Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Kerbala
College of Medicine
Department of Chemistry and Biochemistry

Role of Adiponectin and Oxidative Status in The Pathogenesis of Polycystic Ovarian Syndrome in Iraqi Women

A Thesis

Submitted to the Council of the College of Medicine, University of Kerbala in Partial Fulfillment of the Requirements for the master's degree in

[Clinical Chemistry]

By

Yasmeen Imad Kadhum

B.Sc. in Clinical Laboratories-College of Applied Medical Sciences

University of Kerbala / 2017

Supervised by

Prof. Dr

Fadhil Jawad Al-Tu'ma

College of Medicine

University of Kerbala

2024 A.D.

Assist. Prof. Dr

Nora Sabah Rasoul

College of Medicine

University of Kerbala

1445 H.D.

بسم الله الرحمن الرحيم

وَيَسْأَلُونَكَ
عَنِ الرُّوحِ ۖ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُمْ مِنَ
الْعِلْمِ إِلَّا قَلِيلًا ﴿85﴾

صدق الله العلي العظيم

سورة الأسراء – الآية 85

Supervisor Certification

we certify that this thesis entitled

Role of Adiponectin and Oxidative Status in The Pathogenesis of Polycystic Ovarian Syndrome in Iraqi Women

was prepared by (**Yasmeen Imad Kadhum**) under our supervision at the College of Medicine, University of Karbala, as a partial fulfillment of the requirement for the Degree of Master in (**Clinical Chemistry**).

Prof. Dr.

Fadhil Jawad Al-Tu'ma

Department of Chemistry and Biochemistry

College of Medicine

University of Kerbala

Assist. Prof. Dr.

Nora Sabal Rasoul

Department of Obstetrics and Gynecology

College of Medicine

University of Kerbala

Given the available recommendations, I forward this thesis to debate by the examining committee.

Assist. Prof. Dr. Atheer Hameed Al-Ganimi

Head of Chemistry and Biochemistry

Department College of Medicine – University of Kerbala

Examining committee certification

We, the examining committee, certify that we have read this M.Sc. thesis entitled:

(Role of Adiponectin and Oxidative Status in The Pathogenesis of Polycystic Ovarian Syndrome in Iraqi Women)

We have examined the postgraduate student **(Yasmeen Imad Kadhum)** in its M.Sc. thesis content and our opinion; it meets the standard for the degree of a master in **(Clinical Chemistry).**

Signature:

Assist. Prof. Dr. Maher Abbood Mukheef

College of Medicine

University of Kerbala

Date: / / 2024

(Member)

Signature:

Prof. Dr. Wasan Ghazi Alsafi

College of Medicine

University of Kerbala

Date: / / 2024

(Member)

Signature:

Prof. Dr. Fadhil Jawad Al-Tu'ma

College of Medicine

University of Kerbala

Date: 6 / 3 / 2024

(Member/Supervisor)

Signature:

Assist. Prof. Dr. Nora Sabah Rasoul

College of Medicine

University of Kerbala

Date: 5 / 3 / 2024

(Member/Supervisor)

Signature: Al-Rald

Prof. Dr. Omar F. Abdul- Rasheed

College of Medicine- Al-Nahrain University

Date: / / 2024

(Chairman)

Approved by the College of Medicine- University of Kerbala

Signature:

Date: / / 2024

Prof. Dr. Riyadh Dayhood Al-Zubaidi- Dean of the College of Medicine

Dedication

With love, I dedicate this work to my biggest supporter,

Father

and to my source of inspiration,

Mother

for my brother,

and all my lovely family

Yasameen Imad

Acknowledgments

First, and above all, thanks to the Great Merciful Allah who gave me health, strength, patience, perseverance and facilitated the ways for me to accomplish this work.

Secondly, I would like to thank the patients and all individuals, without their participation, this thesis would not have shown the light.

I would like to express my sincere gratitude to my advisor Prof. Dr. Fadhil Jawad Al-Tu'ma, professor of molecular and clinical biochemistry, College of Medicine, University of Kerbala for his continuous support, patience, motivation, and immense knowledge.

Special thanks then go to my second advisor Assist. Prof. Dr. Nora Sabah Rasoul, Head of the Department of Obstetrics and Gynecology, College of Medicine, University of Kerbala for her suggestions, support, advice, and encouragement.

Moreover, I would like to express my deep thanks to Assist. Prof. Dr. Atheer Hameid Odda, Assist. Prof. Dr. Rana M. Hameed, University of Kerbala, College of Medicine for their valuable help and advice.

Finally, thanks to all my colleagues in the Department of Chemistry and Biochemistry for their nice cooperation, for working together before deadlines, and for all the fun we have had for the last two years.

Yasameen

Summary

Polycystic ovary syndrome is one of the most common endocrine and metabolic disorders among women of reproductive age. Women suffering from PCOS present with a constellation of symptoms associated with menstrual disorder and androgen excess, which significantly impact their quality of life. They may be at increased risk of many abnormalities, including obesity, insulin resistance, type II diabetes mellitus, cardiovascular diseases (CVD), infertility, cancer, and psychological disorders.

Metabolic abnormalities, mainly insulin resistance are evident in a majority of affected individuals, especially among those women who also show obesity. Adiponectin is one of the most abundant adipokines, it plays an important role in energy metabolism and contributes to the pathogenesis of the metabolic syndrome.

Polycystic ovary syndrome is associated with oxidative stress, increased production of free radicals (Malondialdehyde) followed by decreased serum antioxidant levels (Catalase).

This study aimed to explore whether women with PCOS have any difference in adiponectin and oxidative stress levels compared to obese and non-obese control groups. Also, study the correlation between serum level of adiponectin and oxidative stress with other biochemical parameters like hormonal parameters and lipid profiles in PCOS patients.

A case-control study design conducted on 160 women age ranged between (18 – 40) years involving 80 PCOS patients subdivided according to their body mass index (BMI) into 40 obese (BMI≥30) and 40 non-obese (BMI<30) and 80 women as a control, also subdivided according to their body mass index (BMI) into 40 obese (BMI≥30) and 40 non-obese (BMI<30) at the infertility clinic of a gynecological and obstetric teaching hospital, Kerbala health directorate Iraq. Parameters concentration were measured at the College of Medicine, University of Kerbala/Iraq during the duration from Nov. 2022 to Mar. 2023. The Rotterdam criteria-2003 was used to

choose PCOS patients. Patients were interviewed and examined for weight, height, waist circumference, and hip circumference. Five milliliters of venous blood samples were collected in the early morning after an overnight fast. Fasting blood glucose and insulin, additionally to the hormonal levels of each of LH, FSH, Free testosterone, and prolactin were measured by the chemiluminescent automated immunoassay system. The lipid profile of each TC, TG, and HDL were measured by using a Spectrophotometric automatic analyzer (SMART-120). Insulin Resistance was assessed by calculating HOMA-IR using the formula (fasting glucose mg/dl × fasting insulin μU/ml)/405. The SPSS Statistics software, version 28.0 (IBM, SPSS, Chicago, Illinois, USA), was used for all statistical calculations.

The result of this study demonstrated significant elevations in LH concentrations ($p<0.001$), LH/FSH ratio ($p<0.001$) and free testosterone level ($p<0.001$), prolactin level ($p=0.004$), TC ($p=0.050$), TG ($p<0.001$), LDL($p=0.002$), HOMA-IR ($p<0.001$) and MDA level ($p=0.01$) have prevailed in the obese PCOS patient group when compared with the obese control group. While a significant decrease in FSH level ($P<0.001$), ADP level ($P<0.001$), and CAT level ($P<0.001$) during a comparable evaluation between obese PCOS patients and the obese control group.

The optimal diagnostic points for predicting PCOS by MDA were: (sensitivity 60 %, specificity 81.2%) at a level = 4.305 mmol/l. The present study concludes that MDA levels and HOMA-IR increased in PCOS patients while ADP and CAT levels decreased as compared with control.

List of contents

List of Tables

List of Figures

List of Abbreviations

Abbreviations	Full Nomenclature
apM1	Adipose Most abundant Gene transcript 1
AdipoR1	Adiponectin receptor 1
4-AAP	4-aminoantipyrine
AdipoQ	Adiponectin, C1Q, And Collagen Domain Containing
AdipoR2	Adiponectin receptor 2
ADP	Adiponectin
AGEs	Advanced glycation end-product
AMPK	AMP-activated protein kinase
ATP	Adenosine triphosphate
AUC	Area under curve
BMI	Body mass index
CAT	Catalase
CHE	Cholesterol esterase
CVD	Cardiovascular disease
DETBA	1,3-Diethyl-2-thiobarbituric acid
DNA	Deoxy ribonucleic acid
FBS	Fasting blood sugar
FSH	Follicle-stimulating hormone
GBP28	Gelatin-binding protein-28

GnRH	Gonadotrophin-releasing hormone
GK	Glycerol kinase
GPO	Glycerol phosphate oxidase
GPx	Glutathione peroxide
$H_2 O_2$	Hydrogen peroxide
HA	Hyperandrogenemia
HDL	High-density lipoprotein
HOMA-IR	homeostasis model assessment-estimated insulin resistance
IR	Insulin resistance
IRS-1	Insulin receptor substrate 1
LDL	Low-density lipoprotein
LH	Luteinizing hormone
LPO	Lipid peroxidation
MAPK-ERK	mitogen-activated protein kinases- extracellular-signal-regulated kinase
MDA	Malondialdehyde
OD	Ovary dysfunction
OS	Oxidative stress
P38-MAPK	P38 mitogen-activated protein kinase
PCOM	Polycystic ovary morphology
PCOS	Polycystic ovary syndrome
POD	Peroxidase

PPARα	Peroxisome proliferator-activated receptor α
PUFAs	Polyunsaturated fatty acids
RNS	Reactive nitrogen species
ROC	Receiver operating curve
ROS	Reactive oxygen species
SHBG	Sex hormone-binding globulin
SOD	Superoxide dismutase
TAC	Total antioxidant capacity
TBA	Tetramethylbenzidine
TBARS	Thiobarbituric acid reactive substances
TC	Total cholesterol
TCA	Trichloroacetic acid
TG	Triglyceride
Trx	Thioredoxin
TMB	Tetramethylbenzidine
TRIS	Tris aminomethane
VLDL	Very low-density lipoprotein
WHR	Waist hip ratio

Chapter One

Introduction and Literature Review

1. Introduction

Polycystic ovary syndrome (PCOS) is characterized by an unregulated menstrual cycle (oligomenorrhea or amenorrhea), elevated androgenic hormone levels (hyperandrogenism), and many cysts of the ovaries (polycystic ovaries). Other features include male pattern hirsutism, acne, increased skin pigmentation with tags sometimes, and obesity **(Zhang et al.,2020)**. Often comorbiditie with insulin resistance, dyslipidemia, and obesity, it also carries a significant risk for the development of cardiovascular and metabolic sequelae, including diabetes and metabolic syndrome (**Cincione et al.,2021).**

The existence of a fully developed hypothalamic–pituitary–ovarian axis and highly harmonized hormonal feedback circles are essential for the normal ovulatory menstrual cycle. This consists of three phases (follicular phase, ovulatory phase, and luteal phase), the normal menstrual cycle leads to the formation of a mature follicle and release of an oocyte during each cycle, without fertilization menses would occur **(triyeva,2022)**. Every woman of reproductive age exhibits an FSH level rise at the luteal–follicular transition, stimulating a group of follicular growth in the initial follicular phase. The dominant follicle is specified in the mid - follicular phase, and as this dominant follicle grows it progressively secretes inhibin A and oestradiol for a week before ovulation and a later LH surge and oestradiol rise. The progesterone, oestradiol, and inhibin A are secreted by the corpus luteum in response to LH pulses and arrive at their peak in level, vascularization, and secretions **(Mihm et al.,2011).**

1.1. Polycystic Ovary Syndrome

Polycystic Ovary Syndrome (PCOS) is one of the most common endocrine disorders in women of childbearing age **(Hoeger et al.,2021)**. It has been estimated that around 6–10% of women in the reproductive period are affected by this endocrinological disease considering the classical definition of the syndrome and the prevalence rises to 18–20% when using the Rotterdam criteria **(Rocha et al.,2017).**

PCOS is the most common cause of anovulatory infertility. In addition to infertility, patients with PCOS have greater rates of obesity, type 2 diabetes, metabolic syndrome, cardiovascular risk factors such as hypertension, poor body image, eating disorders, anxiety, and depression **(Westerman and Kuhnt, 2022).** The definition of this disorder as a syndrome, not a disease due to the presence of a cluster of symptoms that cannot be identified by a certain etiological factor or as a certificated pathophysiological axis, in addition, it is believed that hormones act on most of the body tissues but in a variant rate which results in a variety of clinical features due to hormonal disturbances and metabolic consequences **(Krug et al.,2019)**.

1.1.1. History and etiopathogenesis:

Stein and Leventhal initially described polycystic ovary syndrome history in 1935 as a mix of amenorrhea, infertility, ovaries that were enlarged, hirsutism, obesity, and persistent anovulation **(Stein and Leventhal,1935).** Before the 10th edition of the International Classification of Diseases was published in 1990, the World Health Organization (WHO) previously combined PCOS with sclerotic ovary- Stein Leventhal syndromes **(Organization,1992)**. The etiopathogenesis of PCOS is a complex genetic,

environmental, and lifestyle interaction. Hyperandrogenism and insulin resistance (IR) are the major characteristics of PCOS (**Mukherjee et al.,2023).** The shape of polycystic ovaries, elevated androgen levels, and insulin resistance with insulin secretion abnormalities are all heritable conditions as shown in **Figure (1.1) (Corrie et al.,2021).**

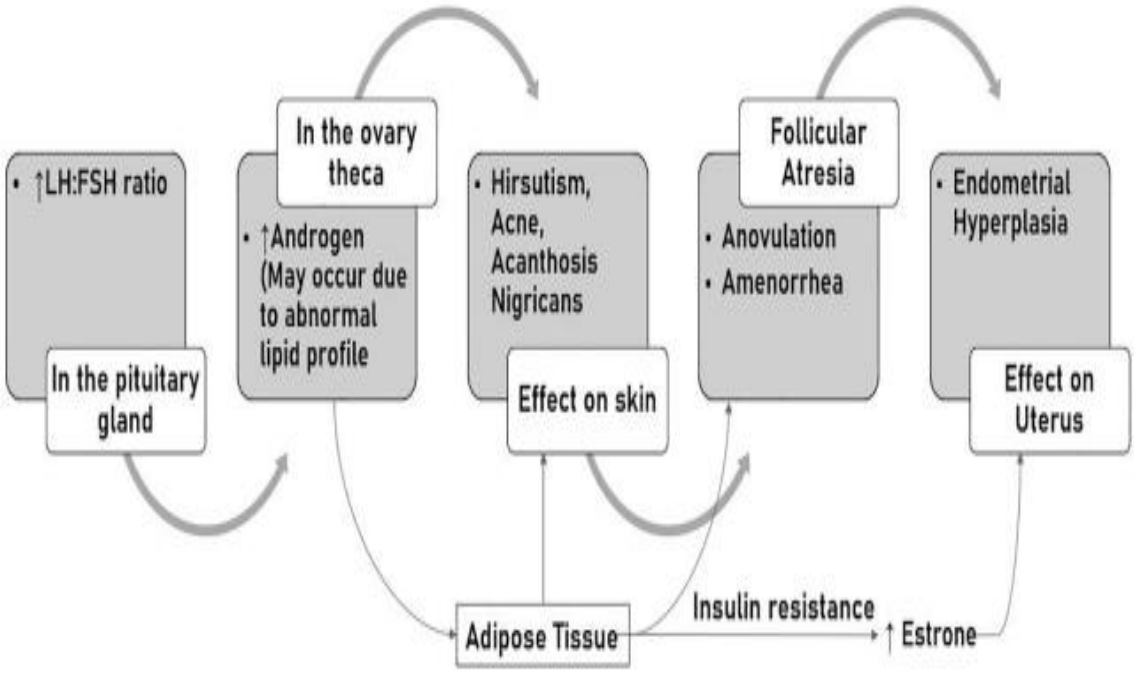


Figure (1.1): Etiopathogenesis of PCOS (Corrie et al.,2021).

Prenatal androgen exposure and the fetus's poor growth are environmental causes, while acquired obesity is a key postnatal issue. The diversity of pathways included and the absence of a common course lead to the multifactorial characteristics and heterogeneity of the syndrome **(Rosenfield and Ehrmann,2016).**

1.1.2. Hyperandrogenism and PCOS:

Hyperandrogenism is an important criterion for the diagnosis of PCOS. In patients with PCOS, the incidence of hyperandrogenism is as high as 60%-80%. Androgen hyperactivation leads to ovulation disorder, menstrual disorder, hair, and acne formation, suggesting that hyperandrogenism is a clinical characteristic of PCOS and an important risk factor **(Ye et al.,2021)**. Women with PCOS are most likely to progress to metabolic syndromes, such as diabetes mellitus type II and cardiovascular disease in the long term. uncontrolled ovarian steroidogenesis develops because of theca cell hyperplasia, which leads to the ovary being a primary source of androgen excess between PCOS women. Anovulation is progress as a result of androgen excess that developed because of the follicular arrest. There is a significant association of decrease maturation rates and developmental competency of oocytes with androgen excess **(Dadachanji et al.,2018)**.

Insulin resistance and hyperinsulinemia can develop endocrine and reproductive features of PCOS. Nevertheless, hyperandrogenemia may affect insulin action directly and/or through many changes that take place in different body sites especially muscle and adipose tissue. Furthermore, excess body fat and obesity itself can aggravate the whole condition as shown in **Figure (1.2) (Abasian et al.,2018)**. Anyhow, there are a lot of heartless circles in this process, with bidirectional relations between hyperandrogenemia, adipose dysfunction, insulin resistance, and different other factors such as inflammation and oxidative stress which crew the picture and make it extremely difficult to understand where the guilt lies **(Moghetti and Tosi,2021).**

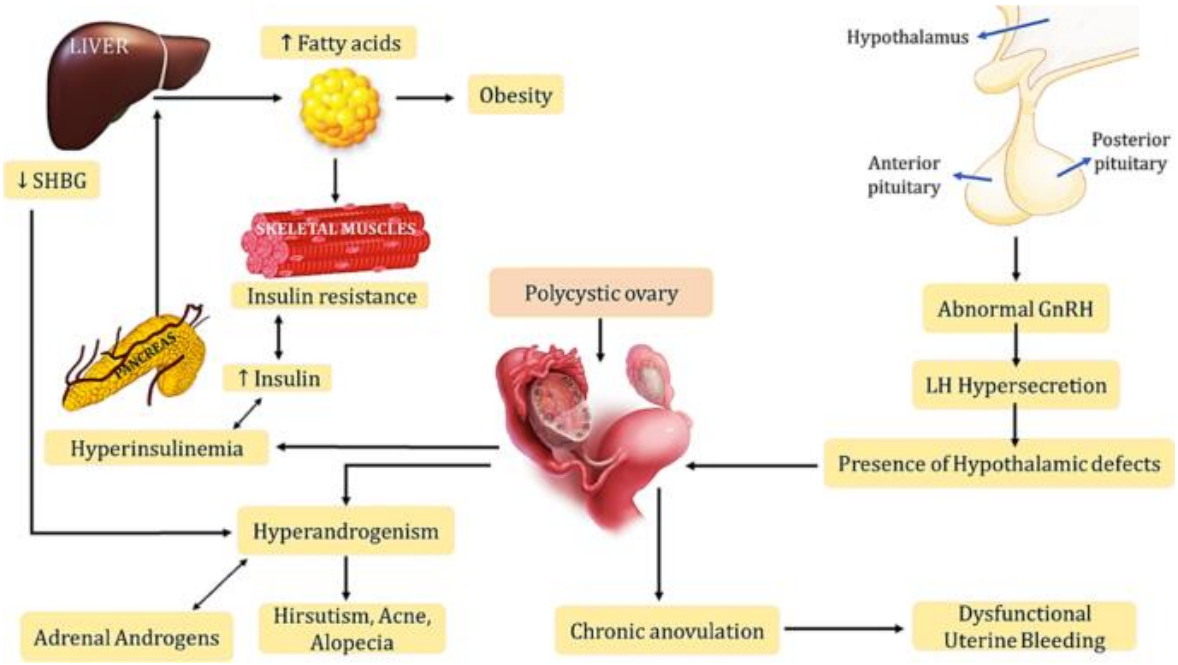


Figure (1.2): The main pathogenic factors in PCOS (Chaudhary et al., 2021).

1.1.3. Prevalence of polycystic ovarian syndrome

The main cause of infertility in Iraqi Arab females is PCOS, which represents about 46% of all infertility cases **(Saeed et al.,2021).** The PCOS prevalence seems to be increasing due to changes in lifestyle, nutrition, and obesity **(Fattah et al.,2021)**. Polycystic ovary is a syndrome without much popular acknowledgment and PCOS women frequently do not request care. It has also just been mentioned that even in the same ethnic group (i.e., Middle Eastern women), there is a huge difference in the prevalence of PCOS depending on different clinical symptoms **Figure (1.3) (Abasian et al.,2018).**

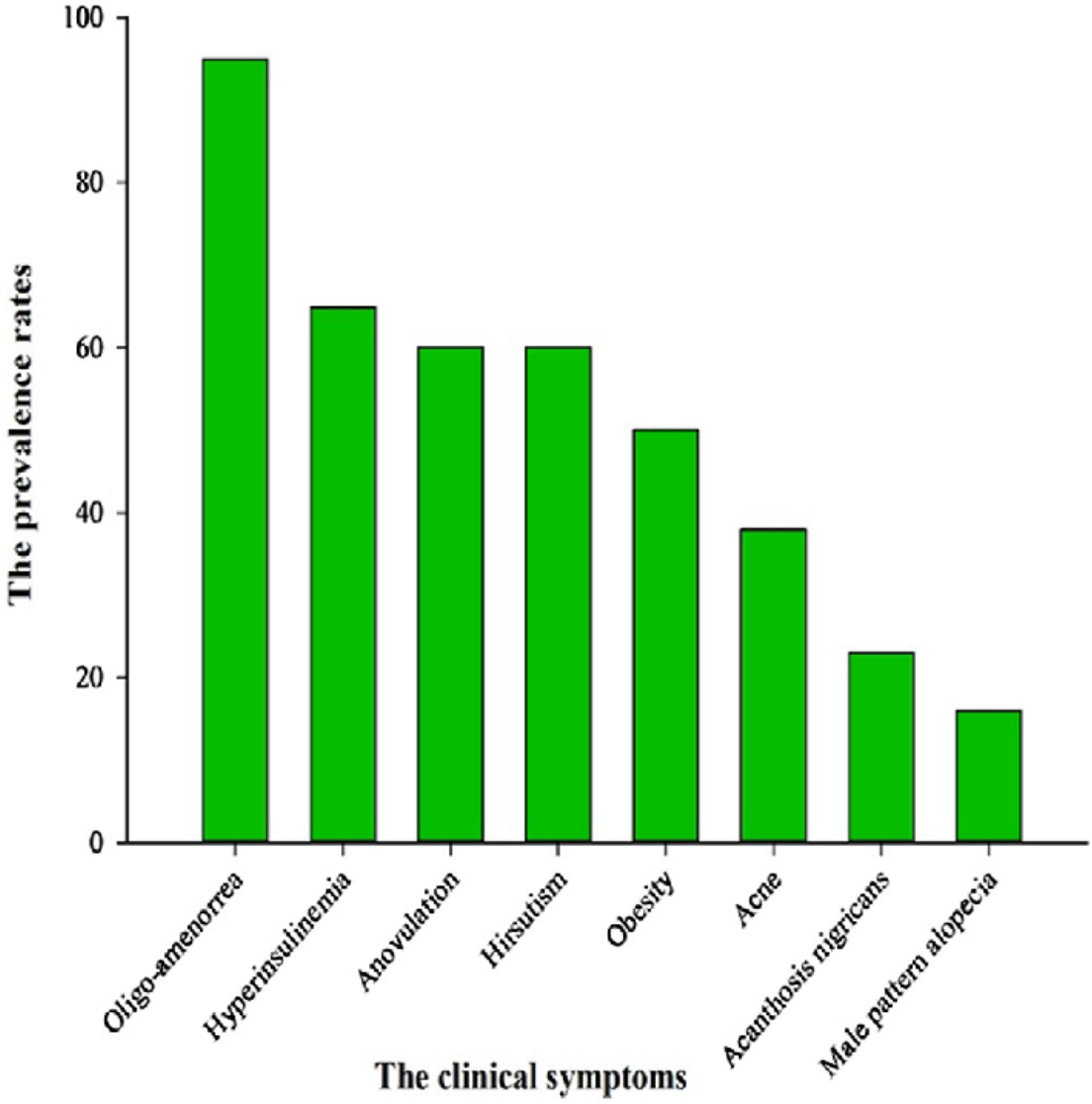


Figure (1.3): The prevalence rates of clinical symptoms in women with polycystic ovary syndrome (PCOS).

1.1.4. Hypothalamic Pituitary Ovarian Axis

Gonadotropin-releasing hormone (GnRH), a neuropeptide that is released into the circulation and travels to the pituitary gland, initiates by the signaling process in the hypothalamus. In response to this signal, the pituitary releases the gonadotropin hormones luteinizing hormone (LH), and follicle-stimulating hormone (FSH). The anterior pituitary gland normally releases

LH and FSH in a pulsatile pattern, in response to GnRH, which also releases in a pulsatile pattern from the hypothalamus (**Speroff and Fritz,2005)**.

FSH and LH bind to the ovarian receptors, leading to estrogen and progesterone secretion. LH is a glycoprotein hormone fundamental for sexual development and reproduction that binds to LH receptors on the theca-cells. Which stimulates theca cells to convert available cholesterol into androgen. This androgen is then carried to the granulosa cells where FSH The glycoprotein hormone binds to an FSH receptor that stimulates the transformation of androgen into estradiol-β-17 using (aromatase) enzyme.

To assist oocyte development, the steroid hormone estrogen steadily rises during the follicular phase of the menstrual cycle. Once the oocyte is ruptured from the ovary, the level of estrogen slowly decreases at the luteal phase of the menstrual cycle and progesterone levels start to increase **(Shaaban et al.,2019)**.

