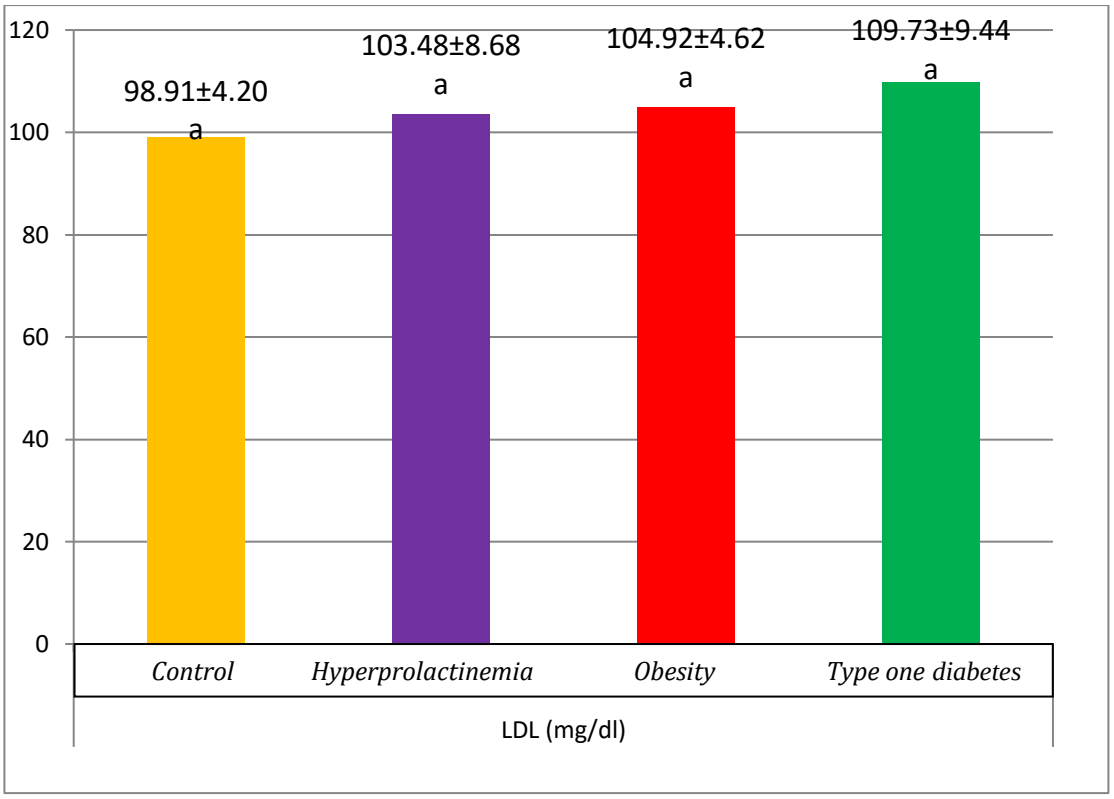
●Values represent mean ±SE.

● Different letters refer to significant differences among groups at level (P≤0.05).

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

3. 5.4 .4 Low-Density Lipoprotein (LDL)

The values of LDL did not differ significantly (P>0.05) in control (98.91±4.20 mg/dl), hyperprolactinemia (103.48±8.68 mg/dl) , obesity (104.92±4.62 mg/dl) and the T1DM (109.73±9.44 mg/dl) groups as shown in figure (3-15) Appendix E.



Figure(3-15): The LDL values in control , hyperprolactinemia , obesity and T1DM women.

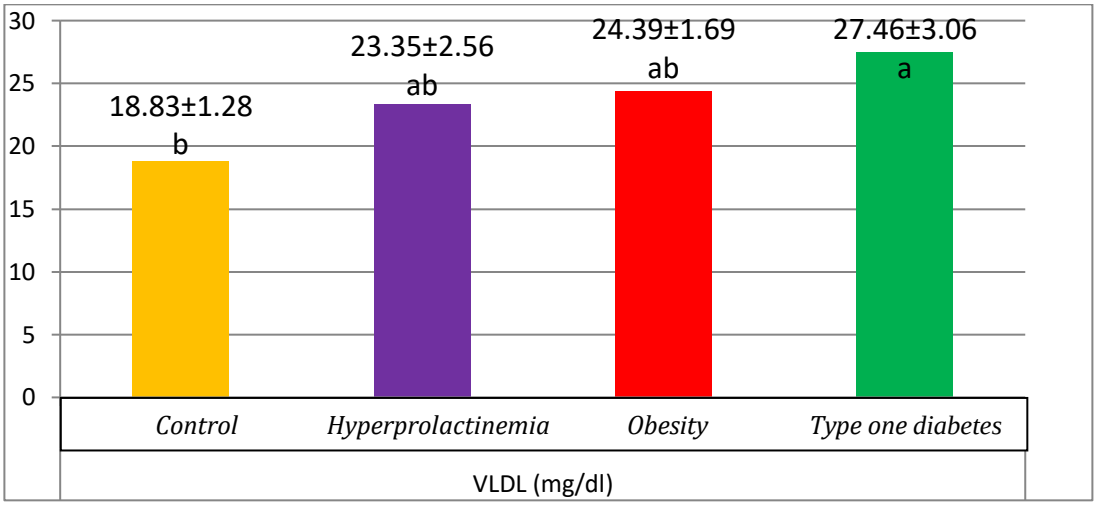
●Values represent mean ±SE.

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

3. 5.4 .3 Very Low-Density Lipoprotein (VLDL)

The values of VLDL in the T1DM group (27.46±3.06 mg/dl) increased significantly (P≤0.05) in comparison with control group (18.83±1.28 mg/dl).

There are no significant (P>0.05) differences in VLDL values among T1DM (27.46±14.69 mg/dl) , obesity (24.39±1.69 mg/dl) and hyperprolactinemia (23.35±2.56 mg/dl) groups. Also, no differences significantly (P>0.05) among obesity , hyperprolactinemia and control groups as shown in figure (3-14) Appendix E.



Figure(3-16): The VLDL values in control , hyperprolactinemia , obesity and T1DM women.

●Values represent mean ±SE.

