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Evaluation of Vaspin Levels and Some Biochemical Variables in poly cystic Ovary Syndrome Patients in Diyala Governorate

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To that who is peerless

My Mother

I dedicate this effort . . . .

Suha

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بسم الله الرحمن الرحيم

وَقُلِ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ ۖ
وَسَتُرَدُّونَ إِلَىٰ عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُمْ بِمَا كُنْتُمْ تَعْمَلُونَ ﴿١٠٥﴾

صدق الله العظيم

سورة التوبة الآية 105

summary

Polycystic ovary syndrome (PCOS) is the most frequent and complex endocrine condition that affects women during their reproductive years, and is characterized by infertility, hirsutism, abnormal follicular development, and increased ovarian androgen production. A variety of different compounds produced by fat tissue or other organs have also been studied as PCOS biomarkers which may be linked to obesity such as, vaspin. The pathogenesis of PCOS might be due to hormonal disorders (ovarian and pituitary gland). The aims of the present study are detection the relation between polycystic ovarian syndrome and adipokine hormone (vaspin hormone). And detection the role of sex hormones in polycystic ovarian syndrome, and whether the PCOS may leads to other complications (cardiac disease, diabetes mellitus) by measuring the lipids profile (cholesterol, Triacylglycerol, High density lipoprotein, Low density lipoprotein) and (Fasting blood sugar, Glycosylated hemoglobin).

This hospital-based comparative study, which is done in Al-Batol hospital in Baaquba city, Diyala province, , included sixty (60) PCOS patients with age mean (28.40±8.17) and thirty (30) healthy women (control group) with age mean (28.33±6.84) included in this study subdivided into two subgroups (normal weight and obese) according to the BMI . Blood samples were aspirated from all individuals from day 2 of menstrual cycle , the women newly detected for PCOS and did not take any treatment in order to evaluate the role of vaspin, prolactin, Luteinizing hormone, Follicle-Stimulating hormone, testosterone, Estradiol , Fasting blood sugar , Glycosylated hemoglobin and lipid profile. Glycosylated hemoglobin

This study revealed a significant elevation in all hormones that included in the study in the two patients subgroups in comparing with the two control subgroups, vaspin levels increased significantly (0.001***) in normal weight patient group (54.83±11.01) in comparing with those of control group

(2.63±0.94), in the same way its levels increased significantly in obese patient group (64.50±3.07) comparing with obese of control group (15.22± 7.45), and an elevation in Fasting blood sugar and Glycosylated hemoglobin, a significant increase in Fasting blood sugar in PCOS patients of the two groups (normal weight and obese) comparing with those of control group, but this not significant increasing in Glycosylated hemoglobin . The cholesterol, triglyceride, Low density lipoprotein , and Very low density lipoprotein are increased significantly in both patients groups comparing with control groups while the High density lipoprotein levels showed no significant increasing. In conclusion the PCOS patients have risks of diabetes mellitus and cardiovascular diseases according to the results of lipid profile and glucose levels. Sexhormones play a role in the formation of PCOS by causing an increase in androgens, which leads to persistent estrogen overproduction and encourages ovarian stromal hyperplasia. The increase in vaspin levels caused fat to accumulate around the waist, which is a symptom of PCOS.

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List of Abbreviations

Abbreviations	Full name
AIP	Atherogenic index of plasma
ASRM	American Society of Reproductive Medicine
BMI	Body Mass Index
CVD	Cardiovascular Disease
DHEA	Dehydroepiandrosterone
DHT	Di hydrotestosterons
E_2	Estradiol
ELISA	Enzyme Linked Immune Sorbant Assay
ESHRE	European Society for Human Reproduction and Embryology
FAI	Free Androgen Index
FSH	Follicle Stimulating Hormone
FBS	Fasting blood sugar
G-3-P	glycerol-3- phosphate
GnRH	Gonadotropin-Releasing Hormone
GOD	Glucose Oxidase
GPO	Glucose peroxidase
HA	Hyperandrogenism
HbA_{1c}	Glycosylated haemoglobin

HCG	**Human Chorionic Gonadotropin**
HDL	**High Density Lipoprotein**
HOMA	**Homeostatic Model Assessment**
hs-CRP	**High Sensitive C-Reactive Protein**
HRP	**Horseradish Peroxidase**
IFG	**Impaired fasting glucose**
IGT	**Impaired glucose intolerance**
IR	**Insulin Resistance**
IU	**International Unit**
LDL	**Low Density Lipoprotein**
LDL-c	**Low Density Lipoprotein-cholesterol**
LDLR	**Low Density Lipoprotein Receptor**
LH	**Luteinizing Hormone**
LPL	**lipoprotein lipase**
^{o}C	**Centigrade**
OD	**Ovulatory dysfunction**
P	**Probability**
PCOS	**Polycystic Ovary Syndrome**
POD	**Peroxidase**
ROC	**Receiver operator curve**

SAT	**Subcutaneous adipose tissue**
SD	**Standard Deviation**
SHBG	**Sex Hormone Binding Globulin**
SPSS	**Statistical Package for Social Sciences**
T	**Testosterone**
T2DM	**Type Two Diabetes Mellitus**
T3	**Triiodothyronine**
T4	**Thyroxin**
TG	**Triglyceride**
TSH	**Thyroid Stimulating Hormone**
TT	**Total Testosterone**
VAT	**Visceral adipose**
VLDL	**Very Low Density Lipoprotein**
WHO	**World Health Organization**
WHR	**Waist Hip Ratio**
WHtR	**waist-to-height ratio**

1.1.Introduction

Polycystic ovarian syndrome (PCOS) is a complex endocrine condition that affects women of reproductive age. PCOS has a variety of causes that aren't fully known, but there's substantial evidence that it's mostly a genetic condition. Anovulation, hyperandrogenism, and the presence of ovarian cysts are the most common symptoms of PCOS(1). PCOS' pathophysiology is poorly understood, but it has been linked to hormone system abnormalities. The main anomaly of PCOS is assumed to be uncontrolled ovarian steroid genesis with thicker theca layers that secrete excessive androgen (2). Polycystic ovary syndrome has a significant impact on the lives of women who are affected, owing to issues such as oligohydramnios, dysfunctional uterine bleeding, infertility, hypothyroidism ,acne, obesity, acanthosisnigricans, hypertension, metabolic syndrome, insulin resistance, type 2 diabetes mellitus (DM), hyperlipidemia, breast cancer ,cardiovascular disease (CVD) and recurrent miscarriages (3).Hormonal disorders such as elevated luteinizing hormone (LH), testosterone levels, and follicle stimulating hormone (FSH) levels have also been discovered to be the main cause of this disease (4). Many studies were based on biochemical phenotyping which offers several advantages over ovarian sonography. First, it can be accomplished with the single blood test assayed. Second, the background rate of abnormal values in the control population can be standardized. Third, biochemical criteria are objective and not subject to operator interpretation (5). Although PCOS is classified as a "complex genetic disease," there is many evidence support a large genetic basis, especially given the syndrome's strong familial nature. Variation in disease manifestation may be influenced in part by ethnicity, environment, or genetic background (6). Also the environmental factors, such as nutrition and activity, probably influence the clinical and biochemical presentation. Given the complicated interconnections of these variables on the PCOS phenotype, a single developmental origin for the diverse PCOS symptoms may appear improbable (7). The initial goal of

biochemical and molecular assays is to identify individuals at high risk by obtaining evidence of associated hormonal, biochemical and genetic parameters, leading to uncover the main cause of PCOS susceptibility (8) .

1.2.Previous studies

AlFaisal and Al-Deresawi, (2013) studied the relationship between the polycystic ovary and thyroid disorders by measuring the level of estradiol hormone (E2), luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone (T),thyroid stimulating hormone (TSH) , tri iodothtronine (T3) and thyroxin (T4) and the body mass index (BMI) and hirsute are also concluded, the results showed that there is a significant decrease in E2 and FSH levels in women having PCOS and fertile women , also, a significant increase in LH levels in infertile women and fertile women, while there is no significant differences in testosterone levels TSH,T3 and T4 levels between women infertile and fertile women .The hormonal profile according to BMI was showed to be significantly decrease in testosterone in obese and overweight in women with PCOS , significant decrease in level FSH in obese PCOS women and no significant differences in E2 and LH levels and a significant decrease in E2 and FSH levels in hirsute women in PCOS (9).

The researcher in (**Kuppusamy *et al***., (2015)) evaluate the cardiovascular risk in PCOS and the relationship between metabolic abnormalities and sympathovagal imbalance (SVI). The study included (BMI), waist-hip ratio, and baseline cardiovascular parameters .The researchers measured fasting plasma glucose, insulin, lipid profile, and testosterone levels. Insulin resistance (HOMA-IR) and lipid risk factors were calculated. The increased HOMA-IR, lipid risk factors and testosterone were significantly elevated in cases PCOS patients . There was a significant correlation of insulin resistance and lipid risk factors. On regression analysis, insulin resistance and lipid risk factors had independent association with PCOS patients have

the potential cardiovascular risks(10).

Malini, N. A., & George, K. R. (2018) suggested a dependence of insulin, LH and testosterone in initiating the hormonal imbalances in PCOS, by their study of seven PCOS subgroups classified on the basis of their LH:FSH ratios, the finding is (LH, FSH, LH:FSH ratio, insulin, HbA1c, estradiol, testosterone and TSH) were significantly increased whereas (progesterone and SHBG levels) were significantly decreased in comparison to control (11).

The study of (**Kim *et al*,**. (2018)) aimed to compare visfatin levels between non-obese women with PCOS and those of matched controls and compare between visfatin levels and various parameters. According to the data of this study, serum visfatin levels were similar between PCOS patients and those of the controls. In women with PCOS, significantly higher serum visfatin levels were observed in the hyper androgenic group than those in the nonhyperandrogenic group. Visfatin levels showed positive correlations with BMI and log free androgen index (FAI) and a negative correlation with HDL cholesterol levels (12).

While in the study of(**Jena *et al*,**. (2018)) comparison done between different adiposity parameters, namely visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) between patients with polycystic ovary syndrome (PCOS) and controls. In addition, correlate these adiposity indices with hormonal parameters as well as cardiovascular (CV) risk factors in patients with PCOS. Recently diagnosed PCOS patients of productive age group according to Rotterdam criteria were included. Age and BMI matched healthy females with normal menstrual cycles were taken as controls. All the study participants undergo detailed clinical, biochemical, and hormonal evaluation. Trans abdominal ultrasound was performed for detailed ovary imaging and assessment of adiposity (SAT and VAT) parameters. A total of

PCOS patients and BMI-matched controls were included. PCOS patients had significantly higher levels of androgens , elevated highly sensitive C-reactive protein, and higher degree of insulin resistance than controls.These parameters were significantly higher than controls , despite both groups having similar BMI. Among PCOS groups (13).

Al Zhang *et al*,.(2019) In this study, a comparison was made between healthy non-obese women and women with polycystic ovaries non-obese women . Non obese women with PCOS showed a higher prevalence of hyperinsulinemia, impaired fasting glucose (IFG), impaired glucose intolerance (IGT), low-HDL, no significant difference of healthy non-obese women was observed and women with polycystic ovaries non-obese women for pre-DM, dyslipidemia, hypercholesterolemia, and hypertension . No study reported specifically an incidence of myocardial infarction, stroke, cerebrovascular accident, arterial occlusive disease, and coronary heart disease in nonobese women with PCOS. Nonobese women with PCOS also suffer from metabolic disturbances and the risk of long-term metabolic complications. Further efforts should be made to elucidate underlying mechanisms and possible interventions in the early phase (14).

Faraj *et al*., (2019) investigate the effect of BMI on ovarian hormones in women with PCOS and healthy women, in which were divided into two groups for testing (BMI less than 25 and more than 25). Hormonal study of Luteinizing hormone (LH), Androgen, Estradiol (E_2) was done for each patient, the results of these hormones in patients compared with controls in both BMI groups with E_2/T ratio showed a significant increase in patients women compared to controls in ($p<0.05$), While the results of Follicle stimulating hormone (FSH) and total Testosterone (TT) showed an increase with not statistically significant. it has been concluded that the increase in BMI doesn't show to have an opposite effect on FSH, Androgen, E_2, and TT levels but the

result of LH shows a highly significant increase in PCOS groups compared to controls when BMI>25 (15).

Al-Juaifari and Al-Jumaili , (2020) This study included women with PCOS and women healthy control women .The analysis of hormones 17B - Estradiol, LH, FSH, T3, Thyroxin and TSH carried out , the results show high significant differences in the levels of serum E2 and LH between two groups and no significant difference in level of serum FSH .The findings also indicate a slight rise in serum concentrations T3 and non-significant between two groups and significant decrease in the level of serum Thyroxin between women with PCOS and control group. The results showed significant positive correlation between E2 and LH which gave and negative correlation with Thyroxin which gave, while other hormones FSH, Tri-iodo thtronine and T3 no significant difference in patient with PCOS and apparently healthy control . This study concluded that the concentrations of estrogen showed rising against ordinary checks in PCOS instances. High concentrations of androgens in peripherally converted PCOS to estrogens may contribute to their enhanced concentration (16)

Dhahaad and Al-Saadi, (2020) Find out in this study a correlation between Aromatase, some sex hormones, and BMI among patients with PCOS was investigated , in which the aromatase relation with PCOS female (without got treatment and got) compared with (E2), testosterone hormones and body mass index. And divided them into three groups with PCOS without getting any treatment, with PCOS with getting treatment and as a healthy females. Aromatase level, serum sex hormones estradiol (E2) and testosterone were measured on the third day of menstrual cycle. Also body mass index was calculated and all variable were compared for the three groups. A negative correlation was found between aromatase with both E2 and testoaterone hormones in female with PCOS without

any treatment. A positive correlation between aromatase and testosterone while negative correlation with E2 in female with PCOS whose got treatment. The correlation between aromatase and BMI gave a positive relation in females with PCOS in both groups (17).

Ibrahim *et al.,* (2020) showed the levels of Apelin, Endoglin, and Transforming(TGFB1) in Women with Polycystic Ovary Syndrome . Levels of apelin, LH, LH/FSH, T, and fasting insulin, as well as homeostatic model assessment of IR (HOMA-IR) in PCOS patients, were significantly higher than in the control group. Correlation analysis showed that apelin level was positively correlated with body mass index and HOMA-IR. Apelin levels and TGF-β1 were significantly increased in PCOS patients while show decrease levels of endoglin (18).

Lin *et al.,* (2020) The concentrations of circulating adipokines in non-obese polycystic ovary syndrome (PCOS) patients had been reported in this study , however, this meta-analysis was done to assess whether the levels of circulating adipokines were changed in non-obese PCOS. And showed that circulating levels of adiponectin decreased statistically in non-obese PCOS women . On the contrary, circulating levels of chemerin , leptin , resistin and visfatin increased significantly in non-obese PCOS females. Besides, there was no statistically significant change in the circulating levels of apelin , irisin , and omentin in non-obese PCOS patients. Scientific evidence suggested that the levels of circulating adipokines altered in non-obese PCOS patients compared with controls. Depending on the degree of obesity, the abnormal change of circulating adipokines levels might play an important role in the occurrence and development of PCOS (19).

Jasim et al., (2020) showed the correlation between serum interleukins levels with Anthropometric data and lipid profiles in obese women with PCOS. Obese non PCOS healthy women .were used to measure serum levels of ,high sensitive C-reactive protein (hsCRP), insulin, total testosterone, and sex hormone binding globulin(SHBG). Also the biochemical measurements of blood glucose and lipid profile were performed by conventional colorimetric methods. The results demonstrated significant serum levels of all interleukins and lipid profile parameters in obese PCOS in comparison with obese on PCOS control women .In addition, PCOS women had higher insulin and free androgen index FAI levels in comparison to non PCOS women. The findings suggest that androgen excess, indicated by high (FAI),might serve as an indicator of a prediabetes status, as it might promote insulin resistance dysfunction in PCOS women. Circulatory interleukin levels in obese PCOS women may act on the development of insulin resistance and androgen oversupply in PCOS, suggesting that high serum ILs levels were related to insulin resistance (IR) and androgen excess but not to body mass index. A high IL level is not an elemental characteristic of PCOS, but it may act in promoting IR and hyperandrogenism of PCOS (20) .

Moin *et al.*, (2021) This study aimed to women with polycystic ovarian syndrome (PCOS) have a hypercoagulable state; however, it is unclear whether this is attributable to PCOS itself or as a result of its metabolic problems. Plasma coagulation pathway protein levels were measured in PCOS women who were enrolled to a PCOS. Plasma protein quantification using a Slow Off- rate modified Aptamer-scan. Patients with PCOS had a higher BMI insulin, but the cohorts were age-matched. Patients with PCOS showed greater levels of plasma kallikrein,

fibrinogen, fibrinogen gamma chain, fibronectin, von Willebrand factor, D-dimer, P-selectin, and plasma kallikrein, but were more likely to develop high blood pressure or heart disease. However, as a sign of inflammation, two anticoagulant proteins, vitamin K-dependent protein-S) and heparin cofactor-II, were enhanced and prothrombin was decreased, and insulin resistance (HOMA-IR) linked with 11 and 6 of the clotting proteins, respectively. There was no association between PCOS and any of the coagulation proteins in a multivariate study that took into consideration inflammation, insulin resistance, and BMI. The hypercoagulable state in PCOS is not unique to the disease, BMI, inflammation, and insulin resistance all have a role (21).

1.3. The aim of the study

The aims of the present study are detect the relationship between polycystic ovarian syndrome and adipokine hormones (vaspin hormone). And detection the role of sex hormones in polycystic ovarian syndrome, whether the PCOS leads to other complications (cardiac disease, diabetes mellitus) by measuring the lipid profile (cholesterol, Triglyceride , High density lipoprotein , Low density lipoprotein) and (Fasting Blood Sugar, Glycated hemoglobin).

2.1.Polycystic ovarian syndrome(PCOS)

Polycystic ovary syndrome (PCOS) is a common hormonal disorder among women of reproductive age with a prevalence of 5–10% the name of the condition comes from the appearance of the ovaries in most, but not all, women with the disorder enlarged and containing numerous small cysts located along the outer edge of each ovary (polycystic appearance) (22). The names for this syndrome include polycystic ovarian syndrome (PCOS), polycystic ovary disease (PCOD), functional ovarian hyperandrogenism, Stein-Leventhal syndrome (original name, not used in modern literature), ovarian hyperthecosis and sclerocystic ovary syndrome (23). In the absence of specific adrenal and pituitary disease, PCOS is characterized as the presence of hyperandrogenism and chronic ovulation (24).The disruption of reproductive hormones such as LH, FSH, estrogen, and testosterone causes abnormalities in the menstrual cycle (like amenorrheaor,irregular,menstrual,periods),Obesity,dyslipidemia,dyemi a,hyperinsulinism (hyperinsulinemia),insulin resistance (IR), and an elevated risk of type 2 diabetes mellitus and cardiovascular disease are among the endocrine and metabolic traits linked to PCOS (25).

Polycystic ovary syndrome PCOS can increase the risk of miscarriage and pregnancy complications including gestational diabetes in women(26). If PCOS is left untreated in the long term, the syndrome may lead to develops chronic risks in adolescents which including obesity, type II diabetes, infertility, endometrial dysplasia, cardiovascular disorders (27),(28). Owing to the intricacy of this

condition, various sets of diagnostic criteria have been initiated for the confirmation of PCOS which are listed below in Figure (2-1) .

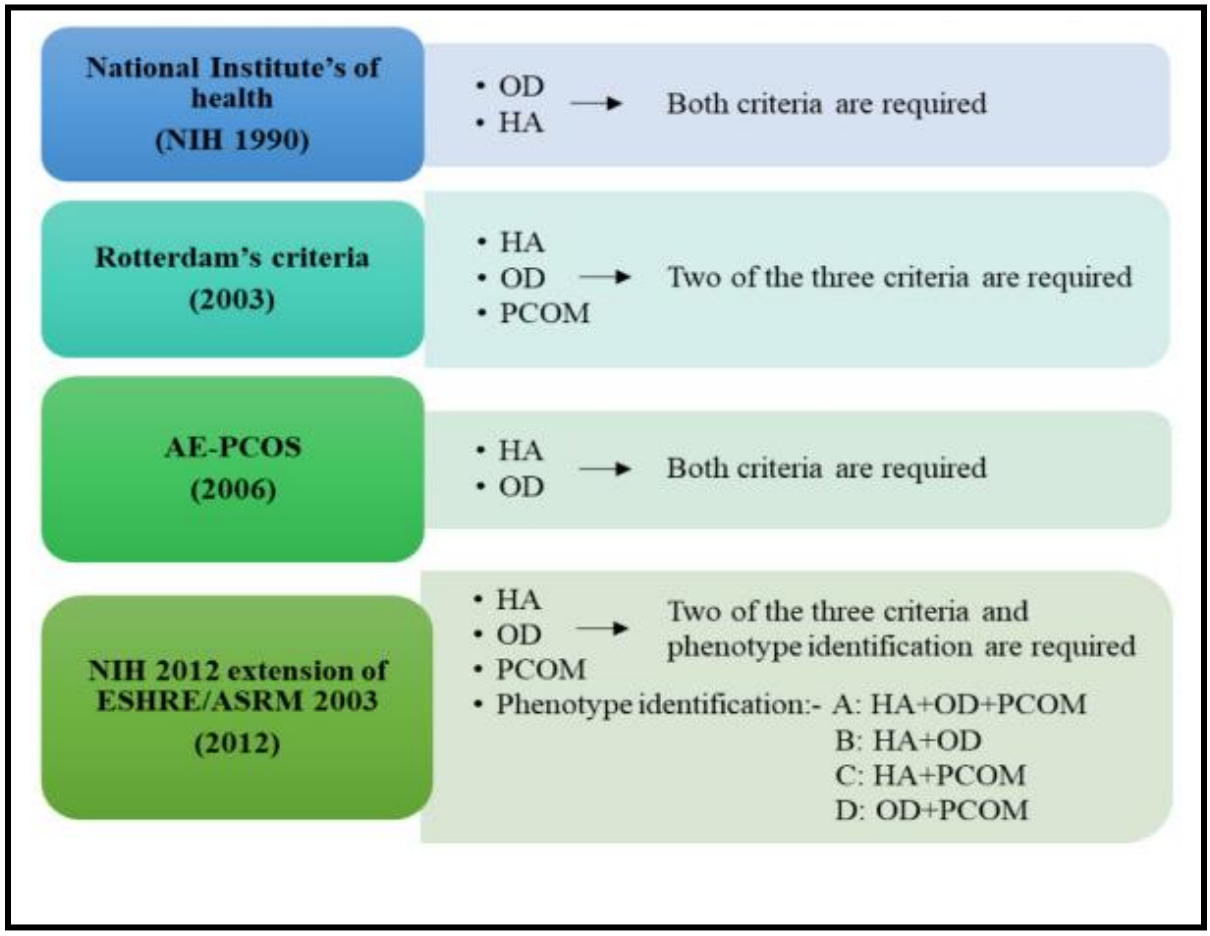


Figure (2-1) Summary of diagnostic criteria for PCOS(29).

