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Chemistry Department

Study of Some Sex Hormones as a monitor for Type II Diabetic premenopausal Women

A Thesis
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By:
Rawnaq Jumaa Kadhim
B. Sc. In Chemistry - Chemistry Sciences Department - University of Baghdad
(2005)

Supervised by

Ass.Prof.Dr. Salman Ali Ahmed

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (١) خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ (٢) اقْرَأْ وَرَبُّكَ الْأَكْرَمُ (٣) الَّذِي عَلَّمَ بِالْقَلَمِ (٤) عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ (٥)

صدق الله العلي العظيم

سورة العلق

الآية(١- ٥)

Dedication

To soft hearted my mother and father who dedicate their lives to illuminating my stays in this world

To My husband Dr. Abd-Al Kareem Al Temeemy and my daughter Halla For their abundant support, for their Patience and understanding, and for their love

To my brothers and sisters who being beside me in their hearts

Rawnaq

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Abstract:

Diabetes mellitus is a disease that affects the metabolic and hormonal status of the human body, and one of these may be the level of sex hormones. The effect may vary according to the duration of disease, degree of control and the age of the patients.

This study was conducted in the specialized Centre for Endocrinology and Diabetes (SCED) in Baghdad city near alkindy hospital from December 2013 to September 2014. Two Hundred premenopausal women (30-49)years during the luteal phase of the menstrual cycle were selected, half of them were diabetic type II and the other half were healthy women. This study was a comparative cross sectional type designed to assess the correlation of sex hormones (Free testosterone, Estradiol, Progesterone, LH, FSH) and HbA1c levels (degree of control of diabetes), age of patients and duration of diabetes, using the data that collected.

Patients assessed for FBG by (Enzymatic Colorimetric method), HbA1C by (Immunoturbidimetric test according DCCT/NSGP protocol), free testosterone was measured by (ELISA), (FSH, LH, Progesterone, Estradiol) using the ELFA technique (Vidas).

Results showed that FBG, HbA1c, free testosterone, LH, FSH were significantly higher, while progesterone and estradiol were significantly lower in type II diabetic women compared with healthy individuals, also free testosterone, FSH levels were significantly increased in women aged (38-49) years, while progesterone and estradiol were significantly decreased. As regards to LH the level increased, but the increment was statistically insignificant. In addition free testosterone and FSH levels were affected by the degree of glycemic control, i.e. in poor glycemic control free testosterone and FSH were significantly increased while estradiol and

progesterone significantly decreased, also LH was increased but statistically was insignificant.

With increase duration of disease, there was an increase of free testosterone and FSH levels. In addition, there was an insignificant increase of LH level. Progesterone and estradiol levels were decreased with increased duration of diabetes mellitus. In conclusion, we found that there is a significant relation between sex hormones and glycemic control, age, and duration of diabetes mellitus in premenopausal women affected by diabetes mellitus type II.

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Abbreviations

ADA	American Diabetes Association
ANOVA	Analysis of Variance
BMI	Body Mass Index
CVD	Cardio Vascular Disease
DCCT	Diabètes control and complications trial
DM	Diabetes Mellitus
ELFA	Enzyme linked Fluorescent assay
ELISA	Enzyme linked immunosorbant assay
FBG	Fasting blood glucose
FSH	Follicular Stimulating Hormone
GDM	Gestational diabetes mellitus
GnRH	Gonadotropine Releasing Hormone
GODs	glucose oxidase
HbA1c	Glycated Hemoglobin
IDDM	Insulin Dependent Diabetes Mellitus
IFG	Impaired Fasting Glucose
IFG	Impaired fasting glucose
IGT	Impaired Glucose Tolerance
LH	Luteinizing Hormone
mIU	milli International Unit
NIDDM	Non Insulin Dependent Diabetes Mellitus
NSGP	National standarization glycohemoglobin program
O.D	Optical Density
Pr_x	Peroxidase
P-value	Probability Value
SD	Standard deviation
SHB	Sex hormone binding globulin
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 diabetes mellitus
WHO	World Health Organization
WHR	Waist-Hip Ratio

Chapter One

Introduction and literature review

1. Introduction & Literature Review:

1.1. Definition of Diabetes Mellitus:

Diabetes Mellitus (DM) is a common metabolic and a multi-system disorder comprising metabolic and vascular abnormalities resulting from insulin deficiency with or without insulin resistance [1, 2].

Diabetes mellitus is a major public health problem in the developed as well as producing nations [3]. It is one of the top five global leading causes of death. In the year 2000, the excess global mortality attributable to diabetes and its late complications was estimated to be 2.9 million deaths, equivalent to 5.2% of all deaths [4].

People with a family history of diabetes mellitus are at high danger of developing the condition and lifestyle change can help cut this risk [5]. The term "Diabetes mellitus" is inferred from the Greek words DIA (=through), Benin (=to go) and diabetes literally means pass through. The disease causes loss of weight as if the body stack is drawn through the urine [6]. Diabetes is a syndrome, initially characterized by a deprivation of glucose homeostasis. The disease is progressive and is linked with oxidative stress with high risk of diabetic dyslipidemia, which is responsible for micro and macro vascular complications of diabetes mellitus [7].

1.2. Signs and Symptoms:

The classical symptoms of untreated diabetes are polyuria (frequent urination), weight loss, polydipsia (increased thirst), and polyphagia (increased hunger) [8]. Symptoms may develop quickly (weeks or months)

in T1DM, while they usually grow a lot more slowly and may be absent or subtle in T2DM. Several other signs and symptoms can mark the onset of diabetes, although they are not specific to the disease. Furthermore, to the well known ones above, they include blurred vision, fatigue, slow healing of cuts, headache, and itchy skin. Diabetes dermatosis refers to the number of skin rashes that can happen in diabetes [9].

1.3. Classification of Diabetes Mellitus:

Diabetes mellitus is classified into four broad categories: type I, type II, gestational diabetes, and "other specific types"[11].

The "other specific characters" are a collection of a few dozen individual causes [11]. The term "diabetes", without qualification, normally refers to diabetes mellitus.

1.3.1. Type 1 Diabetes mellitus:

Type 1 diabetes mellitus is characterized by deprivation of the insulin-producing beta cells of the isles of Langerhans in the pancreas, leading to insulin insufficiency. This type can be also classified as immune-mediated or idiopathic. The majority of T1DM is of the immune-mediated nature, in which a T-cell-mediated autoimmune attack leads to the loss of beta cells and then insulin deficiency [12].

In North America and Europe it causes approximately 10% of D M cases. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Responsiveness and sensitivity to insulin are usually normal, particularly in the early phases. T1DM can affect adults or minors,

merely because a bulk of these diabetes cases were in children, T1DM was traditionally termed "juvenile diabetes [12].

"Brittle" diabetes, too known as labile diabetes or unstable diabetes is a condition that was traditionally used to describe the recurrent and dramatic swings in glucose levels, often happening for no apparent reason in insulin-dependent diabetes. However, this term, has no biological basis and should not be used [13].

Still, T1DM can be accompanied by unpredictable and irregular hyperglycemia, sometimes with severe hypoglycemia, and frequently with ketosis. Other complications include an impaired counter-regulatory response to hypoglycemia, infection, endocrinopathies (e.g., Addison's disease) and gastroparesis (which leads to erratic absorption of dietary sugars). These phenomena are conceived to happen no more frequently than in 1% to 2% of individuals with type 1 diabetes mellitus [14].

1.3.2. Type 2 Diabetes Mellitus:

Type 2 Diabetes Mellitus (T2DM) is the most common type which characterized by insulin resistance, which may be combined with relatively reduced insulin secretion [11]. The defective responsiveness of body tissues to insulin is thought to require the insulin receptor. However, notwithstanding the specific faults are not recognized. In the early stage of type 2, the predominant abnormality is reduced insulin sensitivity, at this stage, hyperglycemia can be lifted by a variety of medications and amounts that reduce glucose production by the liver or improve insulin sensitivity. T2DM is primarily due to genetics and lifestyle factors [15]. A number of lifestyle factors are well-known to be important to the de-

velopment of T2DM, including obesity (defined by a body mass index of greater than thirty), lack of physical activity, stress, poor diet, and urbanization [16]. Excess body fat is associated with 30% of cases in those of Japanese and Chinese descent, 60-80% of cases in those of African and European descent, and 100% of Pima Indians and Pacific Islanders. Those who are not obese often have a high waist–hip ratio [11]. Dietary factors also determine the hazard of developing T2DM. Ingestion of sugar-sweetened drinks in excess is linked with an increased risk [17, 18]. The type of fats in the diet is so important, with polyunsaturated and monounsaturated fat decreasing the risk while saturated fats and trans fatty acids increasing the risk [13]. Besides eating a pile of white rice appears to play a part in increasing risk for developing T2DM [19]. A deprivation of exercises is believed to cause 7% of cases [20].

1.3.3. Gestational Diabetes:

Gestational diabetes mellitus (GDM) resembles T2DM in several respects, requiring a combination of relatively inadequate insulin secretion and responsiveness. It happens in approximately 2-10% of all pregnancies and after delivery may improve or disappear [21]. However, approximately 5-10% of women after pregnancy with gestational diabetes are found to have diabetes mellitus, usually type 2 [21]. Gestational diabetes is completely treatable, but needs careful medical administration throughout the pregnancy. Management may include dietary changes, blood glucose monitoring, and in some cases insulin may be required. Though it may be passing, untreated gestational diabetes can harm the health of the mother or fetus. Risks to the baby include congenital heart,

macrosomia (high birth weight), skeletal muscle malformations and central nervous system anomalies [22].

1.3.4. Other types of diabetes:

Prediabetes indicates a condition that occurs when a person's blood glucose levels are higher than normal but not high enough for a diagnosis of T2DM. Many people who develop T2DM spend many years in a prediabetes state [23].

Some patients cannot be distinctly classified as having type I or type II diabetes. Clinical presentation and disease development may differ significantly in both types of diabetes. The traditional paradigms of type II diabetes happening only in adults and type I diabetes only in children are no longer precise [24].

Some cases of diabetes mellitus are caused by the body's tissue receptors not responding to insulin (even when insulin levels are normal, which is what separates it from type 2 diabetes); this form is very unusual. Genetic mutations (mitochondrial or autosomal) can lead to defects in beta cell function. In some cases, an irregular insulin action may also have been genetically determined. Any disease that causes large damage to the pancreas may lead to diabetes (for example, cystic fibrosis and chronic pancreatitis). Diseases related with immoderate secretion of insulin-antagonistic hormones can cause diabetes (which is typically resolved once the hormone excess is removed). Some toxins damage pancreatic beta cells and a lot of drugs reduce insulin secretion [25].

Other forms of diabetes mellitus include cystic fibrosis-related diabetes, congenital diabetes, which is due to genetic defects of insulin secretion, steroid diabetes induced by high doses of glucocorticoids, and a number of monogenic diabetes [24,25].

1.4. Epidemiology:

The worldwide prevalence of DM has gone up dramatically over the past two decades. Likewise, prevalence rates of impaired fasting glucose (IFG) are likewise increasing. Recent estimates suggest that there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to 366 million by the year 2030 (26). Although the prevalence of both T1DM and T2DM is increasing worldwide, the prevalence of T2DM is expected to rise more rapidly in the future because of increasing obesity and reduced activity levels [27]. At 2000, the prevalence of DM was estimated to be 0.19% in persons < 20 years old and 8.6% in those > 20 years old. In individuals > 65 years old, the prevalence of DM was 20.1%. The prevalence is similar in men and women throughout most age ranges, but it is slightly greater in men > 60 years [27].

Type 2 diabetes is frequently not diagnosed until complications appear. Approximately one-fourth of the U.S. population may have undiagnosed diabetes. Mass screening of asymptomatic individuals has not effectively identified those with prediabetes or diabetes, and rigorous clinical trials to provide such proof are unlikely to occur [28].

In Iraqis the prevalence of hyperglycemia was reported to be 10.4% with an evident increase after the age 45 years [30], in 2008 there is a

study reported that a Diabetes prevalence of 7.43% in Basrah, Iraq [29]. While in 2011, a prevalence rate of 6.8% was accounted in the populations of Sulaimani Iraq [31].

1.5. Etiology of Type 2 Diabetes Mellitus:

Type 2 diabetes is a very heterogeneous syndrome with many potential causal agents. It is due to the interaction of environmental agents with a genetic susceptibility to the disease, and it is clear that the relative contributions of genes and environment can differ considerably even among people whose clinical phenotype is closely similar [10]. Nevertheless, four primary risk factors are seen equally significant in the etiology of T2DM; they are hereditary factors, obesity, lifestyle and early life malnutrition.

i. Genetic Factors

Genetically, T2DM can be monogenic or polygenic. The monogenic forms, although relatively rare, are nevertheless important, and a number of the genes involved have been named and characterized but these subtypes; such as maturity onset diabetes of the young (MODY) are rare and comprise less than 5% of all instances of diabetes [32]. The genes required in the common polygons form or shapes of the disorder have been far more difficult to be identified or characterized [33]. In this regard various genetic loci contribute to susceptibility, and environmental factors (such as nutrition and physical activity) further modulate phenotypic expression of the disease [34, 35]. The strongest evidence for T2DM susceptibility gene is for a locus designated "NIDDM1" on the short arm of chromosome 2. This locus accounts for approximately 30% of the genetic

susceptibility among Mexican-American sibling pairs [36]. The gene in question encodes a proteolytic enzyme, Calpin-10 which is taken in both insulin secretion and action [37].

The danger to develop the disease increases noticeably, if there is a family history, particularly among first-degree relatives. Furthermore, the concordance rate of T2DM among monozygotic twins is between 70 to 90%, which further supports the genetic predisposition of the disease [38].

ii. **Obesity:**

Total body adiposity, a central fat distribution and time-course and the duration of developing obesity are all established risk factors for T2DM in both female and male, particularly in those people who are genetically predisposed [39]. Indeed, delivering a body mass index (BMI), which is computed by dividing weight (in kilos) by squaring the height (in square meters), of > 35 kg/m2 increases the peril of developing diabetes over a 10-year period by a staggering 80-fold, as compared with lean people (BMI < 22 kg/m2) [40]. Obesity may have to be combined with genetic predisposition to β-cell dysfunction or insulin resistance [41], or alternatively, there may be a common genetic predisposition that leads to both obesity and insulin resistance and hence increase the risk of T2DM [41].

iii. **Life Style**

Sedentary lifestyle is associated with high incidence of diabetes in liable populations represented by habitual physical inactivity, smoking and high energy foods [10]. In rural areas, mechanization and the substitution

of physical active occupations may be significant ingredients in bringing up the prevalence of Type II diabetes mellitus [42].

***iv.* Malnutrition Early in Life**

Malnutrition in fetus and during the first year of life has been linked with a consequent development of T2DM. It is suggested that malnutrition in fetus may program metabolic functions, and beta cell development at a critical period, as a result predisposing to T2DM later in life [32].

1.6. Pathogenesis of Type 2 Diabetes Mellitus:

Type 2 diabetes mellitus is characterized by three pathophysiological abnormalities; peripheral insulin resistance, impaired insulin secretion, and immoderate hepatic glucose production [43].

The term "insulin resistance" indicates the presence of an impaired biological response to either exogenously administered or endogenously secreted insulin. This is manifested by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscles and by impaired suppression of hepatic glucose production [32]. Insulin resistance is defined as diminished tissue responses to insulin at one or more sites in the complex pathways of the hormone action, which is related with hyperinsulinemia [47]. This problem, insulin resistance, means that, for the same quantity of circulating insulin, skeletal muscles, liver, and adipose tissue take up and metabolize less glucose than normal. in increase, being less sensitive to insulin, the liver does not respond to the "damping" signal of insulin, so the liver manufactures and releases more glucose than is needed [48].

