Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Karbala /College of Medicine

Department of Microbiology

# Evaluation of Heat Shock Protein 90B1 (HSP 90B1), B-cell Lymphoma 2 (BCL-2) and Bcl-2 Associated X Protein (Bax) and it is relation with Immunopathogenesis of Poly Cystic Ovarian Syndrome

A THESIS

Submitted to the council of the College of Medicine/University of Karbala,

for the fulfillment of the requirement for the degree Master of Science in

Medical Microbiology.

By

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2022 A.D 1444 A.H

**بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ**

**﴿نَرْفَعُ دَرَجاتٍ مِن نَشاءُ وفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ﴾**

[يوسف ٧٦]

**صدق الله العلي العظيم**

## Supervisor Certification

I certify that this thesis " **Evaluation of Heat Shock Protein 90B1 (HSP 90B1), B-cell Lymphoma 2 (BCL-2) and Bcl-2 Associated X Protein (Bax) and it is relation with Immunopathogenesis of Poly Cystic Ovarian Syndrome**" was conducted under my supervision at the department of medical microbiology, College of medicine , University of Kerbala, as a partial fulfillment of the requirements for the degree of Master in medical microbiology .

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**Report of the Head of Medical Microbiology Department**
**Chairman of Postgraduate Studies Committee**

According to the recommendation presented by the Chairman of the Postgraduate Studies Committee, I forward this thesis **" Evaluation of Heat Shock Protein 90B1 (HSP 90B1), B-cell Lymphoma 2 (BCL-2) and Bcl-2 Associated X Protein (Bax) and it is relation with Immunopathogenesis of Poly Cystic Ovarian Syndrome "** for discussion

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We, the examining committee, certify that we have read this thesis and examined the student (**Israa Abdulrasool Abbas Nafty**) in its contents and that in our opinion; it is adequate as a thesis for the degree of Master of Medical Microbiology.

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# *Dedication*

*I dedicate this work*

*To …Whom I carry his name proudly …Who taught us the good manners and the insistence for accomplishing anything we face in life …unfortunately didn't stay in this world long enough…*

*My Dear father*

*To… My strong and gentle soul who taught me to trust Allah…Who helped me so much and did not leave me in this journey*

*My Dear Mother*

*To…The constant source of support and encouragement during the challenges of life…Who I am very thankful to have him in my life…*

*My Dear Husband*

*My Dear daughter, brothers and sister.*

*Israa 2022*

## Acknowledgements

First of all I'd like to praise and offer my gratitude to my Creator, who gave me health and strength to complete this work.

Also, I would like to thank **Dr. Alaa Saad Al-Attabi** and **Dr. Dhamiaa Makki** supervisors of this thesis for their advice and keenness to complete this work in the best possible manner and in the specified time.

Also, I would like to extend my thanks and appreciation to **Assistant Professor Dr.Sawsan Mohammed Jabbar AL-Hasnawi**, Head of the Microbiology Branch of the College of Medicine/ University of Karbala, for her efforts to facilitate the obstacles during this work.

I also extend my thanks and gratitude to all faculty members and staff of the Microbiology Branch of the College of Medicine/Karbala University; I also thank the staff of Karbala gynecological and obstetric teaching hospital especially **Dr. Batool Abdzaid Alsultany** and **Dr. Hameedah Hadi Abdulwahid**

I also would like to thank all of the participant women in this study and the staff of Al-Hussein-medical city hospital for their great efforts in accomplishing this study especially **Dr. Fatema Abdulla Mankhey** and **Msc. Fatima Turky**

Also, I'm extremely grateful to **Prof. Dr. Suhayr easa** college of medicine/ University of Babylon, who supported this study and generously provided knowledge and expertise

I am grateful to my late father, mother and brothers Mohammed, Ali, Hussain, Muntadher and my sister Zahraa, also I thank my dear husband Karar, who have always encouraged me and stood by my side to complete my studies.

Finally, all thanks and appreciation to everyone who helped and advised me to complete this thesis.

## Summary

Polycystic ovary syndrome (PCOS) is one of the most common chronic endocrinopathies affecting between 5 and 10% of reproductive age women. Hirsutism, acne, abnormal menstrual cycle, or obesity are common presenting features in the perimenarcheal stage. PCOS is a multifactorial disorder where several factors have been linked to the development of PCOS, which could lead to female infertility due to failure of follicle maturation, embryo implantation. And combination of genetic, environmental, and endocrine factors, which are the main causes of female ovulatory dysfunction.

The pathogenesis of PCOS has long been controversial. However, evidence accrued over the past 30 years indicates that the immediate pathophysiologic abnormality underlying the vast majority of PCOS is functional ovarian hyperandrogenism and that the insulin-resistant hyperinsulinism found in half of PCOS aggravates it. One of the most common risk factors for the progress of PCOS include family history of PCOS.

Granulosa cells (GCs) play important role in oocyte maturation, fertilization, and subsequent implantation. Many researches revealed a key role of HSP90B1 and Bcl-2/Bax in the syndrome pathogenesis. Increased levels of Hsp90B1 and Bcl-2 which are anti-apoptotic proteins, and decreased levels of Bax which is pro-apoptotic protein, might be involved in the pathogenesis of PCOS.

HSP90B1 is a stress-inducible chaperone protein. Its expression levels significantly affect cell proliferation and survival, cell cycle progression and apoptosis. While the Bcl-2 protein family are the central gatekeepers of the

intrinsic or mitochondrial apoptotic response , And Bax was the first Bcl-2 homologue gene to be identified acting as an apoptosis executer.

Subjects enrolled of the present study were categorized into two groups patients with PCOS and controls. Total 66 patients and 64 controls participant who were attending in Karbala gynecological and obstetric teaching hospital in Karbala, Iraq, were carried out for the period from September 2021 to June 2022. Laboratory tests were done by serological techniques sandwich ELISA for patients, were tested for specific serum human HSP90B1, Bcl-2 and Bax.

The result of the study revealed that HSP90B1 and Bax were significant ($P<0.05$), while Bcl-2 was showed a non-significant ($P>0.05$). The conclusion HSP90B1 and Bax could be candidate as a biomarkers to evaluate the pathogenesis of PCOS.

