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Department of Chemistry

Evaluation of Some Inflammatory Factors in Patients of Polycystic Ovary Syndrome with Insulin Resistance

A Thesis

Submitted to the College of Education for Pure Sciences, University of Kerbala, in Partial fulfillment of Requirement of Degree Master of Science in Biochemistry

by

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Dedication

To... Almighty Allah, who if I ask, he will give me, and if I thank him, he will increase me with blessings.

To…Prophet Muhammad (PBUH), the owner of Israa and Mi'raj, the master of the messengers, the prophet of mercy and the light of the worlds.

To…. Master and waiting imam (may Allah hasten his reappearance),the one for whom I longed and waited for a long time.

To…. my father who taught me everything and expected nothing in return.

To….. my mother who taught me the meaning of sacrifice and devotion, for my beautiful sisters and my dear brother they helps me when I need it, to my husband for his support, encouragement and love,and to everyone who supported me, even with a smile.

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Summary

Polycystic Ovarian Syndrome (PCOS) is one of the most prevalent metabolic and reproductive illnesses, Menstrual irregularities and androgen excess are two symptoms that PCOS-affected women experience, and these symptoms have a substantial negative influence on their quality of life. They may have a higher chance of developing a number of morbidities, such as obesity, type II diabetes, cardiovascular disease (CVD), infertility, cancer, mental health issues and insulin resistance. Insulin resistance (IR) is a failure in insulin-mediated control of glucose metabolism in tissues.It is the most common form of PCOS, and it causes the ovaries to generate more androgen, which causes oligo-ovulation or anovulatory.

This study was designed to evaluate the levels of some inflammatory factors in polycystic ovary syndrome and insulin resistance patients. The study included 80 women between the ages of 16-40 years, divided into 40 patients with polycystic ovaries and insulin resistance, and 40 women as a healthy control group. Each sample measuredthe following parameters LDL, HDL, Triglyceride and Cholesterol using the BS-430 device from Mindray company, while high sensitivity C-reactive protein was measured using BS-200 from Mindray company and human interleukin-6 was measured using MAGLUMI 800 from Snibe company. The results of the statistical analysis found that there was a significant difference in Low Density Lipoprotein(LDL), High Density Lipoprotein(HDL), Triglyceride, Cholesterol, High sensitivity C-reactive protein, Human Interleukin-6 in patients women compared to the control group, As LDL, Triglycerides, Cholesterol, High-sensitivity C-reactive

protein, and Human interleukin-6 increased, HDL decreased in the patients group. A positive correlation was found between triglycerides and each of High-sensitivity C-reactive protein, Human interleukin, and insulin resistance. In addition as a negative correlation was found betweenHDL and Human interleukin-6, and a positive correlation between each of High-sensitivity C-reactive protein and Human Interleukin-6, also it showed that female patients over 30 years of age have anincreased levels of LDL, Cholesterol, High-sensitivity C-reactive protein, and Human interleukin-6 compared to female patients under 30 years of age,while there is no significant value in a Triglycerides, HDL and insulin resistance in relation to the patients' age.The results also showed a significant relationship in the body mass index, exist in the two study groups. Obesity was more prominent in the patients group. The effect of blood pressurecompared between the two groups. The was no significant relationship shown between Interleukin-6, High Sensitivity C-Reactive Protein, Insulin resistance, and disease duration.

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Abbreviations

The Term	Definition
µl	Microliter
A	Absorbance
BMI	Body Mass Index
BPA	Bisphenol A
CRP	C-reactive protein
CVD	Cardiovascular diseases
dL	Deciliter
EDCs	Enocrin-disrupting chemicals
FFAs	Free fatty acids
FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin Releasing Hormone
HDL-C	High density lipoprotein cholesterol
HMGCR	3-Hydroxy-3-methylglutaryl coenzyme a reductase
HOMA-IR	Homeostatic model assessment for insulin resistance
HPO	Hypothalamic-pituitary-ovarian
HS-CRP	High sentivitiy C-reactive protein
IGFBP-1	Insulin-like growth factor binding protein 1

IL-6	Interlukin-6
IMCL	Intramyocellular lipids
IR	Insulin resistance
K	Kilo
kDa	Kilo dalton
Kg	Kilogram
KU	Kilo unit
L	Liter
LDL-C	Low density lipoprotein cholesterol
LDLR	Low density lipoprotein cholesterol receptors
LH	Luteinizing Hormone
LPL	Lipoprotein lipase
m^2	Square meter
mg	miligram
mL	Milliliter
N	Number
NAFLD	Nonalcoholic fatty liver disease
ºC	Degrees Celsius
PCOS	Polycystic Ovary Syndrome
pg	picogram
P-value	Probability level of statistical
r	Correlation coefficient
RA	Rheumatoid arthritis
SHBG	Sex hormone binding globulin
SREBP-2	Sterol regulatory element-binding protein-2
T2DM	Type2 diabetes mellitus
TG	Triglyceride

TRH	Thyrotropin Releasing Hormone
TSH	Thyroid Stimulating Hormone
VAT	Visceral adipose tissue
VLDL	Very low density lipoprotein cholesterol
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

AND

LITERATURE REVIEW

1. Introduction

Interest in polycystic ovaries (PCO) and its related syndrome (PCOS) has changed from a "gynaecological curiosity to a multisystem endocrinopathy" since Stein and Leventhal's first observation in 1935. Given that it accounts for the majority of cases of hirsutism, menstrual irregularities, and anovulatory infertility in women, it is most likely the most prevalent endocrine condition. It is also one of the least understood endocrinological disorders, with a complicated pathophysiology that has sparked a great deal of scholarly discussion[1].Today, it is understood that polycystic ovarian syndrome (PCOS) is characterized by insulin resistance (hyperinsulinemia). Its significance in the pathophysiology of PCOS has just lately been realized. Understanding the function of insulin resistance in PCOS has allowed for the successful use of insulin sensitizing medications in the treatment of this condition. Both obese and lean women with PCOS appear to have some degree of insulin resistance[2].

About 65–80% of people with PCOS also have obesity and insulin resistance (IR) [3], and it is well established that the disease's hyperinsulinemia, hyperandrogenism, and obesity all reinforce one another. However, PCOS is also characterized by a state of chronic inflammation [4], which is partly brought on by an excess of visceral adipose tissue and the pro-inflammatory pathways it possesses. However, normal-weight PCOS patients also experience chronic low-level inflammation [5], according to known evidence, increased levels of C-reactive protein (CRP) and Interlukin-6 (IL-6) have been linked with patients of PCOS[6].

1.2Literature Review

1.2.1 Polycystic Ovary Syndrome

The most prevalent endocrine condition in women, polycystic ovary syndrome (PCOS), has a wide range of clinical and biochemical characteristics. One in fifteen women worldwide are afflicted by PCOS, a diverse and complex female endocrine condition. About 5% to 10% of women between the ages of 12 and 45 who are fertile experience symptoms of PCOS. One of the most frequent symptoms may be an irregular menstrual cycle [7].

The root causes of PCOS are intricate and multifaceted. They could be environmental or genetic. In addition to insulin resistance brought on by the buildup of adipose tissue, lipid toxicity, and oxidative stress, this leads to hormonal abnormalities that encourage hyperandrogenism from the ovaries and adrenal glands [8], Symptoms of polycystic ovaries include irregularity Menstrual cycle, hirsutism, acne, alopecia, dermatitis and its thickness, insomnia or sleep disturbance [9],Also increases Luteinizing hormone (LH)/ Follicle-stimulating hormone (FSH) ratio and androgen (male hormone) levels [10].

The most frequent cause of unovulation in infertile women is polycystic ovarian syndrome (PCOS), which accounts for 70% of infertility problems in women who have trouble ovulating [11]. It is a collection of tiny, fluid-filled sacs the size of pearls that surround immature eggs in the ovary. This condition causes the ovaries to enlarge due to the growth and enlargement of the follicles [12], as shown in figure (1.1).

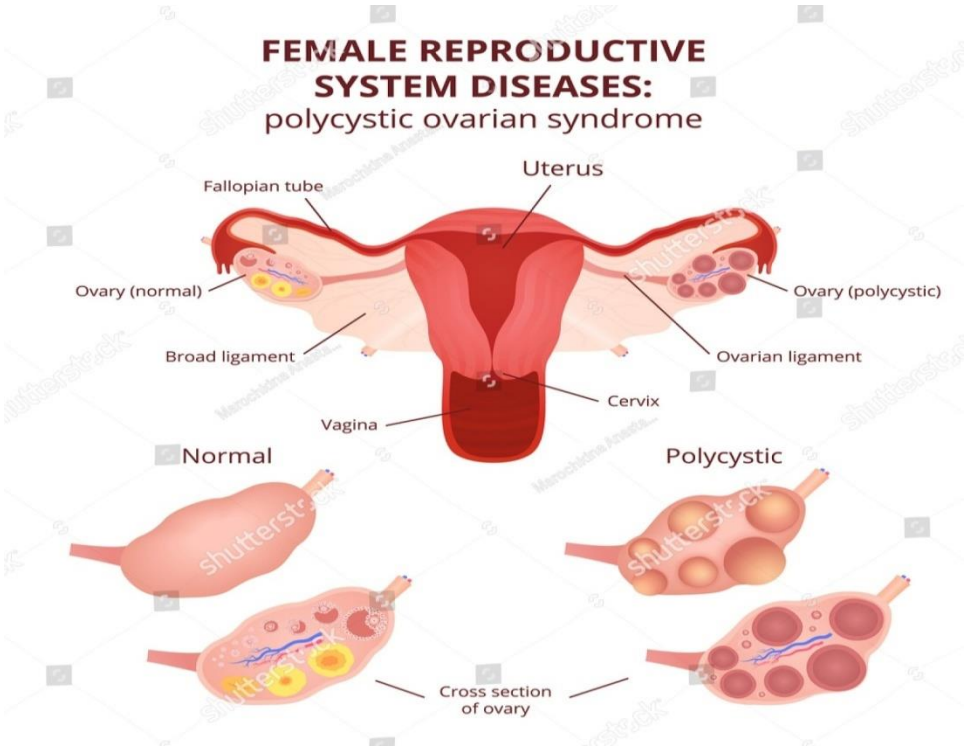


Figure 1.1 Schemethe uterus and ovaries, women reproductive system [13]

1.2.1.1 The Causesof thepolycystic ovary syndrome

According to the diagnostic criteria, PCOS affects 8% to 20% of women worldwide who are of reproductive age each year [14].The pathophysiology of this disease is affected by modifications in steroidogenesis, neuroendocrine function, ovarian folliculogenesis, metabolism, adipose cell activity, insulin production, inflammatory factors,insulin sensitivityand sympathetic nerve function [15]. High carbohydrate intake, hyperandrogenemia,hyperinsulinemia and ongoing low-grade inflammation are seen by Barre et al. as the four main causes of pathophysiological changes in PCOS [16], In Figure (1.2).

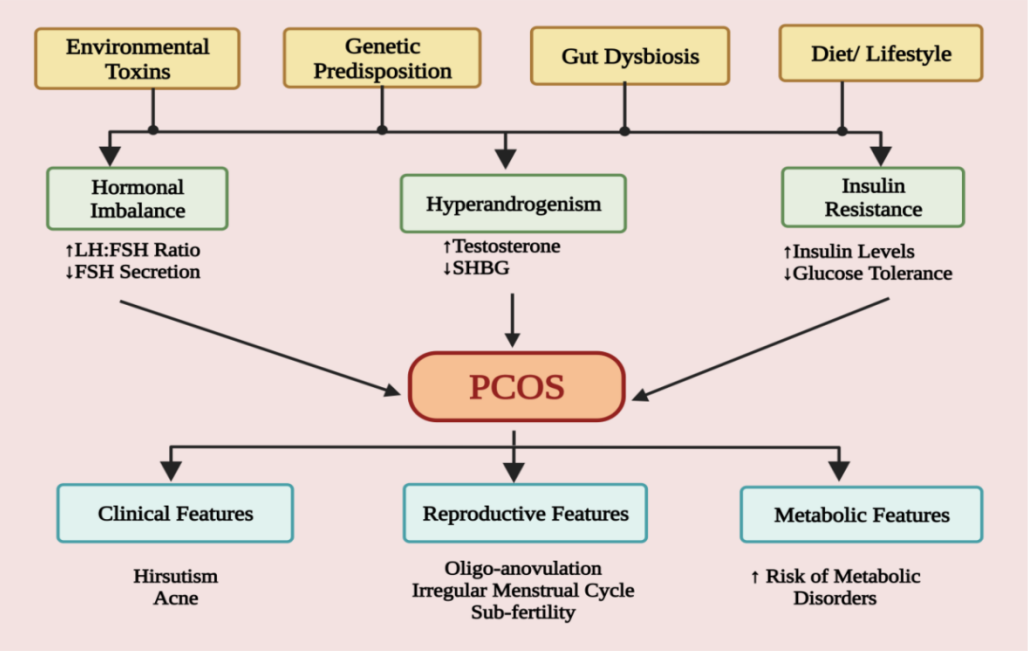


Figure 1.2 Potential pathogenic factors of PCOS[17]

Hyperandrogenemia, which shows up clinically as hirsutism, acne, and alopecia, is the biochemical hallmark of PCOS. 75–90% of PCOS individuals with oligomenorrhea have high levels of androgens, and these levels usually rise as the phenotypic becomes more severe. Hyperandrogenism is a result of excessive androgen production by the adrenals and ovaries [18]. Hyperandrogenismis indicated by elevated levels of free (unbound) testosterone, a key hormone involved in the pathophysiology of PCOS. The overproduction of androgens is caused by abnormal ovarian or adrenal function. Excess androgens' initial impact on normal androgen production in PCOS is poor folliculogenesis. At the early gonadotropin stage, excess androgens encourage the growth of primordial follicles and a rise in antral follicles [19].

The primary hormone in charge of maintaining glucose homeostasis and lipogenesis is insulin. In addition to having an effect on the metabolism of carbohydrate, lipids, and proteins,In addition, insulin has a mitogenic effect. Insulin acts through insulin receptors, which are present in many tissues in the HPO axis. Insulin promotes steroidogenesis by potentiating the appropriate trophic hormones in steroidogenic organs, such as the ovary and adrenal cortex [14].

Hyperinsulinemia is the main factor for increased androgen production since insulin directly imitates the effect of LH and indirectly increases GnRH. Insulin reduces Sex hormone binding globulin (SHBG), a vital circulatory protein that controls testosterone levels. Because free androgens are what produce the clinical signs of PCOS, such as hirsutism, alopecia, and acne, larger levels of free androgens would result from lower SHBG levels [19]. Numerous studies have demonstrated that decreasing insulin resistance will eventually lead to a decrease in androgens and an improvement in the illness state[20, 21].

PCOS is exacerbated by environmental factors [22].Numerous studies have demonstrated the negative effects of environmental pollutants on human health and reproduction, including heavy metals, pesticides, and endocrine-disrupting chemicals (EDCs). In fact, there is growing evidence that environmental toxins play a role in PCOS development. Serum BisphenolA(BPA) levels in hyperandrogenic women with PCOS were higher than those in non-hyperandrogenic women with PCOS and healthy controls, according to research by Takeuchi and Kandaraki et al.[23, 24]. BPA, also known as bisphenol A (2,2-Bis propane), is a synthetic chemical that is used in many everyday products, including food packaging,

baby bottles, medical gadgets, and personal care items. It is found in polycarbonate plastics, epoxy resins, and dental sealants[25]. Increases in blood BPA levels were found to be favorably correlated with serum Testosterone levels in PCOS women as opposed to healthy women in a different investigation [26].

It has been demonstrated that certain genes, gene-gene interactions, or interactions between genes and the environment may alter a person's predisposition to develop PCOS [27]. PCOS is a polygenic and multidimensional illness. There are multiple probable genes having single-nucleotide polymorphisms or mutations that have been linked to a variety of PCOS symptoms, according to several genetic research. All genes and mutations that affect the ovaries either directly or indirectly are associated with PCOS [28]. Genes that encode signaling components for steroidogenesis, steroid hormone action, gonadotrophin action and regulation, insulin action and secretion, energy metabolism, and chronic inflammation are frequently implicated in the pathogenesis of PCOS [28, 29].

1.2.1.2 Symptoms and Diagnosis of Polycystic ovary syndrome

Signs and symptoms of PCOS can vary somewhat and include:-

1- Hormonal issues (Menstrual irregularities,Endometrial cancer ovulation failure,Infertility, and latemenopause) .

2- Metabolic issues (High blood pressure,Type 2 diabetes,Insulin resistance, and cardiovascular disease).

3- Physical issues (Acne, Central obesity,Hair loss and baldness).

4- Psychological problems (Depression, anxiety , and stress).

The two basic criteria for identifying this disease are clinical or laboratory hyperandrogenism and hypomenorrhea. One or two clinical symptoms are what most PCOS-afflicted women suffer. Menstrual disturbances are the most prevalent clinical finding, they typically start during or immediately after menstruation and might manifest as hypomenorrhea, amenorrhea, or polymenorrhea until menstruation returns to normal [30].Adults with PCOS can be diagnosed using one of three different criteria, which are listed in Figure (1.3) :

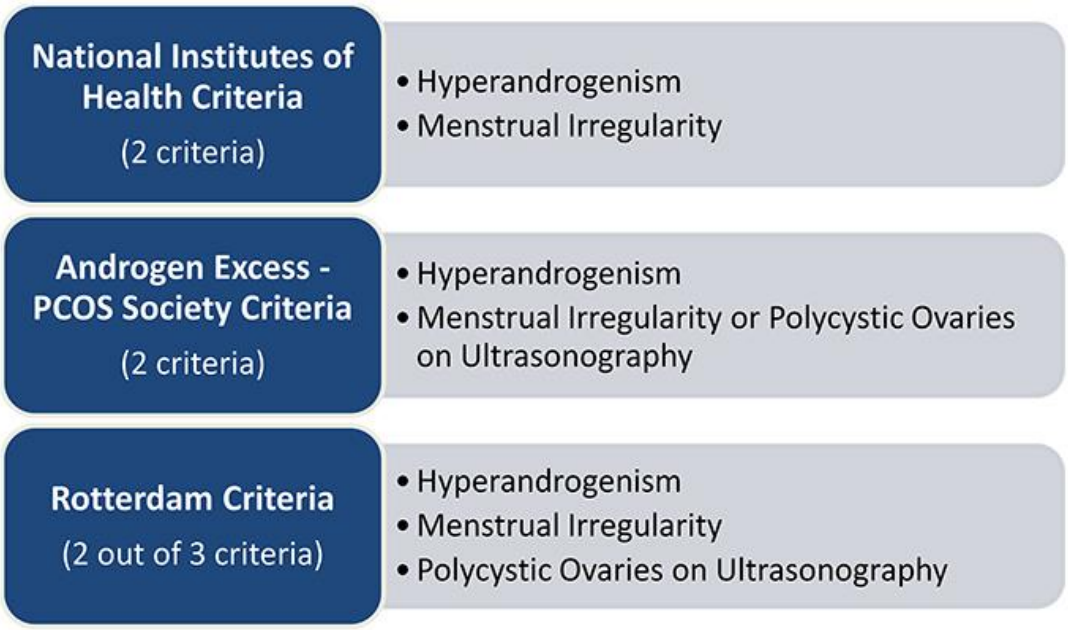


Figure 1.3 Guidelines for the diagnosis of PCOS [31]

1.2.1.3 PCOS subtypes

Based on a number of variables, there are four forms of PCOS:-

1- Metabolic syndrome or pre-diabetes are additional names for insulin resistance. An increased level of the hormone insulin is what is meant by the term. Measuring insulin is one of the preferred techniques for determining insulin resistance.Approximately 65 to 70% of women with polycystic ovarian syndrome have insulin resistance and compensatory hyperinsulinemia [21, 32].

2- Adrenal PCOS is a subtype ofpolycystic ovarian syndromethat affects the adrenal glands: adrenal hyperandrogenism in PCOS may be caused by non-classical adrenal hyperplasia, tumors that produce androgen in the adrenal glands, Cushing's syndrome, or heredity of androgen secretion in the adrenal glands [33].The adrenal cortex produces one-fourth of all circulating testosterone. Excessive levels of adrenal androgen in polycystic ovarian syndrome could be a sign of adrenocortical abnormalities, hypothalamic-pituitary dysfunction, or abnormal cortisol metabolism in the liver [34].

3- Patients with polycystic ovarian syndrome who have thyroid dysfunction: Figure (1.4) illustrates how elevated thyroid stimulating hormone (TSH) and prolactin are caused by elevated (TRH) hormone in primary hypothyroidism.Increased prolactin, increased TSH, and increased dehydroepiandrosterone from the adrenal gland all contribute to polycystic ovarian morphology by preventing ovulation as a result of the altered balance between follicle stimulating hormone (FSH) and luteinizing hormone (LH), as well as the increase in dehydroepiandrosterone. Increased

collagen deposition in the ovaries has also been connected to hypothyroidism [35].

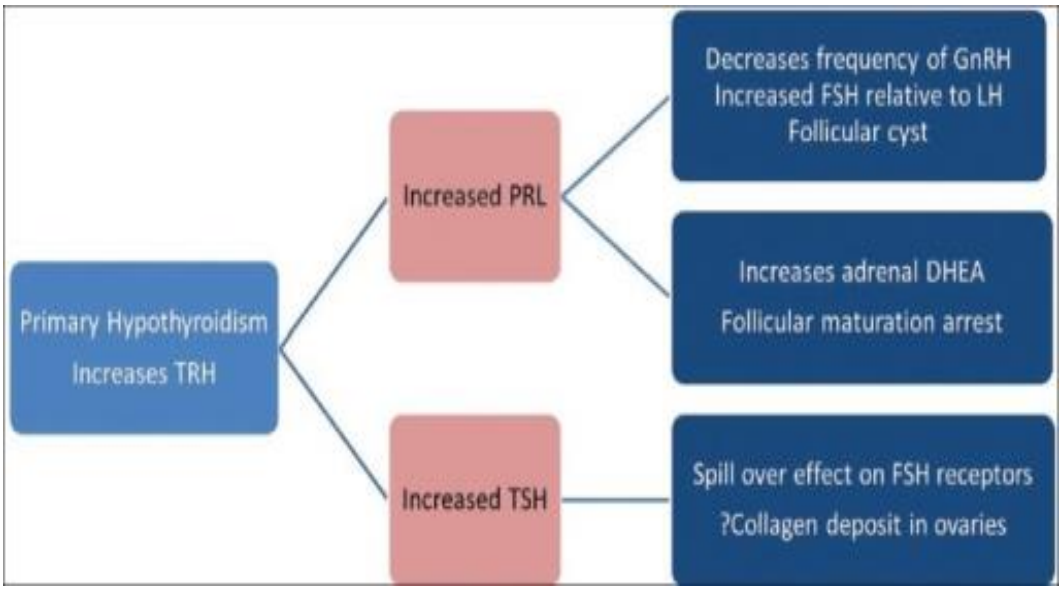


Figure 1.4 Path physiology of Primary Hypothyroidism in PCOSpatients [36]

4- Inflammatory PCOS: Chronic inflammation brought on by chlamydial infections can result in disease processes that cause metabolic and hormonal issues, leading to polycystic ovarian syndrome[37]. Many markers, such as C-reactive protein, white blood count, interleukin-18, and increased oxidative stress, are elevated in this type of PCOS [38-40].