Insulin resistance is present in a person many years before the appearance of type 2 diabetes. People inherit a propensity for producing insulin resistance, and a routine of other health problems worsen the status. For instance, when skeletal muscle cells are bathed in excess free fatty acids," the cells preferentially use the fat for metabolism while taking up and using less glucose than normal, yet when there is a bunch of insulin available. In this manner, the effectiveness of insulin decreased by high degrees of blood lipids. Leading to the conclusion that, high blood lipids increase insulin resistance [49]. The insulin sensitivity is influenced by: age, weight, ethnicity, body fat (especially abdominal), physical bodily function and medication [43].

Insulin resistance takes on a major function in T2DM development and evidences for this is shown in the succeeding:

a- The presence of insulin resistance 10-20 years before the onset of disease [44].

b- Cross-sectional studies demonstrated that insulin resistance is a uniform finding in patients with T2DM [45].

c- Prospective studies confirmed that insulin resistance is the best predictor of whether or not an individual will later become diabetic [44, 45].

In T2DM there is a moderate decrease in the entire volume of pancreatic islet tissue, which is reproducible with a measurable drop in plasma insulin concentration when related to the blood glucose level. Some pathological changes are typical of T2DM, and the most consistent of which is the deposition of amyloid. While beta cell members are reduced by 20-30% of T2DM, alpha mass are unchanged and glucagon secretion is increased, which may contribute to the hyperglycemia [32]. The rela-

tive contributions of insulin resistance and β-cell dysfunction in the pathogenesis of T2DM vary among patients as well as during the course of the disease and there is also a dynamic interaction between insulin resistance and β-cell function. Hyperglycemia can impair both insulin secretion and action; the so called "glucose toxicity" [46].

1.7. Complications of diabetes:

Individuals with diabetes are at increased risk for macrovascular disease includes coronary artery disease, cerebrovascular disease and peripheral vascular disease and are due to atherosclerosis of large vessels; and microvascular disease, including nephropathy and retinopathy; peripheral and autonomic neuropathies; and lower extremity disease [51, 52]. Type I and type II diabetes mellitus may develop complications, which are split into two main groups [53].

1. Severe metabolic complications: These include diabetic ketoacidosis, coma, hyperosmolar nonketonic and hypoglycemia [54,55].

2. Late systemic complications: These are atherosclerosis, diabetic retinopathy, diabetic microangiopathy, diabetic neuropathy and infections. Diabetes is also kept company by a fundamental increase in atherosclerotic disease of large vessels, including cerebral, cardiac, and peripheral vascular disease (cardio vascular diseases) [56].

1.8. Glycated hemoglobin:

Glyco hemoglobin or glycosylated, HbAlc, hemoglobin Alc, or Hblc; sometimes also HbA1c) is a form of hemoglobin used primarily to distinguish the mean plasma glucose concentration over prolonged periods of time. It is made in a non-enzymatic pathway of hemoglobin's normal exposure to high plasma levels of glucose [57].

Glycosylated hemoglobin (HbA1C) is the main target of glycemic control. In this regard, desirable value for HbA1C is valued below 7 [58]. HbA1c is an essential to ascertain the optimal care of diabetic patients and is the gold standard in the analysis of patients' status [59]. HbA1C is the index that indicates the average blood glucose during the past 3 months. One percent change in HbgA1C is equivalent to an approximately 35 mg/dl change in average plasma glucose [85, 60]. Smaller values of HbA1C indicate better glycemic control [61].

According to the American Diabetes Association guidelines the glycosylated hemoglobin test can be done at least two times a year in patients with diabetes who are meeting treatment goals (and who have stable glycemic control) and quarterly in patients with diabetes whose therapy has changed or who are not meeting glycemic goals [62]. Glycated hemoglobin , as an important marker in assessing a patient's risk of microvascular complications. Hence, measurement of both HbAlc and blood glucose levels are directly practiced in the everyday management of patients with type 1 and type 2 diabetes [62]. HbAlc regarded as an index of mean blood glucose over the preceding few weeks to months [63].

1.9. Glycemic control:

Glycemic control is essential in diabetes management, since the lower degree of blood glucose leads to decreased rates of morbidity and death rate, maintaining glycemic control is a goal for all patients with diabetes [64]. Epidemiological studies and prospective randomized clinical trials have demonstrated that women with type II diabetes mellitus generally have poorer glycemic control [65-66].

Glycemic control is seen as the major therapeutic goal for prevention of organ damage and other problems of diabetes [67].

A large balance of patient's remains poorly controlled [68]. Achieving optimal glycemic control in clinical preparation is difficult and the reasons for its poor control are complex. A variety of factors are identified in influencing glycemic control, including age, gender, education, matrimonial status, BMI, smoking, diabetes duration, and type of medications [67, 69, 70].

1.10. Menopause:

Menopause this term means literally "pause in the menses" and refers to the cessation of menstruation and ovarian activity that occurs at about the age of 50 [71]. In a woman with an intact uterus the date of menopause is the day after the last episode of menstrual flow finishes. The term "Perimenopause" refers to the menopause transition years, the time both before and after the last period ever, while hormone levels are still fluctuating erratically. "Premenopause" refers to the years leading up to menopause [72]. It is a phenomenon that all women know about, though

very few have a name for. About 50 million women are going through premenopause right now, and most of them have received some form of premenopause syndrome, which is a collection of symptoms experienced by women for ten to twenty years before menopause [159].

Postmenopause refers to the part of a woman's life that occurs after the date of menopause; once a woman with an intact uterus (who is not pregnant or lactating) has gone a year with no flow at all she is considered to be one year into post menopause [72].

During the postmenopausal years. Which account for about third of a woman's life span, the ovaries are depleted of follicles and stop secreting estradiol and inhibin. The decline in estradiol is due to alterations in the ovaries. Not in the pituitary; actually, FSH and LH secretion by the pituitary is elevated because of a lack of negative feedback from estradiol and inhibin [71]. Previous surveys showed that menopause pretends to be associated with increased risk of glucose intolerance and type II diabetes [73, 74].

1.11. Gonadotropins and Ovarian Steroid Hormones:

1.11.1. Luteinizing Hormone (LH):

Luteinizing Hormone (LH) is a heterodimeric glycoprotein. Each monomeric unit is a glycoprotein molecule; one alpha and one beta subunit compose the full, functional protein [177]. Luteinizing hormone plays a significant part in reproductive Physiology, secreted from the anterior pituitary gland below the consequence of Hypothalamic GnRH, it is set up that the gonads (Ovaries and Testis) suffer from atrophy after a legal in-

jury or amputation occur in the pituitary Gland, it is responsible for the maturity of the ovarian follicles and secretion of estrogen from these follicles [75]. And it's important in the formation of Corpus leutum after ovulation [76]. Besides it helps in secretion of enzymes responsible for the loss of ovarian wall opposite to the follicle consisting the (stigma) in the ovarian follicle (the stigma) is the side of ovulation [77]. Normal ovarian function depends on the concerted action of FSH and LH [178]. Studies in rodents have shown an age-related LH release and decrease in pulsatile GnRH and a loss of positive feedback to estradiol, leading to ovarian arrest despite the presence of sufficient numbers of functional ovarian follicles , In contrast, a study in primates has indicated that pulsatile GnRH release becomes increased at the age of approximately 30 yr (concomitant with the perimenopause for this species), whereas positive feedback responses to estradiol treatment appeared maintained [78].

1.11.2. Follicle stimulating hormone:

Follicle stimulating hormone (FSH) is hetrodimeric glycoprotein consist of two noncovalently linked polypeptides the alp (a) subunit protein and beta (p) specific for its produced within pituitary gonadotroph cells and it is a vital part of the procreative process [79]. Both subunits are necessary for biological activity. FSH has a beta subunit of 111 amino acids (FSH β), which gives its specific biologic action, and is in charge of for interaction with the receptor of follicle-stimulating hormone [179]. It accepts an extensive mixture of actions in both male and female reproductive tissues, including growth, division, and differentiation of Sertoli cells, FSH is directly implied in the production of both male and female gametes, as good as the output of hormones (estradiol and inhibin) that

feed back to influence the secretion of FSH from the pituitary [79]. In both female and male FSH stimulates production of gamete (egg or sperm) [80].

FSH it stimulates the division of the granulose cells and the formation of cellular layer surrounded these cells [76].

The increment in serum estradiol levels causes a decrement in FSH production by inhibiting GnRH production in the hypothalamus [81].

Inhibins are dimeric polypeptides, which have inhibin A and inhibin B. Both are granulosa cell products [118,119]. With inhibin B secreted mostly during the follicular phase [120]. By the developing cohort of antral follicles. Although inhibin A has the capacity to repress FSH secretion by the pituitary, for inhibin B the same has not been presented [121,122]. However, inhibin B may have paracrine functions influencing folliculogenesis in the ovary itself [123,124]. Inhibin B is considered to offer a direct assessment of ovarian reserve because it is mainly made by the FSH-sensitive cohort of antral follicles. The reduction in the secretion of inhibin B, as a outcome of a reduction in cohort size with aging, is linked with elevated FSH levels and with a decreased oocyte quality and fertility potential [120].

Moreover, the levels of inhibin B do not show a gradual decline with increasing female age and are oftentimes seen as a rather late marker of reduced follicle numbers [120,125].

Probably inhibin B is a more dependable index of ovarian activity than of ovarian reserve, due to its direct link with growing follicles, and is much

more influenced by the waning and waxing of ovarian function often seen throughout the menstrual cycle and during late ovarian aging [126].

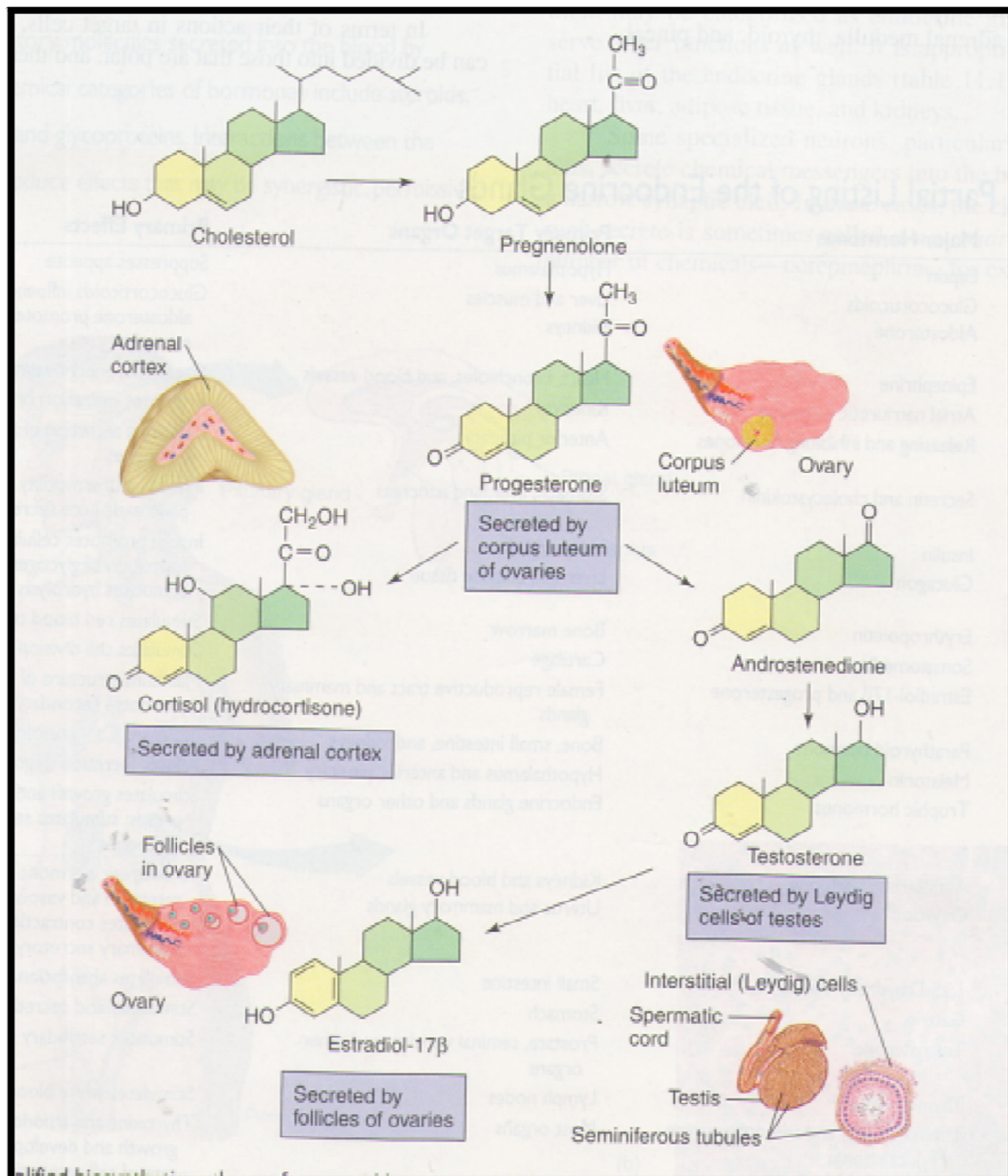


Figure 1.1: Simplified biosynthetic of pathways for steroid hormones Boston et al. [71]

1.11.3. Testosterone hormone:

Testosterone hormone is the principle male hormone, it is synthesized By Leydig cells of the testes from cholesterol, chemically it consists of (19) carbon atoms with (OH) group on 17th carbon atom [75]. Testosterone hormone also released from the adrenal cortex in small quantities about 5% of testosterone concentration [82]. After secretion by the testes about 97 per cent of the testosterone becomes either loosely bound with balsam albumin or more tightly bound with a beta globulin called sex hormone binding globulin and circulates in the blood in these states for 30 minutes to several hours .By that time, the testosterone either is transferred to the tissues or is degraded into inactive products that are subsequently excreted [83]. Androgens may modulate the physiology of vaginal tissue and take part to female genital sexual arousal [180]. There is a time lag effect when testosterone is administered, on genital arousal in women. Also, a continuous increase in vaginal sexual arousal may result in higher genital sensations and sexual appetitive behaviors [182]. In women Testosterone supplementation is effectual in the short term for hypoactive sexual desire disorder [183]. When females have a higher baseline level of testosterone, they have higher increases in sexual arousal levels but smaller increases in testosterone, indicating a ceiling effect on testosterone levels in females [184].

In adult females, increased androgen levels induce insulin resistance [84]. And increase the risk of T2D and CVD [85]. An elevated Androgen levels in women with central obesity and perhaps T2D [86].

Also in premenopausal and postmenopausal women increased free testosterone is associated with higher glucose and insulin concentrations [87].previous study in a small California community that reported a positive association between testosterone and diabetes risk in women [88].

In addition women with NIDDM had somewhat higher free testosterone, lower SHBG, and higher plasma insulin concentration values than did control subjects with similar body mass indicator (BMI) [89]. Further, several clinical trials indicate that androgen deprivation improves insulin sensitivity in adult females, independent of changes in BMI [90].

1.11.4. Estrogen:

Estrogens are family of hormones includes estrone (E1), estradiol(E2), and estriol (E3) synthesized in a diversity of tissues.The primary estrogen of ovarian origin is 17β- Estradiol and about 10 times as potent as estrone and about 80 times as potent as estriol in its estrogenic effect [91]. Except through the early follicular phase of the menstrual cycle, during the reproductive years, estriol serum levels are slightly higher than that of estrone. Therefore it is the predominant estrogen during reproductive years, both in conditions of absolute serum levels as well as in terms of estrogenic activity [92].

