Ministry of Higher Education &

Scientific Research

University of Sulaimani

College of Medicine

Evaluation of Serum Kisspeptin Levels in Obese and Non-obese Patients with polycystic Ovarian Syndrome in Kirkuk Governorate (A Case-Control Study)

A Thesis Submitted to the Council of College of Medicine - University of Sulaimani in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Medical Physiology

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2020 A.D 2719 K 1441 H

Supervisor Certification

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Examining Committee Certification

We, the examining committee, certify that we have read this thesis entitled (Evaluation of Serum Kisspeptin Levels in Obese and Non-obese Patients with polycystic Ovarian Syndrome in Kirkuk Governorate (A Case-Control Study)) and have examined the student (Razaw Omar Ibrahim) in its context and in our opinion it is adequate as a thesis for the degree of Doctor of Philosophy in Medical Physiology

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Dedication

To…

My Mother and Father with Deepest Respect…

Who Providing Me, the Support, Encouragement and the Power to Continue My Postgraduate Study with My Warmest Love and Appreciation…

My Husband with Genuine Thanks…

My Children Varo & Kurdo

All My Teachers and Friends with Deepest Appreciation…

Razaw

Acknowledgements

Praise is to our almighty Allah gracious for enabling me finish what I started and for helping me to present this work.

I would like to express my heartfelt gratitude and appreciation to my honorable supervisors Assist Professor Dr.Shirwam H. Omer (Chairman of Department of physiology, College of Medicine-University of Sulaimani) and Assist Professor Dr. Chro N. Fattah (Department of Obstetrics and Gynecology, College of medicine-University of Sulaimani) for their guidance, valuable advice, discussions and suggestions throughout my study.

My genuine thanks to Assistant Professor Dr. Kosar Mohamed Ali the Dean of the College of Medicine-University of Sulaimani for his support.

I am deeply indebted to the doctors and staff of Azady Teaching Hospital, for their continuous cooperation. Specially Dr.Tara Hameed Khorsheed. And my special thanks to the Scientific Research Labrotary Department at Kirkuk Medical College; where the samples separated, frozen, and stored.

The author would like to acknowledge the staff members of MediaMed Lab in the Kirkuk city, especially their Lab Manager Assist Professor Dr.Sallih Baban for his help and advice

I express my gratitude to all the staff members of Al.Manarah Lab in the Kirkuk city; especially, the Lab Manager Dr.Ahmad Matar for his continuous help .

I am indebted to the family members, friends, and the hospital personnel and their relatives for volunteering in this study, without them, the study could not have been completed.

Lastly, I would like to express all my appreciation and grateful feel to my family for their patient kindness and support throughout my entire life and to all kind, helpful, and lovely people who helped me directly or indirectly to complete this work.

Abstruct

Background

Kisspeptin is a neuropeptide that up-regulates Gonadotropin-releasing hormone (GnRH) secretion. It is an essential element for the Luteinizing hormone (LH) surge and ovulation. This neuropeptide is essential at the onset of puberty and maintenance of normal reproductive function .Women with polycystic ovary syndrome (PCOS) expose alteration in both GnRH & LH secretion.

Objective

This study aimed to evaluate serum kisspeptin levels in healthy and polycystic ovarian syndrome women. Furthermore, it investigates the effect of obesity and age on circulation kisspeptin level in both normal and PCOS women. Moreover, it points out the correlation between kisspeptin and other hormonal parameters. Aim of this study also include comparing serum Sex Hormone Binding Globulin (SHBG) levels between PCOS & healthy women .In addition to that, to provide information about the effect of age on serum SHBG levels in both groups of the study .

Study designe

This case –control study involved one hundred and twenty women. (60 were subfertile with PCOS, 20 were subfertile with high FSH and 40 were normal) were enrolled in the study. All subjects were examined clinically and biochemically. Five ml samples of blood from all the patients and control women were obtained twice during the same menstrual cycle (follicular and preovulatory) except (sufertile patients with high FSH) were blood obtained only once. Serum levels of kisspeptin, SHBG, free testosterone, estradiol, FSH, LH & Antimullerian Hormone were measured by using ELISA technique. The studied women

were divided in to subgroups according to their ages & the women with PCOS were sub divided in to four subgroups according to their clinical & biochemical markers. The values presented as (mean ± SD) and the Kolmogorov-Smirov test was used to test the normality of distribution. A student t-test was used to compare the two group's means. One-way analysis of Variance (ANOVA) was performed to estimate the differences between the groups. Then, Tukey's post-hoc test was used to evaluate the relationship between the two groups .

Results

Kisspeptin levels were higher in PCOS patients than that in the normal group. Kisspeptin correlated with serum free- testosterone level (r =0 .26) (P<0.05). In healthy women, preovulatory kisspeptin levels were higher than follicular kisspeptin level (P<0.05); while this difference was insignificant in PCOS patients. The variation in serum kisspeptin levels between overweight and normal-weight women in both groups was insignificant. In normal women, serum kisspeptin levels were higher in women (> 35 years) than those (< 24 years) with (P = 0.03). Kisspeptin serum level in subfertile women with high FSH was higher than healthy women. There was no statistically significant difference in follicular phase serum level of SHBG between subfertile PCOS & control women. Its levels were significantly lower in women with BMI ≥25 than normal weight women in both groups of the study. Serum level of SHBG in normal weight (BMI <25) subfertile PCOS women were significantly lower than normal weight healthy women p=0.009. In control group weak negative correlation between age &SHBG observed r=-0.33 while, this correlation was insignificant in subfertile PCOS women. No correlation between SHBG & other hormonal parameters recorded. Also, insignificant difference in SHBG level among PCOS subgroups found F=2.061,p=0.116 .

Conclusions

Kisspeptin is higher in subfertile PCOS women. Kisspeptin involved in feedback mechanisms, a disturbance in reproductive axis could disturb kisspeptin normal rhythm. Although kisspeptin not correlate with serum LH level, it correlates with testosterone level which is a common feature of PCOS. Similar to gonadotrophic hormones kisspeptin serum level increase with advancing age. Both obesity & PCOS affect serum level of SHBG but the effect of obesity is more potent. Serum levels of SHBG decline with age.

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List of Abbreviations and Acronyms

AGE	Advanced Glycation End Product
ANOVA	Analysis of variance
AVPA	Antero ventral preventicular Area
AMH	Antimullerian Hormone
AFC	Antral Follicular count
ARC	Arcuate nucleus
BMI	Body Max Index
BMP	Bone morphogenetic protein
CYP	Cytochrome P
DAG	Diacyl glycerol
DYN	Dynorphin
ELISA	Enzyme Linked immunosorbant assay
ERβ	Estrogen B receptor
ERα	Estrogen α receptor
FSH	Follicle stimulating Hormone
FOXO protein	Fork head box protein
FIGLA	Germ cell specific basic helix-loop helix transcription Factor
GnRH	Gonadotropin-releasing hormone
GPr	G-protein coupled receptor
GDFs gene	Growth Differentiation Factor gene
iHH	Hypogonadotropic Hypogonadism
HPG axis	Hypothalamic-pituitary-gonadal axis
IGF	Insulin Like Growth Factor
IRS-1	Insulin Receptor Substrate
Jak-sTaT	Janus Kinas-signal transducer and activator
KD	Kilo Dalton

P234	Kiss, antagonist
KP	Kisspeptin
KNDY	Kisspeptin Neurokinin and dynorphin
LH	Luteinizing Hormone
MAPKS	Mitogen-activated protein kinase
NKB	Neurokinin B
Ob	Obesity gene
PTEN	Phosphatase & tensin homolog
PIP3	Phosphatidylinositol (3,4,5) triphosphate
PIP2	Phosphatidylinositol (4,5) biphosphate
PCOS	Poly cystic Ovarian Syndrome
POA	Pre optic are
POA	Pre optic area
AKt	Protein Kinase B
PKC	Protein Kinase C
SNPS	Single nucleotide poly morphism
SHBG	Sex Hormone Binding Globulin
STAR	Sterogenic acute regulatory protein
NK3R	Tachykinin neurokinin 3 receptor
TAG 3	Tachykinin receptor

Chapter One

Introduction & review of litreture

1.1 Introduction:-

Pulsatile gonadotropin-releasing hormone (GnRH) release from the hypothalamus captains pituitary secretion of LH & FSH, which in turn regulates the production of gonadal sex steroids and gametes (ova and sperm). GnRH emission is markedly affected by a complex cluster of higher-level afferent inputs, such as neurons secreting kisspeptin, neurokinin B, and dynorphin. Feedback signals communicate the status of gonadal function and other aspects of whole-body homeostasis to the neural frame works controlling GnRH release and to pituitary gonadotropes, rendering a facilitated and firmly regulated feedback system that keeps up fitting of gonadal work (Plant, 2019).

Kisspeptin is a neuropeptide that up-regulates Gonadotropin-releasing hormone (GnRH) secretion. Besides, it is a fundamental component for Luteinizing hormone (LH) surge and ovulation. In a normal monthly reproductive cycle, there is an introductory rise in follicle-stimulating hormone (FSH) level that causes progressive follicular maturation. Before the follicles develope, they begin to release estrogen that may stifle the kisspeptin level at this stage. This negative feedback may alter to positive feedback during estrogen further raise and LH surge (Nejad et al .,2917). Hence, kisspeptin is an essential element in creating LH surge and ovulation. Furthermore, its expression increments just before ovulation and LH surge (Trevisan et al.,2018). It is critical to know that GnRH secreting neurons do not express estrogen receptor; while kisspeptin neuron has this receptor (Smith et al., 2006, Haung, 1993, Clarkson et al., 2009). It is well acknowledged that kisspeptin directly stimulates

GnRH secretion via Kiss1-derived peptide recepter (GPR54) expressed in GnRH neurons in rodents. Indeed, the targeted deletion of GPR54 in GnRH neurons of mice resulted in infertility, though the reintroduction of GPR54 to GnRH neurons in GPR54-null mice resulted in fertility (Uenoyama et al., 2019). The most common cause of an ovulatory subfertility is polycystic ovarian syndrome. Women with this syndrome also suffer from menstrual irregularity, increase hair growth, acne and are most commonly overweight, but it also frequently seen in normal-weight women (Dennett & Simon 2015). Alteration in GnRH secretion is a feature of PCOS (Chaudhari et al., 2018) .GnRH expresses two types of pulse generation, slow and fast for stimulation of FSH and LH respectively (Tsutsumi &Webster 2009). In PCOS women LH is commonly increased, FSH is typically in the lower range, this may be related to decrease the sensitivity of GnRH pulse generator to steroid feedbacks and enhance LH secretion (Mccartney & Marshel 2016). Kisspeptin levels are higher within PCOS populace, which underpins the speculation that an over-active KISS1 system causes HPG-axis over activity, driving to unpredictible menstrual cycles and excessive androgen release (Tang et al., 2019) .

At late reproductive age the kisspeptinogeneic cells in the infundibular nucleus hypertrophied. Moreover, dynorphin secreting mRNA neurons is decreased (Rance et al 2009, Rometo & Rance, 2007) that both may contribute in hoisting serum kisspeptin level. Increasing percentages of KP-expressing NKB perikarya, NKB axons, and NKB inputs to GnRH neurons raise the captivating possibility that a significant subset of NKB neurons begins to cosynthesize KP as aging progresses (Hrabovszky et al., 2019).

The role of kisspeptin in conveying metabolic signals to brain centers responsible for reproduction control had been under the spotlight. Lacking kisspeptin signaling in female mice had showed to cause increase body weight, adiposity & glucose tolerance. In women,

a positive relationship between BMI & KISS 1 was found in omental adipose tissue (Tolson et al 2014 & Nyagolova et al 2016).

SHBG is a glycoprotein that synthesis by liver cells, it binds to sex steroid hormones & regulate their bioavailability. Recently reverse correlation between SHBG & obesity, PCOS and insulin resistance has recorded. Eighty percent of circulating testosterone is bound to SHBG, 19% is bound to albumin only 1% is free in the circulation. SHBG is influenced by androgen /estrogen balance, nutritional status, BMI, sex & Insulin resistance (Nelson et al 2001 & Muka et al., 2017).

1.2 Aims of the Study

1-To evaluate serum kisspeptin levels in healthy and polycystic ovarian syndrome women.

2-To investigate the effect of obesity and age on circulation kisspeptin level in both normal and PCOS women.

3-To determine the correlation between kisspeptin and other hormonal parameters.

4-To evaluate serum kisspeptin level among PCOS phenotypes

5-To compare serum kisspeptin levels between infertile PCOS & infertile women with elevated FSH serum level.

6- To compare serum SHBG levels & other parameters between PCOS & healthy women.

7-To determine the effect of BMI on SHBG level

8-To find the correlation between SHBG & other hormonal parameters. In addition to that, to investigate the effect of age on serum SHBG levels in both studied groups.

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Review of the literature:-

1.3 Neuro –endocrine regulation of the reproduction:-

Reproduction is regulated by an intricate interplay of neural and hormonal adverting that converges on hypothalamic neurons that achieve the pulsatile secretion of gonadotropin-releasing hormone (GnRH) (Marques et al., 2018) .

Fertility is started at puberty by the pulsatile secretion of gonadotropin releasing hormone from several neurons in the hypothalamus, which in turn is released into hypophyseal portal blood system from nerve terminals in the palisade layer of the median eminence of the hypothalamus. The GnRH, in turn, acts on the anterior pituitary gland to promote the secretion of FSH &LH hormone (gonadotropic hormones) that stimulates gonads for the secretion of steroid hormones, which is crucial for reproduction (Constantin 2017).

Adequate pulsatile secretion of GnRH is essential for proper attainment and maintenance of productive function, the synchronized release of GnRH bursts is the result of the integral function of the so-called GnRH pulse generator and hypothalamic network (Knobil et al., 1980). Recent shreds of evidence proposed that GnRH secretory patterns are not exclusivly dictated by the intrinsic activity of GnRH neurons, that is located in the preoptic area (POA) in the hypothalamus , but needs the commitment of other hypothalamic afferents (Maeda et al., 2010 , Terasawa et al., 2010).

There are many central and peripheral signals that effect and target GnRH neurons in the hypothalamus , in last decades numerous excitatory and inhibitory inputs to GnRH axis have been discovered, for instance, excitatory amino acids, noradrenalin, neuropeptide (NPY), gamma-aminobutyric acid(GABA) and opoid peptides (Ojeda and skinner , 2005). GnRH neurons affect by several internal & external environmental signals, it integrates signals from sex steroid, stress, glucocorticoid, prolactin, nutritional & metabolic status & other peptides to control gonadotropin emission & hence gonadal

capacity (Marques et al 2018). Recently it had been discovered that there are hypothalamic networks that control GnRH secretion & steroid feedbacks, the neuropeptides that involve in this network include (kisspeptin, Neurokinin & Dynorphin) (KNDy). Neuron –neuron & neuron-glia communication via gap junctions contribute to the synchronized activities among KNDy neurons (Ikegami et al 2017). Neurokinin is a member of the tachykinin family of peptides it stimulates kisspeptin neurons, which lead to GnRH secretion (Navaro et al 2015). While, Dynorphin inhibit kisspeptin secretion (Goodman et al 2004). In this way these neuropeptides coordinate pulsatile GnRH secretion (Navaro et al 2009). Figure (1.1).

Reproductive function is gratly sensitive to the energy status, signaled via the adipose-tissue derived factor .And also to the peripheral hormones and metabolic signals (Casanueva & Deiguez, 1999, Tena-Semper & Huhtaniemi, 2002). Leptin is a 167 amino acid, which produced by obesity gene (Ob), is secreted from adipocyte to the blood stream, it regarded as a signal of energy status in the body that in turn act on the hypothalamus to decrease feeding & enhance energy expenditure (Evans & Anderson 2017).The Leptin receptor is located in the arcuate nucleus of the hypothalamus. It's a member of the interleukin -6 receptors. It works through activation of the janus kinase-signal transducer & activator of (Jak-STaT) system which affect the expression of hypothalamic neuropeptides (Sam & Dhillo 2010).There is no leptin receptor in GnRH releasing neurons But , exogenous leptin administration increase plasma levels of LH , FSH & testerone in fasted mice (Barash et al 1996). Leptin indirectly regulate & affect GnRH secretion via hypothalamic kisspeptin neurons that express the leptin receptor (Smith et al 2006).

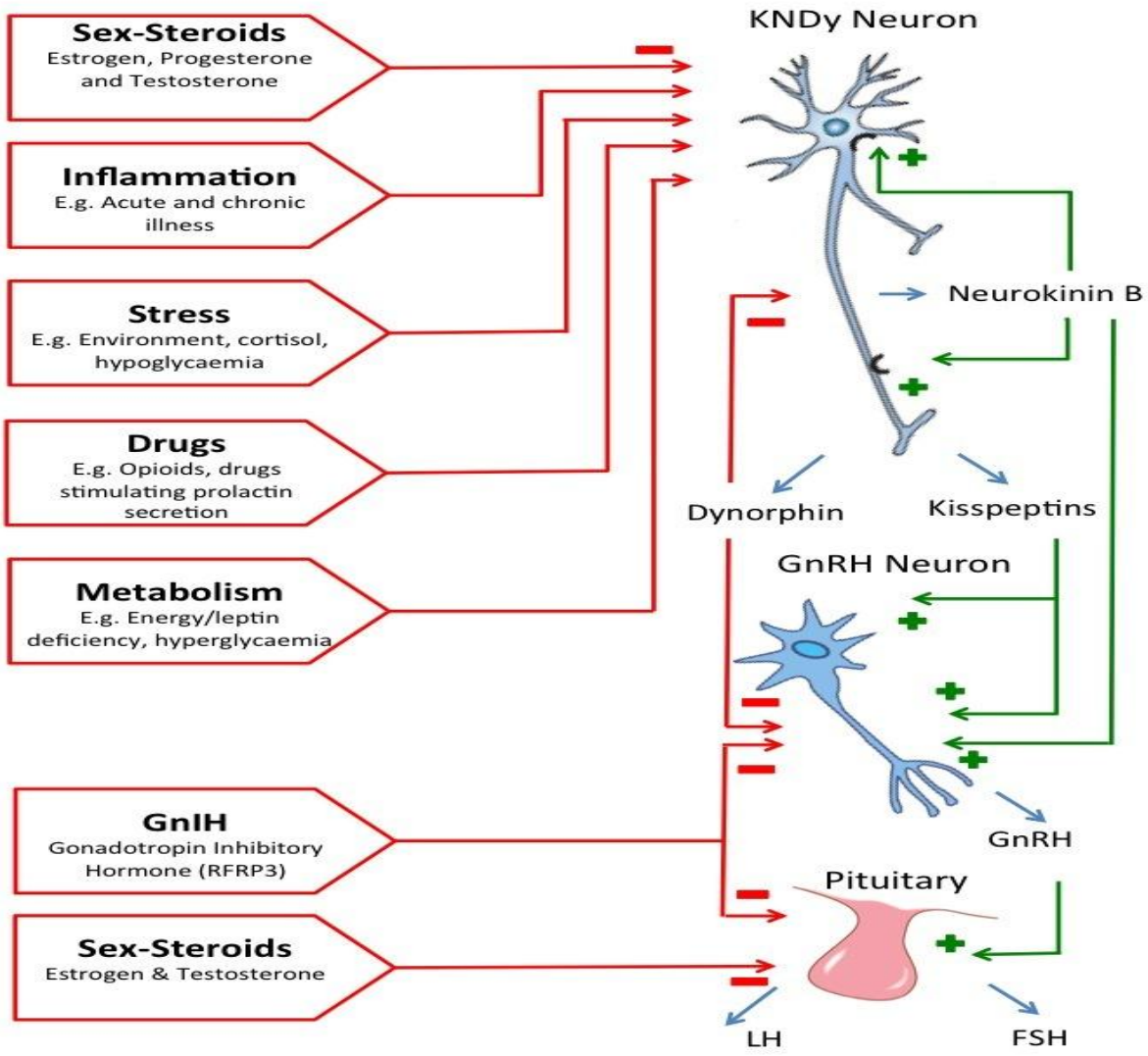


Fig (1.1) shows that KNDy cells are major target for steroid hormones that form a reciprocally interconnected network & have direct projection to GnRH cell bodies. (Marques et al 2018).

Insulin regulates glucose metabolism, energy balance, fat metabolism & sterogenesis. In vitro studies showed that insulin has-dose dependent effect on steroid synthesis, cell proliferation & gene expression for instance (STAR, (sterogenic acute regulatory protein), CPY11 A1 & CPY17A gene) (Munir et al 2004). In addition to that, it works centrally where its receptor exists in the arcuate, ventromedial hypothalamic nucleus & preoptic

area that certain its involvement in the regulation of reproduction (Plum et al 2005, Bruning et al 2000). Another lipokinen that participate in female reproduction is adiponectin, that derived from white adipose tissue & enrolled in central energy metabolism, increase insulin sensitization also it has anti-inflammatory, anti-atherogenic properties, and its receptor is expressed in the hypothalamus that regulates KISS, GnRH secretion (Barbe et al 2019). It found that adiponectin inhibits GnRH secretion beside the suppression of KISS1 mRNA transcription (Wen et al 2012, Wen et al 2008). Low level of adiponectin observed in obese (Arita et al 1999) & PCOS women (Saarela et al 2006, Carmina et al 2005, Toulis 2009). Energy metabolism & its related signals and hormones are tightly connected with female reproduction. Obesity & insulin resistance is considered a potent factor that may disturb sterogenesis & ovulation. Figure1. 2 .

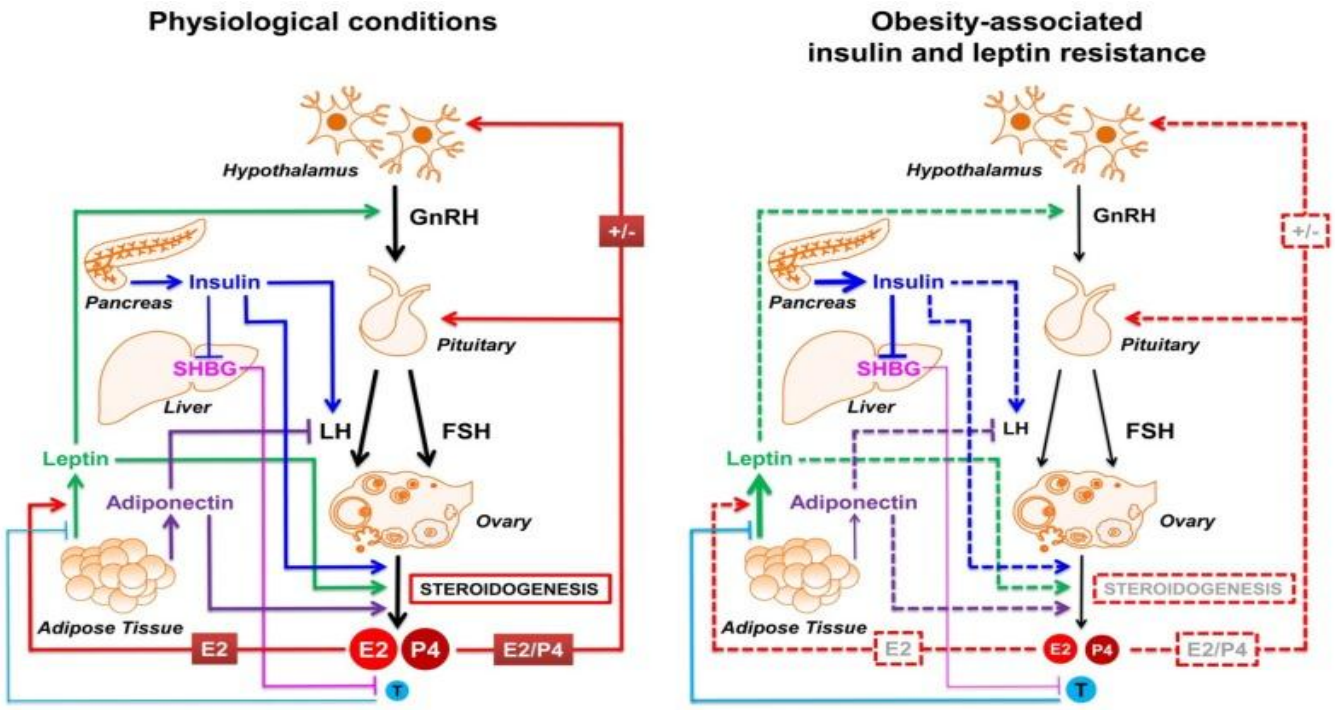


Figure1.2 shows that obesity & hormonal disturbance in obese patients negatively influence on sterogenesis (Frontana & Torre 2016).