Abbreviations: AE-PCOS: Androgen Excess and PCOS society; ASRM: American Society of Reproductive Medicine; ESHRE: European Society for Human Reproduction and Embryology; HA: Hyperandrogenism; OD: Ovulatory dysfunction; PCOM: Polycystic ovarian morphology (12 follicles and 2-9 mm in each ovary).The complex nature of PCOS makes it difficult to pinpoint a single cause.

2.1.1 Poly cystic ovary syndrome and Prevalence of Infertility

The failure of a couple to conceive after 12 months of regular intercourse without the use of contraception in women younger than 35 years of age is the most widely accepted clinical definition of infertility (30). Infertility affects roughly 10% of the world's population. The WHO ranks infertility in the women population as the fifth highest serious global disability according to the Maternal Health Task Force,50 million couples worldwide are infertile (31). Infertility has significant societal and personal for affected couples, resulting in a diminished sense of well-being. Fertility illnesses like polycystic ovarian syndrome (PCOS), which have a wide range of symptoms, all of which impair female identity, might cause specific psychological difficulties (32). PCOS was first characterized in 1935 and has been the subject of much research since then, despite the fact that its etiology and pathogenesis are still poorly understood (33). In comparison with study , there has been an alarming increase in infertility in couples, particularly in Babylon, Iraq. The incidence of infertility was about 12% of total couples, and the incidence of PCO in general females was about 35% (married or unmarried, possibly due to a late in discovery, especially with unmarried group) and 45% in married women decreasing with age (34).

2.1.2 Epidemiology

Poly cystic ovary syndrome is the most common endocrine illness in women, with a prevalence of 5% to 15%. Symptoms include oligomenorrhoea, hirsutism, acne, and hair loss. It produces major psychological disorders in adolescence, such as anxiety and depression (35). Family history was found to have a strong association in incidence and manifestation of the disorder (36). According to the World Health Organization (WHO) estimation revealed over 116 million women (3.4%) are affected by PCOS worldwide PCOS prevalence estimates vary widely around the world , although there is no reliable statistical evidence on the condition's prevalence (37). (38) showed the precision and sensitivity of the procedures for assessing the individual features (and thus the phenotypes) of PCOS are critical in epidemiologic investigations.

2.1.3.Etiology

According to researches many gene loci and the family history of PCOS and its health consequences have been connected to the development of PCOS (39).Overweight, obesity, low socioeconomic status, a family history of PCOS, insulin resistance ,hyperinsulinemia, and hyperandrogenism are all risk factors for the development of PCOS in women (40). Overconsumption of sugar-rich, fructose-rich, transfat-rich, animal-fat-rich, and processed-food-rich diets and beverages are thought to be the major causes (41). Studied that menstrual cycle issue, bad mood, family history of diabetes, family history of infertility, mother's menstrual irregularity, and lack of physical activity were all found to be risk factors for PCOS (42). (43) mentioned that the cardiovascular risk factor in PCOS include obesity, impaired glucose tolerance, diabetes,

hypertension, mood disorders and metabolic syndrome are play role in appearance PCOS.

2.1.4.Clinical features

There are many signs and symptoms for patients with PCOS includes (44). ;

- Heavy bleeding.
- Irregular and missed periods.
- Pelvic pain.
- Infertility
- Excess body hair.
- Difficulty getting pregnant.
- Acne and oily skin.
- Dark, thickened skin (hirsutism).
- Bleeding without ovulation.
- Obesity.
- Depression.
- Hyperandrogenism

2.1.5 Diagnostic Criteria for PCOS

A comprehensive history and physical examination should be the first step in the diagnostic process. Clinicians should pay attention to the patient's menstrual history, weight changes and their impact on PCOS symptoms, as well as cutaneous abnormalities (e.g., terminal hair, acne, alopecia, Acanthosisnigricans, skin tags) (45). (46) demonstrates that individuals with PCOS had more irregular menstrual periods and hirsutism ($P< 0.001$) but fewer climacteric symptoms ($P< 0.05$). Many researchers estimate that 25% of

women with PCOS do have regular menstrual cycles, despite the fact that these times of menstrual bleeding could be anovulatory cycles. Although the recommendations are vary between standards, the Endocrine Society recommends that practitioners diagnose PCOS using the 2003 Rotterdam criteria. The presence of the following three features, according to the Rotterdam criteria, is required for diagnosis: pcos of irregular menstrual cycles and anovulation; hyperandrogenism that can be measured chemically. Ultrasound, transvaginal, and abdominal, are all used to assess enlarged ovaries. (table 2-1) (ESHRE and ASRM-Sponsored PCOS Consensus Workshop Group, (2004).

Excessive acne, androgenic alopecia, or hirsutism (terminal hair in a male-pattern distribution) can all be signs of hyperandrogenism, as can increased serum levels of total, bioavailable, or free testosterone, or dehydroepiandrosterone sulfate (47). In the rare case where an androgen-secreting tumor is suspected, measuring androgen levels can aid (e.g., when a patient has marked virilization or rapid onset of symptoms associated with PCOS) as an Ovulatory dysfunction refers to oligomenorrhea (cycles more than 35 days apart but less than six months apart) or amenorrhea (absence of menstruation for six to 12 months after a cyclic pattern has been established (48).

A polycystic ovary is described as an ovary with 12 or more follicles measuring 2 to 9 mm in diameter (or 25 or more follicles using modern ultrasound technology) or an ovary with a volume more than 10 mL on ultrasonography (49). A single ovary that meets one or both of these criteria is enough to diagnose polycystic ovaries. However, unless imaging is required to rule out a tumor or the patient meets only one of the other Rotterdam criteria

for PCOS, ultrasonography of the ovaries is not indicated (50). The Endocrine Society advises women with suspected PCOS to rule out pregnancy, thyroid dysfunction, hyperprolactinemia, and nonclassical congenital adrenal hyperplasia (45).

suggest that adiposity rebound was linked to PCOS diagnosis and a high BMI in adulthood, according to the findings. PCOS signs, such as prolonged irregular periods and hirsutism, should be checked in adolescent girls with early Adiposity rebound and persistent obesity measurement of LH, TSH, and follicle-stimulating hormone (FSH) levels to calculate a serum ratio of LH/FSH are further procedures that may be useful for diagnosis. A ratio greater than 2 is generally indicative of PCOS, however no clear cutoff values exist due to the variety of assays utilized (51). The FSH level is more useful in determining whether or not you have ovarian failure (52). A study(53) suggests that high levels of anti Müllerian hormone are linked to premature birth in people with polycystic ovarian syndrome . A research (54) reveal that increased vaspin hormone levels are linked to preterm birth in polycystic ovary syndrome women.

2.1.6 Pathophysiology and risk factors

The amount of androgen observed in PCOS individuals is a distinguishing aspect of the illness. Increased levels of free (unbound) testosterone in the bloodstream, a critical hormone in the pathogenesis of PCOS, indicate hyperandrogenism. The essential path for physiological aspects of this complex disorder are broken down (55).

Several genome-wide association studies showed certain locations and alleles that are important in identifying the PCOS phenotype (56).

Environmental factors such as physical activity, lifestyle, and diet might differ greatly depending on the population (57). Endocrine-disrupting chemicals and glycotoxins are also environmental variables that can cause genetic variation and disruption of metabolic and reproductive pathways, leading to PCOS phenotypes and problems (58). Androgen exposure can impair hormone levels, causing GnRH pulse frequency to rise, altering the LH: FSH ratio and resulting in follicular arrest and dysplasia (59). Genetics, neuroendocrine, lifestyle/environment, and obesity are predisposing risk factors that lead to the development of polycystic syndrome, as shown in Figure (2-2).

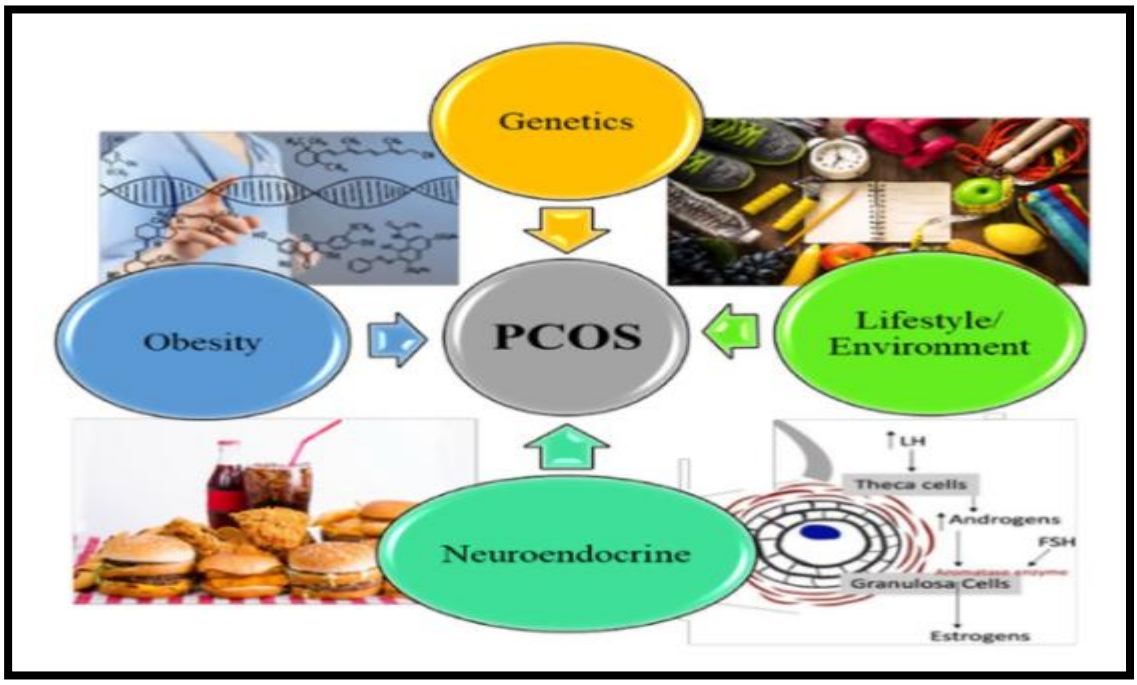


Figure (2-2) PCOS risk change factors.(60)

These factors lead to the cause of hyperinsulinemia, hyperandrogenism, oxidative stress, and irregular factors eventually up surging the metabolic syndrome. Thus, it leads to a defect in the function of the ovaries Figure (2-3).

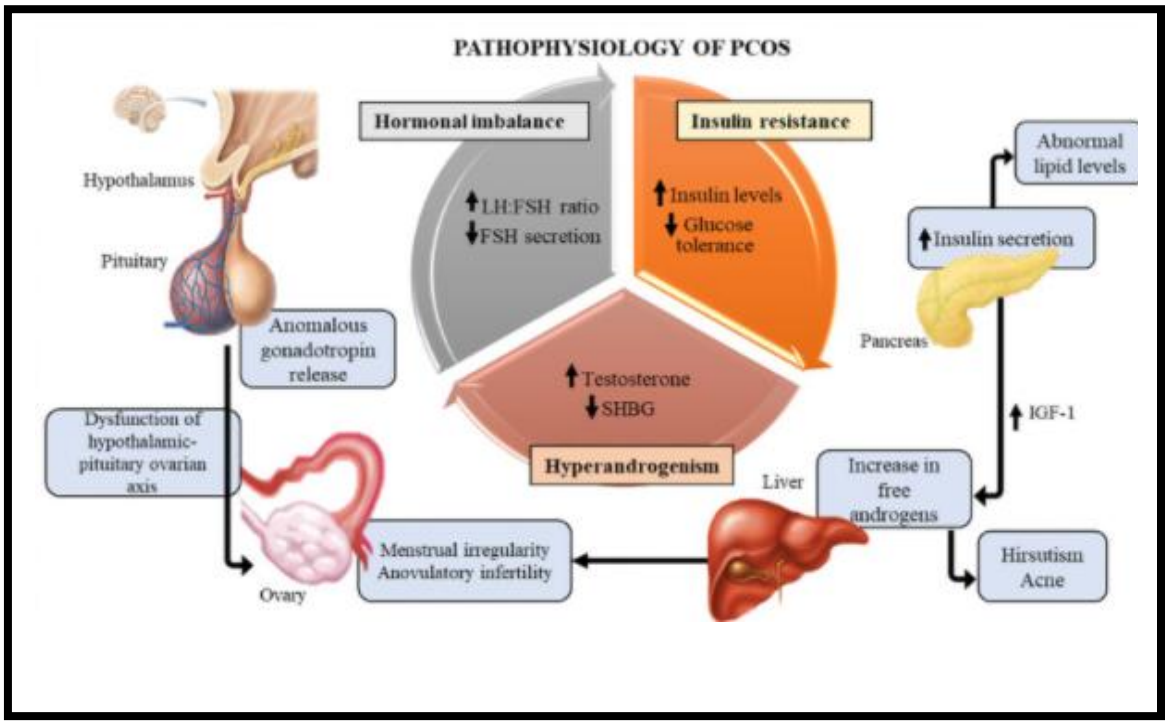


Figure (2-3) Schematic depiction of PCOS linked mechanism. Abbreviations - IGF-1- insulin-like growth factor, LH-luteinizing hormone, FSH-follicle stimulating hormone.(61).

2.1.6.1Poly cystic ovary syndrome and Hyperandrogenism

The clinical and biochemical signs of androgen excess in PCOS result from increased synthesis and release of ovarian androgens(62). Elevated luteinizing hormone increase androgen production(63). reduces Sex Hormone_ binding globulin(SHBG) and raises free circulating testosterone and together, hyperandrogenism impairs ovarian follicle development (64). It is defined as the presence of hair in androgen-dependent sites; in which hair does not normally appear in women (upper lip, chin, chest, upper abdomen back). This is to be distinguished from hypertrichosis that involves a more uniform, whole

body distribution of fine hair (65). Androgens are the primary determinants of hair distribution; however, these androgens must first be converted to testosterone or dihydrotestosterone (DHT) by the 5-α-reductase before they can bind to the receptor of target cells and trigger an androgenic response acne related to hyperandrogenism may be difficult to distinguish from normal pubertal acne in an adolescent with PCOS (66). As a result, a female teen with moderate to severe acne should be checked for PCOS. Furthermore, acne that develops or persists into adulthood is rare and should be noted. The degree of any of these manifestations is highly variable, and it may be influenced by genetic and ethnic variances in androgen sensitivity (67). The most common cause of hirsutism is persistent anovulation and excessive androgen production by the ovaries and adrenal cortex but adrenal causes are most uncommon (68). Measurement of biochemical androgens in PCOS is limited by poor accuracy and reproducibility of assays, which are designed for significantly higher male androgen levels. Free androgen index measurements are generally recommended, derived in the lab from decreased sex hormone binding globulin (SHBG) and total testosterone measurements (69).

2.1.6.2 Menstrual abnormalities

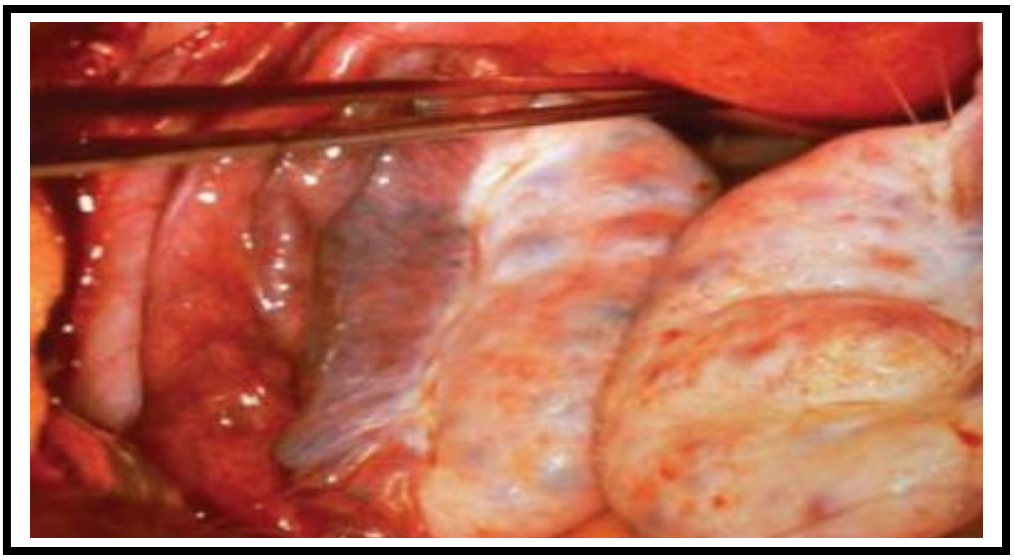
Menstrual disorder, specifically oligo-anovulation, is a common symptom in PCOS patients. Amenorrhea affects 20-50 percent of PCOS women, while only 5-10 percent have normal menstrual cycles (70). On the other hand, Regular cycles, do not always imply regular ovulation the average menstrual cycle lasts between 21 and 35 days. It's safe to presume that a woman is suffering oligo-anovulation if her cycles are consistently longer than 35 days. In women who have normal menstrual cycles, measuring midluteal serum progesterone can assist identify ovulation from anovulation (71).

2.1.6.3 Acanthosisnigricans

Acanthosisnigricans is a rather frequent skin condition. Its cause is unknown, hence the term "idiopathic," however there are a number of related abnormalities that might be noticed, such as hormone alterations and cancers. The most common way to diagnose this disease is to look for hyperpigmentedverrucous plagues with a velvety texture that are symmetrically distributed throughout the intertriginous areas, such as the neck, axilla, groin, and umbilicus, and on rare occasions, the oral and an genital mucosa (72).

2.1.6.4 Cystic ovaries

The ovaries of women with PCOS exhibit either 12 or more follicles measuring 2–9 mm in diameter with many cysts (fluid-filled sacs), or increased ovarian volume (>10 cm3). If there is a follicle >10 mm in diameter, the scan should be repeated at a time early ovarian in order to calculate volume and area. The presence of a single PCO is sufficient to provide the diagnosis. but it has been shown that the measurement of ovarian volume (or area) is a good surrogate for quantification of in clinical practice will noticed by the ultrasonographer ovarian volume Polycystic ovaries are usually 1.5 to 3 times larger than normal (64).



Figure(2-4): Enlarged ovaries in a woman with polycystic ovary syndrome(73).

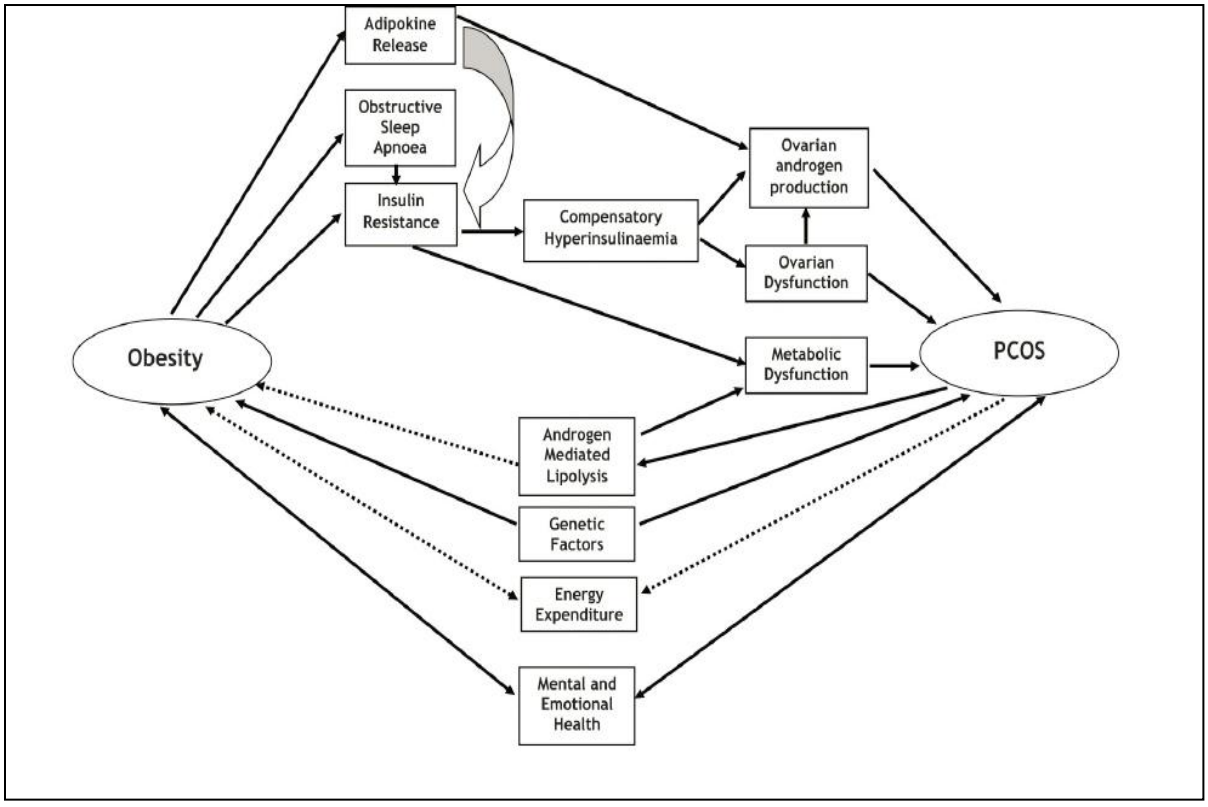
2.1.6.5 Early pregnancy loss

Early miscarriage has been linked to elevated LH and testosterone levels (both of which are linked to PCOS) (74). According to studies, women with PCOS lose at least 40% of their pregnancies early. Studies have established a link between elevated serum LH levels, which are common in women with PCOS, and early pregnancy loss (75).

2.1.6.6 Obesity and PCOS

The worldwide obesity epidemic's evolution over the previous last 50 years is likely to be multi-factorial and complicated, whatever its actual cause, obesity accounts for a huge component of global ill health and is associated with at least 50 obesity-related co morbidities (76). Obesity affects the phenotype of PCOS patients and may play a role in the physiopathology of hyperandrogenism, prolonged anovulation, and the metabolic system. Infertility, metabolic syndrome, and cardiovascular disease have all been

linked to obesity (77). Obesity is linked to hyperinsulinemia which further increases the lipid profile, glucose intolerance in PCOS patients. Obesity augments the androgen production by stimulating LH, which in turn leads to hyperandrogenism (78). As a result, the increase in weight and obesity play a role in the development of PCOS. However, the onset of PCOS can lead to further weight gain and attempts to achieve effective weight control are necessary. The mechanisms through which weight gain and obesity contribute to the development of PCOS, Premenopausal overweight and obese women have a five-fold higher prevalence of PCOS than women of normal weight in the general population. So, following up the new strategies for weight-loss to prevent obesity for women with PCOS is recommended in Figure (2-5).



Figure(2-5). Summary of mechanisms linking obesity with PCOS(79).