Estrone is the predominant circulating estrogen during menopause, and Estriol is the predominant circulating estrogen in terms of serum levels during gestation. During a woman’s reproductive life, which commence with the onset of menstruation and continues until menopause estrogen can be split down into three distinct compounds estrone, estradiol and estriol. [93].Estrogens play significant roles in growth, reproduction,

growth, and upkeep of a diverse range of mammalian tissues [94]. In addition to their important role in reproduction, estrogens also affect the cardiovascular, immune, skeletal, and nervous systems and play a part in the induction and progression of breast cancer [95].

In women, estradiol acts as a growth hormone for tissue of the reproductive organs, further the growth of female secondary sexual characteristics, such as breasts, and are also involved in the thickening of the endometrium and other aspects of regulating the menstrual cycle, supporting the lining of the Fallopian tubes, and the facing of the cervical glands [96]. The steroid hormone estrogens are present in a detectable amount during fetal development, at puberty, estrogen levels rise to initiate secondary sexual characteristics [97]. Estrogen is the term employed to refer to the female sex hormones estrone, estradiol and estriol [98]. These hormones are present in the blood stream of female and male; although male produces a great deal smaller quantities. Estrogen levels increase and fall at different times of the menstraual cycles of a female. The falling estrogen and progesterone levels that follow trigger LH and FSH, initiating the cycle all over once more [97]. Sometimes, hormones like estrogen are called "chemical messengers" because they carry instructions and data from one group of cells in the body to another. Estrogen circulates in the blood stream and affects not only female sex organs such as the uterus, but besides the head, bones, heart and liver [99].

In female the development of secondary sex characteristics is driven by estrogens, estradiol, to be specific. These modifications are initiated at puberty time, most are raised during the reproductive years, and get less pronounced with decreasing estradiol support after the menopause. Thus,

estradiol enhances the development of breast, and is responsible for alterations in the body shape, affecting bones, fat deposition and joints. Fat structure and skin composition are modified by estradiol [92].Various fields in women have observed positive associations between insulin resistance and estradiol [100,101,102]. Whereas results from another study were conflicting [103] . Other studies indicated that there is a lower danger of type II diabetes among postmenopausal women who used estrogen therapy [104,105].

Nevertheless, it has been reported that exogenous estrogen may have different physiological effects depending on duration, dosage, and mode of exposure [106,107]. Especially in similarly, an inverse effect of estrogen on decreasing insulin sensitivity has also been reported among healthy Premenopausal women randomized to oral contraceptives (ethinyl estradiol) [108].

1.11.5. Progesterone:

Progesterone is an endogenous steroid hormone concerned in the pregnancy ,menstrual cycle, and embryogenesis of humans and other species [242] and one of the two main reproductive hormone groups, estrogens are the other one, made by the ovaries of menstruating women [109].

It's primarily a hormone of pregnancy and fertility and its effects on breast epithelium [110]. Also progesterone appears to be involved in the growth of breast cancer, though its role, and whether it is an inhibitor or promoter of breast cancer risk, has not been fully elucidated [243].

The major uses of progesterone in the body are:

*To promote the growth of the embryo and survival and fetus

*To provide a full scope of core biologic effects

*To act as a construction block of other steroid hormones

Progesterone is a precursor (or building block) to many other steroid hormones such as Testosterone, cortisol, and estrogen (estriol, estradiol, estrone). Because it is a modular, its use cans greatly confirm overall hormone balance. Progesterone supplementation will stimulate bone building and help protect against osteoporosis, not overlooking the numerous positive roles it plays in the body. For women who suffer hormonal imbalance, but are not necessarily menopausal, progesterone is equally important [109]. Progesterone hormone plays a critical part in moderating blood sugar levels. Blood sugar regulation is one of the many uses of progesterone in all vertebrates [111]. Also, progesterone plays important parts in the physiology of insulin [112]. Foster and Balfour have indicated that progesterone has reducing effect on serum insulin level in postmenopausal women [113]. On the contrary, it has been not found appreciable decrease or increase in serum insulin level among users of oral progesterone [114]. Previous study was also reported that there is an opposite effect of estradiol and progesterone on the insulin level [115]. In addition, it has been shown that progesterone and estradiol have different effects on insulin signaling [112]. Recent studies also have found the effects of progesterone or estrogens on insulin signaling [116,117].

1.12. Hormonal Changes occurring during the Menstrual Cycle:

Menstrual cycle is a physiological phenomenon during reproductive life of women. Its phases are influenced by variation in the concentration of hormones such as estrogen and progesterone [127].

The ovary secretes variable amounts of progesterone and estradiol under strict hypothalamus-pituitary control [128]. There are two distinguished phases: The follicular phase is characterized by rising levels of estrogens and low levels of progesterone, leading to the maturation of a dominant follicle and ovulation, pronounced by a peak of luteinizing hormone (LH). The luteal phase prepares the uterus for potential implantation. The phenotypic consequences during these two phases depend on timing and concentrations [128]. Actually, the remarkable changes in hormonal levels, albeit within physiologic ranges, cause important alterations in target organs such as the vagina, uterus (already evident on a simple pap smear), breast, and ovary, as well as changes in thermoregulation [129]. In women the reproductive system depends on repetitive cyclic follicle recruitment, single dominant follicle selection, followed by ovulation (release of the oocyte) and the formation of a corpus luteum. In case fertilization and subsequent implantation fail to occur, the corpus luteum recants, with endometrial shedding and menses as a result [130].

The subsequent initiation of follicle recruitment completes the phase known as the “luteofollicular transition.” Such a regular menstrual cycle pattern demands integrative functioning of hypothalamus, pituitary, and ovaries. The GnRH pulse generator within the hypothalamus ensures the

pulsatile release of both LH and FSH from the pituitary. The intercycle rise in FSH concentrations is initiated by decreased steroid and inhibin A concentrations with the demise of the corpus luteum of the preceding cycle [131]. Comparatively high FSH levels in the luteofollicular transition enable cyclic recruitment of a cohort of FSH-sensitive antral follicles, as well as estradiol synthesis and the production of inhibin A and B. Decrease FSH levels (due to negative feedback by rising inhibin B and estradiol) are essential for single dominant follicle selection from the recruited cohort. This process is referred to as the FSH threshold/window concept [132,133]. Inhibin A follows the estradiol serum types across the cycle. Inhibin B is mainly secreted by smaller antral follicles and decreases with the increase of the dominant follicle [134].

1.13. Hormonal Control of Ovarian Cycle:

The elevation of estrogen level in the blood act a negative feedback control for the anterior pituitary gland to control FSH secretion , high level of estrogen in the blood also lead to sadden secretion of GnRH from the hypothalamic, this will leads to secretion of LH from the anterior pituitary gland causing ovulation, LH also helps in the development of corpus leutum which secrete progesterone, high level of progesterone in the blood acts as a negative feedback control for the anterior pituitary gland leading to decrease of LH secretion, after that corpus leutum suffer from leutiolysis [135].

FSH from pituitary gland is responsible for the early maturation of the ovarian follicles; FSH and LH together are responsible for their final ma-

turation. A burst of LH secretion is responsible for ovulation and the initial formation of the corpus leutum.

A smaller mid cycle burst of FSH secretion also occur, LH stimulate the secretion of estrogen and progesterone from the courpus leutum [83].

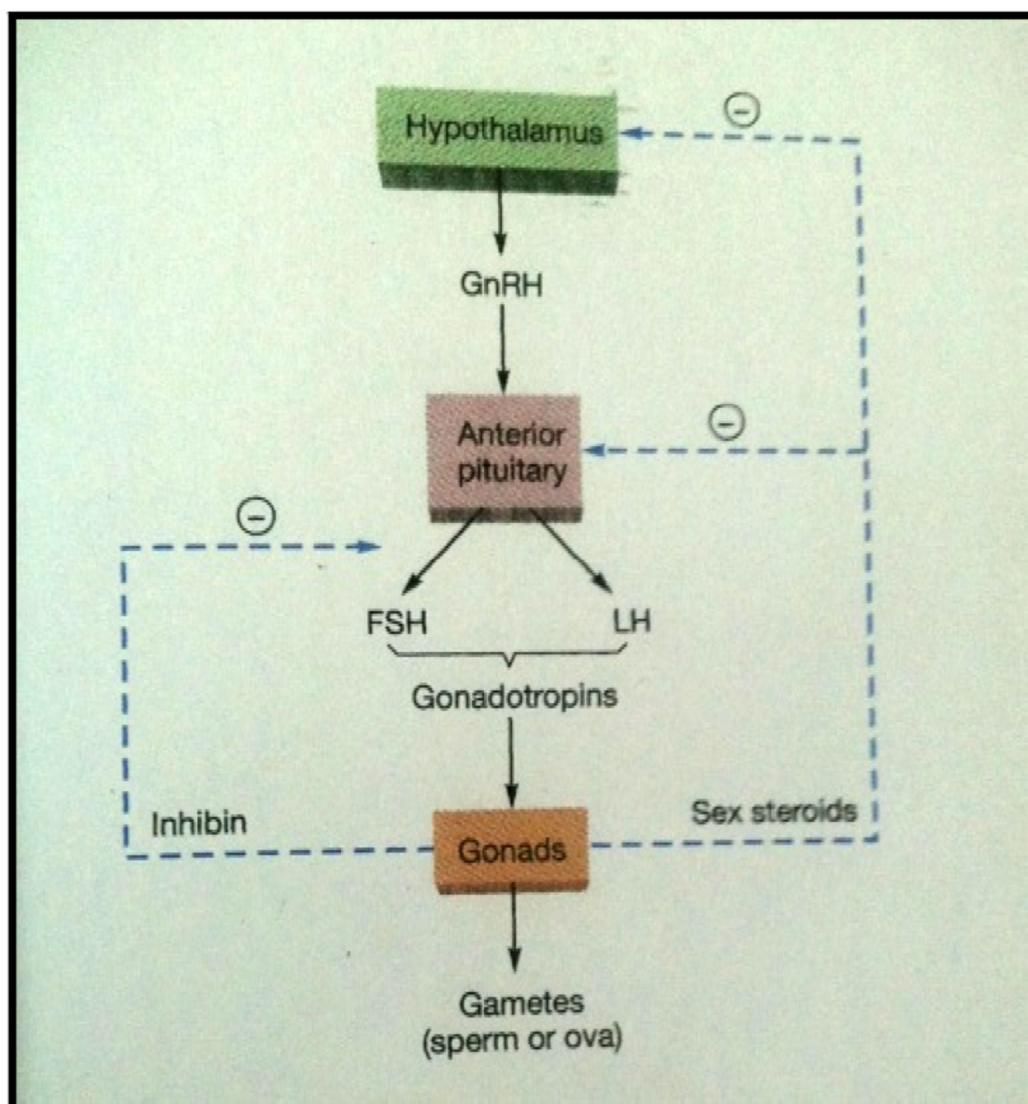


Figure1. 2: Interactions between the hypothalamus, anterior pituitary and gonads Boston et al. [71].

1.14. Sex hormone binding globulin:

Sex hormone binding globulin (SHBG) is a glycoprotein consists of two 373-amino-acid subunits, each with a single steroid binding site. The corpus of its role has traditionally been thought to be that of a transport protein for sex steroids, regulating circulating concentrations of free (unbound) hormones and their transfer to target tissues. Sex hormone binding globulin is synthesized in the liver. Metabolic clearance from the intravascular compartment is biphasic with the first clearance occurring over hours and further clearance over a half-life of several days. glycosylation extends serum half-life [136].

The various sex steroids have different binding affinities for SHBG; with the affinity of testosterone roughly doubles that of estradiol. Estradiol has the highest affinity of human circulating estrogens, being four times greater than estrone and 100 times greater than estriol. Each protein of SHBG contains two steroid binding sites. It is possible that at any one time, zero, one or both binding sites may be occupied and also that different sex steroids may be bound to the same SHBG protein simultaneously. Ligand binding induces conformational change in the hormone binding pocket of the SHBG protein. SHBG contains oligosaccharide side-chains which is not necessary for steroid-binding, may be necessary for the SHBG-steroid complex to interact with plasma membrane receptors in target tissues [137].Several studies reported that SHBG has been inversely associated with insulin resistance and type 2 diabetes in male and female [138,139,140,141].

In both sexes studies showing that SHBG is associated with insulin resistance and glucose levels independent of adiposity [142,143,144].

1.14.1. Functions of Sex hormone binding globulin:

Sex hormone binding globulin (SHBG) has been believed to serve as a transporter of sex steroids, controlling circulating free hormone concentrations. Sex hormones may circulate in one of three patterns, namely, bound to SHBG, and bound to albumin or other plasma proteins or in a free (unbound) state. The free sex hormone hypothesis proposes that only sex hormone in the free (unbound) state is biologically active, and that being lipophilic, unbound hormone gains access to the cell via free diffusion [145].

Sex steroid bound to albumin may dissociate, becoming active and the sum of the concentrations of free and albumin-bound sex steroid may be considered to represent the concentration of active sex steroid. This concentration is sometimes referred to as the bioavailable concentration of sex steroid. The evidence suggests that SHBG has functions in addition to those of transporting sex steroid. SHBG-bound sex steroids have been located inside the cell and megalin has been shown to facilitate the endocytosis of SHBG-bound testosterone [146].

Liganded SHBG does not bind to the SHBG receptor, suggesting that SHBG may have effects via binding to the SHBG receptor that are independent of sex steroids [147].

1.15. Mechanisms of Action of Hormones, Hormone Receptors and Their Activation:

The beginning of a hormone's action is to bind to specific receptores on the target cell. Cells that need receptors for the hormones do not reply. Receptors for some hormones are situated on the target cell membrane, whereas other hormone receptors are situated in the cytoplasm or the nucleus. When the hormone combines with its receptor, this usually initiates a cascade of reactions in the cell, with each stage becoming more powerfully activated so that even when a small concentration of the hormone is found it can have a large effect.

Receptors of hormones are large proteins, and each cell that is to be stimulated, usually has some 2000 to100.000 receptors. Likewise, each receptor is usually highly specific for a single hormone; this specifies the case of hormone that will act on a particular tissue. Only the target tissues that are contained the specific receptors of the hormone that affected by that hormone [83].

1.16. Age and hormonal changes:

The hormonal changes happening during a woman’s reproductive life span are well recognized. Menstrual irregularity is common at both terminals of a woman’s reproductive lifetime. In adolescence, menstrual disorders may stem from an immature gonadal axis [128]. During the menopause transition years (perimenopause), the irregularity reflects a progressive ovarian failure and is correlated with a spectacular decrease in fertility [148]. The suboptimal hormonal setting may produce poor pregnancy outcomes in premenopause and adolescence, even though in

teenage mothers the outcome also may be influenced by reveres economic state of affairs [149].

The premenopausal changes are related to a decline in ovarian inhibin B secretion, and a rise in FSH (follicle stimulating hormone) levels, interests, fertility becomes compromised even before cycle irregularity, likely because of oocyte dysfunction [150]. The rise in FSH actually was specifically related to a decline in the circulating concentrations of INH-B was shown by Klein et al. [151], who compared a group of older reproductive women, selected to have increased circulating concentrations of FSH, with a group of young, regularly cycling subjects. Klein found that Serum FSH was significantly elevated as expected and INH-B levels were significantly lower in the older women compared to younger women, while INH-A was unchanged and estradiol was actually higher in the older women. The present studies have more-recently confirmed the progressive increase in circulating FSH levels with increasing age in females with regular menstrual cycle, though the sublime degree of FSH was seen predominantly in some women over the age of 40 [152]. In regularly cycling women estradiol concentrations appear to be preserved, at least until around age 50, FSH rises and INH-B declines. It is hard to demonstrate fundamental changes in androgen concentrations in the immediate perimenopausal period, though levels postmenopausally appear to be lower than those of young, regularly cycling women, maybe as a function of increasing age during the reproductive years rather than menopausal status. Hormonal measurements are of small diagnostic value during the perimenopause, other than for purposes of physiological study [153].