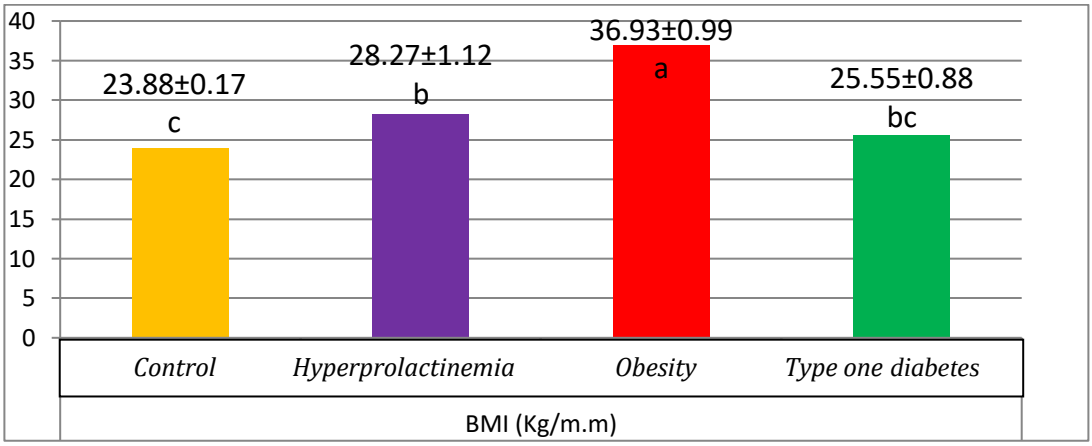
● Different letters refer to significant differences among groups at level (P≤0.05).

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

3.7 Body Mass Index (BMI)

The values of BMI in the obesity group (36.93±0.99) increased significantly (P≤0.05) in comparison with hyperprolactinemia (28.27±1.12), T1DM (25.55±0.88) and control groups (23.88±0.17). The values of BMI in the hyperprolactinemia group increased significantly (P≤0.05) in comparison with control group .

There are no differences significantly (P>0.05) between control and T1DM group. Also, no significant (P>0.05) differences between hyperprolactinemia and T1DM groups as shown in figure (3-17) Appendix E.



Figure(3-17): The BMI values in control , hyperprolactinemia , obesity and T1DM women.

●Values represent mean ±SE.

● Different letters refer to significant differences among groups at level (P≤0.05).

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

Chapter four

Discussion

4 Discussion

4.1 Kisspeptin

The values of KP did not differ significantly (P>0.05) in the HPE, obesity and T1DM and control groups (Figure, 3-1).

The absence of significant differences between the groups may be due to the patients did not suffer from a functional defect in the KP system. This belief is confirmed by study of Arslan and his team (2022) they found KP levels in HPE group does not differ from control group and this agrees with our results , where the researcher conclude HPE does not effect on circulating KP levels but they recorded positively correlates between KP and HPE .

Hoskova and his colleagues (2022) investigated the effects of exogenous KP administration on GnRH pulse generation in the cases with HPE, KP (112–121 ,0.24 nmol/kg) was administered 10 hours every each hour . At hour 11, one intravenous doses of GnRH (75 ng/kg) was given . for the all patient in group , PRL levels did not change significantly before and after administering . Thus, the increase in KP did not change the level of PRL .

Mancini and his team (2021) found did not significant differences in plasma concentrations of KP among normal, overweight and obese group, and this agrees with our study , and the results of Rafique and latif (2015) found serum KP levels in overweight and obese young females did not differ significantly compared to the normal-weight females, and

there not be any correlation between serum KP and anthropometric indices , and this agreement with our result. While , other studies found different results , where they are observed the relationship between KP and obesity.

Arıkan and Sagsoz (2023) noted that the serum KP concentrations were determined to be higher in the control group than in the class-1 (BMI 30.0–34.9) obese group. Moreover, a negative correlation was identified between the participants' BMI and KP concentrations. Pruszyńska-Oszmałek and his team (2021) study the level KP in mothers' blood (MB) from obese and non-obese volunteers revealed an increase in this peptide in the obese group and higher concentration of KP was found in the cord blood (CB) of obese mothers compared to no obese mothers .Also, a strong positive correlation between the concentrations of KP in MB and CB.

Kołodziejski and his team (2018) showed that serum levels of KP are low in obese female compared to controls. These results indicate that KP could be involved in pathophysiology of human obesity . Majeed and his team (2017) observed that serum KP level of obese women was highly significantly increased in comparison to that of normal weight women. The Obese/overweight girls had higher KP levels, and there was a positive correlation between KP and FSH and LH and obesity-related parameters in both gender (Pan *et al* .,2016).

Izzi-Engbeaya (2023) noted the a biologically active dose of KP did not affect self-reported appetite and food intake in women with overweight

or obesity. A comparison study was made between patients with T1D, obesity and healthy subjects , they found the KP levels was lower in controls compared to the obesity subject , but did not reach to significance levels compared to T1D .Also, no significant difference between subjects with obesity and T1D , this agrees with our results (Calcaterra *et al* .,2021). While ,another study on T1D patient have a different KP levels compared to controls; higher KP levels were associated with IR (Hussain *et al* .,2015).

Abbara and his colleagues (2022) found there was no difference in the rate of increase in plasma KP between GDM and healthy control pregnancies .Also, there were no differences in plasma KP levels at any trimester for both pregnancies (GDM and healthy). Plasma KP levels were not significantly altered in GDM pregnancies, both in invariable analysis and after adjustment for maternal age, ethnicity, BMI, smoking status, and parity and this agree with our results .

Perhaps the reason for our difference in results from other researchers is due to the difference in the timing of the blood draw during the menstrual cycle, the difference in the patient's age, the difference in the duration of the infection, the difference in the average body mass, and the difference in the number of samples.

4.2 Gonadotropin releasing hormone

The values of GnRH in a HPE , obesity and T1DM increased significantly ($P \leq 0.05$) in comparison with groups control group as shown in figures (3-2) .

The studies below found that high GNRH is an influential factor in the occurrence of HPE, obesity and T1DM. In study done by Iancu et al (2023) discovered that temporary HPE, characterized as fluctuations in PRL levels during in vitro fertilization (IVF), were recorded. Administration of gonadotropins and GnRH agonists, elevated estradiol levels, and procedure-related stress were suggested as potential reasons ,and this agree with our results.

Adipocytes had GnRH receptor expression. The expression of GnRH receptors gradually increased during adipocyte maturation. The GnRH a promotes adipocyte maturation, encourages the creation of lipid droplets in mature adipocytes, and prevents adipocytes from activating the AMPK pathway. It also stimulates the proliferation of human preadipocytes-subcutaneous (HPA-s) (Li *et al* .,2022) ,and this agree with our results.

Büyükinan and Kurku (2019) observed in patients with central precocious puberty (CPP), the prevalence of obesity was higher in CPP ,and BMI increased with GnRH a treatment. The weight of the patients at the beginning of the treatment are affected and BMI change with GnRH a treatment. Those patients with CPP were more prone to weight gain and BMI increase, and this agree with our results.

Grattan and his team (2007) found that mice treated with five injections of ovine PRL over 48 h showed a 4-fold increase in the number of GnRH neurons , PRL suppresses LH levels in the mouse, as it does in other

species, and indicate that it acts centrally to regulate intracellular signaling within GnRH neurons ,and this agree with our results.