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# List of abbreviations

| | |
|---|---|
| AES | Amino-terminal enhancer of split |
| AMH | Anti-mullerian hormone |
| AMH | Anti-Mullerian hormone |
| APCs | Antigen presenting cells |
| ASRM | American society of reproductive medicine |
| ATM | Adipose tissue macrophages |
| AUB | Abnormal uterine bleeding |
| AUC | Area under the curve |
| BAD | Bcl-2/Bcl-x-associated death promoter |
| BAK | Bh3 homologous agonist killer |
| BAX | B-cell associated X protein |
| BCL-2 | B-cell lymphoma 2 |
| BiD | BH3 interacting-domain death agonist |
| BiK | Bcl-2-interacting killer |
| Bmf | Bcl-2-modifying factor |
| BMI | Body mass index |
| BOK | Bcl-2 related ovarian killer |
| COCP | Combined oral contraceptive pill |
| CRP | C-reactive protein |
| DHEA | Dehydroepiandrosterone hormone |
| EDC | Endocrine disrupting chemicals |
| ELISA | Enzyme-linked Immunosorbent Assay |
| ER | Endoplasmic reticulum |

| | |
|---|---|
| ESHRE | European society of human reproduction and endocrinology |
| FF | Follicular fluid |
| FFA | Free fatty acid |
| FOH | Functional ovarian hyper and organism |
| FSH | Follicle-stimulating hormone |
| GC | Granulosa cells |
| GnRHR | Gonadotropin releasing hormone receptor |
| GWAS | Genome-wide-association studies |
| HMGB1 | High mobility group box 1 |
| hsCRP | High-sensitivity C-reactive protein |
| HSP | Heat shock protein |
| HSP90B1 | Heat shock protein 90 B1 |
| IFN-γ | Interferon-gamma |
| IQR | Inter quartile range |
| IR | Insulin resistance |
| IRF | Interferon regulatory factor |
| iTregs | Induced regulatory T cells |
| IUGR | Intrauterine growth restriction |
| LH | Luteinizing hormone |
| LTi | Lymphoid tissue inducer |
| MMP | Mitochondrial membrane potential |
| MOMP | Mitochondrial outer membrane permeabilization |
| MTHFR | Methylene tetra hydro folate reductase |
| NGS | Next generation sequencing |

| | |
|---|---|
| NIH | National institutes of health |
| NKc | Natural killer cells |
| NLR | NOD like receptors |
| NLRP | NOD like receptor protein |
| NO | Nitric oxide |
| NOD | Nucleotide-binding oligomerization domain proteins |
| nTregs | Naturally occurring regulatory T cells |
| OS | Oxidative stress |
| OSA | Obstructive sleep apnoea |
| PB | Peripheral blood |
| PCO | Polycystic ovary |
| PCOD | Polycystic ovarian disease |
| PCOS | Polycystic ovary syndrome |
| PCR | Polymerase chain reaction |
| POR | Poor ovarian response |
| PPAR-γ | Peroxisome proliferator-activated receptor-γ |
| ROR | Retinoic-acid-receptor-related orphan nuclear receptor |
| SD | Standard deviation |
| SFA | Saturated fatty acids |
| SHBG | Sex hormone binding globulin |
| SHBG | Sex hormone binding globulin |
| SREBP1 | Sterol regulatory element binding protein-1 |
| StAR | Steroidogenic acute regulatory protein |
| T2D | Type-2 diabetes |

| | |
|---|---|
| TLR | Toll-like receptor |
| TNF-α | Tumor necrosis factor- α |
| VAT | Visceral adipose tissue |
| VDR | Vitamin D receptor |

# Chapter One
# Introduction
# and
# literature Review

## 1.1. Introduction

Polycystic ovary syndrome (PCOS) is the most common gynecological disease in women at reproductive age, which is characterized by metabolic and endocrine abnormalities, as well as chronic inflammation (Lambertini, Saul *et al*. 2017) (Seyyed Abootorabi, Ayremlou *et al*. 2018).

PCOS can have a wide range of prevalence due to genetic and environmental factors (Merkin, Phy et al. 2016) . It ranged between 8 and 13% of women of reproductive age and 6–18% of adolescent girls (Bozdag, Mumusoglu et al. 2016). Oligo-/anovulation, irregular menstruation, obesity, and hyperinsulinemia/insulin resistance are the main clinical manifestations of PCOS (Dahan and Reaven 2019). The most common risk factors for the progress of PCOS include family history of PCOS, fast food, diet habits, lack of physical exercise and body mass index (Begum, Shariff *et al.* 2017).

Women with PCOS are more likely to develop many metabolic and reproductive health complications that include gestational diabetes, dyslipidaemia, insulin resistance, miscarriage, prediabetes, type 2 diabetes, obesity, breast cancer, endometrium cancer and others (Escobar-Morreale 2018). Diagnosis of the syndrome is based according to the Rotterdam criteria (2003), when 2 out of 3 characters is found, while other etiologies are excluded (Eshre and Reproduction 2004). These findings are: intermittent or absent menstrual cycles, high circulating levels of testosterone (T) or hirsutism (Gorsic, Dapas et al. 2019) and ultrasonographic findings of the ovary (12 or more small follicles that are between two and nine mm in diameter in both ovaries) (Kadri, Izhar et al. 2021; Alattabi and Aljabery 2021).

More studies about the pathogenesis of PCOS are still unknown. PCOS itself nowadays is considered a condition of chronic inflammation with elevated levels of leukocytes, pro-inflammatory cytokines, elevated white blood count and markers such as the C-reactive protein being detectable and also affects women with a normal BMI (Regidor, Mueller et al. 2020, Ismael, Alattabi et al. 2021).

HSP90B1 is a stress-inducible chaperone protein. Its expression levels significantly affect cell proliferation and survival, cell cycle progression and apoptosis (Sun, Xiao et al. 2015). It has been reported that high expression levels of HSP90B1, also known as gp96 and Grp94, are correlated with cancer cell survival and epithelial ovarian cancer (Block, Maurer et al. 2015). Recent studies in animal models indicated that adrenocorticotropic hormone or cold stress-induced PCOS were associated with increases in HSP90B1, indicating the possible function of the up regulated genes in the pathogenesis of PCOS (Sun, Xiao et al. 2015).