Another complication with type II diabetes is that menopause causes estrogen and progesterone levels to fall. This decrease in hormones can cause fluctuations in blood sugar levels and incidents of low blood sugar. Insulin resistance triggers by estrogen, so low estrogen takes the adverse effect and allows the body to rapidly use up insulin [154].

1.17. Deleterious Effects of Hormonal Imbalance in Adults:

Normal ranges for hormone levels are generally defined as those levels not associated with pathological symptoms. These ranges are used to set thresholds suitable for clinical use and diagnostic or therapeutic decisions. However, as is true for most or all physiological functions, a continuum connects the pathologic and physiologic levels, which predominating overlap. This explains the growth of dynamic tests that assess the reaction to hormonal challenge, though they are too bound by arbitrary normal response criteria [128].

Furthermore, it is easily proven that sexual function and menstrual cycles may be indirectly disturbed in many endocrine conditions, for example, hyper- or hypothyroidism [155], diabetes [156], and pituitary tumors that secondarily affect the gonadal axis. This can take place in women as well as in men [157], and illustrates the interrelationship of the different endocrine systems. Nevertheless, the individual susceptibility to gonadal imbalance caused by other endocrinopathies is variable.

For instance, some women have adverse gonadal effects caused by only mild endocrine disease, whereas others are still productive in spite of major imbalance [128].

1.2. Aims of study:

In premenopausal female

- Evaluate the relation between the control of DM type II and sex steroid hormone levels.
- Find the relation of age group with sex hormone levels in type II DM.
- Explore the relations of duration of diabetes type II to sex hormone level.

patients and Methods

2.1. Chemicals:

Kites used in this study are listed in table 2.1 with their suppliers.

Table 2.1: Kites and suppliers companies.

NO.	Kit	Suppliers Companies
1	ELISA Free Testosterone Kit	DiaMetra/ Italy
2	FBG Kit	Biomaghreb/Tunisia
3	HbA1c Kit	Turbitex /Germany
4	VIDAS Estradiol Kit	Biomerieux/France
5	VIDAS FSH Kit	Biomerieux/France
6	VIDAS LH Kit	Biomerieux/France
7	VIDAS Progesterone Kit	Biomerieux/France

2.2. Apparatus:

Table 2.2: Instruments and their companies

NO.	Apparatus	Suppliers Companies
1	Auto vortex	Stuart Scientific, U.K
2	Biolyzer instrument	Biolyzer 300/ Germany
3	Centrifuge	Hettich/Germany
4	ELISA System	Biokit/ France
5	Incubator	Memmert/Germany
6	Refrigerator	Arcelik, Turkey
7	UV-Vis Spectrophotometer	Shimadzu UV-Vis/Japan
8	VIDAS instrument	VIDAS/France
9	Water bath	Techne junior TE-8J/England

2.3. Ethical clearance:

1. Approval to run the research was taken from the specialized center for Endocrinology and Diabetes (SCED) / Baghdad.
2. Permission was taken from the Ministry of Health (Appendix II).
3. The purpose of the study was explained to the patients.

2.4. Research design:

To achieve the aim and objectives of the present study, a comparative Cross-sectional study design has been chosen.

2.5 .Study setting:

The study was conducted at the specialized center -for Endocrinology and Diabetes (SCED) in Baghdad city. It is a specialized center for Diagnosis, treatment, follow up and research of diabetes. It is located in the center of Baghdad city near alkindy hospital. It provides care and treatment for diabetic patient at all ages and from all governorates. The total number of samples was 200 since its establishment in December 2013 to September 2014. The daily center activities include clinical examination, laboratory investigations, management, following up of diabetic patients and health education.

2.6. Study period:

The study period was ten months from December 2013 to September 2014 devoted for actual study data collection. Collection of data was done by working for five days per week for four hours daily from 8:30 am to 12:30 pm.

2.7. Study population:

Study population was chosen from diabetic premenopausal women attending the specialized center -for Endocrinology and Diabetes (SCED), Normal premenopausal women were selected from the same center.

2.8. Definition of cases:

The cases were defined as women with diabetes type II at age group (30-49) years who were attending to the specialized center for Endocrinology and Diabetes. Type II diabetes was defined According to American Diabetes Association (ADA) criteria HbA1C value of (≥ 6.5%) or FPG≥126 mg/dl (7.0 mmol/l) or 2-hour plasma glucose≥200mg/dl (11.1mmol/l) or in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis a random plasma glucose ≥200 mg/dl (11.1mmol/l), is strongly predicted as pharmacotherapy for diabetes [196].

Patients categorized according to their HbA1c level into three groups in line with the classification used by ADA [196]:

Group 1: Less than 7 were 15 women.

Group 2: Between 7to 8 were 17 women.

Group 3: More than 8 were 68 women.

At the time of this study the patients were on diet and under oral treatment of diabetes, patients categorized according to their age, Group 1 (30-38) years, Group II (38-49) years, and their duration of diabetes

Group I (>4years) and Group II (≤4 years).

2.9. Definition of comparative group:

The control group was defined as normal women at the same age group of cases from the specialized center for Endocrinology and Diabetes.

2.10. Study sample:

A convenient sample included 200 women's (100 cases and 100 healthy women) whose ages were (30-49) years, and agreed to participate in the study and fitted the inclusion criteria.

I. Inclusion criteria

- Samples:

a. Premenopausal women with diabetes type II.

b. Married.

c. Fertile.

d. In luteal phase of menstrual cycle.

e. Regular menses.

f. On treatment(daonil)

Comparative:

- control group: premenopausal women with the same inclusion criteria

2.Exclusion criteria:

- Samples:

a. Pregnant women.

b. Menopausal women.

c. Lactating.

d. Hormonal therapy.

e. Singles.

f. Infertile women.

g. Poly cystic ovarian syndrome.

h. Smoking.

i. Endocrine disease.

j. No family history of Diabetes.

k. Irregular menstrual cycle

2.11. Method for data collection:

The questionnaire for the study was constructed so as informations needed are included in (appendix I). All the information was kept confidential.

2.12. Anthropometric measures:

1. Body mass index (BMI) was obtained by calculating weight by kg/ square height by meter (kg/m2) [158].

BMI (Kg/m^2) = weight (Kg)/ height (m^2)

Table 2.3: Categorization of BMI.

BMI(Kg/m^2)	Categories
<18.5	Under weight
18.5-24.9	Normal weight
25 -29.9	Over weight
30 -34.9	Obese class I
35-39.9	Obese class II
>40	Obese class III

2. Waist-to-Hip ratio:

The waist of women is measured at the narrowest part of the waist, between the iliac crest and lowest rib, and the hip circumference is measured at the widest area of the hips at the greatest protuberance of the buttocks. Then simply calculate the Waist-to- Hip Ratio by dividing waist (cm) to Hip (cm) [254,246].

WHR = Waist (cm)/ Hip (cm)

2.13. Collection of blood samples:

From each patient 10 ml of blood was obtained by vein puncture, using a 10 ml disposable syringe between 9:00 and 11:00 A.M, after 8-12 hours of fasting, 0.5 ml whole blood is mixed with EDTA regent (anticoagulant) and kept for HbA1c estimation. The remaining sample was dispensed in a plain tube and left to clot at room temperature. Then, it was centrifuged at 3000 rpm for 10 minutes to collect serum. The serum was divided into aliquots (250μl) in Eppendroff tubes and stored in a deep freezer (-20) until used.

2.14. Laboratory investigations:

The patients were investigated for the following parameters:

1. FBG (mg/dl).
2. HbA1c%.
3. LH (mIU/ml).
4. FSH (mIU/ml).
5. Free-Testosterone (pg/ml).
6. Progesterone (ng/ml).
7. Estradiol (pg/ml).

2.15. Laboratory methods:

2.15.1. Determination of serum glucose.

Method:

Fasting serum Glucose was measured by enzymatic colorimetric assay method.

Principle:

Glucose is oxidized by glucose oxidase (GOD) enzyme to gluconate and hydrogen peroxide. The formed hydrogen peroxide reacts, under the catalysis of peroxidase (POD), with phenol and 4- amino - antipyrine to form a red - violet quinomine dye (an indicator) according to the following equations :-

$$Glucose + O_2 + H_2O \xrightarrow{GOD} H_2O_2 + Gluconate$$

$$2H_2O_2 + Phenol + 4\ Amino\text{-}antipyrine \xrightarrow{Peroxidase} 4H2O + Quinonimine$$

Table 2.4: Reagents for assessing fasting serum glucose level.

Reagent 1	Tris buffer pH 7	100 mmol/l
Buffer solution	Phenol	0.3 mmol/l
Reagent 2	Glucose oxidase	10 000 U/l
	Peroxidase	1 000 U/l
	4 -amino-antipyrine	2.6 mmol/I
Reagent 3	Standard glucose	100 mg/dl
Standard		5.56 mmol/l
		1g/l

Procedure:

Wave length: ....505 nm (490-550)
Temperature :...(20, 25, 37°C)
Cuvette :...1 cm light path

Table 2.5: Preparation of blank, standard and sample for assessing fasting serum glucose level.

	Blank	Standard	Sample
Standard Sample Working reagent	- - 1 ml	10 µl - 1 ml	- 10µl 1 ml
Then all tubes mixed well and incubated for 30 mn at room temperature (20°C - 25°C). Or 10 mn. At 37°C The stability of color 30 mn.			

Calculation:

Glucose cone. = (O.D. Sample/ O.D. Standard) × n

mg/dl: n = 100
g/l: n = 1
mmol/l: n =5. 56

2.15.2. Determination of Glycated hemoglobin (HbA_{1c}):

Immunoturbidimetric test according DCCT/NSGP protocol.
The concentration of HbA1c and the concentration of total hemoglobin are both measured. The reported HbA1c result is calculated as a % of the total hemoglobin concentration.

The values of HbA1c and total hemoglobin generated in this assay are intended for utilization in the computation of the HbA1c total hemoglobin ratio (%HbA1c) and must not be used individually for diagnostic purposes.

(a) Sample Pre-treatment

The pre-treatment of the whole blood sample is the first step of the procedure. This step causes lyses red blood cells and hydrolysis of the hemoglobin by the action of a protease enzyme in the hemoglobin denaturant reagent.

(b) Determination of Total Hemoglobin

The Total Hemoglobin reagent was used to calculate the concentration of total hemoglobin. The method as described by Wolf et al (1984), involves the conversion of all the hemoglobin derivatives into haematin in an alkaline solution of a non-ionic detergent. The reaction was started by the cessation of the pre-treated sample to the total hemoglobin reagent, resulting in a green solution. The conversion of different hemoglobin species into alkaline haematin with one defined absorption spectrum allows to measurement of the endpoint of total hemoglobin at 600 nm.

(c) Determination of HbA1c

A latex agglutination inhibition assay was used to determination of HbA1c. The agglutinator, which consists of a synthetic polymer containing multiple copies of the immunoreactive part of HbA1c, causes agglutination of latex covered with HbA1c specific mouse monoclonal antibodies. When HbA1c absence in the sample, the agglutinator in the HbA1c R3b Reagent and the antibody-coated micro particles in the HbA1c R3a Reagent will agglutinate, resulting in an increase in optical density. HbA1c, which presence in the sample will slow the rate of agglutination as it competes with the HbA1c agglutinator for antibody binding sites on the latex. Thus, the raise in absorbance is inversely proportional to the concentration of HbA1c in the sample.

An increase in optical density absorbance due to agglutination is measured at 700 nm and the degree of agglutination is used to estimate the concentration of HbAlc from a Calibration Curve. Then, the percentage HbAlc is calculated using the g/dl HbAlc and Total Hemoglobin values.

Working solution concentration:

R1: Hemoglobin Denaturant	
Porcine Pepsin	
Buffer	pH 2.4
Preservative	
R2: Total Hemoglobin Reagent	
Sodium Hydroxide . PH 13	0.4% w/v
Triton	2.5% w/v
Octylphenoxypolyethoxyethanol	2.5% w/v
R3a: HbAlc Antibody Reagent	
HbA1 c Antibody (mouse) coupled particles	<0.1% w/v
Bovine Serum Albumin	
Buffer	pH 8.1
Non ionic Surfactant	0.6% w/v
Proclin 150	0.1% w/v
R3b: HbAlc Agglutinator Reagent	
HbAlc hapten covalently attached to the polymer	
Bovine Serum Albumin	
Buffer	pH 2.0
Proclin 150	0.1% w/v
Non ionic Surfactant	0.2% w/v

Testing procedure:

Materials provided
• Working solutions as described supra.
Additional materials involved.
• Calibrators and controls as designated below
• 0.9% NaCI

Calculation:

The computation of the hemoglobin A1C concentration was generated using the following equation:

$$\% \text{ HbAlc} = \frac{\text{HbA1c [g/dl]}}{\text{Hb [g/dl]}} \text{x100}$$

2.15.3. Determination of Luteinizing hormone (LH):

Principle:

The assay principle combines between an enzyme immunoassay sandwich method with a final fluorescence detection (ELFA). The Solid Phase Receptacle (SPR) serves every bit the solid phase as well as the pipetting device for the assay. reagents for the assay are pre- dispensed in the sealed reagent strips and ready to use. All steps of the assay are performed automatically by the instrument. The medium of reaction is cycled in and out of the SPR several times.

The specimen is taken and transferred into the well containing alkaline phosphatase-labeled anti-LH antibodies (conjugate). To increase the reaction speed the sample/conjugate mixture is cycled in and out of the SPR several times. The antigen binds to the antibodies coated on the SPR and to the conjugate forming a "sandwich". During the washing steps unbound components are eliminated. During the

final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR.

The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone) the fluorescence of which is measured at 450 nm. The fluorescence intensity is proportional to the concentration of antigen present in the sample. The results are automatically calculated at the end of the assay, by the instrument in relation to the calibration curve stored in memory, and then printed out.

Procedure

1. One "LH" strip and one "LH" SPR for each sample were used, control or calibrator was tested, and the storage pouch was carefully resealed after the required SPRs were removed.
2. The test was identified by the "LH" code on the instrument. The calibrator was identified by "S1" and tested in duplicate.
3. The samples were clarified by centrifugation.
4. The calibrator, control and samples were mixed using a vortex- type mixer (for serum or plasma separated from the pellet.
5. The calibrator, control, and sample test portion is 200µl.
6. The "LH" SPRs and MLHM strips were inserted into the instrument, and the color labels with the assay code on the SPRs and the Reagent strips should be matched.
7. The assay was initiated as directed in the User's Manual. All the assay steps were performed automatically by the instrument.
8. The vials were reclosed and returned to the required temperature after pipetting.
9. The assay was completed within approximately 40 minutes. After the assay was completed, the SPRs and the strips were removed from the instrument.

10. The used SPRs and reagent strips should be disposed in a suitable recipient.

2.15.4. Determination of follicle stimulating hormone (FSH):

Principle:

The assay principle of (FSH) hormone is the same as the assay principle of (LH) hormone (section 2.15.3).