In comparison to patients undergoing stimulation using only human menopausal gonadotropins, the use of leuprolide acetate, a GnRH agonist, in conjunction with human menopausal gonadotropins for oocyte retrieval was related with greater serum levels of estradiol and PRL (Meldrum *et al.*,1992) ,and this agree with our results.

The paracrine effects of the alpha and beta subunits of pituitary gonadotropins on lactotroph cells are thought to be the cause of this increase in PRL level caused by GnRH and its agonistic analogues, according to the scientists (Meldrum *et al*.,1992) ,and this agree with our results.

PRL elevation was shown to significantly increase in women stimulated with GnRH agonist long protocol and human menopausal gonadotrophin (HMG) compared to patients treated with short protocol, and it was positively connected with estradiol levels but independent of mid-cycle LH surge (Kamel *et al* .,1994) ,and this agree with our results.

Other studies below have found results that do not agree with us.

Obese women have been shown a reduced responsiveness to exogenous GnRH, this study further examined the LH response to an exogenous GnRH bolus. In particular, the investigators showed decreased LH pulse amplitude and a reduced response to GnRH induced by hyperinsulinemia combined with hyperlipidemia, implicating a pituitary origin of the low

gonadotropin levels (Zippl *et al*.,2021) ,and this don't agree with our results.

The use of a 5-min sampling interval and measurement of gonadotropin free a-subunit (FAS) as the primary marker of GnRH pulse generator activity indicate that GnRH pulse frequency in younger postmenopausal women (PMW) is faster than previously reported, but not increased that during the late follicular phase and mid cycle surge in women with intact ovarian function (Hall *et al*.,2000) ,and this don't agree with our results.

Suppression of ovarian hormones by GnRHAG therapy for 20 weeks did not exaggerate the hypothalamic-pituitary-adrenal axis response to corticotrophin-releasing hormone (CRH), but E2 reduced HPA axis activity compared to pre-treatment levels in premenopausal women (Gavin *et al*.,2018) ,and this don't agree with our results .

Kadhim and Ahmed (2015) found that HbA1c and gonadotropin Levels (FSH and LH) were higher in diabetic premenopausal women compared with control individuals ,and this don't agree with our results. Obesity occurs at a high rate among children with CPP, but does not appear to be related to long term pituitary-gonadal suppression induced by GnRHa administration (Palmert *et al*.,1999) ,and this don't agree with our results.

In study doing by Lin and his team (2021) observed diabetes control was worse in men with GnRH vs. prostate cancer (PCa)-free men as well as compared with PCa men without GnRH ,use of GnRH in T2DM men

with PCa was associated with worse glycemic control. Misztal and his team (2005) found, several-hour-long infusions of PRL into the CNS prior to the next spontaneous ovulation in ewes had no direct effect on the secretory activity of GnRH neurons, and/ or the synthesis, accumulation, or tonic release of LH from the pituitary gonadotrophs ,and this don't agree with our results .

Perhaps the reason for our difference in results from other researchers is due to the difference in the timing of the blood draw during the menstrual cycle, the difference in the patient's age, the difference in the duration of the infection, the difference in the average body mass, and the difference in the number of samples.

4.3 Reproductive hormones

The values of progesterone in a HPE , obesity and T1D`M increased significantly ($P \leq 0.05$) in comparison with groups control group as shown in figures (3-7) .The values of FSH and LH did not differ significantly ($P > 0.05$) in all groups as shown in figure (3-3) and (3-4).The values of PRL in the HPE group increased significantly ($P \leq 0.05$) in comparison with other groups (Figure,3-5) .The values of estradiol in HPE and obesity increased significantly ($P \leq 0.05$) in comparison with groups control group as shown in figure (3-6) . Whereas , The values of testosterone in obesity and T1D increased significantly ($P \leq 0.05$) in comparison with groups control group as shown in figure (3-8) .

In this study the differences in the levels of reproductive hormones in the women with HPE ,obesity and T1DM compared to control and

compared to each other , probably belonged to the intertwined hormonal interactions. So that, In the HPE group, this may be due to the feedback resulting from the increase in PRL and its connection with dopamine .

Dopamine is the inhibitory factor for PRL. Dopamine is important in maintaining a healthy level of PRL. In the event of an increase in PRL secretion, dopamine flows from the hypothalamus into the circulatory system, and thus leads to decrease in PRL secretion and its return to normal level in the healthy state. Thus, any imbalance in this dopamine balance will affect most of the hormones that have feedback with PRL (Nappi *et al* .,2021) .

The results in this study agree with other study such as Arslan and his team (2022) noted that PRL level was found to be significantly higher in the HPE group. No significant differences were detected between the LH levels and FSH .But , Estradiol levels were decrease significantly , this don't agree with our results, in the HPE group compartmented with control.

Hoskova and his team (2022) noted after KP (112–121 ,0.24 nmol/kg) was administered every 1 hour, for 10 hours. At hour 11, one intravenous doses of GnRH (75 ng/kg) was given for women with HPE ,recorded increased total number of LH pulses in the setting of HPE. The LH and estradiol levels increased significantly while FSH and PRL levels did not change significantly.

Posawetz and his team (2021) found the Women with PRLoma had significantly higher levels of PRL this ,agree with our results. and lower

of LH , FSH and estradiol the women age with PRLoma in their study ranging from 35 to 45 year.

Al-Ttaie and his team (2021) observed high level of the PRL and this agree with our results regarding to the PRL , LH and estradiol significantly increased while Progesterone and FSH decreased significantly in the infertility (HPE women) group compared with the controls.

Other studies below have found results that do not agree with us.

The study of Elbardisi and his team (2021),they found in HPE patient that a significant increase in LH levels and a significant decreased in PRL levels . While, there was no differ in the testosterone levels ,and this results don't agree with our results

Elnour and his team (2021)found that PRL concentration significantly higher ,while as ,values of LH , FSH and estradiol don't no significant difference between women with HPE and women with normal PRL ,and don't agree with our results . Owiredu and his team (2019) found the levels of FSH and estradiol lower in females with secondary infertility due to HPE and this results are not consistent with our results.

Khan and his team (2021) found strong association between serum FSH levels with secondary infertility due to HPE . The mean levels of FSH were very decrease .Also, they failed to find significant association between serum LH levels and secondary infertility status ,though, the levels of LH were also decrease in patients with secondary infertility as compared to those without secondary infertility. Infertile women with

normal weight presented high significant medians of serum progesterone than both ranges of overweight patients, progesterone gradually decrease as the weight of patients increase (Giviziez et al.,2022).

In the obese group, the reason may be the outputs of adipose tissue and its effect, Adipose tissue has proven its primary role as an endocrine gland, as it is considered an active and important endocrine tissue . It has soluble products called adipocytokines or adipose tissue hormones such as leptin, which has been shown to have an effect on raising the estrogen hormone, especially in women, and therefore the rise in estrogen may lead to a change in the levels of other reproductive hormones (Triantafyllou *et al*.,2016).