The Bcl-2 protein family are the central gatekeepers of the intrinsic or mitochondrial apoptotic response. The family is comprised of structurally-related proteins with opposing functions that either promote or inhibit apoptosis by interacting with one another (Kale, Osterlund et al. 2018).

Bax was the first Bcl-2 homologue gene to be identified acting as an apoptosis executer. Bax protein is expressed in various tissues, as multiple alternative splice variants, normally localized in the cytosol or loosely attached to the mitochondria (D'Orsi, Mateyka et al. 2017). Previous studies showed that the process of apoptotic cell death was accelerated by Bax

overexpression and that the apoptotic repressor activity of Bcl-2 was countered by Bcl-2 homo dimerization in vivo (Guo, Lv et al. 2020).

## 1.2. Aim of the study

The aim of this study was planned to evaluate associations of apoptotic markers heat shock protein 90B1 (HSP 90B1), B-cell lymphoma 2 (BCL2) and B-cell associated X protein (Bax) with pathogenesis of polycystic ovarian syndrome in a group of Iraqi women, the following objectives achieved this work:

1. To determine some demographic data in patients and control.
2. To determine HSP90B1, Bcl-2 and Bax serum levels by ELISA test.

## 1.3. Literature Review

### 1.3.1. Definition of Polycystic Ovarian Syndrome

Polycystic ovary syndrome (PCOS) is a common reproductive and endocrine disorder. The main features of PCOS are hyperandrogenism, polycystic ovaries, and ovulatory dysfunction (Shi, Huang et al. 2021). PCOS can have a wide range of prevalence due to genetic and environmental factors (Merkin, Phy et al. 2016). It ranged between 8 and 13% of women of reproductive age and 6–18% of adolescent girls (Bozdag, Mumusoglu et al. 2016). Oligo-/anovulation, irregular menstruation, obesity, and hyperinsulinemia/insulin resistance are the main clinical manifestations of PCOS (Dahan and Reaven 2019).

depending on the diagnostic criteria used and the population studied (Ibáñez, Oberfield et al. 2017) (Teede, Misso et al. 2018). Several factors have

been linked to the development of PCOS, which could lead to female infertility due to failure of follicle maturation and embryo implantation (Fessler, Natterson-Horowitz et al. 2016). Individual genes, gene–gene interaction, or gene–environment interactions have been reported to influence predisposition to PCOS development (Khan, Ullah et al. 2019). The cause of PCOS is unknown, and it is consider a complex genetic trait with a high degree of heterogeneity (Subramaniam, Tripathi et al. 2019).

## 1.3.2. History of the disease

The polycystic ovary syndrome, then called the Stein–Leventhal syndrome, was first described in 1935, Stein and Leventhal published their report of seven women with amenorrhea, hirsutism, obesity, and enlarged polycystic appearing ovaries. Since then, much has been learned about this complex disorder (King 2006).

Originally, diagnosis required pathognomonic ovarian findings and the clinical triad of hirsutism, amenorrhea, and obesity .The next diagnostic milestone occurred 30 years later, when researchers in the late 1960s and early 1970s noted derangements in the hypothalamic–pituitary axis. This focused the diagnosis on endocrine criteria, such as elevated levels of serum luteinizing hormone or ratio of luteinizing hormone to follicle-stimulating hormone. With the advent of pelvic ultrasonography in the 1970s and 1980s (first, abdominal sonography and, later, vaginal sonography), The recognition of a characteristic polycystic ovary complicated the diagnosis.

It was also quickly realized that polycystic ovaries can occur in some “normal” women and in women with well-defined endocrinopathies as varied as hypothalamic amenorrhea and congenital abnormal hyperplasia .In normal

ovulatory women with no other typical endocrine features, we prefer to call this finding as polycystic-appearing ovary to distinguish it from the term "PCO," which is often used synonymously, but incorrectly, with PCOS . In 1990, the National Institutes of Health formed a group to investigate PCOS. No consensus was reached regarding the naming of the disorder (Lobo and Carmina 2000).

It is now well recognized that women with this syndrome not only have reproductive health issues but their metabolic and cardiovascular health is also affected (King 2006). Until recently, there has been no universally accepted definition for PCOS. In 2003, an international consensus group proposed that the diagnostic criteria for PCOS are ovarian dysfunction evidenced by oligomenorrhea or amenorrhea and clinical evidence of androgen excess (e.g., hirsutism and acne) in the absence of other conditions that can cause these same signs and symptoms (King 2006).

After the first description of PCOS by Stein and Leventhal, the diagnostic criteria of PCOS have evolved over the years. The 1990 National Institutes of Health (NIH) conference proposed the diagnostic criteria of oligo/anovulation and biochemical and/or clinical hyperandrogenism. In 2003, Rotterdam conference, organized by the European Society of Human Reproduction and Endocrinology (ESHRE) and American Society of Reproductive Medicine (ASRM) broadened the definition of PCOS by adding PCO morphology, which divided PCOS into four subtypes: IM/PCO/HA, IM/PCO, IM/HA, and HA/PCO. The Rotterdam criteria do not delineate the essential features of PCOS.

In 2006, the Androgen Excess Society taskforce on the phenotypes of PCOS emphasized HA as the cornerstone of PCOS and excluded the IM/PCO subgroup. The second criterion essential to diagnose PCOS according to the AES is either anovulation or polycystic ovarian morphology (Thathapudi, Kodati et al. 2014).

Panel members considered the various consensus statements that had been issued by several studies over time, from the NIH 1990 consensus conference, the Rotterdam 2003 conference, and the Androgen Excess Society statement from 2006. They also agreed that because the underlying cause of PCOS is still not determined and maybe multifaceted (Wang and Alvero 2013).

## 1.3.3. Epidemiology

Polycystic ovarian syndrom is the most common endocrine disorder in reproductive-aged women worldwide. Depending on the diagnostic criteria, the prevalence ranges between 5% and 15%. Based on the disorder characteristics, may be present more than 16 phenotypes with various metabolic and reproductive consequences (Leon, Anastasopoulou et al. 2021). Rotterdam criteria include a wider prevalence than the National Institute of Health 1990 Criteria and not accepted by all (Ning, Balen et al. 2013). with calls for them to be updated (Dewailly, Obstetrics et al. 2016). When the PCOS is diagnosed according to the Rotterdam criteria, the prevalence of PCOS is estimated to be about 4 to 21 percent (Lizneva, Suturina et al. 2016). The PCOS prevalence is estimated to be around 4 percent–6.6 percent, based on the report from the NIH workshop of 2012 (Lizneva, Suturina et al. 2016, Ismael, Alattabi et al. 2021).