2.2. Long-term Health Risks of PCOS

2.2.1. Abdominal obesity-metabolic syndrome (Mets)

Obesity, has a significant role in the clinical and metabolic symptoms of PCOS patients. , on the other hand, is critical. The central fat distribution is more important than the increased BMI. Abdominal obesity has long been seen as a health risk factor (80). When BMI (a measure of general obesity) and waist-to-weight ratio (WHtR) (a measure of abdominal obesity) are compared, WHtR is found to be more significantly related with T2DM, metabolic disease condition (Mets) and cardiovascular disease (CVD) occurrences (81). Obesity combined with hyperinsulinemia raises bioavailable androgen levels. In PCOS women, hyperandrogenemia may also contribute to abdominal obesity(82).

2.2.2.Cardiovascular Disease(CVD)

Despite the fact that PCOS is increasingly being linked to a clustering of cardiovascular risk factors (83). Women with PCOS may have several cardiovascular risk factors such as insulin resistance, high LDL-C, central adiposity, hypertension, and homocysteine levels (84). There is no conclusive evidence that PCOS causes an increase in cardiovascular events. Postmenopausal women with histological evidence of PCOS have a(3 to 7) fold greater risk of ischemic heart disease (85). Furthermore, a higher prevalence of myocardial infarction and cardiac catheterization studies have shown more extensive coronary artery disease in patients with PCOS than in women with normal ovaries (86). Previous investigations, on the other hand, found no difference in mortality and morbidity from coronary heart disease in women with a history of PCOS and numerous cardiovascular risk factors compared to age-matched controls (87). Several studies on the markers of

subclinical disease have been focused on the relationship between PCOS and CVD (88). Figure (2-6) summarizes potential pathways through which the cardiovascular risk factors associated with PCOS may translate into clinical CVD.

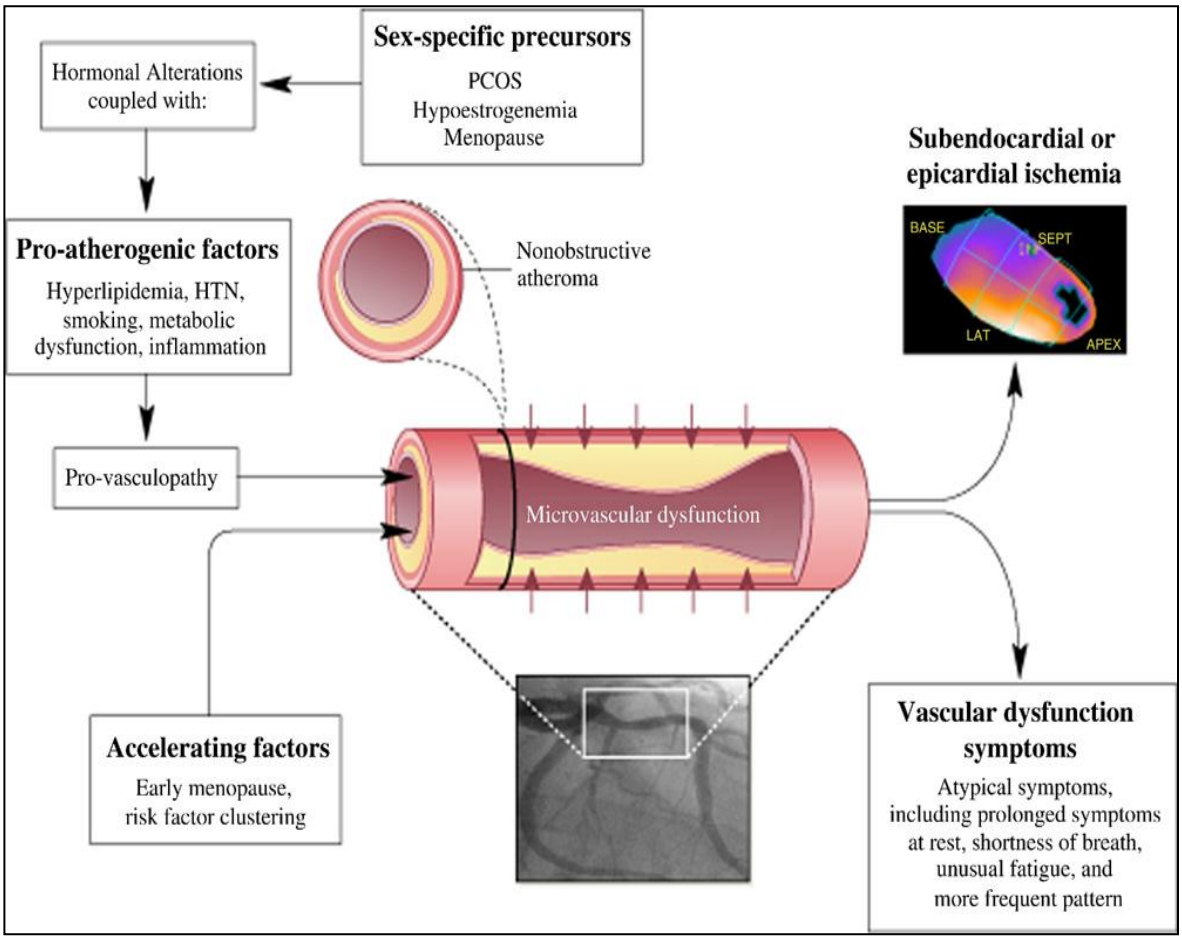


Figure (2-6): Model of Micro vascular Angina in Women (89).

2.2.3.Dyslipidemia

In PCOS patients, anovulation has been linked to dyslipidemia with a prevalence of up to 70%, dyslipidemia may be the most frequent metabolic abnormality in PCOS (90). PCOS is classically associated with an atherogenic. Lipoprotein profile, characterized by elevated levels of cholesterol, TG and LDL- cholesterol and low levels of HDL- cholesterol (91). Obesity could lead to dyslipidemia, irregular ovarian cycles and anovulation in women of reproductive age, a study showed that high levels of TGs,FFAs and oxidized LDLs (oxLDLs) in serum resulted in mitochondrial dysfunction with the augmented release of reactive oxygen species (ROS), in turn leading to ovarian damage and an increased rate of follicular (92). These findings suggest that hyperandrogenism is associated with fat distribution. In PCOS High testosterone levels may further increase abdominal obesity in PCOS(93). These changes suggest that dyslipidemia influences the development of follicles in PCOS, which results in infertility (94) . Figure (2-7) summarized the association between PCOS and other metabolic disorders

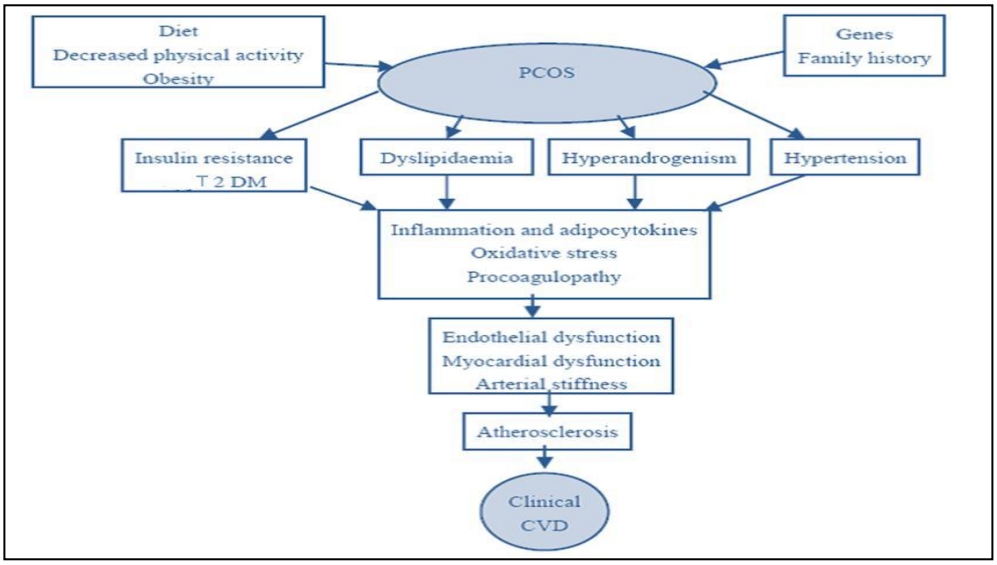


Figure (2-7) :Hypothetical scheme for the pathogenesis of CVD in PCOS (95).

2.2.4.Hypertension

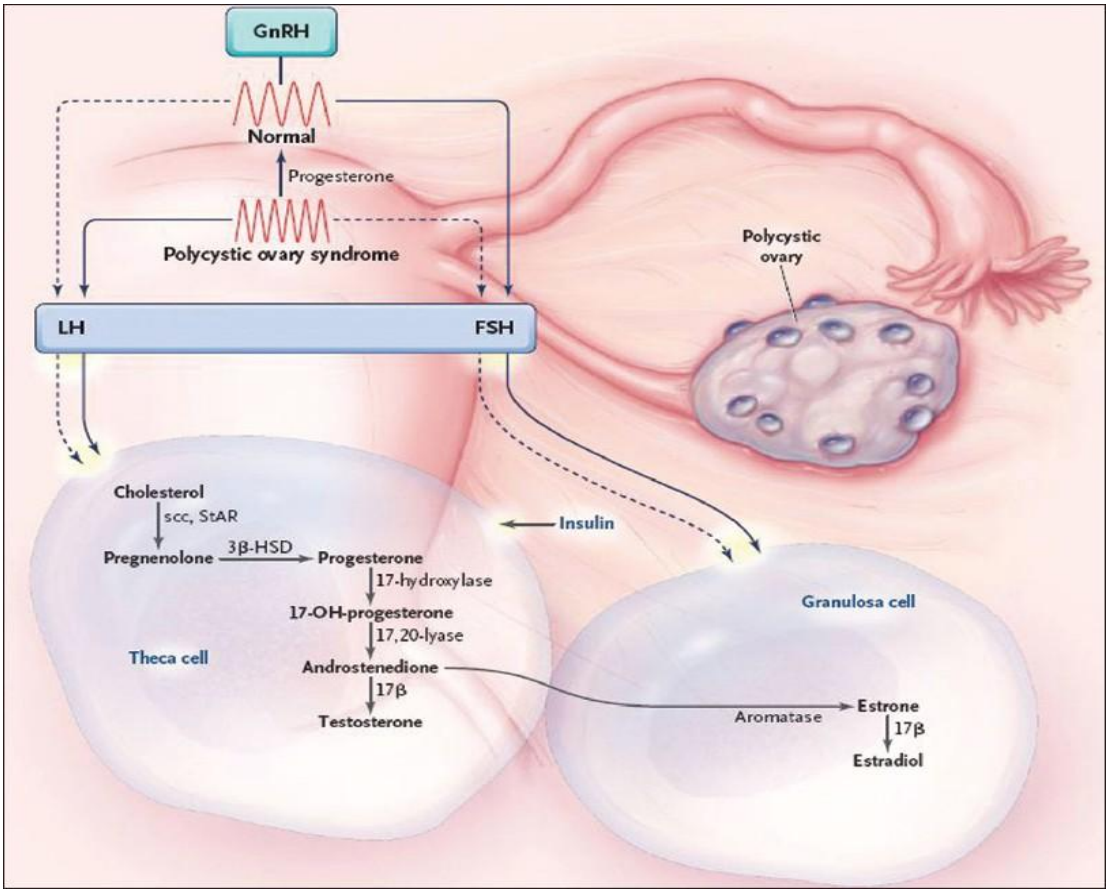
Hypertension is uncommon in young women with PCOS, which affects about 30% of adults, but its frequency rises with the onset of menopause. As a result, multiple studies have shown that women with PCOS have a higher prevalence of hypertension than those without the condition (96).

There is some debate on whether PCOS is linked to hypertension. This can be explained in part by the fact that women with PCOS are more likely to be obese. Hyperinsulinemia's stimulatory effects on the sympathetic nervous system and vascular smooth muscle (97). And changes are noticed in arterial function might also play a role. in a higher daytime systolic arterial mean blood pressure persisting after adjustment for BMI, insulin sensitivity and body fat distribution has also been documented in women of fertile age with PCOS (98).

2.2.5.Insulin resistance and Type 2 diabetes

Hyperinsulinemia is the root cause of excess androgens as insulin directly simulates the action of LH and raise the GnRH indirectly (99). Insulin reduces the amount of sex hormone binding globulin (SHBG), a key circulatory protein that regulates testosterone levels. Reduced SHBG causes an increase in free androgens, which causes clinical symptoms such as hirsutism, alopecia, and acne (100). Insulin resistance can cause dyslipidemia and the patients with PCOS are at high risk for cardiovascular disease and diabetes (101). PCOS is associated with hyperinsulinemia, insulin resistance and obesity, putting women at a high risk of developing type 2 diabetes. Type 2 diabetes is five to ten times more common in women with PCOS than in women without the condition (102) . Insulin can boost ovarian androgen production in both healthy and PCOS women. In vitro, study noticed, ovarian cells from women with

PCOS are more susceptible to insulin-stimulated androgen production. As a result, a hypersensitivity of the intra-ovarian insulin androgen signaling pathway is likely to produce PCOS in women. The identification of this possible flaw could have significant consequences for the development of more specific and effective PCOS treatments (103).



Figure(2-8):Hypothalamic-pituitary-ovarian axis and the role of insulin in PCOS (102) .

2.3. Relation of PCOS with biochemical parameters

2.3.1. Hormones

Hormones are biochemical messengers that are secreted directly into the blood, which carries them to cells of tissues and organs of the body to exert their functions. Adrenal complex hormones, gonadal hormones, and gastrointestinal hormones are examples of hormones that are engaged in high-level activities in an organism such as growth, reproduction, and digesting. Hormonal variables might be paracrine, endocrine, or autocrine (104). A study (105) indicate that gonadal hormones are essential for both males and females to retain sex-typical risk-taking profiles, and that estradiol is adequate to produce risk aversion in both genders.

2.3.1.a. Luteinizing hormone (LH)

Luteinizing hormone (LH) is a glycoprotein hormone released by the gonadotrophin cells in the adenohypophysis(anterior pituitary)together with follicle-stimulating hormone. The pituitary gland, which produces luteinizing hormone, is part of a neurological circuit that includes the hypothalamus the gonads, and the pituitary gland. LH release is increased by gonadotropin-releasing hormone (GnRH) released from the hypothalamus and inhibited by estrogen (in females) and testosterone (in males) in this route (106). LH has a variety of roles that vary between men and women. LH aids in the maturation of primordial germ cells in both sexes. LH causes the testes' Leydig cells to create testosterone in men. LH stimulates the ovaries to produce steroid hormones in women (107). LH also plays a function in both ovulation and the implantation of an egg in the uterus, which serves to regulate the duration and order of the menstrual cycle in females (108). LH and FSH are made from

similar genes and thus have similar properties. They are both glycoproteins made up of an alpha and beta subunit. The alpha subunit is the same between the two hormones, and the beta subunit of each is different and gives each hormone its biological specificity (109). The alpha subunit of LH is made up of 92 amino acids, while the beta subunit has 120 amino acids. These two subunits have a combined mass of 28 kDa (110).

2.3.1.b.Follicle-stimulating hormone (FSH)

The anterior pituitary produces follicle-stimulating hormone (FSH) in response to gonadotropin-releasing hormone (GnRH) from the hypothalamus(111). Both males and females are affected by FSH, which is involved in sexual development and reproduction. The alpha and beta subunits of FSH make form a glycoprotein dimer. FSH has a distinct beta subunit, whereas FSH, hCG(human chorionic gonadotropin) , and LH all have same an alpha subunit (112). FSH is stimulated by GnRH. GnRH is produced in the hypothalamus and released to act on G-protein-coupled receptors in anterior pituitary gonadotropic cells. FSH and luteinizing hormone (LH) are produced by these gonadotropic cells and released into the peripheral circulation (113). GnRH is released in a pulsatile fashion, with low pulse frequencies stimulating more FSH and high pulse frequencies stimulating more LH (114). Continuous usage of GnRH decreases ovulation and estrogen production in women by suppressing the release of FSH and LH from the anterior pituitary. GnRH agonists, such as leuprolide, function through this mechanism in the clinic (115). In females, at the time of menstruation, FSH initiates follicular growth, specifically affecting granulose cells (116). FSH production is inhibited in women due to negative feedback from estrogen levels (117). The pituitary gland is located behind the nasal bridge in the "sella turcica," a protected boney

structure that connects it to the hypothalamus via the infundibular stalk. The hypothalamus is in charge of controlling the production and secretion of a variety of hormones in the pituitary gland Figure (2-9) .

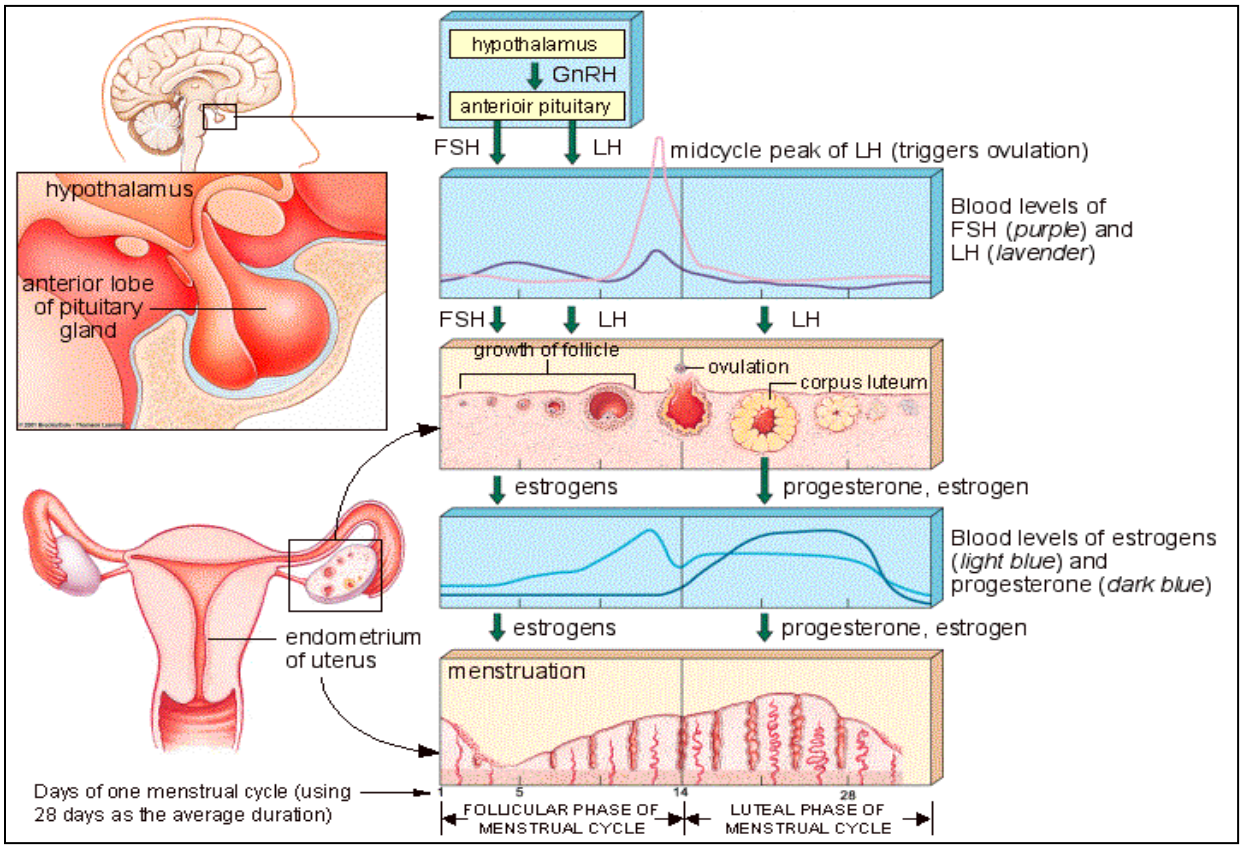


Figure (2-9):Normal regulation of menstrual cycle (118).

2.3.1.c. Prolactin

Prolactin (PRL) is a polypeptide hormone synthesised and secreted by lacto trope cells in the adenohypophysis (anterior pituitary gland). It is also produced in other tissues including the breast and the deciduas (119) . Prolactin causes breasts grow and make milk during pregnancy and after birth. Prolactin levels are normally high in pregnant women and new mothers.

While the levels are normally low for non pregnant women. Prolactin is involved in regulation hundreds of physiologic tasks, but two of the most important are milk production and the formation of mammary glands in breast tissues. Prolactin induces the breast alveolar epithelial cells (where the actual milk production takes place) to produce lactose and other milk components. (i.e., the carbohydrate of milk), casein (i.e., the protein of milk), and lipids during periods of high progesterone (during pregnancy) (120). Estrogen and progesterone levels are higher during pregnancy, and they help to encourage the formation of breast tissue . Estrogen has small effect on prolactin synthesis or secretion (121). PRL's metabolic activities include promotion of food intake and lipogenesis in adipose tissue and hepatocytes, according to experimental findings the PRL secretion has increased in Obese premenopausal women have a visceral fat depot that is proportionate to their BMI, visceral fat are the causes of increased PRL production (122). PRL release is inhibited by dopamine. However, PRL is produced in the endometrium, brain, breast, prostate, lymphocytes, and adipocytes in humans. The no pituitary PRL operates as a cytokine and is controlled in a cell-specific manner, independent of estrogen and neuropeptides (123) .

2.3.1.d . Testosterone

Testosterone is a sex hormone (androgen) generated by the testes and the adrenal cortex in males and promotes the development of male sexual characteristics, enhances the activity of male secondary sex characteristics, and inhibits alterations in secondary sex characteristics after castration During the first 6 weeks of development, the reproductive tissues of males and females are identical. At around week 7 in utero, the SRY (sex-related gene on the Y chromosome) initiates to the development of the testicles (124).

Women often have testosterone levels that are three to four times lower than healthy men. Testosterone in the female body is secreted by the adrenal glands and the ovaries .The majority of testosterone production comes from peripheral conversion of the prohormones and rostenedione and Dehydroepiandrosterone (DHEA) (125). The ovary is responsible for approximately half of the circulating testosterone, whereas the remaining part is produced by the adrenal gland. The circulating testosterone is bound to albumin and sex-hormone binding globulin (SHBG), only around 1% of testosterone circulates freely and is biologically active in women (126). Testosterone also controls secondary masculine qualities. Male hair patterns, vocal alterations, and voice deepening and the development of skeletal muscle (testosterone stimulates protein synthesis) are examples of secondary sex traits, and take place during adolescent growth spurts. Testosterone also increases erythropoiesis, resulting in males having a greater hematocrit than females (127).