1.2.2 Insulin resistance

Insulin resistance, which is defined as a failure in insulin-mediated control of glucose metabolism in tissues, is one of the first signs of a number of illnesses that affect people, and it is linked to a variety of metabolic abnormalities, which basically means that the body's cells do not react to the insulin hormone, reduced sensitivity of cells to insulin signaling, and failure to take up glucose. The body retains glucose because it is not utilised for energy production, which causes weight gain [41]. In this situation, type 2 diabetes may or may not occur in patients with insulin resistance. diabetes likely has enough insulin, the cells it should act on are not typically sensitive to its effects.

Complications of insulin resistance include (IR) include metabolic syndrome, diabetes, impaired glucose tolerance, hypertension,obesity, inflammation, heart disease, and dyslipidemias. Population study indicates that IR is complicated and has heritable elements, such as anomalies in the insulin-signaling pathway (such asdecreased signaling activation or serine phosphorylation on insulin substrate).IR is associated with fat storage, oxidant overproduction, and abnormalities of the mitochondria [42]. Despite the fact that the precise underlying cause of IR is still not entirely understood, a number of significant explanations have been put forth, including oxidative stress, inflammation, insulin receptor mutations, endoplasmic reticulum stress ,and mitochondrial malfunction [43]. Figure (1.5) summarises the main components of IR.

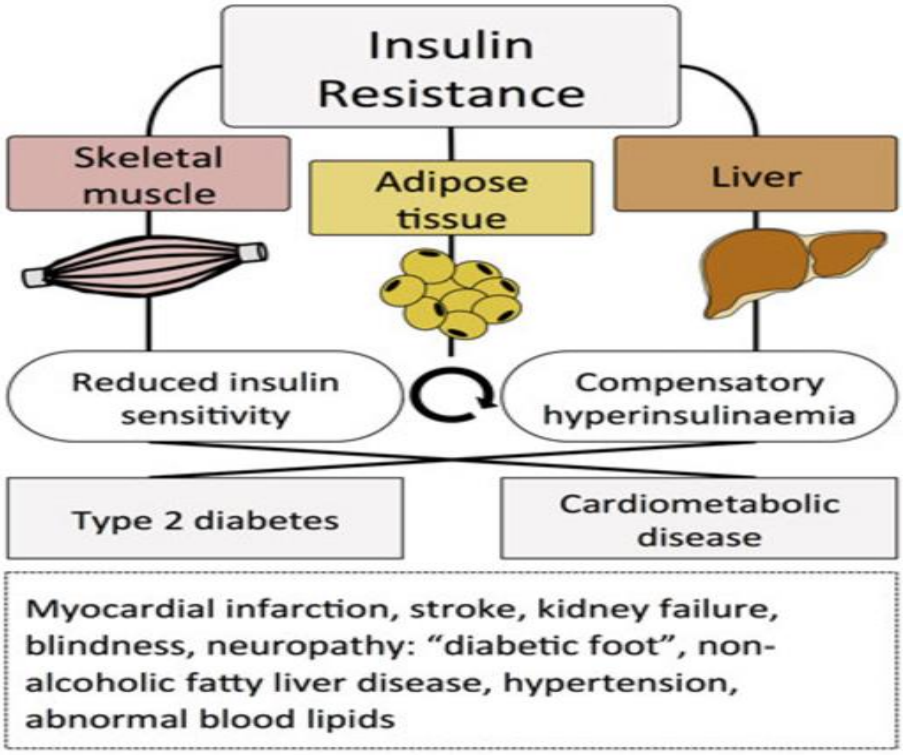


Figure 1.5 Components and consequences of IR[44]

It is difficult to evaluate insulin resistance. The physiological factors that contribute to the assessment of insulin resistance vary depending on whether a person is fasting, eating, resting, or exercising. Glucose metabolism is quick and depends on body composition and dietary patterns[45, 46].

According to the HOMA-IR hypothesis, there is a feedback loop between pancreatic insulin secretion and hepatic glucose release during a fast [47], Serum glucose and insulin levels can be used as a straightforward indicator of insulin resistance. Utilizing HOMA-IR is common in epidemiological studies. In its simplest form, it can be calculated as the result of fasting plasma glucose and insulin levels divided by a fixed amount[48].

1.2.2.2 Insulin resistance in the PCOS

Insulin resistance is the most common form of PCOS, and it causes the ovaries to generate more androgen, which causes oligo-ovulation (abnormal ovulation) or anovulatory (the absence of ovulation) [49]. Depending on the insulin sensitivity index being utilized, the detection rate of insulin resistance varies [50], Type 2 diabetes and the accumulation of abdominal fat are both influenced by high androgen levels[49, 51, 52].

Insulin resistance and the body relative to levels of LH and FSH are the main PCOS patient problems [49]. People with PCOS have more adipocytes, less lipoprotein lipase activity, and less lipolysis [53].

Additionally, the two main hormones were pituitary hormones that control fundamental changes in the body for reproduction, luteinizing hormone (LH) and follicle stimulating hormone (FSH).In most cases, FSH and LH are higher during the start of the menstrual cycle.This happens in PCOS because the hypothalamic release of gonadotropin-releasing hormone (GnRH) can occasionally be aberrant, where there is initially an increase in LH rather than FSH secretion. Increased LH causes an overflow of androgen substrates to be released, which raises testosterone levels, which are linked to thick body and facial hair [49].

Through changing one's lifestyle, the ecological factors that contribute to PCOS are impacted. Increased body weight, eating more saturated fat, having metabolic and reproductive issues, etc. Weight loss has been observed to decrease hyperandrogenism and hirsutism, restore

ovulation, and lessen metabolic and ovulatory dysfunction associated with PCOS [54].

Several genes that regulate insulin action and androgen production have been connected to PCOS.A woman's odds of developing PCOS are typically approximately 30% to 50% due to genetic and inheritance factors, which demonstrate a strong familial association [49].

Seen in Figure (1.6), excessive androgens stimulate visceral adipose tissue (VAT) to create FFAs, which heightens insulin resistance. By directly impacting ovarian cells and indirectly by preventing the hepatic generation of SHBG and insulin-like growth factor binding protein-1 (IGFBP-1), these occurrences act in tandem to keep PCOS at bay [55].

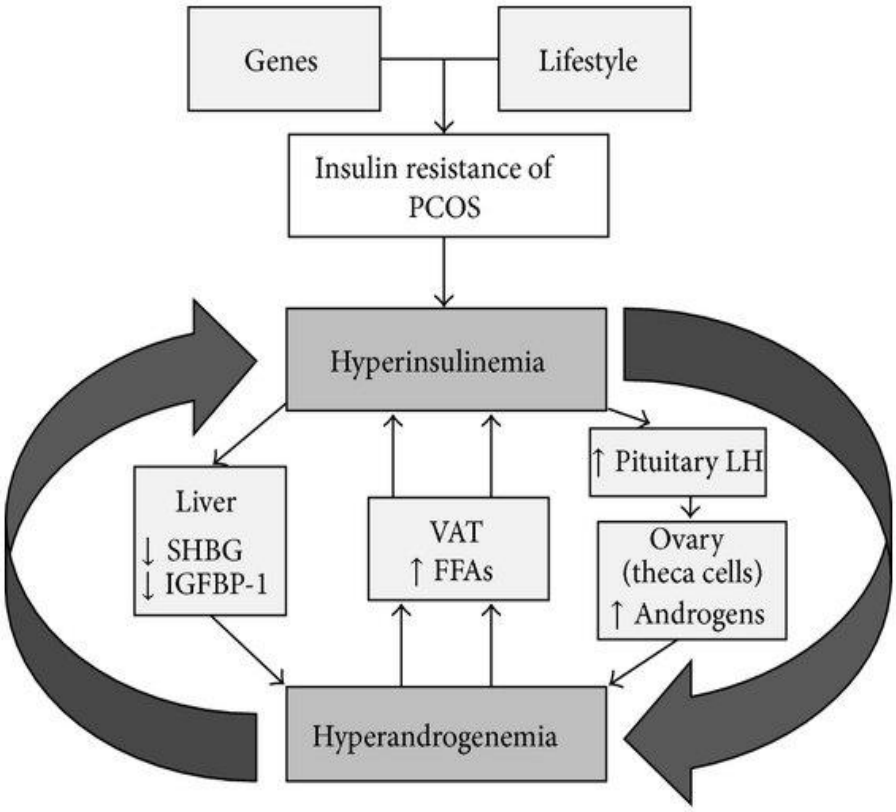


Figure 1.6 The Vicious Cycle between Hyperinsulinemia and Hyperandrogenemia in PCOS development [55]

Women with PCOS are more likely to have lipid problems. The risk of cardiovascular disease is increased by a number of lipid abnormalities that are present in PCOS, including lower levels of high density lipoprotein and higher levels of triglycerides, total cholesterol, and low-density lipoprotein cholesterol (LDL-C). A recent study indicated that consuming a high-fat diet stimulated metabolic and ovarian conversion in those with PCOS, which had an impact on the hormonal profile [56].

Infertility in PCOS is associated with dyslipidemia because of ovarian abnormalities, an increase in polycystic follicles, and an increase in follicular layer thickness. Up to 70% of people with PCOS have

dyslipidemia. There was a higher percentage of IR diagnoses among obese women than among women with a normal BMI, pointing to a link between IR and dyslipidemia [56, 57].

For overweight/obese women with PCOS, an aerobic exercise training intervention enhanced cardiorespiratory fitness and cardio metabolic health [58]. Therefore, chronic inflammation may be another component of the hyper-inflammation associated with PCOS, as has also been proposed for diabetes [59].

1.2.3 Lipids and Lipoproteins

1.2.3.1 Lipids

There is no universally accepted definition of lipids due to the wide variety of substances that fall under this category. Rather than chemical characteristics, hydrophobicity is the primary requirement for inclusion in this category, Lipids are typically referred to be organic substances with bound fatty acids and molecules with more than three carbon atoms [60]. Triacylglycerol (triglycerides) make up 16% of plasma lipids, phospholipids 30%, cholesterol 14%, cholesterol esters 36%, and a significantly lower portion of free fatty acids (4%), which are long-chain fatty acids that have not been esterified[61].

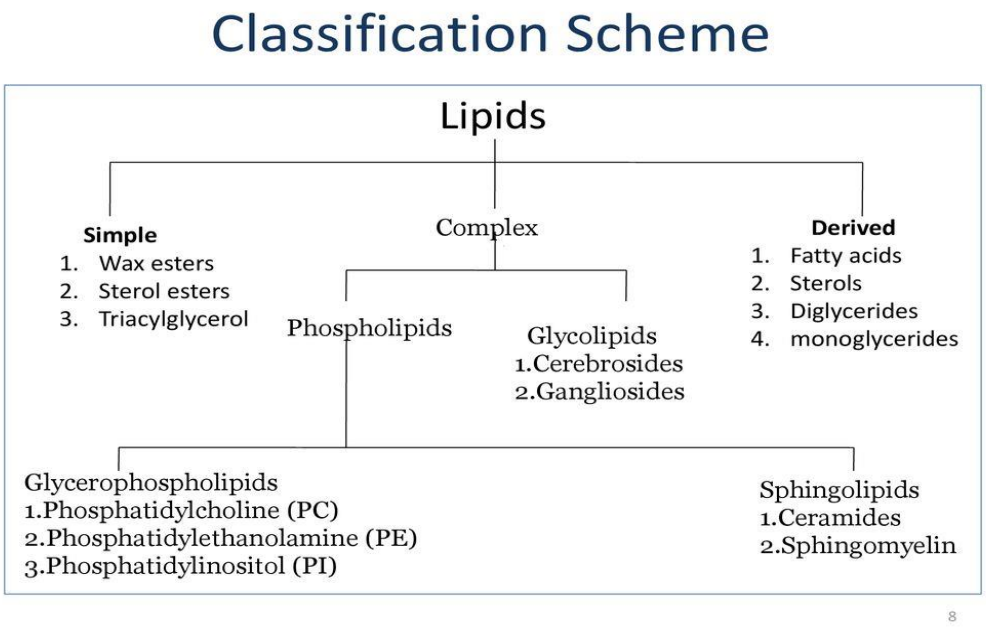
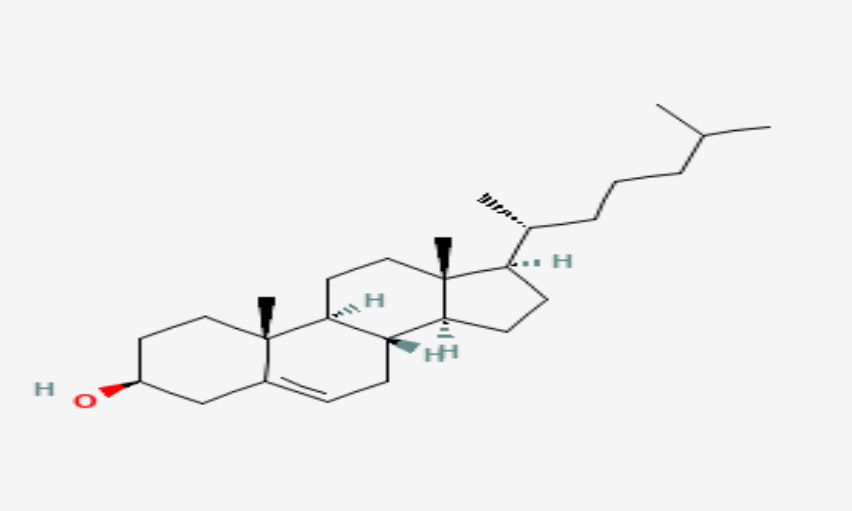


Figure 1.7 The classification of clinically important lipids [62]

1.2.3.1.1 Cholesterol

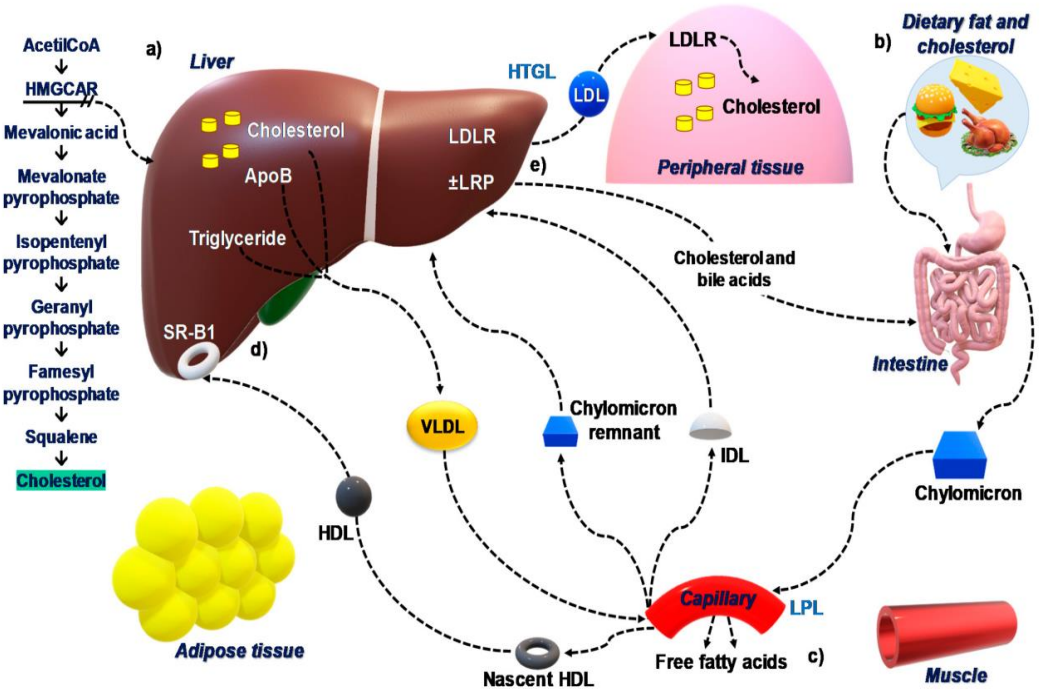
Almost every cell in the human body contains the biomolecule cholesterol. In the body, cholesterol has both positive and negative roles [63, 64], has a molecular formul ($C_{27}H_{46}O$) [65],a Four hydrocarbon rings, a hydrocarbon tail, and a hydroxyl group make up the amphipathic sterol molecule that is cholesterol [66],As shown in the Figure (1.7). Cholesterol plays a variety of crucial physiological activities, including those of being a necessary part of all cell membranes, the building block of steroid hormones, the precursor bile acids, and a signaling molecule in the central nervous system[67].

Only a minor portion of the cholesterol in our bodies is obtained from external, food sources; the majority is produced internally by the liver[68].



Figuer 1.8 Chemical Structure of Cholesterol[65]

The quantity of cholesterol in cells and the rate of small intestine absorption have a significant impact on the liver's rate of cholesterol production. Sterol regulatory element-binding protein-2 (SREBP-2) controls this The subsequent rate-limiting step in the production of cholesterol is the creation of mevalonate, which is catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR). In the small intestine, dietary cholesterol and triglycerides are absorbed and turned into chylomicrons. Chylomicrons are broken down by lipoprotein lipase (LPL) in the plasma to produce fatty acids and chylomicron residues that are absorbed by the liver. such as VLDL, produces the nascent HDL.When the amount of free cholesterol in the hepatocytes decreases due to the import of lipoproteins or increases due to the conversion to bile acids, the cholesterol synthesis enzyme, HMGCR, is "sensed" by SREBP-2, which then activates it. The production of LDL receptors (LDLR) is also activated by SREBP-2, which speeds up the absorption of cholesterol from LDL and then encourages the storage of cholesterol in the liver [69], As shown in Figuer (1.8).

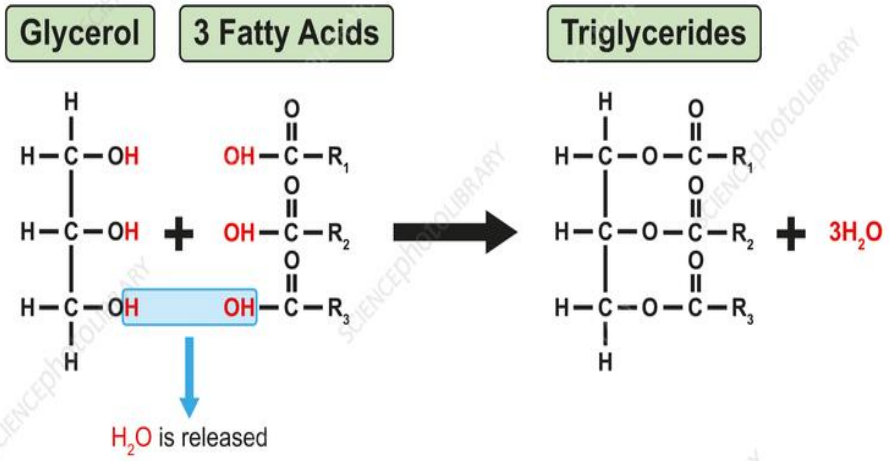


Figuer 1.9 Cholesterol homeostasis in the body [69]

1.2.3.1.2 Triglycerides

A glycerol esterified to three fatty acid chains forms the basis of the non-polar lipid molecule known as a triglyceride,As shown in the Figuer(1.9). The characteristics of these lipid molecules fatty acids can be used to further describe them. Triglycerides can be classed as saturated (no C=C) or unsaturated (one or more C=C) depending on how many double-bonded carbon (C=C) molecules they contain. Triglycerides can also be further divided based on where the C=C molecules are located along the fatty acid chain. In adipose tissue, triglycerides are a very significant source of energy; when they are metabolized, their fatty acid chains are released through hydrolysis and go through fatty acid oxidation where they are transformed into acetyl coenzyme A (acetyl- CoA) for use in the Krebs cycle

and mevalonate pathway. Triglycerides are mostly obtained from exogenous, dietary sources [68].



Figuer 1.10 Formation of triglyceride [70]

Triacylglycerides (TG), a kind of fat that is primarily stored in adipose tissue, are also found in the liver and skeletal muscle. The metabolic syndrome is linked to excessive hepatic TG storage, also known as nonalcoholic fatty liver disease (NAFLD), which is characterized as a TG concentration >5% of liver weight. Similar to this, increased skeletal muscle TG, also known as intramyocellular lipids (IMCL), is associated with insulin resistance in individuals with type 2-diabetes and obesity [71].

1.2.3.2 Lipoproteins

The most significant complex lipids are lipoproteins, which are made up of both proteins and lipids. The non-polar lipids (cholesterol esters and triacylglycerols) in the center of lipoprotein particles are surrounded by polar lipids (free cholesterol, phospholipids), which help bind lipids to proteins. Serum lipoproteins are the lipoproteins that are tested the most [60].

Generalized structure of a plasma lipoprotein.

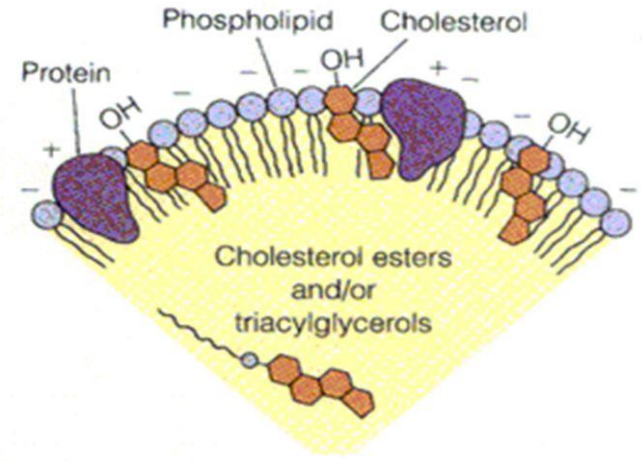


Figure 1.11 The generalized structure of plasma lipoprotein [61]

1.2.3.2.1High Density Lipoprotein (HDL)

High-density lipoproteins (HDL) help move too much cholesterol from the body's outer tissues to the liver [72], due to its apparent inverse correlation with future CVD risk, high-density lipoprotein cholesterol (HDL-C) has been known for a long time as "good cholesterol, Greater than 55 mg/dL is the recommended number for women, and greater than 45 mg/dL for men. Extra cholesterol that has built up in blood vessel walls can be

removed by the liver through the digestive system with the help of HDL cholesterol. Better blood flow is encouraged by HDL cholesterol's role in maintaining dilated blood vessels. Along with other properties, it also has an anti-inflammatory and antioxidant impact that lessens blood vessel damage. As "old" cholesterol is eliminated by cells, HDL cholesterol transports it back to the liver for excretion or recycling [73]. However, more recent studies have questioned whether HDL-C plays a causative role in this association because neither genetic studies nor several large-scale randomised controlled trials have discovered any proof of a cardiovascular protective effect when HDL-C levels are increased. Instead, attention is now being paid to the HDL particle's functional characteristics. Evidence suggests that an inflammatory environment may cause considerable changes in HDL's composition and function, changing it from a vasoprotective anti-atherogenic particle to a toxic pro-atherogenic counterpart [74].

1.2.3.2.2Low density Lipoprotein (LDL)

The term "bad cholesterol" is frequently used to describe low-density lipoprotein. related with CVD and plays a significant role than 130 mg/dL is the recommended range for LDL, most recent recommendations emphasize lower numbers (less than 100 mg/dL). levels differ amongst agencies, and more recent guidelines [75, 76].One of the primary laboratory measurements, LDL-C confers a high risk of cardiovascular disease when inappropriately raised; these molecules have also been the main focus for prescription medication [77].