According to population samples assessed in the United States, Western Europe, the Middle East, East Asia, and Australia, the prevalence of PCOS in females of reproductive age varies by geographic region, ranging from 1 to 19 percent (Merkin, Phy et al. 2016). PCOS can have a wide range of prevalence due to genetic and environmental factors. A lower socioeconomic status is also linked to poorer health, which can result in hormonal changes and/or activate a genetic predisposition to disease development. Inadequate healthcare conditions also lead to lower rates of accurate diagnosis and appropriate treatment (Merkin, Phy et al. 2016; Alattabi and Aljabery 2021).

## 1.3.4. Etiology of Polycystic Ovarian Syndrome

The etiological factors associated with PCOS are not yet so clear and still under debate. There are different factors that could be contributes to the etiopathology of PCOS such as genetic, biochemical, environmental and immunological. Many genes have been shown to be crucial contributors to PCOS , However, to date, none of these factors could be implicated as the main cause (Glueck and Goldenberg 2019; Khan, Ullah et al. 2019; Shaaban, Khoradmehr et al. 2019).

### 1.3.4.1. The Genetic Factor

PCOS is a multifactorial disorder where individual genes, gene–gene interaction, or gene–environment interactions have been reported to influence predisposition to PCOS development, Studies on PCOS reported multiple relatives and siblings in families with autosomal dominant inheritance. The prevalence of PCOS in the first-degree relative of the proband that was found in nearly 55–60% in several small families supported the hypothesis of autosomal dominant inheritance of PCOS. Later on, monogenic causes of

hirsutism and oligomenorrhea in PCOS women and male-pattern baldness were identified (Khan, Ullah et al. 2019).

The most interesting hypothesis was proposed by Franks et al. , who defined PCOS as a genetically determined ovarian pathology characterised by over-production of androgens and manifesting heterogeneously according to the interaction of this genetic "predisposition" with other genetic and environmental factors (De Leo, Musacchio et al. 2016). Genome-wide association studies (GWAS) in women of Han Chinese and European ancestry have reproducibly identified 16 loci. The observed susceptibility loci in PCOS appeared to be shared between NIH criteria and self-reported diagnosis ,which is particularly intriguing. Genetic analyses of causality (by Mendelian Randomization analysis) among women of European ancestry with self-reported PCOS suggested that body mass index (BMI), insulin resistance, age at menopause and sex hormone binding globulin contribute to disease pathogenesis (Day, Karaderi et al. 2018).

Polymerase chain reaction (PCR) and next generation sequencing (NGS) have been used to genotype several of these causative genes. Polymorphisms in the cytokine gene have been discovered in PCOS patients (Patel et al., 2018). PCOS is highly likely to affect the daughters of women who have the condition. The AMH level, which is high in PCOS mothers, is the cause of this predisposition (Olszanecka-Glinianowicz et al., 2016).

### 1.3.4.2. Obesity

Obesity is a metabolic condition characterized by chronic inflammation state with higher pro-inflammatory cytokines, chemokines, and oxidative stress (OS) markers levels (Rudnicka, Suchta et al. 2021). obesity particularly

significant in the pathophysiology of PCOS because it leads to insulin resistance, which exacerbates functional ovarian hyperandrogenism (FOH). It generates testosterone from circulating androstenedione while suppressing gonadotropin production (Rosenfield and Ehrmann 2016; Alattabi and Aljabery 2021).

Insulin signaling is of major importance to the size and function of the adipose tissue depot: it stimulates adipogenesis (development of preadipocytes into adipocytes) and lipogenesis while inhibiting lipolysis. (Rosenfield and Ehrmann 2016). It has been proposed that androgens stimulate the differentiation of pre-adipocytes to adipocytes, especially in the abdomen area, facilitating the development of visceral-type obesity (Rudnicka, Suchta et al. 2021).

Adipose tissue expansion is a consequence of both hyperplasia (adipogenesis), which is driven by proliferation of preadipocytes and their differentiation into adipocytes, and hypertrophy, which is driven by accumulation of lipid in differentiated adipocytes; both processes are major determinants of metabolic dysfunction. However, women with PCOS have lower levels of adiponectin than healthy controls (Schiffer, Kempegowda et al. 2017).

### 1.3.4.3. Environmental Exposure Risks

Environmental toxins are defined as chemical pollutants in the environment that have adverse effects on biologic organisms. These pollutants may be inhaled, absorbed through skin and mucous membranes, or ingested. Scientific evidence has emerged showing significant and lasting effects of environmental toxins on human reproductive health. Intrauterine exposure to

excess androgens or glucocorticoids during certain critical periods of fetal development may lead to the development of PCOS symptoms as well as determine the phenotypic expression of PCOS during adulthood (Merkin, Phy et al. 2016).

The industrialized food system has been recognized as a major contributor to the introduction of toxic chemicals into the environment that may influence reproductive health and possibly affect the development of PCOS. While not widely recognized as ‘‘toxins’’ certain food sources such as starch based and dairy foods have been found to promote exaggerated insulinogenic responses in women with PCOS (Merkin, Phy et al. 2016).

postnatal lifestyle factors like inadequate nutrition accompanied by a lack of physical exercise promote the development of the disease as they often result in obesity and disturbances of the glucose metabolism. In fact, hyperinsulinemia, independent of BMI, is a key contributor to PCOS pathogenesis (Regidor, Mueller et al. 2020). Moreover, Environmental factors such as diet and obesity appear to contribute to the phenotype (Louwers and Laven 2020).