1.4 Kisspeptin

1.4.1 The history of kisspeptin:

Kisspeptins role in the reproductive system discovered lately in 2003, before that period it was well known for its ability for suppressing tumor that's why its termed (Metastin). The First discovery of kisspeptin was in 1996 as a tumor suppressor in melanoma (Tanco et al., 2006). Four years later its receptor (GPR 54) was identified (De Anglemontde & De Tassing, 2010). In 2003 two isolated studies (De-Roux et al., 2003, Siminara et al., 2003) reported the presence of deletions & inactivating mutation of the GPR54 gene in Hypogonadotropic hypogonadism patients (iHH). These two studies were reinforced by two other studies in 2006, 2008 (Castelo et al., 2006, Votsi et al., 2008) they confirmed the key roles of kisspeptin in reproduction. As well as, these studies paved the way for further studies in different branches (Molecular Biology, Neuroanatomy, Physiology and pharmacology) for the analysis of physiologic relevance and mechanism of action of kisspeptin in reproduction. Table (1, 1)

Table 1, 1 illustrates the history of kisspeptin in reproductive field from 2005 to 2011 (Pinilla et al., 2012).

Year of the study	The discovery
2003	The mutation of GPR54 in mice & man are shown to cause IHH
2004	KISS1 mRNA was identified in (ARC) at hypothalamus. Kisspeptin are proved to cause stimulation of GnRH in Rodent GnRH neuron are shown to express Kiss mRNA receptor
2005	The of two different area in the hypothalamus that have kisspeptin neuron in Rodent

	KISS1 neuron in ARC are associated with negative feedback and, AVPV KISS1 neuron with positive feedback Kisspeptin are shown to stimulate gonadotropin secretion in men.
2006	Changes in KISS1 neuron at time of puberty causes increase in GPR54 signaling in rodent Increase in activity of kiss neuron at AVPV is reported during preovulatory surge. Kiss m RNA is shown to increase by leptin.
2007	Hormonal mechanism for sexual differentiation of population KISS 1 neuron in rat are first exposed in rat Kisspeptin are shown to cause GnRH stimulation in women
2008	Link between precocious puberty and kisspeptin was reported Crucial role of kisspeptin in negative & positive feedback are evaluated by functional genomice. Inactivating mutation of TAC3 are shown to cause HH in human
2009	For the first time kisspeptin antagonist, tested in different mammals sp. ARC neuron are shown to have fundamental in GnRH pulse generation
2010	By Using of kisspeptin antagonist the role of kisspeptin in puberty and preovulatory surge was documented. Mapping of KISS1 neuron in human hypothalamus at infandibulus and priventricular region.
2011	Most of the studies were on mouse models by elimination and selective ablation of KISS1 and GPR 54 expressing neuron.

1.4.2 Kisspeptin receptor:-

It has recently been documented that hypothalamic kisspeptin acts as stimulator of GnRH & mediates sex hormone feedback. Moreover, this neuropeptide is essential at the onset of puberty and maintenance of normal reproductive function (Nejad et al., 2017). The kiss 1 gene is localized to chromosome 1q32 & comprise of three exons, which only a portion of the second and third exons are finally translated into precursor 145 amino acid peptide, which is, cleaved in to three shapes of kisspeptin containing 54, 14, or 13 amino acids. The three peptides display the same likn for the GPR 54 receptor (Umayal et al., 2019b).

Kisspeptin is composed of several amidated peptides that are produced because of differential proteolytic processing derived from 145 amino acids encoded by KISS1 gene (Kotani et al., 2001). There are also, other peptide fragments of kisspeptin precursor like kisspeptin -14, kisspeptin-13, kisspeptin-10, that have the same COOH–terminal region of kisspeptin ,all the types of Kisspeptin act collectively via G protein coupled receptor GPR54 (Tena-Semper etal., 2006). GPR54 is a seven transmembrane domain, Gq/11-coupled receptor its stimulation leads to anincrease in intracellular ca2 level (Muir et al., 2001). The increase of ca ion is resulted from the activation of phospholipase c and hydrolysis of PIP3 to PIP3, which in turn moves the Ca ion from the intracellular store. Furthermore, the rise in PIP2 Leads to the formation of Diacyle glycerole (DAC) & Protein Kinase C (PKC) (Ringel et al., 2002). PKC is responsible for phosphorylation of mitogen-activated protein kinase (MAPKS) which has been also involved in the signaling cascade (Tena-Semper et al., 2006).

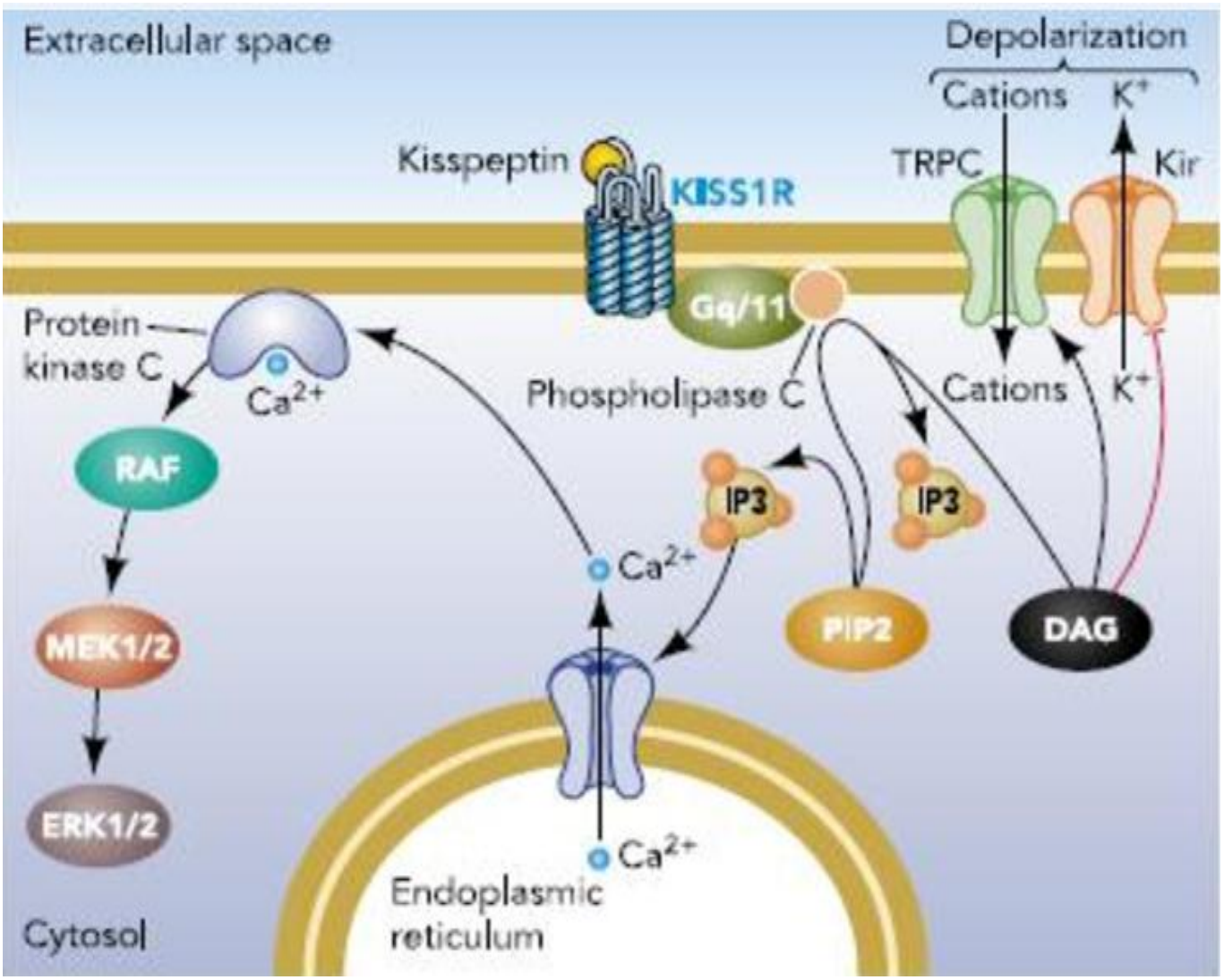
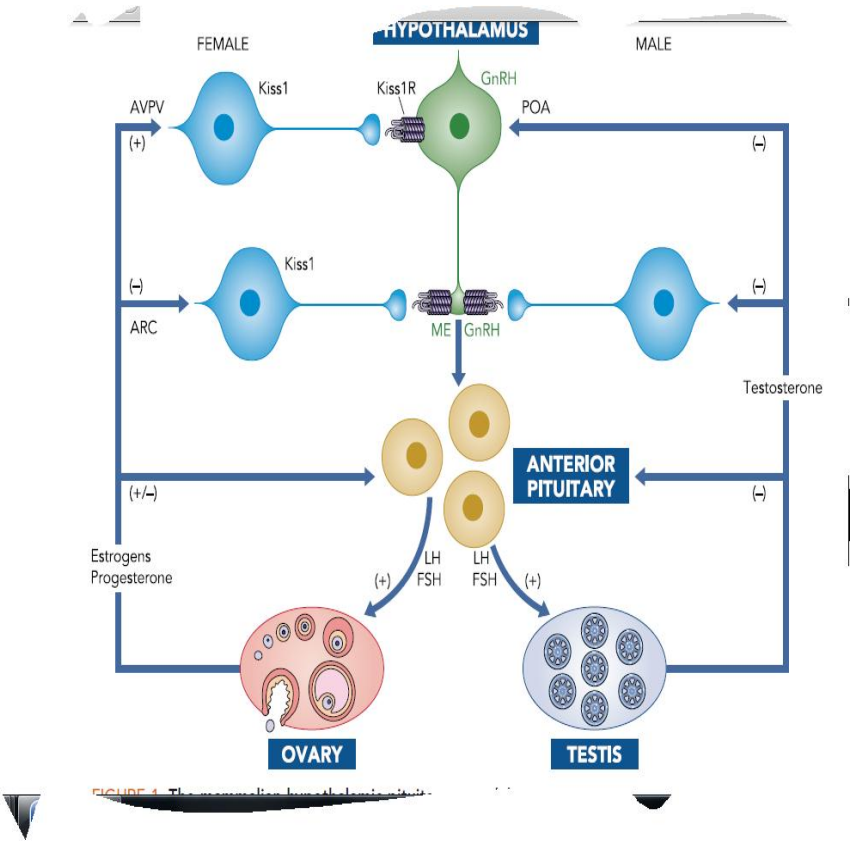


Figure 1. 3 The Cellular action of kisspeptin on GnRH neuron (Tassigny et al., 2010).

1.4.3 Distribution of kisspeptin:-

The location of kisspeptin vary according to species type in the human transcript the receptor's ligand (*KISS1* mRNA) has been recognizes in the placenta, testis, pancreas, liver, and small intestine (Kitada et al., 2002). Within the hypothalamus, KISS1 neurons are present transcendently in the infundibular nucleus (which is the homolog of the ARC in rodents and some other animals, including sheep) and in meager and indiscrete foci in the medial preoptic area (Mikkelsen et al., 2008). In Rodents, there are two major areas in the hypothalamus that contain kisspeptin neurons which is pre-optic area (the rostal one) and Arcuate nucleus (Caudal one), with proportinal more kisspeptin neurons in ARC region more than POA region (DeAngelmont and DeTassing, 2010, Kauffman et al., 2008).). kisspeptin fiber have been found in the preoptic area in close association with GnRH neurons cell bodies so, they are proposed that this association is responsible for modulating the preoulatoryvary GnRH surge in females (Gu et al.,1997). In an immunoreactive study in Monky, the kisspeptin fiber that is originated from ARC has a close opposition to GnRH axon in the median eminence for this reason they proposed to cause the pulsatile release of GnRH (Ramaswamy et al., 2008) fig 1.2.



Figure

1.4 the mammalian hypothalamic pituitary gonadal axis (Pinilla et al., 2012)

1.4.4 Kisspeptin & reproductive axis:-

As mentioned previously the kisspeptin neuron in the hypothalamus is located in two main area, the antero ventral preventricular (AVPA) and arcuate nuclei (ARC), both AVPA & ARC kiss1 population are differentially regulated by testosterone and estradiol (E2) (Kauffman et al., 2007, Han et al., 2006).

It has been shown that GnRH neurons do not express the steroid receptor for both negative and positive feedback regulation. While the kiss1 neuron express the sex steroid receptor. The positive feedback that causes LH surge and subsequently ovulation thought to be mediated by AVPV KISS1 neurons in female rats (Harter &Kavanagh 2018). On the

other hand, the expression of kiss1 in the ARC nucleus is inhibited by steroids, which gives a clue that these neurons have a role in negative feedback regulation of gonadotropin secretion (Nejad et al., 2017) Fig 1, 3. At the time of ovulation & LH surge, the Kiss1mRNA expression increased in the AVPA region but it decreased in ARC area (Smith etal, 2006).

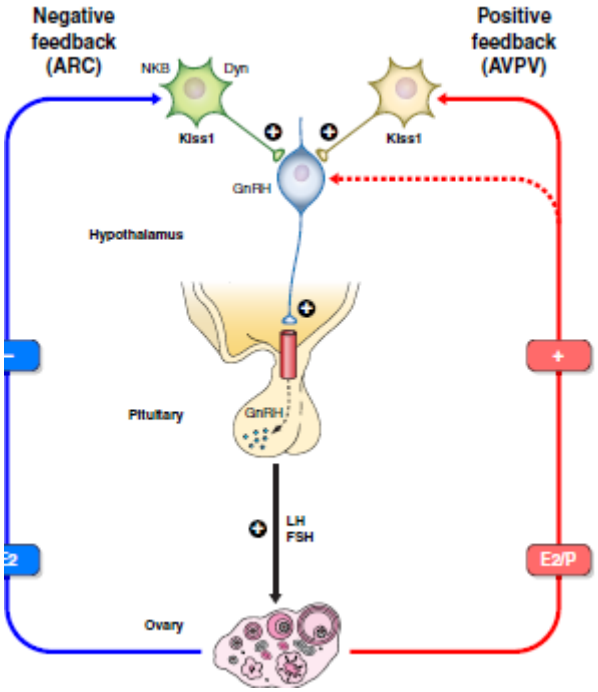


Fig.1.5 shows differential regulations and actions of ARC & AVPV Kiss1 neurons in the control of GnRH in Rodent (Pinilla et al., 2012).

In a normal mice Estradiol –negative feedback increases glutamergic transmission to ARC kisspeptin neurons & decrease it to AVPV neurons, while estradiol positive feedback had the antipode effect (Wang et al., 2018). Estradiol elevation exits the AVPV kisspeptin neurons by increasing the number of depolarization –induced bursts & rebound bursts (Wang et al., 2016). Knocked alpha estradiol receptor in the ARC kisspeptin neurons associated with cycle disruption, while knocked ERα in AVPV kisspeptin neurons

associated with normal cycle but blunted LH surge (Kauffman AS, 2009). In human both negative & positive kisspeptin feedbacks occur in the infundibular nucleus (Rometo et al., 2007).

Normally estrogen level increase abruptly just before ovulation to cause LH surge that triggers ovulation via positive feedback to GnRH neurons, but this neuron doesenot express the estrogen receptor (Huang & Harln, 1993) .While, kisspeptin neuron express this receptor (Clarkson et al., 2008). Furthermore, a recent study demonstrated that progesterone also involved in the positive feedback & LH surge (Mittelmam-smith et al 2017). Kisspeptin neurons express progesterone receptor (Gal et al., 2016) this evidences strongly praises the kisspeptin role in reproductive physiology. Kisspeptin neuronal cell bodies exist in ARC & AVPV area .Moreover, AVPV neurons project to ARC neuron & ARC neurons project to AVPA neurons (Harter e al., 2018). GnRH neuron terminals in the median eminence are exposed to projection from kisspeptin neurons forming anaxo-axonal method for controlling GnRH secretion (de Tassing et al., 2008).

Human studies demonstrated that serum level of kisspeptin like steroid hormones fluctuates during the menstrual cycle its level in the follicular phase is lower than the preovulatory phase suggesting that kisspeptin stimulate LH secretion & enhance ovulation (Latif & Rafique 2015, Zhai et al., 2017) .fig 1.6. In spite of these facts kisspeptin serum concentration shows inter-subject variations, this variation may be due to episodic release of kisspeptin that could be due to its short half-life or due the fact that there are multiple sources of serum kisspeptin, as kisspeptin gene has been reported to be expressed in several peripheral organs(ovary, testes,uterus& pacenta) (Bacopoulou et al., 2017, Kanasaki et al., 2013, Ohtaki et al., 2001).

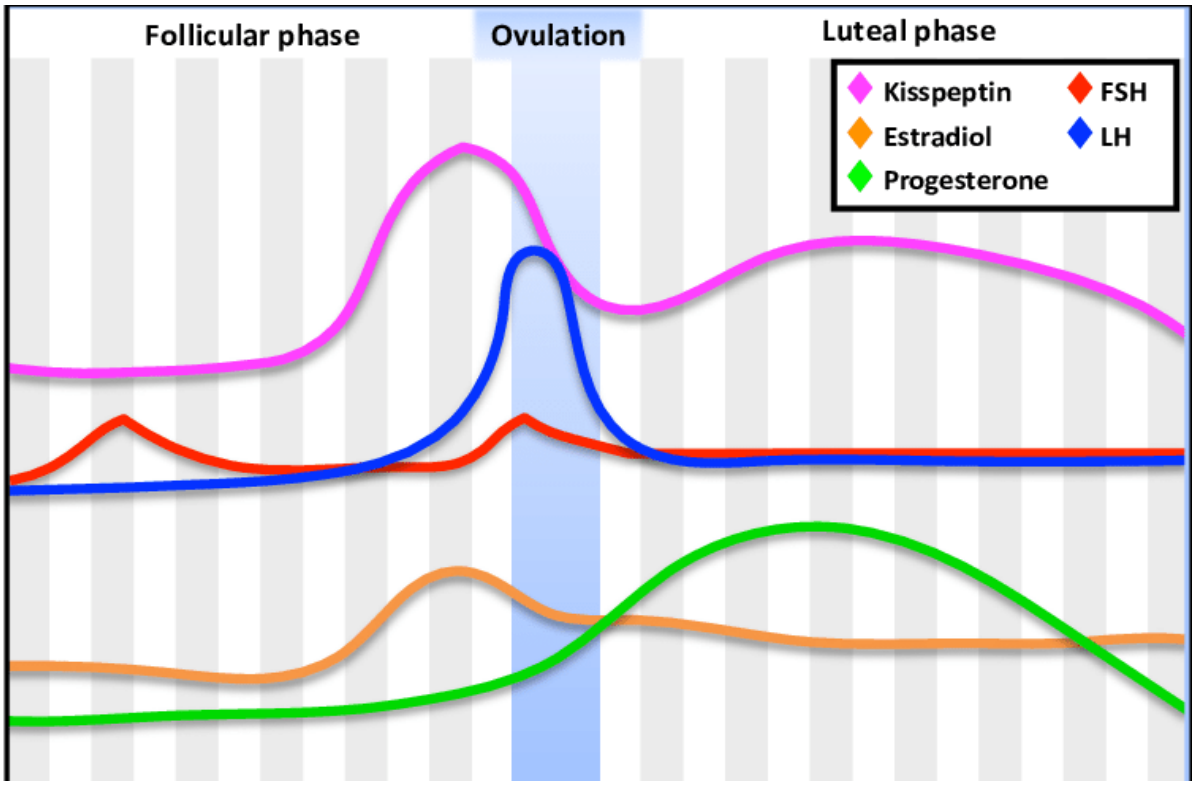


Fig1.6 demonstrates that serum kisspeptin levels rise before ovulation & dropdown after ovulation (Trevisan et al., 2018).

1.4.5 Role of ovarian kisspeptin in ovulation:-

In previous years all the focuses were on the kisspeptin in the hypothalamus, KISS1R is one of the most importantG-protein coupled receptor (GPCRs) in the neuroendocrine control of reproductive function, and its ligand kisspeptin has a significant effect on the hypothalamus (Shahab et al., 2005) .However, it has been reported that, the kisspeptin expression also existes in the ovary (granulose, lutuim and theca cells) (Castellano et al., 2006, Gaytan et al., 2009). The expression of ovarian Kiss1 mRNA shows a distinctive cell- and stage-specific pattern under regulation of LH, whereas Kiss1r mRNA expression remains low and does not significantly fluctuate with the oestrous cycle or gonadotropin treatment in rats (cao etal.,2019).

The precise physiological role of ovarian kisspeptin & detailed mechanism in controlling ovulation is still unclear. Besides there is also controversy, it was illustrated that humans & rodents with impaired GPR 54 signaling can ovulate with artificial stimulation by GnRH (Seminara et al.,2003, Roseweir et al., 2009). In spite of mutation in specific kisspeptinogenic neuron in female mice the ovulation can occur (Mayr et al., 2011). Moreover, in female mice with Kiss Knockout gene & receptor, newly formed corpora lutea could be preserved in the ovaries, and cumulus-oophorus complexes could be found in the oviduct after GnRH administration but, with no or few ovulated oocytes (Gaytan et al., 2014). This observation confirmed the crucial role of ovarian kisspeptin in reproduction. In vivo administration of the kiss1 opponent P234 to the ovarian bursa of 22- to 50-day-old rats postpone vaginal opening and disrupted estrous cyclicity (Ricu et al., 2012). The Cumulus cell expantion & extraction of the first polar body in sheep oocyte is higher when incubated in medium supplemented with 10 Mg/ml kisspeptin (Byril et al., 2017). The immature ovary shows negligible Kiss1 expression (Castellano et al.,2006) and there is no significant difference in ovarian weight between Kiss1/Kiss1r-deficient mice and normal mice before puberty(Garcia et al .,2012) However, after puberty, the ovaries in Kiss1r−/− and Kiss1−/− mice shrink compared with those in control mice, likely due to the loss of kisspeptin-mediated regulation of follicular development, not defects in gonadotropin secretion because follicular development cannot be rescued by gonadotropin replacement(Cao et al.,2019) .Recently in women who underwent assisted reproductive technology it found that intrafollicular kisspeptin participate in oocyte maturation. Also, its level is significantly higher than the circulating kisspeptin level (Taniguchi et al., 2017).