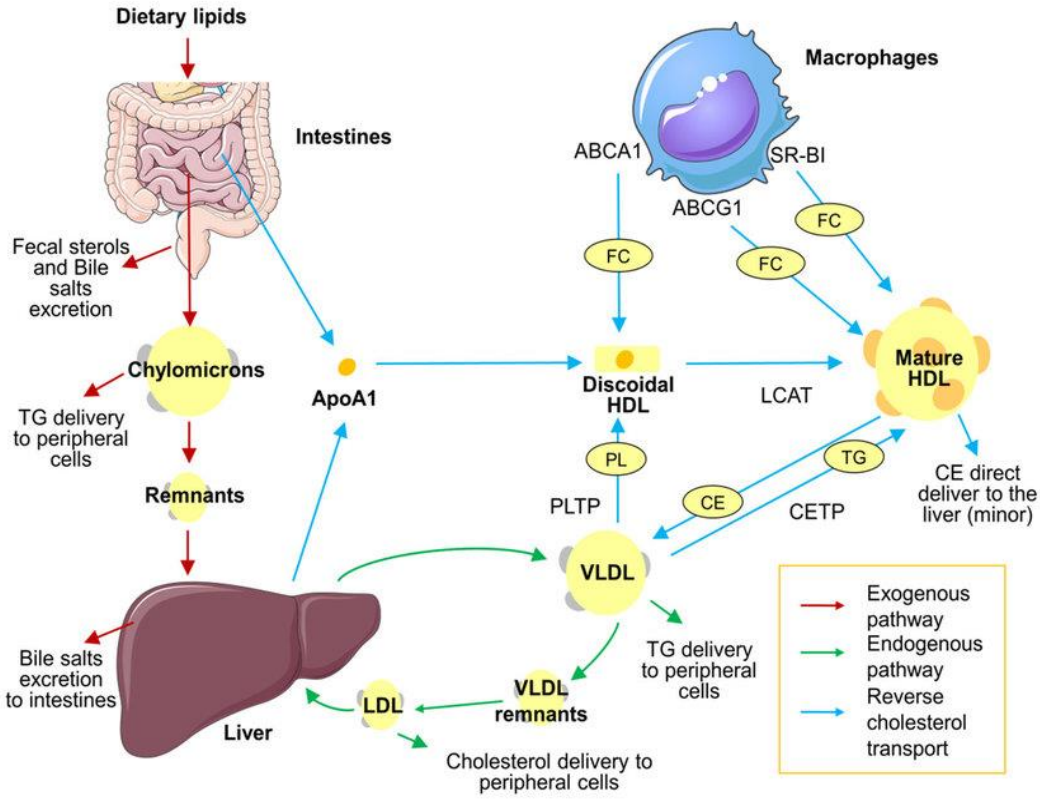


Figure 1.12 Lipoprotein metabolism overview [78]

The most common metabolic abnormality associated with PCOS is dyslipidemia, which affects 70% of patients and is most frequently characterized by hypertriglyceridemia, low HDL-C levels, and small dense LDL-C particles atherogenic dyslipidemia, typical of states of insulin resistance [79],as show in Figuer (1.12).

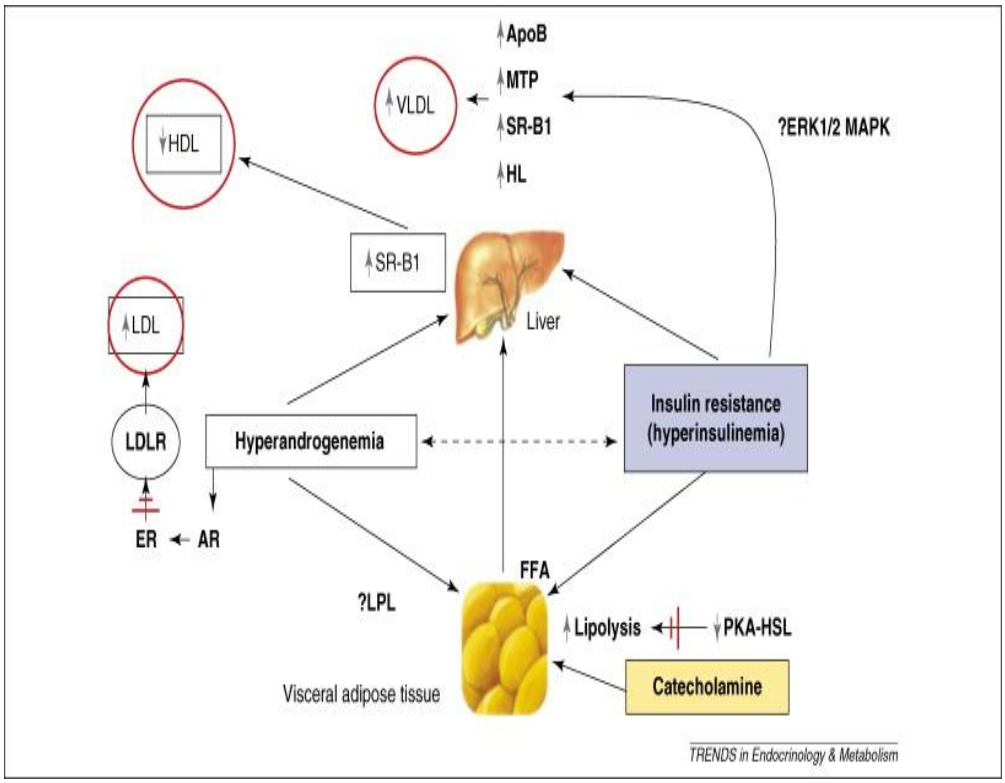


Figure 1.13 Dyslipidemia in PCOS[80]

1.2.4 Inflammation Factors

Factors associated with inflammation include C-reactive protein (CRP), interleukin-6, and other inflammatory markers. It has been observed that these factors enhance the risk of CVD [81]. The blood interleukin-6 (IL-6) level is associated with an increased incidence of CVD [82], and high sensitivity C-reactive protein (HS-CRP) is a well-established independent predictor of CVD [83].

1.2.4.1 Interleukin-6 (IL-6)

A key component of acute inflammation is the cytokine lattice type IL-6. Weissenbach and colleagues first identified IL-6 in 1980 [84]. Because interleukin-6 was the sixth interleukin to be identified, it was given that name[85], IL-6 is a pro-inflammatory cytokine that plays a significant role in cell proliferation and differentiation in humans. It stimulates the production of several proteins involved in acute inflammation [86]. A brief introduction to IL-6 is shown in Figuer (1-13).

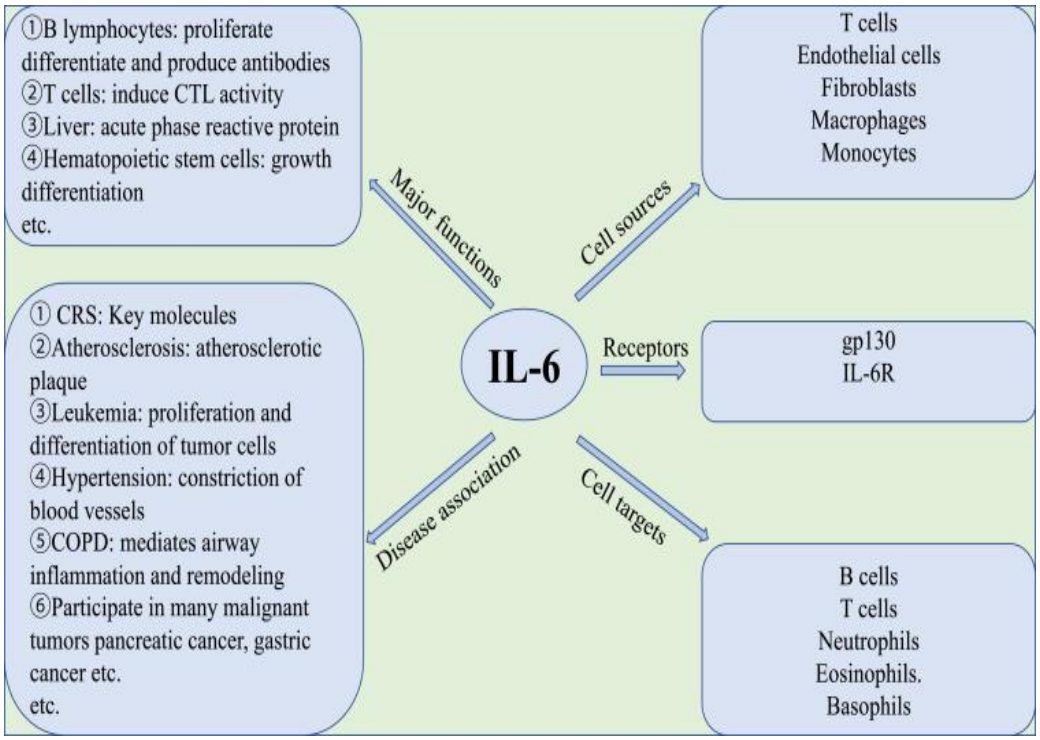


Figure 1.14 Functions of interleukin-6 (IL-6) [84]

Interleukin-6, often known as IL-6, is a short polypeptide with four helices, as shown in Figuer (1.14), which describes its structure and properties. Its isoelectric point is 5.0, it has 184 amino acid residues, a molecular weight of 19–28 kDa, glycosylation sites, and two disulfide

linkages [87]. It is typically found in monomer form. Nearly every stromal cell, immune system cell, and non-lymphocytic cell can produce IL-6, including fibroblasts, endothelial cells, keratinocytes, glomerular mesangial cells, tumor cells, and B-, T-, macrophages, monocytes, dendritic cells, and mast cells [88]. Additionally, IL-6 affects a variety of cell types and controls a broad range of biological processes, including hematopoiesis, the immune-inflammatory response, and reactions in the nervous system[85].

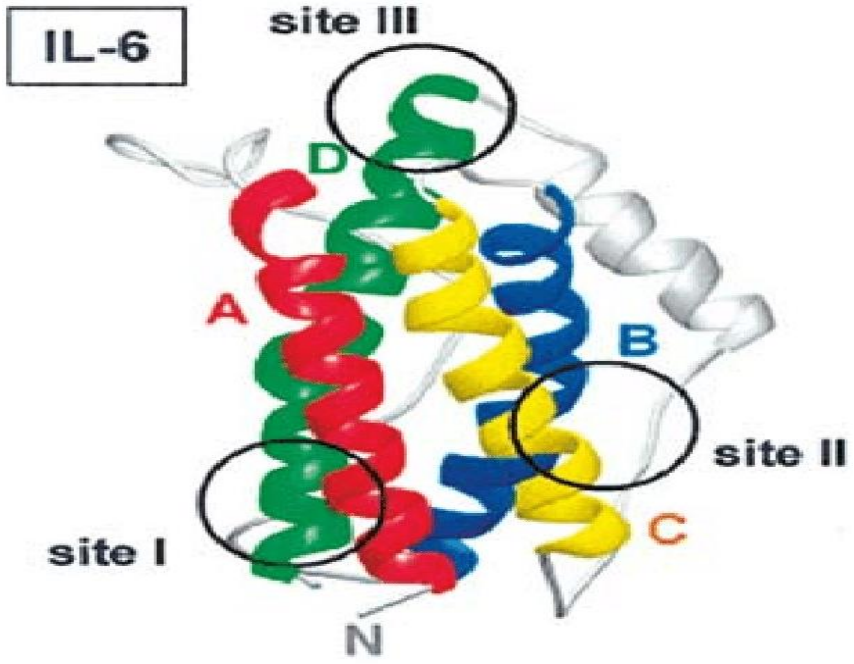


Figure 1.15 IL-6 Structure [89]

The strongest predictor of cardiovascular diseases, including heart failure, myocardial infarction, and mortality from cancer patients with stable coronary artery disease, IL-6 also includes all other causes of death [82]. In terms of reproductive physiology, interleukin 6 (IL-6) is essential. Numerous human diseases and pathophysiological processes, including as atherosclerosis development, ovarian steroid production, fertilization and

implantation, coronary heart disease, osteoporosis, and allergic reactions are all thought to be impacted by IL-6[90].

IL-6 is may play an important role in the etiology of PCOS[91].It has been shown to have an impact on the processes of fertilization, implantation, and ovulation, all of which are impacted in PCOS-affected women [92]. According to one study, IL-6 is a critical mediator related with cardiovascular risk and T2DM in PCOS-affected. As a result, IL-6 could be a valuable biomarker for PCOS diagnosis and therapy of T2DM and cardiovascular illness in PCOS patients [93]. However, the findings of recent investigations on changes in IL-6 levels in PCOS patients are contradictory. Although some research have found significant increases in IL-6 levels in women with PCOS when compared to controls[94-96].

1.2.4.2 High Sentivitiy C-Reactive Protein (HS-CRP)

C-reactive protein (CRP) is a polypeptide molecule that is a member of the pentraxin family. It is made up of five identical subunits, each containing 206 amino acids, and has a molecular mass of 120,000 Daltons. The liver is the primary site of CRP synthesis in response to specific pro-inflammatory cytokines. Innate immunity, opsonization due to its characteristics, complement activation, and immunoglobulin receptor binding are all significant functions it plays [97]. CRP is routinely assessed in rheumatoid arthritis (RA) as a sign of systemic inflammation. Additionally, it serves as an immunological regulator, an important role in the inflammatory pathways associated with RA that promote atherogenic effects. In RA, systemic inflammatory comorbidities are common, and CRP

has been linked to a higher risk of depression, diabetes, metabolic syndrome, cardiovascular disease, and pulmonary conditions [98].

Since CRP is a protein of acute systemic inflammation, it is a key indicator of inflammation [99]. The high sensitivity CRP (HS-CRP) assays are more sensitive and can detect extremely low CRP values [97], It is an inflammatory biomarker that provides predictive information on cardiovascular risk akin to blood pressure or cholesterol [100],predicts recurrent events and mortality in patients with acute or stable coronary syndromes as well as incident myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death in healthy individuals without a history of cardiovascular illness [101]. Lower, average, or higher relative cardiovascular risk are indicated, respectively, by values 1, 1 to 3, and >3 mg/L [100]. Immunonephelometry or immunoturbidimetry are used to measure the CRP. There is no established method. The quantification of HS-CRP is based on "immunolatex" immunonephelemetry sensitized methods [97].

1.2.5 Aims of the Study

This study addresses the following aims:

1- Evaluate of the level of inflammatory factors (HS-CRP, IL-6) in patients of polycystic ovarian and insulin resistance, and compared with a healthy group.

2- Demonstate of the level of lipid profile in patients of PCOS and insulin resistance, and compared with a healthy group.

3- Examine the correlation between biomarkers (Lipid profile and HS-CRP,IL-6)(HS-CRP and IL-6) (HS-CRP, IL-6 and insulin resistance)

4- Identify the risk of this syndrome by evaluating some biomarkers(LDL,HDL,Cholesterol, Tirglceraide,HS-CRP,IL-6).

CHAPTER TWO

MATERIALS AND METHODS

2. Materials and Methods2.1 Subjects and Study Design

A case-control study has been conducted at Gynecological and Obstetric Teaching Hospital in Karbala city and the maternity outpatient clinic, the diagnosis was made by Dr. HamidaHadiAbdWahed . All samples were collected from October 2022 until February 2023. The study was conducted on 80 Iraqi women, women's their age ranged (16-40) years within reproductive age.40 samples were collected from PCOS patients with insulin resistance, the second group included 40 healthy women without PCOS and insulin resistance. Women received a medical examination and a short questionnaire that asked about the woman's age, weight, height, duration of illness, regular or irregular menstrual cycle, sugar test (insulin resistance) and blood pressure. Table (2.1) contains a list of all the data collected from women.

Table 2.1 Questionnaire of this Study

Patient and Control profile
Name
Sample number
Date
Age
Weight
Height
Disease durations (years) :- 0-1 1-2 2-3 3-5
Menstrual cycle: regular irregular
Diabetes screening(insulin resistance): Normal Abnormal
Blood pressure(amount of pressure)

2.2 Groups of this study

In thetwo study groups، the following was confirmed:

1. Controls group (healthy): The healthy group include 40 women, their ages range between (16-40) years.

2. Patients group: The group consists of 40 women with PCOS and insulin resistance, their ages range between (16-40) years.

All of the female population gave verbal informed consent , and Kerbala University approved the research procedure.

2.3 Exclusion criteria

Women suffering from PCOS without insulin resistance, heart disease, kidney disease, thyroid gland diseases were excluded from the study.

2.4Collection Blood Samples

Fasting venous blood samples were collected from women in Gynecological and Obstetric Teaching Hospital and outpatient clinics during 2^{nd} – 3^{rd} day of the menstrual cycle (early follicular phase) for those of normal cycle in order to do the tests:(LDL, HDL, Triglyceride, Cholesterol, high sensitivity C-reactive protein, Human IL-6). 5 ml of blood was drawn using a medical syringe with a capacity of 5 ml, and the blood was then placed in gelatine tubes (often referred to as gel tubes) free of anti-clotting agents since these tubes include a gelatinous component that aids in the serum separation process after centrifugation. After 15 minutes of standing time at room temperature, the samples were centrifuged for 10 minutes at a speed of 3000 rounds per minute to separate the serum, which was stored at -20° C until it was required.

2.5 The Chemicals

The chemicals used in this study are described in Table (2.2).

Table 2.2 The chemicals Kits

Chemicals	Company and Origin
HOMA-IR Kits	Abbott,USA
LDL-Cholesterol Kit	Mindray, China
HDL-Cholesterol Kit	Mindray, China
Total Cholesterol Kit	Mindray, China
Triglycerides Kit	Mindray, China
High sensitivity C-reactive protein Kit	Mindray, China
Human Interleukin 6 CLIA Kit	Snibe,China

2.6 Instrument Analysis and Equipments

Equipments and instruments that are used in the this study are shown in table (2.3).

Table 2.3 Instrument analysis and equipments

Instruments	Supplied Company
Disposable syringe 5 ml	DMK KOLOING- P.R.C
Gelatin tubes (Jell tube) 5 ml	China
Disposable Eppendrof tube 1ml	Afco/Jorden
Centrifuge	Heraeus-Germany
Refrigerator	Samsung (Korea)
MAGLUMI 800	Snibe-China
BS-430	Mindray-China
BS-200	Mindray-China

Figure 2.1 SinbeMaglumi 800

Figure 2.2 Mindray BS-430

Figure 2.3 Mindray BS-200

2.7 Methods 2.7.1 Measurement of body mass index (BMI)

Body mass index (BMI) was calculated in accordance with World Health Organization (WHO) guidelines. It is calculated by dividing a person's weight in kilograms by their height in meters squared (kg/m2) [102].The body mass index is expressed in the following equation:-

BMI = Weight (Kg) / Height (m^2)

BMI were classified into three groups:

1. The BMI of Normal weight range is (18.5-24.9) Kg/m^2
2. The BMI of Overweight range is (25-29.9) Kg/m^2
3. The BMI of Obese is ≥ 30 Kg/m^2

2.7.2 Determinations Total Serum Cholesterol

vitro test for the quantitative determination of TC concentration in serum and plasma on photometric systems is used.

Method

Cholesterol oxidase- peroxidase (CHOD-POD) method

Reaction Principle

Cholesterol esters $\xrightarrow{\text{cholesterol esterase}}$ Cholesterol + Fatty acids

Cholesterol + $O_2 \xrightarrow{\text{Cholesterol oxidase}}$ 4-Cholestenone + H_2O_2

$2H_2O_2$ + Phenol + 4-aminoantipyrine $\xrightarrow{\text{Peroxidase}}$ Quinoneimine (Pink) + $4H_2O$

Cholesterol ester is catalyzed by CHE and CHO to produce H2O2, which oxidizes 4-aminoantipyrine with phenol to make a colored quinoneimine dye. The content of cholesterol is directly proportional to the increase in absorbency.

Reagents

Components and concentrations

R: Phosphate buffer (100mmol/L)

Phenol (5mmol/L)

4-Aminoantipyrine (0.3mmol/L)

Cholesterol esterase (>150KU/L)

Cholesterol oxidase (>100 KU/L) Peroxidase(5KU/L)

Reagent Preparation

Single reagent is ready to use.

Table 2.4 Assay Procedure Cholesterol

Materials	Blank	Sample
R:	1000 µL	1000 µL
Distilled water	10 µL	--------
Sample		
	----------	10 µL

Mix thoroughly at 37°C، and read the absorbance 10 min later.

ΔA= [ΔA sample] – [ΔA blank]

Calculation

The analyzer calculated the TC concentration of each sample automatically after calibration.

Conversion factor: mg/dL x 0.026=mmol/L or: C sample = (ΔA sample/ΔA calibration) x C calibration

The standard curve of Cholesterol determination was plotted in Figure (2.4) and the Cholesterol level in each sample was determined.

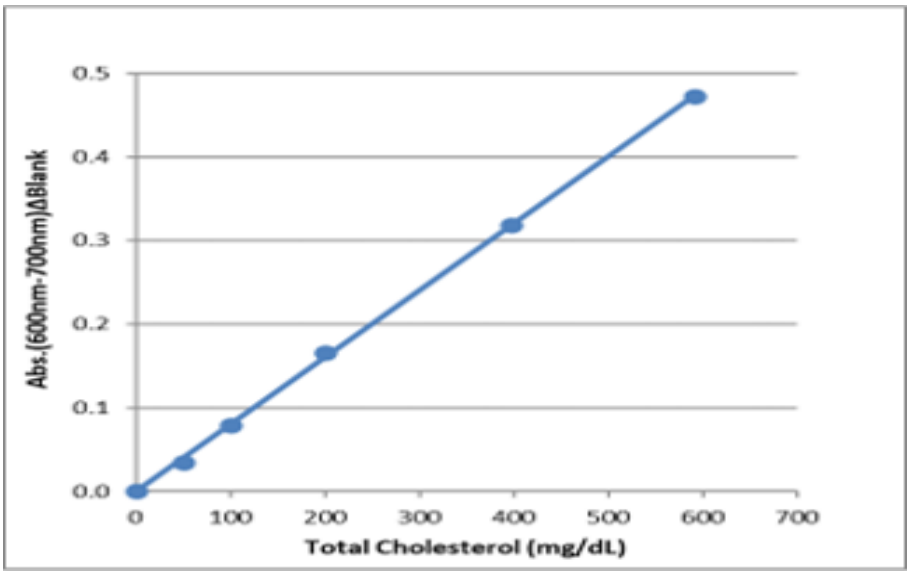


Figure 2.4 Standard Curve of Determination of Cholesterol Concentration.

2.7.3 Determinations Total Serum Triglyceride

Vitro test for the quantitative determination of TG concentration in serum and plasma on photometric systems is used.

Method

Glycerokinase Peroxidase- Peroxidase Method.

Reaction Principle

Triglycerides + $3H_2O$ $\xleftrightarrow{\text{Lipase}}$ Glycerol + fatty acid

Glycerol + ATP $\xleftrightarrow{\text{Glycerol kinase}}$ Glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 $\xleftrightarrow{\text{Glycerol-3-phosphate oxidase}}$ Dihydroxyacetone Phosphate + H_2O_2

$\longleftrightarrow$

H_2O_2+4-Aminoantipyrine + 4-Chlorophenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + HCl + $2H_2O$

Lipase, GK, and GPD catalyze the oxidation of triglycerides to produce H2O2, which then oxidizes 4-Aminoantipyrinel to produce a colored quinoneimine dye. The increase in absorbency is inversely related to the triglyceride levels.