Procedure

1. One "FSH" strip and one "FSH" SPR for each sample were used, control or calibrator was tested, and the storage pouch was carefully resealed after the required SPRs were removed.
2. The test was identified by the "FSH" code on the instrument. The calibrator was identified by "S1" and tested in duplicate.
3. The samples were clarified by centrifugation.
4. The calibrator, control and samples were mixed using a vortex- type mixer (for serum or plasma separated from the pellet.
5. The calibrator, control, and sample test portion is 200µl.
6. The "FSH" SPRs and MLHM strips were inserted into the instrument, and the color labels with the assay code on the SPRs and the Reagent strips should be matched.
7. The assay was initiated as directed in the User's Manual. All the assay steps were performed automatically by the instrument.
8. The vials were reclosed and returned to the required temperature after pipetting.
9. The assay was completed within approximately 40 minutes. After the assay was completed, the SPRs and the strips were removed from the instrument.
10. The used SPRs and reagent strips should be disposed in a suitable recipient.

2.15.5. Determination of Free Testosterone:

Principle

Testosterone in the blood is bound to SHBG (60%) and in lower quantity to other proteins (for example albumin). Only the measurement of Free Testosterone (<1% of Total Testosterone) permits the estimating of the hormone biologically active. Free Testosterone (antigen) in the sample competes with the antigenic testosterone conjugated with horseradish peroxidase (HRP) current in the conjugate for binding to the antibodies anti-Testosterone coated on the microplate (solid phase).The Testosterone bound to proteins does not participate into this reaction, so it is washed away through the washing step (for total Testosterone measurement the ELISA Diametra "Testosterone" kit is available). After incubation, the bound/free separation is done by a simple solid-phase washing. After that the enzyme HRP in the bound-fraction reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blue color that converts to yellow when the Stop Solution (H_2SO_4) is added. The intensity of the color is inversely proportional to the Free Testosterone concentration in the sample. Free Testosterone concentration existent in the sample is calculated throughout a calibration curve.

Procedure

i. The Calibrators should be prepared by mixing the required reagent for 5 minutes with a rotating mixer before use. The Calibrators were mixed and had the following concentration of Testosterone (standard curve).

The concentration of free testosterone was read from the standard curve.

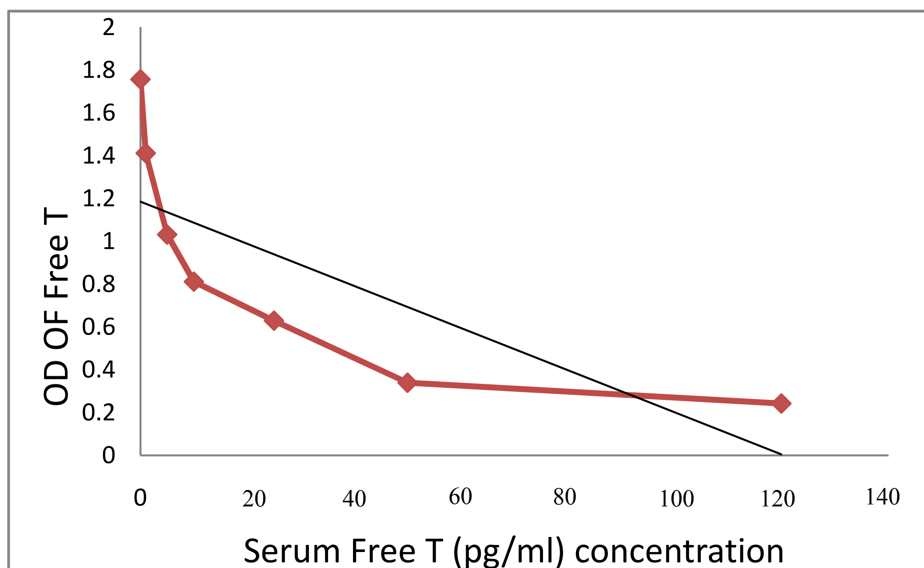


Figure (2.1): Standard Curve of Free testosterone kit at 450nm.

ii. **Preparation of the Washing Solution**

The content of the vial "10X Cone. Wash solution" were diluted. With distilled water to a final volume of 500 ml before used. For smaller volumes respect the 1:10 dilution ratio. The wash solution that diluted was stable for 30 days at 2-8°C. It was possible to notice the presence of crystals in concentrated wash solution, in this case should be mixed at room temperature until complete dissolution of crystals; for more accuracy the whole bottle of concentrated wash solution was diluted to 500 ml carefully also transfer the crystals, then mix until crystals were completely dissolved.

Procedure

• All reagents were allowed to reach room temperature (22-28°C).

• Unused coated microwell strips were released securely in the foil pouch containing desiccant and were stored at 2-8°C.

• Unused reagents should be never being transferred into the original vials to avoid potential microbial and/or chemical contamination.

• As it is required to achieve the determination in duplicate in order to improve accuracy of the test results, two wells were prepared for each point of the calibration curve (C0-C5), two for each control, two for each sample, one for Blank.

Table 2.6: Preparation of calibrator, sample and blank for assessing free testosterone level.

<table>
<tr><td>Reagent</td><td>Calibrator</td><td>Sample/Control</td><td>Blank</td></tr>
<tr><td>Calibrator
C0-C5</td><td>20 μl</td><td></td><td></td></tr>
<tr><td>Sample/Control</td><td></td><td>20 μl</td><td></td></tr>
<tr><td>Conjugate</td><td>100 μl</td><td>100 μl</td><td></td></tr>
<tr><td colspan="4">Incubated for 1 hour at 37ºC
The contents Removed from all well; the wells washed 3 times with 300 μL diluted wash solution.</td></tr>
<tr><td>TMB Substrate</td><td>100 μl</td><td>100 μl</td><td>100 μl</td></tr>
<tr><td colspan="4">Incubated at room temperature (22-28°C) in the dark for 15 minutes.</td></tr>
<tr><td>Stop Solution</td><td>100 μl</td><td>100 μl</td><td>100 μl</td></tr>
<tr><td colspan="4">Shaked the meicroplate softly.
At 450 nm the absorbance read against Blank within 5 minutes</td></tr>
</table>

2.15.6. Determination of Progesterone:

Principle

The principle of assay combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR) serves as the solid phase and the pipetting device for the assay. The assay Reagents are ready-to-use and pre- dispensed in the sealed reagent strips.All steps of the assay are done automatically by the device. The medium of reaction is cycled in and out of the SPR several times.

After dilution of the sample, the progesterone in the sample binds with the specific monoclonal antibody coating the interior of the SPR. During the washing stages, unbound components are eliminated. Then the conjugate is cycled into the SPR. The residual free antibody sites are saturated by the conjugate. The progesterone maintained is detected by the conjugate, which is a derivative of alkaline phosphatase-labeled progesterone. Unbound conjugate is removed by washing, during the final detection step; after that the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The hydrolysis of this substrate catalyzes by the conjugate enzyme into a fluorescent product (4-Methyl-umbelNferone), at 450 nm the fluorescence of which is measured. The density of the fluorescence signal is inversely proportional to the concentration of progesterone current in the sample. The results are calculated automatically at the end of the assay by the instrument in relation to the calibration curve stored in memory, and then printed out.

Procedure

1. The calibrator, control and samples were mixed using a vortex- type mixer (for serum or plasma separated from the pellet).
2. The calibrator, control, and sample test portion is 200 µl.
3. The "PRG" SPRs and "PRG" strips were inserted into the instrument, and should be checked to make sure the color labels with the assay code on the SPRs and the Reagent strips match.
4. The assay was essentially initiated according to the User's Manual. All the assay steps were done automatically by the instrument.
5. The vials were reclosed and return to the required temperature after pipetting.
6. The assay was completed within approximately 40 minutes. After the assay was completed, the SPRs and strips were removed from the instrument.
7. The used SPRs and reagent strips should be disposed in a suitable recipient.

2.15.7. Determination of Estradiol:

Principle:

The principle of assay combines a competition method with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR) serves as the solid phase and the pipetting device for the assay. The assay reagents are ready-to-use and pre-dispensed in the sealed reagent strips. All steps of the assay are done automatically by the device.

The medium of the reaction is cycled in and out of the SPR several times. Then the sample is transferred to the well containing the conjugate, which is an alkaline phosphatase-labeled estradiol derivative. The estradiol present in the estradiol derivative in the conjugate and the serum compete for the anti-estradiol specific antibody sites coated to the inner surface of the SPR. During the washing Steps

unbound components are eliminated. The substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR during the last detection step. The hydrolysis of this substrate catalyzes by the conjugate enzyme into a fluorescent product (4-Methyl-umbelliferone), at 450 nm the fluorescence of which is measured. The Fluorescence density is inversely proportional to the concentration of antigen current in the sample. Results are calculated automatically at the end of the assay by the instrument in relation to the calibration curve stored in memory, and then printed out.

Procedure

1. The calibrator, control and samples were mixed with a vortex type mixer (for serum or plasma separated from the pellet) in order to improve results reproducibility.
2. The calibrator, control, and sample tests portion is 200 µl.
3. The "E2II" SPRs and "E2II" Reagent strips were inserted into the instrument and should be checked to make sure the color labels with the assay code on the SPRs and the reagent strips match.
4. The assay was essentially initiated according to the User's Manual. All the assay steps were done automatically by the Instrument.
5. The vials were restopped and returned them to 2-8°C after pipetting.
6. The assay was completed within approximately 40 minutes. After the assay was completed, the SPRs and the strips were removed from the instrument.
7. The used SPRs and reagent strips should be disposed in a suitable recipient.

2.16. Statistical analysis:

Minitab (version 16) was used to analyze the collected data. These data analyzed using the following measure:

1. Descriptive statistics: frequency, percentages, mean and standard deviation.
2. Inferential statistics: student t-test was used to define the difference between the means of two groups in quantitative variables. ANOVA test (one way) was used for mean difference in more than 2 groups.

P value less than 0.05 considered statistically significant.

Chapter Three

Results and Discussion

3.1. Results and Discussion:

3.1.1. Demographic Characteristics of the Population under Study.

The prevalence of diabetes mellitus is rising rapidly in the world at an alarming rate [160].

Worldwide over 246 million people suffer from diabetes; 122 million of them were women. By the year 2025 this number is expected to rise to 192 million [161]. Complications of diabetes are uniquely and often more severely, affect Women [162]. Previous studies revealed that women affected by T2D disproportionately [163].

Sex hormones are important for differentiation of male and female reproductive systems, regulating not only fertility and sex characteristics, but also metabolism and adipose tissue [240]. Sex hormones had a widespread range of effects on the pathophysiology of women [241].

So, this cross sectional study was designed to assess the correlation of sex hormones (Progesterone, Testosterone, Estradiol, LH, FSH) with HbA1c levels (degree of control of diabetes), age of patients and duration of diabetes in premenopausal women.Using the data that collected.

From 200 samples 100 diabetic premenopausal women and 100 healthy premenopausal women as control the result in table 3.1 shows that the Mean± SD value of age was 38.31±4.08 year in diabetic women and 37.45±4.97 year in healthy women with *p* value of 0.183, also the mean value of BMI and WHR in diabetic women were 27.429± 2.20 Kg/m^2, 1.113±0.104 respectively compared to 27,18± 1.38 Kg/m^2, 1.1368± 0.0865 respectively in control group with *p* value of 0.362, 0.085respectively,these findings means that there is insignificant differences observed in the age, BMI and WHR between the two groups, So they are matched.

Table 3.1: Demographic Characteristics of the Population under Study.

Parameters	**Control No=100**	**Diabetic patients No=100**	***P* value**
	Mean± SD	**Mean± SD**	
Age (year)	37.45 ± 4.97	38.31± 4.08	0.183
BMI (Kg/m^2)	27.18 ± 1.38	27.42± 2.20	0.362
WHR	1.1368±0.0865	1.113±0.104	0.085

P value <0.05 considered significant

3.1.2.Fasting blood glucose and Glycated hemoglobin.

As shown in Table 3.2 that the Mean± SD of FBG and HbA1C were164±19. 8 (mg/dl) and 9.42±2.33 % respectively in our patients compared to 82.5±17.1 (mg/dl) and 4.657±0.447 % respectively in the control group with a *p* value of 0.000 (highly significant) and this is alike to the findings in a group of patients examined by other researchers [167,168,169,170,171]. When these groups were investigated, the values of FBG and HbA1C were higher in diabetic premenopausal women.

Table 3. 2: Fasting blood glucose and Glycated hemoglobin levels in diabetic and healthy women.

Parameters	Control No=100	Diabetic No=100	*P* value
	Mean ± SD	Mean ± SD	
FBG (mg/dl)	82.5±17.1	164±19.8	0.000
HbA1C (%)	4.657±0.447	9.42 ± 2.33	0.000

P value <0.05 considered significant

Serum level of glucose is variable during the day in diabetic patients, so measuring HbA1c is an effective way to estimate the degree of control of diabetes mellitus in preceding 2-3 months [164].

Glycated hemoglobin (HbA1C) measurement is utilized safely as one of diagnostic tool and can be of a great help in therapeutic management of diabetic patients. The study prepared in Malaysia reported that an HbA1C value of 6.5% is an adequate marker to diagnose diabetes because of its high specificity. A borderline (5.6-6.4%) or higher (≥

6.5%) level of HbA1C highly predicted as future pharmacotherapy for diabetes mellitus [165]. The other important study indicated that the important role of HbA1C is not solely for diagnosing diabetes, but it is also as an effective marker indicating the demand for acute intervention [166].

Glycated hemoglobin is made in a non-enzymatic pathway of hemoglobin's normal exposure to high plasma levels of glucose [57]. So, when blood glucose levels are high, glucose molecules bind in red blood cells to the hemoglobin. The longer hyperglycemia occurs in blood, the more glucose binds to hemoglobin in the red blood cells and the higher the glycated hemoglobin.This is also confirming by data from practice showing that HbA1c levels improved significantly by 20 days from the start of the strengthening of glucose-lowering treatment [244].

3.1.3. Gonadotropin hormones levels in diabetic and healthy women.

Table 3.3 has shown that the levels of LH and FSH in diabetic patients were 5.37±0.87 (mIU/ml) and 5.66±1.25 (mIU/ml) respectively, compared with the levels in the control 0.576±0.092 (mIU/ml) and 1.855±0.353 (mIU/ml) respectively, and the dispute is highly important due to the *p* value of 000, this comparison demonstrates that there is a substantial gain in the tier of these hormones in diabetic patients.

Table3.3: Gonadotropin hormones levels in diabetic and healthy women.

Parameters	Control No=100	Diabetic No=100	*P* value
	Mean± SD	Mean± SD	
LH(mIU/ml)	0.576±0.092	5.37±0.87	0.000
FSH(mIU/ml)	1.855±0.353	5.66±1.25	0.000

P value <0.05 considered significant

The development and the reproductive activities of the gonadal tissue are manipulated by the gonadotropin hormones LH and FSH of the anterior pituitary gland, LH promotes production of gonadal hormones, while FSH stimulates gamete (sperm or egg) production [172].

Gonadotropin releasing hormone GnRH from the hypothalamus stimulates the secretion of both LH and FSH, which are subjected to feedback loop regulation by the ovarian hormones [173].

Furthermore, when corpus leutum starts lysing during the last period of leuteal phase (in cases no pregnancy)the progesterone and estrogen levels will decrease while LH, FSH levels increase regularly, also the decrease of progesterone and estrogen may cause a positive feed –back mechanism at the pituitary gland level [75].

And a result of this, an increment of gonadotropin (LH, FSH) will be occurring [174].

Diabetic women showed a decline in the reproductive hormones like inhibin and progesterone. The decrement in inhibin and progesterone causing an increment in FSH and LH secretion level [175].

Furthermore, diabetes mellitus could be the cause of increasing gonadotropin hormones (LH, FSH) in patients under investigation, that is because insulin is easily recognized to facilitate gonadotropin-releasing hormone secretion by hypothalamic neurons, in vitro, possibly that this syndrome is an appearance of insulin resistance at the neuronal level, which results in subnormal secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus [176].