### 1.3.4.4. Insulin Resistant

Insulin resistance (IR) is a major pathophysiologic mechanism in developing clinical symptoms and other metabolic complications of PCOS (Shirazi, Khodamoradi et al. 2021). Insulin resistance and defective insulin secretion are both genetic features of PCOS, raising the risk of type 2 diabetes (Yilmaz, Vellanki et al. 2018). And leads to an increase in free androgen availability, and consequently alteration in follicular development and granulosa cell function (Glueck and Goldenberg 2019). IR and

hyperinsulinemia are metabolic traits characteristic of lean and obese women with PCOS (Sanchez-Garrido and Tena-Sempere 2020; Ismael, Alattabi et al. 2021).

PCOS is often associated with obesity and impairs reproductive health. Even though several theories have been proposed to explain the pathogenic mechanism of PCOS, the role of insulin resistance (IR), independently of obesity, is a key etiological component. Additionally, obesity-related inflammation may have potential implications for ovarian physiology due to the dysregulated adipokine secretion, affecting insulin sensitivity (Aboeldalyl, James et al. 2021). Increased insulin concentration in PCOS patients reduces the serum level of SHBG, which enhances the bioavailability of free testosterone level; Also, these women have abnormal gonadotropin concentration and great androgen biosynthesis from the adrenal and ovaries, stimulated by high level of insulin (De Leo, Musacchio et al. 2016; Alattabi and Aljabery 2021).

Insulin resistance and steroidogenesis are directly modulated by pro and anti-inflammatory cytokines and lipopolysaccharide produced by circulating adipose or intestinal monocytes (Fox, Zhang et al. 2019), (Qi, Yun et al. 2019). Emerging evidence suggests that oxidative stress and chronic low-grade inflammation underpin the genesis of insulin resistance and accelerated atherogenesis in PCOS (González, Considine et al. 2020). Intake of glucose or saturated fat exacerbates insulin resistance and FOH by raising serum levels of several proinflammatory factors, often more so in PCOS than obesity (González, Considine et al. 2019; González, Considine et al. 2020).

### 1.3.4.5. Hyperandrogenism

Hyperandrogenism has been recognized as a contributor to aggravate the reproductive symptoms and the development of metabolic syndrome in PCOS. It is believed that the excessive androgen is primarily from ovary and the adrenal gland. Although adrenal hyperandrogenism affects 20%–30% of PCOS patients (Rosenfield and Ehrmann 2016). it does not exert any effect on metabolic disorder in PCOS patients (Paschou, Palioura et al. 2017). However, excessive androgen from ovaries is considered to be the most important inducer of PCOS (Rosenfield and Ehrmann 2016). Hyperandrogenism in women with PCOS clinically presents as hirsutism, acne, and androgenic alopecia. Other manifestations like weight gain, menstrual irregularities, acanthosis nigricans, and insulin resistance are also manifested by increased androgen excess, depicted in Figure (1.1) (Ashraf, Nabi et al. 2019).

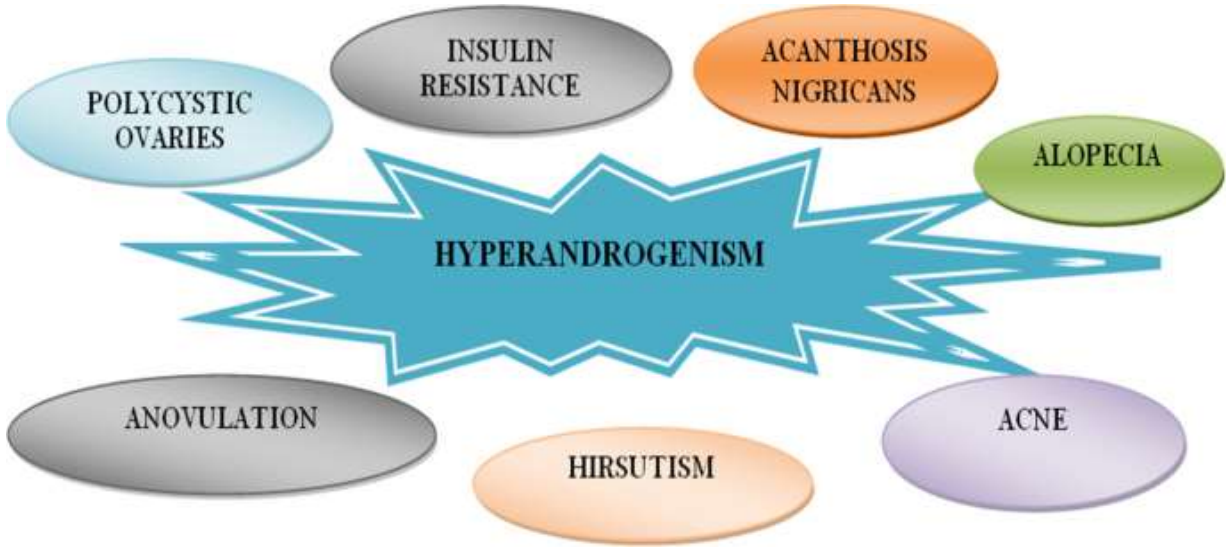


**Figure (1.1):** Clinical implications of hyperandrogenism in PCOS (Ashraf, Nabi et al. 2019).

The neuroendocrine system is thought to be dysregulated, resulting in an imbalance in the hypothalamic-pituitary-ovarian axis and an excess of gonadotropin. The production of LH rather than FSH is stimulated by an increase in GnRH, resulting in a significant increase in the LH:FSH ratio in PCOS. The follicles evolve from primitive follicles, but development was halte at an early stage due to ovarian dysfunction as shown in Figure (1.2) (Walters, Gilchrist et al. 2018). Luteinizing hormone acts primarily on the ovarian theca cells carrying LH receptors and induces the production of androgens. Concomitantly, FSH acts on the ovarian granulosa cells and converts the androgens formed in theca cells into estrogens, principally estradiol, which is responsible for the development of follicles. However, in women with PCOS, it has been hypothesized that dysregulation in the neuroendocrine system leads to an imbalance in the hypothalamic–pituitary–ovarian axis, leading to the overproduction of gonadotrophins (Ashraf, Nabi et al. 2019; Alattabi and Aljabery 2021).