Reagents

Components and concentrations are given below:

Phosphate buffer 50 mmol/L

4-Chlorophenol 5 mmol/L

ATP 2 mmol/L

Mg^{2+} 4.5 mmol/L

Glycerokinase ≥0.4 U/mL

Peroxidase ≥0.5 U/mL

Lipoprotein lipase ≥1.3 U/mL

4-Aminoantipyrine 0.25 mmol/L

Glycerol-3-phosphate-oxidase≥1.5 U/Ml

Table 2.5 Assay Procedure Triglyceride

Materials	Blank	Sample
R:	1000 µL	1000 µL
Distilled water	10 µL	--------
Sample	----------	10 µL

Mix thoroughly at 37°C, and read the absorbance 10 min later.

∆A= [∆A sample] – [∆A blank]

Calculation

The analyzer calculated the TG concentration of each sample automatically after calibration.

Conversion factor: mg/dL x 0.0113=mmol/L or: C sample = (ΔA sample/ΔA calibration) x C calibration

In Figure (2.5), the glyceride level in each sample was calculated using the standard curve for glyceride determination.

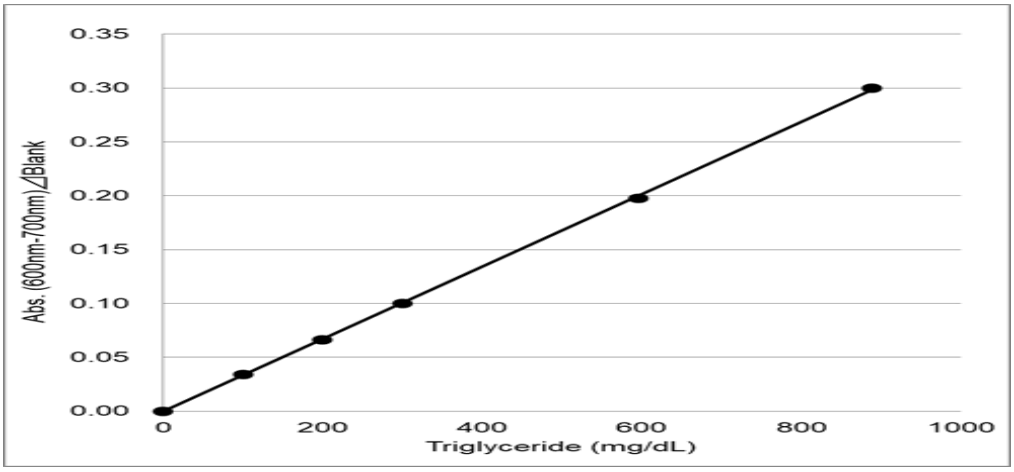


Figure 2.5 Standard Curve of Determination of Triglyceride Concentration.

2.7.4 Determinations Serum HDL-Cholesterol

Vitro test for the quantitative determination of HDL-Cholesterol (HDL-C) concentration in serum on photometric systems is used.

Method

Direct method was used.

Reaction Principle

(1) LDL, VLDL, Chylomicron $\xleftrightarrow{\text{cholesterol esterase + cholesterol oxidase}}$ Cholestenone + H_2O_2

$$2H_2O \xleftrightarrow{\text{Catalase}} 2H_2O+O_2$$

(2) HDL $\xleftrightarrow{\text{cholesterol esterase + cholesterol oxidase}}$ Cholestenone + H_2O_2

$$H_2O_2 + \text{HDAOS} + \text{4-aminoantipyrin} \xleftrightarrow{\text{peroxidase}} \text{Quinonimine}$$

The System monitors the change in absorbance at 600 nm. This change in absorbance is directly proportional to the concentration of cholesterol in the sample and is used by the System to calculate and express the HDL-cholesterol concentration.

Reagents

Components and Concentrationsare given below:

R1: Good's buffer (100 mmol/L) Cholesterolesteras(600U/L)
Cholesteroloxidase(380U/L)
Catalase(600 KU/L)

HDAOS (0.42 mmol/L)

R2:Good'sbuffer (100mmol/L)
4-aminoantipyrine(1.0mmol/L)
Peroxidase(>2.8U/mL)
Surfactant(<2%)

Table 2.6 Assay procedure of HDL-Cholesterol

Materials	Blank	Sample
Reagent 1	900 µL	900 µL
Distilled water	12 µL	--------
Sample	----------	12 µL

Mix, Incubate for 5 min. at 37°C, then add

Reagent 2	300 µL	300 µL

Mix thoroughly, incubate at 37°C for 5 min., and then read theabsorbance change value.

∆A= [∆A sample] – [∆A blank]

Calculation

The standard curve of HDL-Cholesterol determination was plotted in Figure (2.6) and the HDL-Cholesterol level in each sample was determined.

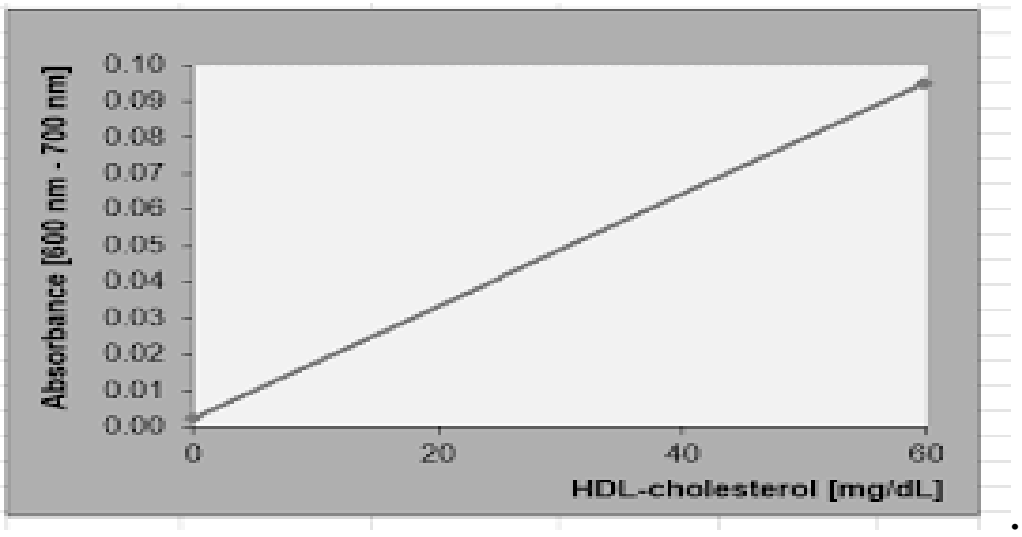


Figure 2.6 Standard Curve of Determination of HDL-Cholesterol Concentration.

2.7.5 Determinations Serum LDL-Cholesterol

Vitro test for the quantitative determination of LDL-Cholesterol (LDL-C) concentration in serum on photometric systems is used.

Method

Direct method was used.

Reaction Principle

(1) HDL, VLDL, Chylomicrons $\xrightarrow{\text{cholesterol esterase + cholesterol oxidase}}$ Cholestenone + H_2O_2

$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O+O_2$

(2) LDL $\xrightarrow{\text{cholesterol esterase + cholesterol oxidase}}$ Cholestenone + H_2O_2

H_2O_2 + TOOS + 4-aminoantipyrin $\xrightarrow{\text{peroxidase}}$ Quinonimine

The System keeps track of any variations in absorbance at 600 nm. The system uses this change in absorbance, which is directly inversely proportional to the sample's cholesterol content, to determine and express the LDL-cholesterol concentration.

Reagents

R1: Good’s buffer (50 mmol/L)

Cholesterol esterase (600 U/L)

Cholesterol oxidase (500 U/L)

Catalase (600 KU/L

TOOS (2 mmol/L)

R 2 : Good's buffer (50 mmol/L)

4-aminoantipyrine (4 mmol/L)

Peroxidase (4 U/mL)

Table 2.7 Assay procedure of LDL-Cholesterol

Materials	Blank	Sample
Reagent 1	900 µL	900 µL
Distilled water	12 µL	--------
Sample	----------	12 µL

Mix, Incubate for 5 min. at 37°C, then add;

Reagent 2	300 µL	300 µL

Mix thoroughly, incubate at 37°C for 5 min, and then read theabsorbance change value.

$\Delta A = [\Delta A \text{ sample}] - [\Delta A \text{ blank}]$

Calculation

The LDL-Cholesterol level in each sample was calculated using the standard curve for determining LDL-Cholesterol, which was depicted in Figure (2.7).

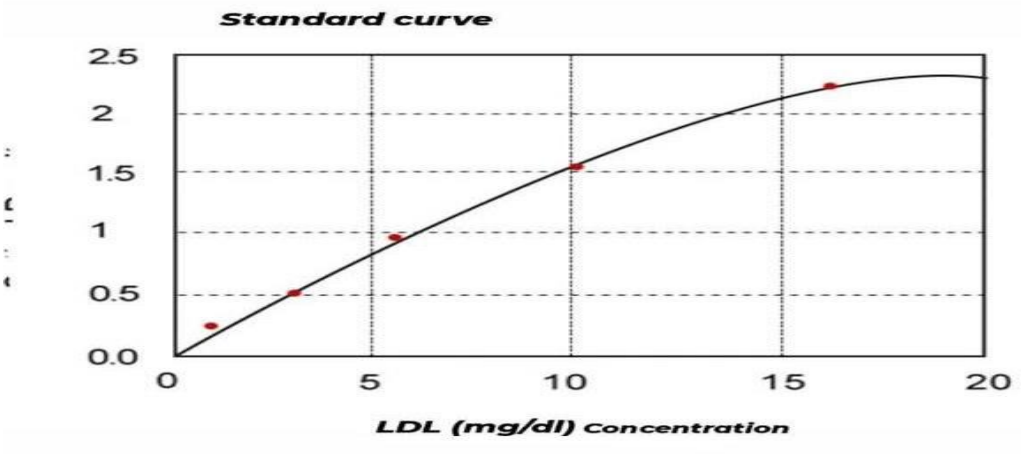


Figure 2.7 Standard curve of determination LDL-Cholesterol of Concentration.

2.7.6 Inflammation Parameters

2.7.6.1 Measurement of Serum IL-6 Concentration

The kit is an in vitro chemiluminescence immunoassay for the quantitative determination of IL-6 in human serum and plasma using the MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.

Principle of the Test

The IL-6 assay is a sandwich chemiluminescence immunoassay. The sample (or calibrator/control, if applicable), buffer, ABEI labeled with anti-IL-6 monoclonal antibody and magnetic microbeads coated with another

anti-IL-6 monoclonal antibody were mixed thoroughly and incubated, forming sandwich complexes. After precipitation in a magnetic field, the supernatant is decanted and then a wash cycle is performed. Subsequently, the Starter 1+2 were added to initiate a chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light unit (RLUS), which is proportional to the concentration of IL-6 present in the sample (or calibrator/control, if applicable).

Table 2.8 Components of IL-6 Kits

Components	Contents	100 test
Magnetic microbeads	Magnetic microbeads coated with anti-IL-6 monoclonal antibody (mouse), containing BSA, NaN_3, (<0.1%)	2.5 mL
Calibrator Low	Containing IL-6 antigen (recombinant) and BSA, NaN_3,(<0.1%)	2.0 mL

Calibrator High	Containing IL-6 antigen (recombinant) and BSA, NaN_3,(<0.1%)	2.0 mL
Buffer	Containing BSA, NaN_3, (<0.1%).	8.5 mL
Diluent	0.9% NaCl	15.0 mL
ABEI Label	Anti-IL-6 monoclonal antibody (mouse) labeled with ABEI, containing BSA, NaN_3, (<0.1%)	8.5 mL
Control 1	Containing IL-6 antigen (recombinant) and BSA, NaN_3,(<0.1%)	2.0 mL
Control 2	Containing IL-6 antigen (recombinant) and BSA, NaN_3,(<0.1%)	2.0 mL

All reagents are provided ready-to-use.

Preparation of Reagent

Resuspension of the magnetic microbeads takes place automatically when the kit was loaded successfully, ensuring the magnetic microbeads were totally resuspended homogenous prior to use.

To ensure proper test performance, strictly adhere to the operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer. Each test parameter was identified via a RFID CHIP on the Reagent. For further information please refer to the operating instructions of MAGLUMI series Fully-auto chemiluminescence immunoassay analyzer.

Calculation

The analyzer automatically calculated the concentration in each sample by means of a calibration curve which was generated by a 2-point calibration master curve procedure. The results were expressed in pg/mL.

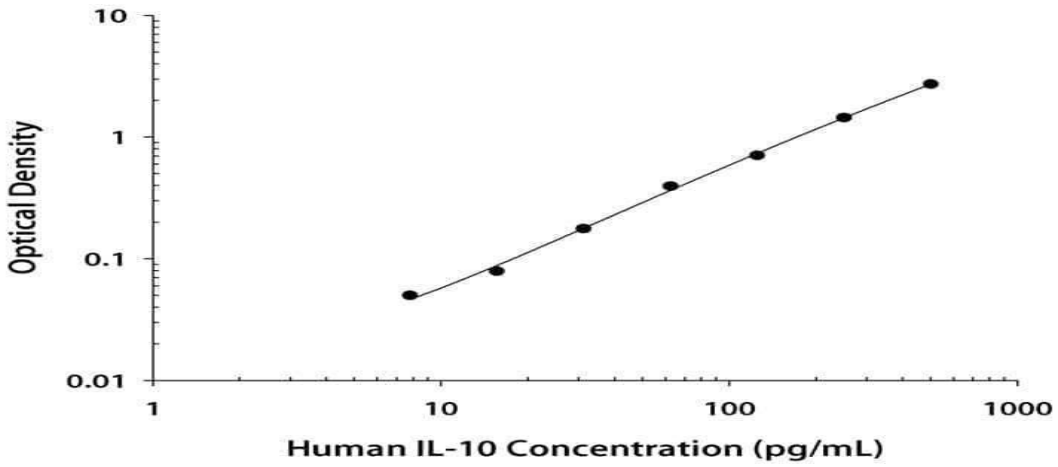


Figure 2.8 Standard Curve of Interleukin-6 for Determination the Concentration of IL-6

Interpretation of Results

The expected range for the IL-6 assay was obtained by testing 275 healthy individuals in China, and gave the following expected value: ≤7.00 pg/ml

2.7.6.2 Measurementof Highsensitivity C-reactive protein

For the latex particle enhanced immunoturbidimetric assay for the measurement of High Sensitivity C-Reactive Protein in Serum or Plasma utilizing the Mindray BS- 200 Analyzer. Only for use in in vitro diagnostics.

Principle of the Test

In the presence of human CRP from the sample, latex particles coated with antibody-specific to that protein clump together to create immunological complexes. According to the level of CRP in the serum, the immune complexes produce an increase in light scattering. Turbidity (absorbance) at 570 nm is read to determine the amount of light scattering. A calibration curve was created using CRP standards with established concentrations is used to calculate the CRP concentration.

Reagents

R-1: Buffer Reagent Glycine buffer: 170 mm

R-2: Latex Suspension

Latex particles were coated with rabbit anti-human CRP antibodies: 0.20% (w/v)

Reagent Preparation

Reagents are prepared for usage and don't need to be reconstituted. Before using, carefully combine.

Calculations

The level of High Sensitivity C-Reactive Protein in each sample was calculated using the standard curve of High Sensitivity C-Reactive Protein Determination shown in Figure (2.9).

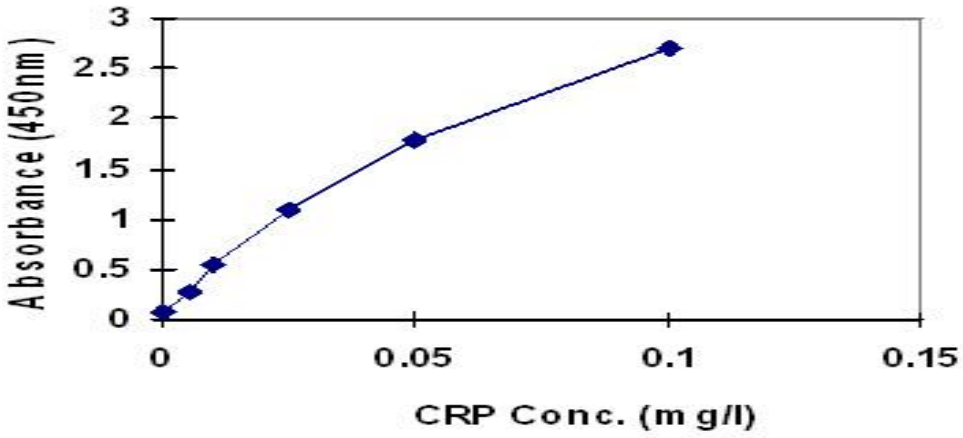


Figure 2.9 Standard Curve of Determination of the High Sensitivity C-reactive Protein Concentration.

Expected Values

The expected level of CRP in healthy people is less than 3.0 mg/L. It is advised that every lab determines its own predicted range.

2.8 Data Analysis

Statistical analysis was carried out using SPSS version 27. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SD). Pearson Chi-Square test and Fisher's Exact test were used to find the association between categorical variables. Correlation coefficient (r) was used to assess the relationship between two continuous variables. A *p*-value of ≤ 0.05 was considered as significant.

CHAPTER THREE

THE RESULTS

3. The Results

Figure 1 shows the distribution of patients with polycystic ovarian syndrome with insulin resistance according to age (years) including (< 30 years and ≥ 30 years). The majority of patients (N=29, 72.5%)were presented with age below 30 years and only eleven patients with polycystic ovarian syndrome and insulin resistance were presented with age and ≥ 30 years (27.5%) . Mean age of patients was (25.88 ± 6.35) years.

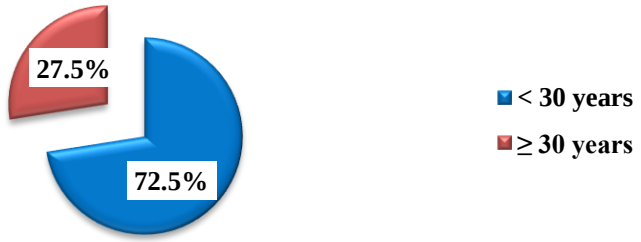


Figure 3.1 Distribution of patients with polycystic ovarian syndrome with insulin resistance according to age (N=40)

Table 3.1: Given the mean differences of age (years) according to study group including (polycystic ovarian syndrome with insulin resistance, control group)

Table 3.1 The mean differences of age according to study group

Study variables	Study group	N	Mean ± SD	P-value
Age (years)	Polycystic ovarian syndrome and insulin resistance	40	25.88 ± 6.35	0.303
	Control group	40	27.40 ± 6.79	

3.1Comparison of groups in lipid profile

Table 3.2: Given the mean differences of lipid profile including (LDL, HDL,triglyceride andcholesterol) according to the study group including (polycystic ovarian syndrome with insulin resistance, control group).

Table 3.2 The mean differences of lipid profile according to study group (N=80)

Lipid profile	Study group	N	Mean ± SD	P-value
LDL (mg/dl)	Polycystic ovarian syndrome and Insulin resistance	40	105.20 ± 22.94	**<0.001***
	Control group	40	75.10 ± 21.95	
HDL (mg/dl)	Polycystic ovarian syndrome and Insulin resistance	40	43.11 ± 12.95	**<0.001***
	Control group	40	53.35 ± 10.49	
Triglyceride (mg/dl)	Polycystic ovarian syndrome and insulin resistance	40	144.48 ± 75.61	**<0.001***
	Control group	40	89.80 ± 29.58	
Cholesterol (mg/dl)	Polycystic ovarian syndrome and insulin resistance	40	176.31 ± 29.96	**<0.001***
	Control group	40	146.29 ± 25.04	

*P value ≤ 0.05 was significant.

3.2 Comparison of Groups inInflammation Factors

Table 3.3: Shows the mean differences of High Sensitivity C-Reactive Protein according to study group including (polycystic ovarian syndrome with insulin resistance, control group).

Table 3.3 The mean differences of High Sensitivity C-Reactive Protein (mg/l) according to study group (N=80)

Study variable	Study group	N	Mean ± SD	P-value
High Sensitivity C-Reactive Protein (mg/l)	Polycystic ovarian syndrome and insulin resistance	40	2.16 ± 2.94	**<0.001***
	Control group	40	0.33 ± 0.15	

*P value ≤ 0.05 was significant.

Table 3.4: Shows the mean differences of Human Interleukin-6 according to study group including (polycystic ovarian syndrome and insulin resistance, control group).

Table 3.4 The mean differences of Human Interleukin-6(pg/ml) according to study group (N=80)

Study variable	Study group	N	Mean ± SD	P-value
Human Interleukin-6 (pg/ml)	Polycystic ovarian syndrome and insulin resistance	40	2.39 ± 1.73	**<0.001***
	Control group	40	1.24 ± 0.92	

*P value ≤ 0.05 was significant.

3.3 Correlation between Lipid Profile and HS-CRP, IL-6 and Insulin resistance in Patients Group

Table 3.5: Demonstrate the correlation of lipid profile including (LDL,HDL,triglyceride andcholesterol) and study markers including (High Sensitivity C-Reactive Protein, Human Interleukin-6 and Insulin resistance) among patients polycystic ovarian syndrome with insulin resistance.

Table 3.5 The correlation of lipid profile and study markersamong patients polycystic ovarian syndrome with insulin resistance (N=40)

Lipidprofile	**High Sensitivity C-Reactive Protein (mg/l)**		**Human Interleukin-6 (pg/ml)**		**Insulin resistance**	
	r	**P-value**	**R**	**P-value**	**r**	**P-value**
LDL (mg/dl)	0.19	0.241	0.461	0.352	0.346	**0.029***
HDL (mg/dl)	-0.216	0.18	-0.414	**0.008***	-0.004	0.979
Triglyceride (mg/dl)	0.485	**0.002***	0.618	**<0.001***	0.553	**<0.001***
Cholesterol (mg/dl)	0.311	0.051	-0.222	0.169	-0.018	0.912

*P value ≤ 0.05 was significant.

3.4 Correlation between HS-CRP and IL-6 in patients group

Figure (3.2) shows the correlation between High Sensitivity C-Reactive Protein and Human Interleukin-6 among patients polycystic ovarian syndrome with insulin resistance (N=40, r=0.686, P<0.001*).