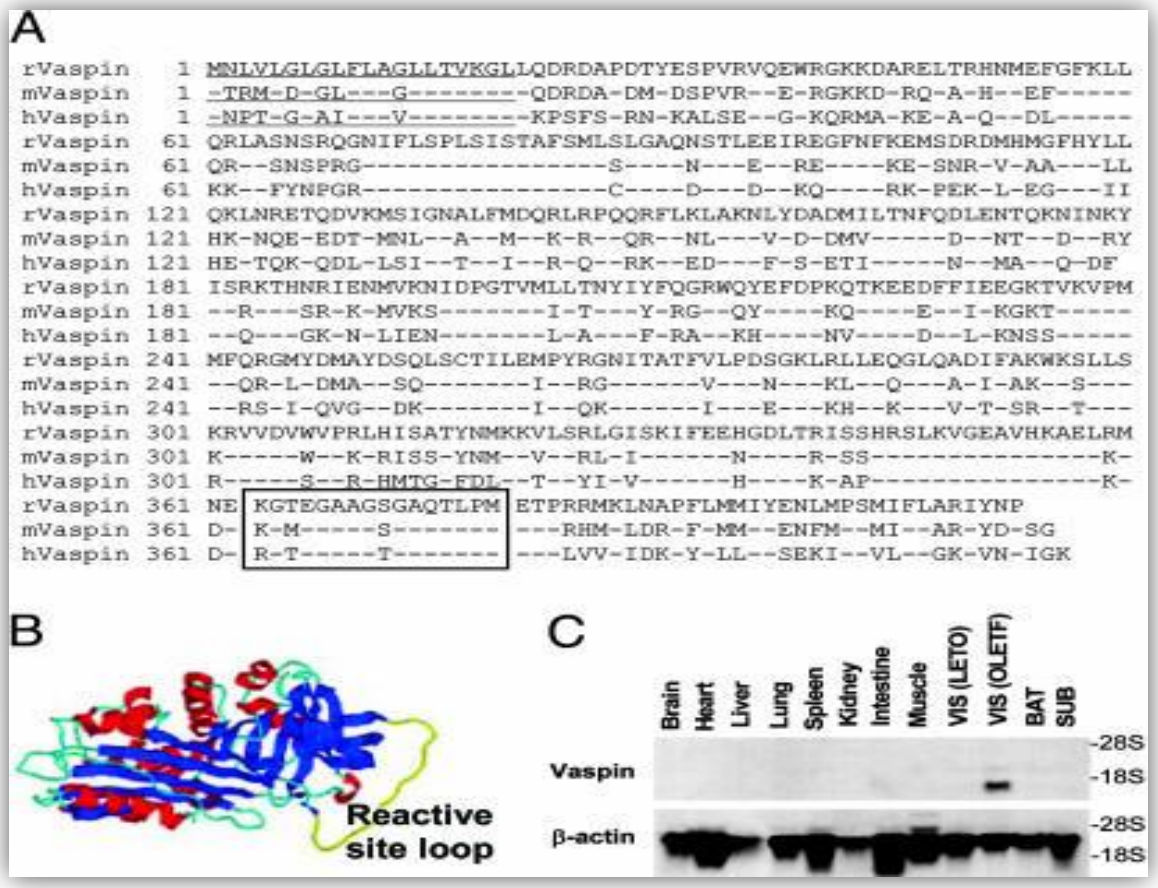
2.3.1.e. Estradiol

Estradiol (**E2)** is a hormone produced predominantly in the ovaries. E2 is the most common estrogen in the body during reproductive years, and it is responsible for the development of female sexual traits and preparing the body for pregnancy. Estradiol is a testosterone metabolite that has been found to play a role in early female sexual development as well as adult female sexual function (128).

2.3.1.f. Vaspin

Vaspin is a new adipokine is mostly expressed in visceral white adipose tissue (Vaspin = visceral adipose tissue-derived serine protease inhibitor; also known as Serpin A12), while it is also found in serum .Vaspin is a protein of 415 amino acids that belongs to the serine protease inhibitors (serpins) family (129). Serpins super family, that though all the serpins share the same tertiary structure, but they have different functions. Serpins consist of three β-sheets (A, B and C), 8-9 α-helices (termed Ha -hI) and the reactive center known as reactive center loop (RCL) (130) . Serpinopathies are disorders that are caused by mutations in serpins These disorders include those affecting the clotting, fibrinolytic, and complement systems; however, the role of the majority of serpins in various path biologic processes awaits further investigations ,obesity and insulin resistance increase both visceral adipose tissue vaspin expression and serum vaspin levels (131). This increase in vaspin production could be a compensatory reaction to counteract the effect of yet-unidentified proteases coming from fat or other tissues that block insulin action . As a result, up-regulation of vaspin expression could be a protective strategy against insulin resistance (132). Both visceral and subcutaneous adipose tissue have been found to express vaspin in adult humans, with depot-specific regulation thought to be influenced by body fat level or insulin sensitivity (133). A variety of different compounds produced by fat tissue or other organs have also been studied as PCOS biomarkers, which may or may not be linked to obesity. Visfatin, vaspin, apelin, retinol-binding protein 4, kisspeptin, copeptin, irisin, and zonulin are only a few of them (134).Vaspin improves insulin sensitivity while also being anti-inflammatory and anti-atherogenic. In comparison to other adipokines, circulating vaspin levels are low. Women have higher

amounts than men, and increase in obesity (135). Recently, growing evidence has demonstrated that vaspin is actively involved in the female reproduction system. Indeed, vaspin is constitutively expressed in the hypothalamus , placenta , and ovary (136). Adipose tissue regulates energy balance as well as feeding, thermo genesis, immunity, and Neuroendocrine function by responding to nutritional, neuronal, and hormonal cues and secreting adipokines (137). Through a number of cirlulating adipocyte -derived hormones, adipose tissue modulates skeletal muscle insulin sensitivity (138). Adipocytes, according to studies ,generate and secrete a number of physiologically active chemicals. Adipose tissue is increasingly regarded as more than just a fat storage organ, but also as a metabolically active endocrine organ (139).



Figure(2_10): Amino acid sequence, structural analyses, and gene expression of vaspin in various tissues. (*A*) Amino acid sequence of rat, mouse, and human vaspin. Signal peptides are underlined, and reactive site loop is boxed. (*B*) Automated protein structure homology modeling by swiss-model predicted the presence of three β-sheets (blue), nine α-helices (red), and one reactive site loop (yellow). (*C*) Northern blot analyses of vaspin in various organs of obese 30-wk-old OLETF and visceral adipose tissue of lean 6-wk-old LETO rats. A single transcript is observed in visceral (VIS) fat of OLETF rats(130).

2.3.2. Lipids Profile Test

Lipids are hydrophobic or amphiphilic molecules that are insoluble in water but soluble in non-polar organic solvents. They include sterols, waxes ,neutral fats, and phospholipids. Fatty acids, neutral fats, waxes, and steroids all fall under the category of lipids (like cortisone). Lipoproteins, glycolipids, and phospholipids are compound lipids (lipids that are complexed with another type of chemical component).and structural support. Lipids are a crucial component of living cells. Lipids, together with carbohydrates and proteins, are the most important components of plant and animal cells. Lipids are easily absorbed by the body and stored. They serve as important source of energy and are an essential component of cell signaling and structure (140).The major plasma lipids are not circulating free in the blood. They are bound to a specific protein (apoproteins) to form large spherical complex molecule called lipoprotein, which are transported through plasma There are four kinds of lipoproteins that are resulted from its ultracentrifugation namely; chylomicron, very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL) (141). PCOS exerts effect on lipid metabolism, so the concentration of serum lipid called lipids profile (include total cholesterol, HDL, LDL, VLDL, and triglyceride) are another important index of the overall metabolic control in diabetic patients (142).

2.3.2.a .Cholesterols

Cholesterol is a component of the plasma membrane of cells and is the essential precursor of all steroid hormones, bile acids, vitamin D, and cholesterol esters. Free Cholesterol and cholesterol esters make up total cholesterol. Serum cholesterol is produced in the liver and is derived from the diet (143). Despite the fact that cholesterol is mostly a byproduct of animal metabolism, and found in trace amounts in plants. The main pathway for cholesterol removal from the body is through the bile (144). Cholesterol can desorbs from peripheral cells, specifically macrophage membranes, and diffuse to external acceptors like HDL particles. Cholesterol measurements can provide supportive evidence in some diseases (145). Plasma cholesterol levels are positively correlated to the development of atherosclerosis The serum total cholesterol is the sum of several types of cholesterol, including LDL-cholesterol, HDL cholesterol and VLDL-cholesterol (146).

2.3.2.b .Triglyceride TG

Triglyceride (Triacylglycerol) is present in dietary fat (exogenous TG), and can be synthesized in the liver and adipose tissue(endogenous TG) to provide a source of a stored energy, which can be mobilized when required, for example, during starvation . Exogenous (dietary) TG is transported in intestinally derived chylomicron, while TG of endogenous origin circulates in hepatic cally derived VLDL (147). In the humans body, high levels of triglycerides in the blood stream have been linked to diseases. Levels of serum triglycerides have also been linked positively to lower levels of HDL-cholesterol and high levels of small dense LDL- cholesterol particle size. Both of these conditions have been linked to an elevated risk of developing

atherosclerosis (148). Insulin resistance, obesity, and Type 2 diabetes mellitus are all common symptoms of hypertriglyceridemia (HTG), which is referred to as the "metabolic syndrome." This environment promotes increased VLDL secretion, which is amplified when FA and insulin levels are high (149). Increased chylomicron production can be caused by insulin resistance, which is characterized by increased circulating FAs and impaired insulin signaling (150).

2.3.2.c. High-density lipoprotein-Cholesterol (HDL-C)

The most complex lipoprotein class is high-density lipoprotein (HDL). HDL is divided into various subclasses, each with its own size, protein and lipid composition, physiological roles, and pathological implications. Several roles of HDLs have been discovered, which may help to explain the epidemiologic link between high HDL- cholesterol and a lower risk of coronary heart disease (151). HDL has a potential antiatherogenic effects and protective effects on endothelial cells. HDL transport the cholesterol from peripheral body cells to liver or steroidogenesis organs such as adrenal, ovary and tests for synthesis steroid hormone, thus protecting against cardiovascular disease (152). Therefore abnormal and reduced vasoprotective effects of HDL are closely associated with atherogenesis and an increased risk of cardiovascular disease (153).The risk of developing manifestations of ischemic heart disease is inversely related to the serum concentration HDL-cholesterol (154).

2.3.2.d. Low density lipoprotein- Cholesterol (LDL-Cholesterol)

Low density lipoprotein -cholesterol, or LDL-cholesterol, is a type of fat that circulates in the blood, transporting cholesterol across the body to where it's needed for cell repair and depositing it inside artery walls. Therefore, role of LDL-Cholesterol in the blood is to transport Cholesterol to the peripheral tissue, making it available to the tissue cells for membrane or hormonal synthesis and for storage for later use. Because cholesterol and triglycerides are water insoluble, they must be bound to proteins in order to pass through the hydrophilic bloodstream (155).This type of lipid is considered harmful as it transports a large amount of cholesterol .The lower density of this lipoprotein allows easy attachment to the inner wall of the blood vessel, thus stimulating the atherosclerotic process (156).

2.3.2.e .Very low -density lipoprotein-Cholesterol (VLDL-C)

These particles are synthesized by the liver and are triglyceride rich. VLDL transports endogenous triglyceride from the liver to other tissues. Similar to chylomicron, the size of the VLDL particles can vary depending on the quantity of triglyceride carried in the particle. When triglyceride production in the liver is increased, the secreted VLDL particles are large. However, VLDL particles are smaller than chylomicron (157). When there are excess fatty acids and cholesterol, they are converted to Triacylglycerol and Cholesteryl esters respectively in the liver and packaged with apolipoprotein into VLDL. Excess carbohydrates can also be converted to Triacylglycerol and can be transported as VLDL. VLDL is transported from the liver to various tissues. In the tissues, lipoprotein lipase (LPL) activated, catalyzes the release of free fatty acids from

the Triacylglycerol present in the VLDL like with chylomicron, by this process VLDL are converted to IDL (158).

2.3.2.f .Atherogenic index of plasma (AIP)

Atherogenic index of plasma (AIP) is a novel index composed of triglycerides and high-density lipoprotein cholesterol (159). It has been used to quantify blood lipid levels and commonly used as optimal indicator of dyslipidemia and associated diseases (e.g., cardiovascular diseases) (160).The use of atherogenic indices may be recommended to identify patients with an increased risk of atherosclerotic cardiovascular disease, especially in male patients (161). Higher AIP level was positively and strongly associated with obesity. AIP is a novel and better biomarker associated with obesity. Controlling the AIP level would be more helpful for the prevention of obesity (162).

2.4.a . Fasting Blood Sugar (FBS)

This measures your blood sugar after an overnight fast (not eating)(163). A fasting blood sugar level of 99 mg/dL or lower is normal, 100 to 125 mg/dL indicates you have prediabetes, and 126 mg/dL or higher indicates you have diabetes (164).

2.4.b . Hemoglobin A1c (HbA1c)

A Glycosylated Hemoglobin or called hemoglobin A1c (HbA1c) test is measures the amount of blood sugar (glucose) attached to hemoglobin. Hemoglobin is the part of red blood cells that carries oxygen from lungs to the rest of body. An HbA1c test shows what the average amount of glucose attached to hemoglobin has been over the past three months. It is a three-month

average because that is typically how long a red blood cell lives(erythrocyte life span 120 days). It is may be used to check for diabetes or prediabetes in adults (165). HbA1c is a blood test that measures the amount of Glycosylated hemoglobin (hemoglobin that was chemically linked to glucose) in the blood. Steps of non enzymatic glycosylation of Hb have been proposed (166).

1. The amino acid valine at N-terminal of the B-chain combines with glucose to form a Schiff base.

2. During the circulation of the human erythrocyte, much of the aldimine undergoes amadori rearrangement to form a more stable keto amine. The steps of this reaction shown in Figure(2-11).

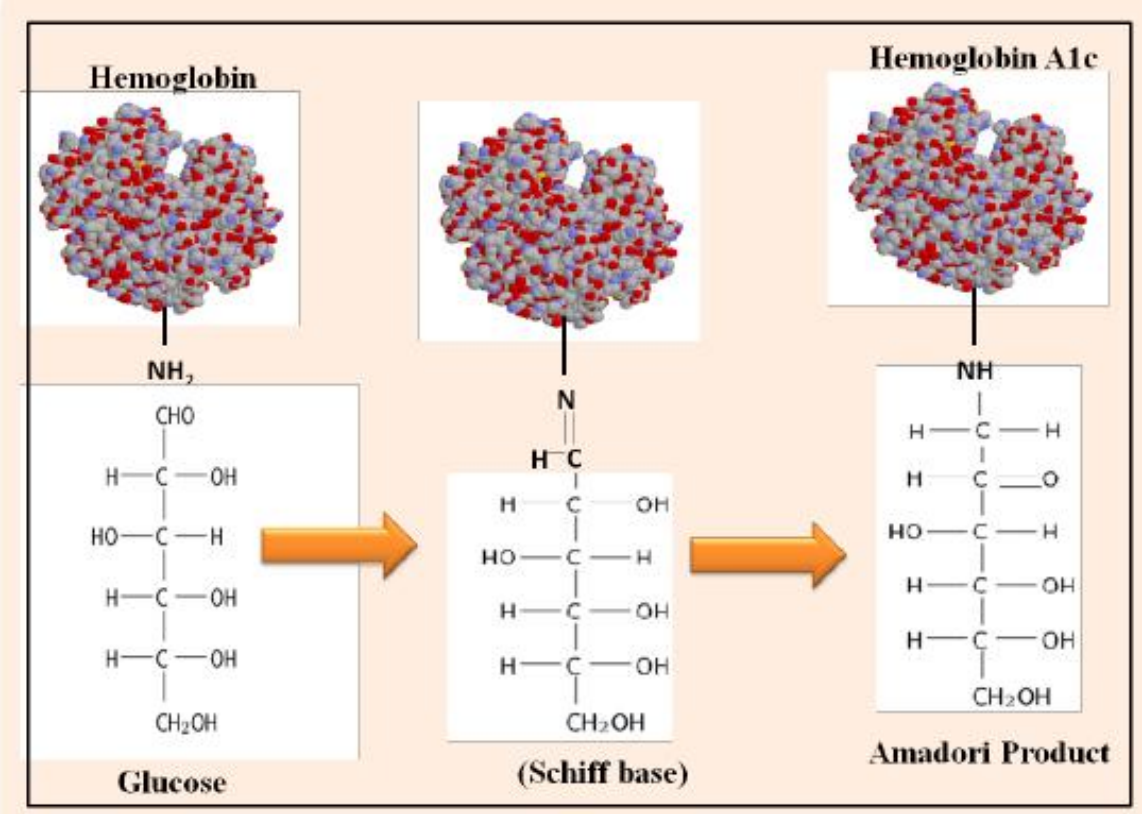


Figure (2-11) Chemical reaction involved in glycation of hemoglobin (167).

Chapter Three

Materials ,Subjects and Methods

3.1. Chemicals and instruments

Kits (chemicals) and instruments employed in the present study are as following:

3.1.1. Chemicals

Table (3-1) Chemicals and their suppliers

Item	Company	Origin
Cholesterol kit	DIRUI	Turkey
Estradiol Kit	Roche	Japan
FSH Kit	Roche	Japan
Glucose kit	DIRUI	Turkey
HbA1C kit	AFIAS	Korea
HDL kit	DIRUI	Turkey
LH Kit	Roche	Japan
Prolactin Kit	Roche	Japan
Testosteron Kit	Roche	Japan
Triglyceride kit	DIRUI	Turkey
Vaspin ELISA Kit	Bio-Tik	China

3.1.2.Equipments and Instruments

Table (3-2) Equipments and Instruments used and their companies suppliers.

Item	Company	Origin
AFIAS	Boditech Med	Korea
Cobas e 411	Roche	Japan
CS-T240 Auto-Chemistry Analyzer	DIRUI	Turkey
ELIZA	BioTek	Germany
Eppendorf tubes	AFICO - DISP	Jordan
Incubator	Prodit	Italy
Micropipettes	Gilson	France
Multichannel micropipettes	Gilson	France
Shaker BS-11	JEIO TECH	Korea
Tips	AFICO - DISP	Jordan
Vacuum EDTA tubes	AFICO - DISP	Jordan
Vacuum Gel tubes	Xinle	Chaina

3.2. Subjects

Ninety (90) samples were collected, (60) samples of patient women with polycystic ovary syndrome with age mean(28.40±8.17) , and (30) samples of healthy women with age mean (28.33±6.84), in Al-Batol hospital –Baaquba city in Diyala province, during the period from November 2020 to January 2021. The two groups are subdivided into obese (BMI ≥ 30 kg m^2) and normal weight (BMI 18.5-24.9kg m^2) (168) .The body mass index (BMI) was measured according to the following equation: dividing the weight in kilograms by the height in squared meters (kg/m^2) (169).

The clinical assessment of patients with PCOS was evaluated by the Physician .All patients are newly detected for PCOS and have no drugs received .PCOS patients had been already diagnosed and the diagnosis had been confirmed according to European society of human reproduction and embryology and American society for reproductive medicine criteria: PCOS is diagnosed if there are any two of the following:

- Presence of polycystic ovary on ultrasound examination.
- Clinical or biochemical hyperandrogenism.
- Menstrual dysfunction with an ovulation.

Informed consent was obtained from all patients and healthy controls. Individual questions to female patients and controls included: age, married or not, number of children, personal medical history, levels of physical exercise, previous history of high blood pressure or diabetes mellitus and, gynecological diseases, regular or irregular menstrual cycle ,amenorrhea or oligomenorrhea and acne or hirsutism. Family histories of high blood pressure, diabetes mellitus and dyslipidemia were also ascertained (see appendix). In all participants, body weight and height, BMI were measured using standard methods. The controls were selected among subjects who were healthy in terms of regular cycle, normal hormonal assay, non-diabetic, non-hypertensive, no other endocrine disorders and were free of acute illness or infection at time of sampling.

3.3. Samples Collection

Samples of blood were collected from each subjects in the morning by drawn 7mL of vein blood using venipuncture by using butterfly syringes, 2ml of blood were collected in lavender top tube (K_2EDTA tube) for measuring HbA1C, and 5 ml of blood collected in gel tube for serum separation by centrifuging at 3000 rpm for 5minutes and the resulting serum was transferred into eppendorf tubes in order to measuring the hormones (vaspin, LH, FSH, prolactin, testosterone and estradiol), lipid profiles, and fasting serum glucose.

3.4.Determination Methods of the Study Parameters

3.4.1. Determination of Hormones

All hormone Parameters of the study (LH, FSH, Testosteron, prolactin, and Estradiol) are estimated in serum of all patient by using an automated quantitative COBAS 411 which shown in Figure (3.1), except vaspin which is estimated by using ELISA kit supplied by Bio-Tek company.

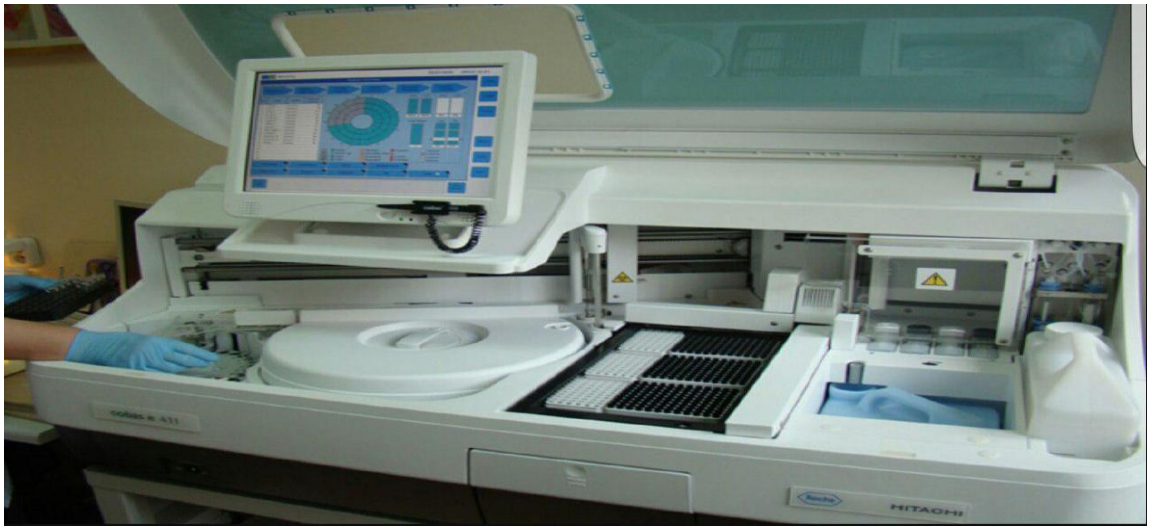


Figure (3. 1) COBAS 411

3.4.1.1. Luteinizing Hormone(LH)

Principle of Assay

Sandwich principle. Total duration of assay: 18 minutes.

▪ 1st incubation: 20 µL of sample, a biotinylated monoclonal LH‑specific antibody, and a monoclonal LH‑specific antibody labeled with a ruthenium complex form a sandwich complex.

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

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

▪ Results were determined via a calibration curve which was instrument specifically

generated by 2‑point calibration and a master curve provided via the reagent barcode.

Reference Value: (2.4-12.6 m.IU/ml)

3.4.1.2.Follicle-Stimulating Hormone (FSH)

Principle of Assay

Sandwich principle. Total duration of assay: 18 minutes.

▪ 1st incubation: 40 µL of sample, a biotinylated monoclonal FSH‑ specific antibody, and a monoclonal FSH‑specific antibody labeled with aruthenium complex a) form a sandwich complex.

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

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

▪ Results were determined via a calibration curve which was instrument specific allygenerated by 2‑point calibration and a master curve providedvia the reagent barcode.