Normally folliculogenesis depends on the transformation of intra-ovarian androgens for estradiol synthesis, and excess androgen production as a consequence of folliculogenesis disordered results in poor maturation of follicles and increased follicular atresia (**Balen et al.,2005)**. Increase LH levels enhance the production of androgen and causes negative feedback at FSH levels, which may limit the transfer of androgen to estrogen and aggravate the excess androgen in the ovaries **(Nardo et al.,2008)**. This pattern of secretion gives rise to an abnormal LH/FSH ratio in many patients, making it likely a valuable diagnosis marker of PCOS **(Le et al.,2019)**. Hypothalamic-pituitary gonadal positive and negative feedback representative by **Figure(1.4).**

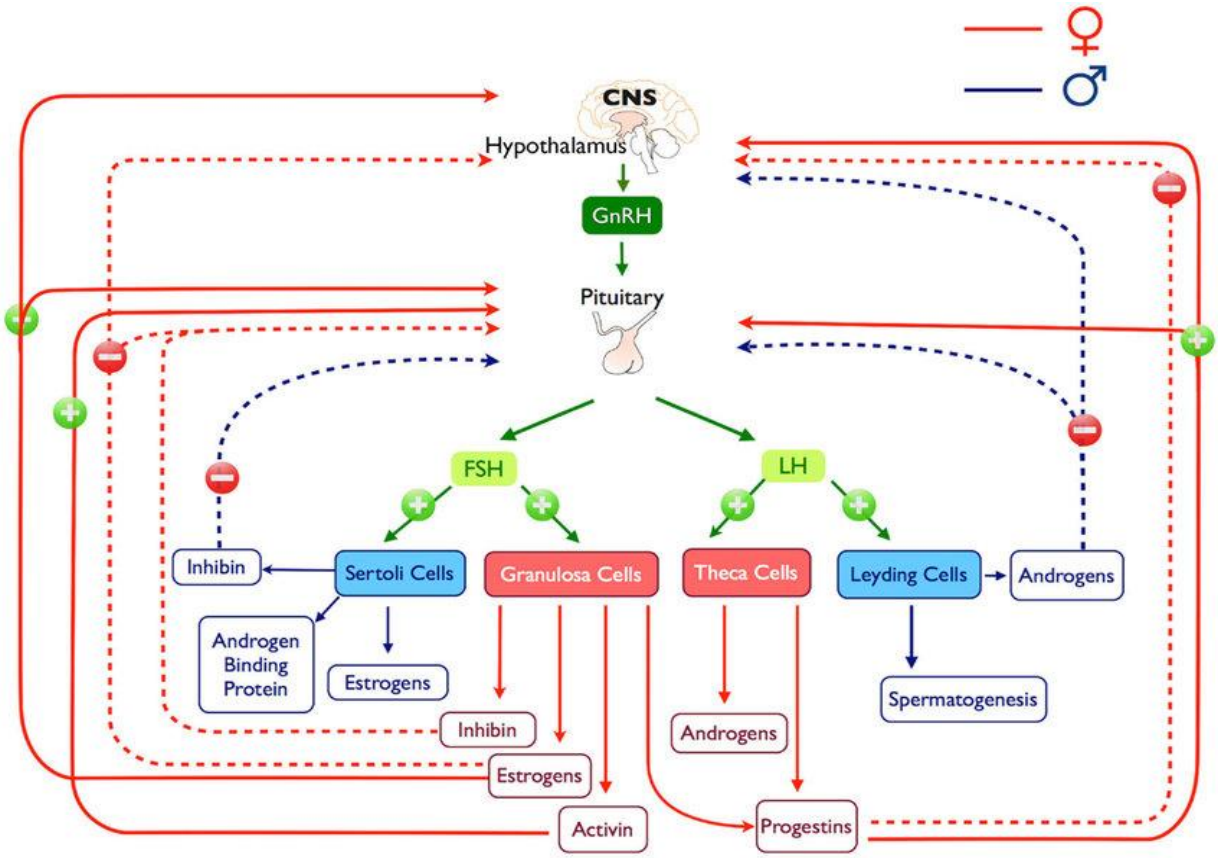


Figure (1.4): Hypothalamic_pituitary gonadal positive and negative feedback mechanism (Durán-Pastén and Fiordelisio,2013).

1.1.5. Polycystic Ovary Syndrome Diagnostic Criteria

For the diagnosis of PCOS in premenopausal women, there are three distinct sets of diagnostic criteria in **Table (1.1) and Table (1.2) ((Mumusoglu and Yildiz, 2020)**

Table (1.1): Polycystic ovary syndrome diagnostic criteria sets

PCOS features	(NIH) 1990/ National Institutes of Health	Rotterdam 2003	Androgen Excess 2006
Hyperandrogenism	✓	±	✓
Ovarian dysfunction	✓	±	±
Polycystic ovarian morphology	–	±	±

✓Essential diagnostic criteria; ± (optional) It may present or not; - Not identified

Table (1.2): Diagnosis statement of (PCOS)

National Institutes of Health (NIH) 1990	Rotterdam 2003	Androgen Excess and PCOS Society 2006
Hyperandrogenism and Ovarian Dysfunction are Essential clinical features	Any 2 of 3 features for diagnosis (Hyperandrogenism, Ovarian Dysfunction, Polycystic Ovarian Morphology)	Hyperandrogenism is the essential clinical feature and Ovarian Dysfunction and/or Polycystic Ovarian Morphology should be accompanied.

1.1.5.1. Ovulatory Dysfunction

Menstrual cycles are irregularly defined as having cycle durations of 35 days or having 8 menstrual cycles annually which are a clinical manifestation of ovulatory dysfunction. While amenorrhea means the absence of menstruation, more than 3 years post-menarche **(Mumusoglu and Yildiz,2020)**.

1.1.5.2. Hyperandrogenism

Clinical and/or biochemical hyperandrogenism characterizes the majority of PCOS patients. Hirsutism is the main symptom of clinical hyperandrogenism. The modified Ferriman-Gallwey scoring system involves the observer rating nine different body regions (the upper lip, chin, chest, upper and lower abdomen, thighs, upper and lower back, and upper arms) on a scale of 0 to 4, which should be used to assess hirsutism. This system takes into account the degree of excessive terminal hair growth. The cutoff for hirsutism is a total mFG score of 4-6, depending on ethnicity. A less precise indication of hyperandrogenism is acne and alopecia. Biochemical hyperandrogenism is the term used to describe elevated serum androgen levels (**Mumusoglu and Yildiz,2020)**.

1.1.5.3. Polycystic Ovarian Morphology

To diagnose polycystic ovary changes using ultrasound there were criteria like the presence of 12 follicles or more in ovaries with diameter measuring between 2 and 9 mm or increase in ovarian volume more than 10 cm^3 in one ovary at least. A classical features of PCOS ultrasound are the arrangement of follicles in periphery of ovaries giving the image of ‹string of pearls ‹**(Bozdag et al., 2016).**

1.1.6. Phenotypes of Polycystic Ovary Syndrome

Four phenotypes can be observed in PCOS: phenotype A, phenotype B, phenotype C, and phenotype D **(Lizneva et al., 2016) Table (1.2).** Type A is the most severe phenotype, and D is the least severe one. Types A and C are the most prevalent types **(Cree-Green, 2017).**

1.1.6.1. Classic Polycystic Ovary Syndrome (phenotype, A and B)

Phenotype A is frequently referred to as (Complete) form of PCOS, while phenotypes A and B are collectively referred to as (Classic) PCOS. Compared to ovulatory or non-hyperandrogenic phenotypes C and D, patients with classic PCOS appear more obese and hirsute, have more irregular menstrual cycles, and are more likely to have insulin resistance and dyslipidemia **(Lim et al.,2019)**.

1.1.6.2. Ovulatory Polycystic Ovary Syndrome (Phenotype-C)

It is known as "ovulatory" PCOS since the 2003 Rotterdam criteria and the 2006 AE-PCOS Society standards both include an additional phenotypic, phenotype -C (HA and PCOM, but without OD). Patients with phenotype C have hirsutism, testosterone, and lipid levels that fall between those of phenotypes A-B and D **(Lizneva et al., 2016).**

1.1.6.3. Non-hyperandrogenic Polycystic Ovary Syndrome (Phenotype D)

The 2003 Rotterdam criteria added the Phenotype -D (OD and PCOM, without HA), known as "non-hyperandrogenic PCOS." These individuals have normal levels of testosterone and the slightest form of endocrine malfunction, such as insulin resistance **(Lizneva et al., 2016).**

Table (1.3): Classification of polycystic ovary syndrome phenotypes (Lizneva et al., 2016)

Phenotype	Hyperandrogenism	Ovarian Dysfunction	Polycystic Ovarian Morphology
Type A	+	+	+
Type B	+	+	
Type C	+		+
Type D		+	+

1.1.7. Biochemical Changes and Polycystic Ovarian Syndrome

Clinical and biochemical androgen excesses are major characteristics of women with PCOS. Studies have reported an association of PCOS with multiple endocrinal, reproductive, and even metabolic risks that reduce the quality of life through the lifelong of the affected female **(Ajmal et al,2019**).

For the diagnosis of PCOS, the basic parameters that must be considered are FSH, LH, and androgen levels. Raised LH level leads to an increase in androgen level that gives rise to the progression of PCOS. PCOS is strongly associated with the future development of type-2 Diabetes mellitus and hyperinsulinemia. The strongest association between abnormalities of ovulation associated with PCOS and elevated levels of the anti-Mullerian hormone (AMH) in the syndrome has been reported to the elevated LH concentration. It was proposed that a GnRH stimulation test could lead to LH excess. Additionally, an increased LH: FSH ratio with GnRH stimulation may be a very helpful supplementary diagnostic technique for identifying PCOS **(Akram and Roohi, 2015).**

1.1.8. Complications of Polycystic Ovary Syndrome

PCOS is understood to be a chronic illness. Menstrual abnormalities, hyperandrogenism, and/or infertility are the predominant symptoms throughout the first years of reproductive life. However, the lifelong implications of this disease must be understood by both women and doctors. Indeed, in roughly 50% of individuals, metabolic abnormalities will eventually replace reproductive failure **(Peigné and Dewailly,2014**). Obesity, glucose intolerance, type 2 diabetes, cardiovascular disease, and endometrial cancer can all develop as people become older in age. Pregnancy problems like gestational diabetes and gestational hypertension are more common in women with PCOS, and there is also a higher risk of preterm birth and perinatal mortality mentioned in **Figure (1.5) (Wang et al.,2013)**.

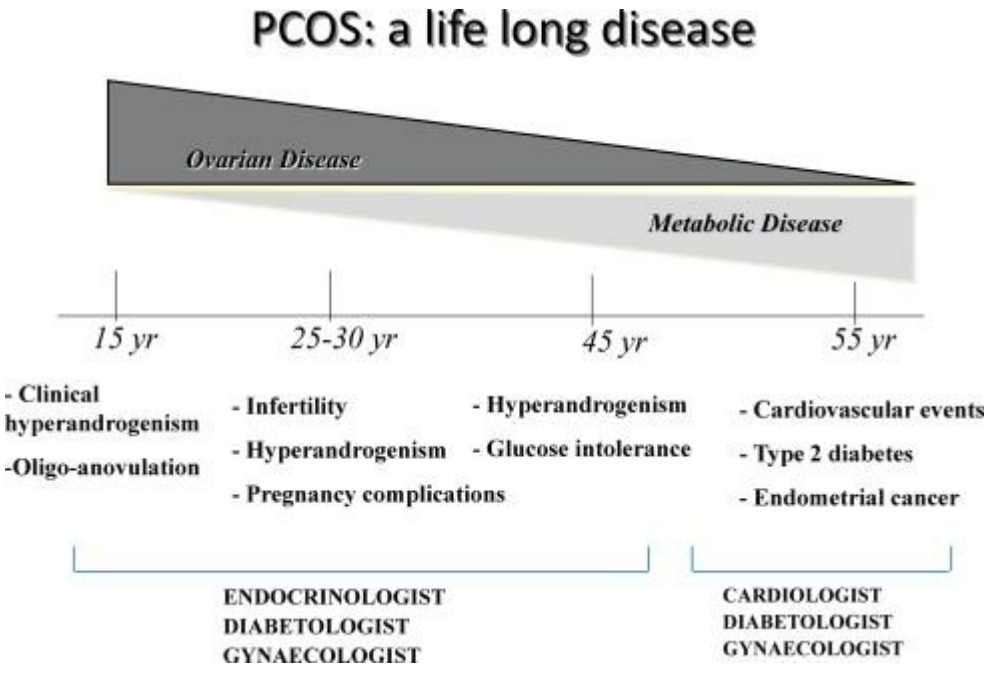


Figure (1.5): PCOS symptoms and complications for life long (Peigné and Dewailly,2014).

1.2. Oxidative stress

Oxidative stress (OS) is a general term usually used to describe an imbalance between the production of free radicals and the ability of the body to defend against their harmful effects by antioxidants that cause DNA damage and/or cell apoptosis. Disturbances in the normal oxidation reaction of cells and the production of free radicals and peroxides can cause toxic effects that damage the cell. The effects of oxidative stress depend on the percentage of these changes from DNA damage and trigger apoptosis and necrosis to cell death **(Burton and Jauniaux, 2011).**

1.2.1. Oxidant _ Antioxidant

1.2.1.1. Free Radicals

Free radicals can be defined as molecular entities or molecular fragments, capable of independent existence (hence "free"). They contain one or more unpaired electrons in an outer atomic orbital or molecular orbital ("radical"). The unpaired electron of a free radical is denoted by a point on the atom or group in which it predominantly resides. The odd number of electron(s) of a free radical makes it unstable, short-lived, and highly reactive. This characteristic is responsible for chain reactions. Free radicals attempt to bond with other molecules, atoms, or even individual electrons to create a stable compound. They either donate or accept an electron from other molecules, acting as oxidizing or reducing agents **(Halliwell and Gutteridge, 2015).** They are produced during many different endogenous and exogenous processes. Mitochondria are the main source of endogenous reactive oxygen species (ROS) produced at the cell level **(Napolitano et al.,2021).**

The overproduction of free radicals can damage macromolecules such as nucleic acids, proteins, and lipids. This leads to tissue damage in various

chronic and degenerative diseases. There are two major types of free radical species: reactive oxygen species (ROS) and reactive nitrogen species (RNS) **(Martemucci et al.,2022**). As shown in **Table (1.4)**.

Table (1.4): Reactive oxygen species and Reactive nitrogen species in the human body (Halliwell,2006).

Free Radicals	Non-Radicals
Reactive Oxygen Species (ROS)	
Superoxide, $O_2^{\bullet-}$	Hydrogen peroxide, H_2O_2
Hydroxyl, $^{\bullet}OH$	Ozone, O_3
Hydroperoxyl, $HO_2^{\bullet}$ (protonated superoxide)	Singlet, $^1O_2$
Carbonate, $CO_3^{\bullet-}$	Organic peroxides, ROOH
Alkoxyl, $RO^{\bullet}$	Peroxynitrite, $ONOO^-$
Peroxyl, $RO_2^{\bullet}$	Nitrosoperoxycarbonate, $ONOOCO_2$
Carbon dioxide radical, $CO_2^{\bullet-}$	
Reactive Nitrogen Species (RNS)	
Nitric oxide, $^{\bullet}NO$	Nitrous acid, HNO_2
Nitrogen dioxide, $^{\bullet}NO_2$	Peroxynitrite, $ONOO^-$
	Peroxynitrous acid, ONOOH
	Alkyl peroxynitrites, ROONO
	Alkyl peroxynitrates, RO_2ONO

Lipid peroxidation (LPO) is a process under which oxidants such as free radicals attack lipids containing carbon–carbon double bond(s), especially PUFAs. LPO products include MDA and hydroxyl radicals, which accumulate due to intracellular and cell wall damage involving PUFAs, with increased levels of ROS serum **(Valgimigli, 2023)**. Malondialdehyde (MDA) is a highly reactive compound that occurs as an enol and is one of the final

products of polyunsaturated fatty acids (PUFAs) peroxidation in the cells. An increase in free radicals causes the overproduction of MDA. Its level is a marker of OS and antioxidant status in cancerous patients. Interestingly, the level of MDA increases both in PCOS and obesity but also in hyperandrogenism and IR (**Uçkan,2022)**.

1.2.1.2. Antioxidant

Antioxidants are chemical compounds or substances that inhibit or retard the oxidation of other molecules. Antioxidants act as free radical scavengers and protect cells or repair the damage done by free radicals. Antioxidants decrease the effect of oxidants by binding together with these harmful molecules. However, antioxidants are effective at low concentrations, may act as oxidants, and become adverse by increasing concentration **(Tebboub and Kechrid, 2021).**

Antioxidants are divided into enzymatic and non-enzymatic antioxidants based on their catalytic activity **(Haida and Hakima, 2019)**:

- Enzymatic antioxidants are produced in cells and protect the body against free radicals via some enzymes that form a distinctive group, Glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) is the key enzyme antioxidants of this defense system by which free radicals that are generated during metabolic reactions are removed **(Jeeva et al.,2015)**.
- Non-enzymatic antioxidants mainly include polyphenols (flavonoids, phenolic acids, and anthocyanins), carotenoids (carotenes, xanthophylls), selenium and zinc which act as cofactors for many antioxidant enzymes and vitamins (vitamins A and C). These types of antioxidants scavenge free radicals by donating hydrogen ions to stabilize the free radicals **(Haida and Hakima, 2019).** In the

antioxidant system of cell protection, along with key antioxidant enzymes, an important role is played by the thioredoxin (Trx)-a dependent system that is involved in the processes of cellular redox-dependent regulation **(Patwardhan et al., 2022)**. The combination of antioxidant properties and the ability to activate the transcription of genes, including some antioxidant enzymes, as well as to inhibit redox-dependent pathways of apoptosis activation, indicates an important contribution of this system to the antioxidant defense system, which increases the resistance of cells to oxidative stress **(Balsera and Buchanan,2019).**

Antioxidant levels decrease in response to weight reduction, caloric restriction, and diets rich in antioxidants **(Valenzano et al.,2019)**. Oxidative stress impairs glucose uptake in muscle and adipose tissue, and reduces insulin secretion from pancreatic b cells, studies have demonstrated that activation of stress-sensitive intracellular signaling pathways results in insulin resistance and impaired insulin secretion. In addition, antioxidant treatments may improve insulin sensitivity in patients with insulin resistance or type 2 diabetes (**Newsholme et al.,2016)**.

1.2.2. Oxidative stress and polycystic ovary syndrome

Oxidative stress (OS) is caused by an imbalance between pro-oxidants and antioxidants. This ratio can be altered by increased levels of reactive oxygen species (ROS) and/or reactive nitrogen species (RNS), or a decrease in antioxidant defense mechanisms. Excessive ROS production, however, may overpower the body's natural antioxidant defense system, creating an environment unsuitable for normal female physiological reactions (**Wen et al., 2023)** ,**Figure (1.6)**.

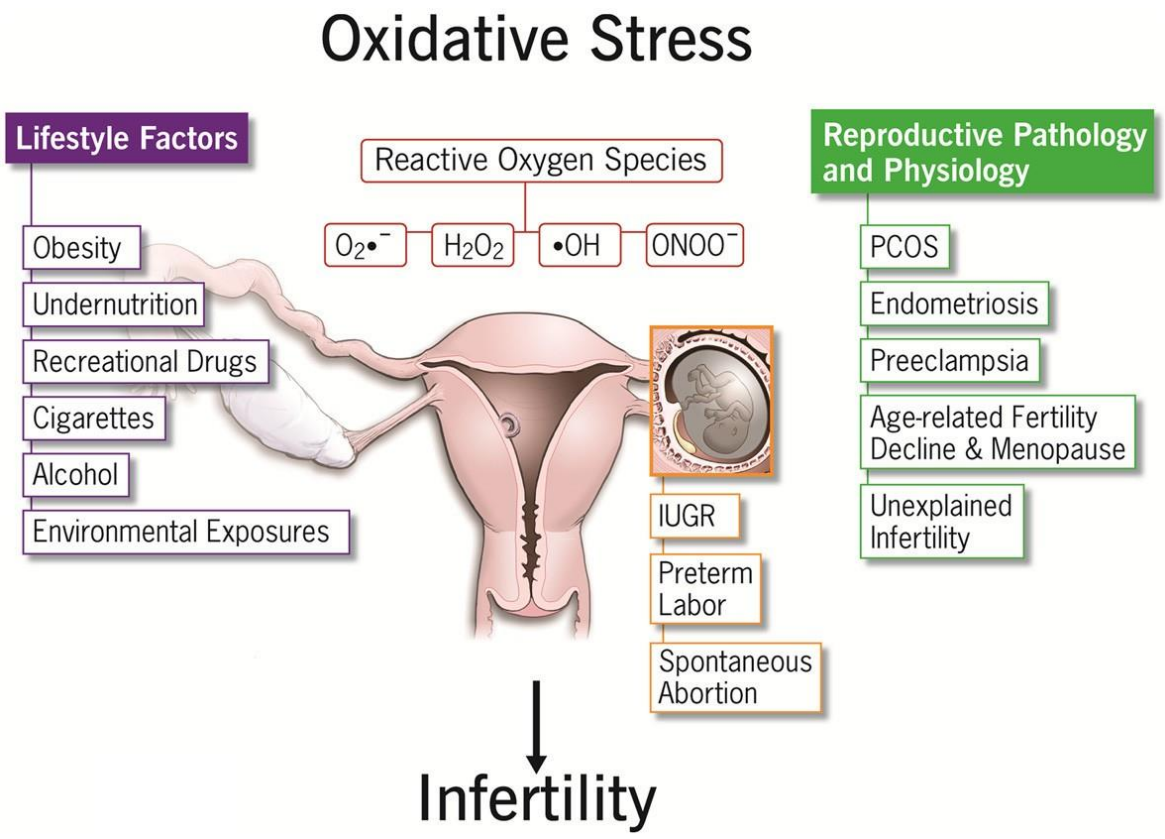


Figure (1.6): Factors contributing to the development of oxidative stress and their impacts on female reproduction (Agarwal et al.,2012).

Oxidative metabolism is also an essential intraovarian regulator of folliculogenesis. Each month, a cohort of follicles begins to grow and develop in the ovary, but only one develops into the dominant follicle **(Agarwal et al.,2012)**. This process is controlled by an increase in ROS and inhibited by antioxidants, while antioxidants support the progression of meiosis II. ROS affects meiosis II progression, diminishes gonadotropin secretion and DNA damage, and inhibits ATP production **(Behrman et al., 2001).**

Free radicals and antioxidants play a crucial role in the ovarian environment during the oocyte maturation and luteal phases. PCOS is associated with

decreased antioxidant concentration. It is one of the states with increased OS, leading to disturbance in the ovarian follicular and luteal phase cycle. Follicular fluid in women with PCOS demonstrated increased levels of ROS and MDA. It decreased TAC, which was directly associated with reduced oocyte maturation and fertilization rates, poor embryo quality, and lower pregnancy rates. Also, AGEs affect the ovarian cells directly in women with PCOS **(Agarwal et al.,2012)**. It was investigated that PCO ovaries displayed an increased concentration of AGE deposition in granulosa, theca, and ovarian endothelial cells **(Merhi, 2014)**. In addition, many clinical manifestations of PCOS such as hyperandrogenism, obesity, and IR may contribute to the development of the local and systemic OS, which may then reciprocally worsen these metabolic abnormalities **(Herman et al.,2020)**, **Figure (1.7)**.

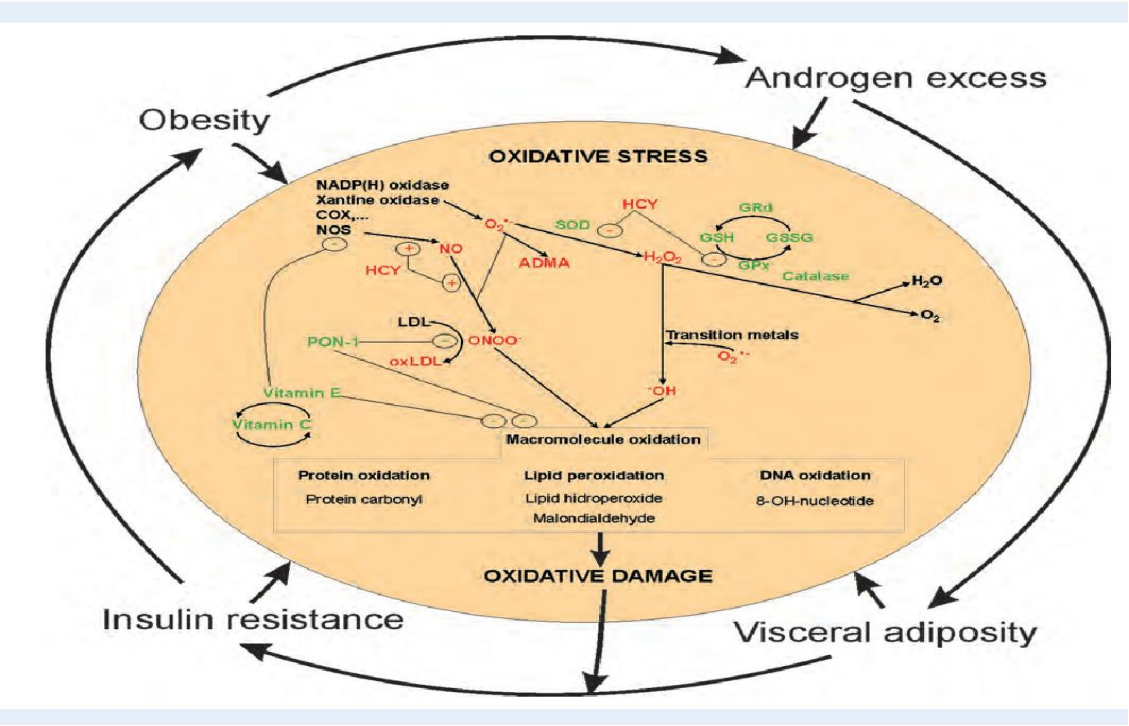


Figure (1.7): Schematic representation of mechanisms and pathways that may contribute to oxidative stress in polycystic ovary syndrome (PCOS) (Murri et al.,2013).

Furthermore, it has been shown that even lean women with PCOS exhibit oxidative stress **(Desai et al., 2014)**.

1.3. Insulin Resistance in polycystic ovary syndrome

Insulin resistance (IR) was first introduced in 1960 by Dr. Yalo and Bresson.IR is a metabolic state with too much insulin production against the normal response because insulin sensitivity reduces under such conditions. One of the most important features of PCOS is type 2 diabetes with IR, which is associated with obesity and CVD **(Victor et al., 2016)**. IR in obese PCO women can disrupt the synthesis of androgens or through the adipocytokines can directly or indirectly, affect the brain-ovarian axis **(Rojas et al., 2014)**.

Women with PCOS often have coexisting IR. Overall, 75% of lean women and 95% of obese women with PCOS have IR **(Stepto et al., 2013)**.