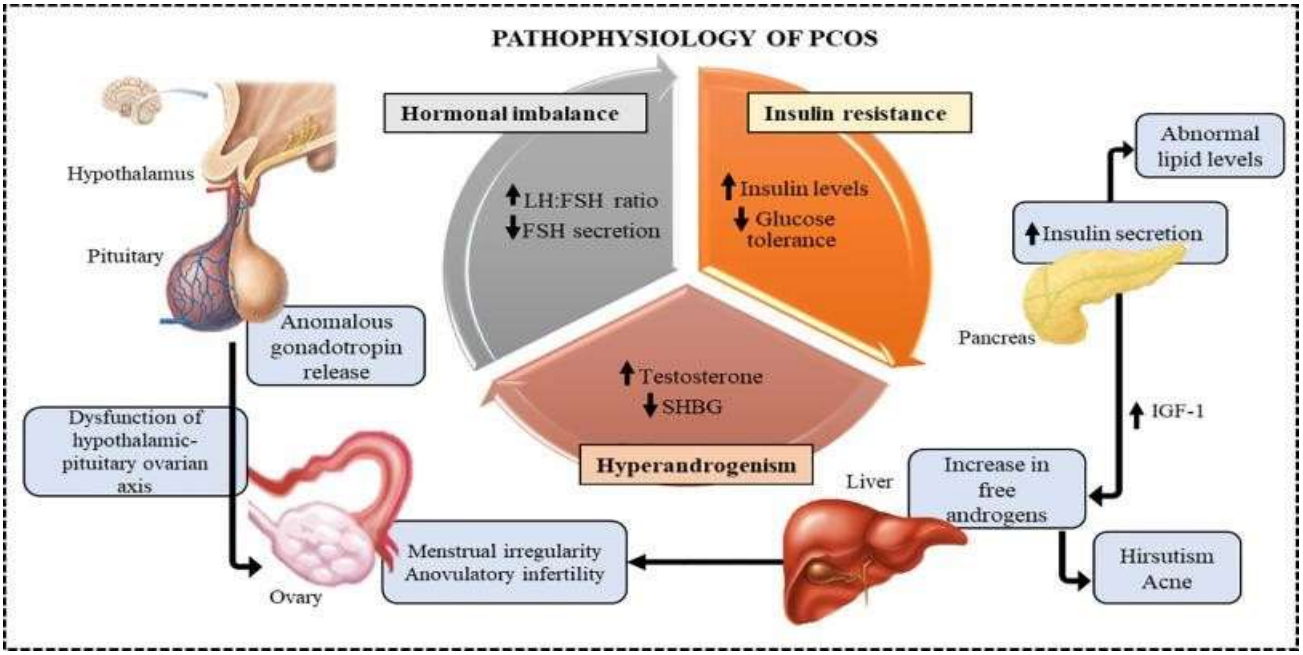


**Figure (1.2):** Schematic depiction of PCOS linked mechanism. (Walters, Gilchrist et al. 2018).

### 1.3.4.6. Inflammation

PCOS is characterized by chronic inflammation (Patel and biology 2018), with elevated levels of leukocytes, pro-inflammatory cytokines, elevated white blood count and markers such as the C-reactive protein being detectable and also affects women with a normal BMI, which is caused in part by excess visceral adipose tissue (Regidor, Mueller et al. 2020). It is considered to be important in PCOS (Shermin, Noor et al. 2019). Another important mediator in chronic low-grade inflammation is CRP. CRP is an acute-phase protein produced in hepatocytes by stimulation of IL-6 (Rostamtabar, Esmaeilzadeh et al. 2021). Mast cells and the classically activated M1 macrophages secrete different kinds of inflammatory cytokines including TNFα, IL-1 and IL-6 all of them being highly inflammatory substances that drive the generation of the classical inflammatory symptoms: redness, heat, swelling, pain, and loss of function (Regidor, Mueller et al. 2020; Alattabi and Aljabery 2021).

Obesity is a metabolic condition characterized by chronic inflammation state with higher pro-inflammatory cytokines, chemokines, and oxidative stress (OS) markers levels (Rudnicka, Suchta et al. 2021). BMI and insulin resistance are the main predictors of increased levels of CRP and white blood cells (Rudnicka, Kunicki et al. 2020). Furthermore, hyperandrogenaemia in PCOS not only contributes to enhanced visceral adiposity but was also shown to contribute to inflammatory processes (Regidor, Mueller et al. 2020). Excessive inflammation has been linked to higher androgen levels (Mukerjee and Research 2020). Recent findings have revealed that inflammation processes are involved in ovulation and play an important role in ovarian follicular dynamics. Of note, the visceral adipose tissue is linked to the development of inflammation (Rostamtabar, Esmaeilzadeh et al. 2021).

Increased levels of free fatty acids are present in obese individuals, As these molecules represent primary ligands for Toll-like receptors, they are an important trigger for innate and adaptive immune response. Furthermore, Macrophages constitute a considerable fraction of adipose tissue and are mainly representing the activated M1 phenotype. Hence, they secrete pro-inflammatory cytokines like TNFα and IL6 that may also induce inflammatory responses in other tissues including the ovary (Regidor, Mueller et al. 2020, Ismael, Alattabi et al. 2021).

## 1.3.5. Pathogenesis of PCOS

Given its heterogeneous clinical presentation, different and eventually non-exclusive mechanisms are involved in the pathogenesis of PCOS. particularly, in the generation of its common metabolic complications. PCOS is closely linked to metabolic disorders such as obesity and insulin resistance (IR). A large proportion of women with PCOS are obese or overweight and exhibit IR with associated compensatory hyperinsulinemia (Sanchez-Garrido and Tena-Sempere 2020).

Hyperandrogenemia is considered the main clinical hallmark of PCOS. It is estimated that more than 80% of women who exhibit signs or symptoms of hyperandrogenism, including hirsutism, acne or alopecia, have PCOS (Sanchez-Garrido and Tena-Sempere 2020). Environmental factors have been also implicated in the pathogenesis of PCOS. As previously discussed, insulin resistance and associated hyperinsulinemia are considered key pathological factors in the development of PCOS, and obesity is the most common cause of insulin resistance. Consequently, all environmental factors that can lead to overweight or obesity and alter insulin action may be involved in the etiology

of this endocrine disorder. Diet and lifestyle are the main factors that may cause or exacerbate the metabolic and reproductive abnormalities of PCOS (Alattabi and Aljabery 2021).