1.5 Infertility:-

Infertility is defined as a failure to conceive after 1 year of unprotected intercourse in women < 35 years old and after 6 months in women > 35 years old. It can be subdivided in to primary infertility that is no prior pregnancies, and secondary infertility referring to infertility following at least one-year prior conception

Causes of Infertility:-

1-Male factor

2-Ovulatory factor

3-Pelvic factor

4-Cervical Factor (Anwar & Anwar., 2016)

Causes of female infertility involve-

1-Ovulatory disorders 25%

2-Endomateriosis 15%

3-Pelvic adhesion 11%

4-Tubal blockage 11%

5-Other tubal abnormalities 11% (Weise & Clapauch, 2014).

Locally it was reported that the most common subfertlity cause in Baghdad was related to ovulatory failure 68.2%, while 20% of the cases were related to unknown causes and 11.89 was related to fallopian tube dysfunction (Haleem et al., 2014).

The etiology of ovarian dysfunction can be classified according to the:

1-Genetic factors (Endometriosis, uterine fibroid, age at menarche, age at menopause)

2-Modifiable factors (age, obesity, smocking, intercourse time).

3-Endocrine disorders that include:

A-Hypothalamic amenorrhea

B-Functional pituitary adenomas (prolactinomas, acromegaly, Cushing's disease)

C-Thyroid disorder (Hyperthoyridism, hypothyroidism, subclinical hypothyroidism)

D-Adrenal disorders (Congenital adrenal hyperplasia, Adisons disease)

E-Ovarian disorders (polycystic ovary syndrome, premature ovarian failure. (Weise & Clapauch, 2014)

According to the World Health Organization abnormal ovarian function is classified (based on FSH level) into three subgroups:

1-Hypogonadotropic conditions that indicate disturbance at hypothalamic-pituitary levels

2- The normogonadotropic oligo-or amenorrheic state is associated with pituitary –ovarian

3-Hypergonadotropic status coincides with diminished ovarian reserve (Dhont, 2005).

Ovulation assessment:-

1-Hormonal assay

Blood or urine analysis can be performed to investigate ovulation in two or more time of the menstrual cycle.

1-Early Follicular phase FSH. FSH level less than 10 IU/ml.

2-Mid cycle LH & estrogen level. Ovulation occurs after 8-20hr after the LH peak. Estrogen is essential for the LH surge. Mid cycle LH (6.6- 20) IU/l indicate ovulation

3- Luteal phase (around 21 day of the cycle) progestrone level.

Serum progestrone < 3ng/l indicate unovulatory cycle, While level (3-10 ng/ml) indicate luteal insufficiency and progestron level more than 10 ng/ml indicate ovulation

2- Physiological changes: several physiological changes occur at ovulation for instance lower abdominal pain, clear smooth slipper vaginal mucous and increase basal body temperature.

3- Transvaginal ultrasound: - It is usually performed at 9th-13th day of the period according to the duration of the cycle. The follicles grow 1-2mm per day, it mature when it reaches 18-24mm. Visualizing follicular disappearance &anendometerial thickness is essential step in detecting ovulation.

1.5.1 Kisspeptin & infertility

Recently it has proven that kisspeptin has fundamental role in reproduction field, from the time of puberty till the end of reproductive period. Evidence has been collected that kisspeptin co work with other neuropeptides like Neurokinin (NKB) & Dynorphin (DYN) to complete its role as a stimulator of GnRH secreted neuron (Goodman et al.,2014).

Puberty is started when anterior pituitary begins to produce and secrete LH &FSH hormone under the effect of pulsatile release of GnRH. At time of puberty Kisspeptin & Neurokinin B are coexpresed, along with dynorphin, in sex hormone responsive neuron in the arcuate nucleus, and their coordinate activity appears to regulate GnRH secretion (Lehman et al., 2010). Pubertal failure has resulted from inactivation & Mutation in gene

encoding GPR 54 receptors (Topaloglu et al., 2012). As well as, Activating autosomal dominant mutation in the kisspeptin receptor gene was identified in a girl with precocious puberty (Teles et al., 2008) suggesting the critical role of kisspeptin in puberty. Mutation in TAG 3 or TAGR (encoding neurokinin B and its receptor) were observed to cause the same problem (Tapaloglu et el., 2009). NKB depends on kisspeptin to modulate its action, infertility that was caused by inactivating mutation of NKB or NK3R can be treated by continuous kisspeptin infusion (Young et al., 2013).

At time of puberty there are the following complex phenomenons:-

1-Full activation of GnRH system occurs by increase in the hypothalamic KISS mRNA /kisspeptin content during the beginning of puberty.

2-Increase in the sensitivity to the excitatory signals from kisspeptinogenic neuron toward GnRH Neurons

3-An enhancement of GPR 54 signaling efficiency in GnRH neuron

4-Desensitization to kisspeptin stimulation is suppressed

5-Up stream of kisspeptin –positive neuron and their projection to GnRH neuron (Castellana & Sempere, 2017).

The kisspeptin secreted neurons (Pre optic area & arcuate nucleus) secret kisspeptin at a certain time of the menstrual cycle , at the end of the follicular phase when FSH level begins to decrease by negative feedback from estrogen and inhibin B, estrogen also exert negative feedback effect on kisspeptinergic neuron of the arcuate nucleus. While, at times LH surge when estrogen exerts positive feedback to the hypothalamus, it also activate kisspeptinogenic neurons in the preoptic area via ER α (Estrogen receptor) to secrete kisspeptin. (Hameed et al 2011).

Estrogen receptors are located on kisspeptinergic neuron, which has two isoforms (Erα & Erβ). ERα is believed to involve in the feedback mechanism and ovulation (Wintermantel et al., 2006) .

Both estrogen and kisspeptin work together for producing LH surge and ovulation. LH surge failed to occur in ovariectomised GPR 54 & KISS null mice that are treated with estrogen and progesterone (Dungan et al., 2007, Clarkson et al., 2008). Also Blockage of estrogen receptor in rodent treated with kisspeptin -10 was observed to cause ovulation failure (Roa et al., 2008). In addition to that ovariectomised pubertal monkey with impaired kisspeptin can partially recover after estrogen replacement (Guerriero et al., 2012).

Male and female mice with mutant Kisspeptin receptor gene are infertile. Female mouse dose not show normal estrus cycle and fails to ovulate (Nejad et al.,2017). In human mutation of GPR 54 KISS1R cause hypogonadal hypogonadisim (de Anglemont & de Tassing, 2010). Furthermore, kisspeptin is also important for oocyte maturation and ovulation loss of NTrk/ kiss 1r signaling in oocyte cause premature ovarian failure (Dorfman et al., 2014). .

There are about twenty studies in different species including humans about the effect of a mutation in KISS1/KISSr, all the studies showed that there is a reduction in ovary size and weight compared to non-knockout counterpart (Hu et al., 2018).

Recently the important role of kisspeptin in the reproduction field was explored, this paved away to use kisspeptin as an infertility treatment.The IV infusion of kisspeptin in healthy male subjects caused a rise in the LH, FSH and testosterone levels (Dhillo et al 2005). The same effect also was observed in females (Dhillo et al., 2007). As well as plasma level gonadotropin were significantly increased after administration of kisspeptin twice weekly in infertile women with functional hypothalamic amenorrhea (Jaysena et al., 2009). A nine

fold LH increase with oocyte maturation was observed after 12 of subcutaneous kisspeptin injection in infertile women undergoing IVF (Abbara et al., 2014). All these studies are collectively strong evidence about the ability of kisspeptin to induce LH surge and oocyte maturation .But it is difficult to determine whether this action is caused by the effect of LH or by action of the kisspeptin on oocyte maturation. Recently evidences has been collected that this maturation is mediated by up regulation of the growth factors involved in follicugenesis (Hue et al., 2018).

1.6 Polycystic ovary syndrome (PCOS)

PCOS is a common endocrinopathy Characterized by oligo-ovulation- or anovulation, sings of androgen excess, and multiple small ovarian follicles. These signs and symptom may vary widely between women as well as with individuals over time (Witchel et al., 2019).

The most common cause of anovulatory subfertility is PCOS. PCOS women also suffer from menstrual irregularity, increase hair growth, acne and most commonly overweight, but it also frequently seen in normal weight women (Dennet & Simon., 2015).

PCOS is classified into three types:-

1-Classic PCOS that involved patients with hyperandrogenism and chronic anovulation but normal ovaries

2- Include patient with polycystic ovaries and higher LH

3-Normogenic phenotype, patient have normal BMI, insulin sensitivity, free androgen index but increasing level of LH and LH/FSH ratio (Weiss and Clapauch, 2014).

1.6.1 Causes of PCOS

PCOS is a multifactorial disorder that may be caused by combination of genetic, environmental and intrauterine factors (Fig1.9)

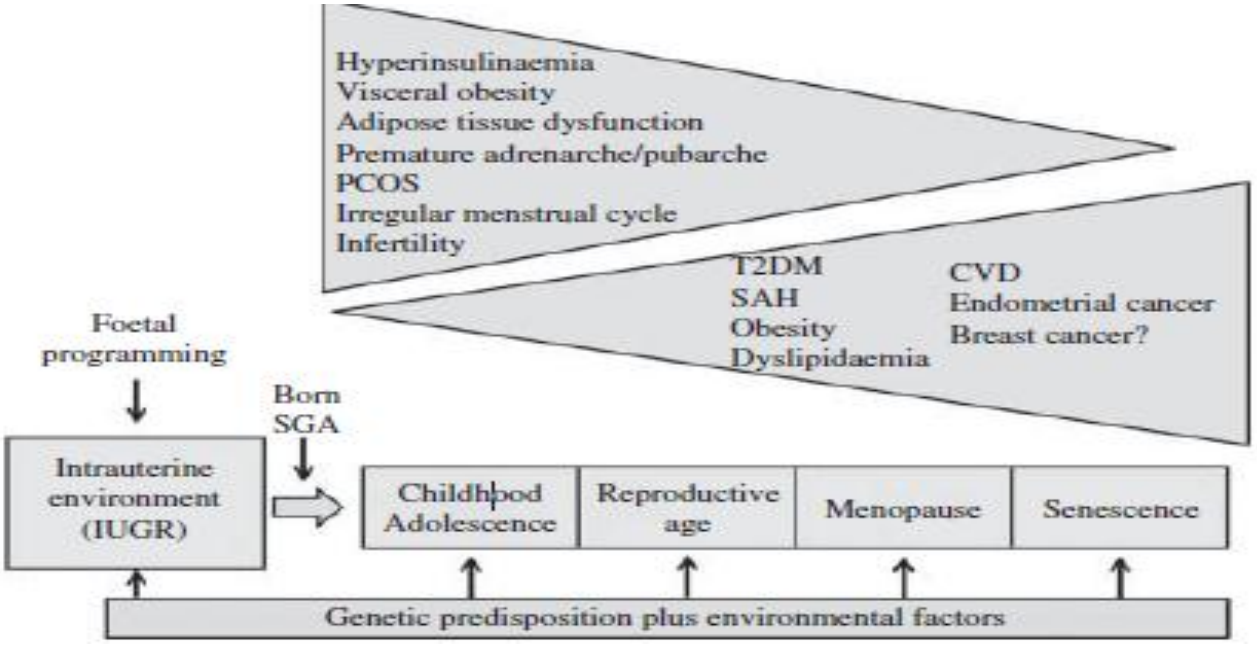


Fig (1.7) shows the etiology of PCOS. Alterations in gene expression occur in intrauterine life because of excess glucocorticoids and or androgens during fetal life, this change is related to several disorders during post fetal life including PCOS. On the other hand, normal mothers with a normal pregnancy may have the disease because of genetic factors. (de Melo et al.,2015).

Different factors may participate in determining PCOS pathogenesis. A retrospective study on PCOS, patients had suggested the existence of specific prenatal risk factors for post – pubertal expression of the PCOS phenotypes. Hirsute women with high testerone levels were borne from over-weight women while normal- weight women with high LH & normal testerone were borne after term. (Kandaraski et a., 2005).

Having PCOS may run in families and there is evidence of genetic cause. Yet, there it's a single gene that causes this disease (Prapas et al., 2009). PCOS is like obesity and type 2 DM which may be caused by several genetic factors. However, women with PCOS have two major genetic alterations in androgen synthesis and in insulin action higher incidence of different gene polymorphisms (Kandraski et al., 2005). Environmental factors include both prenatal and postnatal factores, at adulthood obesity ,diet , sedentary life style, environmental toxin ,stress and drugs are the most common factors (de Malo et al ., 2015).

The prevalence and distribution of PCOS are different in a different area it may be related to racial background. Table 1.2

Country	Prevalence of PCOS
India	8.20 % (Cupta et al.,2018)
USA	4-12 %
Iran & Turkey	7.1 %
Caucasian Spain women	6.5%
China	5.6 %

Table (1.2) illustrates the prevalence of PCOS in different country. Caucasian women living in USA and Europe are less likely to develop PCOS compare with females residing in Middle East, black women are at higher risk for developing PCOS, and Chines women are at lower risk (Ding et al., 2017)

The prevalence of PCOS among 320 infertile Kurdish women in north Iraq was 33% (Hussien & Alalaf, 2013).The environmental factors like a high carbohydrate and high saturated fat intake, sedentary lifestyle may contribute to development of the disease in high

prevalence area. Advanced glycation end products (AGE) are the end products of a chemical procedure called Maillard reaction in which the carbonyl group of carbohydrates reacts non-enzymatically with primary amino groups of proteins such as lysine or arginine (Buccala & Cerami 1992). Kendarakis et al., 2005 found that there were positive correlation between AGEs and insulin resistance indices and hyperandrogenemia. AGEs can be formed both endogenously & exogenously. Elevated blood sugar levels participate in endogenous AGEs production. While exogenous sources are from tobacco and food. In food, AGEs are produced in the process of industrial packaging or home cooking (extensive cooking, baking, grilling & firing also during long term storage (Nicholl & Bucala 1998), (O' Brien & Morrissey 1989).

1.6.2 Pathogenesis of PCOS:-

1-Androgen excess:-

Normally the ovaries and adrenal glands produce testosterone in an equal amount, half of testosterone is produced directly by ovaries and adrenal gland and the other half is produced by peripheral conversion of circulating androstendione. The ovary produces and releases androgens, including 20% of DHEA, 50% of androstenedione and 25% of circulating testosterone (Meek et al.,2014). Steroid hormones are derived from lipid cholesterol, the first hormonal product of cholesterol is pregnenolone, several important synthetic pathways diverge from it, and the final product depends upon tissue and its enzyme fig 1.8

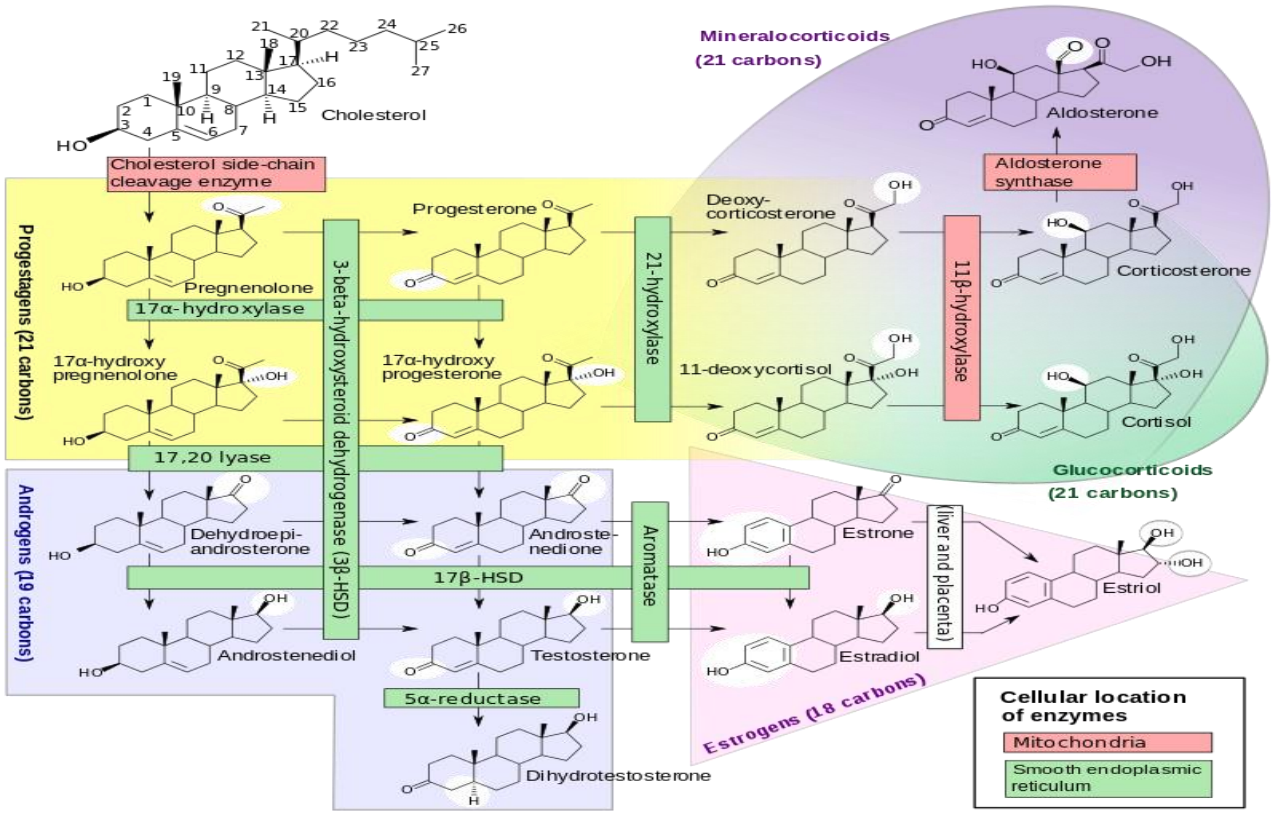


Fig (1.8) the biosynthesis of androgen hormones (Häggström et al., 2014)

Aromatazation occurs in the muscle and adipose tissue in which testosterone and androstendione are converted to estrogen, estron, and estradiol .While in the skin testoste rone is converted to dihydrotestesterone by 5α- reductase, in PCOS patients there isan increase in activity of this enzyme also there is adefect in aromatazation (Steward et al., 1990). Moreover, polycystic ovaries have thickened thecal layer that secrets excessive androgen in vitro under basal condition or in response to LH stimulation (Smith et al., 1994) .Elevated levels of androgen participate in the formation of small immature follicles, ovarian PCO morphology and anovulation. Also, decreasing androgen levels with ovarian aging cause' decrease in the prevalence of amenorrhea in PCOS patients (Takai et al., 1991, Elting et al., 2000). .

Heterogeneity in clinical features, as well as genetic variations observed in PCOS, is associated with hyperandrogenic condition indicating the possible involvement of abnormalities associated with the steroidogenic pathway (Reddy et al.,2014).Genes that code for enzymes involved in the steroidogenic pathway are considered as candidates for PCOS. Among those, the most extensively studied genes are the CYP11A gene (cytochrome P450 side-chain cleavage enzyme gene), CYP17 gene (cytochrome P450 17hydroxylase/17, 20-desmolase gene), and CYP19 gene (aromatase). Studies have shown that ovarian theca cells of PCOS women overexpress enzymes involved in androgen biosynthesis (Nelson et al.,2001) resulting in an increased production of 17-hydroxyprogesterone, testosterone, and androstenedione compared with theca cells from non-hyperandrogenic women (Nelson et al.,2002) Moreover, there is decreased activity of aromatase enzyme, further increasing the androgens. Therefore, abnormalities in androgen production lead to hyperandrogenism in PCOS (Ashraf et al.,2019) .

Hirsutism is a condition characterized by excessive hair growth in androgen-dependent areas of the body. Hairs become coarse and are distributed over the androgen dependent areas in male pattern. The etiology of hirsutism is mainly classified as: idiopathic, androgenic and nonandrogenic. Eighty percent of the females of reproductive age group, who present to clinical settings with hirsutism, most commonly have PCOS. It has been documented that about 70-80% of hirsute women have PCOS while non-androgenic factors are relatively rare (Khan etal., 2019).

Normally, ovarian functions are controlled by both LH &FSH, FSH act on granulosa cells; it stimulates the synthesis of estrogen, inhibin, activin and follistatin released by granulose cell. Sex hormone binding globulin, IGF modulates the amount of androgen made in response to LH (Yarak et al., 2005).

Sex hormone binging globulin (SHBG), a homodimeric glycoprotein of 95 KD, is synthesized in the liver. It has a half-value time of 7 days in plasma. Its main function isto

transport is sex hormone transport within the circulation and to extra-vascular target tissue (Selby, 1990). SHBG plays a role in regulating the bioavailablity of sex steroid concentration through competition of this steroid for available binding sites (Kahn et al 2002). 80% of circulating testosterone is bound to SHBG, 19% is bound to albumin only 1% is free in the circulation (Nelson et al., 2001). SHBG shows high inter individual variation and it's influenced by androgen /estrogen balance, nutritional status, BMI, sex & Insulin.

SHBG concentration increased in an older man , pregnancy, hormone replacement therapy, liver cirrhosis, hyper thyrodisim, hypogonadism , androgenation in females (Elimlinger et al., 2002). , it has a negative correlation with bioavailability of testerone in premenopausal women (Crowford et al.,2015).There is a link between PCOS hallmarks and SHBG level ,where its level is lower in obese , hyperandrogenic women , but its level shows a significant improvement after treatment (Dawsal et al., 2017). More than half of PCOS patients are insulin resistance, but the extent of this resistant is unrelated to obesity, it depends on body fat distribution (ovalee & azziz. 2002) (Castro et al., 2014). yet, insulin resistance and hyperandrogenemia in PCOS patients are interconnected. SHBG is the main serum transporter of testerone meanwhile, serum insulin had found to inhibit SHBG (Nestler & Stauss 1991).