The association between insulin resistance and hyperandrogenism in PCOS is based mainly on two essential concepts:

- That insulin resistance is an important regulatory factor of ovarian synthesis of the androgens, by using synergic action with LH brought about by the P450c17a enzymatic system and, in the presence of hyperinsulinism, acts as a true gonadotropic hormone **(Kosova and Urbanek., 2013)**.

That a condition of hyperinsulinism, occurring as a form of compensation for a state of insulin resistance, may be responsible both for increased androgenic production and greater values of free androgens(testosterone), by using the reduction of hepatic synthesis of the sex hormone binding globulin SHBG **(Gonzalez, 2012)**. **Figure (1.8) (Orbetzova, 2020)**.

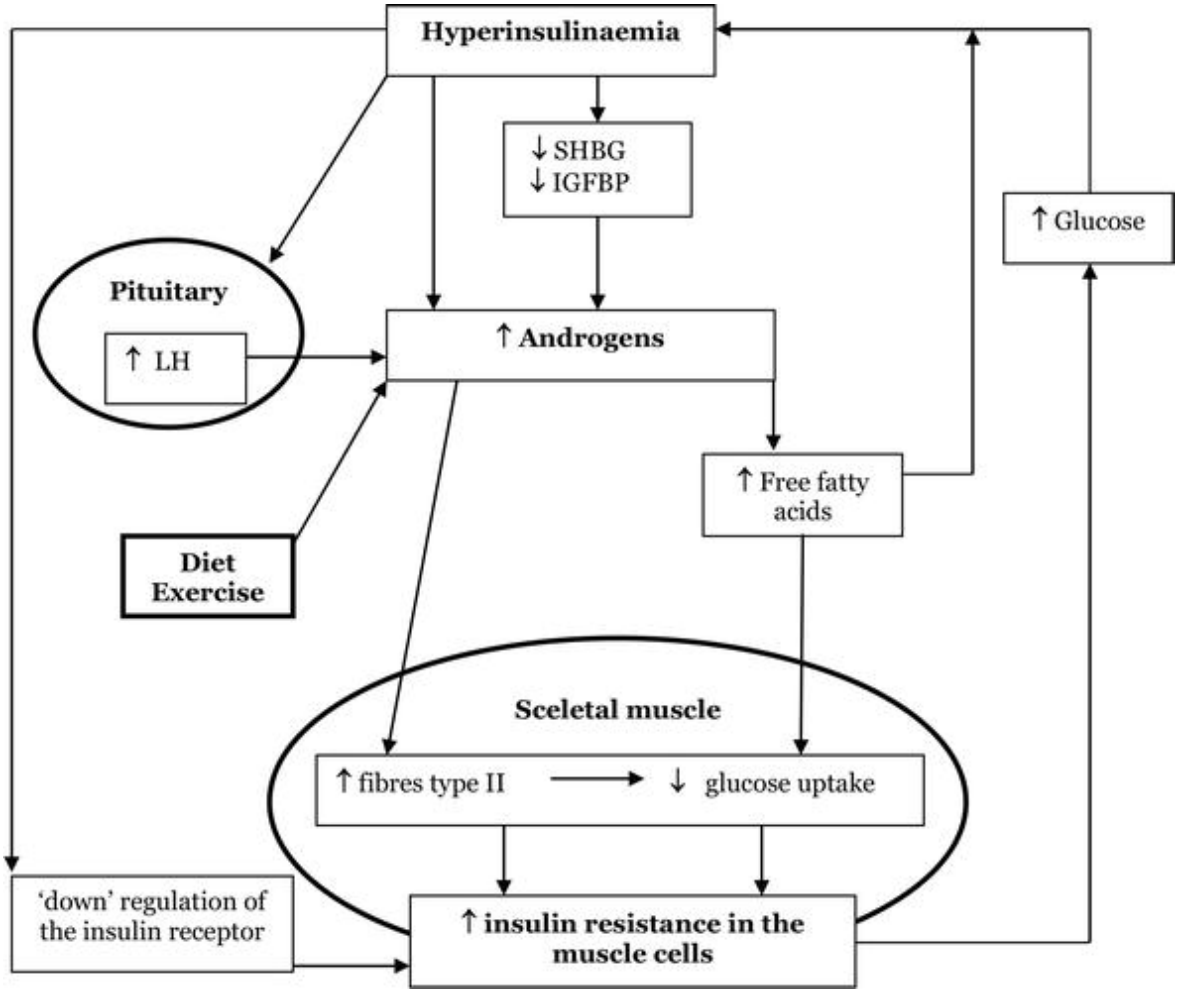


Figure (1.8): Role of insulin in the pathogenesis of PCOS (Orbetzova, 2020).

1.4. Adiponectin

Adiponectin is the most abundant plasma adipokine. It mainly derives from white adipose tissue and plays a key role in the control of energy metabolism, anti-inflammatory, and antiatherogenic properties. It is well known that white adipose tissue is no longer the main storage compartment of triglycerides, but it is a real endocrine organ releasing several biologically active proteins, also known as adipokines. Adipokines are considered as main regulators of the whole body's energy homeostasis. One of these adipokines, named adiponectin, is recognized to play a major role in the regulation of insulin sensitivity and the pathogenesis of the metabolic syndrome. In recent years, its role in the modulation of reproductive functions has become increasingly important **(Barbe et al., 2019)**.

Adiponectin (a protein consisting of 244 amino acids and characterized by a molecular weight of 28 kDa), This hormone was first identified in 1995 and the gene coding for this protein is located on the 3q27 chromosome. The adiponectin structure is made up of single-chain trimers, namely, a variable N-terminal domain, a collagen domain, and a C-terminal globular domain homologous to the immune complement C1q **(Khoramipour et al., 2021)**. In 1996, Adiponectin was named AdipoQ, apM1 (a product of the most abundant gene of adipose, transcript-1), and GBP28 (gelatin-binding protein-28) respectively **(Parida et al., 2019)**.

Adiponectin has two major receptors, AdipoR1 and AdipoR2. The AdipoR1 receptor has a higher affinity for the globular form, while the AdipoR2 receptor preferentially binds to the high molecular weight form. It was initially shown that the AdipoR1 receptor was mainly expressed in skeletal muscles and AdipoR2 in the liver. Subsequently, the expression of these receptors has been identified in other tissues, such as the myocardium, macrophages, brain tissue, endothelial cells, lymphocytes, and adipose

tissue, and in pancreatic β cells where the level of expression of AdipoR2 is even equivalent to that of its expression in the liver, and the level of expression of AdipoR1 higher than that of its muscle expression**, (Kharroubi et al., 2003), Figure(1.9)(Nguyen, 2020)**.

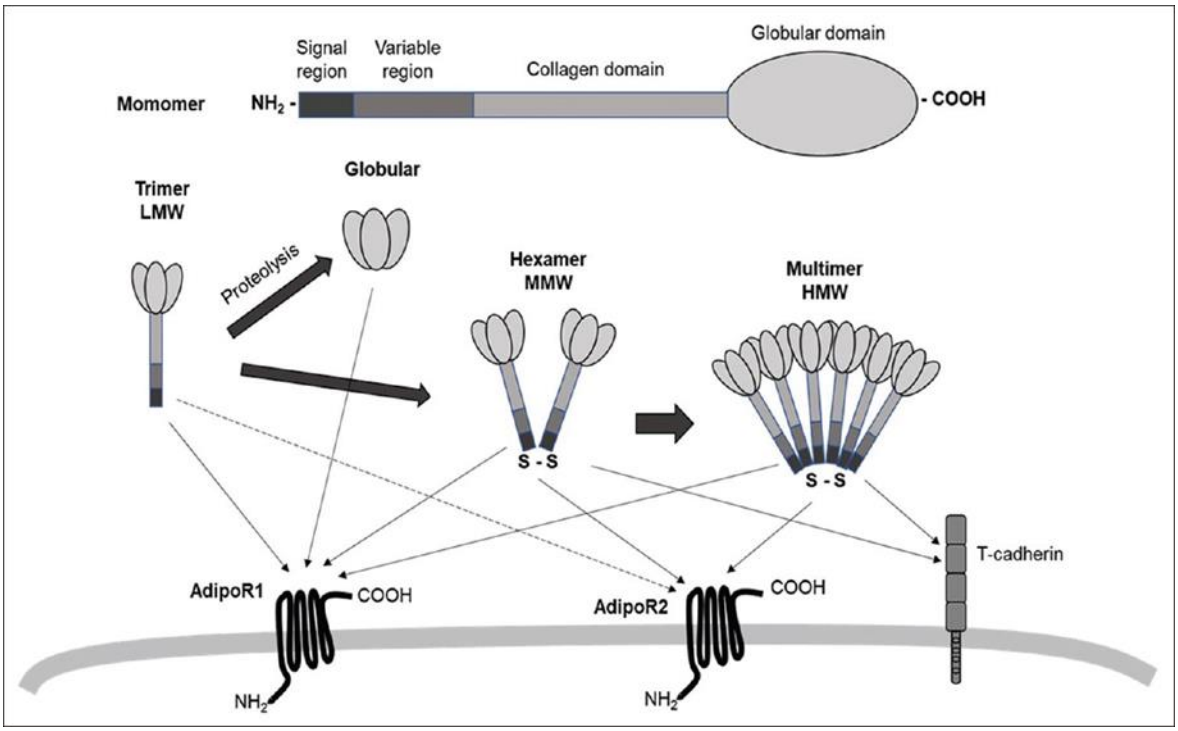


Figure (1.9): Adiponectin structure and receptors (Nguyen, 2020).

1.4.1. Adiponectin and insulin resistance in polycystic ovary syndrome

Insulin resistance commonly exists in women with PCOS and along with the dysfunctional glucose metabolism contributes to the pathogenesis of the syndrome. However, the mechanism of its action is not completely understood **(Rocha et al., 2019)**. Insulin resistance results from a post-binding failure of the receptor insulin signaling pathway in the ovaries, probably because of the enhanced phosphorylation of insulin receptors and insulin receptor substrate-1 (IRS-1) serine **(Diamanti-Kandarakis and Dunaif, 2012)**. It was demonstrated that insulin resistance in the skeletal muscle of PCOS patients might result from the stimulation of serine kinases in the early steps of the insulin signaling pathway, which are involved in the

serine phosphorylation of IRS-1 within mitogen-activated protein kinases-extracellular-signal-regulated kinase (MAPK-ERK) pathway **(Diamanti-Kandarakis and Dunaif, 2012)**. On the other hand, adiponectin is an insulin-sensitizing hormone, which regulates ovarian function and acts through the interaction with the adiponectin receptor 1 (AdipoR1) and AdipoR2 **(Dobrzyn et al., 2018)**. The binding of different forms of the adiponectin to AdipoR1 and AdipoR2 leads to the activation of different signaling pathways through the peroxisome proliferator-activated receptor α (PPARα), AMP-activated protein kinase (AMPK), P38-MAPK, the ERK1/2-MAPK, and Akt **(Tebrani et al.,2013).**

Depending upon the tissue, connecting the adiponectin to its receptors culminates in the modulation of the different biological events such as steroidogenesis, glucose uptake, cell survival, fatty acid oxidation, and vascular expansion as well as cell protection **(Deepa and Dong, 2009)**, **Figure (1.10).**

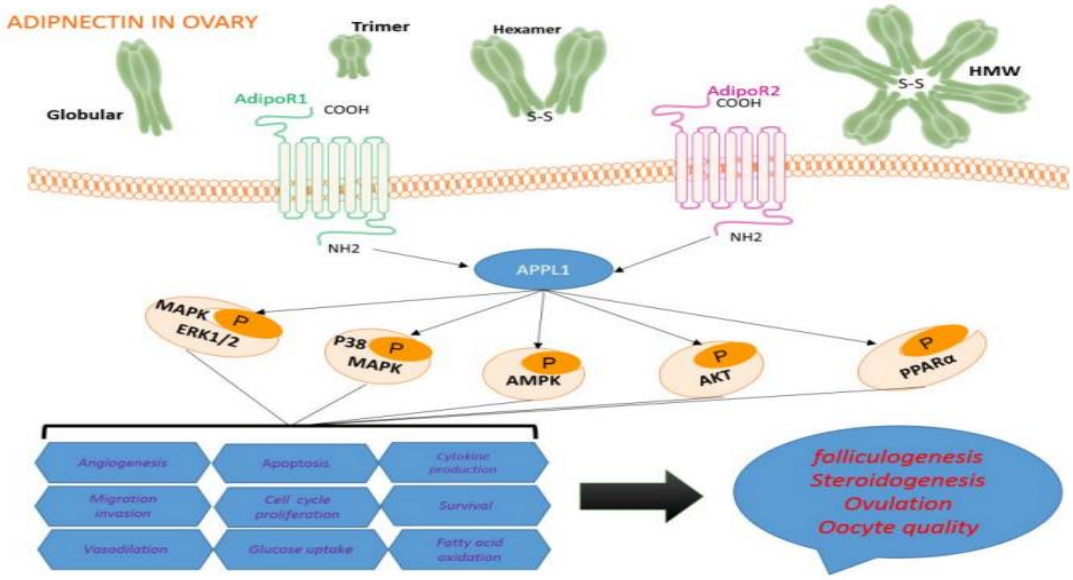


Figure (1.10): Adiponectin signaling pathway in ovarian.

1.4.2. The function of Adiponectin.

Adiponectin exhibits anti-diabetic, anti-inflammatory, and anti-atherogenic effects and it also functions as an insulin sensitizer that enhance insulin sensitivity. Adiponectin has been shown to increase insulin sensitivity by several pathways, including increased glucose absorption by the glucose transporter 4 in skeletal muscle **(Llanos and Palomero, 2022)**. In addition, adiponectin activates the "nuclear hormone receptor" AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor alpha, which leads to the regulation of numerous lipid and glucose metabolism genes, and increases the oxidation of fatty acids and the uptake of glucose, each of which can help to promote insulin sensitivity **(Fisman and Tenebaum, 2009)**. Adiponectin also increases insulin sensitivity by inhibiting the expression of phosphoenolpyruvate carboxykinase and glucose-6- phosphatase by suppressing hepatic gluconeogenesis, thus suppressing gluconeogenesis **(Combs and Marliss, 2014)**, **Figure (1.11) (Menzaghi et al.,2007)**.

By activating ceramidase, which is associated with an increase in sphingosine, adiponectin decreases cellular ceramide levels, leading to decreased hepatic ceramide levels and increased insulin sensitivity **(Parida et al., 2019)**.

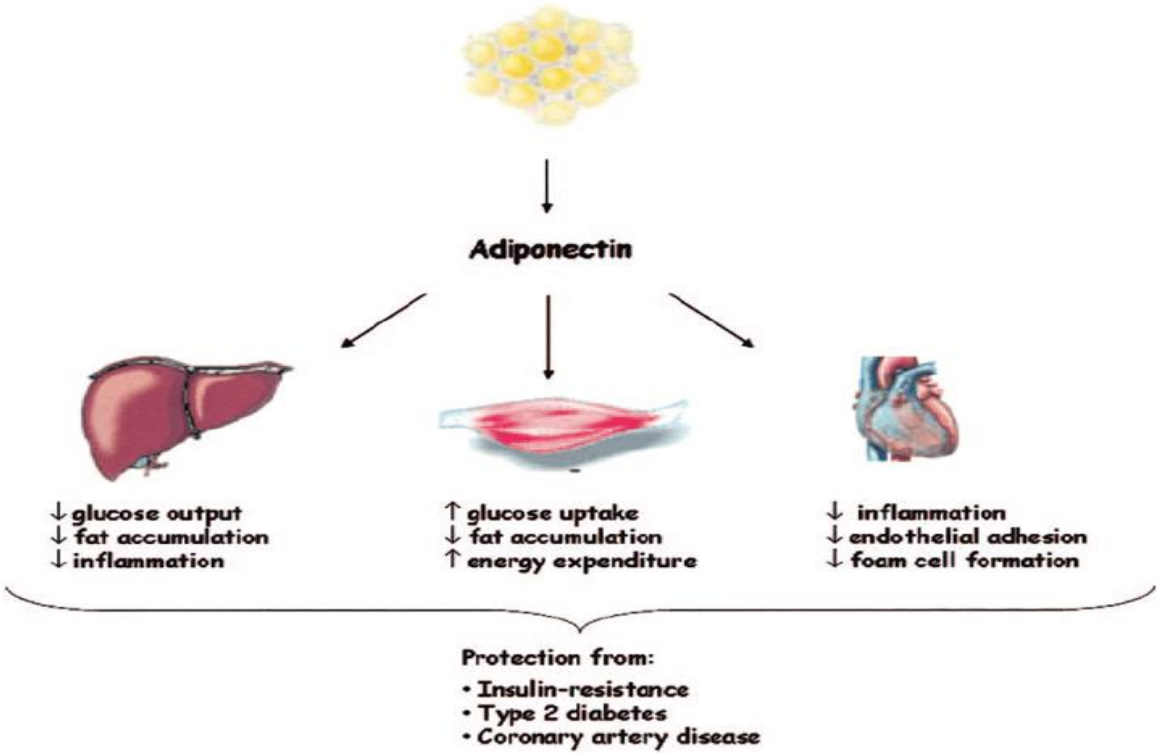


Figure (1.11): Proposed salutary effects of adiponectin (Menzaghi et al.,2007).

1.5. Aim of the study:

1. Estimating the level of adiponectin, catalase and malondialdehyde in patients with PCOS with obese and non-obese PCOS women.
2. Studying the correlation of adiponectin, catalase and malondialdehyde in PCOS patients with other biochemical parameters like lipid profile, hormones levels and HOMA-IR.

Chapter Two

Materials and Methods

2. Materials and Methods

2.1. Subjects

2.1.1. Study Design and Ethical Approval

A case-control study design on 160 women was conducted in the Department of Biochemistry, College of Medicine, University of Kerbala. The subjects were collected throughout the period from (Nov. 2022 to Mar. 2023). The College of Medicine, University of Kerbala, and Kerbala Health Directorate validated the study's ethical approval. The approval was also taken from the administration of the gynecological and obstetric teaching hospital and each patient after explaining the nature and purpose of the study. as shown in **Figure (2.1).**

2.1.2. Patients

From eighty PCOS patients of childbearing age women were selected at the infertility clinic of Kerbala Teaching Hospital for Obstetrics and Gynecology, Karbala Health Directorate, their ages ranged between (18 – 40) years. An exhaustive interview addressing personal history, family history, demographic information, and laboratory examination was performed. A questionnaire was formed to get the data of the patient set which contains the age, weight, height, hip circumference, waist circumference, hip/waist ratio, menstrual regularity, fertility, and hirsutism. The measurements in these studies included anthropometry: waist-to-hip ratio (WHR) and body mass index (BMI). The Scheme of the study is shown in **Figure (2.1).**

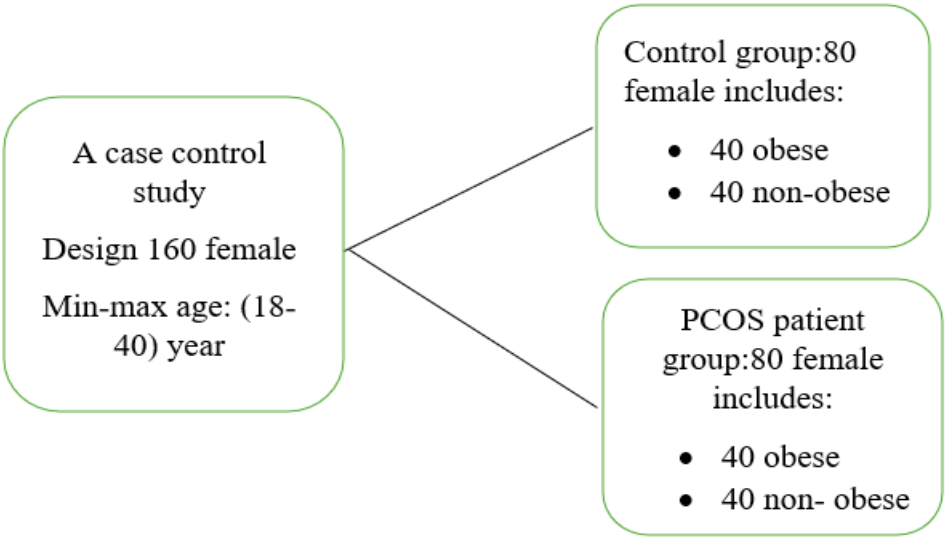


Figure (2.1): Scheme of the study.

- **Inclusion criteria**

The Rotterdam criteria-2003 was used, eighty PCOS females (N=80) with ages reached between (18 - 40 years). It is possible to diagnose patients with any two of the subsequent three conditions: Hyperandrogenemia, Ovarian Dysfunction, ovarian volume greater than 10 cm^3 on ultrasonography, and more than 12 follicles measuring 2 to 9 mm in diameter in at least one ovary **(Freeman et al.,2022)**.

- **Exclusion criteria**

Women suffering from diseases (autoimmune disease, diabetes mellitus, thyroid disease, cardiovascular disease, hypertension, chronic renal failure, adrenal disease, ovarian tumors, and malignant diseases) were excluded and history of receiving any other medication (lipid reduction, ovulation

stimulation, corticosteroids, antidiabetic and antihypertensive medications) within 6 months were excluded also.

2.1.3. Control

Eighty women were selected as the control group, with ages ranging between (18-40 years). They have regular menstruation periods, with normal ovaries as they were observed by the ultrasound. The questionnaire for the control group included the age, weight and height, hip and waist circumferences, and menstrual regularity with fertility and hirsutism. Women should not be smokers with no renal disease, liver disease, cancer, strokes, acute or chronic inflammatory disease, Cushing's syndrome, cerebrovascular accidents, alcoholics, rheumatoid arthritis, autoimmune disease, patients with type 1 and 2 diabetes mellitus, and no history of contraceptive drugs**.**

2.1.4. Blood samples collection

Blood specimen was collected after fasting (at least 8 hours); 5 ml venous blood samples were taken at 08:00 – 12:00 after morning during the 2^{nd} – 5^{th} day of the menstrual cycle (early follicular phase) for PCOS women and the control group. Blood samples were left at least 30 minutes at room temperature. After coagulation, the sera were separated by centrifugation at (4000 rpm) for 5 minutes for separation of serum divided into 4 parts and put in Eppendorf tubes then stored at - 40˚C till examination of the biomolecules in the current study.

2.2. Chemicals and Kits

The Kits used in the current study are summarized in **Table (2.1).**

Table (2.1): Chemicals and kits used in the study and their suppliers.

No.	Chemicals and Kits	Company and Country
1	Adiponectin Kit	Biont/ China
2	Cholesterol Kit	GIESSE/Italy
3	Follicular Stimulating Hormones Elecsys reagent kit	Roche / Germany
4	Free Testosterone Hormone kit 5325-300	Monobind/ USA
5	Glucose kit	Biorex /UK
6	HDL-Cholesterol Kit	GIESSE/Italy
7	Hydrogen peroxide	BDH/Pritish
8	Insulin Elecsys Kit	Roche/USA
9	Luteinizing Hormone Elecsys reagent Kit	Roche/Germany
10	Phosphate buffer	BDH/British
11	Prolactin II Hormone Elecsys reagent Kit	Roche/Germany
12	Thiobarbituric acid (TBA)	BDH/Pritish
13	Triglyceride kit	GIESSE/Italy
14	Trichloroacetic acid (TCA)	BDH/British
15	Tris- HCL	BDH/British
16	Vanadium reagent	BDH/British

2.3. Instruments and Lab Equipment

The instruments and laboratory tools used in the study were summarized in **Table (2.2).**

Table (2.2): Instruments and Lab Equipment that used in the study.

No	Instruments and Lab Equipment	Company and Country
1	Auto analyzer SMART-120	Geno Lab TEK/ USA
2	Centrifuge	Kokusan/ Germany
3	Cobas e411 analyzer	Roche/ Germany
4	Deep freezer	Fisher Scientific/ USA
5	ELISA instrument system	Bio Tek/ USA
6	Gel tube (6ml)	Arth AL-Rafidin/ China
7	Hitachi cups	Arth AL-Rafidin/ China
8	Incubator, TPM- 900	Siroca crossline/Japan
9	Micropipette	Bioasic/ Canada
10	Refrigerator	Concord/ Lebanon
11	Sensitive balance	A&D/ Japan
12	Syringe (5ml)	Arth AL-Rafidin/ China
13	Vortex- mixture	Clay Adams/ Germany
14	Water path	Memmert/ Germany

2.4. Methods

2.4.1. Body Mass Index calculation

Obesity was categorized using the body mass index (BMI) which was calculated from the following equation **(Donini et al., 2020)**:

BMI (Kg/m²) = Weight (kg) / Height (m^2).

WHO classification was used for BMI evaluation. Normal BMI level range between (20-24.9) kg/m2 while overweight ranges between (25- 29.9) kg/m2 and when BMI ≥ 30 kg/m^2, the woman is considered obese **(Namjou et al.,2021).**

2.4.2. Waist Hip Ratio Measurement

The Waist Hip Ratio (WHR) > 0.85 diagnostic standard for abdominal obesity in women **(Lee et al., 2022**).