Izzi-Engbeaya and his team (2023) found that KP injection in obesity women increased circulating LH levels confirming bioactivity of the peptide, as with its known reproductive effects in women with normal weight, but estradiol levels were similar during KP infusions. According to the study by Arikan and his team (2022) they noted the mean LH , FSH, AMH and estradiol did not differ significantly in the class-2 obese (BMI 35.0–39.9) , the class-1 obese (BMI 30.0–34.9) and control groups, don't agree with our results .

Glucose utilization in human adipocytes in late postmenopausal women was inhibited by E2 . Changes in glucose utilization over time since menopause may be explained by a lower ESR1:ESR2 ratio (Ahmed *et al*., 2022). The overweight or obese in women may have altered endocrine profiles in them such as ,high LH , abnormal ratio of FSH and

LH, low progesterone in the luteal phase, and low levels of sex hormone-binding globulin (Lash and Armstrong, 2009).

A high BMI is associated with decreased estradiol levels in all phases of ovarian cycle ,and has a negative impact on pregnancy outcome in obese women (Rehman *et al*., 2012). In obesity women ,significantly higher concentrations of total testosterone this agree with our results, were observed in subjects with metabolic syndrome (MS) when compared with women without MS (Fatani *et al*., 2018).

Serum estradiol level in obese women was highly significantly increased in comparison to the normal weight women and this agree with our results (Majeed *et al*,.2017). Stárka and his team (2020) found positive correlations between lowering overall testosterone, SHBG and increased BMI in obesity.as well as , there were no significant changes to levels of free androgens, estradiol or the gonadotropins LH and FSH ,this don't agree with our results.

Other studies below have found results that do not agree with us

A significant reduction in the concentration of testosterone, LH and AMH hormones , while the concentration of estradiol ,and leptin hormones were significantly higher in obese women compared with control group, also there was no significant difference for progesterone hormone concentration ,this result don't agree with our results according to study by (Salman and Yser , 2022) .

The metabolic syndrome group had a significantly lower mean total testosterone concentration compared to the non-metabolic syndrome

group as well as significant negative correlations between age , systolic blood pressure , and TGs with total testosterone concentration .Also , weight , BMI , WC , body fat mass , and body fat percentage showed significant positive correlations with total testosterone concentration (Lee and Lee.,2021) .

Giviziez and his team (2022) noted that overweight increases more than twice the chance of anovulation ,among infertile women with regular menstrual cycles serum.

While ,the differences of the hormones in the T1D group may be due to the absence , decrease or increase in insulin ,especially, all patients in our study were treated with insulin , high insulin levels in women cause testosterone to rise, while low insulin levels affect progesterone , and therefore may lead to a change in the levels of other reproductive hormones (Melmer *et al* .,2021)

Kang and his team (2021) don't agree with our results ,they observed that the levels of FSH, LH, and Total testosterone levels in young men with T1D were higher than those in the control subjects. Calcaterra and his team (2021)found the concentration of the LH, FSH, LH/FSH and estradiol were similar within obesity, control and T1D subjects , and this agree with our results .Also , KP and AMH were lower in those patients with obesity or T1D.

Women with T1D onset before menarche have a shorter reproductive period compared with non-diabetic women, exhibiting delayed menarche and earlier natural menopause. Women with T1D thus experienced 2.5

fewer reproductive years compared to those without diabetes (Yi *et al*,.2021). The metabolic imbalance plays an important role in reproductive functions, such as the duration and irregularity of the menstrual cycle, the increase in HbA1c increases the duration of the menstrual cycle in women of childbearing age who suffer from T1DM(Gaete *et al*.,2010).

Perhaps the reason for our difference in results from other researchers is due to the difference in the timing of the blood draw during the menstrual cycle, the difference in the patient's age, the difference in the duration of the infection, the difference in the average body mass, and the difference in the number of samples.

4.4 8-isoprostance

The values of 8-isoprostance in obesity , HPE and T1D groups increased significantly ($P \leq 0.05$) in comparison with control groups (Figure,3-9).

In the case of HPE the reason for the increase in 8-isoprostance may be a hormonal imbalance and its effect on oxidative stress, Kani and his team (2019) reached similar results where the increase in 8-isoprostance in infertile women had higher PRL levels than controls. Increasing in F2-isoprostanes are marker of oxidative stress harmfully ; and this increased as a mechanistic link between obesity and cardiovascular disease risk (Keaney *et al*.,2003; Otani ,2011; Spahis *et al* .,2016) .Also, The results support the hypothesis that hyper in blood glucose can increased oxidative stress (Esposito et al .,2002; Ceriello et al .,2007)

Valente and his team (2021) found that no significant differences between the T1D and control groups for gender distribution, 8-iso-PGF-2α. While ,other studies found high levels of 8-isoPGF-2α in T1DM compared to healthy subjects and this agree with our results (Wentholt *et al* .,2008; Meng *et al*., 2015; Altıncık *et al* .,2016).

Pękala-Wojciechowska and his team (2018) found that blood levels of 8-isoprostane in patients with T1DM without complications and those with T1D with advanced complications were significantly higher compared to the control group ,and this agree with our results .While , concentration of 8-isoprostane in exhaled breath condensate (EBC) was lower in diabetic patients with type one have advanced complications, than in patients with T1DM without advanced complications and in the control group.

Saleem and his team (2023) observed that F2 -IsoPs may be associated with obesity-induced cardiovascular risk , they noted that BMI was positively correlated with plasma F2-IsoPs and a positive correlation between F2-IsoPs and percent fat .There was an inverse correlation between F2-IsoPs and HDL , while there was a positive correlation between F2-IsoPs and LDL.

In obese rats, peri-pancreatic white adipose tissue (pWAT), but not epididymal white adipose tissue (eWAT), released less amounts of 5-F2t isoprostanes, 15-F2t-isoprostanes, 4-F4t-neuroprostanes and 10-F4t-neuroprostane compared to lean animals .also , 15-F2t isoprostane epimers, but not 5-F2t-isoprostanes, were able to decrease glucose-

induced insulin secretion in pancreatic islets from Wistar rats (Laget *et al*.,2022).

African Americans women have lower levels of systemic F2 -isoprostane levels despite their predisposition to obesity , T2DM and BMI was inversely correlated with insulin sensitivity in obese women. Addition, they found that plasma F2 -IsoPs levels were significantly associated with reduced insulin sensitivity (Il'yasova *et al*.,2017).

Perhaps the reason for our difference in results from other researchers is due to the difference in the patient's age, difference in the duration of the infection, difference in body mass index, and difference in the number of sample

4.5 Glucose and HBA1C

The values of HBA1C and glucose in T1D group increased significantly ($P \leq 0.05$) in comparison with obesity, HPE , and control groups (Figure,3-10 and 3-11).