2-Insulin Resistance:-

Hyperinsulinemia affect approximately 65-70 % of women with PCOS (De Ugatte et al., 2005).There is a link and interaction between hyperandrogenism and insulin resistance in PCOS patient fig (1.9)

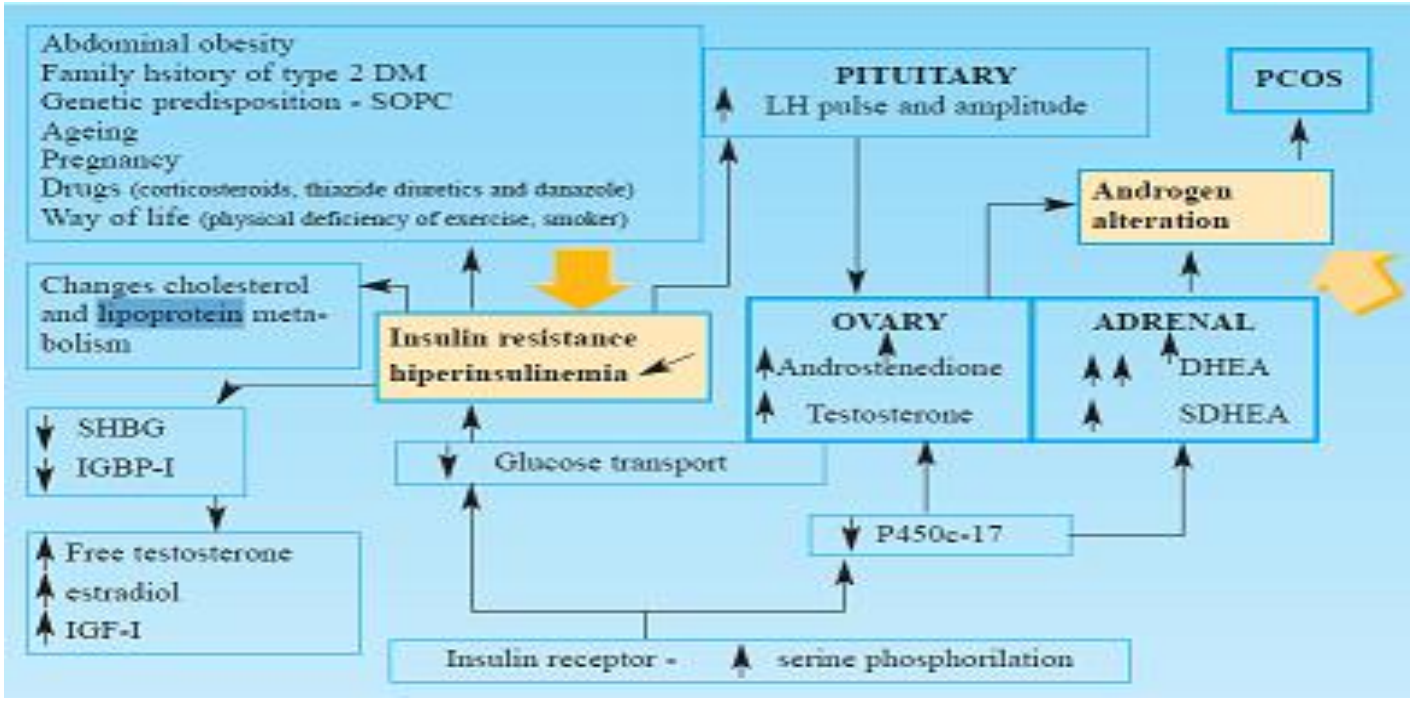


Fig (1.9) shows the interaction between insulin resistance and hyperandrogenism in PCOS women.

Insulin resistance cause alteration in cholesterol metabolism that cause decrease sex hormone binding globulin that leads to increase testerone and estrogen .Excess estrogen cause chronic feedback and increase in the LH that cause further androgen excess.Serin phosphorelation occurs in insulin- resistant due to defects in a certain gene that cause a defect in CPY 17 enzyme that leads to increase in both adrenal and ovarian testerone. (Yarak et al., 2005)

The molecular foundation of PCOS pathogenesis is for the most part identified with FOXO proteins that are essentially expressed in the granulosa cells of the ovary, cystic follicles & oxidative stress-induced apoptosis in the granulosa cells of rat ovary (Shi et al., 2003). Insulin can initiate several cellular pathways, the most significantt pathway is PI3K-AKt pathway, and this pathway is important intracellular signal for cellular metabolism, multiplication& glucose homeostasis. At the point when insulin or IGF-1 binds to its receptor, insulin receptor substrate-1 (IRS-1) is phosphorylated. Phosphorylated IRS-1 recruits PI3K, which enhaces Akt, and the enhanced Akt regulates the activation of many downstream proteins, including FOXO proteins. The phosphatase and tensin homolog (PTEN) antagonizes Akt activation and phosphorylation of FOXO proteins (Nteeba et al.,2013). It is sensable to hypothesize that over-activation of the PI3K-Akt pathway is the most powerful explanation behind the development of PCOS-like phenotypes in the IGF-1 overexpressing mice (Ryu et al., 2019).

3-Disorders in the neuroendocrine system:-

Alterations in GnRH secretion are of the features of PCOS. GnRH express two type of pulse generation, slow and fast for stimulation of FSH and LH respectively. In PCOS women LH is commonly increased, FSH is typically in the lower range, this may be related to decrese the sensitivity of GnRH pulse generator to steroid feedback and enhance LH secretion (Mc Cartney et al.,2002). Recently it is believed that elevated level of GnRH &LH in PCOS women may be related to the cumulative effect of altered GnRH stimulatory & inhibitory neurotransmitters in hypothalamic-pituitary center (Mc cartney et al., 2018).

1.6.3 Kisspeptin & PCOS:-

Kisspeptin trigger GnRH &stimulate LH, secretion for inducing ovulation. In addition ovarian kisspeptin expression was documented in many species including humans, the exact

mechanism for involving kisspeptin in ovulation is still not fully known. PCOS is a neuro-endocrine disorder characterized by ovulatory dysfunction, hyperandrogenism and metabolic alteration.

It had proven that LH & GnRH is high in PCOS patient, so we speculated that the level of kisspeptin is high also. There are many studies about the role of kisspeptin in the pathogenesis of PCOS, same studies recorded elevated kisspeptin level in PCOS patient in compare to normal women (Chen et al., 2010, Nyagolova et al., 2016, Yarolinskaya et al., 2017, Jeon et al., 2013, Gorkem et al., 2018). While other studies demonstrated that there were no different in kisspeptin levels (Panidis et al., 2005, Emerkci et al 2016, Yarlikaya et al 2013). Yilmaz et al. 2014) reported that, based on ROC (receiver operating characteristic), the best cut-off point for the serum kisspeptin levels was 1.87 ng/ml. Serum levels over 1.87 ng/ml exhibited sensitivity and specificity of 59% and 93.8%, respectively, for the diagnosis of PCOS. The positive predictive value was 92.5% and the negative predictive value was 63.8%. These results, therefore, indicate that kisspeptin may be an independent marker for PCOS (Araujo et al., 2020).

The detailed mechanism for the involvement of kisspeptin in the pathogenesis of PCOS is still pending, but recently it has proven that metabolic disorders in PCOS may contribute to the alteration of kisspeptin level. The heterogeneous nature of the genetic etiology of PCOS may play a role in any variation in serum kisspeptin level. Sequencing the GPR 54 gene revealed 5 single nucleotide polymorphism (SNPs) while sequencing the Kiss1 gene revealed 2 SNPs. All identified SNPs showed no significant difference in frequency between PCOS patients & controls. While the heterozygous allele for GPR 45 gene chr 19:918686 is probably associated with serum kisspeptin concentration, which suggests the potential role in the etiology of PCOS (Umayal et al 2019b).

Insulin resistant is manifested in obese & even lean PCOS patients, who usually display signs interstice insulin resistance (Stepto et al 2013) . As well as hyperinsulinemia can cause a significant increase in LH level where it has a role in regulation of GnRH secretion (Moret et al.,2009). In spite, this fact it has been shown that there is insignificant changes in kisspeptin level in non-obese PCOS patients & control group (Yerlikaya et al., 2013, Emekci et al 2016).

Recently, it was recorded that kisspeptin correlated positively only with the free androgen index (Jeon et al 2013). This finding will not belittling the role of insulin resistance and obesity in alteration of kisspeptin level, where hyper insulinemia stimulates androstenedione secretion (Tosi et al.,2012). .

To the best of our knowledge, leptin has been documented in the regulation of kisspeptin secretion GnRH neurons don't express liptin receptor but ,leptin receptor (ob-Rb) mRNA is found in 40% of kiss 1 m RNA expressing cells of the ARC . Not only but also , low expression of Kisspeptin was observed in the case of mutation of leptin gene (Smith et al., 2006).In sheep adminstration of leptin can enhance hypothalamic kiss expression (Backcler et al 2010). There is a positve correlation between kisspeptin and leptin level in PCOS women (Emerkci et al ., 2016).

Obesity causes anelevation in leptin level, in the case of PCOS its unknown that the obesity cause elevation of leptin level or there is other defects in the syndrome that cause its elevation, but Jeon et al recorded high level of leptin level in lean PCOS patients.

PCOS is a complex endocrine disorder that results from the interaction of susceptible and protective genomic variants in several genes under the influence of environmental factors (Umayal et al., 2019a).

1.7 Ovarian Aging

The process of reproductive aging varies considerably among women, it depends on several factors for instances parity, lactation, contraceptive pills, genetic, for that reason some women remain highly fertile until the fifth decade of life, while others show decline fertility in their mid –thirties (Eijkemans et al.,2014). Ovaries undergo much more serious effects of aging than any other tissues of the female body. Ovarian aging mainly depends on the number & quality of follicles in the ovary. Healthy female baby borns with 1-2 million of small ovarian follicles (Billari et al.,2011). At the time of puberty, several follicles (5-9) released with each menstrual cycle. Only one follicle mature & released from the ovary the rest undergo apoptosis, which accelerated significantly 10-15 years before menopause (Amanvermez & Tosun, 2016). Decreasing numbers of follicles coinciding with diminished oocyte qualities that, dictate the gradual changes in the menstrual cycle regularity and decreasing the ability to produce offspring (Broekman et al., 2009).

Genetic factors are involved in menopausal age determination. Some of these genes exert hormonal effects like FSH, FSH R, LH, LHR, CYP 17 & CYP19, whereas others affect the recruitment rate & primordial follicle formation & growth factors such as germ cell specific basic helix-loop transcription factor (FIGLA), bone morphogic protein (BMP)& growth differentiation factor gene (GDF)a gene (Hartge 2009).Genome-wide linkage scanning showed that two related chromosomal regions (9q 21.3 & p 21.3) are associated & linked to premature ovarian failure. Meanwhile, other chromosomes (5, 6, 13, 19 & 20) are also involved in ovarian aging (Li Qi et al 2012) .

Along with the decrease in follicle number, oocyte quality also affected (at least after age 31 year) fig 10. The loss of oocyte quality is believed to be due to an increase in meiotic non disjunction, as a result of increase the rate of aneuploidy in aged females

(Kulieve et al., 2005, Hunt et al., 2008). Non- disjunction and the premature separation of chromosomes contribute to aneuploidy during either meiosis I or II (Peterson et al 1999) . Beside this, there is an increase in oocyte mitochondrial mutation (Woods et al., 2016). Mitochondrial DNA is maternally inherited. Luteinizing granulosa cells in women above 38 years old contain higher levels of mtDNA deletion (Seifer et al 2002). In abnormal Mitochondria ca^{2+} oscillation fails to trigger ATP production & alter spindle formation, a normal chromosomal alignment that affects fertility (Li Qi 2012).

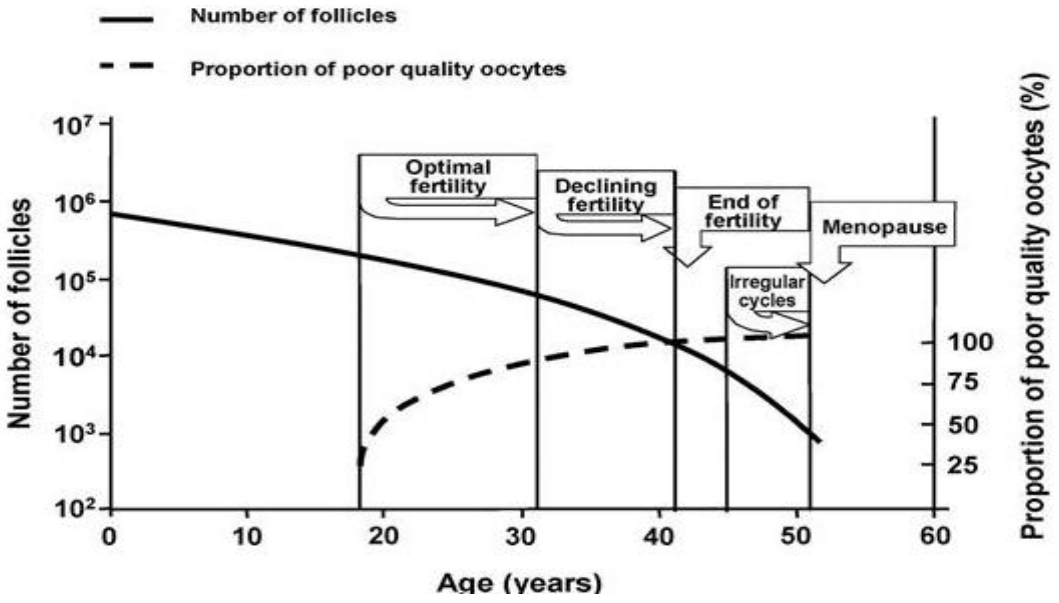


Figure 10 shows the number of primordial follicles present in the ovaries and the chromosomal quality of oocytes in relation to female age (Brun et al., 2001).

1.7.1 Markers of ovarian reserve:-

Ovarian reserve is the pool (number) of antral follicles in the ovaries that are capable of growing in the response to Gonadotropins. Ovarian reserve markers include (antral follicle count, inhibin B, AMH and FSH).

Antral follicle account which is done by transvaginal sonography detects fluid filed follicles which measured (2-10 mm) in diameter, but more than half of detected antral follicles could be in the early or late stage of atresia. For that reason, the clinical response to ovarian stimulation is the only method to assess the actual size of the follicle cohort (Broekmans et al., 2009)

Inhibin B is a polypeptide hormone that is secreted by ovarian granulose cells during the follicular phase of the menstrual cycle it prevent follicular growth by the negative feedback of FSH hormone. Decrease Oocyte quality & fertility potential with aging is associated with a decrease in inhibin secretion and FSH secretion (Hall, 2015).

The antimullerian hormone(AMH) that is secreted by small primordial follicles is a good marker for ovarian reserve, with no fluctuation during menstrual cycle. It is secreted by follicles range 2-6 mm (Weenen et al., 2014). The number of small antral follicle decrease when primordial pool decrease with age which leads to the decrease serum level of AMH which becomes undetectable at menopause (Sower et al.,2008).

The hypothalamic –pituitary- ovarian axis is maintained via negative steroidal feedback. In rodent age related pulsatile decrease in GnRH causes ovarian arrest despite the the presence of ovarian reserve (Wise et al.,2002).While the pulsatile secretion of GnRH decrease with age in primate (Nozaki et al.,1995). In postmenopausal women compared with premenopausal women there is a dramatic increase in LH & FSH levels and pulse

amplitudes but without a change in pulse frequencies (Hall et al.,2000). During few years after menopause both LH & FSH increase steadily (pulse & frequency). Yet the level of steroid hormones stays normal (Rossmanish et al., 2005). In other words, the hypothalamic pituitary unite undergo functional changes independent of feedback signals (Weiss et al.,2004). .

Kisspeptin regarded as a key player in the regulation of reproductive function. It acts in both the hypothalamus & ovary (Coa et al., 2019). In mammals, the infundibular nucleus in the hypothalamus is the main area that controls reproduction where, surgical removal of arcuate nucleus, median eminence and part of the ventromedial nuclei and premammillary areas from the brain doesn't interfere with estrogen negative & positive feedback (Herbison, 2008). It has been demonstrated that the human kisspeptin neurons in this area undergo robust aging-related plasticity (Hrabovsky et al., 2019). It is expected that the serum level of kisspeptin increase at late reproductive age many factors may participate in this increasing the most important one is that, the kisspeptinogeneic cells in the infundibular nucleus hypertrophied at this age (Rance et al., 2009) fig (1.11). Also, dynorphin secreting mRNA neurons is decreased (Rometo & Rance 2007).

The increase in kisspeptin levels may be due to the lack of estrogen feedback. While other studies proposed that this increase is related to increase in ovarian sympathetic & adrenergic stimulation that occurs with aging & cause unovulation (Heider et al.,2001),

(Acuna et al., 2009), (Ricu et al., 2012).

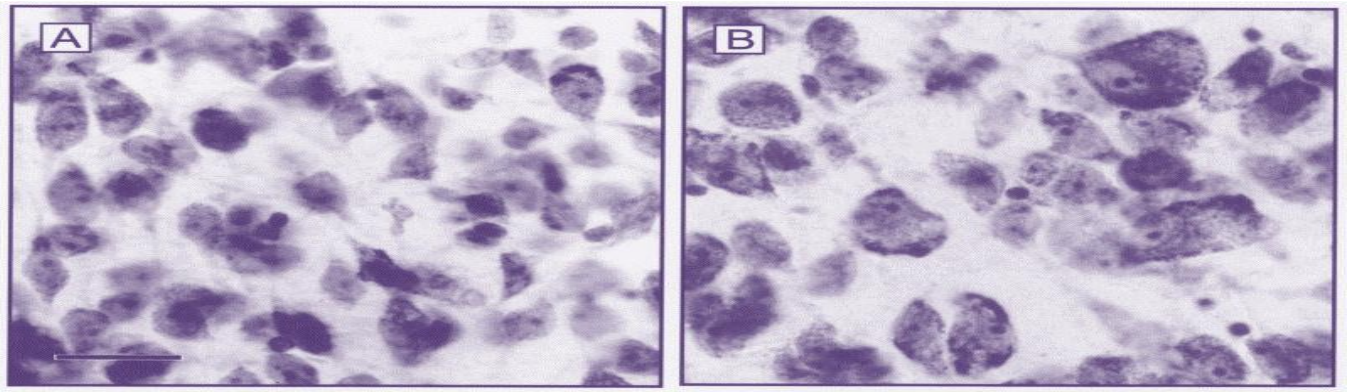


Figure (1.11) violet-stained sections of the infundibular nucleus of young, premenopausal (normal nucleus) (A) and older, postmenopausal (atrophied nucleus) (B) women. There was a significant increase in the size of neurons expressing KiSS-1 mRNA and the number of labeled cells and autoradiographic grains per neuron (Abel & Rane 2009).

In previous years pieces of evidences in rodents have been demonstrated that there is a positive correlation between kisspeptin level & age (Fernandois et al., 2016, Merhi et al., 2016). Kisspeptin administration in postmenopausal women cannot stimulate LH secretion, but postmenopausal women receiving estradiol replacement therapy are only resistance to kisspeptin initially and then they demonstrate a remarkable increase in LH pulse amplitude (Lippincott et al., 2017). Progesterone also involved in kisspeptin regulation it has been demonstrated that progesterone receptor co-localised in KNDy neurons in the hypothalamus & it increase dynorphin concentration, as mentioned befor, dymorphin mRNA neurons decrease in post menauposal & ovariectomized ews, but progesterone administration can restore its normal concentration (Foradori et al., 2005).

Chapter Two

Subjects & Methods

This case-control study was conducted in Kirkuk governorate during the period from April 2018 to March 2019. The study samples collected at the family planning center (infertility clinic) at Azady Teaching Hospital. The ethical approval was obtained from ethical committee of the college of Medicine /University of Sulaimani.

2.1 Subjects

2.1.1 Control Group

Forty women were healthy with a regular menstrual cycle.Their age ranged from 20-40 years (31±7.1). All the membersof this group underwent clinical assessment to confirm they were free from any sign of Poly cystic ovarian syndrome and history of infertility.They attended Family Plancy Center for obtaining Contraception pills.

2.1.2 The patients

Eighty patients were infertile (ovulatory causes) were unable to conceive after one year of regular unprotected intercourse for those bellow 35 years old and, 6 months for those patients above 35 years old. .

Sixty of infertile patients were diagnosed as polycystic ovarian syndrome patients, their age were (30±8.6) years. The diagnosis was based on the Rotterdam Consensus Meeting on PCOS in 2003. It defines the syndrome of PCOS as presence at least two of the following criteria:-

1-Ultra sonic appearance of polycystic ovaries

2-Menestural disturbance

3-Evedance of hyperandrogenism, acne and hirsutism (Balen A, 2004)

Menstrual disturbance involved amenorrhea which ismarked by anabsence of menstrual cycle for more than 6 months and oligomenorrhea which defined as a delay in the menses of > 35 days to 6 months.

The Ultrasound study was performed in the Azady Teaching hospital/ Ultrasound department. Tran'svaginal ultrasonic screening with 3.5 MHC vaginal transducer (Siemens, Model AG 50149, Germany) was performed twice for all infertile women. Ovaries are described as Poly cystic ovaries if there were 12 or more follicles with 2-9 mm in one or both ovaries (Balen&Laven., 2003). The second transvaginal ultrasound was performed at (12th, 13th, 14th) day of the cycle for detecting mature follicle (more than 16 mm) and ovulation.

The third group involved infertile women with high FSH levels, their mean age were (38.14±7.7) years. FSH >10mIu/ml were cosidared high.

Inclusion Criteria:-

All the patients involved in the study fulfilled the following criteria:-

1-The patient agreed to participate in the study

2-The patients had basic investigations for diagnosing type of infertility:

Ovulatory assessment

Hysteo salphengeography

Seminal fluid analysis

Patients with abnormal investigations apart from ovulatory assessments were excluded from the study.

Exclusion Criteria:-

Patient refusal

None of the patients took any medication for at least 3 months before they participating in the study.

Abnormal thyroid function test

Elevated prolactin level

Cushing syndrome

Inability to follow up

2. 2 The Study Protocol: -

The participants were asked to fast on the day of investigations and to come in the morning at 9:00 - 11:00 am for blood drawing. Five ml of blood was obtained from all patients and the study groups by vein puncture at two times of the same cycle (except the infertile women with high FSH, one sample were obtained) the first sample was taken at day 2 of the cycle, the second sample was obtained at preovulatory phase day (12th, 13th, 14th) of the same cycle, all the samples were incubated at room temperature for two hours for completing the clotting process. Serum was separated by centrifugation for 20 minutes at 3000 RPM, then it transferred to plain tubes and stored at – 20 C until the ass ay process.Serum kisspeptin, SHBG, LH, FSH, Estradiol, AMH & free testosterone were measured. The fertile and infertile PCOS women were subdivided into four subgroups according to their ages

(1) 20–24 years old
(2) 25–29 years old
(3) 30–33 years old
(4) 35–40 years old

2.2.1 Medical History (questionnaire)

All the patients and control groups interviewed and examined by the researcher for the sake of the consistency of the data. Well, the structural Questionnaire developed for the study and filled for every patient and control. The questionnaire covered the most important medical history relevant to female fertility including age, age of menarche, marital age, gravidity, parity, mode of delivery, miscarriage. The questionnaire also involved asking about any medical disease complicating pregnancy for instance (Hypertension, Diabetic, Rhumatic disease, Thyroid disease, and anti-phospholipid syndrome). It also included surgical history including (ovarian cysctectomy, myomectomy, evacuation of retained product or dilatation and curetting of endometrial, laparoscopy, curettage), irregularity of menstrual cycle (oligo, poly), drug history, type of contraception (Coitus, lUD, condom, contraceptive pills), ultrasound, semin analysis, hormonal analysis.