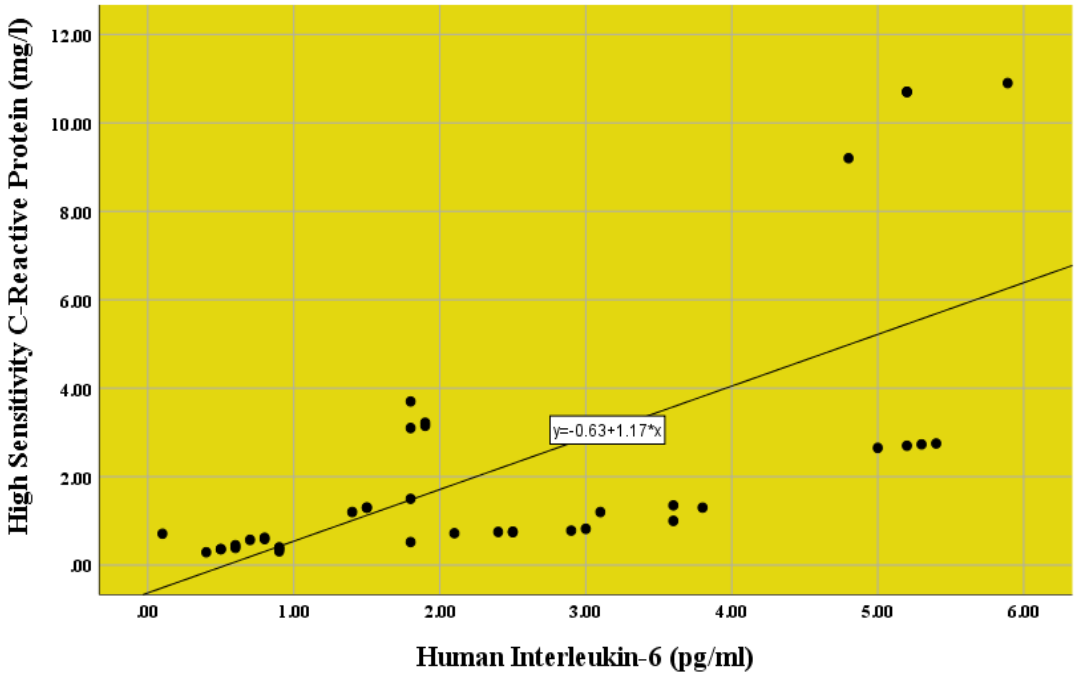


Figure 3.2 Positive linear correlation between High Sensitivity C-Reactive Protein (mg/l) and Human Interleukin-6 (pg/ml) among patients polycystic ovarian syndrome with insulin resistance (N=40, r=0.686, P<0.001*)

3.5 Correlation between HS-CRP, IL-6 and Insulin resistance in patients group

Table 3.6: Given the correlation of study markers including (High Sensitivity C-Reactive Protein and Human Interleukin-6) and Insulin resistance among patients polycystic ovarian syndrome with insulin resistance.

Table 3.6 The correlation of study markers and Insulin resistance among patients with polycystic ovarian syndrome and insulin resistance (N=40)

Study markers	Insulin resistance	
	r	P-value
High Sensitivity C-Reactive Protein (mg/l)	0.091	0.576
Human Interleukin-6 (pg/ml)	0.219	0.174

*P value ≤ 0.05 was significant.

3.6 Study the Differences in the Biomarkers level based on the Age in Patients group

Table 3.7: The mean differences of lipid profile including (LDL, HDL, triglyceride and cholesterol) according to age including (< 30 years and ≥ 30 years) among patients polycystic ovarian syndrome with insulin resistance.

Table 3.7 The mean differences of lipid profile according to age among patients polycystic ovarian syndrome with insulin resistance (N=40)

Lipid profile	Age (years)	N	Mean ± SD	P-value
LDL (mg/dl)	<30	29	99.67 ± 23.85	**0.011***
	≥ 30	11	119.76 ± 11.76	
HDL (mg/dl)	<30	29	43.65 ± 14.90	0.677
	≥ 30	11	41.70 ± 5.39	
Triglyceride(mg/dl)	<30	29	131.95 ± 79.05	0.089
	≥ 30	11	177.52 ± 56.05	
Cholesterol (mg/dl)	<30	29	168.47 ± 28.64	**0.006***
	≥ 30	11	196.97 ± 23.58	

*P value ≤ 0.05 was significant.

Table 3.8 shows the mean differences of study markers including (High Sensitivity C-Reactive Protein, Human Interleukin-6 and Insulin resistance) according to age including (< 30 years and ≥ 30 years) among patients polycystic ovarian syndrome with insulin resistance.

Table 3.8 The mean differences of study markers including (High Sensitivity C-Reactive Protein (mg/l), Human Interleukin-6 (pg/ml) and Insulin resistance) according to age among patients polycystic ovarian syndrome with insulin resistance(N=40)

Study markers	Age (years)	N	Mean ± SD	P-value
High Sensitivity C-Reactive Protein (mg/l)	<30	29	1.10 ± 0.92	**0.015***
	≥ 30	11	4.98 ± 4.40	
Human Interleukin-6 (pg/ml)	<30	29	2.04 ± 1.66	**0.038***
	≥ 30	11	3.30 ± 1.62	
Insulin resistance	<30	29	3.11 ± 0.77	0.808
	≥ 30	11	3.04 ± 0.72	

*P value ≤ 0.05 was significant.

3.7 The Differences in the Study Groups based on the Body Mass Index (BMI)

Table 3.9: shows the association between body mass index and study group including (polycystic ovarian syndrome with insulin resistance, control group).

Table 3.9 The association between body mass index (kg/m^2) and study group (N=80)

Studyvariables	Study group		Total (N=80)	P-value
	PCOS&IR (N=40)	Control group (N=40)		
Body mass index (kg/m^2)				
Normal (18.5-24.9)	10(25.0%)	31(77.5%)	41(51.3%)	
Overweight (25-29.9)	12(30.0%)	9(22.5%)	21(26.3%)	**<0.001***
Obese (≥ 30)	18(45.0%)	0(0.0%)	18(22.4%)	
Total	40(100.0%)	40 (100.0%)	80(100.0%)	

*P value ≤ 0.05 was significant.

3.8.1 The Effect of Body Mass Index (BMI) of Patients Women

Figure (3.3) shows distribution of patients polycystic ovarian syndrome with insulin resistance according to body mass index (kg/m^2) including (normal (18.5-24.9), overweight (25-29.9) and obese (≥ 30)). Normal body mass index was represented in (N=10, 25.0%) of patients, overweight represent (N=12, 30.0%) of patients and less than half of patients (N=18, 45.0%) presented with obesity. Mean body mass index of patients was (29.93 ± 5.64) kg/m^2.

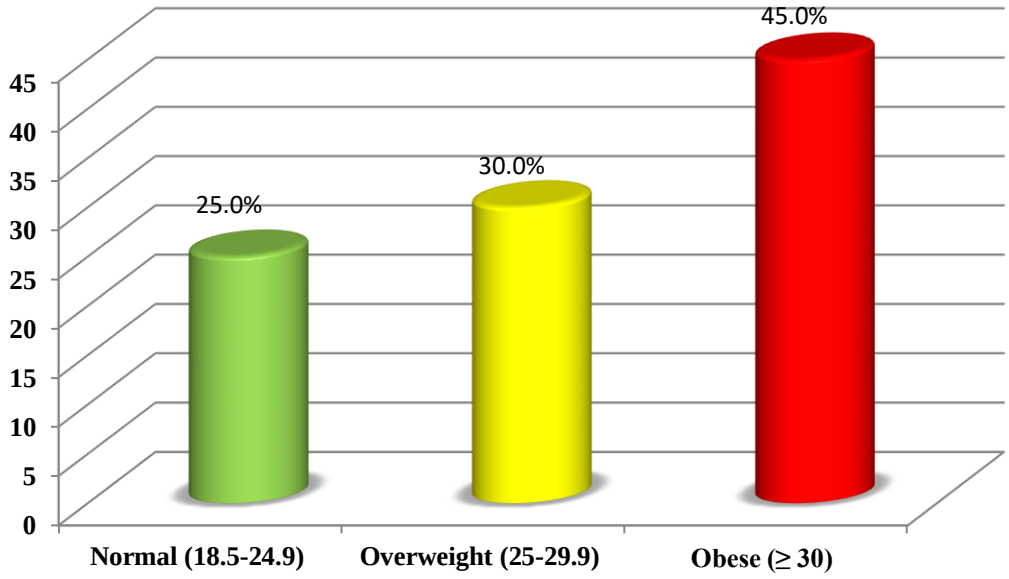


Figure 3.3 Distribution of patients with polycystic ovarian syndrome with insulin resistance according to body mass index (kg/m^2)

3.8 The Differences in the Study Groups based on the Blood Pressure

Table 3.10 The association between blood pressure level (mmHg) and study group (N=80)

Study variables	Study group		Total (N=80)	P-value
	PCOS & IR (N=40)	Control group (N=40)		
Blood pressure measurement				
< 120/ <80	0(0.0%)	22(55.0%)	22(27.5%)	
120-129/ <80	27(67.5%)	13(32.5%)	40(50.0%)	**<0.001***
130-139/ 80-89	11(27.5%)	5(12.5)	16(20.0%)	
≥140/ ≥90	2(5.0%)	0(0.0)	2(2.5%)	
Total	40(100.0%)	40(100.0%)	80(100.0%)	

*P value ≤ 0.05 was significant.

Figure (3.4)shows distribution of patients polycystic ovarian syndrome with insulin resistance according to blood pressure measurement (mmHg) including (<120/<80, 120-129/ <80, 130-139/ 80-89 and ≥140/ ≥90).Patients with blood pressure measurement was (mmHg) (120-129/ <80) represent (N=27, 67.5%), themeasurement of patients with blood pressure was (mmHg)(130-139/ 80-89) represent eleven patients (27.5%) and patients with blood pressure measurement (mmHg)(≥140/ ≥90) represent only two patients (5.0%) .

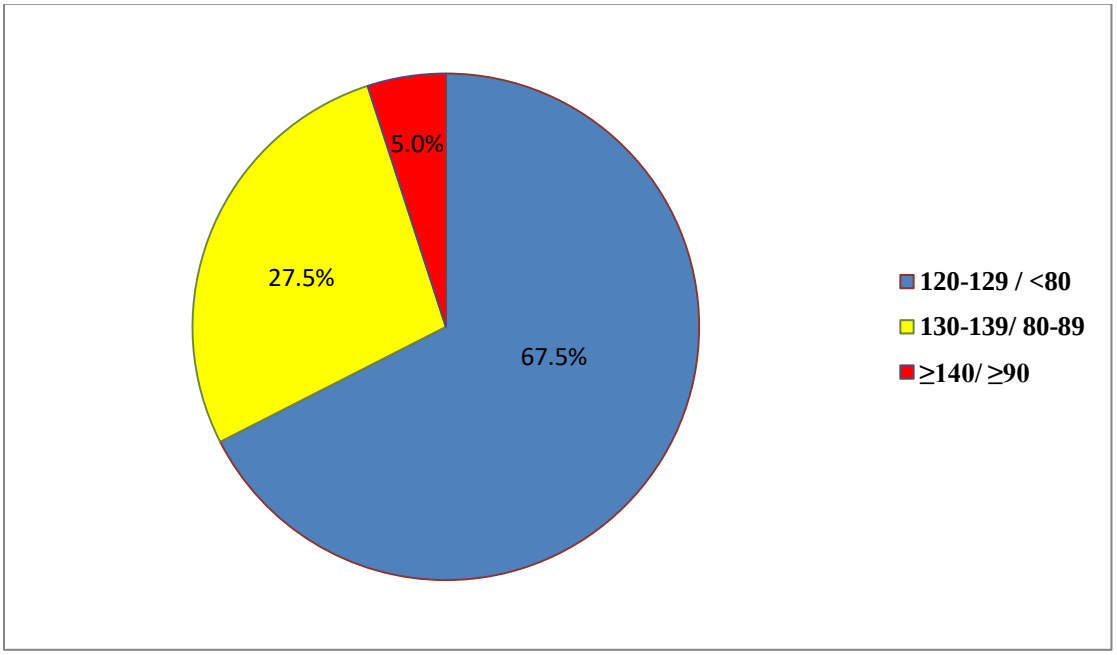


Figure 3.4 Distribution of patients polycystic ovarian syndrome with insulin resistance according to blood pressure measurement (mmHg)

3.9 The Effect of the Duration of Disease in Patients Group

Figure (3.5)shows the distribution of patients polycystic ovarian syndrome with insulin resistance according to duration of disease (years) including (< 1 year, 1-2 years, 2-3 years, 3-5 years and > 5 years). Patients with duration of polycystic ovarian syndrome and insulin resistance less than 1 year represented four patients (10.0%), patients duration of polycystic ovarian syndrome with insulin resistance(1-2) years represented nine patients (22.5%), patients with duration of polycystic ovarian syndrome with insulin resistance(2-3 years) represented ten patients (25.0%), patients with duration of polycystic ovarian syndrome with insulin resistance(3-5 years) represented six patients (15.0%) and patients with duration of polycystic ovarian syndrome with insulin resistance more than 5 years represented eleven patients (27.5%).

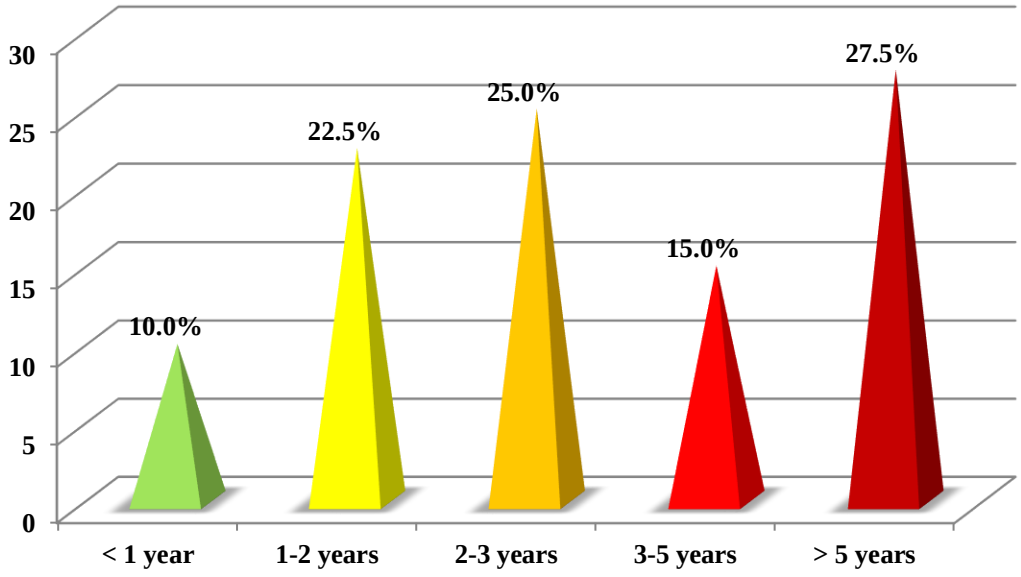
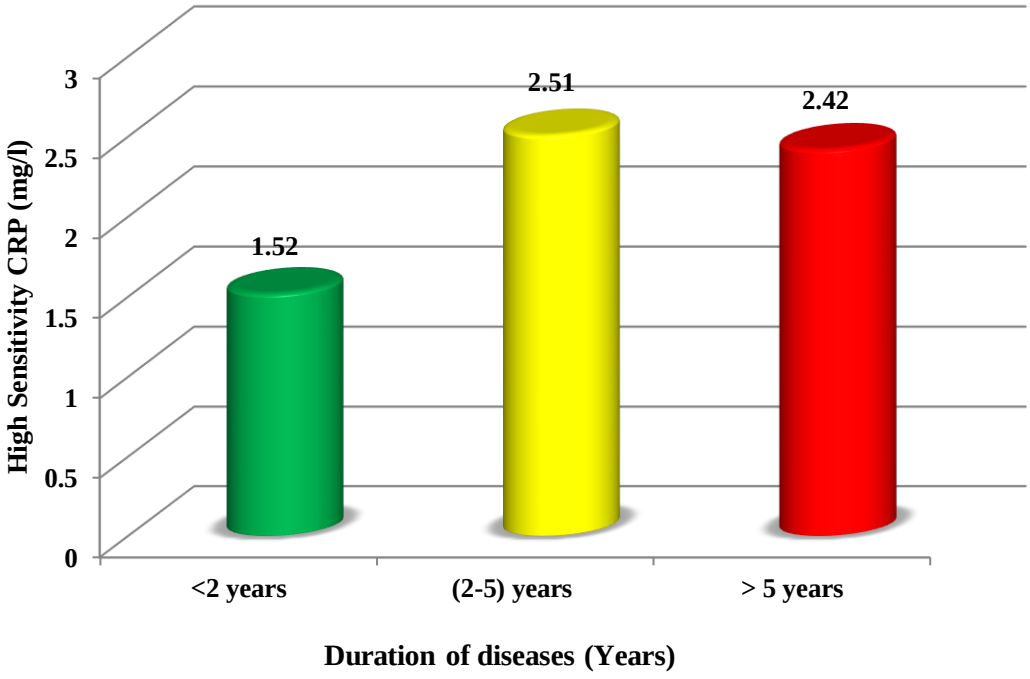


Figure 3.5 Distribution of patients polycystic ovarian syndrome with insulin resistance according to duration of disease (year)

3.9.1 The Effect of the Duration of disease on Biochemical Parameters Levels in Patients Group

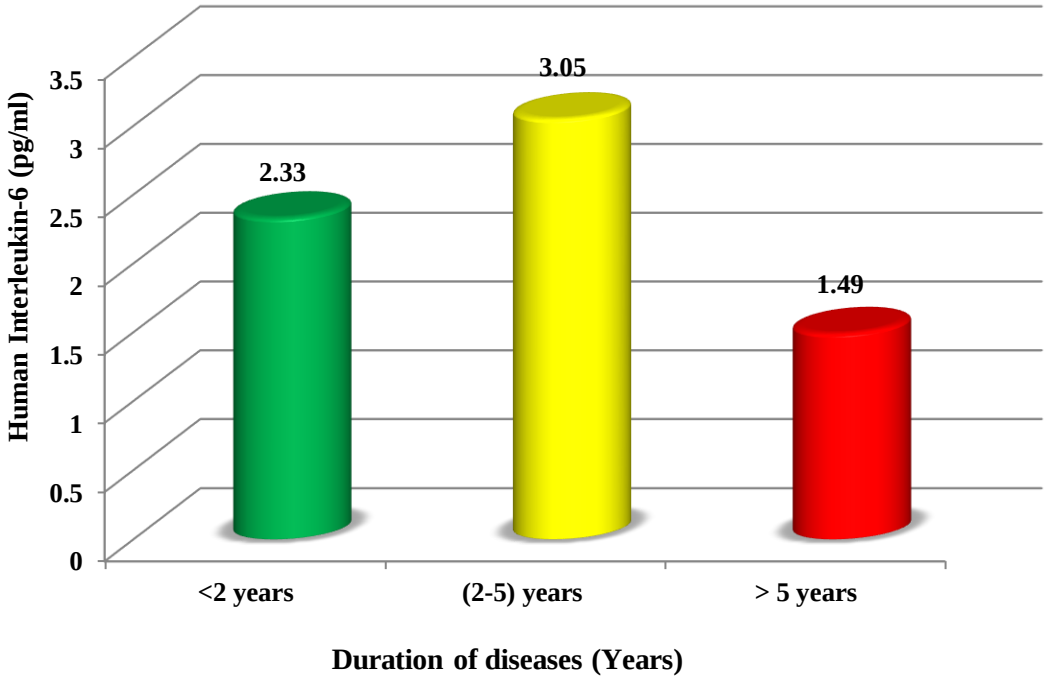
Figure (3.16) Given the mean differences of High Sensitivity C-Reactive Protein according to duration of disease.



° Patients with disease duration (<2 years) equal 13, patients with disease duration (2-5 years) equal 16 and patients with disease duration (>5 years) equal 11.

Figure 3.6 The mean differences of High Sensitivity C-Reactive Protein (mg/l) according to duration of disease (N=40) (F= 0.445, P=0.644)

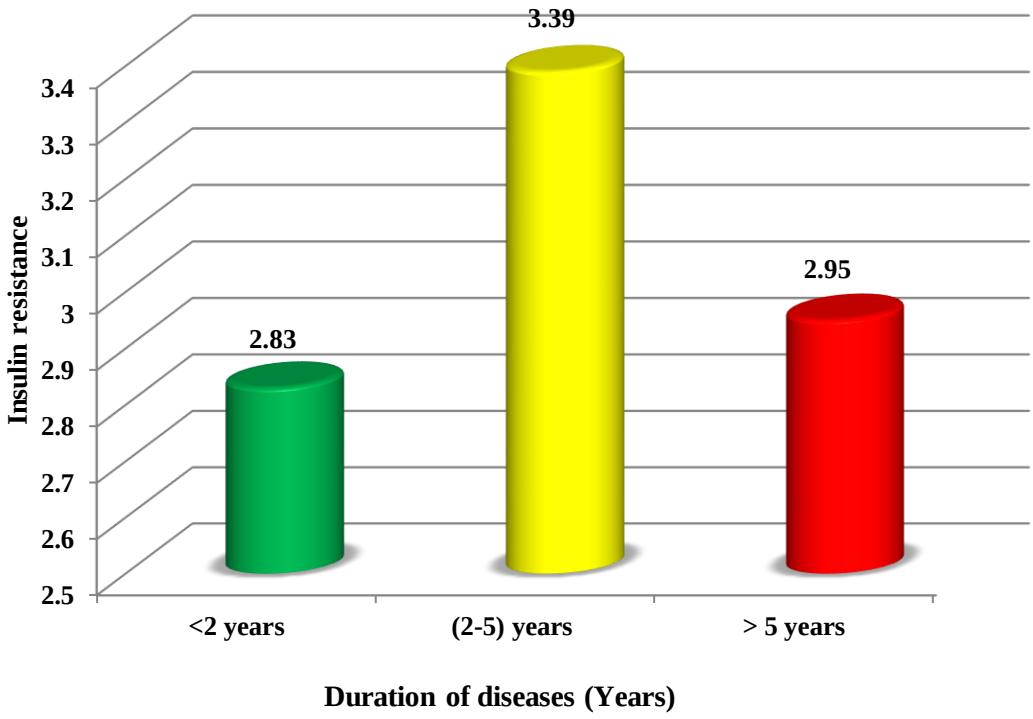
Figure (3.7) Given the mean differences of Human Interleukin-6 according to duration of disease.



° Patients with disease duration (<2 years) equal 13, patients with disease duration (2-5 years) equal 16 and patients with disease duration (>5 years) equal 11.

Figure 3.7 The mean differences of Human Interleukin-6 (pg/ml) according to duration of disease (N=40) (F= 2.93, P=0.066)

Figure (3.8) Given the mean differences of Insulin resistance according to duration of disease.



° Patients with disease duration (<2 years) equal 13, patients with disease duration (2-5 years) equal 16 and patients with disease duration (>5 years) equal 11.

Figure 3.8 The mean differences of Insulin resistance according to duration of disease (N=40) (F= 2.441, P=0.101)

3.10 Menstrual cycleinpatients group

Figure (3.9) shows the distribution of patients polycystic ovarian syndrome with insulin resistance according to menstrual cycle regularity including (regular and irregular). All patients with polycystic ovarian syndrome represented with the irregular menstrual cycle (N=40, 100.0%).