Reference Value: (3.5-12.5 m.IU/ml)

3.4.1.3. Testosterone

Principle of Assay

Competition principle. Total duration of assay: 18 minutes.

▪ 1st incubation: 20 µL of sample are incubated with a biotinylated monoclonal testosterone ‑specific antibody. The binding sites of the labeled antibody become occupied by the sample analyte (depending on its concentration).

▪ 2nd incubation: After addition of Streptavidin-coated microparticles and a testosterone derivate labeled with a ruthenium complex, the complex becomes bound to the solid phase via interaction of biotin and Streptavidin.

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

▪ Results were determined via a calibration curve which was instrument specifically

generated by 2‑point calibration and a master curve providedvia the reagent barcode.

Reference Value: (0.1-0.9 ng/ml)

3.4.1.4. Prolactin

Principle of Assay

Sandwich principle. Total duration of assay: 18 minutes.

▪ 1st incubation: 10 µL of sample and a biotinylated monoclonal prolactin specific antibody form a first complex.

▪ 2nd incubation: After addition of a monoclonal prolactin‑ specific antibody

labeled with a ruthenium complex a (Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)) and streptavidin-coated microparticles, a sandwich complex was formed and becomes bound to the solid phase via interaction of biotin and Streptavidin.

▪ The reaction mixture was aspirated into the measuring cell where the micro particles were magnetically captured onto the surface of the electrode. Unbound substances were then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which was measured by a photomultiplier.

▪ Results were determined via a calibration curve which was instrument specifically

generated by 2‑point calibration and a master curve provided via the reagent barcode.

Reference Value: (1.3-20.0 ng/ml)

3.4.1.5. Estradiol(E2)

Principle of Assay

Competition principle. Total duration of assay: 18 minutes.

▪ 1st incubation: By incubating the sample (25 µL) with two estradiol‑ specific biotinylated antibodies, immunocomplexes are formed, the amount of which was dependent upon the analyte concentration in the sample.

▪ 2nd incubation: After addition of Streptavidin-coated micro particles and an estradiol derivative labeled with a ruthenium complex, the still-vacant sites of the biotinylated antibodies become occupied, with formation of an antibody‑ complex. The entire complex becomes bound to the solid phase via interaction of biotin and Streptavidin.

▪ The reaction mixture was aspirated into the measuring cell where the micro particles were magnetically captured onto the surface of the electrode. Unbound

substances were then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which was measured by a photomultiplier.

▪ Results were determined via a calibration curve which was instrument specifically

generated by 2‑point calibration and a master curve provided via the reagent barcode**.**

Reference Value: (12.5-166 pg/ml)

3.4.1.6. Visceral Adipose Specific Serine Protease Inhibitor(VASPIN)

- **Principle of Assay**

This ELISA kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti- VASPIN antibody was pre-coated onto 96-well plates. And the biotin conjugated anti- VASPIN antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the VASPIN amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of VASPIN can be calculated.

Figure(3- 2)ELISA device

- **Kit Contents**

Reagent	**Preparation**
ELISA Microplate (Dismountable)	
Lyophilized Standard	Stock solution (2000pg/mL) was prepared by adding 1ml of Sample / Standard Dilution Buffer, 1000pg/mL→31.25pg/mL of standard solutions was prepared as follow : 6 Eppendorf tubes were Labeled with 1000pg/mL, 500pg/mL, 250pg/mL, 125pg/mL, 62.5pg/mL, 31.25pg/mL, respectively. 0.3 mL of the Sample/Standard dilution buffer was Added into each tube. 0.3 mL of the above 2000pg/mL standard solution

	was add into 1st tube and mixed them thoroughly. 0.3 mL was transfered from 1st tube to 2nd tube and mixed them thoroughly. 0.3 mL was transfered from 2nd tube to 3rd tube and mixed them thoroughly, and so on.
Sample / Standard Dilution Buffer	
Biotin-labeled Antibody (Concentrated)	Biotin-labeled Antibody Working Solution was prepared as: 100µL of the Biotin-detection antibody was dilute with 10mL of antibody dilution buffer and mix them thoroughly.
Antibody Dilution Buffer	
HRP-Streptavidin	HRP-Streptavidin Conjugate (SABC) Working Solution was prepared: Dilute the SABC with SABC Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1µL of SABC into 99µL of SABC Dilution Buffer.
Conjugate(SABC)	
SABC Dilution Buffer	
TMB Substrate	
Stop Solution	
Wash Buffer (25X)	30mL of Concentrated Wash Buffer was dilute into 750 mL with distilled water.

- **Assay Procedure:**

1. The plate was washed 2 times before adding standard, sample and control (zero) wells.
2. 100µL of standard was added, sample to their spaitialized well and incubate for 90 minutes at 37°C.
3. The plates was aspirated and washed 2 times.
4. 100µL Biotin-labeled antibody working solution was added to each well and incubated for 60 minutes at 37°C .
5. The plate was aspirated and washed 3 times.
6. SABC Working Solution 100µL was add into each well and incubated for 30minutes at 37°C.
7. plate aspirated and washed 5 times.
8. TMB Substrate 90µL was added, and incubated 15 -30 minutes at 37°C.
9. Stop Solution50µL was added.
10. The absorbance was read at 450nm immediately .

- **Calculation:-**

The concentration of vaspin was read from the standard curve below:

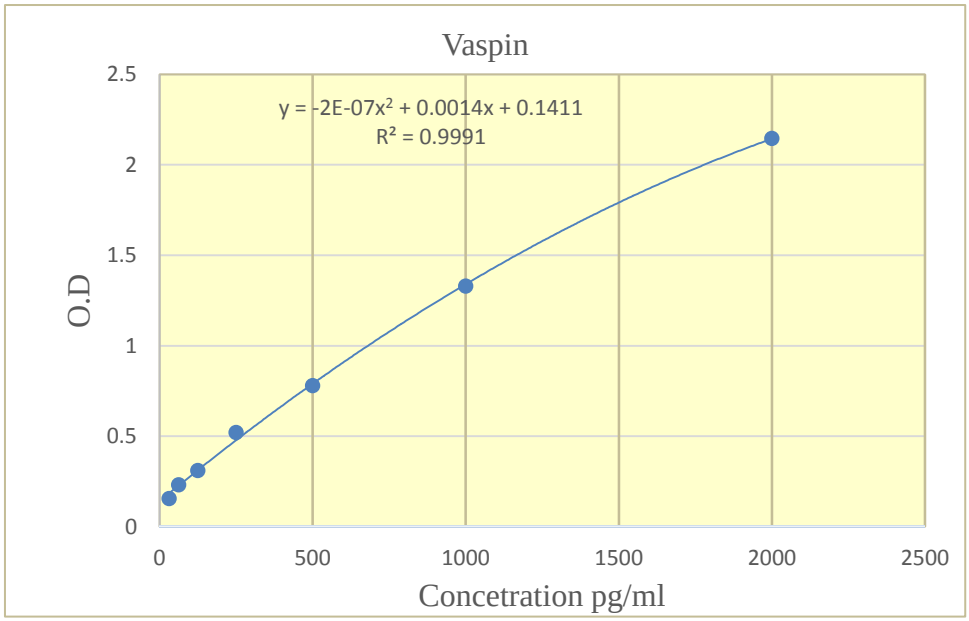


Figure (3-3): Sample of Standard Curve of vaspin at450nm

3.4.2.Determination of Glucose

Glucose is measured by using DIRUIAuto-Chemistry Analyzer .Total duration of assay: 5 minutes.

Principle of Assay

Principle Glucose in the sample , under the catalysis of glucose oxidase (GOD) of the reagent , generate gluconic acid and hydrogen peroxide . Under the existence of peroxidase (POD) , hydrogen peroxide reacts with aniline color of the original material and 4 - aminoantipyrine to produce H , O and quinone imine pigment , the generated volume of quinone imine pigment volume is proportional to glucose content in the sample . By measuring the final pigment volume at specifie wavelength , the glucose concentration in the sample can be calculated

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{aniline color of the original materiall} \xrightarrow{\text{POD}} \text{Quinone imine pigment} + H_2O$$

Figure(3-4) DIRUI Auto-Chemistry Analyzer

3.4.3.Determination of Glycated hemoglobin(HbA1c)

HbA1c is measured by AFIAS a fluorescence Immunoassay (FIA) for the quantitative determination in human whole blood .

Principle of Assay

The test uses sandwich immunodetection method , the detector antibody in buffer binds to antigen in the sample , forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip.

The more antigen in sample forms the more antigen - antibody complex and leads to stronger intensity of fluorescence signal on detector antibody.

3.4.4.Determination of Lipid profile

3.4.4.1.Determination of Triglyceride(TG)

Principle of Assay

Triglyceride is measured by using DIRUIAuto-Chemistry Analyzer. Total duration of assay: 5 minutes

Sample triglycerides is hydrolyzed by lipoprotein lipase (LPL), liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3- phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate (G3P) is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H2O2). In the last reaction, hydrogen peroxide (H2O2) reacts with 4- aminoantipyrine (4-AP) and p-chlorophenol in presence of peroxidase (POD) to give a red colored dye(1):

$$\text{Triglycerides} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerolkinase} + Mg^{+2}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-AP} + \text{aniline color of the original material} \xrightarrow{\text{POD}} \text{Quinone imine pigment} + H_2O$$

3.4.4.2.Determination of Cholesterol

Cholesterol is measured by using DIRUI Auto-Chemistry Analyzer. Total duration of assay: 5 minutes

Principle of Assay

Cholesterol ester in the sample , under the existence of lipoprotein esterase , hydrolyzed into cholesterol and free fatty acid. Total cholesterol oxidized by

cholesterol oxidase to generate cholest -4 -ene-3- ketone and hydrogen peroxide. The generated hydrogen peroxide , under the existence of peroxidase , react with hydroxybenzoic acid and 4 - amino - antipyrine to produce H , O and quinone imine pigments . The generated volume of quinone imine pigment is proportional to total cholesterol volume in the sample , by measuring the generated pigment volume at specific wavelength , total cholesterol concentration can be calculated .

Cholesteryl ester + $H_2O \xrightarrow{LPL}$ cholesterol + fatty acid

Cholesterol + O2 $\xrightarrow{\text{cholesterol esterase}}$ cholesteric-4-en-3-keto + H_2O_2

H_2O_2 + 4-aminoantipyrine +parahydroxy benzoic acid $\xrightarrow{POD}$ quinoneimine pigments + H_2O

3.4.4.3.High density lipoprotein (HDL)

High - density lipoprotein cholesterol is measured by using DIRUI Auto-Chemistry Analyzer. Total duration of assay: 5 minutes

Principle of Assay

Principle High - density lipoprotein cholesterol in the samples, under the existence surfactant in the reagent , is selectively catalyzed and hydrolyzed by cholesterol esterase into cholesterol and free fatty acid . The generated cholesterol oxidized by cholesterol oxidase to produce cholest - 4 - ene - 3 - ketone and hydrogen peroxide . Under the existence of peroxidase , hydrogen peroxide react with aniline color of the original imaterial and 4 - amino - antipyrine to produce H2O and quinone imine pigment , the generated quinone imine pigment volume generate is proportional to high density lipoprotein cholesterol content in the sample , by measuring the final pigment volume at

specific wavelength , high - density lipoprotein cholesterol concentration in the sample can be calculated .

High - density lipoprotein cholesterol + $H_2O \xrightarrow{\textbf{Cholesterol esterase}}$ Cholesterol + Free fatty acid

Cholesterol + O2 $\xrightarrow{\textbf{Cholesterol oxidase}}$ Cholest -4 - ene -3 - ketone + H_2O_2

H_2O_2 + 4-aminoantipyrine +aniline color of the orginal material $\xrightarrow{\textbf{Peroxidase}}$ quinoneimine pigment + H_2O

3.4.4.4.Low density lipoprotein (LDL-Cholesterol)

Low-density lipoprotein-cholesterol was estimated by using formula offriedwald (170).

●Principle

LDL-cholesterol is very difficult to isolate and measure .Hence, LDL level is most usually derived by the friedwalds formulaas follows (170).

LDL-cholesterol = Total cholesterol – [HDL- cholesterol + TG/5]

The reference values of LDL-Cholesterol concentration according to this procedure are (50-150)mg/dl .

3.4.4.5.Very low density lipoprotein (VLDL- Cholesterol)and the index

Very low-density lipoprotein- cholesterol was estimated by using formula of friedwalds (171).

VLDL-Cholesterol = TG/5

Index = cholesterol/HDL (171).

3.5. Statistical analysis

Data of current study were analyzed by using Chi-square (X2) test to compared between percentages. ROC curve used to measured sensitivity and specificity of diagnostic tests (detection the best test for diagnosis). Numeric date were described by (Mean ± SD). Test used to compare between two numeric variables, Pearson correlation (R) accounted to explain type and strength of relationship between variables. A level of significance of α=0.05 was applied to test. (SPSSV.22 and Graph pad prismV.6) programs used to analyze.

*= significant different ($p<0.05$)

**= high significant different ($p<0.01$)

***= very high significant different ($p<0.001$)

N.S = Non. significant

Chapter Four

4.1. Characters of the patients subjects

The results of the current study show the age periods of PCOS women (20-29 and 30-39) represented the highest percentage of patients (45.0% and 25.0%) respectively. While the age periods (≤19 and 40-49) represented the lowest percentage of patients (15.0% and 15.0%) respectively. The differences in the age period of patients were significant (P<0.05).Based on the number of children, women who have no children scored the highest percentage (66.70%), while women who have 2 children scored the lowest percentage (3.30%). The differences among women having children were significant (P<0.05). Related to married status, married women scored a higher percentage (83.30%) than single women (16.70%) with a significant difference (P<0.05) (Table 4.1).

Table (4.1) Personal characters of patients by using chi-square tests.

		Count	Percent	P value
Age_periods	≤19	9	15.0%	0.002**
	20-29	27	45.0%	
	30-39	15	25.0%	
	40-49	9	15.0%	
Children numbers	0	40	66.70%	0.001***
	1	18	30.00%	
	2	2	3.30%	
Marriage	Yes	50	83.30%	0.001***
	No	10	16.70%	

= high significant different (p<0.01), *= very high significant different (p<0.001)

Wendland *et al.,* The current study showed a high incidence of polycystic ovary disease patients in females more than 18 years and these report are compatible with results that showed high prevalence of PCOS patients in

women more than 19 years. Because the diagnostic criteria for PCOS may change, or even normalize, during the course of a woman's reproductive life, the prevalence of PCOS appears to decline with age (172).

A difference between PCOS cases and controls was detected among older women (45 years and older), but not among younger women in the current investigation. Because CVD has such a long time of incubation, metabolic changes seen women with PCOS who are younger appear to have manifest as quantifiable anomalies by middle age. Furthermore, PCOS and aging appear to interact in some way have a considerably higher negative influence on CVD. It is comparable to what we saw in aged ≥45 years PCOS subgroup (173).

Poly cystic ovary syndrome was not shown to be linked with pregnancy loss on its own. But it has many factors in common. However, in the overweight and obese groups, BMI was found to be associated with pregnancy loss. The number of infants born to mothers with PCOS and without PCOS was not significantly different. These results are not compatible with present study that showed high significant among the number of children. ($P<0.05$) (174).

4.2. Parameters of BMI, FBS, HbA1c

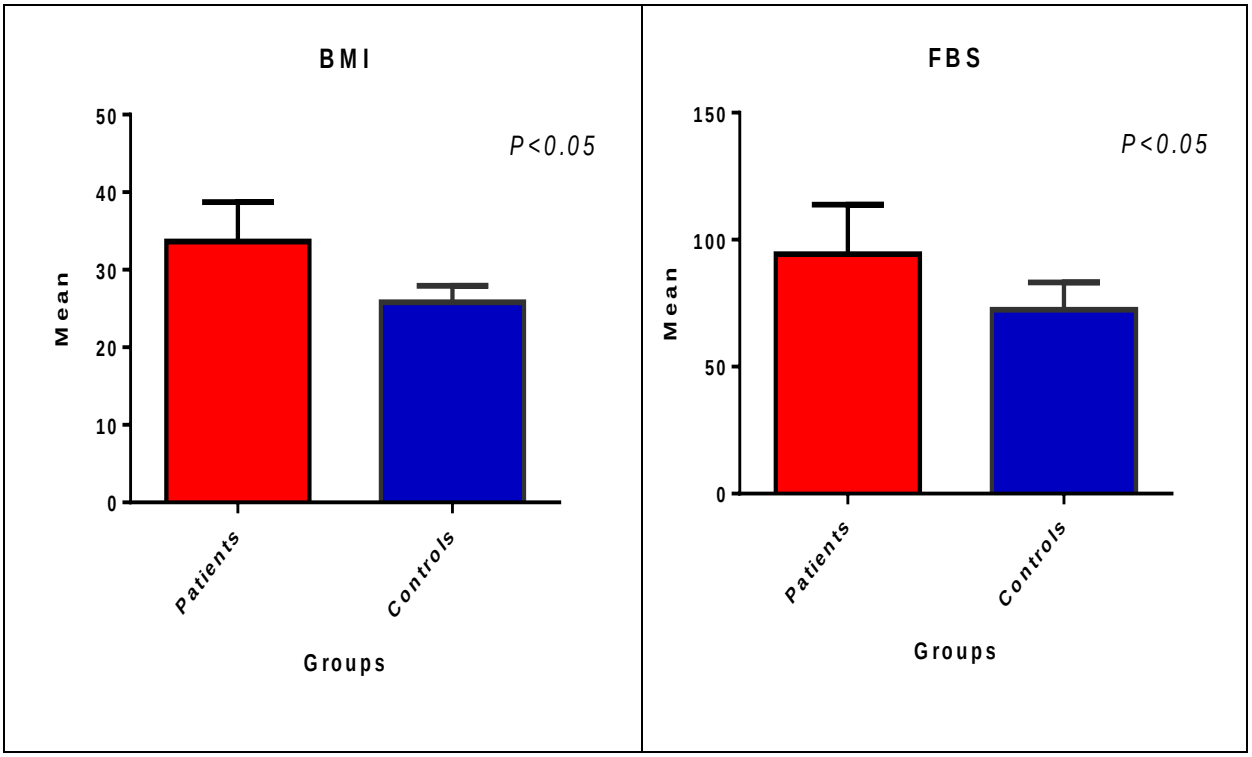
The body mass index is a parameter that can be calculated from weight to square height. The results of the current study(Table (4-2)and Figure (4-1)) show the BMI was increased in patients (33.63±5.10) than in controls(25.80±2.14) with a highly significant difference ($p<0.05$) . The fasting blood sugar (FBS) levels in women with PCOS group (94.35 ±2.14) than controls (72.44±10.70) with a highly significant difference ($p<0.05$) .The results of HbA1c levels in patients (5.25±0.66) than controls

(4.15±0.60)revealed a significant difference ($p<0.05$) between the studied groups in the present study.

Table (4-2):comparison BMI, FBS HbA1c parameters between studied groups by using student T test

Parameter		Mean	Std. Error Mean	p value
BMI (Kg/m^2)	Patients	33.63	0.66	0.01**
	controls	25.80	0.39	
FBS (mg/dl)	Patients	94.35	2.51	0.002**
	controls	72.44	1.95	
HbA1c (%)	Patients	5.25	0.09	0.05*
	controls	4.15	0.11	

*= significant different ($p<0.05$),**= high significant different ($p<0.01$)



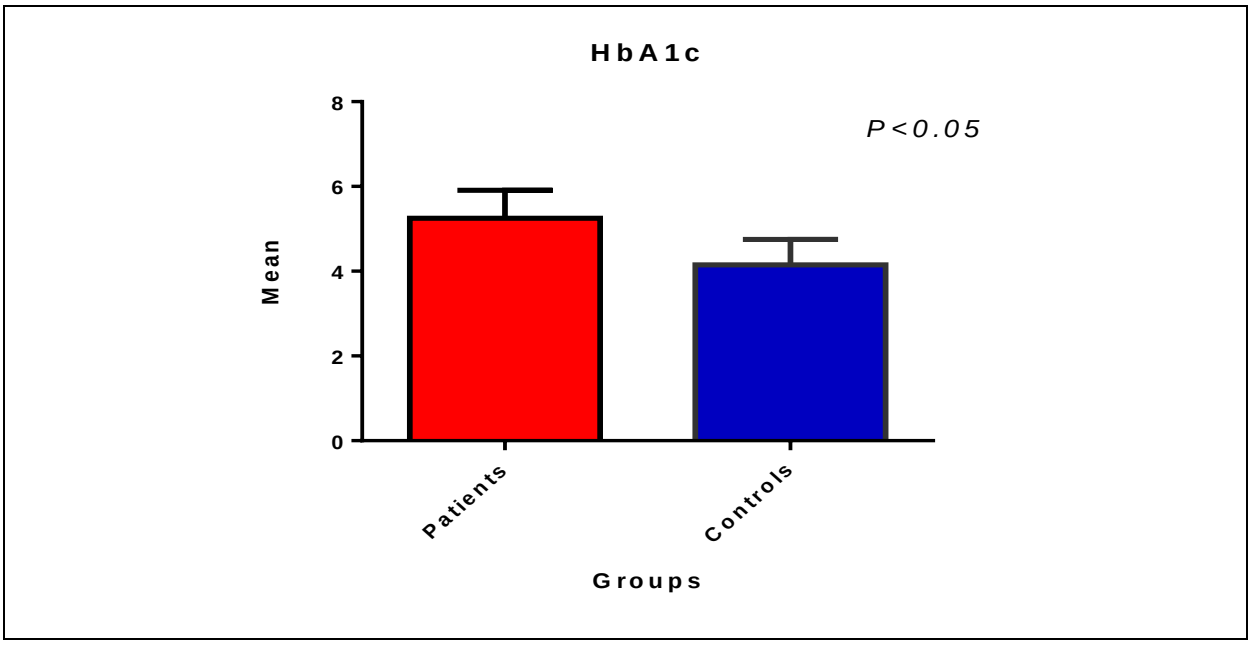


Figure (4-1): comparison BMI, FBS HbA1c parameters between studied groups

Tabassum *et al.*, Showed the BMI of PCOS patients was high than controls with non-significant different .These results are don't agreed with finding of our study that showed high BMI in PCOS patients than controls with significant different ($p<0.05$)(175) .

Lim *et al.*, When comparing women with PCOS to women of normal as presented in the study, it was discovered that those with PCOS had a higher risk of developing hyper fasting plasma glucose, hyperandrogenism, hypertriglyceridemia and HbA1c , which agrees with our findings (176).

Results of current study(Table (4-3) and Figure (4-2)) shows the mean value of BMI was high for obese patients (35.89±2.46) than obese control (29.07±3.17) and BMI for normal weight patients (23.20±1.10) was less than normal weight controls (23.33±0.71) with no significant different ($P>0.05$) between study groups. FBS levels in obese patients group (97.21±19.46) were significantly higher than those in control group (73.81± 17.92), and also the FBS levels in Normal weight patients (87.44± 19.80) were significantly

higher than levels of FBS of controls (76.44±10.55). The HbA1c levels are non-significantly higher in obese (5.30±0.68) and normal weight (5.24±0.59) patients groups comparing with obese (4.07±0.73)and normal weight (4.40±0.45)control groups.