2.2.2 The PCOS classification:-

The PCOS patients were subdivided into four subgroups (according to the clinical and biochemical markers) (Balen et al.,2004) as follows:

Group A: ovulatory dysfunction + hirsutism or hyperandrogenism + PCO feature

Group B: ovulatory dysfunction + hirsutism or hyperandrogenism

Group C: ovulatory dysfunction + PCOS (no hirsutism and normal androgen)

Group D: hirsutism or hyperandrogenism + PCOS with the normal menstrual cycle

2.2.3 Clinical assessment:-

2.2.3.1 Clinical hyperandrogenisim:-

Clinical hyperandrogenism was diagnosed using a modified FerrimanGallwey score for evaluating and quantifying hirsutism in women using nine body areas (upper lip, chin, chest, upper and lower abdomen, thighs, upper and lower back and upper arm) (Goodman et al., 2001). Hair growth was rated from 0 (no growth of terminal hair) to 4 (extensive hair growth) in each of the nine locations; a score of 8 or higher was regarded as indicative of androgen excess.

2.2.3.2 Overweight & Obesity:-

These parameters were defined according to WHO criteria as body mass index (BMI), calculated by dividing the weight in kilograms by height in meters squared. Being overweight was defined as a BMI > 25 kg/m2 and being obese as >30 kg/m2.

2.3 Biochemical assessment:

All the parameters measured by enzyme-linked immunosorbent assay (ELISA) technique.

2.3.1 Hormonal kits with their Remarks:-

Table 2.2 shows hormonal kits & their remarks

Hormonal kits	Company	Country
Human Kisspeptin Elisa Kit	Al.shkairat establishment for medical supply	Jordan
Human sex hormone binding globulin ELISA kit	LDN	Germany
FSH ELISA	Abcam	USA
LH ELISA Kit	Abcam	USA
Estrogen ELISAkit	Biotech	USA
Antimullerian hormone ELISA kit	My Biosource	USA
Testosteron ELISA Kit	Biotech	USA

2.4 Biochemical Assays:-

2.4.1-Human Kisspeptin:-

Principle of the test:

The kit consist of 96-well plates, each well was coated with KISS1Antobody.The biotin conjugated & KISS1 utilized as detection atntibodies.The kit procedure depends on Sandwich enzyme-linked immune-sorbent assay technology.The standard, test samples & bioten conjugated detection antibody were added to the wells subsequently & washed with wash buffer. To picture HRP enzymatic reaction TMB substrate were utilized. The

blue colour appeared as a result of catalyzing TMB by HRP, then the blue colour changed to yellow by adding an acidic stop solution.The density of the produced colour is relative to the KISS1 amount of the sample.Kisspeptin concentration calculated by reading the OD absorbance at 450 nm in amicroplate reader (Platonov etal, 2018).

Assay Steps:-

1-.0.1ml of 10ng/ml, 5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml, 0.3125ng/ml, 0.156ng/ml, standard solutions added into the standard wells.

2- 0.1 ml of Sample / Standard dilution buffer added into the control (zero) well &

0.1 ml of serum added into test sample wells.

3- 0.1 ml of Biotin- detection antibody working solution added into the wells (standard, test sample & zero wells). Incubated for 1 hrs at room temperature.

4-After washing 0.1 ml of SABC working solution added to each well, incubated for 30 minutes at room temperature.

5- After washing 5 times 90 µl of TMB substrate added into each well, the plate covered and incubated at 37°C in dark place for 15-30 min.

6- 50 µl of Stop solution added into each well and mix thoroughly. The color changes into yellow immediately.

7-The O.D. absorbance at 450 nm read by a microplate reader immediately after adding the stop solution.

Standard Curve for calculation of kisspeptin by ELISA:-

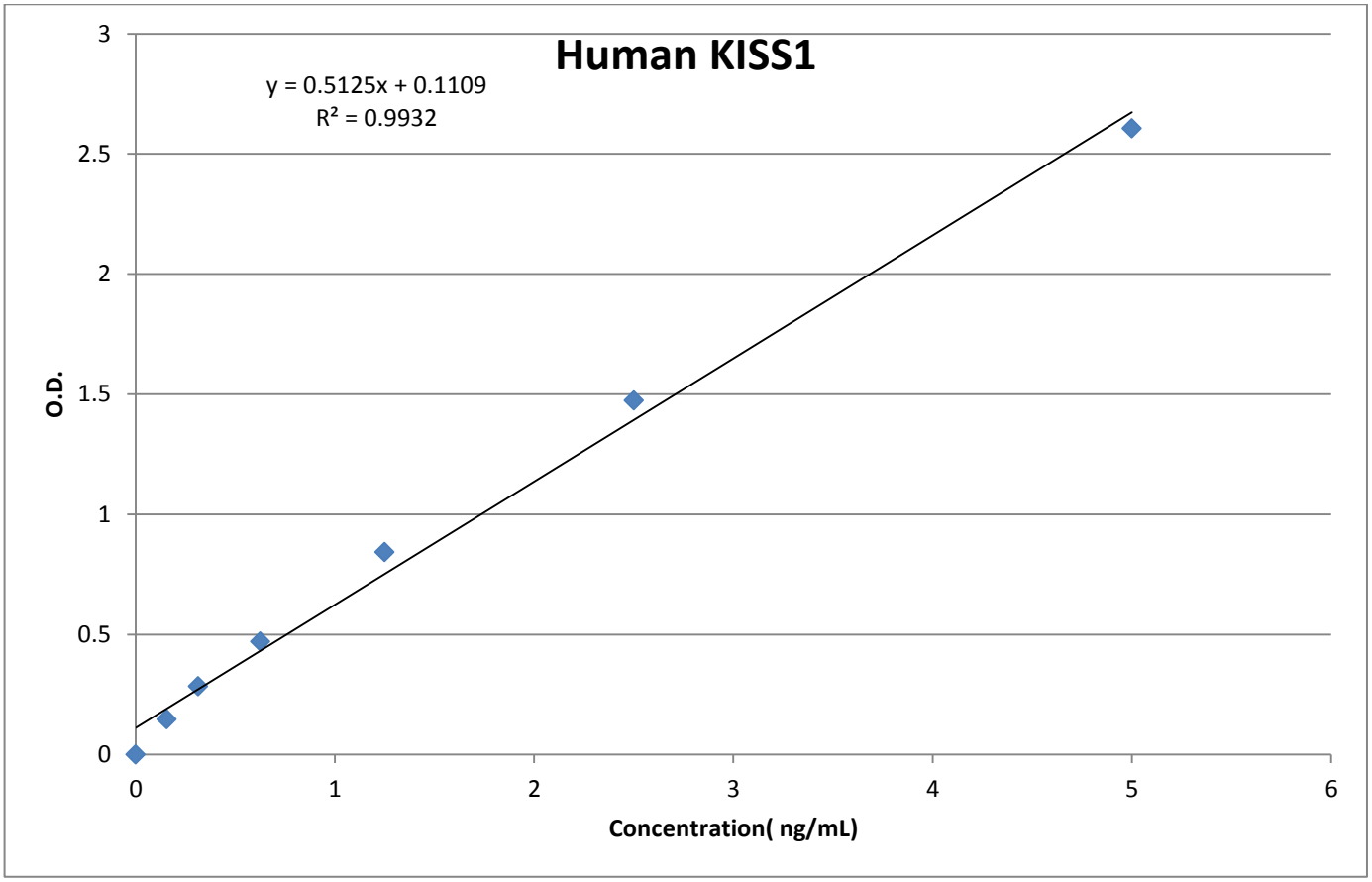


Figure 2.1 shows the standard curve for kisspeptin

2.4.2-Human sex hormone binding globulin:-

Principle of the test:

The microtiter wells are coated with monoclonal mouse antibody for a unique antigenside of SHBG molecule .The samples is incubated in the coated wells with enzyme conjugate which is an anti-SHBG antibody conjugated with horseradish peroxidase.After incubation the unbound conjugate is washed.The amount of peroxidase conjugate is proportional to SHBG in the sample (Elmlingeret al.,2002)

Assay steps:-

1. The Microtiter wells secured in the frame holder
2. Fifty µl of each prediluted Standard, Control and, sample Dispensed into appropriate wells.
3. Incubated for 120 minutes at room temperature.
4. The wells rinsed 3 times with 300 µl - 400 µl diluted Wash Solution per well. Then the wells streaked sharply on absorbent paper to remove residual droplets.
5. 100 µl(Enzyme Conjugate) added into each well.

   Incubated for thirty minutes at room temperature.
7. The contents of the wells shake out the wells rinsed 3 times with 300 µl - 400 µl diluted Wash Solution per well.
8. 100 µl of (Substrate Solution*)* added to each well.
9. Incubated for 15 minutes at room temperature.

The Stop solution (100ml) added to stope the enzymatic reaction.

With a microtiter plate reader .The absorbance (OD) of each well at 450 determined.

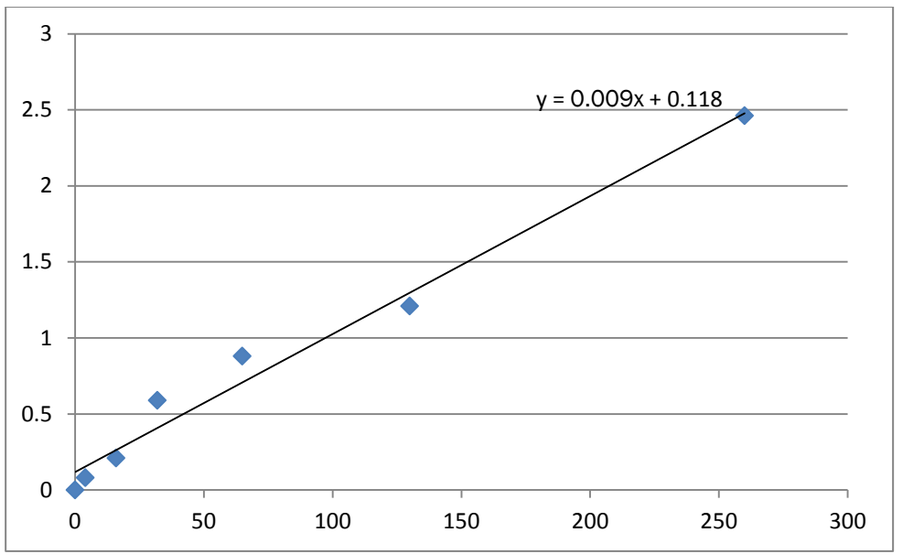


Figure 2.2 Standard curve of SHBG concentration in nmol/l

2.4.3 Anti mullerian Hormone:-

Principle of the test:-

The assay utilizes the competitive restraint ELISA technique. A monoclonal antibody specific to AMH has been pre-coated onto a microplate. A competitive inhibition reaction is propelled between biotin labeled AMH and unlabeled AMH (Standards or samples) with the pre-coated antibody specific to AMH. After incubation the unbound conjugate was washed off. At that point, the reaction between conjugated and Horseradish Peroxidase (HRP) is turned around corresponding to the levels of AMH in the sample. After addition of the substrate solution, the intensity of color created is invert corresponding to the levels of AMH in the sample (Uysal et al., 2017). .

Assay steps:-

1. 50µLof standard & sample was added to each well.

2-50µLof prepared Reagent A added immediately, and incubated for 1 hr at room tempreture;

3. Aspirated and washed 3times;

4. 100µLof prepared Detection Reagent B was added

& incubated 30 minutes at 37^{o}C; aspirated and washed 5 times;

5. 90µL Substrate Solution. Added and Incubated 15-25 minutes at 37^{o}C;

6. 50µL Stop Solution added. Read at 450 nm directly.

2.4.4. Follicle stimulating hormone:-

Principle of the test:

The Follicle stimulating hormone concentration was detected by using ELISA technique.The microplate wells were couted with a mouse monoclonal antiαFSH antibody & another mouse monoclonal anti B-FSH antibody HRP conjugated solution were used.The test sample were permitted to react with antibodies , coming about the FSH molecules being sandiwiched between solid phase and enzyme linked antibodies. The wells washed after 45minute incubation, at that time the unbound antibodies were removed.The solution of TMB reagent was added & incubated for 20 minutes.The blue colour developed, the colour advancement is ceased by adding the stop solution & the colour changed to yellow, which is measured by microplate reader at 450 nm (Berger et al.,1996).The FSH concentration in the samples were calculated

Assay steps

1- The wanted number of coated wells in the holder were Secure.

2- Fifty Ml of standard, specimens, and control added to each wells

3- 100 Ml of of Enzyme conjugate added to each well, shacked and incubated at room temperature for 45 minutes.

4- The microtiter was washed 5 times with distilled water

5-100 Ml of TMB reagent dispensed to each well, and it incubate in the dark 20 minutes.

6- The reaction stopped by adding 100 Ml of stop solution to each well.

7-The optimal density at 450 nm read by microtiter plate reader within 15 minute.

2.4.5 Luteinizing hormone:-

Principle of the test:-

The principle of the test is just like that of FSH hormone but the microplate well coated with a mouse monoclonal anti α-LH antibody and another mouse monoclonal anti β-LH-antibody in the antibody enzyme (horseradish peroxidase) conjugate solution (Berger et al.,1996).

Assay steps:-

1- Fifty Ml of standard, specimens, and control added to each wells

2- 100 Ml of Enzyme conjugate added to each well, shacked and incubated at room temperature for 45 minutes.

3- The microtiter was washed 5 times with distilled water

4-100 Ml of TMB reagent dispensed to each well ,and it incubate in the dark 20 minutes.

5- The reaction stopped by adding 100 Ml of stop solution to each well.

6-The optimal density at 450 nm read by microtiter plate reader within 15 minute.

2.4.6. Estradiol hormone:-

Principle of the test:-

This assay is depends on the Rivalling binding ELISA technique.The microplate wells coated with the gout anti-mouse antibody which bound to estradiol specific monoclonal antibody.The compitision started between estradiol in the sample and with fixed amount of HRP for the sites of the monoclonal antibody.The excess conjugated & sample removed by washing .The estimation of enzymatic activity is done by adding the substrate solution to the wells.

The color pregression is ceased and the absorbance is read at 450 nm. The power of shading is conversely related to the centralization of Estradiol in the sample (Niravath et al.,2017).

Assay Steps

1-400ML of the sample and 100 ML pretreatment E added to a microcentrifuge tube.

1-100 ML of estradiol primary Antibody solution added to each well & incubates for 1 hr.

2- After four times washing 100ML of standard, control added to each well

3-100 Ml of calibrator diluent added to zero standard

4-50 ML of estradiol conjugate was added to all wells, & incubated for 2 hrs at room temperature.

5-After washing 200 ML of substrate solution added to each well, incubated for 30 minutes in dark place at room temperature.

6-100 ML of stop solution added to each well, the color turned yellow.

7- The optimal density of each well determined at 450 nm.

2.4.7 Free Testosterone Elisa kit:-

Principle of the test:-

This assay is depends on the Rivalling binding ELISA technique.The microplate wells coated with the gout anti-mouse antibody which bound to Testosterone specific monoclonal antibody.The compitision started between fre e testosteronein the sample and with fixed amount of HRP for the sites of the monoclonal antibody.The excess conjugated & sample removed by washing .The estimation of enzymatic activity is done by adding the substrate solution to the wells.

The color pregression isceased and the absorbance are read at 450 nm. The power of shading is conversely related to the centralization of free testosterone in the sample (Lee &Chang., 2003).

Assay steps

1- 50 ML of primary Antibody solution added to each well (expect NBS well) incubated for 1 hr at room temperature.

2-After four times washing 100 ML of calibrator Diluent RD5-48 added to the NBS wells & 100Ml of Calibrator diluent RD5-48 to the zero standards

3-100 ML of standard, control, and sample added to the remaining wells.

4-50 ML of the testerone conjugate added to each well & incubated for 3hrs at room temperature.

5-After washing 200ML of substrate solution added to each well & incubated for 30 minutes at room temperature in dark.

6-50 ML of stop solution added to each well the color changed to yellow.

7-The optimal density determined by microplate reader set to 450 nm.

2.5 Statistical Analyses

In this study, the values presented as (mean ± SD) and the Kolmogorov-Smirov test was used to test the normality of distribution. A student t-test was used to compare the mean of the two group means.Chi squared test were used when appropriate. One-way analysis of Variance (ANOVA) was performed to estimate the differences between the groups. Then, Tukey's post-hoc test was used to evaluate the relationship between the two groups. Lastly, the calculation of correlation and Pearson correlation were performed for assaying the correlation between kisspeptin and other biochemical markers in both diseases and healthy women, respectively. P values bellow 0.05 considered significant.

Mann Whitney test used for estimation of estradiol hormone in different studied groups

Chapter Three

The Results:-

The results presented in this chapter were based on the statistical analyses of a case-control study that involved 120 women attending a family planning center at Azady Teaching Hospital. Their ages were ranged between 20-40 years.

3.1 The distribution of study population according to demographic data.

The study groups were divided in to three groups. The first group which involved 60 infertile women with polycystic ovarian syndrome, they diagnosed by clinical, biochemical & ultrasound examinations. The second group included 20 infertile women with ovulatory dysfunction. The third group involved 40 fertile women with a regular menstrual cycle.Their were in significant different in BMI among the studied group.Most of the participants were from inside Kirkuk.Table (3.1).

Table 3.1 shows the distribution of studed group according to demographic data

	Infertile women with PCOS	Infertile women without PCOS	Control	P Value
Number	60	20	40	
BMI Kg/m^2	26.05±3.76	25.93±3.7	25.88±4.01	NS
Mean age Years	30±8.6	38.14±7.7	31±7.1	P<0.05
Residence Inside Kirkuk Outside Kirkuk	 35 25	 16 4	 54 6	P>0.05
Occupation Employer Nonemployee	 15 45	 5 15	 36 4	p<0.05

3.2 Distribution of infertile women according to clinical feature, type of fertility, duration of marriage, duration of infertility& type of menstrual cycle.

More than half of involved infertile women were suffered from primary infertility.The duration of marriage in 43 infertile PCOS women were more than 5 years. Moreover, the duration of infertility in most cases was more than 2 years. Most of infertile women suffered from oligomenorrhea Table (3.2), fig (3.1).

Table 3.2 shows distribution of subfertile women according to the clinical features

	PCOS women	Non PCOS women	P value for Q^2
Type of infertility			
1-Primary	37	14	
2-Secondary	23	6	p>0.05
Abortion	8	3	
Pariety	15	3	
Duration of Marriage			
More than 5 years	43	9	P>0.05
Less than 5 year	17	11	
Duration of infertility			
More than 2 years	31	15	P>0.05
Less than 2 years	29	5	
Hirsutisim			P>0.05
Positive	22	5	
Negative	28	15	

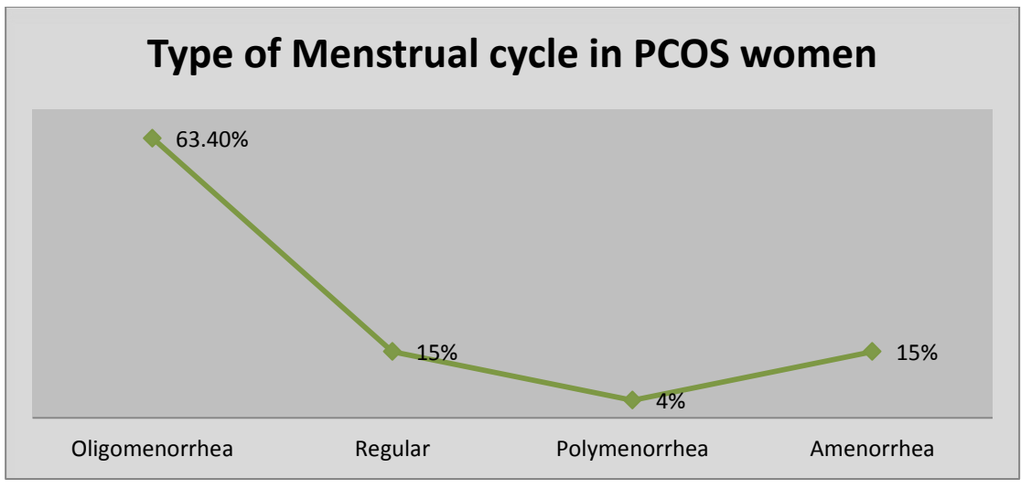


Figure 3.1 Show the distribution of type of menstrual cycle in women with PCOS.

3.3 Comparison between Biochemical markers & BMI in infertile PCOS women & fertile Women.

There were significant difference in serum level of kisspeptin, free testosterone; FSH and LH level between women with PCOS & control group ,while there were no significant difference in BMI between studied group p value ≤ 0.05 Table 3.3

Table 3.3 shows the difference between the biochemical markers & BMI in PCOS & control group

parameters	Infertile PCOSwomen (Mean ±SD)	Control (Mean± SD)	P value
Number of the subjects	60	40	
BMI (Kg/m^2)	26.05±3.76	25.93±3.7	NS
Kisspeptin (ng/ml)	1.79±0.98	1.05±0.86	P^1< 0.05
SHBG (nmol/L)	77.86±7.1	80±2.07	NS
Free Testosteronen(g/ml)	0.66±0.75	0.37±0.22	< 0.05
Estradiol(pg/ml)	47.76±42	45.85±15	P^2 =0.003 Z score=2.89
FSHmIU/ml	5.04±1.33	6.25±2	P^1< 0.05
LHmIU/ml	8.92±5.47	4.57±1.02	P^1<0.05

P^2= p value for Mann-Whitny test .P^1= p value for t test.NS=nonsignificant different

3.4 Kisspeptin, SHBG, Estrogen and Free testosteronere in overweight& non obese PCOS women:-

Table 3.4 showed that only SHBG serum level express a significant statistical difference between PCOS women with BMI more & less than 25kg/$m^{2.}$

Table 3.4 illustrates the comparison in serum (Kisspeptin, SHBG, Estradiol & free testosterone) between overweight & normal weight PCOS infertile women.

Parameters	PCOS BMI ≥25 Mean±SD	PCOS BIM< 25 Mean±SD	P value
Number of the subjects	37	23	
Kisspeptin(ng/ml)	1.59±0.82	1.89±0.9	P^1>0.05
SHBG(nmol/l)	66.09±5.8	72.4±4.64	P^1<0.05
Estradiol (pg/ml)	41.38±30.8	52.25±34	p^2>0.05
Free Testosterone(ng/ml)	0.67±0.54	0.81±1.23	P^1>0.05

P^2= p value for Mann-Whitny test .P^1= p value for t test.pvalue bellow 0.05 is significant

3.5 Kisspeptin, SHBG, Estradiol and Free testosterone in overweight & non obese fertile women:

As shown in Table 3.5 Estrogen serum level was significantly higher in fertile obese women. While, serum levels of SHBG were significantly higher in non-obese fertile women.

Table 3.5 illustrates the comparison in serum (Kisspeptin, SHBG, Estradiol & free testosterone) between overweight&non obese fertile women

Parameters	Fertile women BMI ≥25 Mean±SD	Fertile women BMI<25 Mean±SD	P value
Number of the subjects	23	17	
Kisspeptin(ng/ml	1.38±1.2	0.84±0.9	P^1>0.05
SHBG(nmol/l)	69±5.2	78±5.2	P^1<0.05
Estradiol (pg/ml)	77.1±23	45±30.1	P^2<0.05 Z score =2.1
Free Testosterone(ng/ml)	0.25±0.31	0.31±0.28	P^1>0.05

P^2= p value for Mann-Whitny test .P^1= p value for t test.pvalue bellow 0.05 is significant

3.6 Kisspeptin, SHBG, Estrogen and Free testerone in non-obese fertile women & infertile PCOS women.

As illustrated in Table 3.6 kisspeptin serum level was significantly higher in non-obese PCOS women in comparison with non- obese fertile women.However,SHBG was significantly lower in non-obese PCOS women.