2.4.3. Hirsutism Assessment

The modified Ferriman-Gallwey scoring system is used to determine whether a person has hirsutism. Nine different body parts (the upper lip, chin, chest, upper and lower abdomen, thighs, upper and lower back, and upper arms) are scored from 0 to 4, a score of 0 represents the absence of terminal hair growth, and a score 4 represents extensive growth, **Figure (2.2)**. A total mFG score ≥ of 4-6, is used to define hirsutism (**Mumusoglu and Yildiz,2020).**

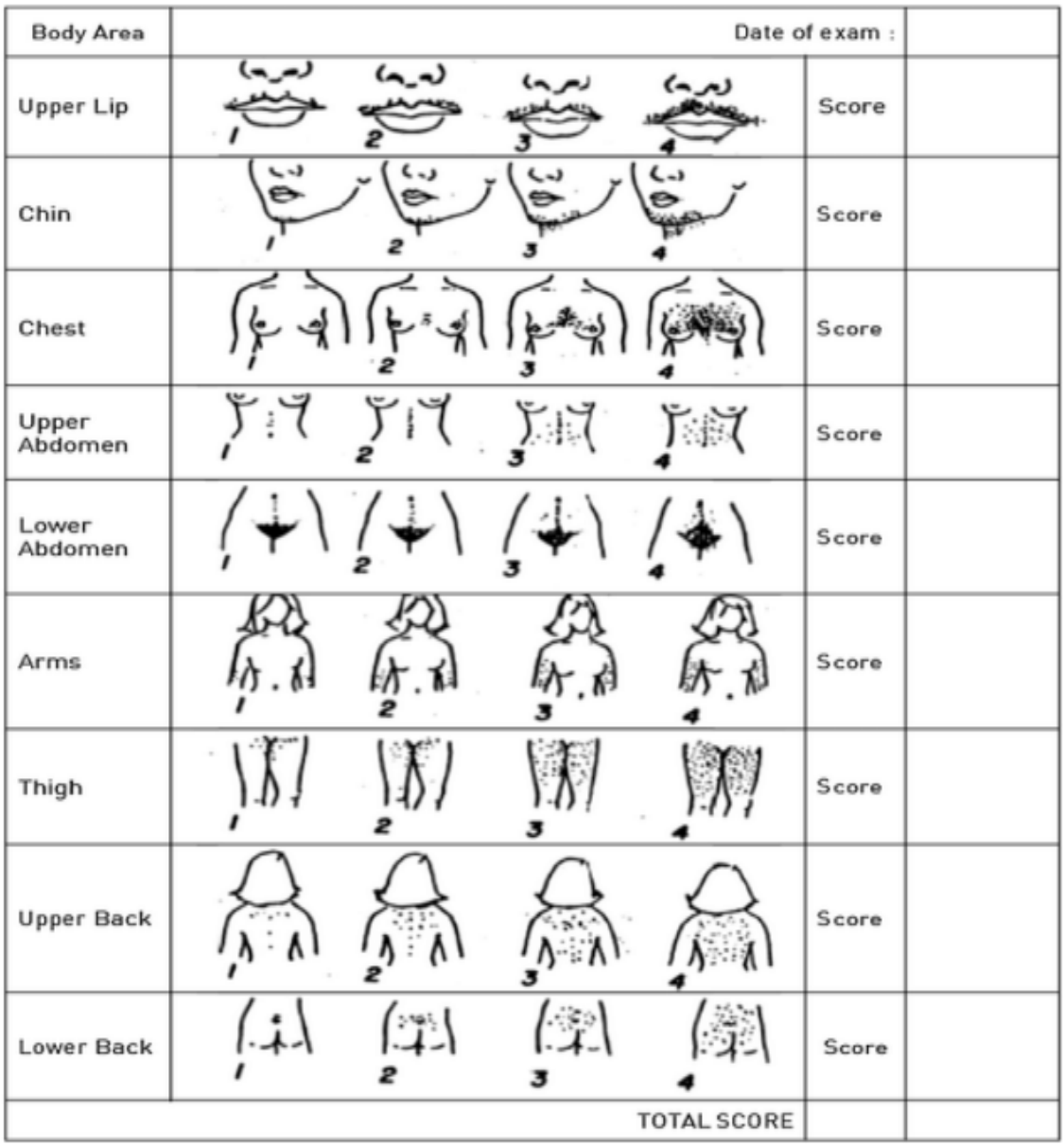


Figure (2.2): The Modified Ferriman-Gallwey Score (Khan et al., 2019).

2.4.4. Determination of Luteinizing, Follicle Stimulating, and Prolactin Hormones

Luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin hormones levels were measured by the chemiluminescent automated immunoassay system (ECL) **(Cobas e 411, Roche Diagnostic, Germany).**

General Assay Principle:

A completely automated, random access, software-controlled system for immunoassay analysis is the Roche Diagnostic Cobas e411 Immunoassay System **(Black and da Silva Costa, 2018).** Three test principles are offered in the system: a bridging principle to identify antibodies in the sample, a sandwich principle (one or two steps) for larger analytes, and a competitive principle for extremely small analytes (**Nivedhita and Brundha,2020)**. Applying voltage to the immunological complexes connected to the streptavidin-coated microparticles causes the chemiluminescent reactions that result in the emission of light from the ruthenium complex to begin electrically **(Toboc et al., 2018)**.

Procedure:

1. Assay cups and assay tips were loaded in the instrument (cobas e411).

2. Regents of each parameter (LH, FSH, PRL), loading in reagent rack.

3. Calibration and control of each reagent were made.

4. The sample of serum was loaded into sample rack then the instrument automatically began analysis.

2.4.4.1. Luteinizing Hormone Level Determination

A. Test Principle

The sandwich principle of the Elecsys LH level assay employs two monoclonal antibodies specifically directed against human LH.

B. working solutions and Reagents

The label on the reagent rack pack reads LH.

M Microparticles with 0.72 mg/mL streptavidin coating.

R1 Anti-LH-Ab~biotin: Mouse monoclonal anti-LH antibody that has been biotinylated 2.0 mg/L in TRIS (Trisaminomethane) buffer at pH 8.0.

R2 Anti-LH-Ab~Ru(by): Mouse monoclonal anti-LH antibody labeled with ruthenium complex at a concentration of 0.3 mg/L in TRIS buffer at a pH of 8.0.

C. Procedure

The assay took 18 minutes to complete.

1. 1st incubation: A sandwich complex was created from 20 µL of the sample, a monoclonal LH-specific antibody that has been biotinylated, and a monoclonal LH-specific antibody that has been labeled with ruthenium complex.
2. 2nd incubation: Following the addition of microparticles coated in streptavidin, the complex was linked to the solid phase by an interaction between biotin and streptavidin.
3. The measurement cell receives the reaction mixture by aspiration, and the microparticles were magnetically attracted to the electrode's surface there. Pro Cell/Pro Cell M was then used to eliminate any remaining unbound materials. A photomultiplier measures the chemiluminescent emission that is caused when a voltage is applied to the electrode.

2.4.4.2. Follicular Stimulating Hormone Level Determination

A. Test principle

The sandwich principle of the Elecsys FSH level assay employs two monoclonal antibodies specifically directed against human FSH.

B. working solutions and Reagents

The label on the reagent rack pack reads FSH.

M Microparticles with 0.72 mg/mL streptavidin coating.

R1 Anti-FSH-Ab- biotin: Mouse monoclonal FSH antibody that has been biotinylated 0.5 mg/L in MES (morpholino)ethane sulfonic acid buffer at pH 6.0.

R2 Anti-FSH-Ab- Ru(bpy): Mouse monoclonal anti-FSH antibody labeled with ruthenium complex at a concentration of 0.8 mg/L in MES buffer at a pH of 6.0.

C. Procedure

The total duration of the assay: 18 minutes.

1. 1st incubation: A sandwich complex was created from 40 µL of the sample, a monoclonal FSH-specific antibody that has been biotinylated, and a monoclonal FSH-specific antibody that has been tagged with a ruthenium complex.
2. 2nd incubation: The complex was bonded to the solid phase after the addition of microparticles coated in streptavidin through the interaction of biotin and streptavidin.
3. The measurement cell receives the reaction mixture by aspiration, and the microparticles were magnetically attracted to the electrode's surface there. Pro Cell/Pro Cell M was then used to eliminate any remaining

unbound materials. A photomultiplier measures the chemiluminescent emission that is caused when a voltage is applied to the electrode.

2.4.4.3. Prolactin Hormone Level Determination

A. Test principle

Two monoclonal antibodies designed specifically against human prolactin were used in the sandwich principle of the Elecsys Prolactin Level Assay.

B. Reagents - working solutions

The label on the reagent rack pack reads "PRL II."

M Microparticles with 0.72 mg/mL streptavidin coating.

R1 Anti-Prolactin-Ab- biotin: Mouse monoclonal anti-prolactin antibody that has been biotinylated, 0.7 mg/L in phosphate buffer at pH 7.0.

R2 Anti-Prolactin-Ab- Ru(bpy): Mouse monoclonal anti-prolactin antibody labeled with ruthenium complex at a concentration of 0.35 mg/L in phosphate buffer at a pH of 7.0.

C. Procedure

The assay took 18 minutes to complete.

1. 1^{st} incubation: A initial complex was formed by 10 µL of material and a monoclonal prolactin-specific antibody that has been biotinylated.
2. 2^{nd} incubation: An interaction between streptavidin and biotin causes a sandwich complex to develop with the addition of a monoclonal prolactin-specific antibody tagged with a ruthenium complex and microparticles coated in streptavidin.

3. The measurement cell receives the reaction mixture by aspiration, and the microparticles were magnetically attracted to the electrode's surface there. Pro Cell/Pro Cell M was then used to eliminate any remaining unbound materials. A photomultiplier measures the chemiluminescent emission that is caused when a voltage is applied to the electrode.

2.4.5. Free Testosterone Hormone Level Determination

A. principle of the test

Enzyme-based Competitive Immunoassay (TYPE 5). Immobilized antibodies, enzyme-antigen conjugates, and natural antigens were the fundamental substances needed for a solid-phase enzyme immunoassay. The natural antigen in serum and the enzyme-antigen conjugate compete for a limited number of insolubilized binding sites and combined with the immobilized antibodies.

B. Reagents – working solutions

1. Icons A-G on Free Testosterone Calibrators, 1 mL/vial Seven vials of free testosterone-containing serum reference with concentrations of 0 (A), 0.2 (B), 1.0 (C), 2.5 (D), 7.5 (E), 20 (F), and 60 (G) pg/mL.
2. Free Testosterone Controls – 1mL/vial – Icons L, M, N three vials of free testosterone serum reference at low, middle, and high established concentrations.
3. Free Testosterone Enzyme Reagent – 13 mL/vial – Icon One vial of Testosterone (Analog)-horseradish peroxides (HRP) conjugate in a protein stabilizing matrix with dye.

4. Testosterone antibody was coated on a 96-well microplate in a free testosterone coated plate that is wrapped in an aluminum bag with a drying agent.
5. One vial of a surfactant in buffered saline makes up the 20 mL-per-vial Wash Solution Concentrate.
6. Tetramethylbenzidine (TMB) in buffer was present in one vial of SubstrateA, which was 7 mL in volume.
7. Hydrogen peroxide ($H_2 O_2$) in buffer was contained in one vial of SubstrateB, which has a volume of 7 mL.
8. Strong acid was contained in one vial of the Stop Solution (8 mL) (1N HCl).

C. Reagent preparation

1. Wash Buffer: Using distilled water and a suitable storage container, dilute the wash solution's contents to 1000 mL.

2. Working Substrate Solution: The contents of the amber vial marked "Solution A" were transferred to the clear vial marked "Solution B."

D. Procedure

1. The microplates' wells were formated for each serum reference, control, and patient specimen to be assayed.
2. In the designated well, 20 µL of the proper serum reference, control, or sample was pipetted.
3. Avolume of (100 µL) of Free Testosterone Enzyme Reagent was inserted into each well.
4. Gently swirled the microplate for 20–30 seconds to mix.
5. At room temperature, covered and incubated for 60 minutes.

6. Decantation was used to discard the microplate's contents.
7. There was decanted and added 350 µL of wash buffer was. For a total of three washes.
8. To each well, 100 µL of the working substrate solution was added.
9. Incubated for fifteen minutes at room temperature.
10. Each well received a volume of (50 µL) of stop solution, which was added and carefully mixed for 15-20 seconds.
11. A microplate reader was used to measure the absorbance in each well at 450nm.

E. Calculation

1. The absorbance obtained from the printout of the microplate reader was recorded**.**

2. On a sheet of linear graph paper, the absorbance for each serum reference was plotted against the appropriate Free Testosterone concentration in pg/mL.

3. A best-fit curve was used to connect the points.

4. The average absorbance of the samples for each unknown was placed on the graph's vertical axis, the point of intersection was determined on the curve, and the concentration (in pg/mL) was read off the horizontal axis to determine the unknown's Free Testosterone concentration.

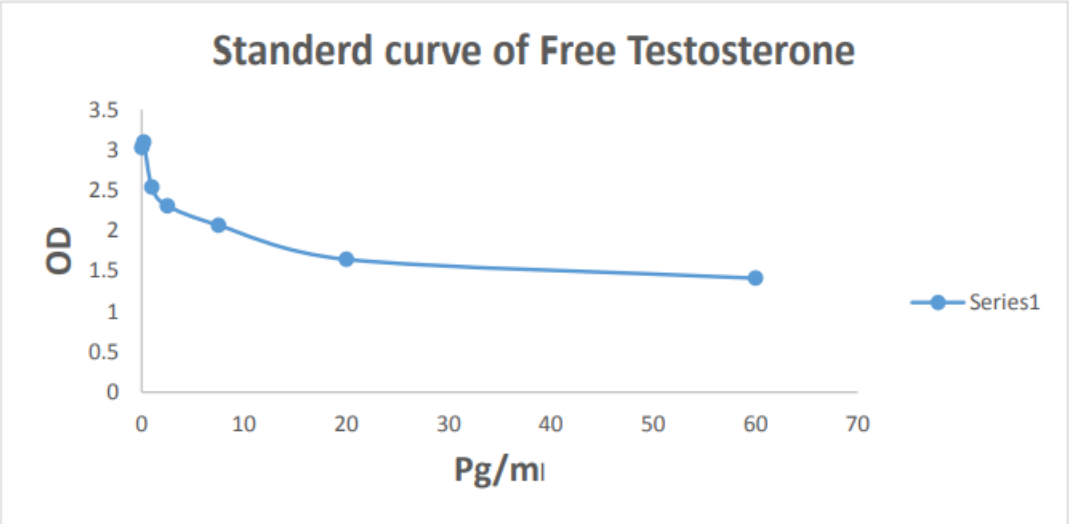


Figure (2.3): The standard curve for free testosterone level determination.

2.4.6. Measurement of Serum Lipid Profile

Total cholesterol, Triglyceride, and HDL were measured by Autoanalyzer Biochemistry (smart-120).

2.4.6.1. Measurement of Serum Total Cholesterol Concentration

A. Principle:

Esterified cholesterol is hydrolyzed into free cholesterol and fatty acid by cholesterol esterase (CHE). 4-Cholesten-3-one and hydrogen peroxide (H_2O_2) are then formed from the released free cholesterol by the action of cholesterol oxidase. In the presence of peroxidase (POD), hydrogen peroxide reacts with a derivative of phenol and 4-aminoantipyrine (4-AAP) to produce a colored complex whose color intensity is directly proportional to the total cholesterol concentration in the sample **(Bishop,2020) (Trinder,1969).**

A schematic representation of the reaction is shown in the following equations:

Cholesterol Esterase

Cholesterol ester ----------►Cholesterol + fatty acids

Cholesterol Oxidase

Cholesterol ----------►4-Cholesten-3-one + H_2O_2

Peroxidase

H_2O_2 + Phenol + 4-AAP ----------►colored complex + H_2O

B. Reagents:

Table (2.3): Reagents used for total cholesterol assay.

Reagent A (100 ml)	Buffer	mmol/l
	4-AAP	1 mmol/l
	CHE	300 U/l
	CHOD	300 U/l
	POD	1500 U/l
	Derivative of phenol	1 mmol/l
Standard (5 ml)	Cholesterol	200 mg/dl

4-AAP, 4-aminoantipyrine; CHE, cholesterol esterase; CHOD, cholesterol oxidase; POD, peroxidase.

C. Procedure

Concentrations of total cholesterol were measured by using a **SMART-120** chemistry analyzer and the procedure for blank, standard, and sample measurement is demonstrated in **Table (2.4).** The mixture was prepared and incubated at 37°C for 5 minutes. The sample-to-reagent ratio was 1:100, and the absorbance of the sample (Ax) and the standard (As) were read against a blank reagent at 510 nm.

Table (2.4): Procedure of total cholesterol assessment.

Pipette	Blank (µl)	Sample (µl)	Standard (µl)
Reagent (A)	1000	1000	1000
Water	10		
Sample		10	
Standard			10

Cholesterol (mg/dl) = Ax/As × 200 (standard value)

2.4.6.2. Measurement of Serum Triglyceride Concentration

A. Principle:

Triglycerides, esters of fatty acids and glycerol, do not circulate freely in plasma but are bound to proteins and transported as macromolecular complexes called lipoproteins. Methods for triglyceride determination generally involve enzymatic hydrolysis of triglycerides to glycerol and free fatty acids followed by enzymatic measurement of the glycerol released (**Kaplan and Pesce,1989**).

The enzyme lipase hydrolyzes triglycerides to produce glycerol and free fatty acids. The glycerol participates in a series of coupled enzymatic reactions, in which glycerol kinase (GK) and glycerol phosphate oxidase (GPO) are involved, and hydrogen peroxide (H_2O_2) is generated. Produced H_2O_2 reacts with TOOS and 4- AAP to form a colored complex, whose absorbance is directly proportional to the concentration of triglycerides in the sample **(Fossati and Prencipe,1982).**

A schematic representation of the reaction is shown in the following equations:

Lipoprotein Lipase

Triglyceride ----------►Glycerol + Fatty acids

Glycerol Kinase

Glycerol + ATP ----------► Glycerol-1-phosphate + ADP

Glycerol Phosphate Oxidase

Glycerol-1-phosphate + O_2 ----------►Dihydroxyacetone phosphate +H_2O_2

Peroxidase

H_2O_2 + 4-AAP + TOOS ----------► colored complex + H_2O

C. Reagents:

Table (2.5): Reagents used for triglycerides assay.

Reagent A (100 ml)	Good buffer	100 mmol/L
	Magnesium chloride	15 mmol/L
	ATP	4 mmol/L
	4-AAP	1 mmol/L
	TOOS	0.1 mmol/L
	LPL	2500 U/L
	POD	1800 U/L
	GK	1000 U/L
	GPO	5500 U/L
Standard (10 ml)	Glycerol	200 mg/dl

ATP, adenosine triphosphate; 4-AAP, 4-aminoantipyrine; LPL, lipoprotein lipase; POD, peroxidase; GK, glycerol kinase; GPO, glycerol phosphate oxidase.

D. Procedure:

Concentrations of triglycerides were measured by using a SMART-120 chemistry analyzer and the procedure for blank, standard, and sample measurement is demonstrated in **Table (2.6).** The mixture was prepared and incubated at 37°C for 5 minutes. The sample-to-reagent ratio was 1:100, and the absorbance of the sample (Ax) and the standard (As) were read against a blank reagent at 510 nm.

Table (2.6): Procedure of triglycerides assessment.

Pipette	Blank (µl)	Sample (µl)	Standard (µl)
Reagent (A)	1000	1000	1000
Water	10		
Sample		10	
Standard			10

Triglycerides (mg/dl) = Ax/As × 200 (standard value)

2.4.6.3. Measurement of Serum High-Density Lipoprotein

A. Principle:

High-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) are the lipoproteins responsible for the vast majority of cholesterol transport in the blood. Of the lipoprotein classes, HDL has the highest proportion of protein relative to lipids, containing slightly more than 50% protein, as well as having the densest and generally smallest size among the lipoproteins. Thus, high LDL levels and low HDL levels are strongly associated with an increased risk of adverse cardiovascular events. Specific polianions in the first phase block the

interfering lipoproteins LDL, VLDL, and chylomicrons (CM), and a specific surface-active agent inhibits the coloration of VLDL, LDL, and chylomicrons in the second phase. The intensity of color is directly proportional to the HDL-C in the sample **(Trinder,1969).**

A schematic representation of the reaction is shown in the following equations:

HDL + LDL + VLDL + CM ----------►HDL + (LDL+VLDL+CM)

Cholesterol Oxidase, Cholesterol Esterase

HDL----------► Fatty acids +H_2O_2

H_2O_2 + 4-AAP + HDAOS ----------► colored complex +H_2O

B. Reagents:

Table (2.7): Reagents used for high density lipoprotein cholesterol assay.

Reagent A (90 ml)	Good Buffer	100 mmol/L
	Polianions	1 mmol/l
	4-AAP	4 mmol/l
Reagent B (30 ml)	CHE	800 U/l
	CHOD	500 U/l
	Peroxidase	1500 U/l
	HDAOS	1 mmol/l
	Detergent	4 mmol/l

4-AAP, 4-aminoantipyrine; CHE, cholesterol esterase; CHOD, cholesterol oxidase.

C. Procedure:

The mixture was prepared and incubated at 37 °C for 5 minutes, and the absorbance of the blank sample (Abx) was read against a blank reagent at 600 nm. In the next step, reagent B was added, mixed, and incubated at 37°C for 5 minutes and the absorbance of the sample (Ax) and the standard (As) were read against a blank reagent. The procedure is demonstrated in **Table (2.8).**

Table (2.8): Procedure of high-density lipoprotein cholesterol assessment.

Pipette	Blank (µl)	Sample (µl)	Standard (µl)
Reagent (A)	300	300	300
Water	4		
Sample		4	
Standard			4
Reagent (B)	100	100	100

HDL (mg/dl) = (Ax – Abx)/ (As – Abs) × (Standard Value)

Low-density lipoprotein cholesterol (LDL) was calculated as:

LDL (mg/dl) = Total Cholesterol – HDL – (Triglycerides/5) (Langsted et al.,2020).

2.4.7. Measurement of Oxidative Stress

2.4.7.1. Assay of catalase Activity

Procedure:

Table (2.9): Procedure for assessments of catalase activity

Reagents	Test µl	Standard µl	Blank µl
Sample	100	------	-----
Phosphate buffer (50mM)	900	1000	3000
Hydrogen peroxide (10mM)	2000	2000	----
After mixing with a vortex and incubating for two minutes at 37 °C, add:			
Vanadium reagent	2000	2000	2000
The tubes were then held at 25 °C for 10 minutes. At 452 nm, the variations in absorbance were measured in comparison to the reagent blank.			

Calculation:

Enzyme activity procedure was elucidated in **Table (2.9).** The rate constant (k) of the first-order reaction equation for catalase activity was calculated using the following formula:

$$\text{Catalase Activity of test kU} = \frac{2.303}{t} * log \frac{S^o}{S} \quad ---(1)$$

where t is time, S^0 is the absorbance of the standard solution, and S is the absorbance of the sample (**Kumar et al.,2017).**

2.4.7.2. Determination of Malondialdehyde

Principle:

Lipid peroxidation in sera was evaluated by thiobarbituric acid reactive substances (TBARS). TBARS test gives a basic, reproducible, and standardized tool for measuring lipid peroxidation in serum. The MDA-TBA adduct designed by the response of MDA and 1,3-Diethyl-2-thiobarbituric acid (DETBA) under high temperature (90-100°C) at acidic conditions is measured calorimetrically at 530-540 nm or fluorometrically at an excitation wavelength of 515 nm and an emission wavelength of 555 nm. This reaction has a much higher sensitivity when measured fluorometrically.

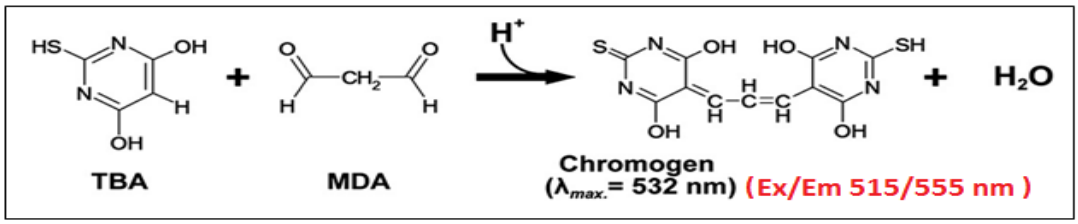


Figure (2.4) Scheme of the adduct MDA–$(TBA)_2$

2.4.8. Measurement of Insulin Resistance

HOMA-IR stands for Homeostatic Model Assessment of Insulin Resistance. The meaningful part of the acronym is “insulin resistance”. It marks for both the presence and extent of any insulin resistance that you might currently express. It is a terrific way to reveal the dynamic between your baseline (fasting) blood sugar and the responsive hormone insulin. HOMA-IR: Based on fasting glucose and fasting insulin concentrations.

Fasting glucose (mg/dl) × Fasting insulin (µU/l)/405 (Bahadur et al.,2021)

Healthy Range:1.0 (0.5–1.4)

Less than 1.0 indicates insulin-sensitive which is optimal.

Above 1.9 indicates early insulin resistance.

Above 2.9 indicates significant insulin resistance, **(Sachdev et al., 2022).**

2.4.8.1. Measurement of Fasting Serum Glucose Concentration

A. Principle:

Enzymatic indicator test based on the Trinder reaction quantified by a pink quinone imine dye formation. In this reaction, glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed is catalyzed by peroxidase and reacts with phenol and 4-amino antipyrine to form the dye indicator. **(Bergmeyer and Graßl, 1983).**

Glucose oxidase

Glucose + O_2 + H_2O----------►Gluconic acid + H_2O_2

Peroxidase

H_2O_2 + 4-aminoantipyrine + phenol----------►quinoneimine + $4H_2O$

B. Reagents:

Table (2.10): Reagent used for Glucose assay.

Glucose Reagent	Glucose Oxidase	>15 U/ml
	Peroxidase	>1 U/ml
	4-aminoantipyrine	0.7 mmol
	Phenol	11 mmol
	MOPS Buffer	0.05 mol
	Phosphate Buffer	0.025 mol

C. Procedure:

The mixture was prepared and incubated at 37 °C for 5 minutes, the absorbance of standard and samples was read against reagent blank at 500 nm.

The procedure of glucose determination is demonstrated in Table (2.11):

Pipette	Reagent Blank	Standard / Sample
Standard / Sample	—	10 µl
Glucose Reagent	1 ml	1 ml

Glucose Conc. = Sample abs./Standard abs. × Standard conc.

2.4.8.2. Measurement of Serum Fasting Insulin

A. Test Principle:

The sandwich principle of the Insulin level assay employs two monoclonal antibodies specifically directed against human Insulin.

B. Reagents - working solutions

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-insulin-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti-insulin antibody (mouse) 1 mg/L; MESb) buffer 50 mmol/L, pH 6.0; preservative.

R2 Anti-insulin-Ab~Ru(bpy) (black cap), 1 bottle, 10 mL: Monoclonal anti-insulin antibody (mouse) labeled with ruthenium complex 1.75 mg/L; MES buffer 50 mmol/L, pH 6.0; preservative.

C. Procedure

The total duration of the assay: 18 minutes.

1. 1st incubation: Insulin from 20 µL sample, a biotinylated monoclonal insulin specific antibody, and a monoclonal insulin specific antibody labeled with a ruthenium complexa) form a sandwich complex.

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

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

4. Results are determined via a calibration curve which is instrument specifically generated by 2 point calibration and a master curve provided via the reagent barcode or e-barcode.

2.4.9. Adiponectin Determination

A. Test Principle

This kit used enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the Human Adiponectin (ADP). Adiponectin (ADP) was added to the wells, which are pre-coated with Adiponectin (ADP)monoclonal antibody and then incubated. After that, anti-ADP antibodies labeled with biotin were added to unite with streptavidin-HRP, which forms an immune complex. Unbound enzymes after incubation were removed and washed. substrate A and B were added. Then the solution turned blue and changed into yellow with the effect of acid. The shades of solution and the concentration of Human Adiponectin (ADP) were positively correlated.