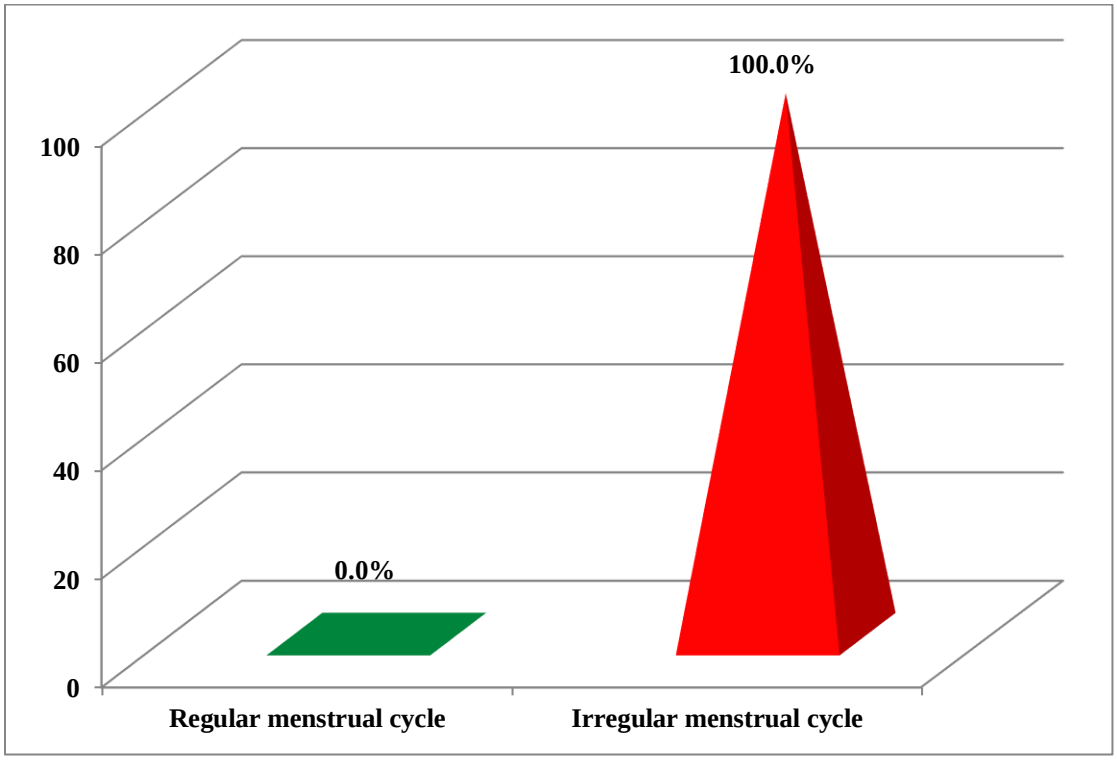


Figure 3.9 Distribution of patients polycystic ovarian syndrome with insulin resistance according to menstrual cycle regularity

CHAPTER FOUR

DISCUSSION

4. Discussion

4.1 Relation between Patients Women and Lipid Profile

The most prevalent endocrine disorder in women of reproductive age is commonly acknowledged to be polycystic ovarian syndrome (PCOS), which can also cause dyslipidemia, hyperandrogenism, hyperinsulinemia, oxidative stress, and infertility. It is now understood that dyslipidemia contributes significantly to the emergence of PCOS[56].The presented study shows an increase in cholesterol, triglycerides, and bad cholesterol (LDL) and a decrease in good cholesterol (HDL) in women patients compared to the control group, as shown in Table (3.2). These results are consistent with earlier research that was published and revealed dyslipidemia patterns [103-106]. A recent study showed that mild hypercholesterolemia is typically observed in women with PCOS [107]. A recent animal study indicated that feeding prepubertal rats a high-fat diet caused metabolic and ovarian changes that are typically seen in PCOS, which raises the possibility that hyperlipidemia may have an effect on the hormonal profile [108]. Oocyte quality and early embryo growth are clearly impacted by obesity, which is brought on by apoptosis, mitochondrial malfunction, and endoplasmic reticulum stress brought on by lipotoxicity[109].According to Shaman A. A. et al.'s findings on lipid profiles based on androgen levels[110], androgens may be a key factor in hyperlipidemia. However, recent research has indicated that hypomethylated genes involved in the synthesis of lipids and steroids may encourage the manufacture of androgen and other steroid hormones, which may help to partially explain the causes of hyperandrogenism in PCOS [111]. According to these research, hyperandrogenism is a significant contributor to lipid abnormalities, whereas alterations in lipid-related genes

favor the development of hyperandrogenism. In comparison to women with PCOS and normal cholesterol levels, those with mild hypercholesterolemia have higher body mass indexes (BMIs), fasting insulin levels, and IR levels [107].

Dyslipidemia was found to be considerably more common in PCOS and insulin resistance patients in the current investigation. A significant amount of free fatty acids are released from adipose tissues into the systemic circulation as a result of insulin resistance, which inhibits the capacity of insulin to control lipolysis [105].The American College of Obstetricians and Gynecologists and the Androgen Excess and PCOS Society both advise that all PCOS patients undergo a thorough fasting lipid and lipoprotein evaluation as part of their cardiovascular risk assessment [104, 105, 112, 113]. Since LDL-C is the lipoprotein biomolecule that causes atherosclerosis the most, it continues to be the main target [104, 105, 114, 115]. After the examination of PCOS patients for dyslipidemia, Patients with normal fasting lipid profiles will need to be evaluated again in two years, orlittle period of time if there has been an increase in body weight[104, 105].

4.2 Relation between Patients Women and Inflammation Factors

PCOS is more common when there are concomitant conditions present, such as metabolic disorders including obesity and insulin resistance. According to Lim and coauthors' meta-analysis's findings, 49% of women with PCOS have an average prevalence of obesity [116]. Furthermore, it is well-known that PCOS is typically made worse when it is linked to obesity. Additionally, it has been noted that women with PCOS had increased

numbers of circulating monocytes and lymphocytes, inflammatory infiltration in the ovarian tissue, and elevated serum concentrations of TNF and C-reactive protein (CRP)[117].This chronic inflammatory disorder is made worse by obesity and hyperinsulinemia, and there are several studies have examined the interactions between obesity, hyperandrogenism, and inflammatory state and hyperinsulinemia [118, 119].The results of the current study revealed an increase in the levels of inflammatory factors high sensitivity C-reactive protein and interleukin-6 in the group of patients, as shown in Tables (3.3)(3.4).

The relation between high-sensitivity C-reactive protein (HS-CRP) levels and PCOS components is poorly understood in this case.Elevated HS-CRP levels indicate a risk factor for cardiovascular disease and type 2 diabetes mellitus[120]. Kelly et al.'s in 2001 study, which is the first to look at low-grade chronic inflammation in women with PCOS, found that CRP concentrations, which are measured using a highly sensitive assay, are significantly higher in PCOS patients compared to healthy women with a regular menstrual cycle and normal levels of androgen. It shows that CRP may be a marker for potential future identification of young PCOS women who are prone to developing CVD because there were only 15–17 participants in each group.According to a 2009 study by Tosi et al. , showed an increase in HS-CRP levels in a group of PCOS patients, it also showed the association PCOS to low-grade inflammation, Insulin resistance is only partially responsible for this outcome, which appears to be mostly determined by body fat, As well as increased androgens play an additional role in this relation[121].There are also several recent studies that agree with the results of the current study[122, 123].In a study carried out in India, by

Ganie MA et al. found no significant difference in HS-CRP levels in the PCOS group.Despite the fact that women with PCOS had higher-trending HS-CRP levels [120].The reason for the difference is that our study focused on patients with polycystic ovaries and insulin resistance, as well as they had an irregular menstrual cycle and of different ages, while a previous study focused on adolescent girls and have a regular menstrual cycle.

Interleukin-6 (IL-6) has long been thought to be a major pro-inflammatory factor[124], which is assumed to be the cause of the obese people have higher endogenous levels of CRP [125]. For reproductive physiology, interleukin 6 (IL-6) is essential. Numerous human diseases and pathophysiological processes, including as atherosclerosis development, ovarian steroid production, fertilization and implantation, coronary heart disease, osteoporosis, and allergic reactions are all thought to be impacted by IL-6 [90, 126].

There are several previous studies that are compatible with the current study[94, 127], and one of these studies is a study conducted by Zheng Peng &Yifan, women with PCOS had considerably greater levels of IL-6 than women with BMI-matched controls. Comparing PCOS-affected women to controls, higher IL-6 levels are associated with IR and total testosterone levels. It's interesting to note that both lean and obese PCOS women had high levels of IL-6. The degree of IR was the primary cause of the notable variation that was seen throughout the studies [93].These results agree with another study bySafa S. M. Al-Shattawi et al. in Iraq, show the relationship between IL-6 and PCOS, Women with PCOS exhibited higher plasma concentrations of IL-6 than controls, who had intermediate values, or normal-weight controls[128, 129].

In addition,the study by Escobar-Morreale et al. discover no connection between PCOS and IL-6 levels [130], The conflicting findings may be due to differences between the current meta-analysis and the prior study, and the current study concentrated on PCOS and IR patients. BMI may also have an impact on inconsistent research results,Considering that obesity is frequently accompanied by higher levels of pro-inflammatory cytokines [128].

4.3 Correlation between Study Biomarkers in Patients Women

Correlations study between High Sensitivity C- Reactive Protein, Human Interleukin-6 and Insulin resistance, when evaluating the data, Pearson's correlation coefficient (r), which measures the strength of a relationship between two variables, the describe as

low (0.2-0.39)

moderate (0.4-0.69)

high (0.7-0.89)

Shown in Tables (3.5)(3.6) the presence of an inverse link between the variables is indicated by a negative correlation coefficient value, whilst a positive correlation coefficient value implies a direct proportion between the variables [131].

In the present study, significantly positive correlation between triglycerides and high sensitivity C-reactive protein. It is findings in line with a recent study byErhan Bozkurt et al.[132]. Another study conducted by Dewa AyuSwastini et al. in Indonesia demonstrated in patients with dyslipidemia,

high triglyceride levels are closely related to high levels of HS-CRP because they both correspond with the amount of atherosclerosis [133].It also due to the association of patients with both PCOS and insulin resistance with dyslipidemia and obesity [134].

The study results also showed correlation of interluken-6 with triglyceride is positive andnegative with HDL, and has a significant value. A previous study similar to our results was conducted by Mert Küçük et al. also found levels were considerably inversely connected with high-density lipoprotein(HDL) levels, but significantly favorably correlated with triglyceride levels,and deduced raise blood IL-6 levels may correlate with BMI and serum lipid levels and serve as a marker for the likelihood of developing cardiometabolic hazards in PCOS patients [96].

It found the correlation betweentriglyceride and insulin resistance his a significant positive correlation in PCOS&IR group. According to the study correspond to the result current study,It was conducted by Dr. J Lord et al. was showed the most important factor associated with metabolic dysfunction in PCOS-affected individuals is visceral obesity. The presence of visceral fat either initiates insulin resistance or manifests as one of its very first symptoms. Furthermore, it suggests that decreasing visceral fat should also decrease insulin resistance, which could explain why exercise and weight loss seem to be more successful therapy than pharmacological ones [135].Another recent study also showed a substantial positive link between TG and HOMA-IR index value using a Pearson linear correlation model and receiver operating characteristic (ROC) curve analyses [136].

The study also revealed the correlation between High Sensitivity C-Reactive Protein and Human Interleukin-6 is a significant positive linear. Numerous studies in various groups of PCOS-afflicted womencongruent with the current study, Including a study by Christelle Chemaga Nkonpawa et al. and another study by Rudnicka et al. showed imply that PCOS is accompanied by a low-grade chronic inflammation [137, 138]. In fact, it appears that hyperandrogenism in PCOS-afflicted women causes adipocyte hypertrophy, which in turn stimulates nuclear factor kappa-B (NF-B), when this factor is activated, a number of inflammatory cytokines are released, which in turn encourage the liver to produce more CRP [139]. The disparity in HS-CRP levels can be attributed to the fact that the cytokine interleukin-6 is primarily responsible for controlling the transcriptional level of CRP induction in hepatocytes [140].

The correlation of High Sensitivity C-Reactive Protein and insulin resistance did not show a significant value as shown in Table (3.6). This is consistent with the results of several studies [120, 128, 137]. These findings conflict with those of some other studies[138, 141]. This discrepancy may be the consequence of diverse population origins, or a difference of the collected samples,where prior research has only looked at individuals with polycystic ovarieswhereas the current study focused on PCOS&IR patients.

The correlation of Human Interleukin-6 and insulin resistance did not show a significant value as shown in Table (3.6). It is consistent with a recent study conducted in India by Soumik Goswami et al. [128].However, in a different investigation, they obtained different results, high IL-6 levels in PCOS were found to be linked with insulin resistance (HOMA-IR) [93].There is still no consensus on IL-6 function in the emergence of T2DM. Although

research suggests that IL-6 may be responsible for the disruption of insulin signaling in adipocytes in vitro, an in vivo demonstration of this finding has not been made. Further research is required in this area because it has been demonstrated that long-term IL-6 stimulation has no effect on skeletal muscle insulin resistance [142].

4.4 The Effect Age on Study Biomarkers in Patients Women

In the present study, The group of polycystic ovaries and insulin resistance patients was divided into two groups according to age (<30years and ≥ 30 years), showed that the results of patients ≥ 30 years old a significant increased the levels of both LDL, Cholesterol, High Sensitivity C-Reactive Protein, and Human Interleukin-6,No significant value was found in Triglycerides, HDL and Insulin resistance, as shown in Tables (3.7) (3.8), The condition of women with PCOS might change with age [143].The prevalence of PCOS appears to decline with aging as the diagnostic criteria for PCOS may diminish or even normalize during the reproductive lifetime [144]. The most significant characteristics of PCOS are irregular menstrual cycles, polycystic ovary morphology, and hyperandrogenism, which may alter by age, It is unknown how long has passed since the onset of a specific symptom before the disease has appeared [145]. In other several studies, it has been shownTriglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and total cholesterol (TC) were found to rise with aging in PCOS-affected women[146-148].

A study conducted in China by Zengwu Wang et al. examined the distribution of HS-CRP and its connection to known risk factors for CVD. Additionally, HS-CRP increase with age [149].Since polycystic ovary

syndrome and insulin resistance are important risk factors for cardiovascular diseases [150], the previous study is identical to the results of our study.

Several studies indicated the amount of IL-6 in the blood that is circulating rises with aging [151, 152], and may be harmful if it is increased for an extended length of time. In particular, IL-6 is strongly linked to a variety of vascular-mediated illnesses (such as cardiovascular disease and atherosclerosis) [153], that make people more susceptible to cerebrovascular accidents [154], and may expedite cognitive decline in aging adults [155]. while a study conducted by Beharka et al. 2001 showed that interleukin did not increase with age [156].

In general, the risk of acquiring metabolic diseases and cardiovascular events increases with age. a malfunction in insulin action that is characterized by decreased whole-body tissue sensitivity to insulin without a change in tissue responsiveness may also be linked to aging [157]. According to a study conducted by Tehrani FR et al. found with get old, the variations in this metabolic marker between PCOS patients and healthy controls became less noticeable[158] , it can be said that IR deteriorates in PCOS patients during the reproductive years, and this deterioration appears to be brought on by rising abdominal fat [159].

4.5 The Effect Body Mass Index (BMI) on Patients Women

The prevalence of obesity among women worldwide has increased 2.5-fold from 6% to 15% over the past 40 years. Over a same period, there has been a corresponding rise in the prevalence of co-morbidities associated with obesity, of which there are more than 50 and which together represent a significant burden on both global health and socioeconomic development.

Insulin resistance, which results from weight increase, compensatory hyperinsulinaemia, and the associated metabolic dysfunction are major mediators in the development of many obesity-related diseases. The metabolic syndrome's symptoms, such as type 2 diabetes (T2D), dyslipidaemia, hypertension, and obesity-related cancers such endometrial carcinoma, as well polycystic ovarian syndrome. Usually throughout adolescence, weight increase causes PCOS to become clinically evident [160].As shown in Table (3.9), when comparing the Body Mass Index group of polycystic ovary patients with insulin resistance and the healthy group has a significant value, and in the patients' group, the dominant characteristic was obesity by 45%, as shown in Figure(3.3), This is a common finding among women with the syndrome, according to many studies that confirmed the current study findings [161-163].

A study conducted by Al-Tu'ma F et al. showed that the physiological, hormonal, and metabolic symptoms of PCOS are made worse by obesity and abdominal fat accumulation, which a large number of females experience [164].Another studywas conducted by NiePolski L et al. discovered that one of the reasons people with PCOS gain weight or struggle to lose weight is because insulin cannot function properly. Numerous serious health complications might develop as a result of obesity, an increase in an ovulation and irregular ovarian cycles are indications of PCOS. The BMI may have an impact on endocrine and metabolic issues. As the condition develops and progresses, obesity has been linked to an increased risk of miscarriages, cardiovascular disease, and infertility [165]. According to a new study conducted by Sánchez-Ferrer ML et al. Stress, anxiety, personal

dissatisfaction and melancholy, are common in women with PCOS, may be exacerbated by by a change in body image brought on by weight gain [166].

4.6 The Effect of Blood Pressure onPatients Women

Elevated blood pressure is a component of the metabolic syndrome [105, 167], Abdominal obesity, insulin resistance, dyslipidemia, and a higher risk of the metabolic syndrome are all characteristics of women with PCOS and may raise the risk of cardiovascular disease (CVD) [167].Insulin resistance and hyperinsulinemia appear to be linked to an elevated risk for hypertension in PCOS women. Both diseases cause vascular muscle wall hypertrophy by interfering with endothelium-dependent vasodilatation processes [168].

The study showed higher levels of blood pressure in Patients of PCOS and insulin resistance compared to the healthy group, as shown in Table (3.10). According to a recent studies whose findings matched those of the current investigation conducted by Mellembakken et al. showed the favorable correlation between blood pressure and waist and lipid status in PCOS-affected women highlights the value of metabolic screening in young, normal-weight PCOS patients [169], also SelçukÖzkan et al. in his studyshows that with PCOS have a higher frequency of hidden hypertension [170]. More than 30% of women with PCOS are thought to have blood pressure that is higher than 130/85 mmHg, and young women with PCOS have a roughly threefold greater risk of hypertension compared to controls [167, 170-173]. Accordingly, the most recent worldwide PCOS guideline advises that all women with PCOS should have their blood pressure checked at the time of diagnosis and then annually after that [148].

4.7 The Effect of the Duration of the Disease

In the current study, a group of patients with polycystic ovarian disease and insulin resistance were separated into three groups based on the length of the disease: Patients with disease duration (2 years equal 13patients), (2-5 years equal 16patients), and (>5 years equal 11patients) were compared with the levels of each of the high sensitivity C-reactive protein, interleukin-6 and insulin resistance, and showed there was no significant value. The duration of the disease was between 2-5 year more prominent , in which the percentage of levels of each of the high sensitivity C-reactive protein , interleukin-6 and insulin resistance increases, as shown in Figures (3.6)(3.7)(3.8).

In a study by Hung et al., a subgroup analysis stratified by how long it had been after the PCOS diagnosis had occurred before the new-onset psychiatric problems had appeared was carried out. The findings showed that in the first year following a PCOS diagnosis, the incidence of depressive disorder, anxiety disorder, and sleep disturbance rose[174].

CHAPTER FIVE

CONCLUSIONS

&

RECOMMENDATIONS

5.1 Conclusions

The study arrived at the following conclusions:

1- High levels of LDL, HDL, TG and decrease HDL are an indicator of atherosclerosis in polycystic ovarian and insulin resistant patients.

2- Elevation of the inflammatory factors interleukin-6 and high sensitivity C-reactive protein is an important predictor for the risk factor in polycystic ovarian and insulin resistance patients.

3- The greater the age, the more cholesterol, LDL, HS-CRP, IL-6, which increases the risk of infections and heart disease.

4- The body mass index for women with PCOS and insulin resistance is higher in obese women by 45%, overweight by 30% and normal by 25%.

5- High blood pressure in women with PCOS and insulin resistance is an indicator of many diseases.

5.2 Recommendations

1- Conducting measurements of the lipid profile of women with polycystic ovaries and insulin resistance from time to time.

2- Conducting continuous examinations for inflammatory factors, especially in patients over 30 years old.

3-It is recommended that are followed Healthy eating habits and exercise regularly to lose weight and reduce the risks of diseases produced by obesity and its consequences.

References

[1] M. S. Bostanci, N. Sagsoz, V. Noyan, A. Yucel, and K. Goren, "Comprasion of Ovarian Stromal and Uterin Artery Blood flow measured by color doppler ultrasonography in polycystic ovary syndrome patients and patients with ultrasonographic evidence of polycystic," *Journal of Clinical Gynecology and Obstetrics*, vol. 2, no. 1, pp. 20-26, 2013.

[2] G. T. Kovacs and R. Norman, *Polycystic Ovary Syndrome*. Cambridge University Press, 2007.

[3] M. Al-Jefout, N. Alnawaiseh, and A. Al-Qtaitat, "Insulin resistance and obesity among infertile women with different polycystic ovary syndrome phenotypes," *Scientific reports*, vol. 7, no. 1, p. 5339, 2017.

[4] S. Patel, "Polycystic ovary syndrome (PCOS), an inflammatory, systemic, lifestyle endocrinopathy," *The Journal of steroid biochemistry and molecular biology*, vol. 182, pp. 27-36, 2018.

[5] E. Carmina, S. Bucchieri, A. Esposito, A. Del Puente, P. Mansueto, F. Orio, *et al.*, "Abdominal fat quantity and distribution in women with polycystic ovary syndrome and extent of its relation to insulin resistance," *The Journal of Clinical Endocrinology & Metabolism*, vol. 92, no. 7, pp. 2500-2505, 2007.

[6] E. Rudnicka, K. Suchta, M. Grymowicz, A. Calik-Ksepka, K. Smolarczyk, A. M. Duszewska *etal.*, "Chronic Low Grade Inflammation in Pathogenesis of PCOS," *International Journal of Molecular Sciences*, vol. 22, no. 7, p. 3789, 2021.

[7] S. H. Guzar, E. S. Jawad, M. A. Altahan, and N. N. Hameed, "The Effects of Trace Element levels on Poly cystic Ovary Syndrome in human female Sat Thi-Qar governorate Iraq," *Research Journal of Pharmacy and Technology*, vol. 12, no. 9, pp. 4447-4453, 2019.

References

[8] T. Zhu and M. O. Goodarzi, "Causes and consequences of polycystic ovary syndrome: insights from Mendelian randomization," *The Journal of Clinical Endocrinology & Metabolism,* vol. 107, no. 3, pp. e899-e911, 2022.

[9] A. E. Joham, R. J. Norman, E. Stener-Victorin, R. S. Legro, S. Franks, L. J. Moran, *et al.*, "Polycystic ovary syndrome," *The Lancet Diabetes & Endocrinology,* 2022.

[10] R. S. Legro, "New directions in polycystic ovary syndrome," in *Seminars in reproductive medicine*,vol. 26, no. 01,pp. 003-004.2008.

[11] M. Zehravi, M. Maqbool, and I. Ara, "Polycystic ovary syndrome and infertility: an update," *International journal of adolescent medicine and health,* vol. 34, no. 2, pp. 1-9, 2021.

[12] A. PRIYADARSHANI, V. Madan, and P. Jayaraj, "Veritable evaluation and inspection of PCOS and its apropos medicaments," *Indian Journal of Biochemistry and Biophysics (IJBB),* vol. 59, no. 11, pp. 1039-1047, 2022.

[13] D. L. Thomas, "Polycystic Ovary Syndrome and Metabolic Syndrome," *News Medical Life Sciences* 2019.

[14] S. F. Witchel, S. E. Oberfield, and A. S. Peña, "Polycystic ovary syndrome: pathophysiology, presentation, and treatment with emphasis on adolescent girls," *Journal of the Endocrine Society*, vol. 3, no. 8, pp. 1545-1573, 2019.