Table 3.6 shows the comparison in serum (Kisspeptin, SHBG, Estradiol & free testosterone) in non-obese fertile & infertile PCOS women.

Parameters	PCOS women BMI < 25kg/m^2	Fertile women BMI<25 kg/m^2	P value
Number of the subjects	23	17	
Kisspeptin(ng/ml)	1.89±0.9	0.84±0.9	^{p1} =0.008
SHBG(nmol/l)	72.4±4.63	78±5.2	p1=0.009
Estrogen(pg/ml)	52.25±34	45±30.1	P^2=0.088
Free Testosterone (ng/ml)	0.81±1	0.31±0.28	P^1=0.122

3.7 Kisspeptin, SHBG, Estradiol and Free testosterone in overweight (fertile & Infertile PCOS) women.

Table 3.7 demonstrate that Kisspeptin and free testosterone serum levels were significantly higher in overweight/obese PCOS women in comparison with overweight/obese fertile women.While estrogen serum level was higher in fertile overweight/obese women.

Table 3.7 illustrates the comparison in serum level of (Kisspeptin, SHBG, Estrogen & free testosterone) between obese fertile & infertile PCOS women

Parameters	PCOS women BMI $\geq$ 25kg/m^2 Mean±SD	Fertile women BMI$\geq$25 kg/m^2 Mean±SD	P value
Number of the subjects	37	23	
Kisspeptin (ng/ml)	1.59±0.82	1.09±0.9	p^1= 0.03
SHBG (nmol/l)	66.09±5.8	69±5.2	NS
Estrogen(pg/ml)	41.38±30.8	77.11±23	P^2=0.003
Free Testosterone(ng/ml)	0.67±0.54	0.25±0.31	P^1=0.006

P^2= p value for Mann-Whitny test .P^1= p value for t test.pvalue bellow 0.05 is significant.NS=nom significant.

3.8 Comparison of Serum Kisspeptinin levels in different age groups:

3.8. 1- In infertile PCOS women

The infertile PCOS women were subdivided in to four subgroups according to their ages. There were no significant variations in Kisspeptin level among age subgroups (F =0.128, p=0.924). Furthermore, there were no significant changes in kisspeptin between age subgroups Table (3.8).

Table (3.8) shows serum Kisspeptin level in different age groups in PCOS women.

Age groups (years)	Number	Kisspeptin level ng/ml(mean ±SD)	P value
1- (20-24)	16	1.68±0.71	1&2 NS
2- (25-29)	13	1.71±0.78	2 &3NS
3-(30-34)	14	1.74±0.80	3 &4 NS
4- (35-40)	17	1.88±1.19	1&4NS 2&4 NS

One-wayANOVAdone for comparison among age groups & tukey test done for multiple comparisons.NS=not significant

3.8. 2- In Control (fertile) women

Unlike infertile PCOS women, the kisspeptin level showed statistical significant variation among age subgroups in fertile women (F= 3.2, p 0.03). Moreover, Kisspeptin level was significantly higher in group 1 in comparison to group 4(p= 0.0166).Table (3.9) fig (3.2), (3.3).

Table (3.9) shows serum Kisspeptin level in different age groups in fertile women.

Age groups (years)	Number	Kisspeptin level ng/ml (mean ±SD)	P value
1- (20-24)	11	1.03±0.81	1&2 NS
2- (25-29)	10	1.2±0.7	2&3 NS
3- (30-34)	9	1.3±0.3	3 &4 NS
4- (35-40)	11	2.01±0.9	1&4 p=0.0166

One-wayANOVAdone for comparison among age groups & post hoc tukey test done for multiple comparisons.NS=not significant

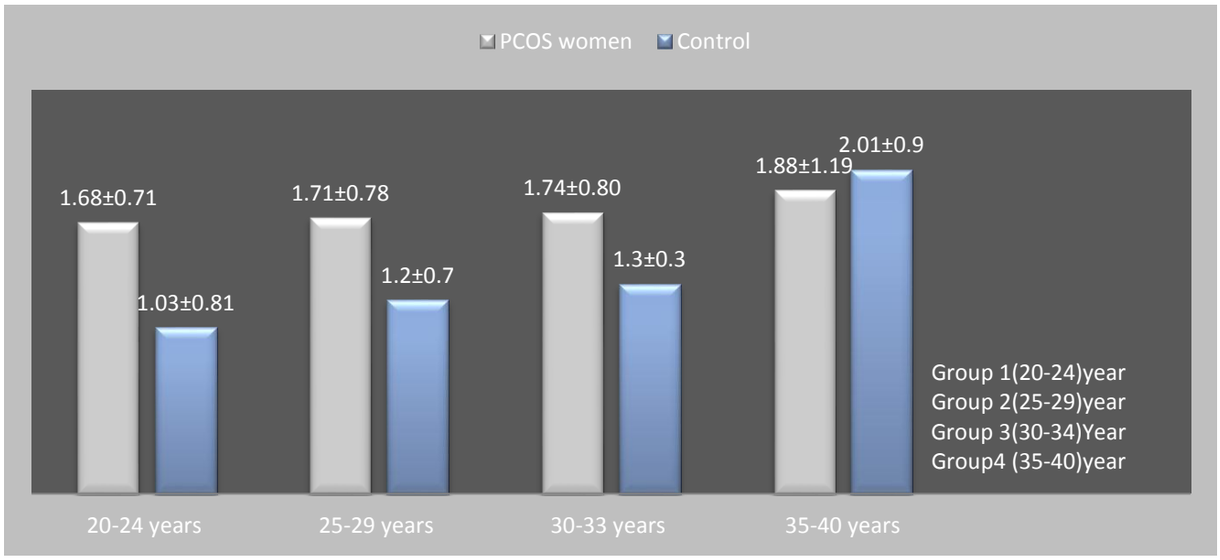


Figure (3.2) Shows the serum kisspeptin levels at different age subgroups in both groups of the study.There were insignificant statistical difference amonge age subgroups in women with PCOS .While in control women kisspeptin serum level in group 1 was significantly higher than group 4.p value <0.05. .

3.8.3 Association of Kisspeptinwith AMH & FSH:-

3.8.3.1- In infertile PCOS patient-

By using the correlation coefficient there was no significant correlation between Kisspeptin &FSH, kisspeptin & AMH. Table 3, 10

Table 3.10 shows correlation of kisspeptin with AMH& FSH in PCOS women.

parameters	Kisspeptin r value	P value
FSH (MIU/ml)	-0.01	0.46
AMH(pg/ml)	-0.11	0.35

3.8.3. 2- In fertile women: -

As shown in Table 3.11 & fig 3.4 there was a significant reverse correlation between kisspeptin serum level &AMH serum level, but the revese correlation between kisspeptin serum level& FSH serum level was statistically insignificant.

Table 3.11 shows correlation of kisspeptin with AMH& FSH in fertile women

parameters	Kisspeptin r value	P value
FSH (MIU/ml)	-0.12	0.35
AMH(pg/ml)	-0.33	0.03

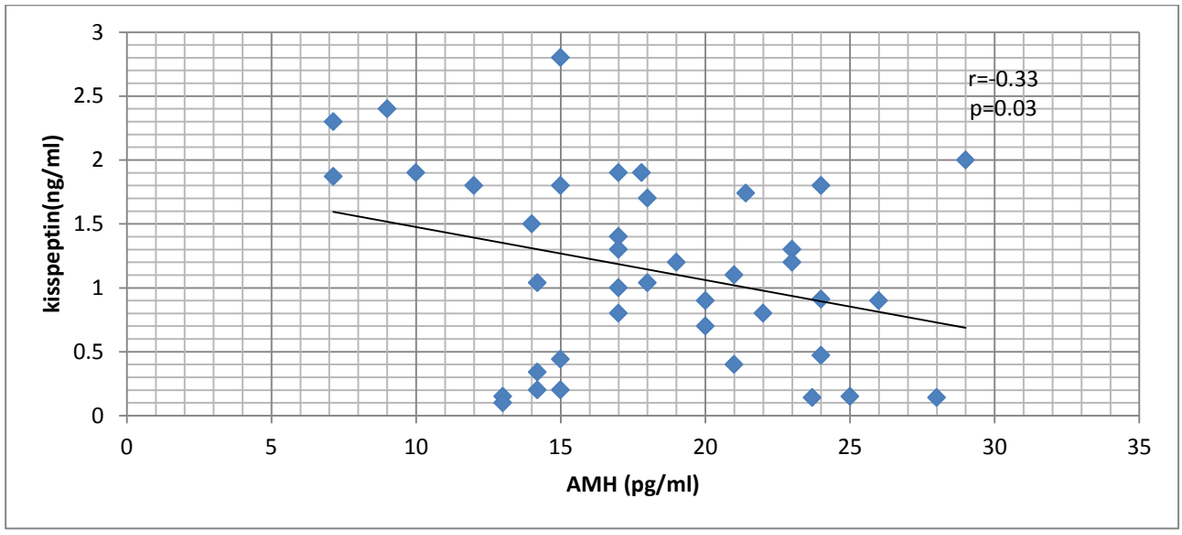


Figure 3.4 illustrates the correlation between Kisspeptin & AMH in fertile women

3.9- Association of SHBG with age, FSH & AMH: -

3.9. 1- In infertile PCOS women: -

No significant correlation between SHBG and (age, FSH, AMH) was found. Table (3.12)

Table 3.12 shows correlation of SHBG (as dependent variable) with (AMH, FSH and age) (as independent variable) in infertile PCOS women.

parameters	SHBG r value	P value
Age (years)	-0.187	NS
FSH (MIU/ml)	-0.02	NS
AMH(pg/ml)	-0.01	NS

3.9.2-In fertile women

There was a weak reverse significant correlation between age &SHBG .While no significant correlation between (SHBG&AMH) & (SHBG &FSH) was observed.
Table 3.13

Table 3.13 shows correlation of SHBG (as dependent variable) with (AMH, FSH and age)(as independent variable) in healthy women.

parameters	SHBG r value	P value
Age (years)	-0.33	0.02
FSH (MIU/ml)	-0.13	NS
AMH(pg/ml)	-0.11	NS

3.10 Correlation of Kisspeptin& SHBG with other biochemical markers in infertile PCOS women:-

There was a positive correlation between Kisspeptin and free testosterone (r= 0.26, p=0.04). While, the results showed no correlation between Kisspeptin and other parameters .Table (3.14) , fig (3.4)

Regarding SHBG there wasn't any correlation between the parameters and SHBG except for a very weak non significant correlation with BMI (r=- 0.162) Table (3.14), fig (3.5)

Table (3.14) shows correlation between Kisspeptin & SHBG with other biochemical markers in infertile PCOS women.

parameters	Kisspeptin ng/ml		SHBGnmol/l	
	r value	P value	r value	P value
BMI (Kg/m2)	-0.13	0.30	-0.162	0.10
LH(mIU/Ml)	0.02	0.8	0.001	0.49
Free testosterone (ng/ml)	0.26	0.04	-0.151	0.12
Estrogen(pg/ml)	0.145	0.26	0.035	0.39
SHBG(nmol/l)	0.078	0.58	------	----
Kisspeptin(ng/ml)	---------	--------	0.078	0.58

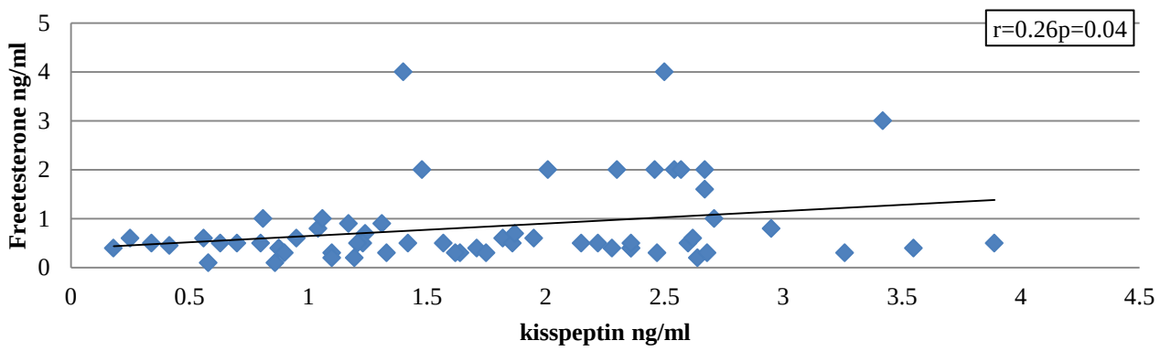


Fig (3.5) shows correlation between free testosterone & Kisspeptin in infertile PCOS women

3.11 Phenotypes of PCOS patients & their kisspeptin levels:-

The PCOS patients were sub divided into four subgroups according to clinical & biochemical markers Fig (3.6).

A: Ovulatory dysfunction + hirsutism or hyperandrogenism + PCO feature

B: Ovulatory dysfunction + hirsutism or hyperandrogenism

C-Ovulatory dysfunction +PCOS (no hirsutism, normal androgen)

D: Hirsutism or hyperandogenism +PCOS with normal menstrual cycle

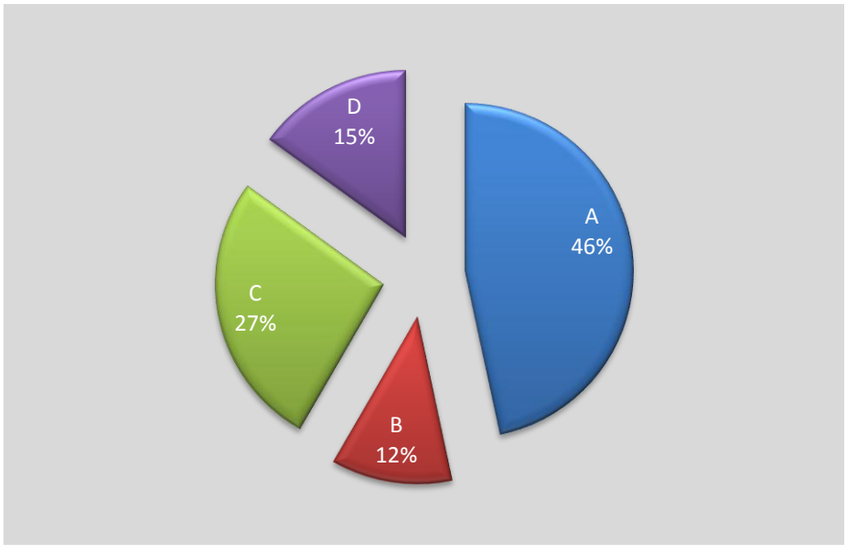


Fig 3.6 shows the PCOS phenotypesof patients involved in the study

The Kisspeptin level was higher in group A as compare to other subgroups but it was statistically insignificant. Table 3.15

Table 3.15 illustrates serum Kisspeptin level in PCOS patients' subgroups

PCOS subgroups	Number of the patients	Kisspeptin ng/ml	F value
A	28	1.92±0.96	F=2.48 P=0.07
B	7	1.71±0.8	
C	16	1.63±0.73	
D	9	1.04±0.65	

3.12 Sex hormone binding globulin in PCOSs subgroups:-

There were no significant difference in SHBG level among the subgroups (F=2.061 p=0.116), Table (3.16)

Table 3.16 illustrates serum SHBG level in PCOS patient's subgroups.

PCOS subgroups	Number of the patients	Kisspeptin ng/ml Mean& SD	F value
A	28	80±33.2	F=2.061 P=0.116
B	7	100±20.2	
C	16	70±14	
D	9	80±25	

3.13 Kisspeptin level in the follicular & pre ovulatory phase of the menstrual cycle:-

Both in fertile & infertile (PCOS) women blood was obtained at two periods of the cycle, the follicular & the pre ovulatory period. In PCOS infertile women the difference between Kisspeptin serum levels was not quite significant while, in normal women, the Kisspeptin level in preovulatory phase was significantly higher than Kisspeptin level in follicular phase Table 3.17.

Table (3.17) shows serum Kisspeptin level in (follicular & pre ovulatory) phase in both Infertile PCOS & control women.

Infertile (PCOS) women		Fertile (control) women	
Menstrual cycle phase	Serum Kisspeptin ng/ml Mean ± SD	Menstrual cycle Phase	Serum Kisspeptin ng/ml Mean ±SD
Follicular phase	1.79±0.98	Follicular phase	1.05±0.88
Pre ovulatory phase	2.1±0.9	Pre ovulatory phase	1.56±0.7
P^1 value	p>0.05	P^2 value	P<0.05

P^1= the difference between follicular phase & preovulatory phase in women with PCOS. p^2=the difference between follicular & preovulatory phase in control women.p<0.05 considared significant.

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3.14 –Comparison of Demographic &Biochemical parameters among the study groups:-

The study also involves 20 infertile women with anovulation; there was a significant variation in serum Kisspeptin level among the three groups of the study. Furthermore, estrogen, free testerone, FSH and LH also showed significant variations. Table 3.18

Table (3.18) shows comparison in age, BMI and other biochemical parameters among the three study groups.

Parameters Mean& SD	infertile PCOS women(1)	infertile Women with high FSH(2)	Fertile women(3)	F&P value
Number of the subject	60	20	40	
Age (years)	30±8.6	38.14±7.7	31±7.1	P=0.001
BMI (kg/m2)	26.05±3.76	25.93±3.7	25.88±4.01	NS
Kisspeptin ng/ml)	1.79±0.98	1.86±0.9	1.05±0.86	F=8.9 P=0.001
SHBG(nmol/l)	77.86±55.27	80±86	80.13	NS
Testosterone ng/ml)	0.66±0.75	0.30±0.09	0.37±0.22	F=4.6 P=0.012
Estrogen pg/ml)	47.76±42	63±30.1	45.85±15	F=2.09 P=0.019
FSH(mIU/ml)	5.04±1.33	13.15±0.09	6.25±12	F=10 P=001
LH(mIU/ml)	8.92±5.47	4.9 ±1.55	4.57±1.02	F=22 P=001

3.14.1 Comparison in biochemical markers between infertile women with high FSH & control women.

Serum Kisspeptin was significantly higher in infertile women in comparison with normal women $p < 0.05$. FSH & estrogen also were higher in infertile women .Table 3.19

Table 3.19 illustrates serum level of (kisspeptin, SHBG, free testosterone, estrogen, FSH and LH) in infertile women with high FSH & controlwomen.

Parameters	Infertile womenwith high FSHMean±SD	Healthy womenMean±SD	P value
Number	20	40	
Kisspeptin ng/ml	1.86±0.9	1.05±0.86	0.001
SHBG nmol/l	80.03±67	80±13	NS
Free testosterone ng/ml	0.30±0.09	0.37±0.22	NS
Estrogen pg/ml	63.075±30	45.85±15	0.002
FSH mIU/ml	13.15±0.9	6.25±12	0.013
LH mIU/ml	4.9±1.55	4.57±1.02	NS

3.14.2 Comparison in biochemical markers between Infertile & Infertile (PCOS) women.

There was no significant variation in both Kisspeptin & SHBG between the two groups. But, FSH was significantly higher in infertile women & LH and testerone in PCOS women. Table (3.20)

Table 3.20 illustrates serum level of (kisspeptin, SHBG, free testosterone, estrogen, FSH and LH) in infertile women with high FSH & infertile PCOS women.

Parameters	Infertile women Mean±SD	Infertile (PCOS) women Mean±SD	P value
Number	20	60	
Kisspeptin ng/ml	1.86±0.9	1.79±0.098	NS
SHBG nmol/l	80.03±67	77.86±55.2	NS
Free testosterone ng/ml	0.22±0.09	0.66±0.75	0.01
Estrogen pg/ml	63.075±30	47.76±42	NS
FSH mIU/ml	13.15±0.9	5.07±1.33	0.01
LH mIU/ml	4.9±1.55	8.92±5.4	0.01

Chapter Four

Discussion

4.1 Biochemical markers in both infertile PCOS & healthy women:-

4.1.1 Kisspeptin in PCOS & control women:-

The present study involved 120 women (60 infertile PCOS, 20 infertile with elevated FSH and 40 healthy women).The follicular phase serum Kisspeptin level was significantly higher in subfertile PCOS women as compared with normal women Table (3.3). In agreement with our result , increase serum Kisspeptin level in PCOS patients were observed in several studies (Jeon et al., 2013,Yimaz et al.,2014, , Presiyana et al., 2016, Yarmolinskaya et al., 2017, Gorkem et al.,2018).While , other studies didn't find this variation (Pandis et al., 2006, Yerlikaya et al., 2013, Emerkci et al., 2016& Albalawi et al., 2018). Penidis et al & Albalawi et al they used different PCOS diagnostic criteria. There was asignificant variation in age & BMI in Albalawi et al study. Panidis et al study was conducted on a small sample size that's why insignificant variation obtained. The largest study was conducted by Emerkci et al., using 250 women for controls (regular menstrual cycles) and relating each phenotype of PCOS to kisspeptin levels .There was no evidence of differences in serum levels between the study groups. A great flaw in their research was the non-inclusion of BMI and age in their pairings in contradistinction to the other studies.Recently, the discovery of Kisspeptin and its receptor paved away for investigations about its role in the pathogenesis of PCOS.

The pharmacokinetics of physiologic kisspeptin in humans and how typically adjusted concurring to disease state remains to be illustrated. But, it has been proven that any disturbance & change in the reproductive axis & hormones mirrors the kisspeptin serum level (Zhu et al., 2016, Demirbilek et al., 2012, Rhie et al., 2011). Bacopoulou et al., 2017 stated that the increased serum kisspeptin level in patients with ovarian disorder may be due to strong output from hypothalamus in order to maintain menstruation.

Kisspeptin cause up regulation of GnRH, it also exist in the ovary and involved in the ovulation and sex hormone regulation. Alteration in kisspepeptin secretion might result in a considerable commotion of the gonadotropic axis similar to that present in PCOS patients. As the PCOS is a heterogeneous syndrome, not all patients share the same endocrine and hormonal alterations. Serum kisspeptin level may be higher in phenotypes with ovulation

dysfunction & hyperandrogenism. As mentioned earlier, the involved infertile PCOS patients divided into four subgroups according to Rotterdam criteria, most PCOS patients in this study were those with ovulatory dysfunction & hyperandrogenism, normal kisspeptin in certain phenotype may disturb the result & obscure the variation.