Many studies have suggested that genetic factors may be important in the etiology of this syndrome (Sanchez-Garrido and Tena-Sempere 2020). Some studies show correlation of the iron overload with PCOS. High levels of ferritin and transferrin leads to a reduction in anti-inflammatory cytokines and anti-oxidant molecule levels. OS and chronic low-grade inflammation are known to be pathways involved in PCOS pathogenesis (Rudnicka, Suchta et al. 2021). Altered mineral status in the form of hypercalcaemia and very low manganese levels in addition to higher levels of zinc and copper was demonstrated in women with PCOS compared to controls. Other mechanisms proposed to have a role in PCOS pathogenesis Some studies have suggested a role of vitamin D deficiency in metabolic abnormalities seen in PCOS women (Ganie, Vasudevan et al. 2019).

Previous studies confirmed that adiponectin exerts wide beneficial effects on inflammation, and reduced adiponectin levels may contribute to an increased risk for cardiovascular complications in obesity, insulin resistance and diabetes (Yan, Ding et al. 2021). The imbalance of the intra-ovarian regulatory system seems to play a role in this increased sensitivity to LH. Exogenous factors, such as insulin resistance and hyperinsulinemia, are examples of extra ovarian modulators that disrupt normal intra-ovarian regulatory mechanisms, Since ovarian steroidogenesis is directly related to the gonadotropic stimulus, alterations of LH are indicated as one of the factors involved in the development of PCOS (Crespo, Bachega et al. 2018). Granulosa cells (GCs) and oocytes have metabolic cooperation for the

continuous supply of glucose and any alteration in this process may have deleterious effects on follicular growth and oocyte maturation. Both FSH and LH stimulate glucose uptake as demonstrated in oocyte-cumulus cell complexes and GCs (Chahal, Geethadevi et al. 2021).

studies of metabolic dysfunction in PCOS primarily focus on impaired glucose disposal and peripheral IR in muscle and the liver (Ezeh, Chen et al. 2020). Previous studies have demonstrated that PCOS share similar properties with many chronic inflammatory disorders, and classical inflammatory mediators such as C-reactive protein (CRP), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) have also been found to be elevated in patients with PCOS (Zhang, Che et al. 2020). IL-6 plays an important role in ovarian maturation and implantation process. IL-1β and TNF-α are important markers that influence ovulation and pregnancy. IL-1 secreted in follicle can regulate granulosa cells to synthesize prostaglandin and control the collagenase activity, which play a crucial role in ovulation (Mohammadi, Kayedpoor et al. 2017).

Inflammasomes are cytosolic multiprotein complexes, identified as a series of NLRs, including NOD1, NOD2 and the NOD like receptor protein (NLRP) .They respond to a variety of endogenous (i.e. damage-associated molecular pattern) and exogenous (i.e. damage-associated molecular pattern) stimuli (Yu and Li 2021). Activation of the NLRP3 inflammasome results in activation of caspase-1, which in turn promotes the production of mature IL-1β and IL-18 from pro-IL-1β and pro-IL-18, respectively. Such cytokines play important roles in regulating ovarian steroidogenesis, maturation of ovarian follicles, and other reproductive processes, and the expression of IL-18 was significantly increased in patients with PCOS (Zhou, Li et al. 2021).

Wang et al suggested that hyperandrogenism is the main cause of infertility as a result of PCOS, hyperandrogenism can drive the generation of the NLRP3 inflammasome, which results in the secretion of inflammatory mediators, and induced low-grade inflammation in mice with PCOS. Some women with PCOS appear to have increased levels of androgen, oxidative stress, free fatty acid (FFA) and highmobility group box 1 (HMGB1), molecules that serve as danger signals to activate the inflammasome pathway, especially the NLRP3 inflammasome pathway (Zhou, Li et al. 2021).

## 1.3.6. Immune Cells and their Role in PCOS

### 1.3.6.1. Macrophage

Macrophages are the most abundant immune cells within the adipose tissue and ovary, both in animals and humans , particularly in the thecal, luteal, and atretic follicles, where they participate in multiple processes in the ovary, such as folliculogenesis and ovulation (Hu, Pang et al. 2020).

Macrophages are key players in the inflammatory immune response and the balance between M1 (inflammatory) and M2 (anti-inflammatory) macrophages can determine the immunological milieu and the ovarian cell fate (Lima, Nivet et al. 2018). PCOS is characterized by a shift in macrophage polarization from an anti-inflammatory M2 to a proinflammatory M1 state (Luan, Zhang et al. 2022). Approximately 85 % of PCOS patients are affected by IR, An abnormally elevated insulin level promotes androgen production in ovarian theca cells, decreases insulin receptor autophosphorylation in ovarian granulosa cells, aggravates the ovarian chronic inflammatory response and IR, and worsens the pathological process of PCOS as shown in figure (1.3) (Luan, Zhang et al. 2022).

The dysfunction of fat cells and accumulation of macrophages can also result in an influx of a plethora of proinflammatory cytokines and chemokines (e.g., IL-1, IL-6, IL-10, IL-12, nitric oxide, and TNFα) into the circulatory system at the same time, leading to a state of systemic, chronic low-grade inflammation that can affect ovarian function (Hu, Pang et al. 2020). The low-grade chronic inflammation in PCOS patients is mainly attributed to accumulated visceral fat, in which adipocytes undergo necrosis after hypoxia and gather a large number of inflammatory cells to produce a variety of inflammatory cytokines. In thin PCOS patients, there were more macrophages and pro-inflammatory factors in adipose tissue, resulting in insulin resistance (IR) (Luan, Zhang et al. 2022).