B. Reagent- Working Solutions

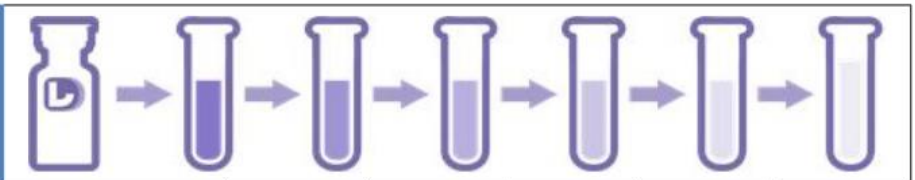
1. ELISA plate.
2. Standard solution.
3. Standard dilution.
4. Washing concentrated buffer.
5. Chromogen solution A.
6. Chromogen solution B.
7. Streptavidin–HRP.
8. Seal plate membrane.
9. Hermitic bag.
10. Anti-ADP antibodies labeled with biotin.
11. Stop the solution.

C. Procedure

1. Standard solutions were diluted as a representative in Table **(2.12):**

Table (2.12): Serial dilution method of Adiponectin standard

32mg/L	Standard No.5	120µl Original Standard + 120µl Standard diluents
16mg/L	Standard No.4	120µl Standard No.5 + 120µl Standard diluents
8mg/L	Standard No.3	120µl Standard No.4 + 120µl Standard diluent
4mg/L	Standard No.2	120µl Standard No.3 + 120µl Standard diluent
2mg/L	Standard No.1	120µl Standard No.2 + 120µl Standard diluent

Stock standard						
Tube	**standard**	**S5**	**S4**	**S3**	**S2**	**S1**
mg/L	64	32	16	8	4	2

2. Sample injection: 1) Blank well: Chromogen solution A and B, and stop solution were only added. 2) Standard solution well: 50µl standard and streptavidin-HRP 50µl were added. 3) Sample well to be tested: 40µl sample was added and then 10µl ADP antibodies and 50µl streptavidin-HRP. Then covered with a seal plate membrane and mixed them up. Incubated at 37°C for 60 min.
3. Washing solution was prepared by Diluting the washing concentration (30X) with 580 ml distilled water to reach 600 ml.
4. Washing: The seal plate membrane was removed, drained the liquid, and shaken off the remaining liquid. Each well is filled with washing solution. After 30 seconds of standing, the liquid was drained.

5. Color development: 50µl chromogen solution A was added first to each well and then added 50µl chromogen solution B to each well. Shaked and mixed them up. Incubated for 10 minutes at 37℃ away from light for color development.
6. Stop: 50µl Stop Solution was added to each well to stop the reaction (the blue color changed into yellow immediately at that moment).
7. . According to standards' concentrations and the corresponding OD values, the linear regression equation of the standard curve was calculated. Then according to the OD value of the samples, the concentration of the corresponding sample was calculated.
8. The concentration of standards the abscissa and OD value the ordinate was made. The standard curve was drawn on the coordinate paper as shown in **Figure (2.4).** According to the OD value of the sample, located its corresponding concentration (which is the concentration of the sample), or calculated the linear regression equation of the standard curve according to the concentration of the standard and the OD value. Then substituted with the OD value of the sample and the concentration of the sample was calculated.

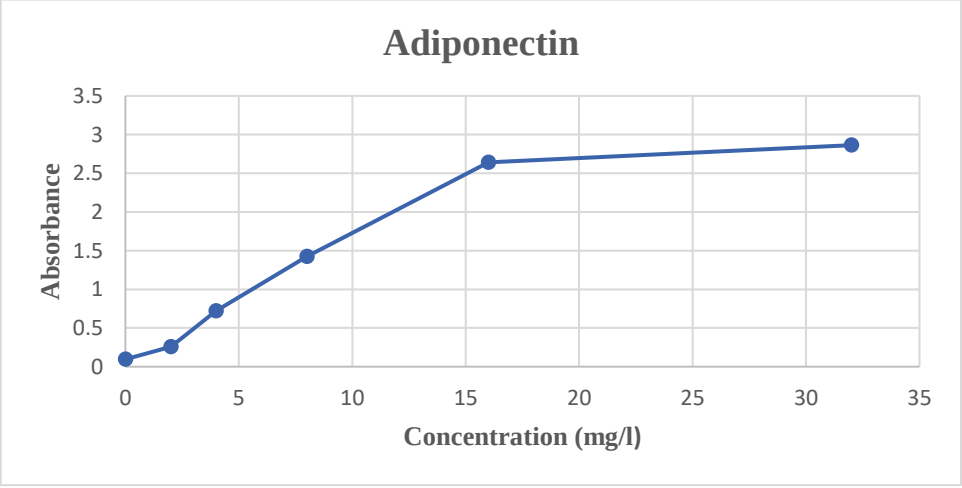


Figure (2-5): Standard curve for Adiponectin.

2.5. Statistical analysis

Information from the questionnaire and all test results from study groups samples were entered into a data sheet. The data analysis for this work was generated using the Statistical Package for the Social Sciences software, version 24.0 (IBM, SPSS, Chicago, Illinois, USA. Descriptive statistics were performed on the data of each group. Values were illustrated by No. for categorical, Scale variables were presented by mean ± standard deviation for normal data. The distribution of the data was checked using Shapiro-Wilk test as a numerical means of assessing normality. For abnormal distribution, the univariate analysis was performed using an independent Kruskal Wallis Test for continuous variables. Mean comparisons were made using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Biomarkers were compared using the Spearman rank test to evaluate the relationship within the case study. Significant differences in categorical variables among the parameters were confirmed through analytical statistical tests. Results of all hypothesis tests] with p values < 0.05 was considered to be statistically significant.

Chapter Three

Results

3. Results

3.1. Demographic Characteristics

The characteristics of the study groups are presented in **Table (3.1)**, and **Table (3.2)**, which consists of the data of both patients with PCOS and the control group. There was a significant difference (p= 0.032) in WHR between patients and the control group. Data reported that PCOS seems to be more prevalent among younger women with an age less than 26 years with a percentage of about (70%) compared to (30%) in patients with an age of more than 26 years. About, (71%) of patients were at high risk (≥ 0.85), (29%) were at low and moderate risk (< 0.85).

The hirsutism score showed that about (68.75%) of the participants had hirsutism, Also the presence and absence of acne were estimated to be 56.25%, and 43.75% respectively.

The investigation of ovarian morphology showed that 71 have PCOS patients and only 9 participants had normal morphology. Smoking is an important modifiable risk factor, it was examined, that most of the patients (58.75%) were confirmed to be passive smokers, while (38.75%) were non-smokers, and the remaining were active.

Table (3.1): Demographic characteristics of patients and control groups.

Characteristics	Group	Patient N= 80	Control N= 80
Age (year)	18-26	56	33
	27-35	23	41
	36-44	1	6
BMI (kg/m^2)	<30	40	40
	≥30	40	40
WHR	<0.85	23	43
	≥0.85	57	37
Hirsutism	Present	55	0
	Absent	25	80
Acne	Present	45	0
	Absent	35	80
Ultrasound	Normal	9	80
	PCOS ovary	71	0
Smoking Status	Passive	47	36
	Active	2	0
	Non-smoker	31	44

WHR, waist-hip ratio; BMI; body mass index.

Table (3.2): The comparison of Anthropometric characteristics between patients and the control group.

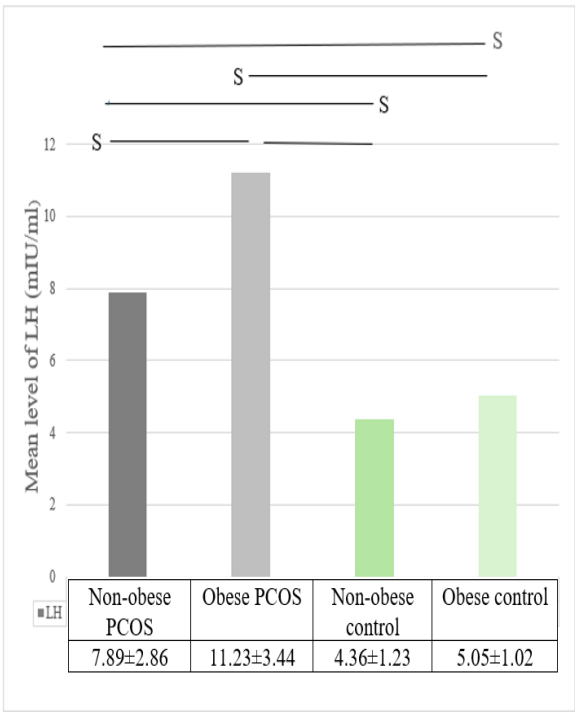
Parameters	Patient group Mean±SD	Control group Mean±SD	P value
Age (Year)	24.21±4.60	27.83±4.73	**≤0.001***
BMI (kg/m2)	29.09±5.32	28.74±5.76	0.694
WHR	0.84±0.05	0.82±0.05	**0.032***

Independent t-test. Data expressed as mean±SD; BMI: body max index; WHR: waist to hip ratio. Statistical significance at *p<0.05.

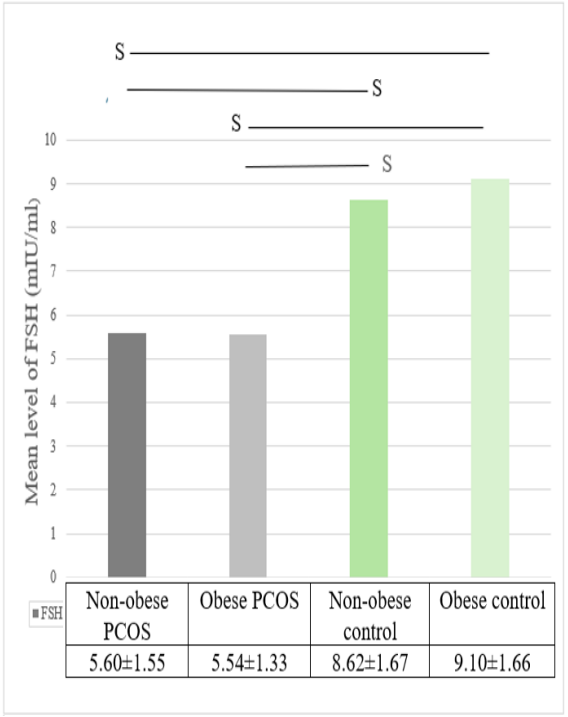
3.2. Examination of The Level of Hormones, metabolic features, Insulin Resistance indices, Adiponectin, and oxidative status for the PCOS and Control subgroups.

3.2.1. Comparison of Hormonal Characteristics among patients PCOS groups and Control groups.

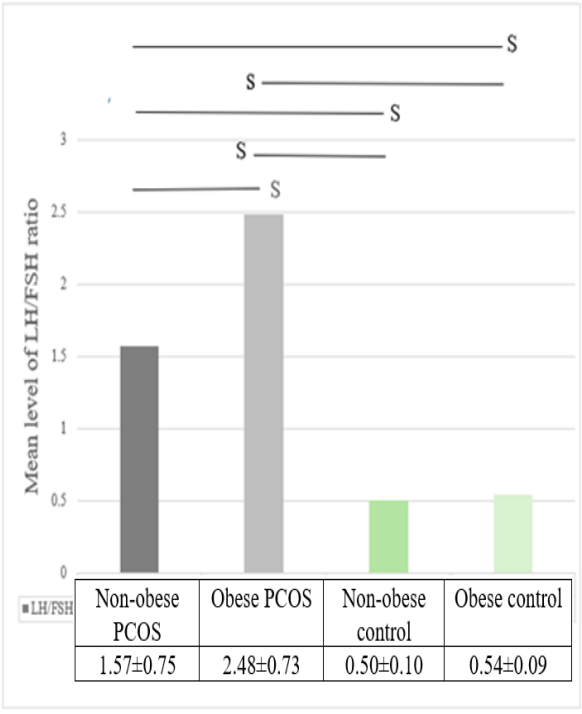
The levels of LH, LH/FSH, Prolactin, and Free testosterone were significantly higher in obese PCOS patients (11.23 mIU/ml, 2.48, 16.72 ng/ml, and 2.74 pg/ml, respectively) compared to the control subgroup. While the level of FSH was significantly decreased in obese PCOS patients (5.45 mIU/ml), **Figure (3.1)**. For obese and non-obese PCOS, there was a significant difference regarding the levels of LH, FSH, LH/FSH, Prolactin, and Free testosterone compared to the obese and non-obese control ($p \leq 0.05$), **Figure (3.1).**



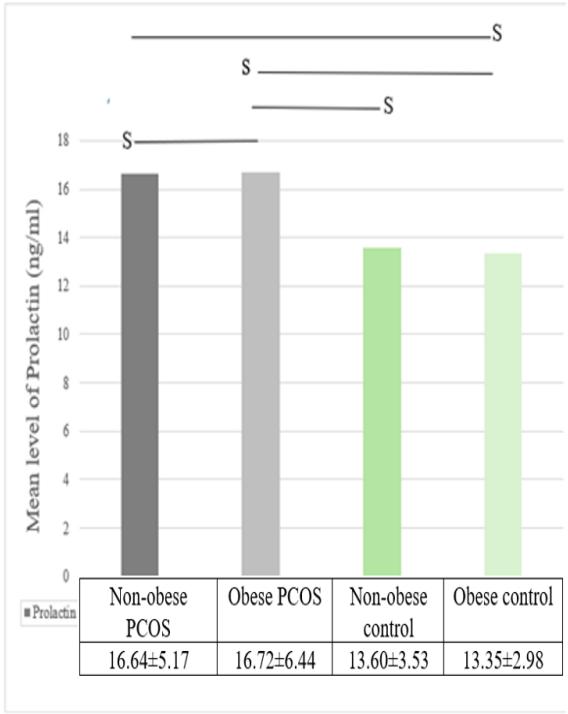
Non-obese PCOS	Obese PCOS	Non-obese control	Obese control
7.89±2.86	11.23±3.44	4.36±1.23	5.05±1.02



Non-obese PCOS	Obese PCOS	Non-obese control	Obese control
5.60±1.55	5.54±1.33	8.62±1.67	9.10±1.66



Non-obese PCOS	Obese PCOS	Non-obese control	Obese control
1.57±0.75	2.48±0.73	0.50±0.10	0.54±0.09



Non-obese PCOS	Obese PCOS	Non-obese control	Obese control
16.64±5.17	16.72±6.44	13.60±3.53	13.35±2.98

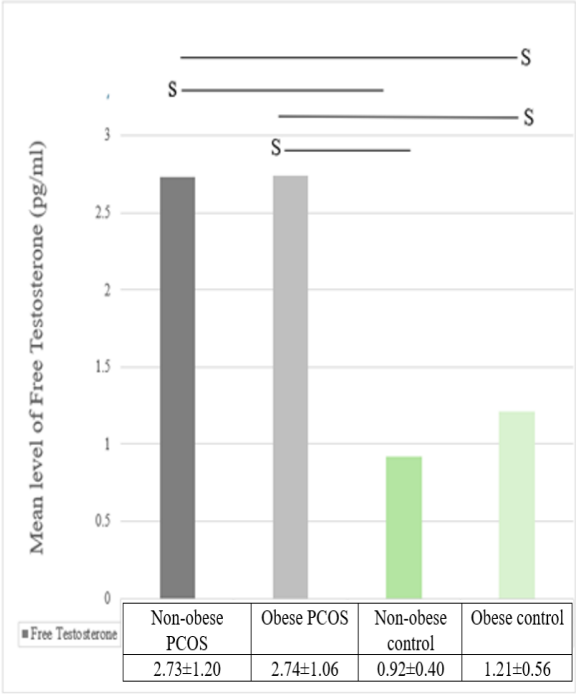


Figure (3.1): Mean Differences in Serum Level of Parameters (LH, FSH, LH/FSH, Prolactin, and Free testosterone) for the PCOS subgroups Compared to the Control subgroups.

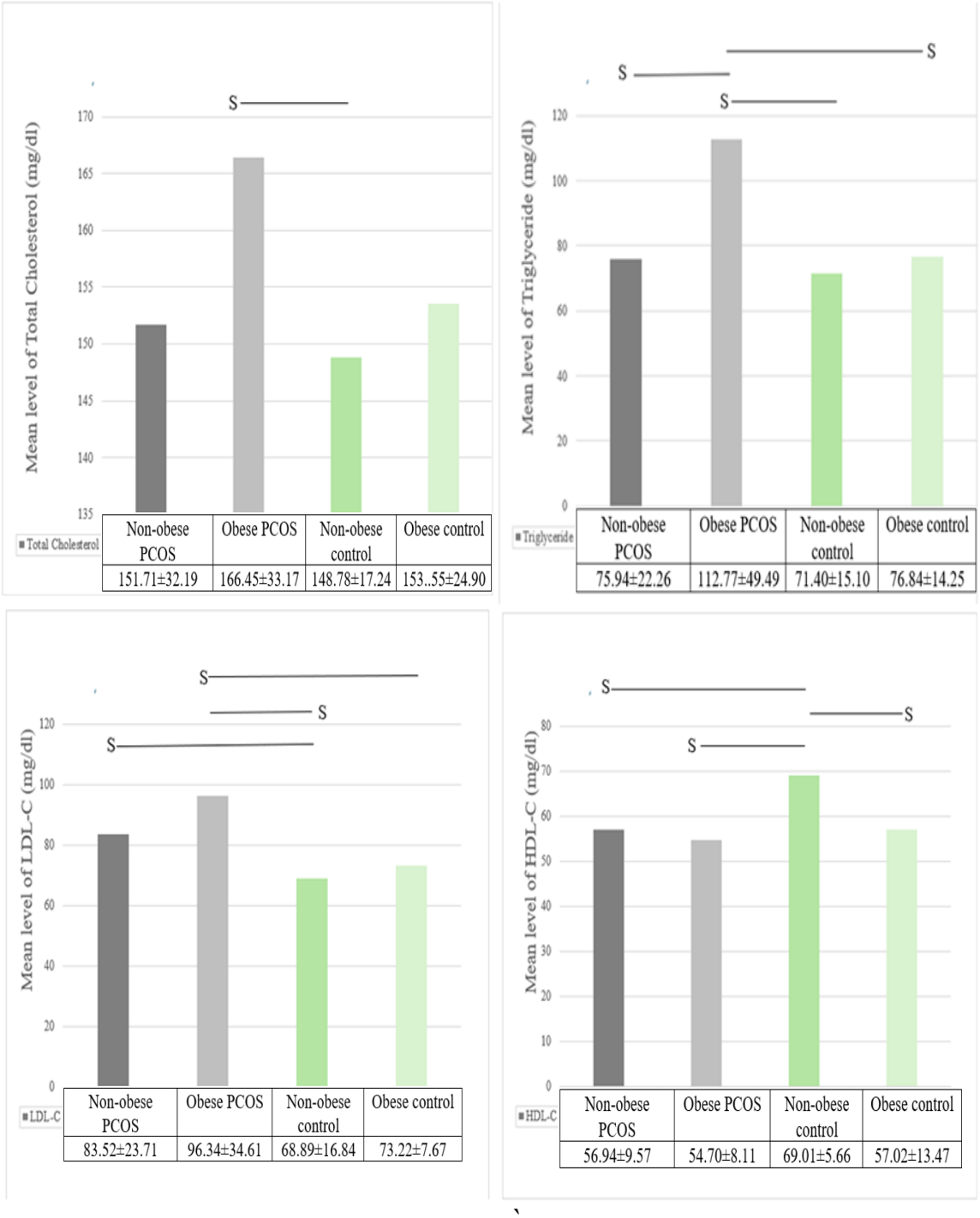
One-way ANOVA test; Data expressed as Mean±SD; LH: luteinizing hormone, FSH: follicular stimulating hormone; S: significant. Statistically significant at $p<0.05$.

3.2.2. Comparison of Metabolic characteristics among patients PCOS groups and Control groups.

The levels of total cholesterol, triglyceride, and LDL were significantly higher in obese PCOS patients (166.45, 112.77, and 96.34 mg/dl, respectively) compared to the controls, whereas HDL levels had reached the statistical significance ($P\leq0.05$) among obese and non-obese PCOS after comparison with non-obese control. For obese PCOS, there was a significant difference regarding the levels of triglyceride and LDL compared to the obese and non-obese control, **Figure (3.2).**

The comparison of fasting glucose and insulin showed that glucose was significantly elevated in both obese and non-obese PCOS women than

controls. Also, there were significant differences between PCOS subgroups and control subgroups ($p \leq 0.05$). Insulin levels were higher in the obese PCOS group (15.7µU/ml) compared with the control subgroup. There was a significant difference after the comparison of PCOS subgroups (obese and non-obese PCOS) and control subgroups ($p \leq 0.05$), **Figure (3.2).**



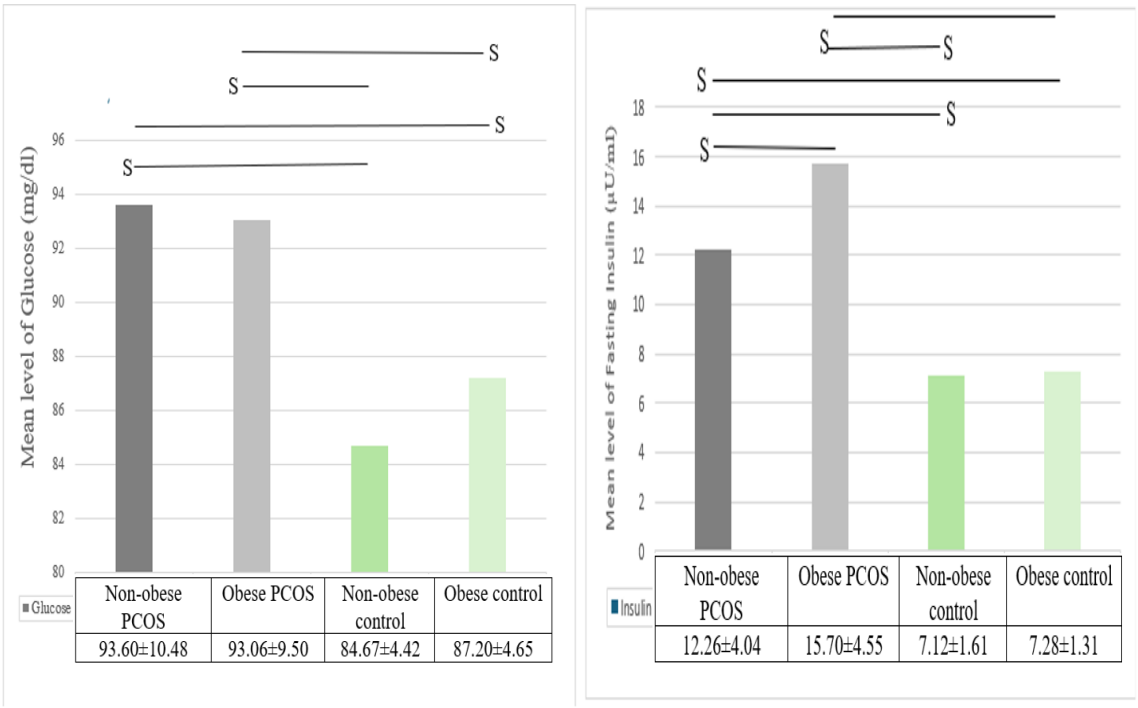


Figure (3.2): Mean Differences in Serum Level of Parameters (Total cholesterol, Triglycerides, LDL-C, HDL-C, Glucose, and Insulin) for the PCOS subgroup Compared to the Control subgroup.

One-way ANOVA test; Data expressed as Mean±SD; LDL-C: low-density lipoprotein- cholesterol; HDL-C: high-density lipoprotein-cholesterol; S: significant. Statistically significant at $p<0.05$.

3.2.3. Comparison of HOMA-IR among patients PCOS groups and Control groups.

The level of HOMA-IR was significantly higher in obese PCOS patients (3.8) compared to the controls. For obese and non-obese PCOS, there was a significant difference regarding the levels of HOMA-IR compared to the obese and non-obese control, **Figure (3.3).**

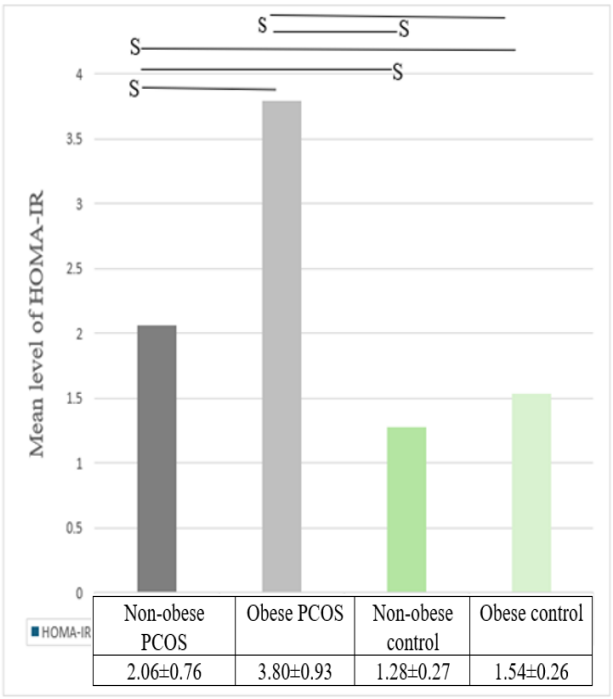


Figure (3.3): Mean Differences in Serum Level of HOMA-IR for the PCOS subgroups Compared to the Control subgroups.

One-way ANOVA test; Data expressed as Mean±SD; HOMA-IR: homeostasis model assessment-estimated insulin resistance; S: significant. Statistically significant at p<0.05.

3.2.4. Comparison of adiponectin among patients PCOS groups and Control groups.

The level of adiponectin was significantly decreased in obese PCOS patients (7.87 mg/l) compared to the controls. For obese and non-obese PCOS, there was a significant difference regarding the levels of adiponectin compared to the obese and non-obese control, **Figure (3.4).**

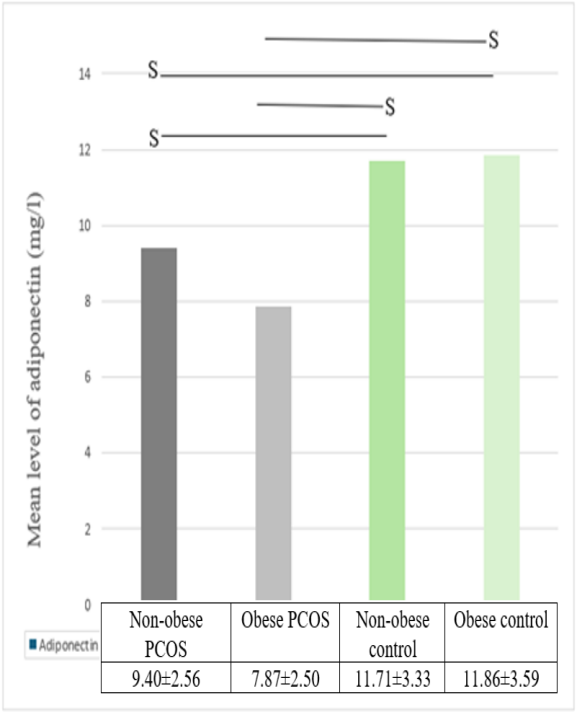


Figure (3.4): Mean Differences in Serum Level of adiponectin for the PCOS subgroups Compared to the Control subgroups.