[15] L. Ibáñez, S. E. Oberfield, S. Witchel, R. J. Auchus, R. J. Chang, E. Codner, *et al.*, "An international consortium update: pathophysiology, diagnosis, and treatment of polycystic ovarian syndrome in adolescence," *Hormone research in paediatrics*, vol. 88, no. 6, pp. 371-395, 2017.

References

[16] L. Barrea, P. Marzullo, G. Muscogiuri, C. Di Somma, M. Scacchi, F. Orio, *et al*, "Source and amount of carbohydrate in the diet and inflammation in women with polycystic ovary syndrome," *Nutrition research reviews,* vol. 31, no. 2, pp. 291-301, 2018.

[17] S. Singh, N. Pal, S. Shubham, D. K. Sarma, V. Verma, F. Marotta, *etal.*, "Polycystic Ovary Syndrome: Etiology, Current Management, and Future Therapeutics," *Journal of Clinical Medicine,* vol. 12, no. 4, p. 1454, 2023.

[18] S. A. Kanbour and A. S. Dobs, "Hyperandrogenism in Women with Polycystic Ovarian Syndrome: Pathophysiology and Controversies," *Androgens: Clinical Research and Therapeutics,* vol. 3, no. 1, pp. 22-30, 2022.

[19] J. Bulsara, P. Patel, A. Soni, and S. Acharya, "A review: Brief insight into Polycystic Ovarian syndrome," *Endocrine and Metabolic Science,* vol. 3, p. 100085, 2021.

[20] H. Ding, J. Zhang, F. Zhang, S. Zhang, X. Chen, W. Liang, *et al.*, "Resistance to the insulin and elevated level of androgen: a major cause of polycystic ovary syndrome," *Frontiers in endocrinology,* vol. 12, p. 741764, 2021.

[21] J. C. Marshall and A. Dunaif, "Should all women with PCOS be treated for insulin resistance?," *Fertility and sterility,* vol. 97, no. 1, pp. 18-22, 2012.

[22] E. Diamanti-Kandarakis, H. Kandarakis, and R. S. Legro, "The role of genes and environment in the etiology of PCOS," *Endocrine,* vol. 30, pp. 19-26, 2006.

[23] E. Kandaraki, A. Chatzigeorgiou, S. Livadas, E. Palioura, F. Economou, M. Koutsilieris, *et al.*, "Endocrine disruptors and

polycystic ovary syndrome (PCOS): elevated serum levels of bisphenol A in women with PCOS," *The Journal of Clinical Endocrinology & Metabolism*, vol. 96, no. 3, pp. E480-E484, 2011.

[24] T. Takeuchi, O. Tsutsumi, Y. Ikezuki, Y. Takai, and Y. Taketani, "Positive relationship between androgen and the endocrine disruptor, bisphenol A, in normal women and women with ovarian dysfunction," *Endocrine journal*, vol. 51, no. 2, pp. 165-169, 2004.

[25] H. H. Le, E. M. Carlson, J. P. Chua, and S. M. Belcher, "Bisphenol A is released from polycarbonate drinking bottles and mimics the neurotoxic actions of estrogen in developing cerebellar neurons," *Toxicology letters*, vol. 176, no. 2, pp. 149-156, 2008.

[26] A. Konieczna, D. Rachoń, K. Owczarek, P. Kubica, A. Kowalewska, B. Kudłak, *et al.*, "Serum bisphenol A concentrations correlate with serum testosterone levels in women with polycystic ovary syndrome," *Reproductive Toxicology*, vol. 82, pp. 32-37, 2018.

[27] R. M. Kumar, S.; Shah, R.; Bhat, A.; Verma, S.; Chander, G.; Bhat, G.R.; Thapa, N.; Bhat, A.; Wakhloo, A.; et al., " Role of genetic, environmental, and hormonal factors in the progression of PCOS," *A review. J Reprod Healthc Med*, p. 3, 2022.

[28] M. J. Khan, A. Ullah, and S. Basit, "Genetic basis of polycystic ovary syndrome (PCOS): current perspectives," *The application of clinical genetics*, pp. 249-260, 2019.

[29] N. Ajmal, S. Z. Khan, and R. Shaikh, "Polycystic ovary syndrome (PCOS) and genetic predisposition: A review article," *European journal of obstetrics & gynecology and reproductive biology: X*, vol. 3, p. 100060, 2019.

References

[30] R. J. Muhammad and A. I. Mahmoud, "Relationship of recurrent miscarriage with polycystic ovary syndrome," *Karbala University, College of Education for Pure Sciences,* 2022.

[31] L. B. Samer El Hayek , Layal H. Hamdar , Fadi G. Mirza, and, G. Daoud, "Poly Cystic Ovarian Syndrome: An Updated Overview," *Sec. Clinical and Translational Physiology,* vol. 7, 2016

[32] D. A. Dumesic, J. D. Phan, K. L. Leung, T. R. Grogan, X. Ding, X. Li, *et al.*, "Adipose insulin resistance in normal-weight women with polycystic ovary syndrome," *The Journal of Clinical Endocrinology & Metabolism,* vol. 104, no. 6, pp. 2171-2183, 2019.

[33] Z. Y. Halimova, G. J. Narimova, G. M. Jabborova, and U. A. Mirsaidova, "Case of Pituitary corticotropinoma-from PCOS to obvious Cushing," in *Endocrine Abstracts*,vol. 63, 2019.

[34] S. A. Paschou, E. Palioura, D. Ioannidis, P. Anagnostis, A. Panagiotakou, V. Loi, *et al.*, "Adrenal hyperandrogenism does not deteriorate insulin resistance and lipid profile in women with PCOS," *Endocrine connections,* vol. 6, no. 8, p. 601, 2017.

[35] C.-W. Ho, H.-H. Chen, M.-C. Hsieh, C.-C. Chen, S.-P. Hsu, H.-T. Yip, *et al.*, "Increased risk of polycystic ovary syndrome and It's comorbidities in women with autoimmune thyroid disease," *International journal of environmental research and public health,* vol. 17, no. 7, p. 2422, 2020.

[36] R. Singla, Y. Gupta, M. Khemani, and S. Aggarwal, "Thyroid disorders and polycystic ovary syndrome: An emerging relationship," *Indian journal of endocrinology and metabolism,* vol. 19, no. 1, p. 25, 2015.

References

[37] L. C. Morin-Papunen, A. J. Duleba, A. Bloigu, M.-R. Järvelin, P. Saikku, and A. Pouta, "Chlamydia antibodies and self-reported symptoms of oligo-amenorrhea and hirsutism: A new etiologic factor in polycystic ovary syndrome?," *Fertility and sterility*, vol. 94, no. 5, pp. 1799-1804, 2010.

[38] F. Duică, C. A. Dănilă, A. E. Boboc, P. Antoniadis, C. E. Condrat, S. Onciul, *et al.*, "Impact of increased oxidative stress on cardiovascular diseases in women with polycystic ovary syndrome," *Frontiers in Endocrinology*, vol. 12, p. 614679, 2021.

[39] A. J. Duleba and A. Dokras, "Is PCOS an inflammatory process?," *Fertility and sterility*, vol. 97, no. 1, pp. 7-12, 2012.

[40] A. Borthakur, Y. D. Prabhu, and A. V. Gopalakrishnan, "Role of IL-6 signalling in polycystic ovarian syndrome associated inflammation," *Journal of Reproductive Immunology*, vol. 141, p. 103155, 2020.

[41] M. Crook, "Lipoprotein X: clinical implications," vol. 50, ed: SAGE Publications Sage UK: London, England, 2013, pp. 93-94.

[42] M. N. Molina, L. Ferder, and W. Manucha, "Emerging role of nitric oxide and heat shock proteins in insulin resistance," *Current hypertension reports*, vol. 18, no. 1, p. 1, 2016.

[43] D. E. James, J. Stöckli, and M. J. Birnbaum, "The aetiology and molecular landscape of insulin resistance," *Nature Reviews Molecular Cell Biology*, vol. 22, no. 11, pp. 751-771, 2021.

[44] C. Nowak, "Insulin Resistance: Causes, biomarkers and consequences," Acta Universitatis Upsaliensis, 2017.

[45] J. K. Kim, "Hyperinsulinemic–euglycemic clamp to assess insulin sensitivity in vivo," *Type 2 Diabetes: Methods and Protocols*, pp. 221-238, 2009.

References

[46] S.-P. Choukem and J.-F. Gautier, "How to measure hepatic insulin resistance?," *Diabetes & metabolism,* vol. 34, no. 6, pp. 664-673, 2008.

[47] R. Turner, N. Oakley, and J. Nabarro, "Control of basal insulin secretion, with special reference to the diagnosis of insulinomas," *Br Med J,* vol. 2, no. 5754, pp. 132-135, 1971.

[48] D. R. Matthews, J. P. Hosker, A. S. Rudenski, B. Naylor, D. F. Treacher, and R. C. Turner, "Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man," *Diabetologia,* vol. 28, pp. 412-419, 1985.

[49] E. Sharif and M. Alwakeel, "New markers for the detection of polycystic ovary syndrome," 2019.

[50] L. Shahin, D. Hyassat, A. Batieha, Y. Khader, M. El-Khateeb, and K. Ajlouni, "Insulin sensitivity indices in patients with polycystic ovary syndrome with different body mass index categories," *Current Diabetes Reviews,* vol. 16, no. 5, pp. 483-489, 2020.

[51] M. Rajkhowa, S. Brett, D. J. Cuthbertson, C. Lipina, A. J. Ruiz-Alcaraz, G. E. Thomas, *et al.,* "Insulin resistance in polycystic ovary syndrome is associated with defective regulation of ERK1/2 by insulin in skeletal muscle in vivo," *Biochemical Journal,* vol. 418, no. 3, pp. 665-671, 2009.

[52] R. Condorelli, A. Calogero, M. Di Mauro, L. Mongioi', R. Cannarella, G. Rosta, *et al.,* "Androgen excess and metabolic disorders in women with PCOS: beyond the body mass index," *Journal of endocrinological investigation,* vol. 41, pp. 383-388, 2018.

References

[53] S. F. de Medeiros, R. J. Rodgers, and R. J. Norman, "Adipocyte and steroidogenic cell cross-talk in polycystic ovary syndrome," *Human reproduction update*, vol. 27, no. 4, pp. 771-796, 2021.

[54] S. S. Merkin, J. L. Phy, C. K. Sites, and D. Yang, "Environmental determinants of polycystic ovary syndrome," (in eng), *Fertil Steril*, vol. 106, no. 1, pp. 16-24, Jul 2016, doi: 10.1016/j.fertnstert.2016.05.011.

[55] R. D. Nuzhat Shaikh, Srabani Mukherjee, , "Genetic Markers of Polycystic Ovary Syndrome: Emphasis on Insulin Resistance," *International Journal of Medical Genetics*, ID 478972 vol. 2014, p. 10, 2014.

[56] Q. Liu, Y.-j. Xie, L.-h. Qu, M.-x. Zhang, and Z.-c. Mo, "Dyslipidemia involvement in the development of polycystic ovary syndrome," *Taiwanese Journal of Obstetrics and Gynecology*, vol. 58, no. 4, pp. 447-453, 2019.

[57] J. Radons, "The human HSP70 family of chaperones: where do we stand?," *Cell Stress and Chaperones*, vol. 21, no. 3, pp. 379-404, 2016.

[58] E. C. Costa, J. C. F. d. Sá, N. K. Stepto, I. B. B. Costa, L. F. Farias Junior, S. d. N. T. Moreira, *et al.*, "Aerobic training improves quality of life in women with polycystic ovary syndrome," 2018.

[59] I. Kyrou, E. Karteris, T. Robbins, K. Chatha, F. Drenos, and H. S. Randeva, "Polycystic ovary syndrome (PCOS) and COVID-19: an overlooked female patient population at potentially higher risk during the COVID-19 pandemic," *BMC medicine*, vol. 18, pp. 1-10, 2020.

[60] J. Velisek, R. Koplik, and K. Cejpek, *The Chemistry of Food*. Wiley, 2020.

References

[61] G. S. Getz, "Lipid Transfer Proteins: Introduction to the Thematic Review Series," *Journal of Lipid Research,* vol. 59, no. 5, pp. 745-748, 2018.

[62] M. H. Stipanuk and M. A. Caudill, *Biochemical, physiological, and molecular aspects of human nutrition-E-book.* Elsevier health sciences, 2018.

[63] J. Fantini, R. M. Epand, and F. J. Barrantes, "Cholesterol-recognition motifs in membrane proteins," *Direct mechanisms in cholesterol modulation of protein function,* pp. 3-25, 2019.

[64] M. S. Sekhar, S. Marupuru, B. S. Reddy, S. J. Kurian, and M. Rao, "Physiological role of cholesterol in human body," in *Dietary sugar, salt and fat in human health*: Elsevier,pp. 453-481, 2020.

[65] "PubChem Compound Summary for CID 5997, Cholesterol.," *National Center for Biotechnology Information*, 2023.

[66] E. Ikonen, "Cellular cholesterol trafficking and compartmentalization," *Nature reviews Molecular cell biology,* vol. 9, no. 2, pp. 125-138, 2008.

[67] F. D. Porter and G. E. Herman, "Malformation syndromes caused by disorders of cholesterol synthesis," *Journal of lipid research,* vol. 52, no. 1, pp. 6-34, 2011.

[68] J. Iqbal and M. Hussain, "Jahangir Iqbal and M," *Mahmood Hussain. Rev. Lit. Arts Am,* vol. 296, pp. 1183-1194, 2009.

[69] G. R. Caponio, D. Q. H. Wang, A. Di Ciaula, M. De Angelis, and P. Portincasa, "Regulation of Cholesterol Metabolism by Bioactive Components of Soy Proteins: Novel Translational Evidence," *International Journal of Molecular Sciences,* vol. 22, no. 1, 2021.

References

[70] A. D. S. P. LIBRARY, "Science Photo Library's website," vol. 50.3 x 24.9 cm · 19.8 x 9.8 in (300dpi), I. o. t. f. o. a. t. f. g. a. f. acids., Ed., ed.

[71] K. M. Beaudry and M. C. Devries, "Sex-based differences in hepatic and skeletal muscle triglyceride storage and metabolism," *Applied Physiology, Nutrition, and Metabolism*, vol. 44, no. 8, pp. 805-813, 2019.

[72] Y. Zhang, I. Zanotti, M. P. Reilly, J. M. Glick, G. H. Rothblat, and D. J. Rader, "Overexpression of apolipoprotein AI promotes reverse transport of cholesterol from macrophages to feces in vivo," *Circulation*, vol. 108, no. 6, pp. 661-663, 2003.

[73] L. E. Smith, D. K. Smith, J. D. Blume, M. F. Linton, and F. T. Billings IV, "High-density lipoprotein cholesterol concentration and acute kidney injury after cardiac surgery," *Journal of the American Heart Association*, vol. 6, no. 12, p. e006975, 2017.

[74] S. T. Chiesa and M. Charakida, "High-density lipoprotein function and dysfunction in health and disease," *Cardiovascular drugs and therapy*, vol. 33, pp. 207-219, 2019.

[75] V. A. Pallazola, J. C. Murray, M. Al Harthy, S. L. Zimmerman, J. Webster, and L. P. Gondek, "Anthracycline-induced acute myocarditis and ventricular fibrillation arrest," *American journal of hematology*, vol. 93, no. 3, pp. 469-470, 2018.

[76] G. M. Baer, *The natural history of rabies*. Routledge, 2017.

[77] H. R. Superko and S. King III, "Lipid management to reduce cardiovascular risk: a new strategy is required," *Circulation*, vol. 117, no. 4, pp. 560-568, 2008.

References

[78] A. Sanllorente, C. Lassale, M. T. Soria Florido, O. Castañer, M. Fitó, and Á. Hernáez, "Modification of High-Density Lipoprotein Functions by Diet and Other Lifestyle Changes: A Systematic Review of Randomized Controlled Trials," *Journal of Clinical Medicine*, vol. 10, p. 5897, 2021.

[79] Y. B. Sverrisdottir, T. Mogren, J. Kataoka, P. O. Janson, and E. Stener-Victorin, "Is polycystic ovary syndrome associated with high sympathetic nerve activity and size at birth?," *American Journal of Physiology-Endocrinology and Metabolism*, vol. 294, no. 3, pp. E576-E581, 2008.

[80] E. Diamanti-Kandarakis, A. G. Papavassiliou, S. A. Kandarakis, and G. P. Chrousos, "Pathophysiology and types of dyslipidemia in PCOS," *Trends in Endocrinology & Metabolism*, vol. 18, no. 7, pp. 280-285, 2007.

[81] T. E. R. F. Coalition, "Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies," *Journal of Vascular Surgery*, vol. 53, no. 2, pp. 548-549, 2011.

[82] C. Held, H. D. White, R. A. Stewart, A. Budaj, C. P. Cannon, J. S. Hochman, *et al.*, "Inflammatory biomarkers interleukin-6 and C-reactive protein and outcomes in stable coronary heart disease: experiences from the Stability (stabilization of atherosclerotic plaque by initiation of darapladib therapy) trial," *Journal of the American Heart Association*, vol. 6, no. 10, p. e005077, 2017.

[83] T. A. Pearson, G. A. Mensah, R. W. Alexander, J. L. Anderson, R. O. Cannon III, M. Criqui, *et al.*, "Markers of inflammation and cardiovascular disease: application to clinical and public health

practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association," *circulation*, vol. 107, no. 3, pp. 499-511, 2003.

[84] J. B. Moore and C. H. June, "Cytokine release syndrome in severe COVID-19," *Science*, vol. 368, no. 6490, pp. 473-474, 2020.

[85] Y. Ohsugi, "The immunobiology of humanized Anti-IL6 receptor antibody: From basic research to breakthrough medicine," *Journal of Translational Autoimmunity*, vol. 3, p. 100030, 2020.

[86] P. Uciechowski and W. C. Dempke, "Interleukin-6: a masterplayer in the cytokine network," *Oncology*, vol. 98, no. 3, pp. 131-137, 2020.

[87] J. Scheller, C. Garbers, and S. Rose-John, "Interleukin-6: from basic biology to selective blockade of pro-inflammatory activities," in *Seminars in immunology*, vol. 26, no. 1: Elsevier, pp. 2-12,2014.

[88] S. A. Jones and B. J. Jenkins, "Recent insights into targeting the IL-6 cytokine family in inflammatory diseases and cancer," *Nature reviews immunology*, vol. 18, no. 12, pp. 773-789, 2018.

[89] C. Toumpanakis, R. A. Standish, E. Baishnab, M. C. Winslet, and M. E. Caplin, "Goblet cell carcinoid tumors (adenocarcinoid) of the appendix," *Diseases of the colon & rectum*, vol. 50, pp. 315-322, 2007.

[90] S. Ferrari, L. Ahn-Luong, P. Garnero, S. Humphries, and S. Greenspan, "Two promoter polymorphisms regulating interleukin-6 gene expression are associated with circulating levels of C-reactive protein and markers of bone resorption in postmenopausal women," *The Journal of Clinical Endocrinology & Metabolism*, vol. 88, no. 1, pp. 255-259, 2003.

References

[91] X. Li, H. Huang, D. Ma, M. Zhu, and J. Lin, "Correlations between adipocytokines and insulin resistance in women with polycystic ovary syndrome," *Zhonghua yi xue za zhi,* vol. 89, no. 37, pp. 2607-2610, 2009.

[92] K. A. Toulis, D. G. Goulis, G. Mintziori, E. Kintiraki, E. Eukarpidis, S.-A. Mouratoglou, *et al.*, "Meta-analysis of cardiovascular disease risk markers in women with polycystic ovary syndrome," *Human reproduction update,* vol. 17, no. 6, pp. 741-760, 2011.

[93] Z. Peng, Y. Sun, X. Lv, H. Zhang, C. Liu, and S. Dai, "Interleukin-6 levels in women with polycystic ovary syndrome: a systematic review and meta-analysis," *PLoS One,* vol. 11, no. 2, p. e0148531, 2016.

[94] A. N. Vgontzas, G. Trakada, E. O. Bixler, H.-M. Lin, S. Pejovic, E. Zoumakis, *et al.*, "Plasma interleukin 6 levels are elevated in polycystic ovary syndrome independently of obesity or sleep apnea," *Metabolism,* vol. 55, no. 8, pp. 1076-1082, 2006.

[95] İ. Tarkun, B. Çetinarslan, E. Türemen, Z. Cantürk, and M. Biyikli, "Association between circulating tumor necrosis factor-alpha, interleukin-6, and insulin resistance in normal-weight women with polycystic ovary syndrome," *Metabolic syndrome and related disorders,* vol. 4, no. 2, pp. 122-128, 2006.

[96] M. Küçük, S. Ö. Altınkaya, S. Nergiz, S. D. Sezer, H. Yüksel, İ. Bağlı, *et al.*, "Interleukin-6 levels in relation with hormonal and metabolic profile in patients with polycystic ovary syndrome," *Gynecological Endocrinology,* vol. 30, no. 6, pp. 423-427, 2014.

[97] M. Moutachakkir, A. Baraou, A. Boukhira, and S. Chellak, "Immunoanalytical characteristics of C-reactive protein and high

sensitivity C-reactive protein," in *Annales de biologie clinique*, 2017, vol. 75, no. 2, pp. 225-229.

[98] J. E. Pope and E. H. Choy, "C-reactive protein and implications in rheumatoid arthritis and associated comorbidities," *Seminars in Arthritis and Rheumatism,* vol. 51, no. 1, pp. 219-229, 2021/02/01/ 2021.

[99] B. Baydaa Hussien, "Salivary High Sensitive C-Reactive Protein and Gingival Health Status among a Group of Women with Polycystic Ovary Syndrome," *MUSTANSIRIA DENTAL JOURNAL,* vol. 13, no. 1, 2016.

[100] P. M. Ridker, "A test in context: high-sensitivity C-reactive protein," *Journal of the American College of Cardiology,* vol. 67, no. 6, pp. 712-723, 2016.

[101] S. S. Bassuk, N. Rifai, and P. M. Ridker, "High-sensitivity C-reactive protein: Clinical importance," *Current Problems in Cardiology,* vol. 29, no. 8, pp. 439-493, 2004.

[102] D. Mohajan and H. K. Mohajan, "Body Mass Index (BMI) is a Popular Anthropometric Tool to Measure Obesity among Adults," *Journal of Innovations in Medical Research,* vol. 2, no. 4, pp. 25-33, 2023.

[103] R. Manikkumar, D. D. Roy, V. Krishnan, and T. Vijayakumar, "Association of DNA damage and dyslipidemia with polycystic ovarian syndrome," *Journal of Medical & Allied Sciences,* vol. 3, no. 1, 2013.

[104] R. A. Wild, M. Rizzo, S. Clifton, and E. Carmina, "Lipid levels in polycystic ovary syndrome: systematic review and meta-analysis," *Fertility and sterility,* vol. 95, no. 3, pp. 1073-1079. e11, 2011.

References

[105] R. A. Wild, E. Carmina, E. Diamanti-Kandarakis, A. Dokras, H. F. Escobar-Morreale, W. Futterweit, *et al.*, "Assessment of Cardiovascular Risk and Prevention of Cardiovascular Disease in Women with the Polycystic Ovary Syndrome: A Consensus Statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society," *The Journal of Clinical Endocrinology & Metabolism,* vol. 95, no. 5, pp. 2038-2049, 2010.