Kisspeptin withen the ovary has critical transient and spatial specificity, recommending that the kisspeptin/ KISS1R system performs different functions at multiplet physiological stages in the ovary (Cao et al., 2019). Animal model with PCOS expressed high level of Kisspeptin m RNA in the ovarian tissue Hue et al., 2018). On the other hand, there is evidence of direct kisspeptin action on the ovary. In fact, there are kisspeptin receptors in the luteal granulosa cells of patients under ovarian stimulation protocol(Araujoet al.,2020),Where The use of kisspeptin-54 as an oocyte maturation trigger augmented expression of genes involved in ovarian steroidogenesis in human GL cells including, FSH receptor (FSHR), LH/hCG receptor (LHCGR), steroid acute regulatory protein (STAR), aromatase, estrogen receptors alpha and beta (ESR1, ESR2), 3-beta-hydroxysteroid dehydrogenase type 2 (3BHSD2) and inhibin A (INHBA) (Owens et al.,2018). Ovarian kisspeptin may also participate in PCOS pathogenesis but it's still unclear if it affects circulating Kisspeptin or not. .

4.1.2Sex Hormone Binding Globulin in PCOS and control women:

In the present study, there were insignificant variations in serum SHBG levels in infertile PCOS& control women Table (3.3).

Dissimilar to our result Jamil et al, 2015 found that serum SHBG levels were significantly lower in PCOS patients in comparison with healthy women. In accordance with this study, a meta analytic study done by Deswal et al 2018 they analyzed the results of 22 cross sectional and 14 case- control studies around the world , in almost all this studies the level of SHBG in PCOS patients were lower than control women . In the same line with these two previous studies Nadaraja et al, 2018 stated that all androgenic parameters except SHBG were higher in PCOS women. In the current study, there was no significant variation in BMI of involved women that is why no significant differences were observed. Body mass index is considered the major determinant of SHBG plasma concentration, with anegative correlation, that may be due to decrease insulin sensitivity & hyper insulinemia caused by obesity (zhu et al., 2019). Another reason is that the involved PCOS women in this study stopped taking medication for three months before they participate in the study. SHBG is very sensitive to

insulin sensitizing agents like (metformin, troglitazone) that had been found to elevate SHBG in PCOS patients (Mehrabian &Agghahi.,2013). In previous years the reverse relation between obesity, insulin resistance and SHBG had been observed. As well as, it had been found that increased insulin level lead to its synthesis suppression which may cause hyperandrogenic symptoms in PCOS patients (Dunaif et al., 1988, Nether et al 1991 and Davison et al., 2007).

4.1.3 Free testosterone in PCOS & control women:-

In the present study the level of free testosterone was significantly higher in subfertile PCOS women as compared with normal women. Table (3.3)

Hyperandrogenemia is the most typical hormonal alteration in PCOS women. Hyperandrogenisim can be detected biochemically by estimation of (Total testosterone, free testosterone, SHBG, androstenedion, 17-hydroxyprogesterone, Dehydro epiandrostenedion and free androgen index) (Deleo et al., 2016).

In agreement with our result Mustafa et al., 2017 also detected high free testosterone level in PCOS patients. Modulation of 17, 20 hydroxylase activities in the ovary are responsible for the regulation of androgen production in both theca and granulosa cells. In hyperandrogenic women there is relative inhibition of 17-20 hyroxylase activity which leads to an increase in 17 OHP and reduction in aromatase activity. Not only this but also androstendion is produced 20 times more in PCOS ovary (De Leo et al., 2016; Gilling-smith et al., 1994). Insulin resistance in PCOS patients also contribute to hyperandrogenism (Bremer & Miller et al., 2008).

4.1.4 Estradiol in PCOS & control women:-

Inspite the fact that, grunulosa cells of PCOS ovaries contain estrogen inhibitor that prevents aromatization even if the level of FSH is adequate. Also, the low estrogen level in PCOS women may contribute to the lowering the responsiveness of inhibin to FSH leading to disorder in follicular function. (Sanjay et al., 1996, Homer et al., 2017). But, in the present study serum level of follicular phase estradiol was significantly higher in PCOS patients (47.76±42 pg/ml) as compared to control women (45.85±15 pg/ml) Table (3.3). Al.Deresawi et al., 20 15 & Al.Mhana. 2011 they also found that serum level of estrogen in PCOS patients was significantly lower than normal women. On the other hand, in agreement with our result Koppali et al., 2017 demonstrated that estrogen level like androgens is higher in PCOS women. Elevated LH in PCOS women may contribute to estrogen & androgen

elevation, where PCOS ovaries are more active than normal ovaries; this activity may lead to overproduction of both estrogen &testosterone. It is worth to mention that, the most predominant biochemical markers in assessing PCOS include androgens, gonado tropic hormones and insulin- resistance related markers. Estrogen levels may be normal in PCOS patients (Chang & Katiz 1999).Its worth to mention , we measured follicular phase esradiol level,where Estradiol levels are constantly in the early to mid follicular range without the normal mid-cycle increases (Dumitrescu et ai.,2015).

4.1.5 FSH & LH in PCOS & control women;-

In this study FSH levels were lower in PCOS women but LH levels were higher Table (3.3). Normally FSH level raise at the beginning of the follicular phase under the effect of GnRH, it stimulates follicles bellow (6-8mm), when these follicles grow it become under the estrogen effect. In PCOS ovaries high frequency and amplitude of GnRH were observed ,this elevation mirrored by LH elevation that causes early luteinisation of granulosa cells of immature small follicles and growth arrest favoring the greater production of steroid hormone that negatively block FSH production.(Mc Kenna et al., 1988, Yen et al .,1999, Deleo et al 2016).

Elevated androgen level wouldn't suppress LH secretion because androgens are not under the neuro- endocrine negative mechanism. Yet, recently it has been observed that androgen hormones also contribute to positive feedback in the follicular phase that causes LH surge; the result is a ceaseless cycle with high LH & androgen secretion (de Melo et al., 2015)

The Neuroendocrine disorders in PCOS patients affect the afferent upstream of GnRH , high androgen level participate in progesterone desensitization and alteration in the negative feedback of estrogen thus elevating LH level. Increasing androgen level in PCOS patients is the main etiological factors in a disturbing hypothalamic pituitary- axis (Witchel & Sempere 2013) .

4.2 Kisspeptin & obesity:-

4.2.1 In fertile women:-

In the present study the serum level of Kisspeptin in over-weigh/obese and non-obese fertile women was insignificant Table (3.5).

This result is in agreement with those of (Rafique & Latif 2015) who obtained almost the same result in normal & overweight Saudis women. Our results also go on line with (Pita *et*

al., 2011) who didn't find any correlation between BMI & Kisspeptin. While Koladzeijski e al., 2018 find that level of Kisspeptin was significantly higher in normal- weight women as compared to obese women, they also demonstrate that there were negative correlations between Kisspeptin & BMI. The reason beyond this decrement may be due to the fact that the obese women in Koladzeijski study were with BMI greater than 30.In this study the BMI of both groups was less than 30. Bacopoulo et al., 2016 stated that in Anorexic adolescent's girls' serum kisspeptin levels were correlated negatively with BMI but there was insignificant variation in kisspeptin level between anorexic & normal- weight girls. This infers that the relation between kisspeptin & BMI may be more obvious in overweight & obese women

Human reproductive function is affected by both extreme nutrition –under nutrition and obesity, kisspeptin is responsible for conveying metabolic information into brain centers that are responsible for reproductive regulation. Kisspeptin receptors also present in non- GnRH brain areas also in peripheral tissue like adipose tissue. The expression of kiss mRNA and GnRH secretion is reduced in fasting mice. Furthermore, High fat diet increases its level (Castellano et al., 2005, Li et al., 2012) .

Kisspeptin levels may be affected by other metabolic and hormonal disorders that company obesity for instance leptin level. Leptin levels increase with increasing body fat, the relation between kisspeptin and leptin had been under the spotlight where female mice lacking kisspeptin signaling displayed higher BMI and leptin levels. Not only have that, but the mutation in leptin receptor caused hypogonadisim in humans. (Tolson et al., 2014, Farooqi & Rahitly 2009). The leptin responsive GABA n ergic neuron may regulate reproduction function by conveying signals of energy balance through kisspeptin neuron, GnRH neurons do not express leptin receptor but it found that kisspeptin neuron expresses this receptor.Yet; knocking out leptin receptor wouldn’t prevent reproduction. (Clark et al., 2015,Chehab, 2014). Zhu et al., 2016 found that both serum kisspeptin & leptin are correlated positively with BMI in 647 chines children & adolescents in the different pubertal stages. In the same line, a strong positive correlation between kisspeptin & liptin was observed in overweight females (Rehman et al.,2018). Rafique & Latif, 2015 returned the association between obesity and kisspeptin to alteration in plasma triglyceride level; they believe that hypertriglycemia induced lipotoxic inflammation in the hypothalamus. In parallel with these Wu et al., 2012 & 2013 found kisspeptin 10-injection in bird liver markedly increase lipid anabolism also, it can elevate the levels of TG & LDL –C in primary cultured hepatocytes of

chickens. Finally interplay between kisspeptin & other metabolic & hormonal parameters may be more important than the body fat mass &BMI.

4.2.2 **Kisspeptin & obesity in PCOS patients: -**

The result of this study showed that there were insignificant variations in serum kisspeptin levels between normal weight & (obese, overweight) PCOS women.Table (3.4). This result is in the same line as Nyaglova et al., 2018, Jeon et al., 2013 and Yerlikaya et al, 2013). Also, they found no relation between BMI and kisspeptin in PCOS patients. But Pandis et al., 2006 demonstrated that Kisspeptin in PCOS women was negatively correlated with BMI & insulin resistance, the numbers of involved women in the Pandis study were only 19; correct estimation cannot be driven by this number. Also the kisspeptin levels were significantly and inversely correlated with IR. Women with PCOS and BMI >25 kg/m^2 being more insulin-resistant with no lean healthy women being included in the control group might have led to an overall increase in IR with a resulting decrease in kisspeptin levels (Umayal et al.,2019). Interestedly in this study, the levels of kisspeptin in lean PCOS patients were significantly higher than non-obese healthy women Table (3.6) suggesting that PCOS is the main etiological factor in raising serum kisspeptin. Several other metabolic markers, the plasma concentration of testosterone, dehydroepiandrosterone sulfate, free androgen index, leptin, retinol-binding protein, high density lipoprotein and sex hormone binding globulin, have been reported to be positively correlated with kisspeptin but It seems that kisspeptin level is not associated with BMI because only one study found that it is negatively correlated with kisspeptin in logistic regression analysis (Tang etal.,2019, Araujo et al.,2020).

4.3.1 SHBG & obesity; -

When the study participants divided according to their BMI, the serum level of SHBG in women with BMI ≥25 was significantly lower than women with BMI <25 in both fertile & infertile (PCOS) women.

These results agree with Akin et al., 2008 who demonstrated that premenopausal women with low SHBG were those who had BMI more than 30 kg/ m2. Also, Dahan & Goldstein, 2006 who measured the serum level of SHBG in obese PCOS & healthy overweight women, they didn't find a significant difference. In the same line, Franik et al 2018 found that PCOS patients with WHR (Waist to hip ratio) more than 0.8 showed lower SHBG serum levels than those with WHR less than 0.8. Futhermore, both overall and central obesity were significantly associated with having an irregular menstrual cycle. This association was

substantially influenced by hormonal factors, particularly SHBG (Wei et al., 2012).

Obesity is related to type 2diabetus, insulin resistance, hyperlipidemia and, hyperglycemia. Serum insulin had been found to inhibit SHBG production in liver cells. Moreover, in vitro studies illustrated that insulin decrease SHBG in cultured liver cells. Treatment with Diazoxide had found to elevate serum SHBG levels (Plymate et al 1988, pasquali et al.,1995). Recently it is believed that SHBG is not just a steroid transporter but it also has several receptors in different target tissue suggesting that the physiological role of SHBG is more complex than what is believed previously (Mohamed et al., 2016). It is critical to understand the impact of obesity as a risk factor for serum SHBG level s in women with PCOS. A low serum SHBG level in PCOS patients is not only an important influencing factor for hyperandrogenemia but is also an important predictor of insulin resistance, as well as a risk factor for glucose and lipid metabolism disorders. Serum SHBG is associated with complications and long -term prognosis in PCOS and it play s an important role in the pathogenesis of PCOS (zhu et al., 2019). .

4.3.2 Estradiol & obesity:-

In premenopausal women, estrogen is produced primarily in the ovaries, corpus luteuim & placenta a small but significant amount of estrogen can be produced by the liver, heart, skin & brain. The enzyme aromatase is responsible for the last step in estrogen synthesis. Aromatase is a member of cytochrome 450 and it's widely expressed in many sites, including liver, gonads, blood vessels, bone & adipose tissue (Santen et al 2009). .

In postmenopausal women estrogen is largely produced by adipose tissue, serum level of estrogen is significantly higher in obese women as compare with normal weight women. A high estrogen level in obese women make adipocyte less sensitive to the lipolytic effect of estrogen or obesity cause high aromatase activity, then subsequently leads to higher estrogen level and possibly estrogen resistance (Colleluri et al 2018). .

In our study estrogen levels in overweight fertile patients were significantly higher than normal-weight women Table (3.5); this result is contrastd with Freeman et al., 2010 who found that estrogen levels are higher in thin women. However Akin et al., 2008 & Pasquali et al., 1997 stated that Low level of SHBG in obese premenopausal women correlates positively with estrogen level. But, Ziomkiewicz et al., 2008 demonstrated that women with medium fat

mass were those with higher level of estrogen in comparison with high and low-fat mass, this go's online with our result where the mean BMI of involved fertile women was less than 30. Moderate fat accumulation (gluteofemeral fat) that is increased by estrogen hormone is necessary to increase metabolic profile, decrease inflammation & lower cardiometabolic risks (Leeners et al 2017). Its worth to mention the pregnancy outcames in assisted reproduction techniques is higher in women with BMI ranged between 23-24.9 (Rehman et al., 2018) .

4.5 Kisspeptin & age:-

4.5.1In fertile women-

In the present study both Fertile & subfertile women were divided into four age sub- groups. In control women, serum follicular phase kisspeptin level was significantly higher in subgroup 4 (35-40Year) as compared with subgroup 1 (20-24 year). Table (3.9).Furthermore, a positive significant correlation between kisspeptin & age were obtained .Table (3.11)

As far as we are aware, this is the first study that compared the serum level of kisspeptin in different age groups in premenopausal women. For estimating the effect of age on kisspeptin and its neurons animal studies were conducted. In ovarictomized lab animals the neurons that express KISS1 & estrogen receptor mRNA in the arcuate nucleus hypertrophied at late reproductive age which cause kisspeptin elevation.(Kinoshita et al.,2005, Rance & Bruce et al.,2004, Roaj et al., 2006, Rance et al., 2009).

As it mentioned in the preceding chapter, there are two areas in the hypothalamus that secret kisspeptin. The first area is the AVPV nucleus that's involve in positive feedback .While, the second area is ARC nucleus which is involved in the negative feedback by estrogen through ERα. Alteration in estrogen level at late and end reproductive age may participate in elevating the kisspeptin level.

The same phenomena may be also true for humans. The kisspeptin level fluctuates during different stages of reproductive life; its level dramatically increases during pregnancy due to placental and fetal secretion of kisspeptin (Guimiot et al., 2012, Desroziers et al., 2012). Moreover, its level declines during lactation. It found that kisspeptin neuron express prolactin receptor, where kisspeptin administration during lactation can restore ovulation (Scott & Browch, 2013, Brown et al., 2014). In the time of puberty, there is an increase in the number and activity of kissneurones. Both negative & positive kisspeptin feedback occurs in

infundibular nucleus, Yet KISS1 expression in the infundibular nucleus has been shown to increase after menopause (Kauffiman et al., 2010, Rometo et al., 2007).

In the present study we observed the elevation of serum, kisspeptin in women below 40 years old. There are several factors that will affect reproductive age in women for instance time of menarche, genetic factors, gravidity and lactation. In spite of all healthy women in this study were with normal menstruation and normal FSH level but the fertility and hormonal changes may be observed in the premenopausal period The level of estrogen may be normal or increased during this period (Weis et al., 2004). If women more than 40 years old were included in this study, the association between age & kisspeptin would be clearer. Many factors may lead to that. The most important factors are that the kisspeptinogeneic cells in the nfundibular nucleus hypertrophied at this age. Also, dynorphin-secreting mRNA neurons are decreased (Rometo et el., 2007). Increase in kisspeptin levels may occur due to the lack of estrogen feedback (Rance., 2009). Other studies proposed that this increase is related to an increase in ovarian sympathetic and adrenergic stimulation that occurs with aging and cause inovulation and kisspeptin elevation (Heider et al., 2001, Acuna et al., 2009, Ricu et al., 2012).

4.5.2-In PCOS patients:-

There were no statistical changes in serum kisspeptin level in different age groups in PCOS patients. Table (3.8).

This result agrees with (Jeon et al., 2015, Emekci etal. 2016 Gorkem et al., 2018).They didn't observe any correlation between age & kisspeptin in PCOS patients. The endocrine& hormonal alterations in PCOS patients may bestrew the normal changes in kisspeptin that occur with ovarian aging.

In the present study AMH correlated weakly (r= - 0.33) with kisspeptin level in healthy women, While no correlation observed in infertile PCOS women. Table (3.9).

AMH is a marker of ovarian reserve; recently it found that its level in PCOS patients is higher than normal women. Piltonen et al., 2005 found that in PCOS women its level remain highs even in the late stages of reproduction. Locally Marbut et al., 2012 found that age-related changes in serum AMH level were insignificant in PCOS patients. But koutlaki et al., 2013 demonstrated that the negative correlation between AMH & age can be detected in PCOS women. This inconsistency may be related to the fact that, some hallmarks of PCOS affect AMH serum level more than others. It had been showed that AMH correlate positively with

total testerone, score of hirsutisim &the antral follicular count (Mahran et al., 2015). In the same line Nardo e*t al*., 2009 found that in PCOS women AMH is related to insulin resistance & androgen but this effect appears to be independent of age.

Regarding normal women weak negative correlation AMH & kisspeptin obtained in this study Table (3.11), this is in agreement with (Mehri et al., 2016) they quantified KISS1, Kiss by using real-time polymerase chain reaction. They obtained their samples from ovaries of young and old mice and, cells from cumulus & mural granulosa of women who underwent IVF were examined .They found that KISS1 & kissr mRNA was significantly higher in older mice than younger one. In the same line, fernadois et al., 2016 also assert this finding they demonstrated that kisspeptin changes inordinate way with epinephrine which increase naturally with aging, they also found that administration of kisspeptin caused an increase in serum AMH. Bneath ordinal nutrient supply, the administration of kisspeptin in the ovary can cause basic changes in the follicles, and these structural changes can be turned around by the administration of the kisspeptin opponent peptide 234 (P234). Besides, kisspeptin administration increments plasma anti-Mullerian hormone (AMH) in 6- and 10-month-old rats. Moreover, P234 administration reduces plasma AMH levels in rats (Baarends et al., 1995). Not only have that, kisspeptin can block the increase in follicle stimulating hormone receptor (FSHR) expression by isoproterenol (ISO, a β-adrenergic agonist). Collectively, kisspeptin adversely directs the development of preantral follicles by actuating thesynthesis of AMH and decrease the affectsbility to FSH by repressing the induction of FSHR expression by the sympathetic activator, in this manner, reducing the recruitment of primary follicles (Cao *et al*., 2019)

4.6 -SHBG & age:-

SHBG correlate negatively and significantly with age r= - 0.33in healthy women tab (3.13), But no correlation observed in infertile PCOS patient.Table (3.12).

The relation between SHBG & age were exposed previously (Anderson et al., 1974, Harman et al., 2001). It also found that its level decline with steroid decline. SHBG is lower in women's in the thirties than those in the twenties. Also, there is a notable decline in its serum level in premenopausal women.(Davidson et al., 2005, Elmlinger et al.,2005 , Maggio et al., 2008).The major alteration in SHBG level occur beyond age 40 exactly at 45 years old ,for that reason a weak correlation between age & SHBG observed in this study .

The bioavailability of steroid hormones depend on the level of SHBG, any decrease in estrogen or androgen may mirror the decrease in SHBG level. Oral Estradiol administrations

elevate its serum level in postmenauposal women. (Dowsett et al., 1985, Ropponen et al., 2005)

No correlation between AMH &SHBG observed .table (3.12). Song el al., 2017 also didn't record any correlation between them. Both AMH & SHBG levels decline with aging, AMH decrease steadily with age, it shows a significant decrease at age 25 and a dramatic decline at age 35.While SHBG level change after age 40. (Maggio et al.,2008)

In infertile PCOS patients, there was no significant correlation between age &SHBG table (3.12). The endocrine disturbances in PCOS patients affect the SHBG level. Hyper androgenesim & insulin resistance lower its level even in young patients. Recently it found that women in the classic phenotype of PCOS exhibit the lower level of SHBG (Nardo et al., 2009, Yan Yue et al., 2018, Song et al., 2017).

No correlation between AMH & SHBG was observed in PCOS patients. AMH in PCOS women is affected by androgens, insulin resistance, and basal follicular status. But it mostly increased due to an increase of follicles beyond 5 mm. Diffrent mechanisms cause alteration in SHBG & AMH level. Women with poly cytic ovary morphology had higher SHBG and AMH than PCOS women (Song et al., 2017)

4.7-Relation between Kisspeptin and other biochemical markers in subfertile PCOS women:-

In the present study Kisspeptin level correlates significantly with only free testosterone level (r= 0.26, p= 0.04). Table (3.14).fig 1

Kisspeptin is a key regulator of GnRH secretion .It also regulates steroid hormones .It is a crucial element in modulating LH surge & ovulation. (Hrabovsky et al., 2014, Meczekalski et al., 2006, Roa et al., 2009, yenoyama et al., 2009).

In PCOS beside steroidal alteration, there is a disturbance in the hypothalamic –pituitary ovarian axis that may affect LH & FSH secretion. In the present study both kisspeptin & LH were higher in PCOS patients but we didn’t find any correlation between them. While, the positive correlation observed in other studies (Nyagolova et 2016, Emerkci et al., 2016).

Regarding free testosterone Gorkem et al., 2017 also found a positive correlation between kisspeptin &testosterone. Moreover, peripheral administration of kisspeptin in adult mice caused a dramatic increase in the free testesterone level. In disagreement with Iwata et al., 2017 found that hyperandrogenenesim in adult rats caused a decrease in the number of KISS

1 expressing cells in both AVPV & ARC nuclus they also suggested that the ARC nucleus that contains estrogen receptor also have androgen receptor which can be affected by excess androgen & cause negative feedback to kisspeptin. Many shred of evidance in human &animal had been collected & accented that hyperandrogenim lead to Increase LH/GnRH secretion (Barnes et al., 1994, Abboett et al., 2013, Padmanabhan & veigar Lopez, 2012, Hogg et., 2012, Apter etal 1994). In Iwata study they used dehydrotetosterone which may act through estrogen negative pathway, not through the androgen pathway.

4.8 Circulating kisspeptin level in different PCOS phenotypes:-

In the present study, the Infertile PCOS patients divided into four subgroups according to the Rotterdam criteria Table (3.15). Kisspeptin level in group A was higher than the other groups but this variation was not statistically quite significant p= 0.07 Table (3.15).