One-way ANOVA test; Data expressed as Mean±SD; S: significant. Statistically significant at p<0.05.

3.2.5. Comparison of Catalase and Malondialdehyde among patients PCOS groups and Control groups.

The level of catalase was significantly decreased in obese PCOS patients (1.64 U/I) compared to the control subgroup. For non-obese PCOS, there was a significant difference regarding the levels of catalase compared to the obese and non-obese control ($p \leq 0.05$), while obese PCOS had no significant difference with obese control ($p > 0.05$), **Figure (3.5).** The level of Malondialdehyde was significantly higher in obese PCOS patients (5.65 mmol/l) compared to the control subgroup. For obese PCOS, there was a significant difference regarding the levels of MDA compared to the obese and non-obese control ($p \leq 0.05$), while non-obese PCOS had no significant difference with non-obese and obese control ($p > 0.05$), **Figure (3.5).**

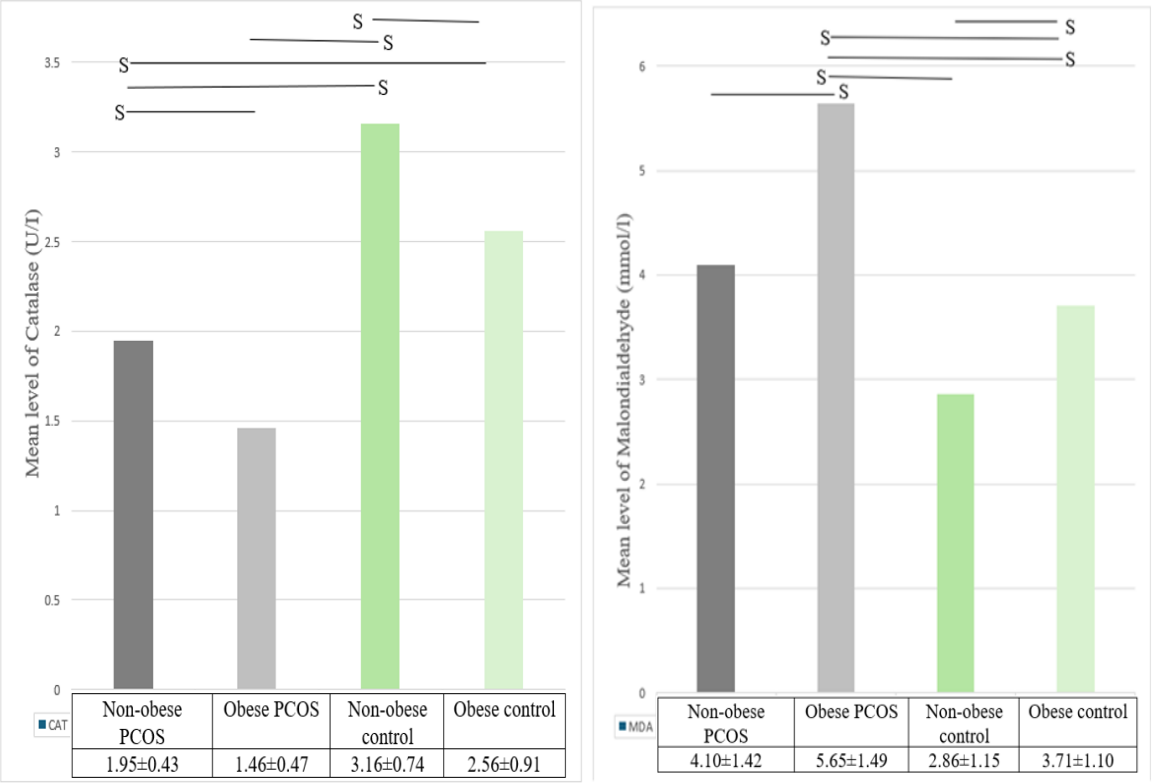


Figure (3.5): Mean Differences in Serum Level of Parameters (Catalase and Malondialdehyde) for the PCOS subgroups Compared to the Control subgroups.

One-way ANOVA test; Data expressed as Mean±SD; CAT: catalase; MDA: malondialdehyde; S: significant. Statistically significant at p<0.05.

3.3. Correlation between Biomarkers and studied parameters in patients' groups.

3.3.1. Correlation between Adiponectin and parameters.

There was a significant negative correlation between levels of adiponectin and BMI (p≤0.001), WHR(p=0.018), TC(p=0.005), TGL (p=0.028), LDL(p=0.008), HOMA-IR(p≤0.001) and MDA(p≤0.001). In addition, there was a significant positive correlation with CAT (p≤0.001), while there was no significant correlation with LH, FSH, LH/FSH ratio, Prolactin, Free testosterone, glucose, and insulin (p>0.05), as shown in **Table (3.3)**

Table (3.3): Correlation of adiponectin with Demographics and laboratory parameters in PCOS patients.

Parameters	Adiponectin	
	r	p
BMI	-0.641**	**≤0.001**
WHR	-0.265*	**0.018**
LH	-0.120	0.290
FSH	-0.113	0.318
LH/FSH ratio	-0.059	0.602
Prolactin	0.025	0.826
Free testosterone	0.003	0.976
TC	-0.308**	**0.005**
TG	-0.246*	**0.028**
HDL	-0.108	0.338
LDL	-0.294**	**0.008**
Glucose	-0.049	0.664
Insulin	-0.132	0.242
HOMA-IR	-0.611**	**≤0.001**
CAT	0.562**	**≤0.001**
MDA	-0.668**	**≤0.001**

Data expressed as Mean±SD; r: Pearson's correlation coefficient; BMI: body mass index; WHR: waist-hip ratio; ; LH: luteinizing hormone; FSH: follicular stimulating hormone; TC: total cholesterol; TG: triglyceride; ; LDL-C: low-density lipoprotein- cholesterol; HDL-C: high-density lipoprotein-cholesterol; HOMA-IR: homeostasis model assessment-estimated insulin resistance; catalase; MDA: malondialdehyde; S: significant. Statistically significant at p<0.05.

3.3.2. Correlation between Catalase and parameters

The results in **Table (3.4)** showed a significant negative correlation between levels of CAT and BMI (p≤0.001), WHR(p=0.004), TG (p **≤0.001**), insulin (p **≤0.001),** HOMA-IR(p≤0.001) and MDA(p≤0.001). In addition, there was a significant positive correlation with ADP (p≤0.001), while there was no significant correlation with LH, FSH, LH/FSH ratio, Prolactin, Free testosterone, TC, HDL, LDL, and glucose (p>0.05).

Table (3.4): Correlation of Catalase with Demographics and laboratory parameters in PCOS patients.

Parameters	CAT	
	r	p
BMI	-0.615**	**≤0.001**
WHR	-0.316**	**0.004**
LH	-0.112	0.322
FSH	0.116	0.308
LH/FSH ratio	-0.218	0.053
Prolactin	0.154	0.172
Free testosterone	0.175	0.120
TC	-0.213	0.058
TG	-0.381**	**≤0.001**
HDL	0.093	0.186
LDL	-0.150	0.352
Glucose	-0.105	0.053
Insulin	-0.217	**≤0.001**
HOMA-IR	-0.615**	**≤0.001**
ADP	0.562**	**≤0.001**
MDA	-0.630**	**≤0.001**

Data expressed as Mean±SD; r: Pearson's correlation coefficient; BMI: body mass index; WHR: waist-hip ratio; ; LH: luteinizing hormone; FSH: follicular stimulating hormone; TC: total cholesterol; TG: triglyceride; ; LDL-C: low-density lipoprotein- cholesterol; HDL-C: high-density lipoprotein-cholesterol; HOMA-IR: homeostasis model assessment-estimated insulin resistance; catalase; MDA: malondialdehyde; S: significant. Statistically significant at p<0.05.

3.3.3. Correlation between Malondialdehyde and parameters.

The results in **Table (3.5)** showed a significant positive correlation between levels of MDA and BMI (p≤0.001), WHR(p=0.001), LH(p=0.019), TC(p=0.025), TGL(p=0.002), HOMA-IR(p=0.050) and insulin(p≤0.001). In addition, there was a significant negative correlation with ADP(p≤0.001) and CAT(p≤0.001), while there was no correlation with FSH, LH/FSH ratio, Prolactin, Free testosterone, HDL, LDL and Glucose (p>0.05).

Table (3.5): Correlation of Malondialdehyde with Demographics and laboratory parameters in PCOS patients.

Parameters	MDA	
	r	p
BMI	0.796**	**≤0.001**
WHR	0.356**	**0.001**
LH	0.262*	**0.019**
FSH	0.081	0.477
LH/FSH ratio	0.208	0.064

Prolactin	-0.032	0.775
Free testosterone	-0.088	0.436
TC	0.251*	**0.025**
TG	0.349**	**0.002**
HDL	-0.116	0.414
LDL	0.210	0.186
Glucose	-0.092	0.352
Insulin	0.244*	**0.050**
HOMA-IR	0.748**	**≤0.001**
ADP	-0.668**	**≤0.001**
CAT	-0.630**	**≤0.001**

Data expressed as Mean±SD; r: Pearson's correlation coefficient; BMI: body mass index; WHR: waist-hip ratio; ; LH: luteinizing hormone; FSH: follicular stimulating hormone; TC: total cholesterol; TG: triglyceride; ; LDL-C: low-density lipoprotein- cholesterol; HDL-C: high-density lipoprotein-cholesterol; HOMA-IR: homeostasis model assessment-estimated insulin resistance; catalase; MDA: malondialdehyde; S: significant. Statistically significant at p<0.05.

3.4. Study the association of biomarkers with patients' groups

The Multinomial logistic regression was performed to analyze the association of the CAT, ADP, and MDA with the PCOS subgroups. It was found that all the parameters were highly significant differences in the PCOS subgroup. MDA was represented as a risk factor in PCOS patients, while CAT and ADP were represented as protective factors, as shown in Table **(3.6).**

Table (3.6): The Multinomial logistic regression of PCOs with levels of biomarkers.

Biomarkers	Groups	OR (Lower-Upper)	P-Value
ADP	**Non-obese PCOS**	0.784 (0.673-0.914)	**0.002[S]**
	Obese PCOS	0.640 (0.534-0.768)	**<0.001[S]**
	Obese control	0.894 (0.885-1.162)	0.836[NS]
	Non-obese control	1[a]	-
CAT			
	Non-obese PCOS	0.097 (0.035-0.274)	**<0.001[S]**
	Obese PCOS	0.035 (0.01-0.13)	**<0.001[S]**
	Obese control	0.346 (0.144-0.836)	**0.002[S]**
	Non-obese control	1[a]	-
MDA			
	Non-obese PCOS	1.144 (0.641-2.041)	**<0.001[S]**
	Obese PCOS	2.165 (1.135-4.127)	**<0.001[S]**
	Obese control	1.843 (1.044-3.2510)	**0.003[S]**
	Non-obese control	1[a]	-

Multinomial logistic regression; ADP: adiponectin; CAT: catalase; MDA: malondialdehyde; OR: odd ratio; [S]= Significant, [NS]= non-significant; 1[a]: The reference category is Control; statistically significant at p<0.05.

3.5. Receiver operating characteristic (ROC)

ROC curve and AUC analysis were performed for the MDA for the patient group. Results of the receiver operating curve (ROC) curve and AUC analysis for the MAD diagnostic parameters showed that MAD has a good performance for prediction patients, data are presented in **Table (3.7) and Figure (3.6).**

For MAD levels: (sensitivity 60 %, specificity 81.2%) at a level = 4.305 (mmol/l). The p-values of the AUC were <0.001 and highly statistically significant. The sensitivity and specificity results were confirmed using Youden's J statistics.

Table (3.7): AUC, optimal threshold, sensitivity, and specificity of MDA obtained by the ROC curve in patients.

Variable(s)	MDA
AUP	76.3%
Sensitivity %	60%
Specificity %	81.2%
Youden index	0.412
Cut-off points	> 4.305 mmol/l
CI (95%)	0.691-0.836
PPV	88.6%
NPV	47.3%
Accuracy	63.4%
P value	**<0.001[S]**

ROC; Receiver operating characteristic; AUC: area under curve; CI; confidence interval; s: significant; PPV: positive predictive value; NPV: negative predictive value. Statistically significant at p <0.05.

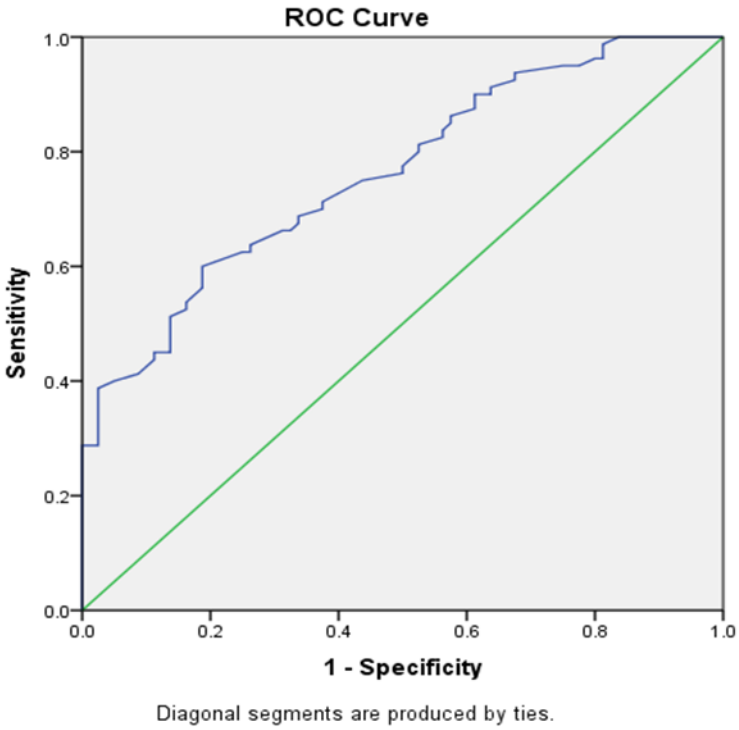


Figure (3.6): ROC curve for Malondialdehyde.

Chapter Four

Discussion

4. Discussion

Polycystic ovary syndrome (PCOS)is the most common chronic reproductive and metabolic endocrine disorder affecting women of childbearing age, with prevalence estimated to be 4%–21%worldwide **(Belenkaia et al.,2019).** In addition to infertility, it can lead to serious complications such as metabolic syndrome, diabetes Mellitus, dyslipidemia, endometrial cancer, and cardiovascular disease **(Nandi et al.,2014).**

The prevalence of PCOS is likely to increase in parallel with the obesity epidemic **(Barber et al., 2006).** BMI represents the sum of skeletal muscle mass, bone, organs and peripheral and visceral adipose tissues. All these components of BMI have different roles in contributing to health status **(Donini et al., 2020)**. Lim et al. in a systemic review and meta-analysis concluded that women with PCOS had a greater risk of overweight, obesity, and central obesity **(Lim et al., 2012).**

Waist to hip ratio in women is an accurate indicator of health, youth, and fertility **(Streeter and McBurney, 2003).** The current study presented evidence showing a clear association between WHR and PCOS. Obese patients were at high risk (≥ 0.86), and non-obese patients were at moderate risk (0.81 - 0.85). Most investigators have found that (30% - 50%) of women with PCOS are obese with a tendency to have an increased WHR or abdominal obesity.

There is also evidence showing that even normal-weight PCOS subjects have increased intra-abdominal fat **(Yildirim et al., 2003)**. Measurement of visceral adipose tissue by WHR may offer a warning of metabolic disorders in PCOS women. A recent study confirmed that PCOS women are more susceptible to increasing WHR regarding the development of IR **(ŠumaracDumanović et al., 2022).**

Hirsutism is common in women with PCOS and is the most consistent and reliable symptom used for evaluating clinical hyperandrogenism. In current study, (69%) of PCOS women were hirsute. In a large study of over 1000 women with androgen excess, 659 presented with hirsutism, and (78%) of the hirsute women were diagnosed with PCOS **(Azziz et al., 2004)**

Hyperandrogenism, in women with PCOS, might be clinically presented as acne. Androgens cause excessive sebum production, which is the precipitating factor for the formation of acne lesions **(Ashraf et al., 2019)**. In the presented study, (56%) of PCOS women were positively affected with acne, whereas (44%) were not. A previous study recorded that the prevalence of acne in PCOS women of Kashmir is (48%) **(Keen et al., 2017).** Another study has estimated the prevalence of acne in patients with PCOS at (10 - 34)% **(Tutakne and Chari, 2003)**

Elevated LH is a frequent finding of polycystic ovarian syndrome (PCOS), however, it is not required for diagnosis. LH is known to enhance ovarian androgen production in addition to inducing ovulation and luteinization, and it is one of the primary factors contributing to hyperandrogenism in PCOS patients. Luteinizing hormone stimulates the production of androgens largely in ovarian theca cells that have LH receptors **(Ashraf et al., 2019)**. Higher LH concentrations appear to be related to more severe types of PCOS.

Previous research has demonstrated a positive association between follicle number and ovarian volume, with higher LH concentrations in PCOS patients being associated with more severe cycle disruptions and a higher likelihood of infertility. LH hypersecretion in PCOS women also reflects how severe the condition is **(Hendriks et al., 2008)**.

Numerous follicles in the theca cells of the ovaries become mostly pre-antral and antral stages arrested due to increased LH stimulation, leading to hyperplasia of theca cells and accumulation of follicular fluid that forms

cyst-like structures along the periphery of the ovary giving it a string of pearls appearance. In contrast, a relative deficit in FSH impairs follicular development **(Ashraf et al., 2019)**. Increased LH pulse frequency impairs estrogen and FSH synthesis, thus inhibiting follicle growth and ovulation and finally contributes to the formation of polycystic ovaries in PCOS patients **(Liao et al., 2021)**. Consistently rapid (GnRH) pulsatility, which favors pituitary LH over FSH synthesis and leads to the elevated LH concentrations and consequently changed LH / FSH ratios typical of PCOS, is thought to be a neuroendocrine feature of the condition. Follicular development is hampered by low FSH levels, while ovarian androgen production is enhanced by high LH levels **(Malini and George, 2018)**. In PCOS, FSH levels may be increase, fall below their normal levels or remain constant **(Mohammed and Qasim, 2021)**. The occurrence of the disorder in the LH and FSH hormones is the result of hypothalamus pituitary gland, which causes the difference in the level of these hormones in the affected women (**Mansour et al., 2020).**

The pathogenesis of polycystic ovary syndrome has been forced to include abnormality of the hypothalamic-pituitaryovarian or adrenal axis. The relative increase in LH to FSH release is caused by a disruption in the gonadotrophin-releasing hormone's (GnRH) pattern of production. The ovarian granulosa cells are affected by FSH, which transforms the androgens produced in the theca cells into estrogens, primarily estradiol, which is necessary for the formation of follicles **(Ashraf et al., 2019).** The aberrant feedback mechanism that increased LH release was brought on by ovarian estrogen. LH to FSH ratios in healthy women typically range between 1 and 2. This ratio is inverted in women who have polycystic ovarian disease, and it may even increase to 2 or 3 **(Saadia, 2020)**.

Either free or coupled to proteins like SHBG and albumin, testosterone is present. Normal testosterone levels are as follows: 80% of it is bound to sex hormone-binding globulin, 19% of it is tied to albumin, and only 1% is free to circulate. According to the Rotterdam Agreement, circulating free testosterone or FAI measures should be used instead of serum total testosterone to detect hyperandrogenism in women with PCOS **(Ashraf et al., 2019)**.

The hyperplasia of theca cells in PCOS women's ovaries and the deregulation of steroid production pathway enzymes are both factors in the rise in androgen. Increased androgens prevent the hypothalamic-pituitary axis from receiving any negative feedback, increasing the frequency of GnRH pulses. LH production is more favored by elevated hypothalamic GnRH than FSH production **(Ashraf et al., 2019)**. The first impact of androgen excess in PCOS is impaired folliculogenesis. Increased androgens in the early gonadotropin-independent stage stimulate the formation of primordial follicles and increase the number of small antral follicles **(Dewailly et al., 2016)**. High LH encourages the ovary to secrete too many androgens in PCOS patients, while increased FSH may prompt the ovary's cells to convert too much androgens to estrogen **(Malini and George, 2018).**

The diagnosis of PCOS and/or phenotypic should be made when the presence of clinical indicators of hyperandrogenemia is uncertain or missing. This can be done by testing calculated free testosterone, free androgen index, or calculated bioavailable testosterone in clinical practice. Even though 78 percent of PCOS patients visiting clinics exhibit biochemical hyperandrogenemia **(Mumusoglu and Yildiz, 2020).** Therefore, The main factor believed to be responsible for this disorder's symptoms and indications developing is an excess of androgen **(Özen and Cander, 2022)**.

Hyperprolactinemia and polycystic ovary syndrome (PCOS) are on the list of the most frequent causes of female infertility **(Dehghan et al., 2021)**. Opinions on the relationship between PCOS and hyperprolactinemia widely vary from the recognition of elevated levels of Prolactin as a disorder characteristic of PCOS on one hand, to the obligatory exclusion of hyperprolactinemia **(Zafar et al., 2021)**. There have been several possible mechanisms increasing Prolactin secretion that may occur in PCOS. The effect of estrogens, which stimulate the synthesis and secretion of prolactin, as well as cell proliferation of lacto tropic pituitary cells. Elevated estradiol levels in PCOS could result in increased Prolactin concentrations **(Szosland et al., 2015)**. The role of Prolactin in promoting insulin secretion in islets is well-demonstrated. According to a research, prolactin can influence the target organs' essential enzymes and transporters involved in the metabolism of glucose and lipids as well as metabolism balance **(Ramirez and de Sá, 2021)**. Therefore, it has been hypothesized that prolactin is intimately linked to insulin resistance, hypertension, thromboembolic stroke, and coronary syndrome **(Chen et al., 2022)**.

A relatively significant subset of infertile women includes those with PCOS. Hyperinflammation, insulin resistance, decreased glucose tolerance, metabolic syndrome, hyperlipidemia, hypertension, and an elevated risk of cardiovascular disease are all intimately related to PCOS. According to a research by Haiyan Yang, PRL is considerably lower in PCOS patients than in controls **(Yang et al., 2020)**. While others showed that the serum Prolactin upper reference limit was almost 1.5 times higher than in controls. The route behind PCOS-related Prolactin elevation may be explained by a decrease in central dopaminergic tone that causes both Prolactin and LH levels to rise **(Mahboobifard et al., 2022)**. Another prior study has reported cases of twin sisters presenting with features of PCOS associated with idiopathic hyperprolactinemia **(Goyal and Ganie, 2018).**

About lipid profile Rizzo et al. concluded that total cholesterol, triglyceride, and LDL concentrations were higher and HDL levels were lower in controls versus PCOS **(Rizzo et al., 2009)**. It was demonstrated that obese women have elevated serum levels of cholesterol and LDL as compared with the corresponding levels in the normal weight group and higher triglycerides and lower HDL than normal or overweight PCOS women **(Castelo-Branco et al., 2010**). However, many women with PCOS still have a completely normal lipid profile and in larger studies of lipid levels in women with PCOS mostly fall within normal ranges as determined by National Cholesterol Education **(Legro et al., 2003)**.

Increases in TG, TC, and LDL-C and a reduction in HDL-C are characteristics of obese patients' aberrant blood lipid profiles **(Durmus et al., 2017)**. Current findings indicated that obese PCOS women had greater abnormality in lipid metabolism than the obese control group, including higher levels of TG, Cholesterol, and LDL-C and no differences in HDL-C levels, like the result reported by **(Ibrahim et al.,2020)**. Hyperinsulinemia and hyperandrogenism may also be linked to elevated blood LDL-C levels in PCOS patients. When IR exists, lipoprotein lipase activity declines, which diminishes the liver's ability to remove LDL-C.

Furthermore, it has been demonstrated that testosterone inhibits the ability of estrogen to cause the liver to produce LDL-C receptors, lowering hepatic LDL-C receptors and raising plasma LDL-C levels **(Feingold et al.,2017).** Additionally, testosterone can stimulate the liver to create TG by changing the catecholamine signal in adipocytes and increasing the release of circulating non-esterified fatty acids. Therefore, in PCOS patients, hyperandrogenism may also result in a rise in TG **(Li et al., 2017)**. However, it seems that different researches had reported contradicting results because of characteristics like race, genetics, lifestyle, and geographic region.

In current study, both obese and non-obese PCOS subjects had significantly higher mean fasting blood glucose levels compared to controls, combined with significantly higher serum levels of insulin and IR. Zuo et al. reported making comparable findings **(Zuo et al.,2016)**. The main mechanism in the etiology of PCOS in both obese and non-obese people is thought to be hyperinsulinemia and IR, Yeon et al **(Yeon Lee et al.,2010)** have demonstrated that obese PCOS women with elevated blood glucose and BMI of over 27 develop diabetes. The percentage of obese PCOS patients was 31% compared to 10.3% of thin PCOS patients and 7.5% compared to 1.5% of PCOS patients who are slim, respectively.

In PCOS patients, insulin signaling that is mediated by a protein tyrosine kinase receptor has been studied, **(James et al., 2021),** revealing increased insulin receptor serine phosphorylation in insulin-resistant PCOS patients, which prevents insulin receptor tyrosine kinase function. It also influences the P450c17 enzyme's activity, which in women with PCOS results in hyperandrogenism. Additionally, hyperinsulinemia amplifies the actions of LH on theca interstitial cells, increasing the synthesis of androgen **(Yeon Lee et al.,2010)**.

Patients with PCOS have an IR rate between 50% and 70%. Because reactive oxygen species (ROS) are produced as a result of hyperglycemia and increased levels of free fatty acids, IR promotes Oxidative stress. According to several studies, the degree of clinical presentation and hyperinsulinemia severity are associated **(Yeon Lee et al.,2010).**

Patients with PCOS have shown signs of oxidative stress brought on by hyperglycemia, IR, and ongoing inflammation. Due to the excess generation of ROS caused by hyperglycemia and greater amounts of free fatty acids, IR increases Oxidative stress. By causing multinucleated cells to produce tumor necrosis factor (TNF a), hyperglycemia also contributes to inflammation.