These results in this study may be attributed to the effect of the absence or deficiency of insulin in T1DM patient , As insulin works to reduce blood sugar levels, and in case of people with T1DM, patients suffer from a defect in the beta cells of the pancreas, which work to produce the hormone insulin, which plays a crucial role in the balance of glucose in the blood.

Posawetz and his team (2021) found don't differ between PRLoma and control groups in value of FG, HOMA-IR and HbA1c, and this

agree with our results. Izzi-Engbeaya and his team (2023) found that administration of KP did not influence pre-prandial ,and postprandial glucose and insulin levels in women with overweight or obesity, and this agree with our results.

Blood glucose was significantly higher in obese and overweight patients compared to normal subjects while there was no difference between obese, and overweight .Also HOMA index values, progressively and significantly higher by going from the normal to the overweight and the obese according to study by Mancini and his team (2021). Azam and his team (2023) measured plasma glucose two hours after eating, as well as fasting glucose, and found that they were not associated with obesity among people with diabetes.

The mean HOMA, serum insulin, and glucose levels were highest in the class-2 obese (BMI 35.0–39.9)group compared with the class-1(BMI 30.0–34.9) obese and control groups and this don't agree with our results (Arikan *et al* .,2022).

Previous studies agree with the results of our study regarding value of glucose and HBA1C.

Alkaabi and his team (2022) found that HbA1c was significantly higher among patient with T1DM than controls.Kang and his team (2021) noted that glucose and average HbA1c increased in T1DM patients compared to controls .Valente and his team (2021) recorded that the glycemic variability was seven times higher in T1DM than in healthy controls .

Ahmad and his team (2022) found that demonstrate that the increase of adipose ESR2 expression, occurring in postmenopausal women is linked to reduced glucose uptake in response to E2 treatment, and this is reversed by presence of an ESR2 antagonist. They suggest that E2 action mediated by ESR2 may contribute to the development of adipose tissue and insulin resistance in postmenopausal women.

The glycemic and T2D risk genetic variants contribute to higher FG and FI levels ,and decreased beta cell function in children ,and adolescents. The causal effects of adiposity on increased insulin resistance are detectable from childhood age (Balkhiyarova *et al* .,2022). Fatani and his team (2018) noted that in obesity women Significantly higher concentrations of glucose ,this don't agree with our results, were observed in subjects with (metabolic syndrome) MS compared with women without MS.

Perhaps the reason for our difference in results from other researchers is due to the difference in the patient's age, difference in the duration of the infection, number of hours of fasting, difference in average body mass, difference in the number of samples.

4.6 Lipid profile and Body Mass Index (BMI)

The values of cholesterol decreased significantly ($P \leq 0.05$) in HPE .While , the values of TG increased significantly ($P \leq 0.05$) in the T1D .But, the values of LDL did not differ significantly ($P > 0.05$) . The values of HDL decreased significantly ($P \leq 0.05$) in the HPE and obesity groups . While , the values of VLDL increased significantly ($P \leq 0.05$) in the

T1D group (Figures 3-12,3-13,3-14,3-15,3-16). The values of BMI in the obesity and HPE group increased significantly ($P \leq 0.05$) in comparison with control groups (Figure 3-17).

These results may be attributed to the effect of the difference in body mass index, the effect of PRL elevation/or the variance in the volume and distribution of fat mass/or the absence or deficiency of insulin in different groups.

Krysiak and his team (2023) noted the hyperPRLemic women differed from normoPRLemic ones in (HDL)-cholesterol and TG. Cabergoline decreased total and monomeric PRL levels, which was accompanied by normalization of HDL-cholesterol, TG whereas a did not effect on PRL levels, combined contraceptives increased TG.

The HPE might have adverse effects on lipid , they tested dopamine agonist treatment with cabergoline has significant effects on blood lipids, in patients with micro- or macroPRLoma. After a median follow up of 9 months, PRL levels decreased. There was a significant decrease in median levels of LDL and total cholesterol , but no change in HDL and TGs . In patients with PRLomas, normal isation of elevated PRL levels by cabergoline treatment was accompanied by significant reductions in LDL and total cholesterol. So, that support the notion that treatment of HPE may have beneficial effects on lipid metabolism (Schwetz *et al*.,2017).

In a study on Infertile group with hyper PRL , they found the total cholesterol, TG, VLDL, and LDL increased significantly compared to the

control group and don't agree with our results, while HDL decreased significantly compared to the control and that agree with our results (Al-Ttaie and Aljawadi ,2021).

Patients with PRLoma had significantly higher BMI than controls; fat mass did not differ between groups, and this don't agree with our results . Only men - but not women - with PRLoma had significantly higher fat mass compared to controls. Levels of LDL was significantly higher, levels of HDL significantly lower than in controls. After a median of 10 weeks after initiation of cabergoline, total and LDL cholesterol had significantly decreased. Analyzing results from men and women separately, this change occurred in men only (Posawetz *et al.*, 2021).

Elevated PRL effects on the lipid metabolism, Bernabeu and Casanueva (2013) found that elevated PRL increased LDL cholesterol and TG levels and this don't agree with our results , while decreased HDL levels and this agree with our results. Pala and his team (2015)showed as improved metabolic profile (reduction in LDL, TGs, body mass index (BMI), and WC) in patients with PRLomas treated with cabergoline (Cabergoline is a drug that stimulates dopamine receptors and reduces the production of the hormone PRL) for six months.

Deusdará and his team (2022) found that the fasting insulin equivalent (FIeqs) for obesity were higher than those for overweight, with early adolescence showing elevated WC for both TG/HDL and TG/glucose (TyG). In teenagers aged 15 to 17 years, the FIeqs for elevated WC were higher than those for obesity and overweight.

In comparison to older adolescents, adolescents between the ages of 12 and 14 were found to have greater relationships between obesity and TG/HDL and TyG. However, WC demonstrated higher relationships with TG/HDL and TyG in adolescents aged 15 to 17 years than obesity did (Deusdará *et al* .,2022).

Azam and his team (2023) study the 3911 patient of DM included, the study recorded an obesity prevalence of 32.9%. The female , age 15-44 years , urban residence , history of hypertension , high diastolic blood pressure , high LDL , and high TGs were the risk factor of obesity among DM subjects; while high HDL higher education and married were protective factors of obesity among DM subjects.

In obesity women observed significantly higher concentrations of LDL, but lower concentrations of HDL and this don't agree with our result, in weman with metabolic syndrome (MS) compared with women without MS (Fatani et al ., 2018) Total Cholesterol, LDL, HDL and TG did no significant difference between overweight, obesity and normal weight and this don't agree with our results (Mancini *et al.,* 2021).

The effect of BMI influenced by blood pressure was significantly higher among Chinese individuals with overweight and obesity (Huang *et al*.,2023). Calcaterra and his team (2021)found that higher values of BMI, WC and WC/H were detected in patients with obesity compared to control and T1DM ,and agree with our results .