In order that the patients of the present study have higher levels of gonadotropin hormones (LH, FSH), and this could be a reasonable explanation for the significant increase in (LH, FSH) in premenopausal type II diabetic women as shown in Figure (3.1). The results of the present study are consistent with other results [181,185], which showed that there is a higher (LH, FSH) levels in diabetic premenopausal women compared with the control patients, although inconsistent with the results of another study [186].

The present study has shown an increase in the level of gondotropin hormones in the diabetic group compared to control this is probably due to the fact that in the other study the group of patients was 28 female, while our group was one hundred, and the Mean± SD of HbA1c in that group was (6.15±1.2)%, likewise their patients were not all premenopausal while in our groupHbA1c was (9.42± 2.33)%, and the patients in the present study were totally premenopausal, and possibly due to a more sensitive assay method.

In the present study used ELFA technique (Vidas) but the other study used ELISA technique. So this is making our findings on the increasing gonadotropin hormones (LH, FSH) in diabetic women appear much decisive and more precise than that study.

So from this , conclude that the results of the present study are fairly different from the other study [186].

3.1.4.Relation between Free testosterone and diabetes mellitus in women.

Table 3.5 shows that the Mean± SD of free testosterone in diabetic women was 3.55±0.84 (pg/ml) compared to 0.443±0.066 (pg/ml) in the control group and the *p* value was 0.00 (highly significant).

Table 3.4: Free testosterone level in diabetic and healthy women.

Parameter	**CONTROL N0=100**	**DIABETIC No=100**	***P* value**
	Mean± SD	**Mean±SD**	
Free testosterone(pg/ml)	0.443±0.066	3.55±0.84	0.000

P value <0.05 considered significant

Previous study showed that sex hormone binding globulin (SHBG); a serum protein that affects free circulating hormone levels, in females has been inversely associated with type II diabetes and insulin resistance [185]. So the highly significant increase in free testosterone in premenopausal diabetic women in the present study compared to control may due to the low level of sex hormone binding globulin in diabetic individuals [185], which leads to an increase in the free testosterone.

In addition to, there are two main sources of producing testosterone in premenopausal women, the adrenal gland and the ovary [187,188].

Hyperandrogenic women have an exalted degree of adrenal androgen secretion. Furthermore, a decreased conversion of testosterone to estradiol by the aromatase enzyme complex is another probability of androgen excess in reproductive-age women [188].

Aromatase is a cytochromeP450 enzyme that catalyzes the rate-determining step in the synthesis of estradiol from androgens and is shown in several tissues, including ovary, testis, brain, adipose tissue, bone, and skin of most vertebrate types [189].

The results of the present study are in agreement with other studies [185,190,191]. Which found elevated levels of testosterone in premenopausal diabetic women compared with the control samples.

3.1.5. Progesterone and Estradiol levels in diabetic and healthy women.

Table 3.6 showed that the values of progesterone and estradiol in diabetic women was 4.28±0.98 (ng/ml) and 67.7±11.1 (pg/ml) respectively, while the values of the control group was 17.52±2.30 (ng/ml) and 196.7±17.6 (pg/ml) respectively, and these differences were highly significant according to their *p* value of 0.00.

Table3.5: Progesterone and Estradiol levels in diabetic and healthy women.

Parameters	CONTROL N0=100	DIABETIC No=100	*P* value
	Mean± SD	Mean± SD	
Progesterone(ng/ml)	17.52±2.30	4.28±0.98	0.000
Estradiol (pg/ml)	196.7±17.6	67.7±11.1	0.000

P value <0.05 considered significant

Progesterone, which is released by the corpus luteum, is present only in the luteal stage of the menstrual cycle in significant quantities. After ovulation, progesterone levels go up gradually, progressing to a coincidental peak at around the same time that estrogen peaks in the luteal phase, around days 21 to 23 of a normal menstrual cycle (28-day) [192, 193].

And then the decreasing in progesterone and estradiol in our diabetic premenopausal women is perhaps due to the negative feedback mechanism of increment of LH and FSH hormones [75], as remarked in the figure (1.2).

Estrogen is synthesized in the ovaries in premenopausal women, and via conversion from testosterone by aromatase in adipose tissue in men and women [194].

Thus, the decreased conversion of testosterone by an aromatase enzyme complex to estradiol in premenopausal diabetic women may be the reason for decreasing estradiol in our patients.

In addition, the results of the present study of decreased progesterone level in premenopausal type II diabetic women compared to control (Figure 3.1) are inconsistent with other studies [190,191]. The patients of one of these studies [191] were also in the leuteal phase as our patients while the patients of the other study [190] were not clearly defined regarding the phase of the menstrual cycle.

Regarding estradiol levels the results of current study are in agreement with other studies [185,167] , but there is a work done by another group [195], showing higher levels of this hormone than our results; and this difference is probably due to that their patients are all diabetic of different BMI levels while our comparison was between diabetic and control subjects and this may affect our results, and that their work was during the pre ovulatory phase of the menstrual cycle while our work was in the post ovulatory phase .

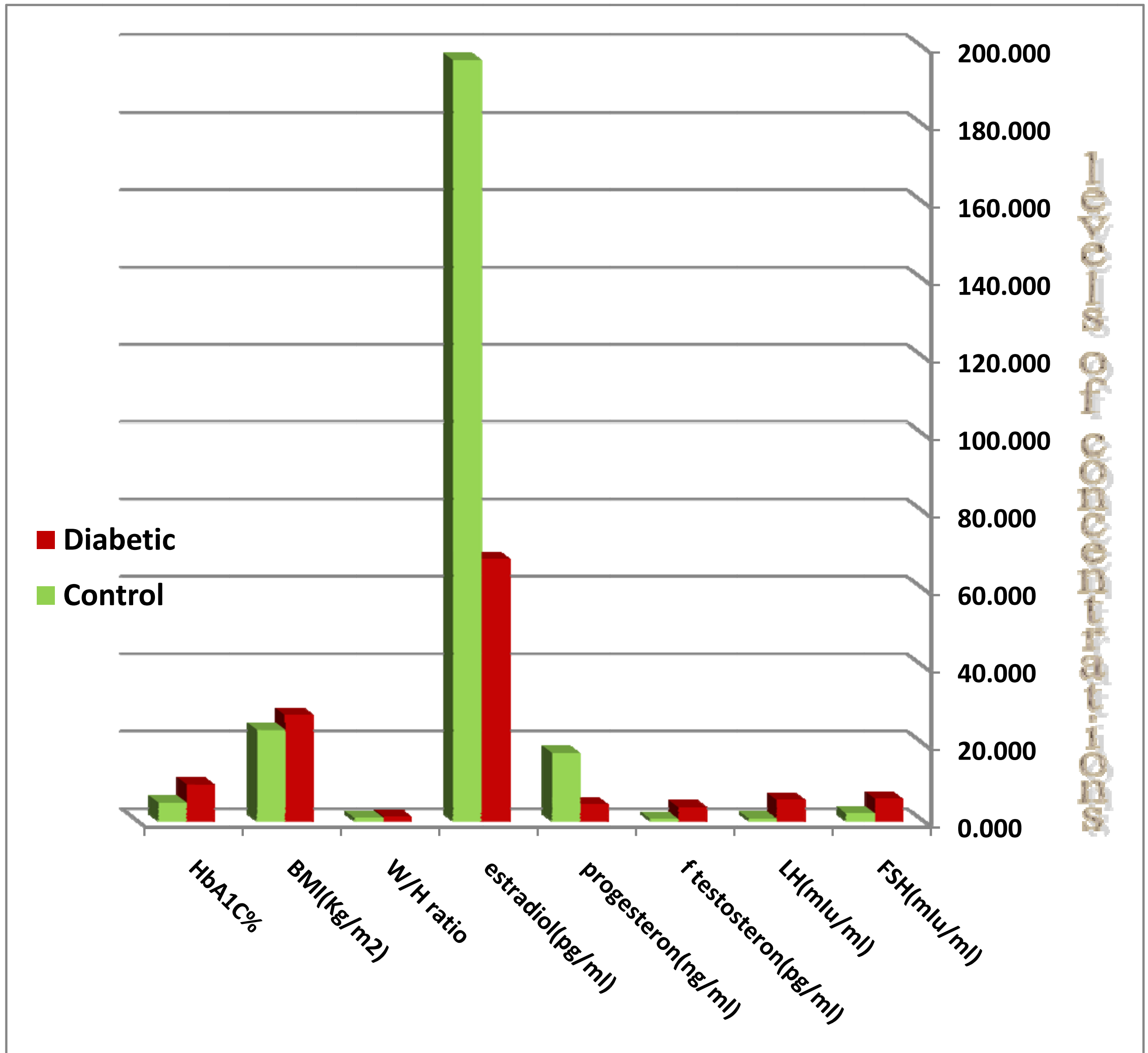


Figure 3.1. Comparison of sex hormones levels in diabetic and control groups.

3.1.6. Comparison of sex hormones according to degree of control of DM.

As shown in Table 3.8 the levels of BMI and WHR were 23.393± 1.730 (Kg/m^2) and 0.9887±0.1161 respectively in good control, 26.671±0.394 (Kg/m^2), 1.0943±0.0733 respectively in fair control and 28.488± 1.251 (Kg/m^2), 1.1457±.0853 respectively in poor control. And the mean value of HbA1c was 6.360±0.424% in good control , 7.459± 0.269% in fair control and 10.588±1.879% in poor control with *p* value of 0.00 (highly significant) ,also it was found that the mean values of both LH and FSH were 2.715±0.768 (mIU/ml) , 4.6533±0.7736 (mIU/ml) respectively in good control compared to 2.733±0.026 (mIU/ml), 4.4276±0.0113 (mIU/ml) respectively in fair, 6.608±0.665 (mIU/ml), 6.1962±1.013 (mIU/ml) respectively in poor control and the *p* values were 0.085(not significant),0.00 (highly significant) respectively, while the mean value of free testosterone was 1.861± 0.623 (pg/ml) in good control , 2.238±0.893 (pg/ml) in fair control , 4.253± 1.419 (pg/ml) in poor control with *p* value of 0.00(highly significant) and for progesterone and estradiol were 10.033±2.877 (ng/ml) , 127.11±19.05 (pg/ml) respectively in good control , 8.100±2.050 (ng/ml), 90.17±14.50 (pg/ml) respectively in fair control and 2.062± 1.298 (ng/ml) , 47.92±9.70 (pg/ml) respectively in poor control and the *p* values were 0.00(highly significant) and 0.04 (significant) respectively.

Table3.6: Comparison of value of mean sex hormones according to degree of control of DM.

Parameters	HbA1c<7 No=15	HbA1c(7-8) No=17	HbA1c>8 N0=68	P Value
	Mean± SD	Mean± SD	Mean± SD	
HbA1c %	6.360±0.424	7.459± 0.269	10.588±1.879	0.00
BMI(Kg/m^2)	23.393± 1.730	26.671±0.394	28.488± 1.251	0.00
WHR	0.9887±0.1161	1.0943±0.0733	1.1457± .0853	0.00
LH(mIu/ml)	2.715 ±0.768	2.733±0.026	6.608±0.665	0.085
FSH(mIu/ml)	4.6533±0.7736	4.4276±0.0113	6.1962±1.013	0.00
Free Testosterone (pg/ml)	1.861± 0.623	2.238±0.893	4.253± 1.419	0.00
Progesterone (ng/ml)	10.033 ±2.877	8.100±2.050	2.062± 1.298	0.00
Estradiol (pg/ml)	127.11±19.05	90.17±14.50	47.92±9.70	0.04

P value <0.05 considered significant

According to Laboratory Evaluation of Diabetes Control (American Diabetes Association Guidelines) for HbA1C, the population was categorized **[196]** as good diabetes control (<7%), fair diabetes control (7-8%) and poor diabetes control (>8%).

Now regular measurement of HbA1c values is the principal means to track and measure glycemic control in diabetes. Because of its importance as a marker of diabetes control, it makes sense that patient knowledge of recent and target HbA_{1c} values might be a useful precondition for involvement in diabetes management. Consequently, in recent years in that location has been an increased focus on encouraging patients to be cognizant of and discuss HbA1c values with their doctors [197].

The present study had shown that out of one hundred type 2 diabetics, 15 women had good, 17 women had fair and 68 women had poor glycemic control on the basis of their HbA1C status. This suggests that about more than 50% of type 2 diabetics had poor control.

Several studies were consistent with the findings of the present study [168, 169,171,198, 199, and 200]. The study done by Kumar et al[199], was about 1000 diabetic patients, they found that about half of them were poorly controlled, also this finding agrees with the conclusion of the study of Mahmood et al[200], they reported that the majority of their patients with type II diabetes had poor control of their glycemic status.

It was found that the level of HbA1C which signifies the level of control of diabetes has a substantial kinship with both the BMI and W/H ratio. This finding is seen clearly in the Graph (3.2) because when the HbA1 C was >8 the BMI was 28.488 (Kg/m^2) and the W/H ratio

was 1.146 compared with 23.393(Kg/m^2) and 0.989 respectively when the HbA1C was <7.

These findings of this cross sectional study are in agreement with previous studies which confirmed the association between obesity and type II diabetic patients attending a diabetes clinic [201,202,203, 204], except for one study of 1000 individuals [198], Which showed that BMI was normal in all their patients regardless of diabetic control, so they concluded that BMI was insignificantly related to glycemic control. The differences between the results of our study and those of the previous study may be due to differences in background characteristics of our and their patients, such as age and difference in serum sex hormone levels.

Insulin resistance of skeletal muscle is the basic defects in type 2 diabetes, hyperinsulinemia in the early stages of the disease followed by insulin deficiency in the later stages, hyperglycemia, elevated hepatic glucose production, and dyslipidemia [205, 206, and 207].

Both types of diabetes mellitus can cause various endocrine disorders by affecting the secretion, metabolism, clearance or bioavailability of hormones. Diabetes mellitus has many effects on the hypothalamus pituitary gonadal axis, including disturbances of gonadotropin release, which may be due to the abnormal generation of gonadotropin releasing hormone (GnRH) pulses in the hypothalamus [208].

Figure 3.2 shows the degree of control of diabetes mellitus estimated by the level ofHbA1c and is classified in three groups (<7means good control, (7-8) means fair control and > 8 means bad control). This graph shows that the level of sex hormones varies according to these groups

signifying the importance of control of diabetes effect on the levels of sex hormones. For example, in the group of high HbA1c (poor control) the level of free testosterone and FSH and their changes significant probably due to the bad control of diabetes causing changes in the level of these hormones. While the levels of progesterone and estrogen are not affected in the same way in other word they are inversely related to the level of HbA1C.

Regarding LH its level was higher than normal, but the increase was not significant. Also, there was an elevation in serum progesterone in type II diabetic females with good glycemic control, this is because good control is associated with good ovulation and when there is good ovulation there is elevation in serum progesterone in luteal phase [209].

Another study [210], suggested that in both pre- and postmenopausal women increased androgenicity as assessed by decreased SHBG and increased testosterone is strongly linked with insulin resistance.

Moreover, Females have estrogen hormone, which is protective for developing diabetes [211,212], Estrogen seems to contribute to glucose homeostasis in women also estrogen makes the body cells more sensitive or receptive to insulin [213]. In other words, in premenopausal woman's estrogen may have a protective effect against insulin resistance[214].

Results of the current study go with the findings of Al-Sharefi [209], who found in his patients who were premenopausal diabetic women that estradiol and progesterone levels were decreased and FSH and LH levels were increased in association with an increase in HbA1c level indicating poor control of diabetes in women.Noticing that part of his patients were comparable to our patients.