[106] C. I. Enechukwu, A. J. Onuegbu, M. J. Olisekodiaka, G. U. Eleje, J. I. Ikechebelu, J. O. Ugboaja, *et al.*, "Oxidative stress markers and lipid profiles of patients with polycystic ovary syndrome in a Nigerian tertiary hospital," *Obstetrics & Gynecology Science,* vol. 62, no. 5, pp. 335-343, 2019.

[107] V. Pergialiotis, E. Trakakis, C. Chrelias, N. Papantoniou, and E. Hatziagelaki, "The impact of mild hypercholesterolemia on glycemic and hormonal profiles, menstrual characteristics and the ovarian morphology of women with polycystic ovarian syndrome," *Hormone Molecular Biology And Clinical Investigation,* vol. 34, no. 3, 2018.

[108] R. Patel and G. Shah, "High-fat diet exposure from pre-pubertal age induces polycystic ovary syndrome (PCOS) in rats," *Reproduction,* vol. 155, no. 2, pp. 139-149, 2018.

[109] D. E. Broughton and K. H. Moley, "Obesity and female infertility: potential mediators of obesity's impact," *Fertility and sterility,* vol. 107, no. 4, pp. 840-847, 2017.

[110] M. Spałkowska, S. Mrozińska, A. Gałuszka-Bednarczyk, K. Gosztyła, A. Przywara, J. Guzik, *et al.*, "The PCOS patients differ in lipid profile according to their phenotypes," *Experimental and Clinical Endocrinology & Diabetes,* vol. 126, no. 07, pp. 437-444, 2018.

References

[111] J.-X. Pan, Y.-J. Tan, F.-F. Wang, N.-N. Hou, Y.-Q. Xiang, J.-Y. Zhang, *et al.*, "Aberrant expression and DNA methylation of lipid metabolism genes in PCOS: a new insight into its pathogenesis," *Clinical Epigenetics*, vol. 10, pp. 1-12, 2018.

[112] L. Mosca, "Guidelines for prevention of cardiovascular disease in women: a summary of recommendations," *Preventive Cardiology*, vol. 10, pp. 19-25, 2007.

[113] A. C. o. P. Bulletins-Gynecology, "ACOG Practice Bulletin no. 108: Polycystic ovary syndrome," *Obstet Gynecol*, vol. 114, no. 4, pp. 936-949, 2009.

[114] A. M. Ahmed Alobaidi AH, Ahmad SS, Alsamarai AM., "Dyslipidemia and oxidative stress in Iraqi women with poly cystic ovary syndroms," *World J Pharm Pharm Sci*, vol. 4, no. 09, 39-48 2015.

[115] J. J. Kim and Y. M. Choi, "Dyslipidemia in women with polycystic ovary syndrome," *Obstetrics & gynecology science*, vol. 56, no. 3, pp. 137-142, 2013.

[116] S. S. Lim, M. Davies, R. J. Norman, and L. Moran, "Overweight, obesity and central obesity in women with polycystic ovary syndrome: a systematic review and meta-analysis," *Human reproduction update*, vol. 18, no. 6, pp. 618-637, 2012.

[117] Y.-l. Xiong, X.-y. Liang, X. Yang, Y. Li, and L.-n. Wei, "Low-grade chronic inflammation in the peripheral blood and ovaries of women with polycystic ovarian syndrome," *European Journal of Obstetrics & Gynecology and Reproductive Biology*, vol. 159, no. 1, pp. 148-150, 2011.

References

[118] F. González, R. V. Considine, O. A. Abdelhadi, and A. J. Acton, "Inflammation triggered by saturated fat ingestion is linked to insulin resistance and hyperandrogenism in polycystic ovary syndrome," *The Journal of Clinical Endocrinology & Metabolism*, vol. 105, no. 6, pp. e2152-e2167, 2020.

[119] F. Gonzalez, "Nutrient-induced inflammation in polycystic ovary syndrome: role in the development of metabolic aberration and ovarian dysfunction," in *Seminars in reproductive medicine*, vol. 33, no. 04,pp. 276-286, 2015.

[120] M. A. Ganie, S. Hassan, S. Nisar, N. Shamas, A. Rashid, I. Ahmed, *et al.*, "High-sensitivity C-reactive protein (hs-CRP) levels and its relationship with components of polycystic ovary syndrome in Indian adolescent women with polycystic ovary syndrome (PCOS)," *Gynecological Endocrinology*, vol. 30, no. 11, pp. 781-784, 2014.

[121] F. Tosi, R. Dorizzi, R. Castello, C. Maffeis, G. Spiazzi, G. Zoppini, *et al.*, "Body fat and insulin resistance independently predict increased serum C-reactive protein in hyperandrogenic women with polycystic ovary syndrome," *European Journal of Endocrinology*, vol. 161, no. 5, pp. 737-745, 2009.

[122] R. A. Jasim, M. A. Umran, and E. H. Humadi, "Correlation Between Serum Interleukinslevels with Anthropometric Data and Lipid Profiles in Obese Iraqi Women With Polycystic Ovary Syndrome," *Iraqi Journal of Science*, vol. 61, no. 1, pp. 68-76, 2020.

[123] S. Sohaei, R. Amani, M. J. Tarrahi, and H. Ghasemi-Tehrani, "The effects of curcumin supplementation on glycemic status, lipid profile and hs-CRP levels in overweight/obese women with polycystic ovary syndrome: A randomized, double-blind, placebo-controlled clinical

trial," *Complementary therapies in medicine,* vol. 47, p. 102201, 2019.

[124] L. L. Lehrskov and R. H. Christensen, "The role of interleukin-6 in glucose homeostasis and lipid metabolism," in *Seminars in Immunopathology*, vol. 41, no. 4, pp. 491-499, 2019.

[125] J.-P. Bastard, C. Jardel, J. Delattre, B. Hainque, E. Bruckert, and F. Oberlin, "Evidence for a link between adipose tissue interleukin-6 content and serum C-reactive protein concentrations in obese subjects," *Circulation,* 1999.

[126] J.-M. Fernandez-Real, M. Vayreda, C. Richart, C. Gutierrez, M. Broch, J. Vendrell, *et al.*, "Circulating interleukin 6 levels, blood pressure, and insulin sensitivity in apparently healthy men and women," *The Journal of Clinical Endocrinology & Metabolism*, vol. 86, no. 3, pp. 1154-1159, 2001.

[127] E. M. Alissa, S. A. Algarni, A. J. Khaffji, and N. M. Al Mansouri, "Impact of interlukin-6 on central obesity measures in women with polycystic ovarian syndrome," *Journal of Obstetrics and Gynaecology*, vol. 40, no. 8, pp. 1133-1137, 2020.

[128] S. Goswami, S. Choudhuri, B. Bhattacharya, R. Bhattacharjee, A. Roy, S. Mukhopadhyay, *et al.*, "Chronic inflammation in polycystic ovary syndrome: A case–control study using multiple markers," *International Journal of Reproductive BioMedicine*, vol. 19, no. 4, p. 313, 2021.

[129] S. S. Al-Shattawi, E. F. Al-Jumili, and M. A. Al-Azzam, "The relationship between obesity and polycystic ovary syndrome in a sample of Iraqi infertile women," *Iraqi journal of biotechnology,* vol. 17, no. 3, 2018.

References

[130] H. F. Escobar-Morreale, M. Luque-Ramírez, and F. González, "Circulating inflammatory markers in polycystic ovary syndrome: a systematic review and metaanalysis," *Fertility and sterility*, vol. 95, no. 3, pp. 1048-1058. e2, 2011.

[131] P. Waldmann, "On the use of the Pearson correlation coefficient for model evaluation in genome-wide prediction," *Frontiers in genetics*, vol. 10, p. 899, 2019.

[132] E. Bozkurt, S. Beysel, A. Vurmaz, E. Atay, S. Gokaslan, and A. Ertekin, "Evaluation of relationship among polycystic ovary syndrome, atherogenic index of plasma, and high sensitive C reactive protein," 2021.

[133] D. A. Swastini, I. A. D. Wiryanthini, N. L. P. Ariastuti, and A. Muliantara, "Atherosclerosis prediction with high sensitivity C-reactive protein (hs-CRP) and related risk factor in patient with dyslipidemia," *Open access Macedonian journal of medical sciences*, vol. 7, no. 22, p. 3887, 2019.

[134] S. Ishrat and M. Hussain, "Prevalence of Insulin Resistance, Dyslipidemia and Metabolic Syndrome in Infertile Women with Polycystic Ovary Syndrome," *Journal of Bangladesh College of Physicians and Surgeons*, vol. 39, no. 4, pp. 225-232, 2021.

[135] J. Lord, R. Thomas, B. Fox, U. Acharya, and T. Wilkin, "The central issue? Visceral fat mass is a good marker of insulin resistance and metabolic disturbance in women with polycystic ovary syndrome," *BJOG: An International Journal of Obstetrics & Gynaecology*, vol. 113, no. 10, pp. 1203-1209, 2006.

[136] Y. Liu, M. Du, Y. Gan, S. Bao, L. Feng, and J. Zhang, "Triglyceride Induced Metabolic Inflammation: Potential Connection of Insulin

Resistance and Recurrent Pregnancy Loss," *Frontiers in Endocrinology*, vol. 12, p. 621845, 2021.

[137] C. C. Nkonpawa, V. J. A. Moor, A. T. Tankeu, A. S. Momo, G. S. Wafeu, F. Amazia, *et al.*, "Inflammation and Insulin Resistance in a Group of Sub-Saharan African Women with Polycystic Ovary Syndrome," *Journal of Inflammation Research,* vol. 14, p. 4643, 2021.

[138] E. Rudnicka, M. Kunicki, K. Suchta, P. Machura, M. Grymowicz, and R. Smolarczyk, "Inflammatory markers in women with polycystic ovary syndrome," *BioMed research international*, vol. 220, 2020.

[139] P. M. Spritzer, S. B. Lecke, F. Satler, and D. M. Morsch, "Adipose tissue dysfunction, adipokines, and low-grade chronic inflammation in polycystic ovary syndrome," *Reproduction*, vol. 149, no. 5, pp. R219-R227, 2015.

[140] S. Black, I. Kushner, and D. Samols, "C-reactive protein," *Journal of Biological Chemistry*, vol. 279, no. 47, pp. 48487-48490, 2004.

[141] W. Hu, J. Qiao, Y. Yang, L. Wang, and R. Li, "Elevated C-reactive protein and monocyte chemoattractant protein-1 in patients with polycystic ovary syndrome," *European Journal of Obstetrics & Gynecology and Reproductive Biology*, vol. 157, no. 1, pp. 53-56, 2011.

[142] V. B. Matthews, T. L. Allen, S. Risis, M. H. Chan, D. C. Henstridge, N. Watson, *et al.*, "Interleukin-6-deficient mice develop hepatic inflammation and systemic insulin resistance,"*Diabetologia,* vol. 53, no. 11, pp. 2431-41, 2010.

References

[143] E. Pavlik, P. DePriest, H. Gallion, F. Ueland, M. Reedy, R. Kryscio, *et al.*, "Ovarian volume related to age," *Gynecologic oncology*, vol. 77, no. 3, pp. 410-412, 2000.

[144] M. P. Lauritsen, J. Bentzen, A. Pinborg, A. Loft, J. Forman, L. Thuesen, *et al.*, "The prevalence of polycystic ovary syndrome in a normal population according to the Rotterdam criteria versus revised criteria including anti-Müllerian hormone," *Human reproduction*, vol. 29, no. 4, pp. 791-801, 2014.

[145] V. A. Kushnir, N. Halevy, D. H. Barad, D. F. Albertini, and N. Gleicher, "Relative importance of AMH and androgens changes with aging among non-obese women with polycystic ovary syndrome," *Journal of ovarian research*, vol. 8, no. 1, pp. 1-7, 2015.

[146] E. Carmina, A. M. Campagna, and R. A. Lobo, "A 20-year follow-up of young women with polycystic ovary syndrome," *Obstetrics & Gynecology*, vol. 119, no. 2 , pp. 263-269, 2012.

[147] S.-J. Liang, C.-S. Hsu, C.-R. Tzeng, C.-H. Chen, and M.-I. Hsu, "Clinical and biochemical presentation of polycystic ovary syndrome in women between the ages of 20 and 40," *Human Reproduction*, vol. 26, no. 12, pp. 3443-3449, 2011.

[148] H. J. Teede, M. L. Misso, M. F. Costello, A. Dokras, J. Laven, L. Moran, *et al.*, "Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome," *Human reproduction*, vol. 33, no. 9, pp. 1602-1618, 2018.

[149] Z. Wang, X. Wang, Z. Chen, L. Zhang, and M. Zhu, "Distribution of high-sensitivity C-reactive protein and its relationship with other cardiovascular risk factors in the middle-aged Chinese population,"

International journal of environmental research and public health, vol. 13, no. 9, p. 872, 2016.

[150] O. Osibogun, O. Ogunmoroti, and E. D. Michos, "Polycystic ovary syndrome and cardiometabolic risk: Opportunities for cardiovascular disease prevention," *Trends in Cardiovascular Medicine,* vol. 30, no. 7, pp. 399-404, 2020.

[151] B. M. Bettcher, C. L. Watson, C. M. Walsh, I. V. Lobach, J. Neuhaus, J. W. Miller, *et al.*, "Interleukin-6, age, and corpus callosum integrity," *PLoS One,* vol. 9, no. 9, p. e106521, 2014.

[152] D. Albani, S. Batelli, L. Polito, F. Prato, M. Pesaresi, G. B. Gajo, *et al.*, "Interleukin-6 plasma level increases with age in an Italian elderly population ("The Treviso Longeva"–Trelong–study) with a sex-specific contribution of rs1800795 polymorphism," *Age,* vol. 31, pp. 155-162, 2009.

[153] M. Y. Abeywardena, W. R. Leifert, K. E. Warnes, J. N. Varghese, and R. J. Head, "Cardiovascular biology of interleukin-6," *Current pharmaceutical design,* vol. 15, no. 15, pp. 1809-1821, 2009.

[154] N. S. Jenny, B. French, A. M. Arnold, E. S. Strotmeyer, M. Cushman, P. H. Chaves, *et al.*, "Long-term assessment of inflammation and healthy aging in late life: the Cardiovascular Health Study All Stars," *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences,* vol. 67, no. 9, pp. 970-976, 2012.

[155] S. P. Mooijaart, N. Sattar, S. Trompet, J. Lucke, D. J. Stott, I. Ford, *et al.*, "Circulating interleukin-6 concentration and cognitive decline in old age: the PROSPER study," *Journal of internal medicine,* vol. 274, no. 1, pp. 77-85, 2013.

References

[156] A. A. Beharka, M. Meydani, D. Wu, L. S. Leka, A. Meydani, and S. N. Meydani, "Interleukin-6 Production Does Not Increase With Age," *The Journals of Gerontology: Series A,* vol. 56, no. 2, pp. B81-B88, 2001.

[157] J. W. Rowe, K. L. Minaker, J. A. Pallotta, and J. S. Flier, "Characterization of the insulin resistance of aging," *The Journal of clinical investigation,* vol. 71, no. 6, pp. 1581-1587, 1983.

[158] F. Ramezani Tehrani, M. Solaymani-Dodaran, M. Hedayati, and F. Azizi, "Is polycystic ovary syndrome an exception for reproductive aging?," *Human Reproduction,* vol. 25, no. 7, pp. 1775-1781, 2010.

[159] D. Panidis, K. Tziomalos, G. Misichronis, E. Papadakis, G. Betsas, I. Katsikis, *et al.*, "Insulin resistance and endocrine characteristics of the different phenotypes of polycystic ovary syndrome: a prospective study," *Human reproduction,* vol. 27, no. 2, pp. 541-549, 2012.

[160] T. M. Barber and S. Franks, "Obesity and polycystic ovary syndrome," *Clinical endocrinology,* vol. 95, no. 4, pp. 531-541, 2021.

[161] T. M. Barber, P. Hanson, M. O. Weickert, and S. Franks, "Obesity and polycystic ovary syndrome: implications for pathogenesis and novel management strategies," *Clinical Medicine Insights: Reproductive Health,* vol. 13, p. 117, 2019.

[162] C. J. Glueck and N. Goldenberg, "Characteristics of obesity in polycystic ovary syndrome: Etiology, treatment, and genetics," *Metabolism,* vol. 92, pp. 108-120, 2019.

[163] L. Zhou, Z. Ni, J. Yu, W. Cheng, Z. Cai, and C. Yu, "Correlation between fecal metabolomics and gut microbiota in obesity and polycystic ovary syndrome," *Frontiers in Endocrinology,* vol. 11, p. 628, 2020.

References

[164] F. Al-Tu'ma, N. HadiFarhan, and W. G. Al-Safi, "Association between fat mass and obesity Geners polymorphism with PCOS women in Iraqi population," *Ijppr. Human,* vol. 5, no. 1, pp. 62-72, 2015.

[165] L. Niepolski and A. E. Grzegorzewska, "Salusins and adropin: new peptides potentially involved in lipid metabolism and atherosclerosis," *Advances in medical sciences,* vol. 61, no. 2, pp. 282-287, 2016.

[166] M. L. Sánchez-Ferrer, E. Adoamnei, M. T. Prieto-Sánchez, J. Mendiola, S. Corbalán-Biyang, M. Moñino-García, *et al.*, "Health-related quality of life in women with polycystic ovary syndrome attending to a tertiary hospital in Southeastern Spain: a case-control study," *Health and quality of life outcomes,* vol. 18, pp. 1-10, 2020.

[167] D. Glintborg, "Endocrine and metabolic characteristics in polycystic ovary syndrome," 2015.

[168] D. Macut, V. Mladenović, J. Bjekić-Macut, S. Livadas, O. Stanojlović, D. Hrnčić, *et al.*, "Hypertension in polycystic ovary syndrome: novel insights," *Current hypertension reviews,* vol. 16, no. 1, pp. 55-60, 2020.

[169]J. R. Mellembakken, A. Mahmoudan, L. Mørkrid, I. Sundström-Poromaa, L. Morin-Papunen, J. S. Tapanainen, *et al.*, "Higher blood pressure in normal weight women with PCOS compared to controls," *Endocrine Connections,* vol. 10, no. 2, p. 154, 2021.

[170] S. Özkan, Ö. Ç. Yılmaz, and B. Yavuz, "Increased masked hypertension prevalence in patients with polycystic ovary syndrome (PCOS)," *Clinical and Experimental Hypertension,* vol. 42, no. 8, pp. 681-684, 2020.

References

[171] D. Glintborg, K. H. Rubin, M. Nybo, B. Abrahamsen, and M. Andersen, "Cardiovascular disease in a nationwide population of Danish women with polycystic ovary syndrome," *Cardiovascular diabetology*, vol. 17, pp. 1-12, 2018.

[172] M.-M. E. Ollila, K. Kaikkonen, M.-R. Järvelin, H. V. Huikuri, J. S. Tapanainen, S. Franks, *et al.*, "Self-reported polycystic ovary syndrome is associated with hypertension: a northern Finland birth cohort 1966 study," *The Journal of Clinical Endocrinology & Metabolism*, vol. 104, no. 4, pp. 1221-1231, 2019.

[173] S. Lim, N. Kakoly, J. Tan, G. Fitzgerald, M. Bahri Khomami, A. Joham, *et al*., "Metabolic syndrome in polycystic ovary syndrome: a systematic review, meta-analysis and meta-regression," *Obesity reviews*, vol. 20, no. 2, pp. 339-352, 2019.

[174] J.-H. Hung, L.-Y. Hu, S.-J. Tsai, A. C. Yang, M.-W. Huang, P.-M. Chen, *et al.*, "Risk of psychiatric disorders following polycystic ovary syndrome: a nationwide population-based cohort study," *PLoS One*, vol. 9, no. 5, p. e97041, 2014.

الخلاصة

تعد متلازمة تكيس المبايض (PCOS) واحدة من أكثر أمراض التمثيل الغذائي والتناسلية انتشارًا. تعد عدم انتظام الدورة الشهرية وزيادة الأندروجين من الأعراض التي تعاني منها النساء المصابات بمتلازمة تكيس المبايض، ولهذه الأعراض تأثير سلبي كبير على نوعية حياتهن. قد يكون لديهم فرصة أكبر للإصابة بعدد من الأمراض، مثل السمنة ومرض السكري من النوع الثاني وأمراض القلب والأوعية الدموية والعقم والسرطان وقضايا الصحة العقلية ومقاومة الأنسولين. مقاومة الأنسولين (IR) هو فشل في التحكم بوساطة الأنسولين في أيض الكلوكوز في الأنسجة، وهو الشكل الأكثر شيوعًا من متلازمة تكيس المبايض، ويتسبب في إنتاج المزيد من الأندروجين، مما يؤدي إلى قلة الإباضة أو عدم التبويض.

صممت هذه الدراسة لتقييم مستويات بعض العوامل الالتهابية في متلازمة تكيس المبايض ومرضى مقاومة الأنسولين. اشتملت الدراسة على 80 امرأة تتراوح أعمارهن بين 16 و40 عامًا، مقسمين إلى 40 مريضة مصابات بتكيس المبايض ومقاومة الأنسولين، و40 امرأة كمجموعة تحكم صحية، ثم تم قياس كل من الدالاتالحيوية التالية LDL وHDLوTriglycerideوCholesterol باستخدام جهاز BS-430 من شركةMindray، بينما تم قياس البروتين التفاعلي C عالي الحساسية باستخدامBS-200من شركةMindray. وتم قياس IL-6 باستخدام MAGLUMI 800 من شركة Snibe. أظهرت نتائج التحليل الإحصائي وجود فرق معنوي فيHS-CRP,IL-6 ,HDL,TG,Cholesterol,LDLفي النساء المريضات مقارنة بالمجموعة الضابطة ، حيث ارتفع كل من Triglyceride,LDL , Cholesterol, IL-6 ,HS-CRP وانخفض HDL في مجموعة المريضات. وتم العثور على ارتباط إيجابي بين Triglyceride وكل من HS-CRP،IL-6 ومقاومة الأنسولين، وإلى ارتباط سلبي بين HDL وIL-6، وعلاقة إيجابية بين كل منHS-CRP وIL-6، وقد أظهر أيضًا أن النساء المريضات الاتي اعمارهن فوق 30 عام قد زادت لديهم مستوياتCholesterol، LDL, IL-6 ,HS-CRP مقارنةً بالنساء المريضات الذين تقل أعمارهم عن 30 عامًا. بينما لا توجد فرقمعنوي كبير في دالات الحيوية ،Triglyceride HDL ومقاومة الأنسولين فيما يتعلق بعمر المريضات. كما أظهرت النتائج وجود علاقة

معنوية في مؤشر كتلة الجسم في مجموعتي الدراسة، وكانت السمنة أكثر بروزًا في مجموعة المريضات . وكذلك أظهر تأثير ضغط الدم وله قيمة معنوية عند مقارنة بين المجموعتين، ولم تظهر علاقة معنوية بينHS-CRP، IL-6 ومقاومة الأنسولين ومدة المرض.

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء

كلية التربية للعلوم الصرفة

قسم الكيمياء

تقييم مستويات بعض عوامل الالتهابات في مريضات متلازمة تكيس المبايض ومقاومة الأنسولين

رسالة

مقدمة إلى كلية التربية للعلوم الصرفة\جامعة كربلاء كجزء من متطلبات الحصول على درجة الماجستير في الكيمياء

من قبل

زهراء عماد حسين

بأشراف

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دكتوراه في الكيمياء الحياتيةبورد في النسائية والتوليد والعقم

1445هـ2023م