Table (4-3): comparison of BMI, FBS, HbA1c parameters between normal weight and obese of the studied groups by using student T test

Parameter		Patients		controls		P value
		Mean	Std. Error	Mean	Std. Error	
BMI (Kg/m^2)	Normal weight	23.20	0.49	23.33	0.24	0.98
	Obese	35.89	0.35	29.07	0.85	0.031*
FBS (mg/dl)	Normal weight	87.44	8.86	76.44	3.52	0.02*
	Obese	97.21	2.81	73.81	4.79	0.009**
HbA1c (%)	Normal weight	5.24	0.26	4.40	0.15	0.13
	Obese	5.30	0.10	4.07	0.19	0.17

*= significant different ($p<0.05$),**= high significant different ($p<0.01$)

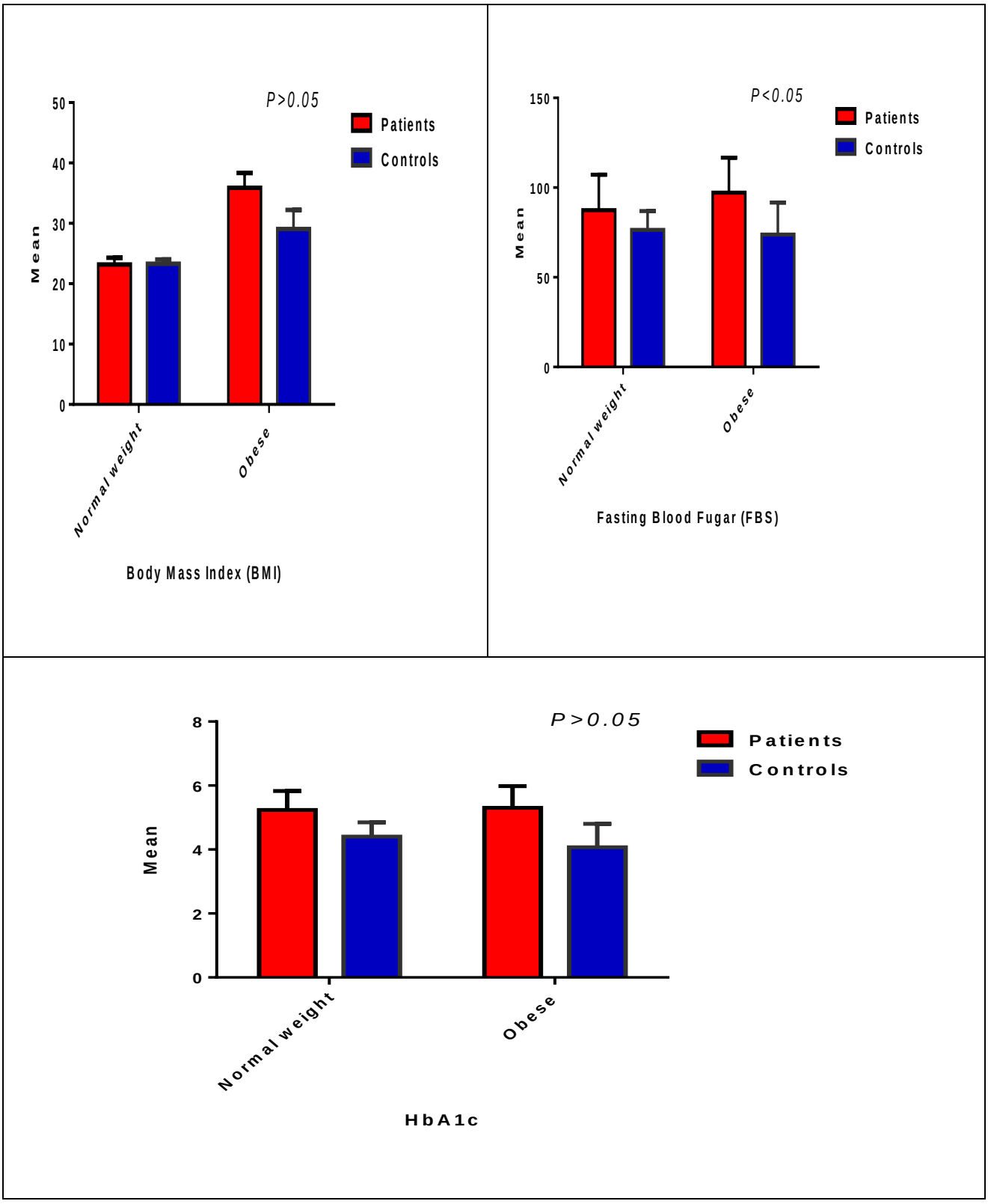


Figure (4.2):Comparison BMI, FBS HbA1c parameters between normal weight and obese of the studied groups

Hassan and Thozhukat, revealed the BMI of subjects from the study group patient was significantly higher as compared with the BMI of the control group (25.1 and 20.5, respectively). Where they found the high obese and normal weight in patients with PCOS. These results somewhat agreed with our results that showed high obese and overweight in patients with PCOS but being significant with control (177).

Nader poor *et al.*, Indication this study Obesity is now a major international health concern. It is increasingly common in young women with reproductive, metabolic, and psychological health impacts. Reproductive health impacts are often poorly appreciated and include polycystic ovary syndrome (PCOS), infertility and pregnancy complications. Obesity exacerbates hormonal and clinical features of PCOS and women with PCOS appear at a higher risk of obesity (178). Showed PCOS is associated with obese , and 40-70% of women with PCOS are either overweight or obese, and the incidence of PCOS in overweight or obese women is four times that of women with normal weight . In China, approximately one-third of PCOS women have a BMI higher than 23 kg/m2 , Additionally, obese has been associated with adverse reproductive outcomes in Chinese women with PCOS Fat is common in women with PCOS, particularly abdominal obese(179).

Obese's effect on PCOS development. Hypotheses result metabolic anomalies linked to obese could also be applied to the function of obese in the development of PCOS. According to the adipokine theory, adipose tissue is an endocrine organ that secretes a variety of hormones (adipokines). PCOS is characterized by hyperandrogenism. PCOS may arise as a result of changes in adipokine levels (172). Obese exacerbates both the metabolic consequences stated above as well as reproductive abnormalities such as menstrual irregularities, poor pregnancy outcomes, and a poor response to infertility treatment(180).

PCOS manifests, is commonly accompanied by obese and is associated with higher risk for type 2 diabetes (T2D), cardiovascular disease, nonalcoholic fatty liver disease (NAFLD), infertility, pregnancy complications, and depression (181).

Layegh *et al.*, and Rashidi *et al.,* indicates the high levels of FBS in obese PCOS than in non-obese. These results were compatible with our study that showed the high levels of FBS in obese PCOS than in non-obese women. In addition to gynecological and hyper androgenic features associated with obese , dyslipidemia, hypertension, and hyperinsulinemia were also associated with obese women (182),(183).

Unluer *et al,*. Mentioned the absence of a difference between obese and non-obese groups in terms of HbA1c values suggests occurring in the obese group may also be important in the non-obese group. These results agree with our results that showed no significant differences between obese and non-obese groups in terms of HbA1c (184).

Suggested In PCOS patients, a HbA1c level of 5.3 percent may be appropriate to begin efforts for early detection of elevated inflammation as a potential indicator of cardiovascular disease risk (185), This is consistent with our study (Table 4.3).

4.3. Parameters of Lipid profile

The results of the current study(Table (4-4) and Figure(4-3)) showed cholesterol and TG they were increased in patients (191.77±36.84) and (182.44±65.01) than in controls (136.27±21.09)and (100.47±35.88) with a highly significant difference ($p<0.05$).The study showed HDL-C levels were increased in PCOS patients (60.96±9.65) than in controls (50.40±7.43) with a highly significant difference ($p<0.05$). Showed LDL and VLDL between they were increased in patients**(**94.76±26.04**)** and **(**36.32±13.10) than in

controls(65.93±16.87) and (20.07±7.15) with a highly significant difference ($p<0.05$). Showed Index the was increased in patients **(**3.17±0.67**)** than in controls (2.68±0.61) with a significant difference ($p<0.05$)**.**

Table (4-4): comparison lipid profile parameters between studied groups by using student T test

Parameter		Mean	Std. Error Mean	p value
Cholesterol(mg/dl)	Patients	191.77	4.76	0.003**
	controls	136.27	3.85	
TG(mg/dl)	Patients	182.44	8.39	0.004**
	controls	100.47	6.55	
HDL (mg/dl)	Patients	60.96	1.25	0.002**
	controls	50.40	1.36	
LDL (mg/dl)	Patients	94.76	3.36	0.001***
	controls	65.93	3.08	
VLDL (mg/dl)	Patients	36.32	1.69	0.001***
	controls	20.07	1.30	
Index	Patients	3.17	0.09	0.02*
	controls	2.68	0.11	

*= significant different ($p<0.05$)**,** **= high significant different ($p<0.01$), ***= very high significant different ($p<0.001$)

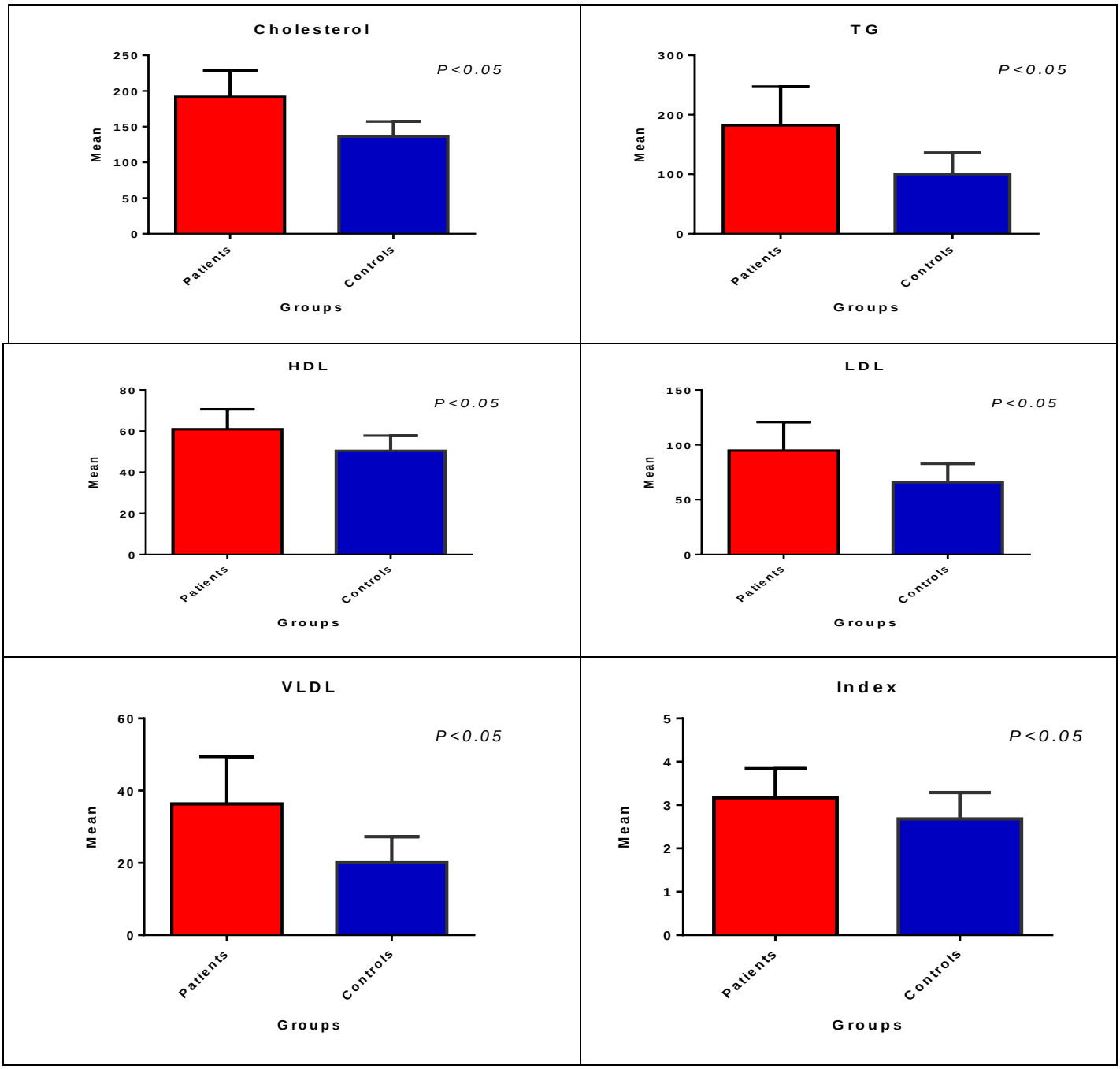


Figure (4-3): comparison lipid profile parameters between studied groups

Kuppusamy *et al*,. suggested there are several metabolic derangements that present in PCOS. Dyslipidemia is metabolic disturbance that most common in PCOS patients which affects around 70% of them. PCOS is peculiarly associated with an atherogenic lipid profile in the form of increased triglycerides, elevated LDL and levels of HDL. In PCOS as clinical observations affirm there is a relationship between dyslipidemia and impaired

glucose (10) . The prevalence of abnormalities of lipid in PCOS patients with type 2 diabetes or impaired glucose was greater 88% in comparison to PCOS patients that had normal glucose 58%. In addition, lipid abnormal itiesappeared in 81% of PCOS patients(45).

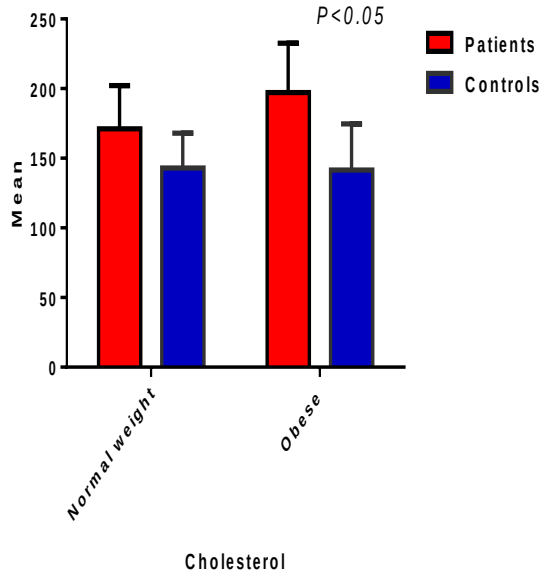
The results of studies by Dipanshu Dinh, & Thompson,. are consistent with the results of the present study which refers to the increase in levels of TG, LDL,HDL and total cholesterol in PCOS patients in compared with control (143).

Result of current study shows the mean value of Cholesterol was higher for obese patients (197.13±35.39) than obese controls (141.50±33.13), so the Cholesterol for normal weight patients (171.20±31.01) was more than of normal weight controls (143.00±24.94) with high significant different ($P<0.05$) between study groups. The other lipid profiles differs significantly TG(178.92±77.99),HDL(57.14±4.33),LDL(78.42±23.24),VLDL(35.78±20.00) in normal weight patient group are higher than those in control group TG (81.78± 23.20), HDL(54.19±8.09), LDL(71.59±19.59), VLDL(16.43±4.73). The lipid profiles differs significantly except HDL in the same way between obese of patients group and obese of control group as shown in Table (4-5).

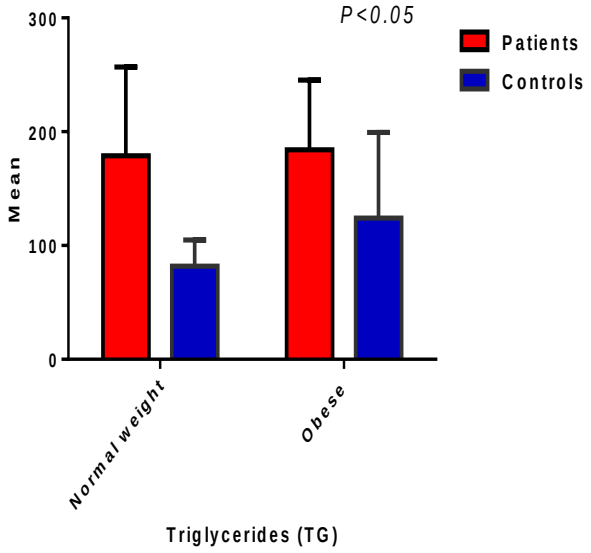
Table(4.5): Comparison lipid profile parameters between normal weight and obese of the studied groups by using student T test.

		Patients		Controls		P value
		Mean	Std. Error	Mean	Std. Error	
Cholesterol(mg/dl)	Normal weight	171.20	13.87	143.00	8.31	0.009**
	obese	197.13	5.11	141.50	8.85	0.001***
TG(mg/dl)	Normal weight	178.92	44.72	81.78	7.73	0.001***
	obese	184.10	8.83	124.07	20.10	0.006**
HDL (mg/dl)	Normal weight	57.14	1.94	54.19	2.70	0.82
	obese	60.68	1.23	48.96	1.37	0.15
LDL (mg/dl)	Normal weight	78.42	10.39	71.59	6.53	0.08
	obese	99.46	3.63	67.93	5.35	0.03*
VLDL (mg/dl)	Normal weight	35.78	8.94	16.43	1.58	0.02*
	obese	36.83	1.77	24.76	4.05	0.03*
Index	Normal weight	2.60	0.24	2.66	0.22	0.82
	obese	3.27	0.09	2.74	0.17	0.311

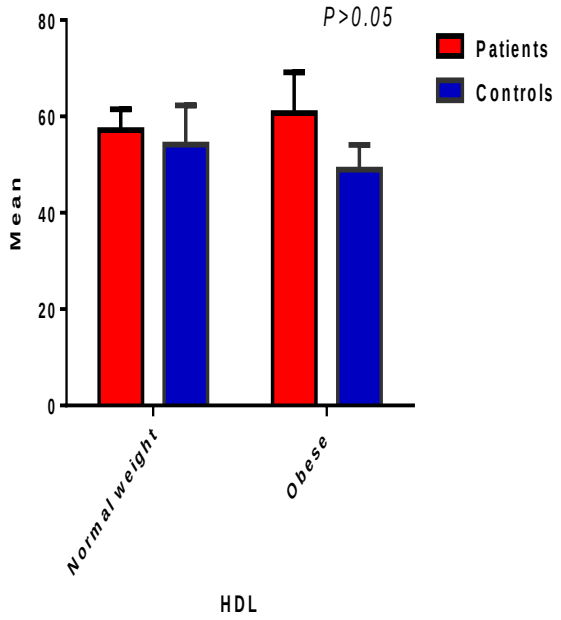
*= significant different ($p<0.05$), **= high significant different ($p<0.01$), ***= very high significant different ($p<0.001$)



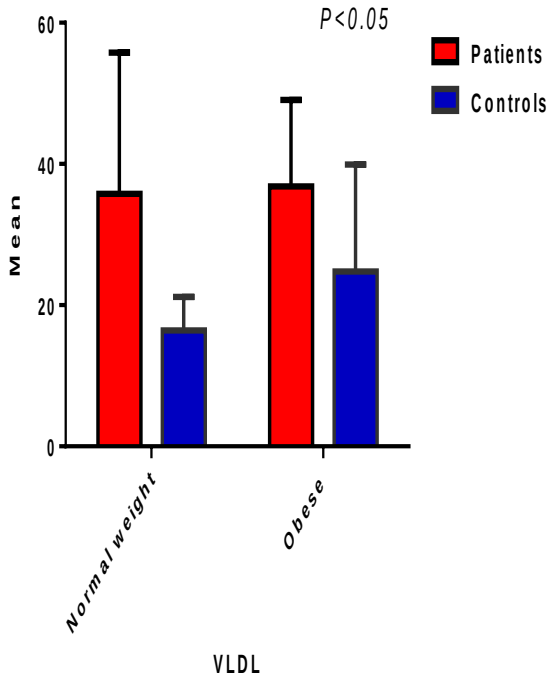
Cholesterol



Triglycerides (TG)



HDL



VLDL

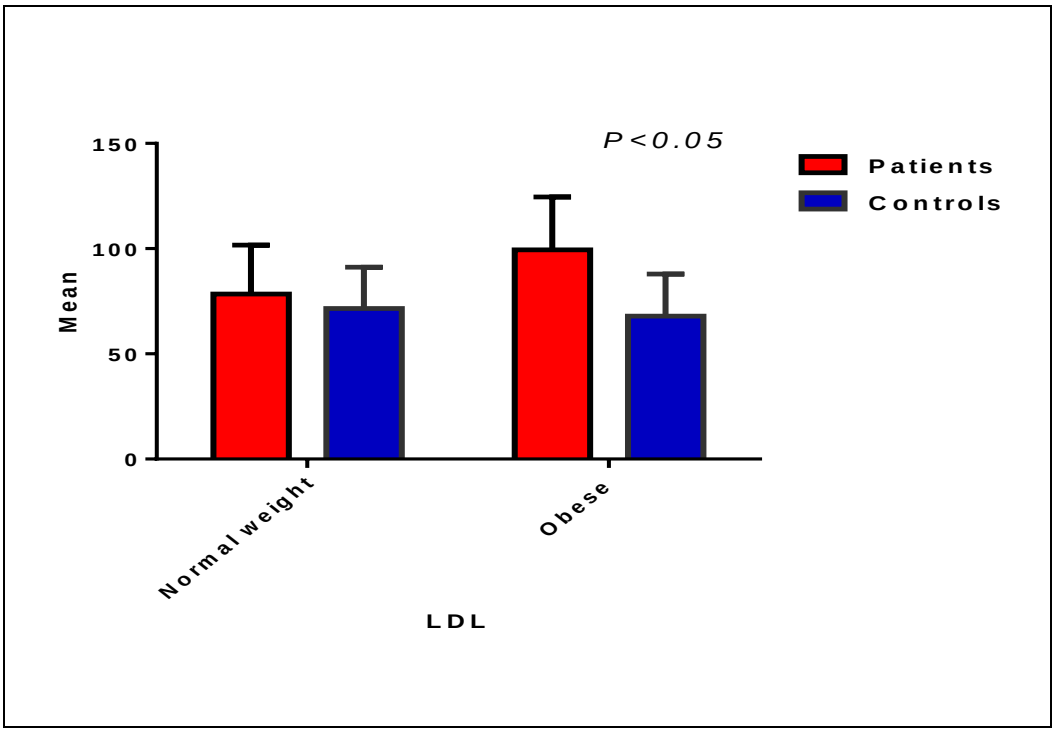


Figure (4-4):Comparison lipid profile parameters between normal weight and obese of the studied groups .

Boshku and Panova., showed the presence of high levels of lipid profile parameters in obese PCOS patients than in non-obese with a highly significant difference ($p<0.05$) These results were agreed with our study, Obese has an important impact on lipid profile (186). Dyslipidemia of obese is characterized by increased TG, low levels of HDL-C, increased subtractions of small, dense LDL, and increased levels of apolipoprotein (187).

Faloia *et al,*. mentioned the effect of obese on lipid profile and hyperlipoproteinemia in PCOS women and Around 50% of PCOS patients are overweight or obese with an accumulation of abdominal fat (188).