The diabetic patients are more prone to develop hyperlipidemia , where was the pattern of lipid abnormalities observed was high TG in

(31%) of patients, high LDL in (19%), low HDL in (11%), high cholesterol (14%) and combined hyperlipidemia (25%) diabetic patients (Uttra *et al*., 2011). High BMI in otherwise healthy adolescents is associated with increased risk for incident T1D in early adulthood (Zucker *et al*.,2022).

In study done by Alkaabi and his team (2022) found that 50% of the T1DM patients were overweight but none were obese. According to the lipid profile, HDL, ApoA, TG, TC , and ApoB levels were significantly higher in patients with T1DM than in controls and this agree with our results except the TC. LDL levels did not differ between the two groups and this agree with our results.

Valente and his team (2021) found that no significant differences between the groups for gender distribution and lipid profile . In the T1DM group short-term glycemic variability (STGV) showed positive correlations with serum total cholesterol , and TG , while they recording an inverse correlation with age . long-term glycemic variability (LTGV) showed a positive correlation with STGV and serum TGs .

Abdoun and his team (2022) observed the hypertension and dyslipidemia were seen in 10% and 31% of children with T1DM respectively. The elevated LDL and BMI were significantly higher in female patients than in males. Besides, there were significant associations between high blood pressure and BMI and elevated LDL levels. Mahmoud Alakkad and his associates (2020) observed high significant in the frequency of dyslipidemia in children and adolescents group with

T1DM compared to of the healthy group .Where , showed that dyslipidemia in children and adolescents with

Otaibi and his team (2017)found that all lipid profiles throughout the five-year follow-up research showed a considerable rise. When compared to the baseline risk ratio (0.8%), the prevalence of the risk TC/HDL ratio increased during the course of the previous year by 52.1%. Average T1D patients had an optimal ratio in the first year of measurement, according to a research comparing the TC/HDL ratio with BMI categories. The frequency of the optimum ratio decreased across all categories at the same pace after a five-year follow-up analysis. In the final year of the trial, risk TC/HDL ratio assessments revealed that the average group had more readings than in the first year.

Kang and his team (2021) observed the total cholesterol, LDL, and TG were decreased in young man with type 1 diabetic patients compared to controls , and this don't agree with our results . The Adolescent patients, which have T1DM, have abnormal lipid profile level and abnormal lipoprotein building. Then after grown up glycemic control is a significant become abnormal. the cholesterol, TG, HDL, LDL was increase in younger patients with T1DM and decrease in VLDL and this don't agree with our results except the TG ,HDL (Ghio *et al*.,2011; Mitrovi *et al*., 2014; Alsultani *et al*.,2022).

Alakkad and his associates (2020) observed high significant in the frequency of dyslipidemia in children and adolescents group with T1DM was significantly more frequent than in control subjects especially among

diabetic females. The most frequent type of dyslipidemia with T1DM was high TG,TC and LDL , while HDL less the classic diabetic dyslipidemia in the form of hyperTGmia was much less frequent and this don't agree with our results except the TG.

Arıkan and Sagsoz (2023) found that the BMI in obese women did not a significant difference compared with controls and this don't agree with our results. Kang, and his team (2021) found that height, weight and BMI, were not significantly different between the young men with T1D and control , and this don't agree with our results.

Perhaps the reason for our difference in results from other researchers is due to the difference in the patient's age, difference in body mass index, difference in the number of samples, difference in the duration of the infection.

Chapter five

Conclusions and Recommendations

Conclusions

The results of the current study showed the following conclusions:

1- Kisspeptin was not an effective agent in the incidence of hyperprolactinemia, obesity, and type 1 diabetes mellitus.

2- The hyperprolactinemia, obesity, and type 1 diabetes mellitus groups showed a difference in fertility, as GnRH and progesterone increased in women with hyperprolactinemia, obesity and type 1 diabetes. The FSH and LH had normal levels in patients with hyperprolactinemia, obesity and type 1 diabetes . The increase in prolactin hormone was restricted to women with hyperprolactinemia. Elevated Estradiol in hyperprolactinemia and obese women while Elevated testosterone in obesity and type one diabetes women

3- Patients with hyperprolactinemia, obesity and type 1 diabetes have elevated levels of 8-isoprostane and this may be due to excessive oxidative stress.

4- Women with type 1 diabetes have elevated levels of HBA1C and glucose, while hyperprolactinemia and obesity levels are normal, and this reflects the functional impact of the diabetic.

5- Metabolic deterioration in groups where hyperprolactinemia patients had low cholesterol and HDL concentrations, obese patients had low HDL concentrations, and type 1 diabetic patients had elevated TG and VLDL levels

Recommendations

Further studies would be recommended and including the following :

1-Adopting the data of this study in developing programs for detecting and treating hyperprolactinemia, obesity, and T1DM.

2-A comparative study of Kisspeptin and GnRH levels before and after puberty, also before and after menopause.

3-A study of the kisspeptin hormone in males and its relationship with semen properties and fertility hormones.

4-A study of reproductive functional differences between patients with type 1 diabetes and healthy in the pre- and post-puberty age groups.

5-A study of reproductive functions among women with hyperprolactinemia , Primary infertility and second infertility.

6-A study of reproductive functions among obese, morbidly obese, and overweight in women of childbearing age.

7-A study of 8-isoprostane levels and its relationship to type 2 diabetes and obesity in men.

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Appendix

Appendix A - Questionnaire

Name mobile

Age/date births

Married ☐ pregnant ☐ children ☐

Is your period regular ? Yes ☐ No ☐

Do you have problems with the reproductive system ?

DO you have diabetes ?