Excess testosterone enhances the production of ROS from leukocytes, the expression of the p47phox gene, and the development of MDA, according to studies done on lean, healthy women of reproductive age who also had hyperglycemia. It's possible that diet-induced Oxidative stress, with hyperandrogenism as the progenitor, is the cause of OS being present in the lack of obesity. Chronic inflammation is exacerbated by Oxidative stress, and vice versa **(Deba et al.,2017).**

One of the major contributing factors to difficulties in PCOS women with increased adiposity is altered adiponectin levels. The current study assessed the levels of adiponectin in women with PCOS who were in reproductive age groups. The serum adiponectin levels of PCOS women were considerably lower than those of control women. Previous research revealed similar results **(Beyazit et al.,2021) (Onyegbule et al.,2022)**.

In contrast, a study found that regardless of body mass index status, patients with PCOS exhibited considerably lower levels of serum adiponectin than controls **(Jahan et al.,2023**). There are several theories as to why women with PCOS have lower adiponectin levels. While some research has indicated that glucose intolerance and insulin resistance are the causes of alterations in adiponectin concentrations **(Zusi et al.,2023)**. Others have shown this insulin resistance has no effect on adiponectin levels, which fluctuates according to the degree of obesity **(Escobar-Morreale et al.,2006)**.

PCOS women in the current study were overweight and almost identical to controls in terms of BMI. According to a study, women with PCOS express considerably less adiponectin messenger RNA (mRNA) than weight-matched women without PCOS. The lower expression of adiponectin mRNA in both subcutaneous and visceral fat tissue was found to be compatible with

the lower levels of circulating adiponectin levels observed in women with PCOS **(Yadav et al.,2013)**.

The greater BMI of PCOS women in the current study suggests a higher degree of adiposity, which may be linked to their lower adiponectin levels. Li et al. have, however, also reported a drop in total adiponectin **(Li et al.,2015)** and HMW adiponectin levels in PCOS women, suggesting that the low levels of HMW adiponectin in PCOS are caused by posttranscriptional/translational changes and that the decreased adiponectin levels happen without regard to BMI or insulin resistance **(O'Connor et al.,2010**).

In the current study, the concentration of malondialdehyde significantly increased at the probability level ($p \leq 0.01$) in the group of obese women with PCOS compared to the obese control group. Based on the findings, it was believed that the source of the elevated oxidative stress was obesity. The findings of this study are in agreement with those of Zimmer and his team's study **(Zimmer et al.,2010)**, which showed a rise in malondialdehyde levels due to an uptick in the lipid peroxidation process of unsaturated fatty acids in various cell types, including egg cells, as a result of an uptick in various active forms of oxygen and nitrogen, and this rise gives a hint. Increased ROS production as a result of excessive oxidative damage in the participants may therefore be the cause of higher MDA concentrations. As a result, these oxygen species have the ability to oxidize a variety of other important biomolecules, such as membrane lipids.

Rasool and his team have determined that women with polycystic ovarian syndrome exhibit symptoms of oxidative stress since the MDA value is one of the markers of the occurrence of oxidation within the body caused by the lipid peroxidation of fatty acids **(Muhammad et al.,2018)** agreed with the results as well, indicating that MDA concentrations were higher in PCOS-

afflicted women than in control women, and Ukan **(Uçkan et al.,2022)** further demonstrated that MDA concentrations were higher in obese women than in non-obese women.

Receiver operating characteristic analysis used to assess our findings to gauge the diagnostic potency of the expression. It was noticed that the AUC of MDA levels was 76.3% and this could aid in distinguishing patients with PCOS from those without PCOS. A study by Rashad et al. supported current findings **(Rashad et al.,2019**).

An intracellular antioxidant enzyme called catalase is mostly found in cell peroxisomes and to a lesser extent in the cytoplasm. It catalyzes the conversion of hydrogen peroxide to water and molecular oxygen. According to the current study, the activity of catalase in serum samples from PCOS patients was significantly lower than that of controls. Al-Azzawie et al. and Kandasamy et al. both observed that PCOS patients have significantly lower catalase activity compared to the control group **(Al-Azzawie and Humadi.,2010).** As a result, the decrease in catalase activity may be caused by the formation of ROS since PCOS patients' oxidative stress reduced catalase function. Additionally, it was believed that hyperinsulinemia and dyslipidemia factors actively contribute to the reduction in antioxidant levels associated with obesity and an increase in oxidative stress.

Due to its involvement in lipid and carbohydrate metabolism as well as insulin sensitivity, adiponectin plays a significant role in maintaining energy balance. The relationship between adiponectin and the factors under investigation was examined in the current study. Adiponectin was found to have a substantial negative correlation with BMI. Shin & others **(Shin et al.,2011),** found that in their study of 60 PCOS individuals, adiponectin was associated with obesity, and Ramanand et al., **(Ramanand et al.,2013)** found that in PCOS women, there was a strong inverse relationship between

adiponectin and age. Rather than indicating a correlation with fat, the study's measurements of adiponectin in PCOS women demonstrated a substantial negative connection with hyperinsulinemia and IR. Sangeeta reported similar findings **(Sangeeta.,2012)**. On the other hand, adiponectin was found to exhibit a non-significant negative correlation with both fasting insulin levels and HOMA-IR in earlier research **(Sangeeta.,2012)**. Different genetic traits and varying patterns of deposition in different groups can account for this variation.

Adiponectin and total cholesterol, LDL, and triglycerides were revealed to correlate in the current study significantly negatively. Izadi et al. obtained similar results **(Izadi et al.,2013)**. The observed inverse relationship between serum apo-CIII and circulating adiponectin suggests that adiponectin-induced decrease in apo-CIII, a well-known LPL inhibitor, is the likely mechanism for TG reduction by adiponectin **(Tsubakio-Yamamoto et al.,2012).**

In the correlation, there were positive associations between MDA and BMI, WHR, HOMA-IR, total cholesterol, triglycerides, and LH **.**The current results are consistent with those of earlier research **(Uçkan et al.,2022)**. Another study that found a negative association between MDA and BMI, WHR, cholesterol, and LH conflicts with current findings **(Yilmaz et al.,2021).** Reviewing the correlation analysis carried out in the patient group revealed that increased oxidative stress exacerbated dyslipidemia and hyperinsulinemia, two indications of PCOS-associated metabolic syndrome. Additionally, a study recommended tracking variables including MDA, SOD, TG, and LDL throughout PCOS patients' recovery periods **(Uçkan et al.,2022)**.

Additionally, a negative association between the waist-hip ratio (cm), BMI, HOMA-IR, and triglyceride levels were discovered. These findings were in

line with earlier research **(Malini.,2023).** Therefore, it can be said that patients who have low catalase levels undergo oxidative stress.

In conclusion, it was found that both PCOS groups reported poor antioxidant levels, particularly the obese PCOS group, and considerably increased MDA values.

Since the findings of hyperinsulinemia and dyslipidemia were linked to oxidative stress and lowered antioxidant markers, it was believed that PCOS patients' oxidative stress conditions may play a role in metabolic syndrome and cardiovascular diseases. Elevated oxidative stress, low antioxidant levels, and insulin resistance, as well as the connections between these variables, lend credence to the theory that oxidative stress contributes to the etiology of PCOS.

Furthermore, increased insulin resistance has given rise to higher HOMA-IR readings in obese PCOS individuals. The HOMA-IR level in PCOS patients who are not obese, in contrast to the control group, has not changed. It most likely results from elevated levels of LDL, triglycerides, and total cholesterol in PCOS groups with obesity.

As a result, in addition to existing risk factors including dyslipidemia, central obesity, and insulin resistance, oxidative stress may further raise the chance of developing cardiovascular problems in women. Future studies on obese and non-obese PCOS patients need to pay more attention to oxidative stress.

Chapter Five

Conclusions and Recommendations

5. Conclusions and Recommendations

5.1. Conclusions

1. The level of adiponectin and catalase were significantly decrease in obese and non-obese PCOS patients compared to control subgroup. While Malondialdehyde level was increase in all PCOS patients compared to control group.
2. Results were indicated a significant correlation between adiponectin and BMI, WHR, HOMA-IR, TC, TG and LDL. A significant correlation of catalase and MDA with BMI, WHR and HOMA-IR. MDA exhibit good diagnostic efficacy according to ROC analysis.

5.2. Recommendations

1. To study genes affecting obesity in PCOS patients considering obesity of the most serious signs.
2. Further studies are needed to standardize measurement units of each biomarker to facilitate comparison across studies and also examine the mechanism of oxidative stress on PCOS.
3. Increase the size of the sample and include other groups, such as patients with diabetics and patients with heart diseases.
4. Increase the research on the effects of PCOS in menopausal women and their increased risk of endometrial cancer.

References

References

- Abasian, Z., Rostamzadeh, A., Mohammadi, M., Hosseini, M., and Rafieian-Kopaei, M. **(2018).** A review on the role of medicinal plants in polycystic ovarian syndrome: pathophysiology, neuroendocrine signaling, therapeutic status, and prospects. Middle East Fertility Society Journal, 23(4), 255-262.
- Agarwal, A., Aponte-Mellado, A., Premkumar, B. J., Shaman, A., and Gupta, S. **(2012).** The effects of oxidative stress on female reproduction: a review. Reproductive biology and endocrinology, 10, 1-31.
- Ajmal, N., Khan, S. Z., and Shaikh, R. **(2019).** Polycystic ovary syndrome (PCOS) and genetic predisposition: A review article. *European journal of obstetrics & gynecology and reproductive biology: X*, *3*, 100060.
- Akram, M. and Roohi, N. **(2015**). Endocrine correlates of polycystic ovary syndrome in Pakistani women. J Coll Physicians Surg Pak, 25: 22-6.
- Al-Azzawie, H. F., and Humadi, E. H. **(2010).** Oxidative Stress and the Antioxidant Mechanisms in a Sample of Iraqi Patients with Polycystic Ovary Syndrome (POS). IRAQI JOURNALOF COMMUNITY MEDICINE, 23(3).
- Ashraf, S., Nabi, M., Rashid, F. and Amin, S. **(2019).** Hyperandrogenism in polycystic ovarian syndrome and role of CYP gene variants: a review. Egyptian Journal of Medical Human Genetics, 20: 1-10.
- Azziz, R., Sanchez, L., Knochenhauer, E., Moran, C., Lazenby, J., Stephens, K., et al., **(2004).** Androgen excess in women: experience

with over 1000 consecutive patients. The Journal of Clinical Endocrinology and Metabolism, 89: 453-462.

- Bahadur, A., Verma, N., Mundhra, R., Chawla, L., Ajmani, M. and Sri, M. S et al. **(2021).** Correlation of homeostatic model assessment-insulin resistance, antiMullerian hormone, and BMI in the characterization of polycystic ovary syndrome. Cureus13(6).
- Balsera, M., and Buchanan, B. B. **(2019).** Evolution of the thioredoxin system as a step enabling adaptation to oxidative stress. Free Radical Biology and Medicine, 140, 28-35.
- Barbe, A., Bongrani, A., Mellouk, N., Estienne, A., Kurowska, P., Grandhaye, J., et al., **(2019)**. Mechanisms of adiponectin action in fertility: an overview from gametogenesis to gestation in humans and animal models in normal and pathological conditions. *International journal of molecular sciences*, *20*(7), 1526.
- Barber, T. M., McCarthy, M. I., Wass, J. A. H., and Franks, S. **(2006)**. Obesity and polycystic ovary syndrome. Clinical endocrinology, 65(2), 137-145.
- Behrman, H. R., Kodaman, P. H., Preston, S. L., and Gao, S**. (2001).** Oxidative stress and the ovary. Journal of the Society for Gynecologic Investigation, 8(1_suppl), S40-S42.
- Belenkaia, L. V., Lazareva, L. M., Walker, W., Lizneva, D. V., &and Suturina, L. V. **(2019).** Criteria, phenotypes and prevalence of polycystic ovary syndrome. Minerva ginecologica, 71(3), 211-223.
- Bergmeyer, H. and Graßl, M. **(1983).** Methods of Enzymatic Analysis, 3rd edn. Verlag Chemie, Weinheim, 2: 267-268.
- Beyazit, F., Hiz, M. M., Turkon, H., and Unsal, M. A. **(2021).** Serum spexin, adiponectin and leptin levels in polycystic ovarian syndrome

in association with FTO gene polymorphism. Ginekologia Polska, 92(10), 682-688.

- Bishop, M. L**. (2020).** Clinical Chemistry: Principles, Techniques, and Correlations, Enhanced Edition: Principles, Techniques, and Correlations. Jones & Bartlett Learning.
- Black, C. and DA Silva Costa, F. **(2018).** Biomarker immunoassays in the diagnosis of preeclampsia: calculating the sFlt1/PlGF ratio using the Cobas® e 411 analyser. Preeclampsia. Springer, 1710: 9 - 26
- Bozdag, G.; Mumusoglu, S.; Zengin, D.; Karabulut, E. and Yildiz, B. O. **(2016).** The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. Human reproduction, 31: 2841-2855.
- Burton, G. J., and Jauniaux, E. **(2011)**. Oxidative stress. *Best practice & research Clinical obstetrics & gynaecology*, *25*(3), 287-299.
- Castelo-Branco, C., Steinvarcel, F., Osorio, A., Ros, C. and Balasch, J. **(2010).** Atherogenic metabolic profile in PCOS patients: role of obesity and hyperandrogenism. Gynecological endocrinology, 26: 736-742.
- Chaudhary, H., Patel, J., Jain, N. K., and Joshi, R**. (2021).** The role of polymorphism in various potential genes on polycystic ovary syndrome susceptibility and pathogenesis. *Journal of ovarian research*, *14*, 1-21.
- Chen, J.; Chen, X.; Dominiczak, A. F.; Carey, R. M.; Laffer, C. L.; Elijovich, F.; et al., **(2022).** A Rare Disease Leading to Hypertension. Hypertension, 79: 1147-1152.
- Cincione, R. I., Losavio, F., Ciolli, F., Valenzano, A., Cibelli, G., Messina, G., et al., **(2021).** Effects of mixed of a ketogenic diet in overweight and obese women with polycystic ovary

syndrome. *International journal of environmental research and public health*, *18*(23), 12490.

- Combs, T. P., and Marliss, E. B. **(2014)**. Adiponectin signaling in the liver. Reviews in Endocrine and Metabolic Disorders, 15, 137-147.
- Corrie, L., Gulati, M., Vishwas, S., Kapoor, B., Singh, S. K., Awasthi, A., et al., **(2021).** Combination therapy of curcumin and fecal microbiota transplant: Potential treatment of the polycystic ovarian syndrome. *Medical Hypotheses*, *154*, 110644.
- Cree-Green, M. **(2017).** Worldwide dissatisfaction with the diagnostic process and initial treatment of PCOS. The Journal of Clinical Endocrinology & Metabolism, 102: 375-378.
- Creinin, M. D., Keverline, S. and Meyn, L. A. **(2004).** How regular is regular? An analysis of menstrual cycle regularity. Contraception, 70: 289-292.
- Dadachanji, R., Shaikh, N., and Mukherjee, S. **(2018).** Genetic variants associated with hyperandrogenemia in PCOS pathophysiology. *Genetics research international*, *2018*.
- Deba, Z., Jambale, T. A., Swamy, P. G., and Murthy, D. J. **(2017).** Study of levels of malondialdehyde, superoxide dismutase and hs-CRP in serum of non-obese patients with polycystic ovarian syndrome. Int J Clin Biochem, 4, 191-194.
- Deepa, S. S., and Dong, L. Q. **(2009).** APPL1: role in adiponectin signaling and beyond. American Journal of Physiology-Endocrinology and Metabolism ,(1)296 ,E22-E36 .
- Dehghan, E.; Namiranian, N.; Ghadiri-Anari, A.; Ratki, S. K. R. and Azizi, R. **(2021).** Evaluation of hyperprolactinemia risk factors in infertile women referred to Yazd Infertility Center: A cross-sectional study. International Journal of Reproductive BioMedicine, 19: 1085.

- Desai, V., Prasad, N. R., Manohar, S. M., Sachan, A., Narasimha, S. R. P. V. L., and Bitla, A. R. R. **(2014).** Oxidative stress in non-obese women with polycystic ovarian syndrome. Journal of clinical and diagnostic research: JCDR, 8(7), CC01…..
- Dewailly, D.; Robin, G.; Peigne, M.; Decanter, C.; Pigny, P. and CatteauJonard, S. **(2016).** Interactions between androgens, FSH, anti-Müllerian hormone and estradiol during folliculogenesis in the human normal and polycystic ovary. Human reproduction update, 22: 709-724.
- Diamanti-Kandarakis, E., and Dunaif, A. **(2012)**. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev, 33(6), 981-1030. doi:10.1210/er.2011-1034
- Dobrzyn, K., Smolinska, N., Kiezun, M., Szeszko, K., Rytelewska, E., Kisielewska, K., et al, **(2018)**. Adiponectin: A New Regulator of Female Reproductive System. Int J Endocrinol, 2018, 7965071.
- Donini, L. M., Pinto, A., Giusti, A. M., Lenzi, A. and Poggiogalle, E. **(2020).** Obesity or BMI paradox? Beneath the tip of the iceberg. Frontiers in Nutrition, 7: 53.
- Dumesic, D. A., Oberfield, S. E., Stener-Victorin, E., Marshall, J. C., Laven, J. S., and Legro, R. S. **(2015)**. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. *Endocrine Reviews*, *36*(5), 487-525.
- Durán-Pastén, M. L., and Fiordelisio, T. **(2013).** GnRH-induced Ca2+ signaling patterns and gonadotropin secretion in pituitary gonadotrophs. Functional adaptations to both ordinary and extraordinary physiological demands. *Frontiers in endocrinology*, *4*, 127.

- Durmus, U., Duran, C., and Ecirli, S**. (2017).** Visceral adiposity index levels in overweight and/or obese, and non-obese patients with polycystic ovary syndrome and its relationship with metabolic and inflammatory parameters. Journal of endocrinological investigation, 40, 487-497.
- Escobar-Morreale, H. F., Villuendas, G., Botella-Carretero, J. I., Alvarez-Blasco, F., Sanchon, R., Luque-Ramirez, M., et al., **(2006).** Adiponectin and resistin in PCOS: a clinical, biochemical and molecular genetic study. Human reproduction, 21(9), 2257-2265.
- Fattah, A.; Hadavi, F.; Bahrami, F.; Khoshkholgh, R.; Ahmadi, A. and Mahmoodabadi, M. **(2021).** Prevalence of Polycystic Ovary Syndrome among Girls‘ Students of Kerman University of Medical Sciences and a Meta-Analysis of the Prevalence of PCOS among Iranian Adolescent Girls. International Journal of Pediatrics, 9: 13957-13969……
- Feingold, K. R., Brinton, E. A., and Grunfeld, C. **(2017).** The effect of endocrine disorders on lipids and lipoproteins.
- Fisman, E. Z., and Tenenbaum, A. **(2014).** Adiponectin: a manifold therapeutic target for metabolic syndrome, diabetes, and coronary disease?. Cardiovascular diabetology, 13(1), 1-10.
- Fossati, P., and Prencipe, L. **(1982).** Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical chemistry, 28(10), 2077-2080.
- Freeman, A. M., Burks, H. R., and Wild, R. A. **(2022).** Diagnostic Criteria and Epidemiology of PCOS. In Polycystic Ovary Syndrome: Current and Emerging Concepts (pp. 3-11). Cham: Springer International Publishing.

- González, F. **(2012)**. Inflammation in polycystic ovary syndrome: underpinning of insulin resistance and ovarian dysfunction. Steroids, 77(4), 300-305.
- Goyal, A. and Ganie, M. A. **(2018).** Idiopathic hyperprolactinemia presenting as polycystic ovary syndrome in identical twin sisters: A case report and literature review. Cureus, 10(7): e3004.
- Haida, Z., and Hakiman, M. **(2019)**. A comprehensive review on the determination of enzymatic assay and nonenzymatic antioxidant activities. Food science & nutrition, 7(5), 1555-1563.
- Halliwell, B. **(2006).** Oxidative stress and neurodegeneration: where are we now?. Journal of neurochemistry, 97(6), 1634-1658.
- Halliwell, B., and Gutteridge, J. M. **(2015).** Free radicals in biology and medicine. Oxford university press, USA.
- Hendriks, M.-L.; Brouwer, J.; Hompes, P. G.; Homburg, R. and Lambalk, C. B**. (2008).** LH as a diagnostic criterion for polycystic ovary syndrome in patients with WHO II oligo/amenorrhoea. Reproductive biomedicine online, 16: 765- 771.
- Herman, R., Jensterle, M., Janež, A., Goričar, K., and Dolžan, V. **(2020).** Genetic variability in antioxidative and inflammatory pathways modifies the risk for PCOS and influences metabolic profile of the syndrome. *Metabolites*, *10*(11), 439.
- Hoeger, K. M., Dokras, A., and Piltonen, T. **(2021).** Update on PCOS: consequences, challenges, and guiding treatment. The Journal of Clinical Endocrinology & Metabolism, 106(3), e1071-e1083.
- Ibrahim, T. A. E. S., Ali, A. E. S., and Radwan, M. E. H. **(2020).** Lipid profile in women with polycystic ovary syndrome. The Egyptian Journal of Hospital Medicine, 78(2), 272-277.

- Itriyeva, K. **(2022).** The normal menstrual cycle. Current Problems in Pediatric and Adolescent Health Care, 6: 101183.
- Izadi, V., Farabad, E., and Azadbakht, L. **(2013).** Epidemiologic evidence on serum adiponectin level and lipid profile. International journal of preventive medicine, 4(2), 133.
- James, D. E., Stöckli, J., and Birnbaum, M. J. **(2021).** The etiology and molecular landscape of insulin resistance. *Nature Reviews Molecular Cell Biology*, *22*(11), 751-771.
- Jahan, I. A., Morshed, M. S., Banu, H., Chowdhury, E. U. R., and Hasanat, M. A. **(2023).** Serum adiponectin in women with polycystic ovary syndrome. Bangladesh Journal of Medicine, 34(3), 206-213.
- Jeeva, J. S., Sunitha, J., Ananthalakshmi, R., Rajkumari, S. and Ramesh, M., **(2015).** Enzymatic antioxidants and its role in oral diseases. Journal of pharmacy & bioallied sciences, 7(Suppl 2), S331.
- Keen, M. A., Shah, I. H. and Sheikh, G. **(2017).** Cutaneous manifestations of polycystic ovary syndrome: A cross-sectional clinical study. Indian dermatology online journal, 8: 104.
- Khan, A., Karim, N., Ainuddin, J. A., and Fahim, M. F. (2019). Polycystic Ovarian Syndrome: Correlation between clinical hyperandrogenism, anthropometric, metabolic and endocrine parameters. Pakistan Journal of Medical Sciences, 35(5), 1227
- Kharroubi, I., Rasschaert, J., Eizirik, D. L., and Cnop, M. **(2003)**. Expression of adiponectin receptors in pancreatic β cells. Biochemical and biophysical research communications, 312(4), 1118-1122.
- Khoramipour, K., Chamari, K., Hekmatikar, A. A., Ziyaiyan, A., Taherkhani, S., Elguindy, N. M., et al., **(2021).** Adiponectin: Structure, physiological functions, role in diseases, and effects of nutrition. *Nutrients*, *13*(4), 1180.

- Kim, J. J. and Choi, Y. M. **(2019).** Phenotype and genotype of polycystic ovary syndrome in Asia: ethnic differences. Journal of Obstetrics and Gynaecology Research, 45: 2330-2337.
- Kim, Y., Lim, J. H., Kim, E. N., Hong, Y. A., Park, H. J., Chung, S., et al**., (2022).** Adiponectin receptor agonist ameliorates cardiac lipotoxicity via enhancing ceramide metabolism in type 2 diabetic mice. Cell Death & Disease, 13(3), 282.
- Kosova, G., and Urbanek, M. **(2013).** Genetics of the polycystic ovary syndrome. Molecular and cellular endocrinology, 373(1-2), 29-38.
- Krug, I.; Giles, S. and Paganini, C. **(2019).** Binge eating in patients with polycystic ovary syndrome: prevalence, causes, and management strategies. Neuropsychiatric disease and treatment, 15: 1273.
- Kumar, P., Raman, T., Swain, M. M., Mishra, R., and Pal, A. **(2017).** Hyperglycemia-induced oxidative-nitrosative stress induces inflammation and neurodegeneration via augmented tuberous sclerosis complex-2 (TSC-2) activation in neuronal cells. Molecular neurobiology, 54, 238-254.
- Langsted, A., Jensen, A. M. R., Varbo, A., and Nordestgaard, B. G. **(2020)**. Low high-density lipoprotein cholesterol to monitor long-term average increased triglycerides. *The Journal of Clinical Endocrinology & Metabolism*, *105*(4), e1657-e1666.
- Le, M. T., Le, V. N. S., Le, D. D., Nguyen, V. Q. H., Chen, C., and Cao, N. T. **(2019).** Exploration of the role of anti-Mullerian hormone and LH/FSH ratio in the diagnosis of polycystic ovary syndrome. *Clinical Endocrinology*, *90*(4), 579-585.
- Lee, S. W., Hwang, I. S., Jung, G., Kang, H. J., and Chung, Y. H. **(2022).** Relationship between metabolic syndrome and follicle-

stimulating hormone in postmenopausal women. Medicine, 101(18), e29216-e29216.

- Legro, R. S., Azziz, R., Ehrmann, D., Fereshetian, A. G., O'keefe, M., Ghazzi, M. N. et al., **(2003).** Minimal response of circulating lipids in women with polycystic ovary syndrome to improvement in insulin sensitivity with troglitazone. The Journal of Clinical Endocrinology and Metabolism, 88: 5137-5144.
- Li, H., Chen, Y., Li, Y., Huang, J., Zhao, X., Chen, X., et al., **(2015).** A case-control study of correlation between serum adiponectin levels and polycystic ovary syndrome. Zhonghua fu Chan ke za zhi, 50(11), 814-818.
- Li, S., Chu, Q., Ma, J., Sun, Y., Tao, T., Huang, R., et al., **(2017).** Discovery of novel lipid profiles in PCOS: do insulin and androgen oppositely regulate bioactive lipid production?. The Journal of Clinical Endocrinology & Metabolism, 102(3), 810-821.
- Liao, B.; Qiao, J. and Pang, Y. **(2021).** Central regulation of PCOS: Abnormal neuronal-reproductive-metabolic circuits in PCOS pathophysiology. Frontiers in Endocrinology, 12: 667422.
- Lim, S. S., Davies, M., Norman, R. J. and Moran, L**. (2012).** Overweight, obesity and central obesity in women with polycystic ovary syndrome: a systematic review and meta-analysis. Human reproduction update, 18: 618-637.
- Lim, S. S., Kakoly, N. S., Tan, J. W. J., Fitzgerald, G., Bahri Khomami, M., Joham, A. E., et al., **(2019).** Metabolic syndrome in polycystic ovary syndrome: a systematic review, meta-analysis, and meta-regression. *Obesity reviews*, *20*(2), 339-352.
- Lizneva, D.; Suturina, L.; Walker, W.; Brakta, S.; Gavrilova-Jordan, L. and Azziz, R. **(2016).** Criteria, prevalence, and phenotypes of polycystic ovary syndrome. Fertility and sterility, 106: 6-15.