Saghafi-Asl *et al,*. Researchers found that lipid and lipoprotein levels were similar in obese, overweight, and non-obese PCOS patients and controls, but that HDL-C levels were lower in obese PCOS women (189) . Ibrahim *et al,*. According to the findings, women with PCOS have an atherogenic lipoprotein

profile with elevated cholesterol, LDL, and triglycerides, especially in the obese group, which could be a risk factor for cardiovascular complications. These results disagreed with our study (190).

The cause of dyslipidemia in PCOS may be hyperinsulinemia and hyperandrogenemia. This allowed adipocytes to undergo increased lipolysis caused by catecholamine and release free fatty acids into the circulation (191). Increased free liver fatty acids cause VLDL secretion, leading to hypertriglyceridemia. Hypertriglyceridemia leads through the reverse cholesterol transfer pathway to reduced HDL cholesterol and elevated LDL cholesterol levels. The further androgenic priming of adipocytes in early life predisposes to PCOS-associated dyslipidemia (192). The study indicates that in PCOS patients, total cholesterol and triglycerides were significantly higher in non-obese women compared to controls (151). Such results are consistent with the analysis by (193) where serum total cholesterol and serum triglycerides were elevated in non-obese PCOS compared to control group whereas HDL, LDL and VLDL were not statistically significantly different in both groups. In addition, showed a statistically significantly increase of total cholesterol and LDL in non-obese PCOS compared to control, while there was no statistically significant difference between triglycerides and serum HDL. Theses above results are compatible with our results that show high levels of lipid profile in non-obese PCOS patients compared to controls (185).

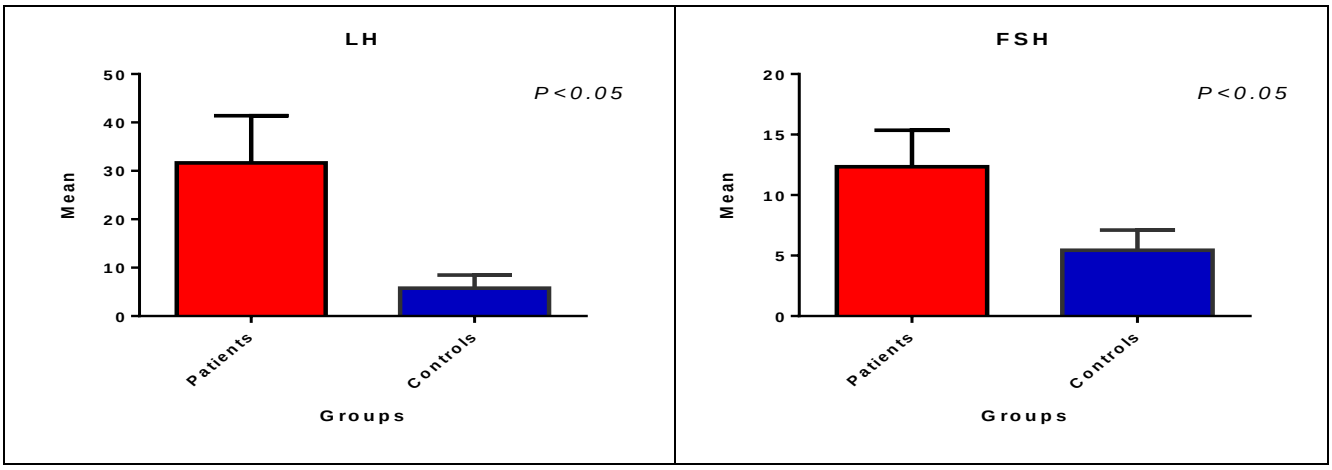
4.4. Parameters of Hormones

The results of the current study(Table (4-6) and Figure (4-5)) showed LH the was increased in patients (31.63±9.75)than in controls(5.79±2.68) with a highly significant difference ($p<0.05$).The results in the levels of FSH and Prolactin between the studied groups patients (12.34±3.02) and (33.32±9.07))than in controls(5.44±1.67) and (14.18±4.86) with a high significant difference ($p<0.05$) . Showed Testosteron the was increased in patients (4.59±1.52)than in controls(0.15±0.11) with a highly significant difference ($p<0.05$).The results in the levels of Estradiol and Vaspin between the studied groups patients (107.21±42.57) and (62.23±21.35))than in controls(53.22±36.85) and (3.41±3.27) with a high significant difference ($p<0.05$)

Table (4-6): comparison hormones parameters between studied groups by using student T test

Parameter		Mean	Std. Error Mean	p value
LH(m.IU/ml)	Patients	31.63	1.26	0.001***
	controls	5.79	0.49	
FSH(m.IU/ml)	Patients	12.34	0.39	0.003**
	controls	5.44	0.30	
Prolactin(ng/ml)	Patients	33.32	1.17	0.002**
	controls	14.18	0.89	
Testosteron (ng/ml)	Patients	4.59	0.20	0.001***
	controls	0.15	0.02	
Estradiol (pgm/ml)	Patients	107.21	5.50	0.002**
	controls	53.22	6.73	
Vaspin(pgm/ml)	Patients	62.23	2.76	0.003**
	controls	3.41	0.60	

= high significant different (p<0.01), *= very high significant different p<0.001)



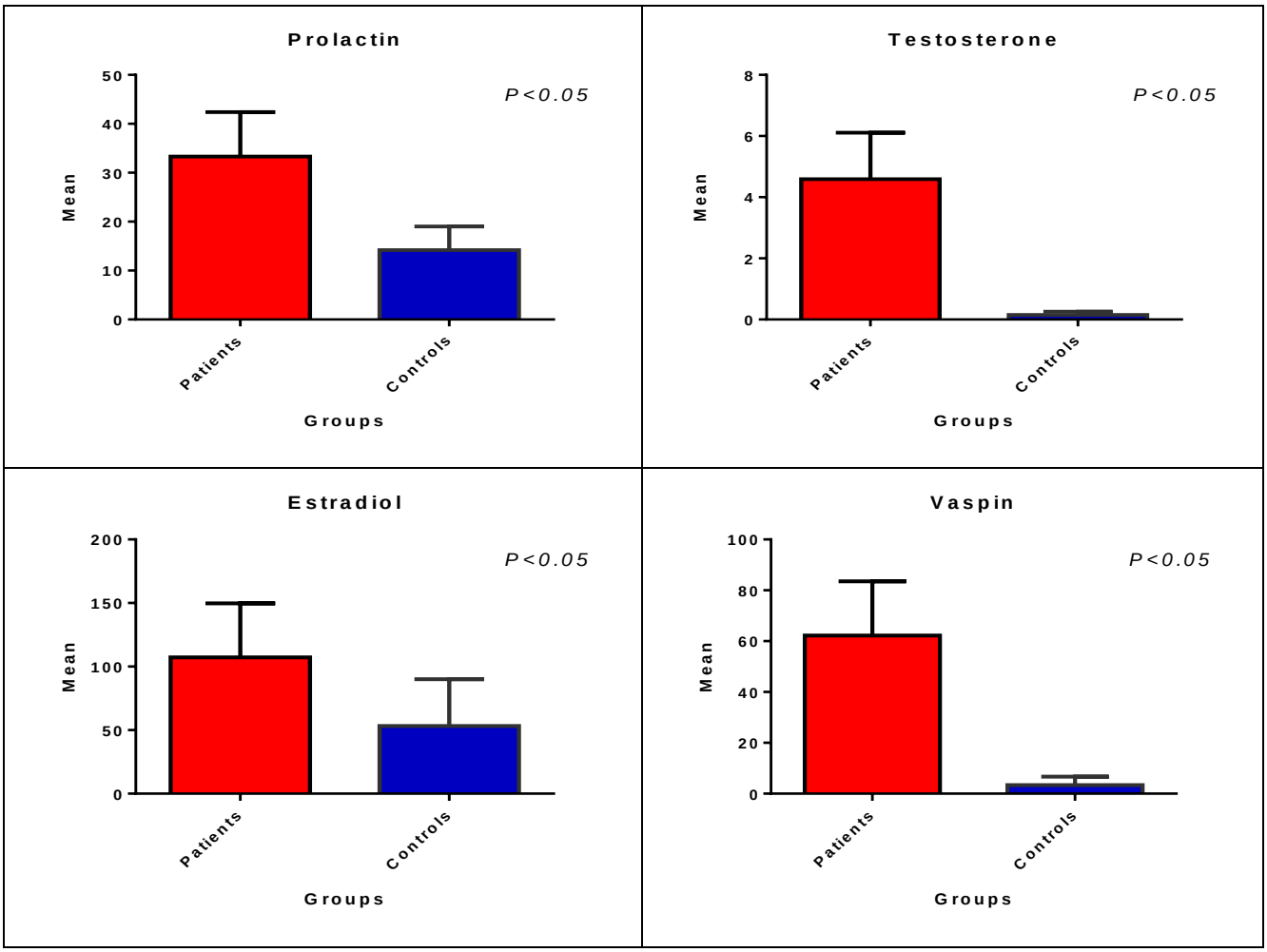


Figure (4-5): comparison hormones parameters between studied groups

There are numerous of hypothesis about the causes of PCOS development and the concurrent presence of several inter dependent disorders. In obese girls this disorder arises as an exaggerated adrenarche, according to suggestion of a group of experts. The combination of elevated levels of adrenal androgen and obesity leads to increased formation of extra glandular estrogen by the way of peripheral aromatization. This high amount of estrogen exerts appositive feedback on LH secretion and reverse in FSH secretion (4).

The results of our study are agree with previous studies; study by Hashemi *et al.*, revealed significant difference between group of polycystic

ovary syndrome compared with the group of control. and increase in LH level increases ovarian androgen production (194).

At present, testosterone is the most common measurement in routine clinical practice for the investigation of female hyperandrogenism That can be produced either directly by the ovaries or generated by the metabolism of its precursor androstenedione in adipose or peripheral tissues Data of the present study agree with the study by that showed increased level of testosterone in PCOS patients and the testosterone plays an important role in PCOS that (124).

There have been several possible mechanisms increasing that may occur in PCOS. Hyperprolactinemia could be a consequence of altered dopamine turnover which might also deteriorate GnRH output, that in turn could result in abnormal ovary function and constitute a common cause for both PCOS and hyperprolactinemia. Out of peripheral hormones, the strongest effect have estrogens, which stimulate the synthesis and secretion of prolactin, as well as cell proliferation of lacto tropic pituitary cells, Hyperprolactinemia and polycystic ovary syndrome (PCOS) are on the list of the most frequent causes of female infertility. Both pathologies are characterized in common by several clinical features. At the same time This study found significant differences between women with polycystic ovaries and the control group, where a significant increase in prolactin hormone was found, and this is consistent with the results we reached(195)

Consistently with early observations that estrogens stimulate release, elevated in PCOS could result in increased estradiol concentrations. However, currently available data on the role of estradiol secretion in humans is controversial. The role of estrogens in excess is a well-known duce in increased estradiol In patients with polycystic ovaries(196).

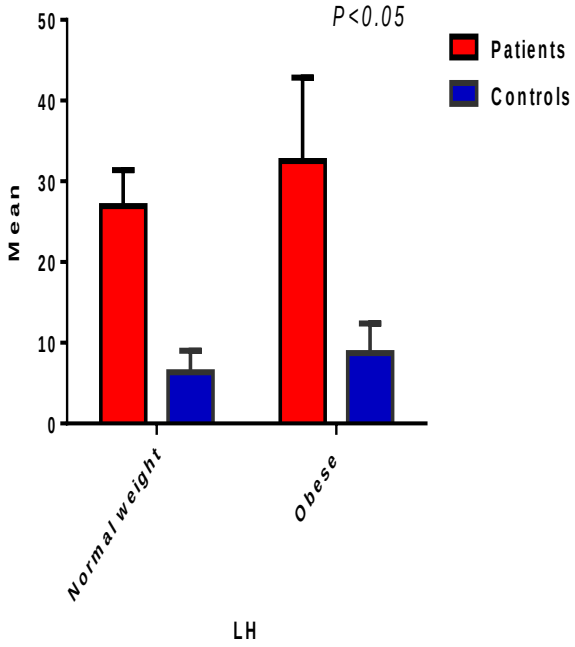
Byung *et al*,. According to other studies, higher circulating vaspin levels in PCOS patients may be a compensatory strategy for glucose metabolism. The detrimental effects of intra-abdominal fat accumulation may be explained, at least in part, by visceral fat depot–specific adipokine production. Differential protein or mRNA expression in fat depots has been described for a number of years. This study found significant differences in vaspin hormone between women with PCOS and the control group, where a significant increase was found, and this is consistent with the results we have reached (197).

The result of the current study shows the mean value of LH was high for obese patients (32.53±10.31) than obese control (8.74±3.66). The LH for normal weight patients (26.94±4.46) was more than normal weight controls (6.34±2.70) with high significant different ($P<0.05$) between study groups. FSH levels are higher in both normal weight (9.26±1.43) and (12.79±3.11) obese of patients groups compared with normal weight (5.22±1.86) and obese (6.65±2.39) of the control group significantly(0.002**). Prolactin levels in both normal weight (27.52± 2.83) and obese (33.91±9.64) of patient groups are higher significantly (0.001***) than those in control groups (14.61±5.83), (16.72±5.83) normal weight and obies respectively. Testosterone increased significantly (0.001***) in the two patient's subgroups (4.13±0.76) , (4.71±1.55) the normal weight and obies respectively compared with those in control subgroups (0.16±0.09), (0.67±0.27) the normal weight and obies respectively. Estradiol and Vaspin levels differ in the same way significantly increase (0.001***) between the two patients' subgroups compared with those in control subgroups as shown in the Table(4.7) .

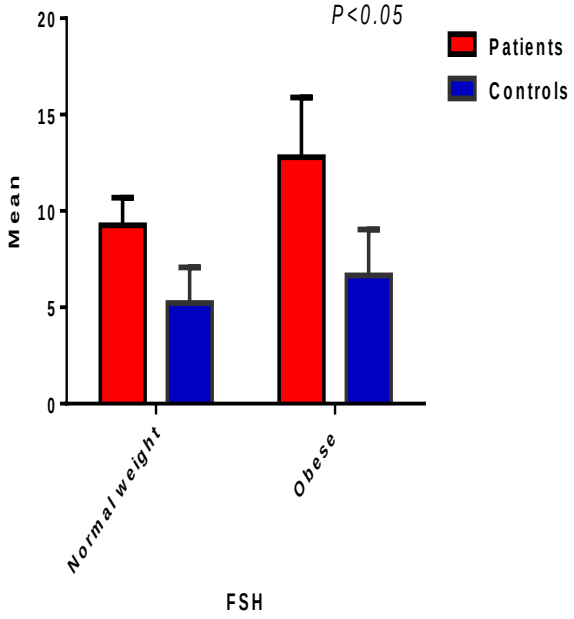
Table (4-7): comparison hormones parameters between normal weight and obese of the studied groups by using student T test

		patients		controls		
		Mean	Std. Error	Mean	Std. Error	P value
LH(m.IU/ml)	normal weight	26.94	1.99	6.34	0.90	0.001***
	Obese	32.53	1.49	8.74	1.78	0.001***
FSH(m.IU/ml)	normal weight	9.26	0.64	5.22	0.62	0.02*
	obese	12.79	0.45	6.65	0.64	0.001***
Prolactin(ng/ml)	normal weight	27.52	1.26	14.61	1.94	0.01**
	obese	33.91	1.39	16.72	2.01	0.004**
Testosteron (ng/ml)	normal weight	4.13	0.34	0.16	0.04	0.001***
	obese	4.71	0.22	0.67	0.34	0.001***
Estradiol (pgm/ml)	normal weight	112.04	11.82	44.04	9.24	0.001***
	obese	104.59	6.25	62.82	10.57	0.001***
Vaspin(pgm/ml)	normal weight	54.83	11.01	2.63	0.94	0.001***
	obese	64.50	3.07	15.22	7.45	0.001***

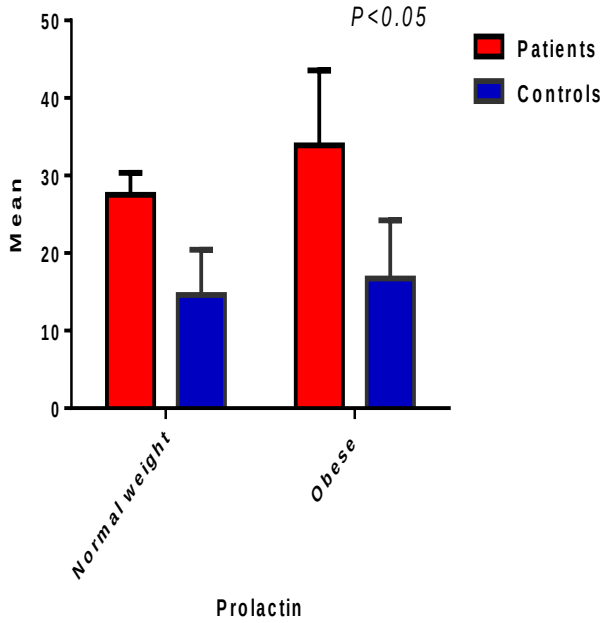
= high significant different ($p<0.01$), *= very high significant different ($p<0.001$)



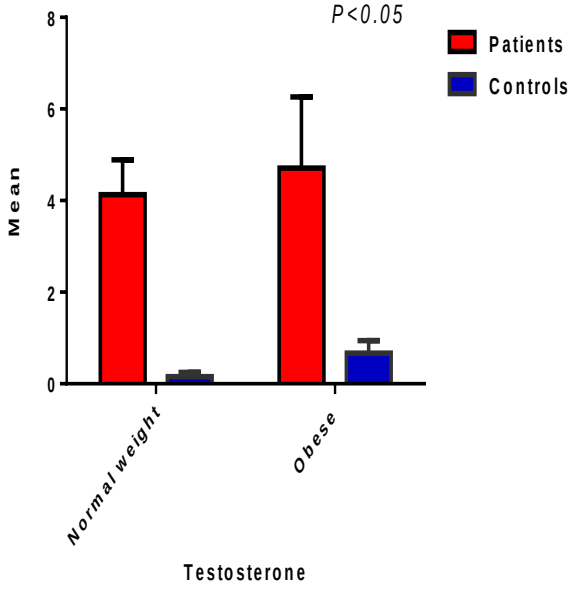
LH



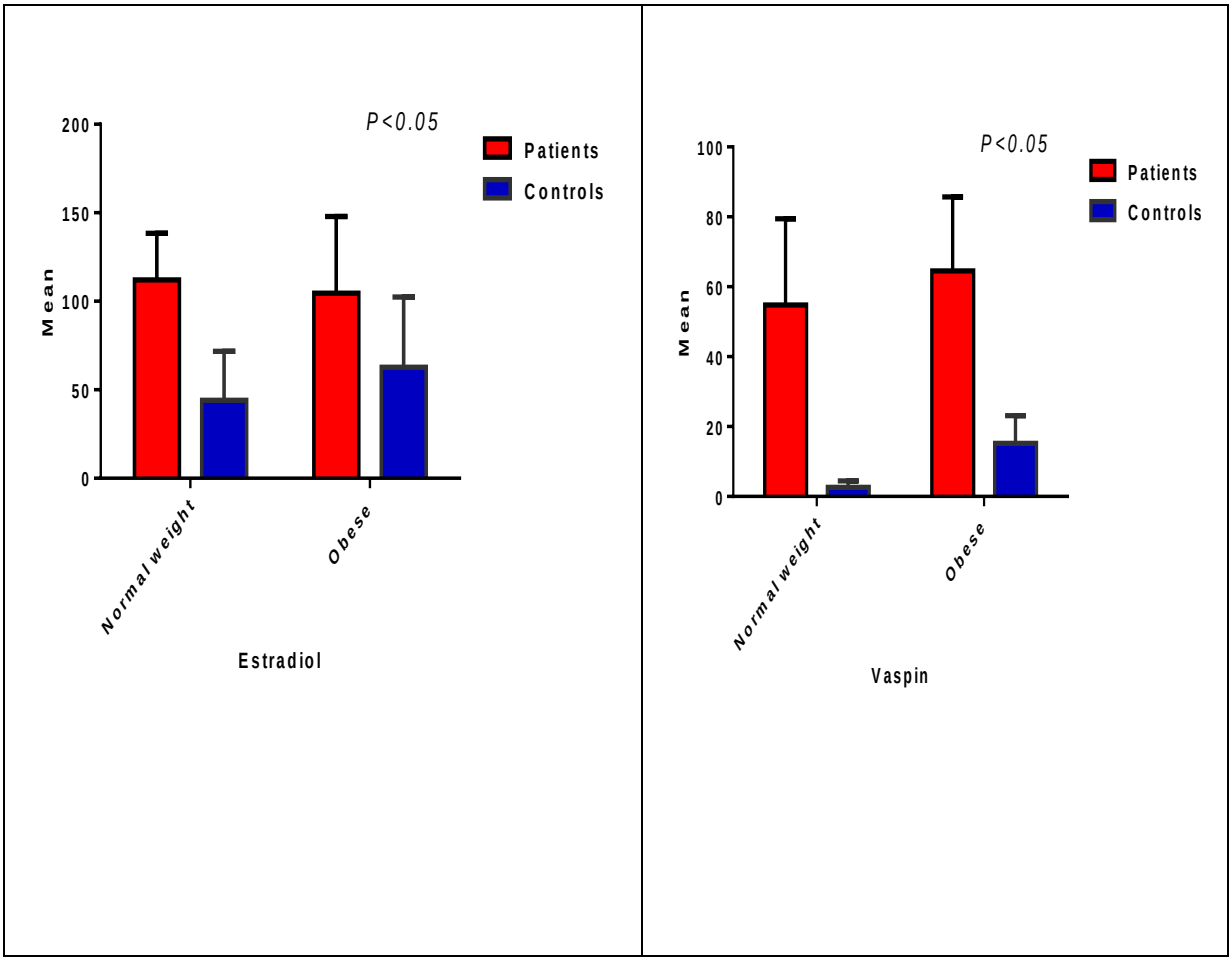
FSH



Prolactin



Testosterone



Figure(4-6): comparison hormones parameters between normal weight and obese of the studied groups

Zeng *et al*,. It has been suggested that the majority of PCOS patients with hyperandrogenism suffer from LH secretion problems, which lead to abnormal folliculogenesis and failed dominant follicle selection (198). (199) In obese PCOS patients on a restricted diet, evidence of estradiol-dependent negative feedback on LH secretion retention can predict follicle maturation and ovulation. In the pathophysiology of polycystic ovarian disease, abnormality of the hypothalamic-pituitary-or adrenal axis has been imposed. In women with PCOS, the pituitary-derived luteinizing hormone (LH) is often usually elevated, which stimulates ovarian androgen overproduction(200).