We expected that serum kisspeptin level in the group D (normal androgen) would be statistically lower than the other groups but due to small sample size & inequality in subgroups sample size. Also, the false positive sonography in the detection poly cystic ovary may contribute to this insignificancy. In line with our speculation (Jamil et al., 2015, Yue et al., 2018) found that the normal androgen phenotype expressed the milder endocrine & metabolic abnormalities. In this study, Most PCOS patients have ovulatory dysfunction and hyperandrogenism. Recently, a variety of experimental animal studies have been conducted to estimate KISS1, KISS r and KISS positive cells in different PCOS induced phenotypes. Aliabadi et al., 2017 & Matsuzaki et al., 2017 found that kisspeptin positive cells & kiss mRNA expression in letrozol injected female rates were higher than normal rats. Additionally, they demonstrated that the enhanced neural cells in PCOS rates might contribute to the hypersecretion of LH. Similarly, Kondo et al., 2016 who studied ARC kisspeptin immune reactivity by using anti-progestin R4486, found that besides the increase in the LH level, there was also an increase in the number of kisspeptin positive cells in the hypothalamus. On the other hand, Marcondes et al., 2017& Iwata et al., 2017 didn’t agree with this. They suggested that low kisspeptin expression in testosterone-treated rats might contribute to anovulation and decrease in LH secretion. However, Bayasula et al., 2016 found that rats treated by dihydrotestosterone showed normal LH levels. These inconsistencies in the effect of androgens on the kisspeptin expression are partly because of different methods employed to evaluate this impact. Besides, the difference in the age of experimental animals may contribute to this controversy. It is worth mentioning that in all previously mentioned studies that evaluate kisspeptin in different PCOS animal phenotypes increase kisspeptin

expression in the hypothalamus, were observed in animals with high LH levels. This is in line with our hypothesis that kisspeptin levels may show variation among PCOS phenotypes.

4.9 Sex Hormone Binding globulin in PCOS Phenotypes:-

In the present study there were no statistical differences in serum SHBG level among different PCOS subgroups Table (3.16).

Our result didn't agree with (Song et al., 2017; Danilowicez et al 2014) they found that normal androgen lean PCOS women had higher SHBG level in comparison with hyperandrogenised women .In the same line Jamil et al., 2015 they suggest that the alteration in the hormonal & other biochemical markers are self-evident in hyperandogenised PCOS women. On the other hand, Women with normal androgen may have low SHBG level. SHBG is a marker for androgen bioactivity, low SHBG and normal testosterone may indicate peripheral androgen activity (Danilowiceze et al., 2014).

In spite of the fact that both testosterone & SHBG are related to obesity and insulin resistance, but many other factors may be located in between, higher testosterone levels & lower SHBG levels were associated with lower adiponectin & higher leptin level in normal women, suggesting that adipose tissue hormones may involve in regulating the both hormones. (Wildman et al., 2012) .Women with normal androgen may have low SHBG level.

4.10 - Kisspeptin level in the follicular & preovulatory phase of both fertile & subfertile PCOS women:-

In the present study, there were significant variations in serum kisspeptin level in the preovulatory phase as compared with follicular phase, but this difference were not quite significant in subfertile PCOS women. Table (3.17).

In normal monthly reproductive cycle, there is an essential rise in FSH level that causes gradual follicular maturation. Before the follicles grow it start to secret estrogen that may suppressthe Kisspeptin level at this stage. This negative feedback may change to positive feedback during estrogen further raises and LH surge. Kisspeptin is an essential element in producing LH surge & ovulation. Not only that , its expression increase just before ovulation & LH surge.(Dugan et al., 2007; Clarkson et al.,2008; Smith et al.,2006).

Subcutaneous injection of kiss 54 in women with hypothalamic amenorrhea caused a potent increase LH & FSH level; this response is more pronounced in preovulatory phase (jayasena et al., 2009; Jayasena et al., 2011, Chan Y et al 2012). In another study the effect of kisspeptin administration in women with and without steroid supplements were investigated, the more potent effect observed in postmenopausal women with no estrogen supplements suggesting the involvement of estrogen in negative kisspeptin suppression (George etal.2012). Rafiq & Latif, 2015 also found that serum kisspeptin in healthy young women were significantly higher in the preovulatory phase.

Regarding PCOS patients the difference between follicular phase and preovulatory phase kisspeptin was not quite significant p= 0.07 (Table). The most common PCOS complication is anovulation, most of PCOS women are oligomeric, and besides this there is a disturbance in GnRH pulse secretion followed by the disturbance in LH & FSH secretion.

As far as we are aware, this is the first study that measured serum kisspeptin levels in two different phase of menstrual cycle in PCOS women. Recently (Katalalski et al., 2018 & Meczekalski et al., 2016) they evaluate the pairing between LH & kisspeptin in both PCOS & normal women They found that in normal women each kisspeptin pulse frequency is followed by LH pulse frequency. But this rhythm were not observed in PCOS women, where kisspeptin & LH was increased independently .Not only this but they also suggest that this irregularity worse when the disease progress.

Our results let us infer that the metabolic and endocrine disturbance that affected the follicular phase kisspeptin level it could be also affects its level in other phases of the cycle.

4.11 Kisspeptin level in women with high FSH level:-

The data obtained from the present study showed that serum kisspeptin were significantly higher in infertile women with elevated FSH level as compared with healthy womenTable (3.18).

In the presiding sections the crucial role of kisspeptin in LH surge & ovulation has been discussed .Ovulation can't occur without kisspeptin & its receptor even in the existence of GnRH secretion. Premature ovarian failure was induced by NTR2 and KISS1 insufficiency (Gyaton et al.,2014; Dorfman et al., 2014).

The stimulatory effect of GnRH on LH & FSH is different. FSH secretions are more complicated than LH. Kisspeptin increase both LH & FSH but its effect on LH is more conspicuous. (Skorupskait et al., 2014, Skorupdkait et al., 2018,Jaysena et al., 2011; Navaro

et al.,2005). Other factors may affect FSH secretion, for instance gonadal peptide (inhibin) that selectively inhibits FSH secretion (Babiker & Al shaikh, 2016).

Estrogen is involved in control both of FSH & LH. Kisspeptin neurons contain alpha & Beta estrogen receptor. Electrophysiological finding ,illustrated that estrogen decrease glutamatergic impute to ARC kisspeptin neurons .Not only that, in the female mice lacking kisspeptin specific estrogen receptor it increases the glutamatergic transmission to the same cells (Wang et al.,2018).

Estrogen negatively inhibits LH secretion through Beta estrogen receptor & positive feedback occurs through the estrogen alpha receptor. While it produce FSH surge by estrogen alpha receptor (Roa et al., 2008a, Roa et., 2008b). In the same line, In postmenopausal women treated with neurokinin receptor antagonist (neurokinin works with kisspeptin in up-regulation of GnRH) decrease LH secretion, but the FSH level remained high (Skorupskaite et al., 2018).

There are limited data about serum kisspeptin level infertile women (with ovarian causes) rather than PCOS women. Mumtaz et al., 2016 found that the serum level of kisspeptin in infertile women with unexplained fertility were lower than normal women. But we obtained a higher level in infertile women with high FSH level. The lower estrogen level may participate in the failure of kisspeptin suppression. When estrogen or estrogen with progesterone was given to ovarictomised monkeys, KISS mRNA reduced to near undetectable levels (Navarro et al., 2004, Smith et al., 2005a, Rometo et al., 2007, Oakley et al., 2009). Administration of kisspeptin to postmenauposal women caused a marked FSH increase in the absent of estrogen & progesterone, while this elevation were not quite significant in those using exogenous progesterone &estrogen (George et al., 2012).

In the present study there were significant difference in age between the third group & the first & second group this could participatetate in elevation of kisspeptin level.

Kisspeptin is located in different foci inside the ovary; it performs multiple functions at different physiological stages (Cao et al., 2019). Kisspeptin acts as a regulator for follicle development, it blocks the effect of adrenergic stimulation on FSHR.Increase in ovarian noradrenergic tone during aging could be complementary to putative increase in ovarian kisspeptin with age (Fernandois et al., 2016)

4.12 Study Limitation:-

This study has a few restrictions. The numbers of participants were not equal between the study groups; a larger sample size can give more precise results.

Another shortcoming of this study is those insulin resistances that involve several tests and biochemical parameters did not checke for PCOS patients. Insulin resistance is a commone hallmark for PCOS & it can have a relation with kisspeptin level.

Another limitation of this study; for hyperandrogenism assessment it was better to check all the types androgen hormones like (Total testosterone, androstenedion, 17 hydroxy progestrone, Dehydroepiandrostenedion & free androgen index).

Selecting patients who did not take medications were not easy. Some patients were hiding this fact which can negatively affect our results.

Chapter Five

Conclusions and Recommendations:-

5.1 Conclusions:-

1-In this study, we evaluated serum kisspeptin levels in healthy and PCOS women. The findings argued that the metabolic and hormonal disturbance in PCOS patients affects serum level of kisspeptin.

2-The results showed that kisspeptin serum level increases with aging. The author concluded that, these physiological changes may be inconspicuous in PCOS patients.

3-In this study we did not obtain significant variation between normal weight & overweight/ obese patient, the author reached the conclusion that a slight change in BMI doese not affect serum kisspeptin level.

4-In this study elevated kisspeptin serum level in subfertile women with high FSH level were obtained, the author concluded that other causes of ovarian subfertility (rather than PCOS) can also affect kisspeptin level

5-The author also concluded that SHBG is an important biochemical marker for diagnosis of PCOS, its levels decline with advancing age and it strongly affected by obesity.

5.2 Recommendations:-

In this study the numbers of participants were not equal between the study groups, so a larger study with equal division is recommended

To estimate the relation between BMI & kisspeptin, a larger sample size is preferable to give a chance to the researcher for classifying the study population into different subgroups (underweight, normal weight, overweight & obese). Also, the measurement of adipose tissue hormones and the total fat mass will help in precise estimation.

It is recommended to evaluate serum kisspeptin levels in different menopausal stages (pre, post and transitional) & correlate it with the clinical and biochemical parameters.

Neurokinin and dynorphin are neuropeptides that work with kisspeptin in the hypothalamus for regulation GnRH secretion. They can be detected in the circulation.

Evaluation of them with kisspeptin in different phases of the menstrual cycle may give further findings.

Evaluation of kisspeptin level in other types of subfertility is recommended

In further work, comparison of kisspeptin serum level between treated and untreated subfertile women is recommended

The author would like to recommend, the investigation of the ability of kisspeptin administration in treatment of fertility disorders

We were unable to find variation in serum kisspeptin among PCOS phenotypes. Therefore, the answer to this issue remains a question for future works. Furthermore, criteria's rather than Rotterdam criteria can be used for diagnosing PCOS

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Addendum

Publications:-

The following article abstract from the thesis content have been published:-

1-Razaw O.Ibrahim, Shirwan H. Omer, Chro N. Fattah. The Correlation between Hormonal Disturbance in PCOS Women and Serum level of Kisspeptin. December 2019. International Journal of Endocrinology. Impact factor according to Clarivate analysis (Thomson Reuters) =2.34 Publisher: Hindawi

2-Razaw O.Ibrahim, Shirwan H.Omer, Chro N. Fattah. Determination of Serum Sex Hormone Binding Globulin in Poly Cystic Ovarian Syndrome & Healthy Women. December 2019. Journal of Sulaimani Medical College.Vol (9) No 4.

Appendix A

Questionnaire

Name:- Date:

ID:-

Day of menstruation:-

Mobile number:-

Address:-

Occupation:-

Menarche

Duration of marriage:-

Primary Infertility;-

Secondary infertility:-

Gravidity:-

Parity: (Normal, caeserian section)

Miscarriage :- 0 1 2 3

Late miscarriage

Last delivery:-

Medical history:- Hypertention Diabetes

Rheumatic Disease( ) , thyroid dis( ) ., Antiphospho lipid syndrome( )

Surgical history: (Ovarian cystecty, myomecty, curtage)

Menstural cycle history :-

Regular , oligo, Poly

Drug history:

Body weight , Height

Ultrasound;-

Sign of hirsutisim:-

Hormonal parameter:-

FSH, LH, Prolactin, Free testosterone/Sex hormone binding Globulin

Kisspeptin Elisa result (1) Follicular phase

Kisspeptin Elisa result (2) preovulatory phase

وەزارەتی خوێندنی بالاو توێژینەوەیی زانستی

زانکۆی سلەیمانی

کۆلیژی پزیشکی

هەلسەنگاندنی هرمونی کیسپێپتین لەنەخۆشی قەلەوو لاواز کەنەخۆشی هێلکەدانی فرە کیسیان هەیە لەپارێزگای کەرکوك

لێکۆلینەوەیەکەپێشکەش بە ئەنجومەنی کولیجی پزیشکی زانکۆی سلەیمانی کراوە ،بۆ تەواو کردنی بەشیك لە پێویستیەکانی پلەی دکتۆرا لە زانستی کارئەندام زانی پزیشکی

لەلایەن

رازاو عمر إبراهیم

ماستەر لە کارئەندام زانی پزیشکی

بەسەرپەرشتی

ی.پ.د.شیروان حمەسالح عومەر

بەشی کۆئەندام زانی کولیژی پزیشکی

زانکوی سلەیمانی

ی.پ.د.چرۆ نەجمەدین فتاح

بەشی ژنان و مندال بوون کۆلیژی پزیشکی

زانکۆی سلەیمانی

پوختە

سەرەتا :هۆرمۆنی کیسپیبتین ئەو نیوروپیپتایدەیە کە گرنگە بو دەرنانی هۆرمۆنی GnRH هەروەها گرنکە بو دەردانی هۆرمۆنی تەنی زەرد (LH) و هیلکە کردنو ؛ دەردانی هیلکۆکەلە هئلکەدان.ئەم نیرۆپیبتیدە گرنگە لەکاتی دەست بیکردنی هەرزەکاریو هیۆشتنەوەی کاری زاوزێی ئساسایی.ئەوئافرەتانەی نەخوشی هیلکەدانی فرە کیسیان هەیە تێکچونی هەردوو ئەو هرمونەیان هەیە .

مەبەستەکان:مەبەستی ئەم لێکولینەوەیە هەلسانگاندنی کلینیکو کیمیاژیانی بو هورمونی کیسپیپتین لە افرەتانی هیلکەدانی فرەکیس بەبەراورد لەکەل او ژنانەی کەوا اسایین.لەگەل اوەشدا کاریگەری قەلەویو تەمەن لسەرئەم هرمونە لێدەکولیتەوە.ئامانجیکی تری ئەم تێژینەوەیە بریتیبوو لە بەراورد کردنی هرمونیSHBG لە افرەتانی فرەکیسوو ئاسای.

رێگای کارکردن: ئەم تۆیژینەوە ئەنجام دراوە بو120ئافرەت کە 60 لەبەژداربووان نەزوك بوون هوکاری تەزوکییەکەیان بەهوی نەخوشی فرەفرەکیسی هیلکەدان بوو.20 لە بەژداربوان نەزوك بوون خاوەنی رادەیەکی بەرزی هرمونیFSH بوون.40 بەژداربوو ئاسای بوونو هیچ نەحوشییەکیان نەبوو.هەموو بەشداربوان دابەاش کرابوون بەپیی تەمەنو قەلەوی .ژنانی تووشبوو بە هیلکەدانی فرەکیسی جاریکیکە دلبەش کراون بو چوار گروپ. پینج مل خوین لەهموو بەژداربوان وەرگیرابوو لەدوو کاتی جیاوازی سوری مانگانەدا (جگە لەوژنانەی کەFSH بەرزبوو یەك جار خوینیان لیوەرگیرا) .ئاستی هەموو پارامیتەرەکان پێورا بە بەکارهێنانی تەکنیکی ELISA.

ئەنجامەکان : ئاستی هورمۆنی کیسپیپتین لەافرەتانی هیلکەدانی فرەکیس زیاتربوو بەبەراورد لەگەل افرەتانی اسایی.ئاستی کیسپێبتین لەگەل ئاستی تۆستیستیرونی ئازاد پەیوەنی راستەوانەیان هەبوو.لە ئافرەتانی ئاسایی ئاستی کیسپیبتین لەکاتی پیش دەرجوندا زیاتربوو بە بەراورد لەگەل ئاستی ئەو هرمونە لەدووەم ڕۆژی سووری مانگانەدا.بەلام ئەم جییاوازیە لە ئافرەتانی فرە کیس بەدی نەکرا.جیاوازی لە ئاستی هرمونی کیسپیپتین لەنیوان افرەتانی قەلەوو لاواز هەردوو گروپی تیژینەوەکە بیبایەخ بوو.ئاستی کیسپیپتین لە ئافرەتی گەنج کەم تربوو بەبەراورد لەکەل ئافرەتانی سەروو چل سال.ئاستی SHBG لە ئافرەتانی قەلەو کەم تربوو بەبەراورد لەکەل ۆافرەتە لاوازەکان.

دەرئەنجامەکان: ئاستی هرمونی کیسپێپتین لەژنانی نەزوك کە فرەکیسی هیلکەدانیان هەیە زیانرە لە ئافرەتانی ئاسایی دا.کیسپێپتین بەژداری دەکات لە ریکخستنی هرمونەکانی کو ئەندامی زاوزێی مێیینە.تیکچوونی ئەم هرمونانە دەبێتە هۆی تیکچوونی ئاستی کیپێپتین.ئاستی کیسپێپتین لەخویندا زیاد دەکات بە تێپەربوونی تەمەن دا.قەلەویو نەخوشی فرەکیسی هیلکەدان کار دەکەنە سەر ئاستی SHBG لەخویندا بەلام کاریگەری قەلەوی زیاترە.

وزارة التعليم العالي والبحث العلمي

جامعة سليمانية

كلية الطب

تقيم مستوى الكيسبيبتين في مصل الدم لمرضى البدينين وغير البدينين الواتي لديهن متلازمة تكييس المبايض في محافظة كركوك (دراسة حالات و شواهد)

اطروحة مقدمة الى مجلس كلية الطب - جامعة السليمانية، كجزء من متطلبات نيل شهادة الدكتورا فلسفة في الفسلجة الطبية

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1442ه

2020م

الخلاصة

المقدمة:-الكيسبيبتين هو بيبتيد عصبي الذي ينظم إفراز هرمون الغدد التناسلية (GnRH). وهو عنصر أساسي لطفرة هرمون اللوتي (LH) والإباضة. هذا الببتيد العصبي ضروري في بداية سن البلوغ والحفاظ على الوظيفة التناسلية الطبيعية. النساء المصابات بمتلازمة المبيض المتعدد الكيسات (PCOS) يكشفان التغيير في كل من إفراز GnRH و LH.

الأهداف:- تهدف هذه الدراسة إلى تقييم مستويات المصل لkisspeptin في النساء العقيمات المصابات بمتلازمة المبيض المتعدد الكيسات و الأصحاء. علاوة على ذلك ، فإنه يبحث عن تأثير السمنة والعمر على مستوى kisspeptin ، وكذلك انه يشير إلى العلاقة بين kisspeptin وغيرها من المعالم الهرمونية المذكورة في الدراسة. تهدف هذه الدراسة أيضًا إلى مقارنة مستويات الغلوبيولين الرابط للهرمون الجنسي SHBG في الدم بين متلازمة تكيس المبايض والنساء الأصحاء. بالإضافة إلى ذلك ، لتوفير معلومات حول تأثير العمر على مستويات SHBG في مصل الدم في كلا المجموعتين من الدراسة. شملت دراسة الحالة والتحكم هذه مائة وعشرين امرأة. (60 كانت عقيمة مع متلازمة تكيس المبايض ، 20 كانت عقيمة مع ارتفاع FSH و 40 كانت طبيعية) التحقوا في الدراسة. تم الحصول على خمس مل من الدم من نساء العقيماتات المصابات بمتلازمة تكيس المبايض والسيطرة مرتين خلال نفس الدورة الشهرية (مسامي وقبل التبويض) ومرة واحدة من النساء العقيمات ذات FSH عالي. تم قياس مستوى المصل من kisspeptin، SHBG، testerone الحرة ، هرمون الاستروجين،FSH،LH و AMH باستخدام تقنية ELISA. تم تقسيم النساء الخاضعات للدراسة إلى مجموعات فرعية وفقًا لأعمارهن ، وتم تقسيم النساء المصابات بمتلازمة تكيس المبايض إلى أربع مجموعات فرعية وفقًا للعلامات السريرية والكيميائية الحيوية. تم استخدام القيم المعروضة كـ (mean ± SD) واختبار Kolmogorov-Smirov لاختبار الحالة الطبيعية للتوزيع. تم استخدام اختبار t للطلاب لمقارنة المجموعتين. أجري تحليل التباين في اتجاه واحد (ANOVA) لتقدير الاختلافات بين المجموعتين. بعد ذلك ، تم استخدام اختبار توكي في مرحلة ما بعد الاختبار لتقييم العلاقة بين المجموعتين.

النتائج:-كانت مستويات Kisspeptin أعلى في مرضى متلازمة تكيس المبايض عن تلك الموجودة في المجموعة العادية. أرتبط كيسبيبتين بمستوى هرمون التستوستيرون الحر في الدم (م = 0,26). في النساء الأصحاء ، كانت مستويات كيسبيبتين في مرحلة قبل التبويض الأعلى من مستواه في مرحلة المسامي (P <0.05) ؛ بينما كان هذا الاختلاف ضئيلاً في مرضى متلازمة تكيس المبايض. وكان الاختلاف في مستويات المصل لkisspeptin بين النساء يعانون من زيادة الوزن والوزن الطبيعي في كلا المجموعتين ضئيلة. في النساء الأصحاء، كانت مستويات كيسبتين أعلى في النساء (< 35 سنة) من تلك (<24 سنة) مع (P = 0.03). كان مستوى المصل Kisspeptin في النساء المصابات بالخصوبة مع ارتفاع FSH أعلى من النساء الأصحاء. لم يكن هناك فروق ذات دلالة إحصائية في مستوى مصل SHBG بين النساء متلازمة تكيس المبايض والتحكم. كانت مستوياتها أقل بكثير في النساء ذوات مؤشر كتلة الجسم أكبرمن 25. في المجموعة الضابطة لوحظ وجود ارتباط سلبي ضعيف بين العمر و SHBG بينما كانت هذه العلاقة ضئيلة في نساءالمصابات بمتلازمة تكيس المبايض. لم يسجل أية علاقة بين SHBG وغيرها من المعلمات الهرمونية

المسجلة. تم العثور على اختلاف بسيط في مستوى SHBG بين مجموعات PCOS الفرعية F = 2.061، p = 0.116. إستنتجنا من هذه الدراسة إن مستوى مصل الدم لكيسبيبتين أعلى في النساء العقيمات المصابات بمتلازمة تكيس المبايض. و إن كيسبيبتين تشارك في آليات التغذية المرتدة ، يمكن للاضطراب في المحور التناسلي أن يختل الإيقاع الطبيعي لكيسبيبتين. على الرغم من أن kisspeptin لم يرتبط بمستوى LH في الدم ، إلا أنه ارتبط بمستوى testerone وهو سمة شائعة عند مرضى PCO.

الأستنتاجات:- على غرار الهرمونات الموجهة للغدد التناسلية مستوى المصل لكيسبيبتين تزداد مع تقدم العمر. تؤثر كل من السمنة ومرض متلازمة تكيس المبايض على مستوى مصل SHBG ولكن تأثير السمنة يكون أكثر فعالية. مستويات مصل SHBG تنخفض مع تقدم العمر