- Llanos, P., and Palomero, J. **(2022).** Reactive Oxygen and Nitrogen Species (RONS) and Cytokines—Myokines Involved in Glucose Uptake and Insulin Resistance in Skeletal Muscle. *Cells*, *11*(24), 4008.
- Mahboobifard, F.; Rahmati, M.; Amiri, M.; Azizi, F. and Tehrani, F. R. **(2022).** To what extent does polycystic ovary syndrome influence the cut-off value of prolactin? Findings of a community-based study. Advances in Medical Sciences, 67: 79-86.
- Mahmood Rasool, R. A., Rizwan, R., Malik, A., Asif, M., Zaheer, A., Jabbar, A., et al., **(2018).** Inter-relationship of circulating biochemical markers of oxidative stress and comorbid condition in polycystic ovary syndrome. Biomedical Research, 29(21), 3779-3783.
- Malini, N. and George, K. R. **(2018).** Evaluation of different ranges of LH: FSH ratios in polycystic ovarian syndrome (PCOS)–Clinical based case control study. General and comparative endocrinology, 260: 51-57.
- Malini, S. S. **(2023).** Insulin resistance and Oxidant-Antioxidant Markers in Young Women with Polycystic Ovarian Syndrome. Bulletin of Pure & Applied Sciences-Zoology, (1).
- Mansour, A.; Sanginabadi, M.; Mohajeri-Tehrani, M. R.; Karimi, S.; Gerami, H.; Mahdavi-Gorabi, A. et al., **(2020).** Effect of Oligopin Supplementation on Polycystic Ovary Syndrome: A Randomized Controlled Trial.
- Martemucci, G., Costagliola, C., Mariano, M., D'andrea, L., Napolitano, P., and D'Alessandro, A. G. **(2022).** Free radical properties, source and targets, antioxidant consumption, and health. *Oxygen*, *2*(2), 48-78.

- Menzaghi, C., Trischitta, V., and Doria, A. **(2007).** Genetic influences of adiponectin on insulin resistance, type 2 diabetes, and cardiovascular disease. *Diabetes*, *56*(5), 1198-1209.
- Merhi, Z. **(2014).** Advanced glycation end products and their relevance in female reproduction. Human Reproduction, 29(1), 135-145.
- Mihm, M.; Ganguly, S. and Muttukrishna, S. **(2011).** The normal menstrual cycle in women. Animal reproduction science, 124: 229-236.
- Moghetti, P. and Tosi, F. **(2021).** Insulin resistance and PCOS: chicken or egg? Journal of Endocrinological Investigation, 44: 233-244.
- Mohammed, Z. I. and Qasim, M. T**. (2021).** Correlation of AMH and LH Levels in PCOS Patients with Pregnancy Rate. Annals of the Romanian Society for Cell Biology, 7: 945-951.
- Mumusoglu, S. and Yildiz, B. O. **(2020).** Polycystic ovary syndrome phenotypes and prevalence: differential impact of diagnostic criteria and clinical versus unselected population. Current Opinion in Endocrine and Metabolic Research, 12: 66-71.
- Mumusoglu, S. and Yildiz, B. O. **(2020).** Polycystic ovary syndrome phenotypes and prevalence: differential impact of diagnostic criteria and clinical versus unselected population. Current Opinion in Endocrine and Metabolic Research, 12: 66-71.
- Murri, M., Luque-Ramírez, M., Insenser, M., Ojeda-Ojeda, M., and Escobar-Morreale, H.F. **(2013).** Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis. *Human reproduction update, 19 3*, 268-88.

- Namjou, B., Stanaway, I. B., Lingren, T., Mentch, F. D., Benoit, B., Dikilitas, O., et al., **(2021)**. Evaluation of the MC4R gene across eMERGE network identifies many unreported obesity-associated variants. International Journal of Obesity, 45(1), 155-169.
- Nandi, A., Chen, Z., Patel, R., and Poretsky, L. **(2014).** Polycystic ovary syndrome. Endocrinology and Metabolism Clinics, 43(1), 123-147.
- Napolitano, G., Fasciolo, G., and Venditti, P. **(2021).** Mitochondrial management of reactive oxygen species. Antioxidants, 10(11), 1824.
- Nardo, L. G., Patchava, S., and Laing, I. **(2008).** Polycystic ovary syndrome: pathophysiology, molecular aspects, and clinical implications. *Panminerva medica*, *50*(4), 267-278.
- Newsholme, P., Cruzat, V. F., Keane, K. N., Carlessi, R., and de Bittencourt Jr, P. I. H. **(2016).** Molecular mechanisms of ROS production and oxidative stress in diabetes. Biochemical Journal, 473(24), 4527-4550.
- Nguyen, T. M. D. **(2020).** Adiponectin: role in physiology and pathophysiology. *International journal of preventive medicine*, *11*.
- Nivedhita, G., and Brundha, M. P. **(2020).** Eclia Test-Review. Indian Journal of Forensic Medicine & Toxicology, 14(4), 5067-5073.
- O'Connor, A., Phelan, N., Tun, T. K., Boran, G., Gibney, J., and Roche, H. M. **(2010).** High-molecular-weight adiponectin is selectively reduced in women with polycystic ovary syndrome independent of body mass index and severity of insulin resistance. The Journal of Clinical Endocrinology & Metabolism, 95(3), 1378-1385.
- Onyegbule, O. A., Meludu, S. C., Dioka, C. E., Okwara, J. E., Obi-Ezeani, C. N., and Njoku, C. M. **(2022).** Serum adiponectin and

metabolic profile in women with polycystic ovarian syndrome in relation to body mass index. Int. J. Res. Med. Sci, 10, 599-603.

- Orbetzova, M. M. **(2020).** Clinical impact of insulin resistance in women with polycystic ovary syndrome. In *Polycystic Ovarian Syndrome*. IntechOpen.
- World Health Organization. **(1992)**. *The ICD-10 classification of mental and behavioural disorders: clinical descriptions and diagnostic guidelines* (Vol. 1). World Health Organization.
- Özen, Ö. and Cander, S. **(2022).** Evaluation of the relationship between dehydroepiandrosterone sulfate-total testosterone ratio and metabolic parameters in patients with polycystic ovary syndrome. Turkish Journal of Internal Medicine, 4: 47-54.
- Parida, S., Siddharth, S., and Sharma, D. **(2019).** Adiponectin, obesity, and cancer: clash of the bigwigs in health and disease. *International journal of molecular sciences*, *20*(10), 2519.
- Parida, S., Siddharth, S., and Sharma, D. **(2019).** Adiponectin, obesity, and cancer: clash of the bigwigs in health and disease. International journal of molecular sciences, 20(10), 2519.
- Patwardhan, R. S., Sharma, D., and Sandur, S. K. **(2022).** Thioredoxin reductase: An emerging pharmacologic target for radiosensitization of cancer. Translational Oncology, 17, 101341.
- Peigné, M. and Dewailly, D. **(2014).** Long term complications of polycystic ovary syndrome (PCOS). Annales d'endocrinologie, 2014. Elsevier, 75: 194- 199.
- Peigné, M., and Dewailly, D. **(2014).** Long term complications of polycystic ovary syndrome (PCOS). In *Annales d'endocrinologie* (Vol. 75, No. 4, pp. 194-199). Elsevier Masson.

- Ramanand, S. J., Ghongane, B. B., Ramanand, J. B., Patwardhan, M. H., Ghanghas, R. R., and Jain, S. S. **(2013).** Clinical characteristics of polycystic ovary syndrome in Indian women. Indian journal of endocrinology and metabolism, 17(1), 138.
- Ramirez, A. V., and de Sá, L. B. **(2021).** Melatonin and its relationships with diabetes and obesity: a literature review. Current Diabetes Reviews, 17(7), 38-50.
- Rashad, N. M., Ashour, W. M. R., Allam, R. M., Saraya, Y. S., and Emad, G. **(2019).** Oxidative stress and risk of polycystic ovarian syndrome in women with epilepsy: implications of malondialdehyde and superoxide dismutase serum levels on female fertility. The Egyptian Journal of Internal Medicine, 31, 609-619.
- Rizzo, M., Berneis, K., Hersberger, M., Pepe, I., Di Fede, G., Rini, G. B., et al., **(2009).** Milder forms of atherogenic dyslipidemia in ovulatory versus anovulatory polycystic ovary syndrome phenotype. Human reproduction, 24: 2286-2292.
- Rocha ,A. L., Oliveira, F. R., Azevedo, R. C., Silva, V. A., Peres, T. M., Candido, A. L., et al, **(2019).** Recent advances in the understanding and management of polycystic ovary syndrome. F1000Research, 8 .
- Rocha, A. L. L., Faria, L. C., Guimarães, T. C. M., Moreira, G. V., Cândido, A. L., and Couto, C. A. **(2017).** Non-alcoholic fatty liver disease in women with polycystic ovary syndrome: systematic review and meta-analysis. Journal of endocrinological investigation, 40, 1279-1288.
- Rojas, J., Chávez, M., Olivar, L., Rojas, M., Morillo, J., Mejías, J., et al, **(2014).** Polycystic ovary syndrome, insulin resistance, and obesity:

navigating the pathophysiologic labyrinth. International journal of reproductive medicine, 2014.

- Rosenfield, R. L., and Ehrmann, D. A. **(2016).** The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. *Endocrine reviews*, *37*(5), 467-520.
- Saadia, Z**. (2020).** Follicle stimulating hormone (LH: FSH) ratio in polycystic ovary syndrome (PCOS)-obese vs. non-obese women. Medical Archives, 74: 289.
- Sachdev, S. S., Jamil, A., Gunabalasingam, P., and Safdar, N. A. **(2022).** The effects of acitretin on insulin resistance, glucose metabolism, and lipid levels in patients with psoriasis. *Indian journal of dermatology*, *67*(4), 349.
- Saeed R. H., Al-Mohaidi A. M. and Ismail N. K. **(2021)**. Association Between Some Risk Factors with Hormonal State in a Sample of Infertile Iraqi Women. International Journal of Drug Delivery Technology 11: 685-689.
- Sangeeta, S. **(2012).** Metformin and pioglitazone in polycystic ovarian syndrome: a comparative study. The Journal of Obstetrics and Gynecology of India, 62, 551-556.
- Shaaban, Z., Khoradmehr, A., Jafarzadeh Shirazi, M.R. and Tamadon, A. **(2019).** Pathophysiological mechanisms of gonadotropins– and steroid hormones–related genes in etiology of polycystic ovary syndrome. Iranian Journal of Basic Medical Sciences, [online] 22(1), pp.3–16.
- Shin, H. Y., Lee, D. C., and Lee, J. W. **(2011).** Adiponectin in women with polycystic ovary syndrome. Korean journal of family medicine, 32(4), 243.

- Stein, I. F., and Leventhal, M. L. **(1935).** Amenorrhea is associated with bilateral polycystic ovaries. *American journal of obstetrics and gynecology*, *29*(2), 181-191.
- Stepto, N. K., Cassar, S., Joham, A. E., Hutchison, S. K., Harrison, C. L., Goldstein, R. F., et al**, (2013).** Women with polycystic ovary syndrome have intrinsic insulin resistance on euglycaemic–hyperinsulaemic clamp. Human reproduction, 28(3), 777-784.
- Streeter, S. A. and Mcburney, D. H. **(2003).** Waist–hip ratio and attractiveness: New evidence and a critique of "a critical test". Evolution and Human behavior, 24: 88-98.
- Šumarac-Dumanović, M., Stamenković-Pejković, D., Jeremić, D., Dumanović, J., Mandić-Marković, V., Žarković, M. et al., **(2022).** Age, body mass index, and waist-to-hip ratio related changes in insulin secretion and insulin sensitivity in women with polycystic ovary syndrome: minimal model analyses. International Journal of Endocrinology
- Szosland, K.; Pawłowicz, P. and Lewiński, A. **(2015).** Prolactin secretion in polycystic ovary syndrome (PCOS). Neuroendocrinology Letters, 36: 53-58.
- Tebboub, I., and Kechrid, Z. **(2021).** Effect of curcuma on zinc, lipid profile and antioxidants levels in blood and tissue of streptozotocin-induced diabetic rats fed zinc deficiency diet. Archives of Physiology and Biochemistry, 127(2), 162-169.
- Tehrani, F. R., Daneshpour, M., Hashemi, S., Zarkesh, M., and Azizi, F. **(2013)**. Relationship between polymorphism of insulin receptor gene, and adiponectin gene with PCOS. Iranian journal of reproductive medicine, 11(3), 18 .5

- Toboc, A.; Stan, C.; Stănescu, A.-C. and Ionică, M. **(2018).** Performance evaluation of immunoassay methods using innovative Westgard method decision chart/six sigma: metric tool. Advanced Topics in Optoelectronics, Microelectronics, and Nanotechnologies IX, 2018. International Society for Optics and Photonics, 109773: 817 - 822.
- Trinder, P. **(1969).** Enzymatic methods for glucose determination. Ann Clin Biochem, 6: 24-26.
- Tsubakio-Yamamoto, K., Sugimoto, T., Nishida, M., Okano, R., Monden, Y., Kitazume-Taneike, R., et al., **(2012).** Serum adiponectin level is correlated with the size of HDL and LDL particles determined by high performance liquid chromatography. Metabolism, 61(12), 1763-1770.
- Tutakne, M. and Chari, K. **(2003).** Acne, rosacea and perioral dermatitis. IADVL Textbook and atlas of dermatology, 2: 689-710.
- Uçkan, K., Demir, H., Turan, K., Sarıkaya, E., and Demir, C. **(2022).** Role of oxidative stress in obese and nonobese PCOS patients. *International Journal of Clinical Practice*, *2022*.
- Valenzano, A., Polito, R., Trimigno, V., Di Palma, A., Moscatelli, F., Corso, G., et al., **(2019).** Effects of a very low-calorie ketogenic diet on the orexinergic system, visceral adipose tissue, and ROS production. *Antioxidants*, *8*(12), 643.
- Valgimigli, L. **(2023).** Lipid Peroxidation and Antioxidant Protection. *Biomolecules*, *13*(9), 1291.
- Victor, V. M., Rovira-Llopis, S., Banuls, C., Diaz-Morales, N., Martinez de Maranon, A., Rios-Navarro, C., et al, **(2016).** Insulin resistance in PCOS patients enhances oxidative stress and leukocyte adhesion: role of myeloperoxidase. PLoS One, 11(3), e0151960.

- Wang, Y., Zhao, X., Zhao, H., Ding, H., Tan, J., Chen, J., et al., **(2013).** Risks for gestational diabetes mellitus and pregnancy-induced hypertension are increased in polycystic ovary syndrome. *BioMed research international*, *2013*.
- Wen, Z. H., Kuo, H. M., Shih, P. C., Hsu, L. C., Chuang, J. M. J. and Chen, N. F., **(2023).** Isoaaptamine increases ROS levels causing autophagy and mitochondria-mediated apoptosis in glioblastoma multiforme cells. Biomedicine & Pharmacotherapy, 160, 114359.
- Westerman, R., and Kuhnt, A. K. **(2022).** Metabolic risk factors and fertility disorders: A narrative review of the female perspective. Reproductive Biomedicine & Society Online, 14, 66-74.
- Yadav, A., Kataria, M. A., Saini, V., and Yadav, A. **(2013).** Role of leptin and adiponectin in insulin resistance. Clinica chimica acta, 417, 80-84.
- Yang, H.; DI, J.; Pan, J.; Yu, R.; Teng, Y.; Cai, Z. et al., **(2020).** The association between prolactin and metabolic parameters in PCOS women: A retrospective analysis. Frontiers in endocrinology, 11: 263.
- Ye, W., Xie, T., Song, Y., and Zhou, L. **(2021)**. The role of androgen and its related signals in PCOS. *Journal of cellular and molecular medicine*, *25*(4), 1825-1837.
- Yeon Lee, J., Baw, C. K., Gupta, S., Aziz, N., and Agarwal, A. **(2010).** Role of oxidative stress in polycystic ovary syndrome. Current women's health reviews, 6(2), 96-107.

- Yeon Lee, J., Baw, C. K., Gupta, S., Aziz, N., and Agarwal, A. **(2010).** Role of oxidative stress in polycystic ovary syndrome. Current women's health reviews, 6(2), 96-107.

- Yildirim, B., Sabir, N. and Kaleli, B. **(2003).** Relation of intra-abdominal fat distribution to metabolic disorders in nonobese patients with polycystic ovary syndrome. Fertility and sterility, 79: 1358-1364.
- Yılmaz, S. K., Eskici, G., Mertoğlu, C., and Ayaz, A. **(2021).** Adipokines, inflammation, oxidative stress: critical components in obese women with metabolic syndrome. Progress in Nutrition.
- Zafar, U.; Amber Zaidi, S.; Syed Muhammad Osama Jafri, H.; Imran, S.; Bhatty, A. and Abidi, F. **(2021).** Effectiveness of Metformin and its Combination with Probiotic in Polycystic Ovarian Disease with Hyperprolactinemia: A Randomized Clinical Trial. Journal of Pharmaceutical Research International, 33(47A): 268 - 274.
- Zhang, Y.; Ho, K.; Keaton, J. M.; Hartzel, D. N.; Day, F.; Justice, A. E.; et al., **(2020).** A genome-wide association study of polycystic ovary syndrome was identified from electronic health records. American journal of obstetrics and gynecology, 223: 559. e1-559. e21.
- Zimmer, K. P., Fischer, I., Mothes, T., Weissen-Plenz, G., Schmitz, M., Wieser, H., et al., **(2010).** Endocytotic segregation of gliadin peptide 31–49 in enterocytes. Gut, 59(3), 300-310.
- Zuo, T., Zhu, M., and Xu, W. **(2016).** Roles of oxidative stress in polycystic ovary syndrome and cancers. Oxidative medicine and cellular longevity, 2016.
- Zusi, C., Csermely, A., Rinaldi, E., Bertoldo, K., Bonetti, S., Boselli, M. Let al., **(2023).** Crosstalk between genetic variability of adiponectin and leptin, glucose-insulin system and subclinical atherosclerosis in patients with newly diagnosed type 2 diabetes. The Verona Newly Diagnosed Type 2 Diabetes Study 14. Diabetes, Obesity and Metabolism.

Appendices

University of Kerbala-College of Medicine

Department of Chemistry and Biochemistry

Student name: Yasameen Imad Kadhum

Supervisors:

Prof. Dr. Fadhil Jawad Al-Tu'ma - College of Medicine – University of Kerbala

Assist. Prof. Dr. Nora Sabah Rasoul – College of Medicine– University of Kerbala

((Experimental Data))

Sample No.
Inclusion criteria: Patients aged (18-40) which diagnosed with any two of the subsequent three conditions: Hyperandrogenemia, Ovarian Dysfunction, ovarian volume greater than 10 ml on ultrasonography, and fewer than 12 follicles measuring 2 to 9 mm in diameter.
Exclusion criteria: autoimmune disease, diabetes mellitus, thyroid disease, cardiovascular disease, hypertension, chronic renal failure, and malignant diseases) and a history of receiving any other medication (lipid reduction, ovulation stimulation, corticosteroids, antidiabetic and antihypertensive medications) within 6 months were excluded also.
Age:

Length:		Weight:	BMI:
Waist:		Hip:	WHR:
Hirsutism	Yes:		No:
Acne	Yes:		No:
Ultrasound	Normal ovary:		PCOS ovary:
Smoking status	Passive:	Active:	Non-smoker:
Hormones	LH:		LH: FSH ratio:
	FSH:		
	Prolactin:		
	Free testosterone:		
Lipid Profile	Cholesterol:		
	Triglyceride:		
	HDL:		
	LDL:		
Glucose:			
Insulin:		HOMA-IR:	
Adiponectin:			
Oxidative stress	Catalase:		
	Malondialdehyde:		

الملخص

تعد متلازمة المبيض المتعدد التكيسات واحدة من أكثر اضطرابات الغدد الصماء و التمثيل الغذائي لدى النساء في سن الإنجاب. تعاني النساء المصابات بمتلازمة تكيس المبايض من مجموعة من الأعراض المرتبطة باضطراب الدورة الشهرية وزيادة الأندروجين، مما يؤثر بشكل كبير على نوعية حياتهن. قد يكونون أكثر عرضة للإصابة بأمراض متعددة، بما في ذلك السمنة ومقاومة الأنسولين ومرض السكري من النوع الثاني وأمراض القلب والأوعية الدموية والعقم والسرطان والاضطرابات النفسية..

اضطرابات التمثيل الغذائي، وخاصة مقاومة الانسولين تكون واضحة في الأشخاص المصابين وخاصة بين النساء المصابات بالسمنة. الاديبونكتين هو واحد من أكثر الاديبوكاينات الشائعة، يلعب دورا مهما في أيض الطاقة ويساهم في إمراضية متلازمة الأيض.

ترتبط متلازمة المبيض المتعدد التكيسات بالإجهاد التأكسدي، أي زيادة إنتاج الجذور الحرة (زيادة بيروكسيد الدهون و المالوند الديهايد) تليها انخفاض مستويات مضادات الأكسدة في الدم ونشاط إنزيم مضادات الأكسدة (الكتليز).

تهدف هذه الدراسة إلى استكشاف ما إذا كانت النساء المصابات بمتلازمة تكيس المبايض لديهن أي اختلاف في مستويات الأديبونيكتين والإجهاد التأكسدي مقارنة بمجموعات الأصحاء البدينين وغير البدينين. كما تم دراسة تحليل الارتباط بين مصل الأديبونيكتين ومستويات الإجهاد التأكسدي مع العوامل البيوكيميائية الأخرى مثل العوامل الهرمونية ومستويات الدهون في مجموعة المرضى.

تم تصميم دراسة الحالات والشواهد لـ ١٦٠ امرأة تتراوح أعمارهم بين (١٨ - ٤٠) عامًا، وشملت ٨٠ مريضة بمتلازمة تكيس المبايض مقسمة وفقًا لمؤشر كتلة الجسم (BMI) إلى ٤٠ يعانون من السمنة المفرطة (BMI≥30) و٤٠ غير مصابين بالسمنة (BMI≥30)) و ٨٠ امرأة كمجموعة الأصحاء، مقسمة أيضًا وفقًا لمؤشر كتلة الجسم (BMI) إلى ٤٠ يعانون من السمنة المفرطة (BMI≥30) و٤٠ غير مصابين بالسمنة (BMI≥30) في سن الإنجاب لدى استشاري الخصوبة الإنجابية في أمراض النساء والتوليد المستشفى التعليمي، مديرية صحة كربلاء العراق وكلية الطب، جامعة كربلاء العراق خلال الفترة من نوفمبر ٢٠٢٢ إلى مارس٢٠٢٣. تم افتراض معايير روتردام-٢٠٠٣ للإناث من متلازمة تكيس المبايض. تمت مقابلة المرضى وفحصهم للتأكد من الوزن والطول ومحيط الخصر ومحيط الورك. تم جمع خمسة ملليلتر من عينات الدم الوريدي في الصباح الباكر بعد صيام طوال الليل. تم قياس نسبة السكر في الدم والأنسولين الصائم، بالإضافة إلى المستويات الهرمونية لكل من هرمون LH وFSH والتستوستيرون الحر والبرولاكتين بواسطة نظام المقايسة المناعية الآلي الكيميائي. تم قياس ملف الدهون لكل من TC وTG وHDL وLDL باستخدام محلل تلقائي (SMART-120) وتقنيات قياس الألوان الأنزيمية. تم

تقييم مقاومة الانسولين عن طريق حساب HOMA-IR باستخدام الصيغة (جلوكوز صائم ملغم / ديسيلتر × أنسولين صائم µU / مل) /٤٠٥. تم إجراء الحسابات باستخدام برنامج SPSS Statistics، الإصدار 28.0 (IBM، SPSS، Chicago، Illinois، USA).

أظهرت نتيجة هذه الدراسة ارتفاع ملحوظ في تركيز ,LH (p<0.001) نسبة LH/FSH (p<0.001) و مستوى التستوستيرون الحر (p<0.001), مستوى البرولاكتين (p=0.004), ,(p<0.001)TGL ,TC (p=0.050) LDL(p=0.002), HOMA-IR (p<0.001) و مستوى MDA (p=0.01) في مجموعة مرضى تكيس المبايض المصابين بالسمنة بعد المقارنة مع مجموعة الأصحاء المصابين بالسمنة.

في حين انخفض مستوى هرمون FSH (P <0.001)، ومستوى ADP (P <0.001)، ومستوى(p<0.001) CATخلال تقييم مماثل بين مرضى متلازمة تكيس المبايض البدينين ومجموعة السيطرة التي تعاني من السمنة المفرطة.

وكانت النقاط التشخيصية الأمثل للتنبؤ بمتلازمة تكيس المبايض بواسطة MDA هي: (الحساسية ٦٠٪، النوعية٨١٪) عند مستوى =٤,٣٠٥. وفقا للدراسة الحالية، لخصت إلى أن مستويات MDA وHOMA-IR

زادت في مرضى متلازمة تكيس المبايض بينما انخفضت مستويات ADP وCAT مقارنة مع الأصحاء.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء - كلية الطب

فرع الكيمياء والكيمياء الحياتية

دور الأديبونكتين والإجهاد التأكسدي في التسبب بمتلازمة تكيس المبايض عند النساء العراقيات

رسالة ماجستير

مقدمة إلى مجلس كلية الطب / جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في

(الكيمياء السريرية)

من قبل

ياسمين عماد كاظم

بكالوريوس تحليلات مرضية-كلية العلوم الطبية التطبيقية – جامعة كربلاء /٢٠١٧

بإشراف

أ. د. فاضل جواد آل طعمة

كلية الطب – جامعة كربلاء

أ.م. د. نورا صباح رسول

كلية الطب-جامعة كربلاء

٢٠٢٤م

١٤٤٥هـ