Marshall *et al,.* showed low levels of LH and FSH in obese PCOS than in non-obese with no significant difference ($p>0.05$). These results disagreed with our results that showed high levels of LH and FSH in obese PCOS than in non-obese with a highly significant difference ($p<0.05$) (201). Lal and Perween proposed high levels of LH, FSH and testosterone in PCOS patients than controls (202). These results are similar to our findings that showed high levels of LH, FSH and testosterone in PCOS patients than controls. When obese PCOS was compared to non-obese PCOS, the levels of LH, FSH, testosterone, and E2 were considerably higher ($P< 0.05$) (203). These results are different to our findings that showed high levels of LH, FSH and testosterone in obese PCOS than in non-obese PCOS(204).

Keyif *et al,.* (205) Suggested that testosterone levels in obese PCOS patients were substantially higher than in non-obese PCOS patients and controls. These findings corroborate our findings .Obese PCOS has higher testosterone levels than normal weight PCOS (205). In comparison to their normal weight PCOS counterparts, obese PCOS patients with high sugar have lower levels of sex hormone-binding globulin (SHBG), resulting in higher levels of free testosterone in their blood (3).

Glintborg *et al,.* (206) showed FSH and estradiol (E2) levels in PCOS patients are normally within (broad) normal ranges, while LH levels can be elevated. Androgen interferes with E2-dependent signaling pathways, causing oocyte maturation to be disrupted (206). Tan *et al,.* They confirmed these findings, finding higher serum and adipose tissue vaspin levels in women with PCOS, as well as reporting that obese subjects had higher serum vaspin levels, which showed a significant positive relationship with BMI and vaspin (207). These results are compatible with our study that showed high levels of vaspin in PCOS patients. Guvenc *et al,.* Mentioned the presence of PCOS is linked to a drop in vaspin levels. These results not compatible to our results

that show high levels of vaspin in obese PCOS patients than non-obese with high significant different ($p< 0.05$), Vaspin levels were found to be associated with body mass index in women with PCOS and waist circumference in controls (208). Vaspin has also been related to metabolic syndrome and ovarian pathology, such as polycystic ovary syndrome (PCOS), due to greater vaspin levels in PCOS patients' blood(54). Heiker, According to the researchers, vaspin could be a new link between obese and metabolic dysregulation in obese PCOS. PCOS patients have a higher quantity of adipose tissue in general, which has previously been connected to this relationship. We discovered that, regardless of BMI, PCOS patients have significantly higher vaspin (209). Byung *et al,*. According to other studies, higher circulating vaspin levels in PCOS patients may be a compensatory strategy for glucose metabolism. The detrimental effects of intra-abdominal fat accumulation may be explained, at least in part, by visceral fat depot–specific adipokine production. Differential protein or mRNA expression in fat depots has been described for a number of years (210). Vaspin is an adipocytokine that was isolated from visceral white adipose tissue These data suggest that the increase in vaspin may be a compensatory response to obesity (211).

Dogan *et al,*. The current study hypothesizes that increased vaspin secretion might represent a compensatory mechanism in response to decreased impairment of glucose metabolism. We therefore sought to provide new mechanistic insight into the relationship between vaspin serum concentrations and obese or its associated metabolic disorders. Recent research has discovered a sexual dimorphism in circulating vaspin, with women having considerably higher vaspin levels. There is currently no information on the control of vaspin by gonadal and adrenal hormones (212).

Described by Chang HM *et al.*, Vaspin concentrations were shown to be positively linked with BMI in the current investigation. Changes in serum vaspin concentration in response to weight loss, and the relationships between changes in serum vaspin concentrations and changes in anthropometric and metabolic characteristics in obese people after weight loss Responders' serum vaspin concentrations dropped dramatically (by 2% of their baseline weight). Body weight, BMI, waist circumference, and hip circumference were all substantially linked with changes in serum vaspin concentrations in the studies (213).

Sex steroid precursors, estrogens, androgens, as well as glucuronidated androgen metabolites, are all elevated in PCOS women. connection has previously been attributed to the generalization of a higher amount of adipose tissue in PCOS patients. However, regardless of BMI, we discovered that vaspin is significantly higher in PCOS patients (214). Khashchenko *et al*,. showed the sensitivity of Testosteron was very high in PCOS . All existing clinical recommendations suggest measuring testosterone, have been shown to aid in the diagnosis of PCOS and provide additional information on metabolic danger (215).

Resist is an adipocytokine that promotes inflammation and, can play a role in the pathogenesis of PCOS . These adipokines can also be used as PCOS biomarkers.(216).

4.5. Correlation among Parameters

Result of current study shows the Vaspin parameter is positive correlate with (BMI (r=.255*), FBS (r=.107), Cholesterol (r= .224), HDL (r= .131), VLDL (r= .188), LH (r= .112), Prolactin (r= .095), Estradiol (r= .007), HbA1c (r= .048), TG (r= .175), LDL (r= .196), Index (r= .154), and Testosteron (r= .393**). In contrast, Vaspin parameter is negative correlate with FSH (r= -.056) (Table 4-8).

Table (4-8): correlation relationship among variables by using Pearson correlation test.

		BMI	HbA1c	TG	LDL	Index	FSH	Testosteron	Vaspin
BMI	r	1	.224	.103	.382**	.387**	.300*	.171	.255*
	p		.085	.435	.003	.002	.020	.193	.050
FBS	r	.385**	.837**	.337**	.287*	.432**	.150	-.216	.107
	p	.002	.000	.009	.026	.001	.253	.097	.416
Cholesterol	r	.350**	.265*	.531**	.784**	.622**	.303*	-.036	.224
	p	.006	.040	.000	.000	.000	.019	.788	.085
HDL	r	-.017	.107	.145	-.048	-.334-**	.069	-.149	.131
	p	.896	.415	.267	.717	.009	.599	.256	.320
VLDL	r	.123	.303*	.995**	.096	.435**	.123	-.115	.188
	p	.351	.019	.000	.466	.001	.349	.382	.150
LH	r	.198	.171	.170	.219	.192	.593**	.221	.112
	p	.130	.192	.195	.093	.142	.000	.090	.394
Prolactin	r	.130	-.011	.079	.210	.284*	.332**	.106	.095
	p	.322	.934	.551	.108	.028	.010	.418	.469
Estradiol	r	-.188	-.034	.019	.009	.019	.046	.041	.007
	p	.150	.796	.888	.943	.887	.727	.758	.955
Vaspin	r	.255*	.048	.175	.196	.154	-.056	.393**	1
	p	.050	.718	.181	.134	.240	.670	.002	

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

The present study showed a difference in serum vaspin between PCOS patients and healthy controls. The most predictive variables for circulating in PCOS patients were vaspin and appeared to be in positive correlation with BMI and testosterone levels in PCOS patients compared with healthy controls, which supports the suggestion that there is a relationship between metabolic syndrome components (e.g. dyslipidemia) and androgen disorders in PCOS patients. It may be one of the early markers in the development of metabolic syndrome and contribute to increased diabetogenic and atherogenic risk in these patients. Monitoring its levels may assist in the management of PCOS patients, particularly with regard to lifestyle changes and diet in obese patients (217).

4.6. Receiver operator curve of patients results

LH, FSH, Prolactin, Vaspin and Testosteron parameters showed a highest sensitivity (100%) when compared with sensitivity of others parameters FBS (91%), HbA1c (95%), Cholesterol (90%), TG (95%), HDL (88%) , LDL (86%). VLDL (95%), Index (88%) and Estradiol (91%) with high significant different ($p<0.05$).

Depending on specificity, Cholesterol , LDL and Index parameters showed a highest specificity (56%, 56% and 60%) respectively, when compared with specificity of others parameters FBS (40%), HbA1c (43%), TG (36%), HDL (37%) , VLDL (36%), Estradiol (36%), LH (50%), FSH (43%) , Prolactin (53%) and Testosteron (46%) with high significant different ($p<0.05$) (Table4-9).

Table (4-9): Receiver operator curve, sensitivity and specificity of variables

Variables	AUC	Std. Error	Asymptotic Sig\	Asymptotic 95% Confidence Interval		sensitivity	specificity
				Lower Bound	Upper Bound		
FBS	.846	.042	.000	.764	.927	91%	40%
HbA1c	.891	.034	.000	.825	.956	95%	43%
Cholesterol	.911	.031	.000	.851	.971	90%	56%
TG	.892	.036	.000	.822	.962	95%	36%
HDL	.808	.050	.000	.710	.905	88%	37%
LDL	.848	.041	.000	.767	.928	86%	56%
VLDL	.890	.036	.000	.819	.960	95%	36%
Index	.676	.061	.007	.556	.796	88%	60%
LH	1.000	.000	.000	1.000	1.000	100%	50%
FSH	.990	.007	.000	.976	1.000	100%	43%
Prolactin	.989	.008	.000	.972	1.000	100%	53%
Testosteron	1.000	.000	.000	1.000	1.000	100%	46%
Estradiol	.854	.048	.000	.760	.948	91%	36%
Vaspin	1.000	.000	.000	1.000	1.000	100%	47%

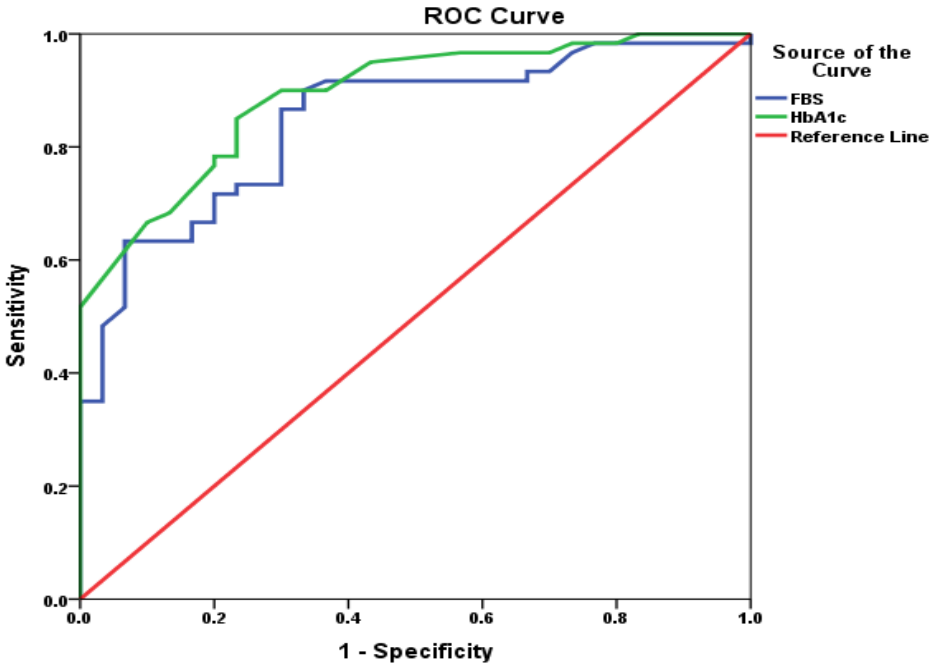


Figure (4-7) ROC curve of FBS and HbA1c parameters.

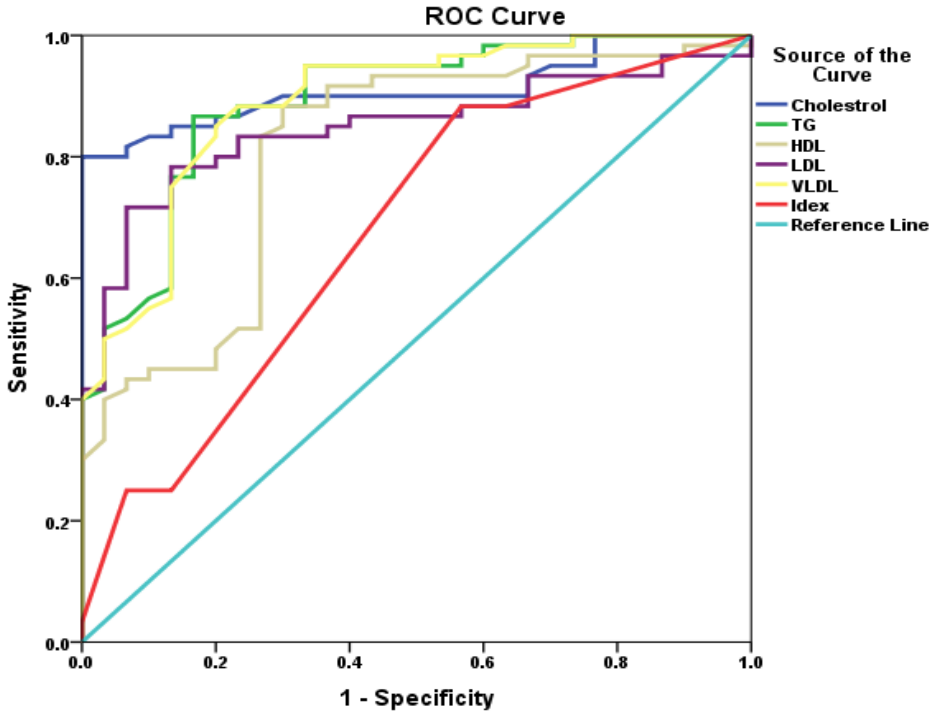


Figure (4-8) ROC curve of lipid profile parameters.

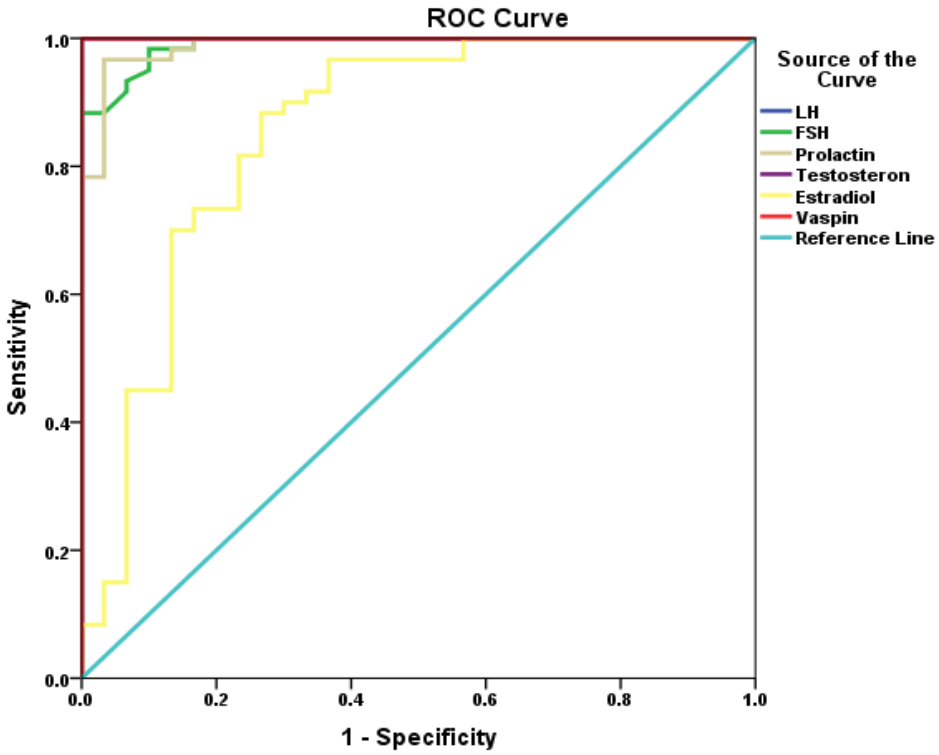


Figure (4-9): ROC curve hormones parameters.

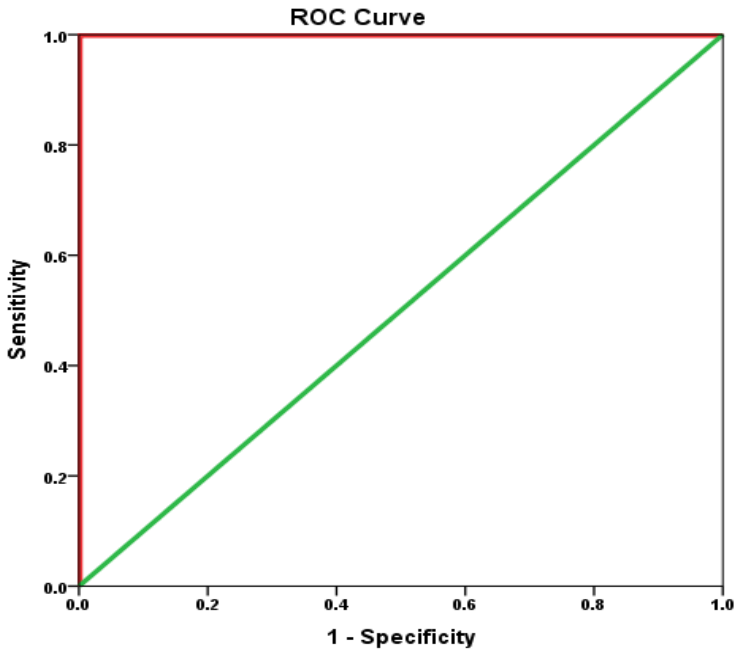


Figure (4-10): ROC curve Vaspin parameter

Conclusions

From the results of the current study it can be concluded that:

1. Sex hormones takes apart in the onset of PCOS where there is increasing in aromatization of androgens to estrogens, resulting in estrogen overproduction on a long-term basis, stimulates ovarian stromal hyperplasia.
2. Increasing in vaspin levels resulted in the accumulation of fats around the waist a characteristic of PCOS, vaspin is related to obesity in Iraqi women.
3. Women with PCOS had a greater risk of diabetes mellitus since the results of glucose significantly increased.
4. Women with PCOS also had a risk of cardiovascular diseases since the results of lipid profile are significantly increased.
5. In the current study vaspin appeared to be in positive correlation with testosterone, since testosterone has a role in the etiology of PCOS the vaspin has the role of developing the fat tissue then obesity.

Recommendations

1. Development of programs to increase women's awareness towards polycystic ovarian syndrome for women to know how to deal with the disease, as well as how to prevent complications.
2. More studies are needed to revels the roles of other adipokine hormones (like Omentin) in PCOS.
3. Other studies are needed for detection the levels of insulin in PCOS patients.
4. More studies with large size of samples and different areas are needed .

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Appendix

Questionnaire

No: **Date:**

Name: **Address:** **Mobile No.:**

Age:

Weight: **Kg** **High** **cm**

Married: **No. of children:**

Family history **PCOS:** **Hirsutism :** **Diabetes:**

Menarche **Oligomenorrhea:** **irregular menses:** **Amenorrhea:**

Utrine Bleeding: **regular menses:** **History of abortion:**

Hypertension:

Diabetes

Acne: **Hirsutism** **Oily skin:**

PCOS treatment

results	test
	LH
	FSH
	prolactin
	Testosterone
	E2
	Vaspin
	Hba1c
	FBS
	TG
	Cholesterol
	LDL
	HDL
	VLDL

Appendix

الخلاصة

ان متلازمة تكيس المبايض (PCOS) هي أكثر اضطرابات الغدد الصماء شيوعًا وتعقيدًا التي تصيب النساء في سنوات الإنجاب ، تتسم بالعقم ، والشعرانية ، والتطور الجريبي غير الطبيعي ، وزيادة إنتاج الهورمونات الذكرية في المبيض. قد يكون التسبب في متلازمة تكيس المبايض ناتجًا عن اضطرابات هرمونية (المبيض والغدة النخامية). تهدف الدراسة الحالية إلى الكشف عن العلاقة بين متلازمة تكيس المبايض وهرمون الأديبوكين (هرمون الفاسبين). والكشف عن دور الهورمونات الجنسية في متلازمة تكيس المبايض ، وما إذا كانت متلازمة تكيس المبايض قد تؤدي إلى مضاعفات أخرى (أمراض القلب والسكري) عن طريق قياس مستوى الدهون (الكولسترول ، الدهون الثلاثية ، الدهون عالية الكثافة ، الدهون واطئة الكثافة) و (FBS، HbAIc).

أجريت هذه الدراسة (دراسة مقارنة) في مستشفى البتول/بعقوبة/محافظة ديالى. تضمنت (60) عينة من مريضات تكيس المبايض بمعدل عمر (8.17±28.40) و (30) عينة من النساء الاصحاء بمعدل عمر (6.84±28.33) و تم تقسيم كل من مجموعة المرضى و الاصحاء الى مجموعتين ثانويتين اعتمادا على مؤشر كتلة الجسم (BMI)(مجموعة البدينات و مجموعة النحيفات). تم جمع العينات في اليوم الثاني من الدورة الشهرية. ان جميع المريضات تم اكتشافهن حديثًا لمتلازمة تكيس المبايض ولم يأخذن أي علاج، لتقييم دور الفاسبين والبرولاكتين و LH و FSH "وهرمون التستوستيرون و E2 و FBS و HbA1c و صور الدهون.

كشفت هذه الدراسة عن ارتفاع في جميع الهرمونات التي تم تضمينها في الدراسة، ارتفعت مستويات الفاسبين على وجه الخصوص معنويا (***0.001)في مجموعة النساء الضعيفات من المريضات (11.01±54.83) مقارنة بمثيلاتها في النساء الاصحاء (0.94±2.63)، و بنفس الطريقة تزداد مستويات الفاسبين في مجموعة البدينات من النساء المريضات (3.07±64.50) مقارنة بمثيلاتها في النساء الاصحاء(7.45 ±15.22).

الخلاصة

و ارتفاع FBS و HbA1c في المجموعتين الثانويتين للمرضى مقارنة بمجموعتي الاصحاء، كذلك زيادة الكوليسترول والدهون الثلاثية و LDL و VLDL بشكل ملحوظ في كلا المجموعتين مقارنة بمجموعات الاصحاء بينما لم تظهر مستويات HDL زيادة معنوية. يستنتج من هذه الدراسة ان مريضات متلازمة تكيس المبايض تعاني من مخاطر الإصابة بمرض السكري وأمراض القلب استنادا الى نتائج تحليل الدهون و نتيجة تحليل مستوى السكر. ان الهورمونات الجنسية تلعب دورا في التسبب بهذه المتلازمة حيث تتفكك الهرمونات الجنسية في بداية متلازمة تكيس المبايض و يتزايد تحويل الأندروجين إلى هرمون الاستروجين ، مما يؤدي إلى زيادة إنتاج هرمون الاستروجين بشكل كبير ، ثم تحفيز تضخم انسجة المبيض. أدت الزيادة في مستويات هورمون الفاسبين إلى تراكم الدهون حول الخصر وهي إحدى سمات متلازمة تكيس المبايض.

وزارة التعليم العالي والبحث العلمي

جامعة ديالى

كلية العلوم

قسم الكيمياء

تقييم مستويات الفاسبين وبعض المتغيرات الكيموحيوية لمريضات تكيس المبايض في محافظة ديالى

رسالة مقدمة الى

مجلس كلية العلوم / جامعة ديالى

وهي جزء من متطلبات نيل درجة الماجستير في علوم الكيمياء

من قبل الطالبة

سها صبري حسن

بكالوريوس في علوم الكيمياء 2006

كلية العلوم للبنات– جامعة بغداد

بأشراف

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1443هجري 2021 ميلادي

CHAPTER ONE
Introduction and Literatures Review

CHAPTER Three Experimental Part

CHAPTER Four

Results and Discussion

CHAPTER Two
Theoretical Part

REFERENCES

CONCLUSION
&
RECOMMENDATIONS

Appendix