NO ☐ TYPE ONE ☐ TYPE TWO ☐

History disease ----------

Type treatment----------

Hereditary----------

DO you have obesity? Yes ☐ No ☐

History disease ----------

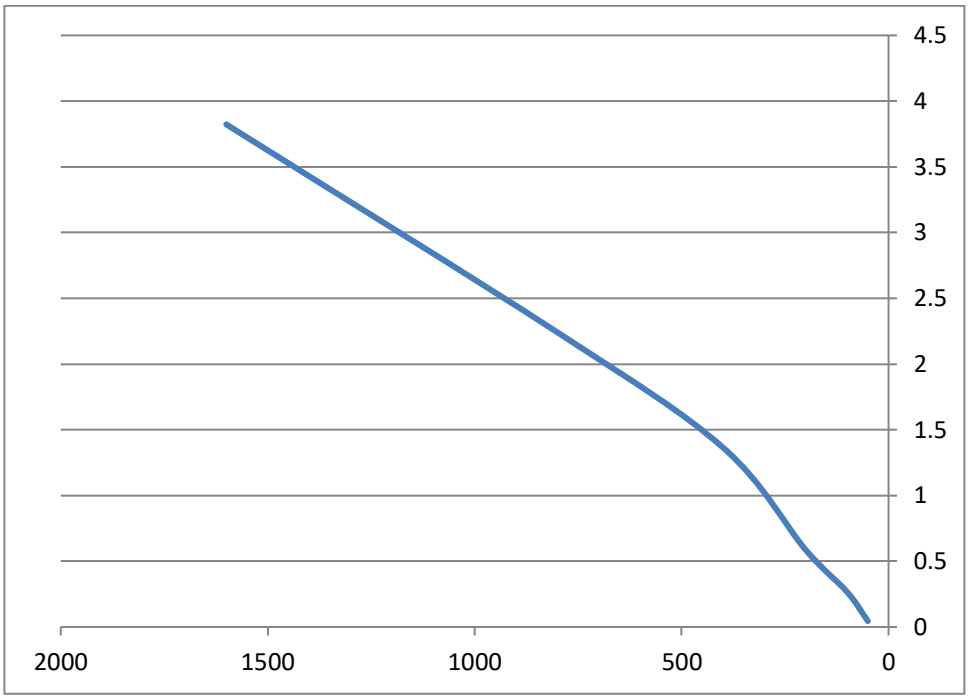
Hereditary----------

DO you use weight loss drugs?

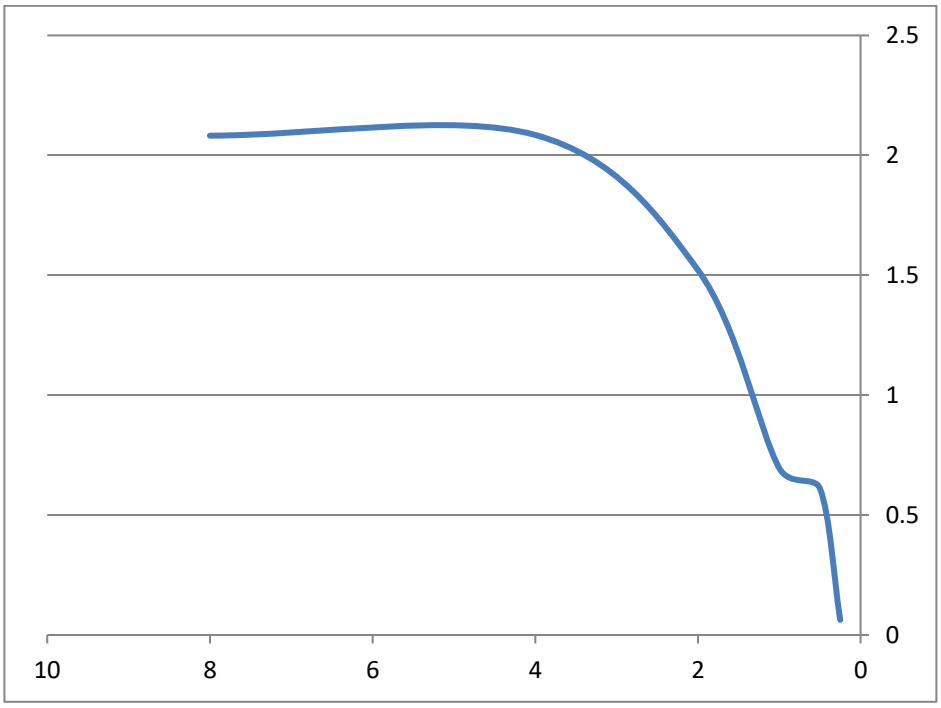
Are you on a diet ?

DO you suffer from other disease?

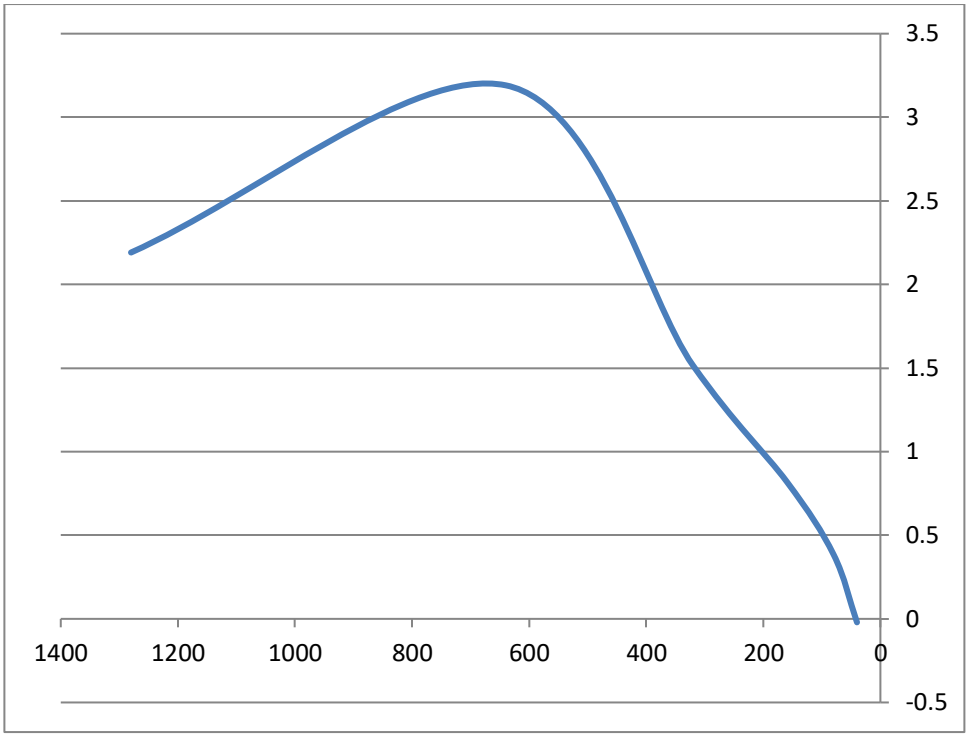
Do you take medication or supplements?