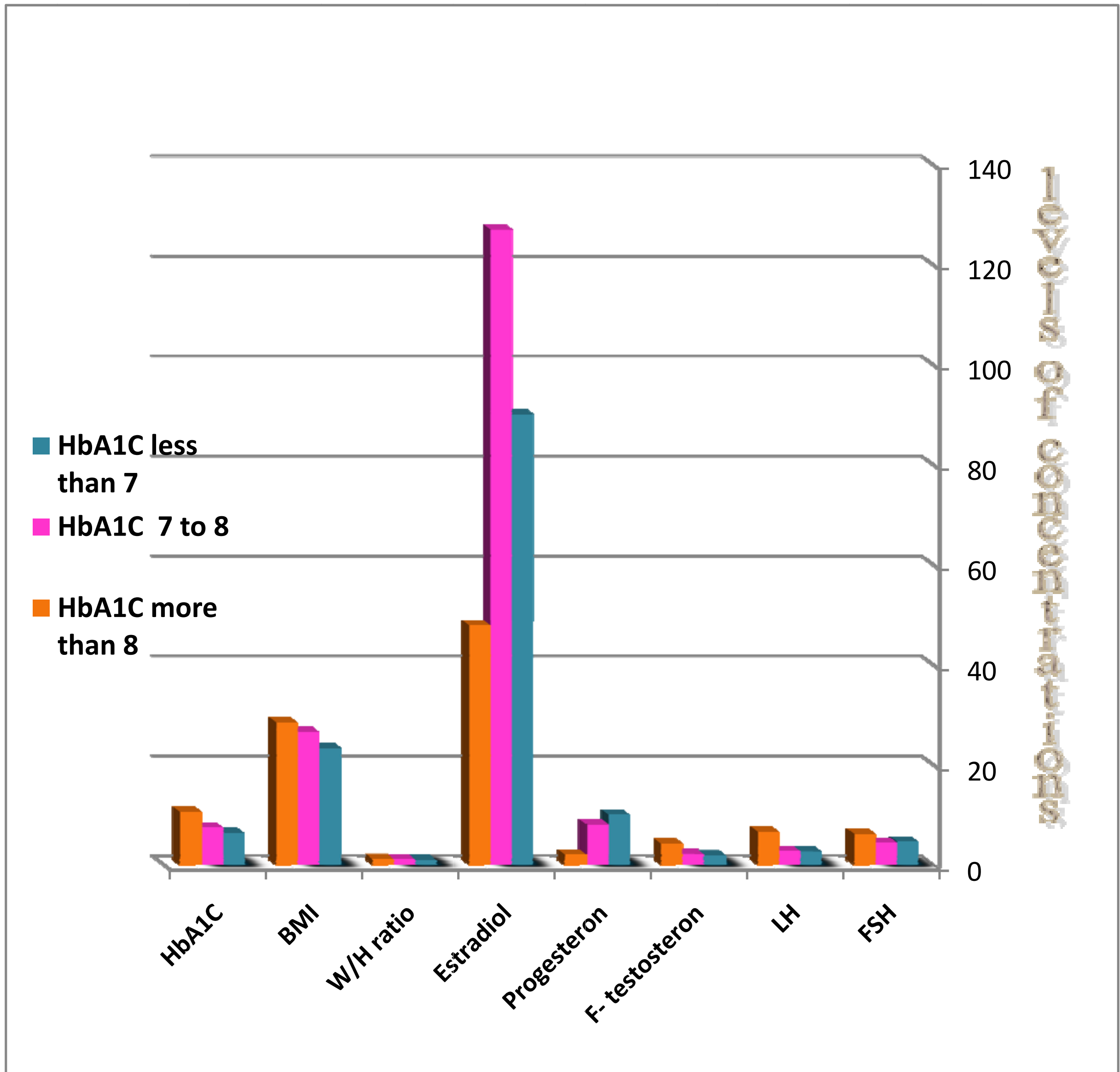


Figure3.2: Comparison of sex hormones levels according to degree of control of DM.

3.1.7: Comparison of mean sex hormones levels according to age groups.

Results in table 3.9 showed that in the group I the mean value of BMI and WHR were 25.83±2.10 (Kg/m^2) and 1.074±0.122 respectively and 28.881±1.72 (Kg/m^2)and 1.1495±0.0679 respectively in the group II with *p* value 0.00(highly significant), also the mean value of HbA1c was 7.490±0.968% in group I and11.21±1.72% in group II while the mean value of LH and FSH were 3.88±0.36 (mIU/ml) and 4.85±1.14 (mIU/ml) respectively in group I and 6.73±0.69(mIU/ml) and 6.414±0.799 (mIU/ml) respectively in group II with *p* value 0.077(not significant) and 0.00 (highly significant) respectively , furthermore ,the mean value of free testosterone, progesterone and estradiol were 2.47±0.78 (pg/ml) and 7.05±1.20 (ng/ml)and 92±12 (pg/ml) respectively in group I and 4.55±1.25 (pg/ml), 4.20±0.818 (ng/ml) and45.40±8.55 (pg/ml) respectively in group II and the *p* values were 0.00,0.00(highly significant) and 0.019(significant) respectively.

Table 3.7: Comparison of mean sex hormones levels according to age groups.

Parameters	Group I Age(30-38) No=48	Group II Age(38-49) No=52	*P* Value
	Mean± SD	Mean± SD	
HbA1c%	7.490±0.968	11.21±1.72	0.00
BMI(Kg/m^2)	25.83±2.10	28.881±0.874	0.00
WHR	1.074±0.122	1.1495±0.0679	0.00
LH(mIU/ml)	3.88±0.36	6.73±0.69	0.077
FSH(mIU/ml)	4.85±1.14	6.414±0.799	0.00
Free Testosterone(pg/ml)	2.47±0.78	4.55±1.25	0.00
Progesterone(ng/ml)	7.05±1.20	1.730±0.818	0.00
Estradiol(pg/ml)	92±12	45.40±8.55	0.019

P value <0.05 considered significant

In the present study it was found that there is a significant relationship between the age of diabetic women and the level of HbA1c, in another word, increased risk of diabetes mellitus type II with aging.

Studies conducted by. Arnetz et al. [215] and Kilpatrick et al. [216] have shown that there is a significant positive correlation between HbA1c and age in diabetic patients. Wolf et al. [217] reported that With aging, there is more serious risks such as non-insulin dependent diabetes mellitus. Iozzo et al. [218] Cowie et al. [219] and Uma et al. [220] suggested an increased incidence of diabetes was observed with age. Meneilly et al. [221] was also found that females suffer from diabetes at an older age, although in women with advancing age, diabetes becomes slightly more frequent, also it was found regarding our patient's body build that their BMI and WHR level varied with the age group so that the BMI was highest in the (46-50) age group.While the WHR was highest in the (41-45) age group which may be related to the long presence of diabetes mellitus disease.

Obesity is associated with an increased risk of developing type II diabetes and insulin resistance. Adipose tissue in obese individuals, releases increment amounts of non-esterified fatty acids, hormones, glycerol, pro-inflammatory cytokines and other elements that are required in the evolution of insulin resistance. When insulin resistance is accompanied by dysfunction of pancreatic islet β-cells, the cells that release insulin failure to moderate blood glucose level results. Abnormalities in β-cell function are so critical in determining the risk and development of type II diabetes mellitus [50]. Also, abdominal obesity may cause fat cells to expel pro-inflammatory chemicals. These chemicals can produce the body less sensitive to the insulin it produces

by disrupting the insulin function responsive cells and their ability to react to insulin [247,248].

The findings of current study agree with Akter et al. [249] who concluded that a high prevalence of both overweight and obesity exists in diabetic women of reproductive age in Bangladesh and it seems to be associated with increasing age.

While several studies indicated the positive association between age and obesity in healthy women Sanchez-Garcia et al. [222] who suggested that age associated with the changes in anthropometric values were identified, 73.7% of women and 19.1% of men (WHR 0.85 and WHR 1) had high central adipose tissue distribution. Sowers et al. [223] founded, a positive correlation of age with WHR, and Wang et al. [224] concluded that the cutoff value of BMI, WHR, were increased with increase age in the Chinese DM patientes.Tyagi and Kapoor. [225] conducted that among the adult urban females of Indian the weight, BMI and WHR showed an increase with advancing age.

In addition, it was found from our study that there were changes in sex hormones in our patients according to their age group. It can be seen from the table (3.9) and figure (3.3) that all hormonal changes between age groups were significant except for LH hormone where the changes between age groups were not significant.

The age of women is one of various factors which restricted the ovarian steroid secretion capacity, these hormones go down around the age of 30 years old, so that gonadotropin (LH, FSH) will increase [226,126].

During the premenopausal period insulin resistance becomes more prevalent and many women observe that their blood sugar levels

increase, due to the issue of sex hormones; furthermore, they note that the physical structure gets more sensitive to insulin like it was before the premenopausal period when a woman gets to menopause, and the stages of estrogen and progesterone decline [190].

Previous studies shed light on the correlation between sex hormones and age in postmenopausal diabetic women. These studies had shown that women with type II diabetes have a high testosterone at an older age [227,228,229,230,231]. And low estradiol [232].Noticing that these findings in postmenopausal women.

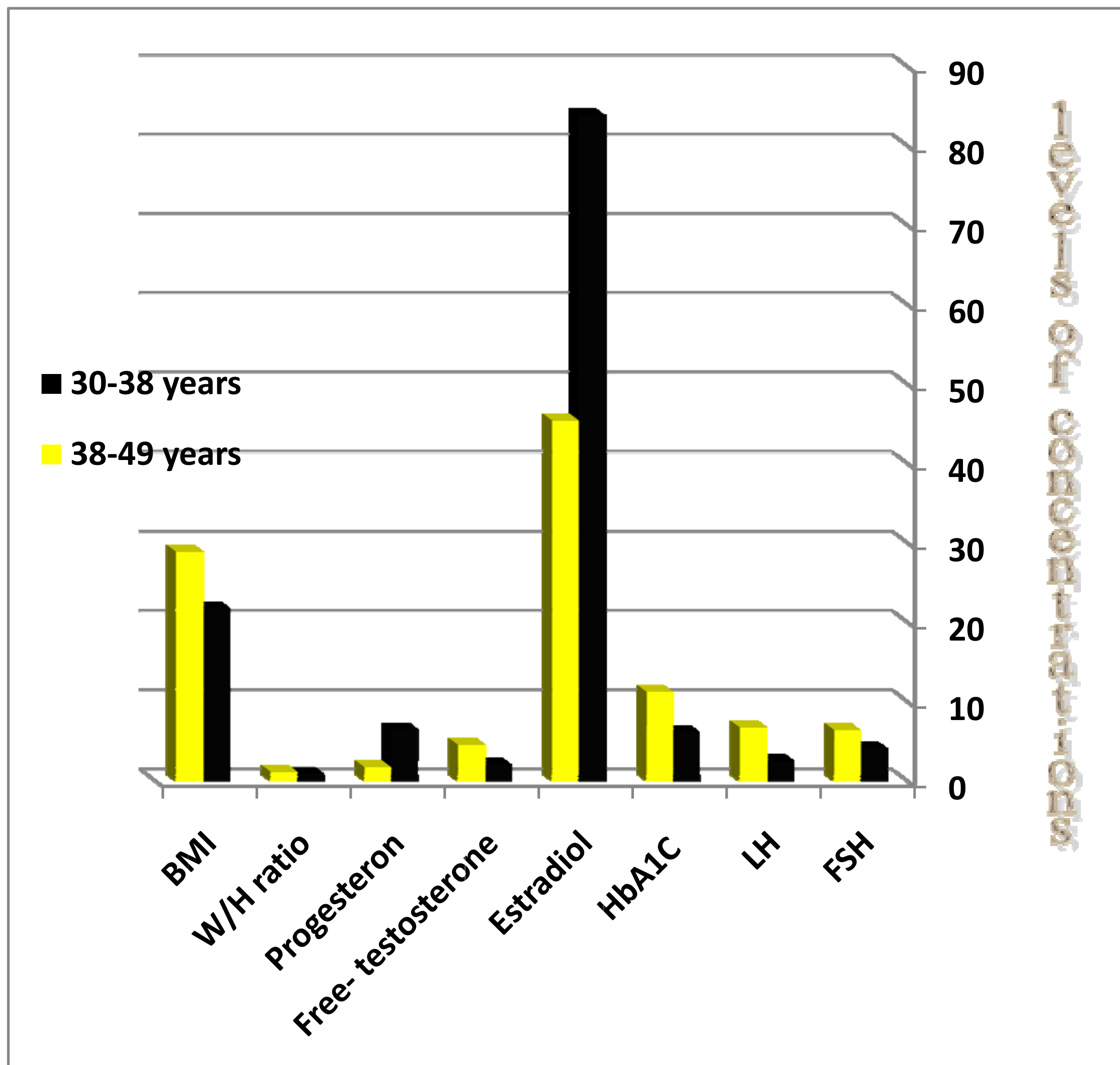


Figure 3.3: Comparison of sex hormones levels according to age groups.

3.1.8: Comparison of mean sex hormones levels according to the duration of diabetes.

Results in Table 3.10 showed that the level of HbA1c was 7.424±0.935 in group I and 11.12±1.73 in group II with highly significant *p* value of 0.00. While the LH level was 3.88±0.16 in group I and 6.63±0.43 in group II and the difference was not significant (*p*= 0.085), according to the levels of FSH and free testosterone were significantly higher in group II than group I (*P*=0. 00) With levels of 4.77±1.09 (mIU/ml), 2.43±1.80 (pg/ml) in group I respectively, and 6.423±0.786 (mIU/ml), 4.51±1.24 (pg/ml) in group II respectively. Whereas levels of progesterone and estradiol were significantly lower in group II than group I (*P*=0. 00) So, the levels were 7.25±1.18 (ng/ml), 94±13.5 (pg/ml) in group I respectively, and 1.761±0.834 (ng/ml), 45.75±8.57 (pg/ml) in group II respectively.

Table3.8: Comparison of mean sex hormones levels according to the duration of diabetes.

Parameters	Group I <4 years No=46 Mean± SD	Group II ≥4years No=54 Mean ±SD	*P* Value
HbA1c%	7.424±0.935	11.12±1.73	0.000
LH(mIu/ml)	3.88±0.16	6.63±0.43	0.085
FSH(mIu/ml)	4.77±1.09	6.423±0.786	0.000
Free testosterone(pg/ml)	2.43±0.80	4.51±1.24	0.000
Progesterone(ng/ml)	7.25±1.18	1.761±0.834	0.000
Estradiol(pg/ml)	94±13.5	45.75±8.57	0.021

P value <0.05 considered significant

The biochemical findings of this study as seen in Table 3.10 and Figure 3.4 revealed in female diabetic patients there is a positive correlation between HbA1c and duration of diabetes type II. Manouchehr et al. [233] Clark et al. [234] Colwell et al. [235] and Kitabchiet al. [236] found that duration of diabetes related with poor glycemic control. Gloria et al. [237] reported that duration of diabetes influence glycemia directly and HbA1c indirectly. Also studies prepared by Kilpatrick et al. (216) and Arnetz et al. (215) have demonstrated a significant positive correlation between HbA1c and duration of diabetes in diabetic patients.

Ronald et al. [238] indicated that the longer duration of diabetes is known to be linked with poor control, possibly because of progressive deterioration of insulin secretion with time because of beta cell failure, which prepares the answer to diet alone or oral agents unlikely.

David et al. [239] indicated that age and poor glycemic control related pathology with duration of diabetes are believed to speed up degenerative changes in a concerted way.