Appendix B - Kisspeptin measure of absorbance



Appendix C - GnRH measure of absorbance



Appendix D - 8-isoprostance measure of absorbance

Appendix E table shows the concertation of parameter in groups

Parameter	Control	Hyperprolactinemia	Obesity	T1DM
Kisspeptin	123.65±2.08 a	127.59±1.86 a	124.50±2.18 a	123.98±2.82 a
GnRH	0.60±0.01 b	0.69±0.02 a	0.66±0.01 a	0.67±0.01 a
FSH	6.63±0.34 a	6.49±0.34 a	6.25±0.36 a	6.55±0.36 a
LH	5.19±0.45 a	5.13±0.35 a	4.33±0.37 a	5.00±0.32 a
Prolactin	11.86±0.97 b	48.61±4.29 a	11.97±0.85 b	13.86±1.66 b
Estradiol	27.45±1.83 c	54.67±6.32 a	39.74±4.18 b	23.71±1.98 c
Progesterone	0.21±0.01 c	1.22±0.07 a	0.93±0.04 b	0.96±0.06 b
Testosterone	0.13 ±0.02 c	0.21±0.03 bc	0.36±0.03 a	0.22±0.03 b
8isoprostance	22.68±1.84 c	103.42±3.77 a	108.69±4.87 a	64.15±5.34 b
HBA1C	4.9±0.11 b	5.28±0.14 b	5.07±0.11 b	9.8±0.54 a
Glucose	96.54 ±2.63 b	94.48±2.78 b	98.11±5.55 b	289.28±24.47 b
cholesterol	151.19±5.25 ab	144.87±7.19 b	170.37±5.79 a	171±11.13 A
Triglyceride	94.20±6.43 b	105.83±9.36 b	121.96±8.48 ab	137.35±15.32 a
HDL	43.92±2.58	35.57±2.00	34.41±1.21	39.18±2.23

	a	b	b	ab
LDL	98.91±4.20 a	103.48±8.68 a	104.92±4.62 a	109.73±9.44 a
VLDL	18.83±1.28 b	23.35±2.56 ab	24.39±1.69 ab	27.46±3.06 a
BMI	23.88±0.17 c	28.27±1.12 b	36.93±0.99 a	25.55±0.88 bc

ملخص

هدفت الدراسة الحالية إلى معرفة الحالة الصحية والإنجابية لدى النساء من خلال تقدير مستويات الكيسبتين وبعض المؤشرات الهرمونية والكيميائية الحيوية لدى النساء المصابات بفرط هرمون البرولاكتين والسمنة ومرض السكري من النوع الأول في محافظة ميسان، والتي أجريت في كل من محافظة ميسان. -مستشفى الصدر التعليمي ومستشفى ميسان للأطفال والولادة ومركز السكري والغدد الصماء ومختبر ابن الهيثم خلال الفترة من حزيران 2022 الى شباط 2023. العينة الكاملة هي 92 امرأة (اعمار 20 - 40 سنة) مقسمة الى اربعة رئيسية المجموعات (23 امرأة / مجموعة) على النحو التالي: المجموعة الضابطة: النساء الأصحاء مع دورات شهرية منتظمة دون أي اضطرابات هرمونية، مجموعة فرط برولاكتين الدم: النساء مع فرط البرولاكتين في الدم، مجموعة السمنة: النساء لديهن مؤشر كتلة الجسم أكثر من 30 كجم / م ^ 2 و مجموعة مرضى السكري: النساء المصابات بـ السكري النوع الاول.

أظهرت النتائج الحالية أن قيم الكيسبتين لم تختلف معنويا ($P>0.05$) بين جميع المجموعات الأربع. كما زادت قيم GnRH في مجموعات فرط برولاكتين الدم والسمنة والسكري النوع الاول معنويا ($P<0.05$) مقارنة مع مجموعة السيطرة.

ولم تختلف قيم FSH وLH معنويا ($P>0.05$) بين المجموعة الأربع. ارتفعت قيم البرولاكتين في مجموعة فرط برولاكتين الدم معنويا ($P<0.05$) مقارنة بمجموعات السكري النوع الاول والسمنة والسيطرة.

ارتفعت قيمة الاستراديول في مجموعة فرط برولاكتين الدم والسمنة بشكل معنوي ($P<0.05$) مقارنة مع مجموعة السكري النوع الاول والمجموعة الضابطة. ارتفعت قيمة هرمون البروجسترون في مجموعات فرط برولاكتين الدم والسمنة والسكري النوع الاول مقارنة مع مجموعات السكري النوع الاول والسمنة والسيطرة. كما زادت قيمة هرمون التستوستيرون في مجموعة السمنة والسكري النوع الاول معنويا ($P<0.05$) مقارنة مع السكري النوع الاول. فرط برولاكتين الدم والمجموعات الضابطة.

ارتفعت قيم 8-isoprotance في مجموعات السمنة وفرط برولاكتين الدم معنويا ($P<0.05$) مقارنة مع السكري النوع الاول ومجموعات السيطرة.

ارتفعت قيم HBA1C والجلوكوز الصائم في مجموعة السكري النوع الاول معنويا ($P<0.05$) مقارنة مع مجموعة السمنة وفرط برولاكتين الدم ومجموعة السيطرة.

انخفضت قيم الكولسترول في مجموعة فرط بروﻻكتين الدم بشكل ملحوظ ($P<0.05$) مقارنة مع مجموعة السكري النوع الاول ومجموعة السمنة. ارتفعت قيم الدهون الثلاثية (TG) في مجموعة السكري النوع الاول بشكل معنوي ($P<0.05$) مقارنة بمجموعتي فرط بروﻻكتين الدم والمجموعة الضابطة. كما انخفضت قيم البروتين الدهني عالي الكثافة (HDL) في مجموعة فرط بروﻻكتين الدم والسمنة بشكل ملحوظ ($P<0.05$) في مجموعة السكري النوع الاول. المقارنة مع مجموعة السيطرة .لم تختلف قيم البروتين الدهني منخفض الكثافة (LDL) معنويا ($P>0.05$) في السيطرة وفرط بروﻻكتين الدم والسمنة ومجموعات السكري النوع الاول. ارتفعت قيم البروتين الدهني منخفض الكثافة جداً (VLDL) في مجموعة السكري النوع الاول معنوياً ($P<0.05$) مقارنة بمجموعة السيطرة.

تمت مناقشة التأثير الفسيولوجي للنتائج وفقًا لتأثير ارتفاع مستوى البروﻻكتين وزيادة تركيز الجلوكوز في الدم وكتلة الدهون في مجموعات فرط بروﻻكتين الدم والسمنة و السكري النوع الاول

لم يكن كيسبيبتين عاملاً فعالاً، فقد اختلفت الخصوبة والإجهاد التأكسدي المفرط وتدهور التمثيل الغذائي في فرط بروﻻكتين الدم والسمنة والسكري النوع الاول.

وزارة التعليم العالي

والبحث العلمي

جامعة ميسان

كلية العلوم

قسم علوم الحياة

تقدير مستويات الكيسبيبتين , الهرمونات التكاثرية و المعايير الكيميائية الحيوية في النساء المصابات بفرط هرمون بروﻻكتين الدم والسمنة والسكري من النوع الاول في محافظة ميسان

رسالة مقدمة الى

مجلس كلية العلوم / جامعة ميسان

وهي جزء من متطلبات نيل درجة الماجستير علوم في علوم الحياة

من قبل

نقاء قاسم غالي

بكالوريوس علوم / علوم حياة 2018

بإشراف

أ . د . زينب عبد الجبار رضا العلي

ا.م .د. صبا جاسم الهاشمي

اب ٢٠٢٣ م

صفر 1445 هـ