According to the duration of diabetes we found that free testosterone and FSH levels were increased significantly. But the increased in LH hormones was insignificant, furthermore, our results suggested that progesterone and estradiol levels decreased significantly with duration of disease.

Poor control of diabetes mellitus type II presented by high HbA1c level associated with increased levels of sex hormones probably due to the effect of poor control on ovarian function.

There is a strong link between duration of DM type II and age [234,235,236] as most of patients with DM2 discovered late in

adulthood. So if we have longer duration of DM we assume that those patients in the higher age group.

Possibly the long term alteration in diabetes control and ovarian exposure to high glucose level may affect hormonal balance, leading to increase free testosterone, FSH and decrease progesterone and estradiol.

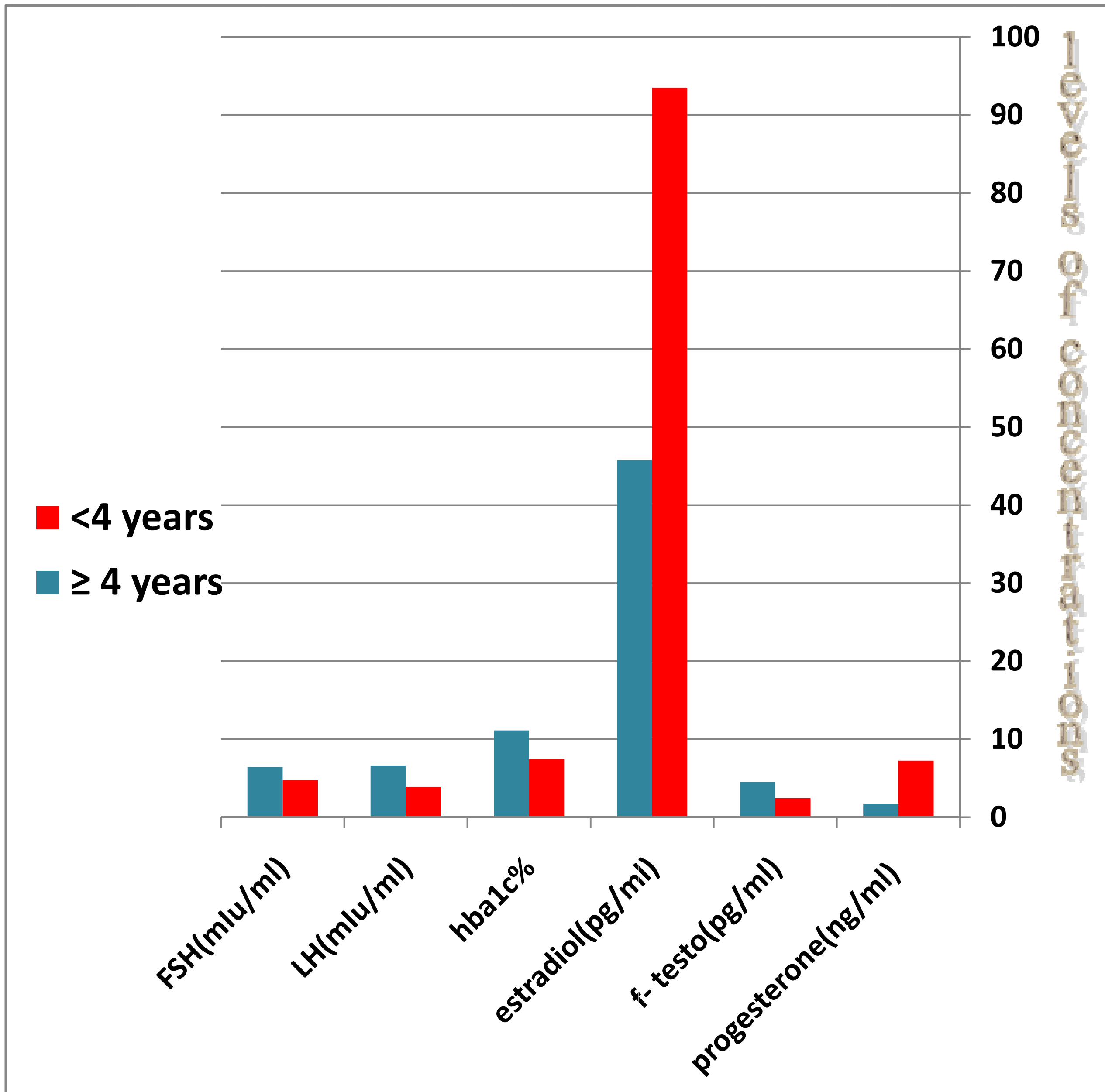
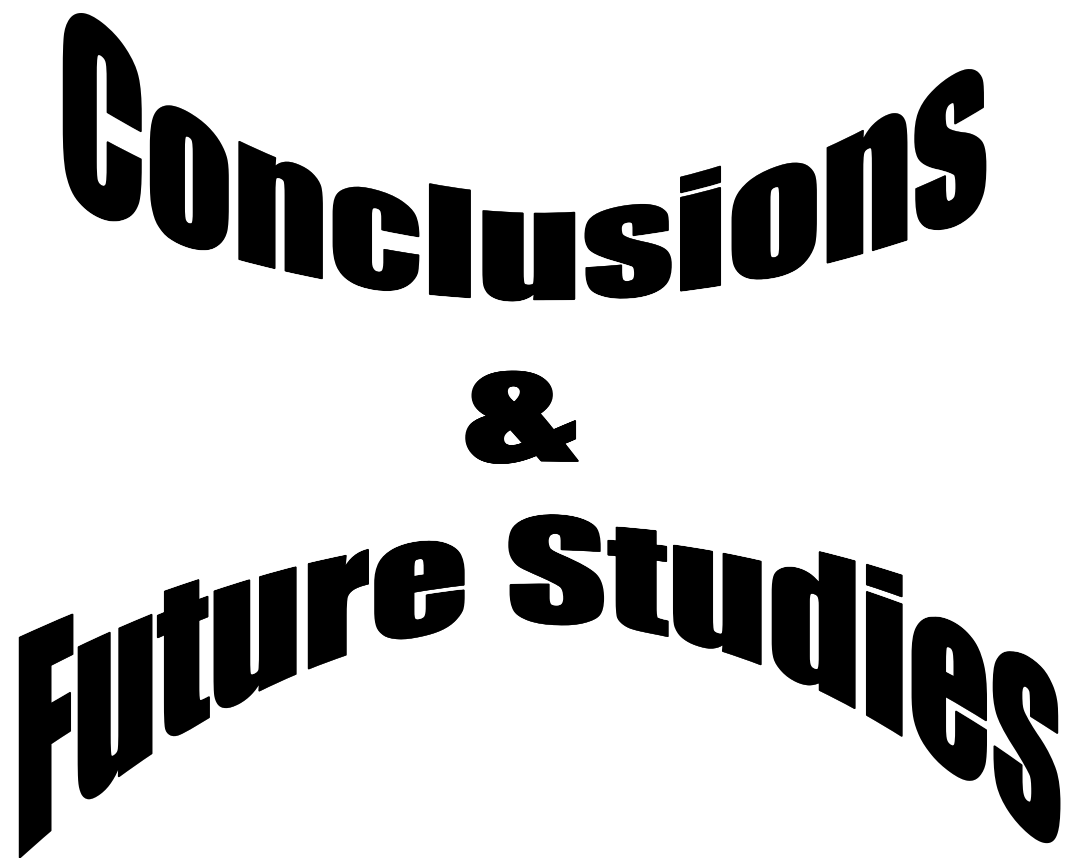


Figure 3.4: Comparison of sex hormones levels according to duration of diabetes.


Conclusions
&
Future Studies

I. *Conclusions*

In conclusion,

1. In type II diabetic premenopusal women an alteration in Free testosterone, FSH, Progesterone and Estradiol hormones levels compared to control group.

2. Women with poor glycemic control have an increase in free testosterone and FSH hormones, associated with a decrease in both progesterone and estradiol compared with good glycemic control.

3. Age of Premenopausal women play an impotant role of variation in anthropometric and reproductive hormones measurement .

4. Duration of diabetes affected levels of reproductive hormones in Premenopausal women.

II. Future studies

According to the results presented in this thesis, the study recommends the following:

1. Measurement serum insulin in diabetic premenopausal women is recommended to predict insulin resistance and its correlation with sex hormones.
2. Study the impact of increasing free testosterone in diabetic women on the other sex hormones.
3. Comparison of sex hormones level in type I and type II diabetic premenopausal women.

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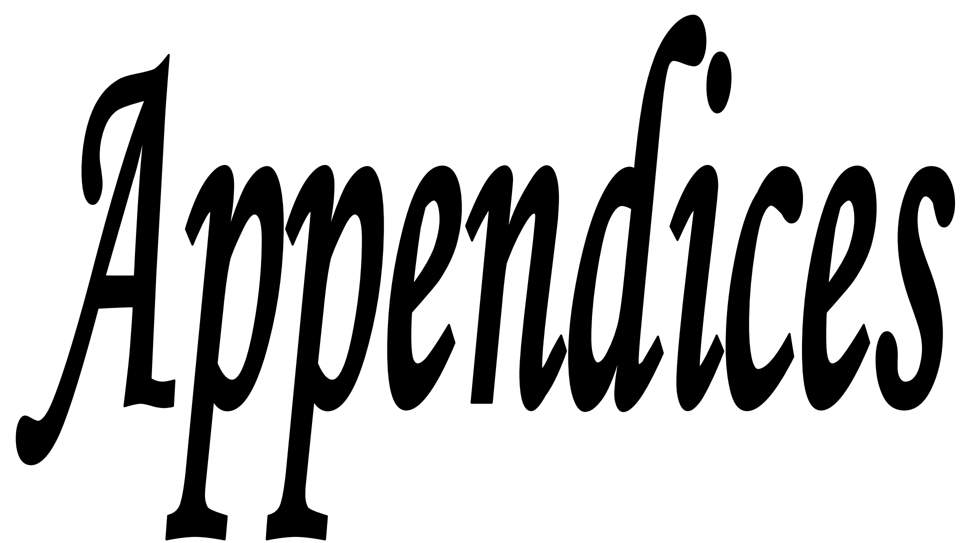
Appendices

Appendix I

Questionnaire

No. : **No. of file:** **Name:**

Age: **occupation:** **diet:**

Medication: (Type of treatment):

Duration of disease: **Family history:**

Pregnancy: **lactating:**

Smoking: **other disease:** **married:** **children:**

Day of menstrual cycle: **regularity of cycle:**

Weight:	Height:	BMI:
Waist:	Hip:	W/H ratio:

BIOCHEMICAL TESTS

Parameters	Level
FBG(mg/dl)	
HbA1C%	
LH(mIu/ml)	
FSH(mIu/ml)	
Free testosterone(pg/ml)	
Progesterone(ng/ml)	
Estradiol(pg/ml)	

Appendix II

جمهورية العراق
وزارة الصحة
دائرة صحة بغداد / الرصافة
مكتب المدير العام
شعبة التدريب والتطوير
وحدة التطوير المهني المستدام
العدد
التاريخ / / ٢٠١٢

م/ تسهيل مهمة

تحية طيبة...

كتاب وزارة التعليم العالي والبحث العلمي/ جامعة النهرين/ كلية العلوم
المرقم في / ١٠ / ٢٠١٢

نرجو الموافقة على تسهيل مهمة الباحث ( ) طالبة الماجستير
لانجاز بحثه الموسوم (( ))

في الحصول على البيانات الإحصائية وتزويده بالمعلومات الواردة في محتويات البحث وحسب الضوابط وسياقات العمل على أن لا تتحمل وزارة الصحة أية تبعات مالية مع التقدير...

مدير شعبة التدريب والتطوير
/ / ٢٠١٢

نسخة منه إلى/
شعبة التدريب والتطوير / وحدة التعليم الطبي المستمر

الخلاصة

داء السكري هو المرض الذي يؤثر على الحالة الأيضية والهرمونية للجسم البشري، واحد هذه التاثيرات هو مستوى الهرمونات الجنسية التي يتأثراختلافها وفقا لمدة المرض، ودرجة السيطرة على المرض وعمر المريض.

أجريت هذه الدراسة في المركز التخصصي لأمراض الغدد الصماء والسكري (SCED) في مدينة بغداد للفترة من ديسمبر 2013 إلى سبتمبر 2014. تم اختيارمائتي امرأة قبل سن اليأس (30-49) خلال الثلث الاخير من الدورة الشهرية ، نصف عدد النساء كن مصابات بداء السكري من النوع الثاني والنصف الآخر كن سليمات. هذه الدراسة من النوع المقطعي المقارن صممت لتقييم علاقة الهرمونات الجنسية (التستوستيرون الحر، الاستراديول، البروجسترون، LH، FSH) مع السكر التراكمي(الذي يمثل درجة السيطرة على مرض السكري)، عمر المرضى ومدة مرض السكري، باستخدام البيانات التي جمعت.

تمت عملية قياس نسبة FBG بالطريقة الانزيمية اللونية، HbA1c بواسطة (الاختبار المناعي)، وقد تم قياس هرمون التستوستيرون الحر بواسطة (ELISA)، (FSH، LH، البروجسترون، استراديول) باستخدام تقنيةELFA بجهاز(Vidas).

أظهرت النتائج أن نسبة HbA1c، التستوستيرون الحر، LH، FSH كانت أعلى، في حين كان البروجسترون والاستراديول أقل في النساء المصابات بداء السكري مقارنة مع النساء السليمات . وزادت مستويات FSH بشكل كبير في النساء الذين تتراوح أعمارهم بين (38-49) سنة، في حين مستويات البروجسترون والاستراديول انخفضت بشكل ملحوظ. فيما يتعلق بهرمون(LH) فقد كانت هناك زيادة في المستوى ولكن الزيادة كانت غير ذات دلالة من الناحية الإحصائية. بالإضافة إلى ذلك فقد تأثرت نسبة التستوستيرون الحر ومستويات FSH مع درجة السيطرة على داء السكر، بعبارة اخرى ،في حالة ضعف السيطرة على داء السكري ارتفع مستوى التستوستيرون الحر وال FSH بينما كان هناك انخفاض في مستوى هرمون البروجستيرون والاستراديول ، وهنا ايضا **كانت هنالك زيادة في هرمون LH لكن الزيادة كانت إحصائيا ضئيلة .**

مع زيادة مدة المرض كان هنالك زيادة في هرمون التستوستيرون الحر وFSH. بالإضافة إلى ذلك كانت هناك زيادة ضئيلة في مستوى هرمون LH. وانخفضت مستويات هرمون البروجسترون واستراديول مع زيادة مدة داء السكري عند المرضى.

يستنتج من هذه الدراسة أن هناك علاقة ذات دلالة بين مستوى الهرمونات الجنسية في الدم وعمر المريضات ودرجة التحكم في نسبة السكر في الدم ومدة داء السكري في النساء قبل سن اليأس المصابات بمرض السكري من النوع الثاني.

جمهورية العراق
وزارة التعليم العالي و البحث العلمي
جامعة النهرين / كلية العلوم
قسم الكيمياء

دراسة بعض الهرمونات الجنسية كدوال لمراقبة مرض السكري من النوع الثاني في النساء قبل سن اليأس

رسالة مقدمة الى
كلية العلوم – جامعة النهرين
وهي جزء من متطلبات نيل الماجستير في علوم الكيمياء الحياتية

من قبل
رونق جمعة كاظم بكلوريوس علوم
قسم الكيمياء– جامعة بغداد
(2005-2004)

بأشراف

أ.م د. سلمان علي أحمد

1436 هـ **2015 م**