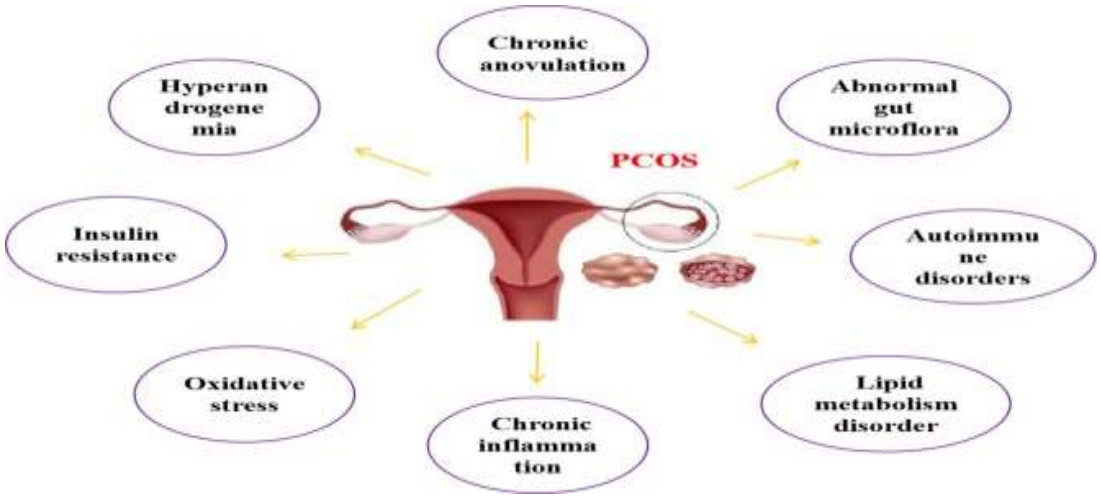


**Figure (1.3):** The influencing factors in the development of PCOS. Hyperandrogenism and insulin resistance, oxidative stress, and cystic follicles are engaged in the pathological process of PCOS. Simultaneously, enhancing inflammatory medium secretion can aggravate chronic inflammation, affect the tissue cell function, and result in ovarian dysfunction and metabolic disorder. The destruction of immune homeostasis is also closely tightly related to the pathological process of PCOS. (Luan, Zhang et al. 2022).

Adipose tissue macrophages (ATMs), ATMs constitute ~10% of the stromal vascular fraction in white adipose tissue, although their population can increase dramatically in pathologic states. Over the past several decades, the endocrine role of adipose tissue has been clearly defined (Rehman, Pacher et al. 2021). Hormones and immune cells appear to play an important role in the progression of PCOS, according to growing evidence, Hyperandrogenemia can stimulate monocyte infiltration in the ovary and increase inflammatory factor secretion, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), resulting in chronic ovarian tissue inflammation, affecting the maturation of developing follicles in the ovary and leading to cystic follicle formation (Luan, Zhang et al. 2022).

On the other hand, Oakley et al. (2011) suggests that the spleen may serve as an immune cell reservoir for the ovary and that splenic monocytes can be mobilized in a cyclic manner to the ovaries where they differentiate into macrophages. Macrophage-derived secretory products such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6 and nitric oxide (NO), among others, exert direct effects on endocrine ovarian cells. These signaling molecules modulate normal reproductive function but are also involved in the pathogenesis of reproductive chronic inflammatory disorders as polycystic ovarian syndrome (PCOS) (Figueroa, Mendoza et al. 2018).

As TNFα plays a crucial role in the apoptosis of the granulosa and luteal endothelial cells, finally leading to follicular atresia and a luteolytic effect, its

concentration determines the quality of the oocyte and eventually promotes PCOS-independent hyperandrogenemia and obesity. Furthermore, As TNFα and IL-6 also potentially induce insulin resistance, stimulate the production of androgen, and cause hypothalamic-pituitary-ovarian axis secretion disorder, a concomitant PCOS condition may result in a vicious cycle. Therefore, it considered that prolonged high androgen levels experienced by PCOS patients might drive macrophages conversion to the M1 phenotype, resulting in the secretion of more proinflammatory cytokines and thereby enhancing PCOS clinical manifestations (Hu, Pang et al. 2020).

### 1.3.6.2. Dendritic Cells (DCs)

DCs are a heterogeneous group of antigen-presenting cells (APCs), which exist in an immature state in the circulation and have potent phagocytotic ability; thus, they can capture and process antigens and present them to T cells in the lymph nodes, serving as a bridge between the innate and adaptive immune responses. After receiving the activation signal associated with the antigen, DCs produce cytokines and inflammatory mediators such as TNFα, IL-6, IL-11, IL-12, and IL-23, which, in turn, induce the proliferation of allogeneic T cells and differentiate them to the Th17 and Th1 subtypes (Hu, Pang et al. 2020; Ismael, Alattabi et al. 2021).

In visceral adipose tissue (VAT), DCs suppress inflammation by activating the β-catenin and PPARγ pathways, which are important regulatory mechanisms for fat expansion (Hu, Pang et al. 2020). The expansion and renewal of adipocytes are tightly regulated at the transcriptional level. In mature adipocytes, peroxisome proliferator-activated receptor-γ (PPARγ) regulates lipid accumulation during hypertrophy , while the Wnt/β-catenin

pathway controls adipocyte hyperplasia (Macdougall, Wood et al. 2018). Subsequently, β-catenin activation triggers PI3K/Akt that, in turn, induces IL-10 production and inhibits IL-6 secretion (Hu, Pang et al. 2020). In comparison, increasing evidence implicates CD11c+HLA-DR+ DCs as necessary follicular fluid cell components, and mature DCs are positively associated with the ovary response to gonadotrophic, implying a role in ovulation linked to aseptic inflammation (Gamble, Purgato et al. 2019).

The number of DCs in the follicular fluid was found to be significantly decreased in PCOS patients as compared to those in healthy controls. Therefore, it considered that with more VAT in PCOS patients, DCs may serve not only to restrain obesity-induced inflammation but also to promote pathogen persistence. Meanwhile, there might not be a sufficient amount of DCs in the follicular fluid to induce the recruitment and activation of T cells (Th17, Th1 cells), resulting in the failure of follicle development and maturation (Hu, Pang et al. 2020).

More recently, Fainaru et al. demonstrated that dendritic cells (DCs) are an important cellular component of the Follicular fluid (FF) and their stage of maturation correlates positively with the ovarian response to gonadotropins. Their function may be associated with the induction of sterile inflammation responsible for ovulation (Zhang, Tian et al. 2017).

### 1.3.6.3. Innate Lymphoid Cells (ILCs)

ILCs are a family of lymphocytes comprising the innate counterparts of T cells. They are poised to secrete cytokines that respond swiftly to pathogenic tissue damage and shape subsequent adaptive immunity (Panda and Colonna 2019). ILCs act early in the immune response by reacting promptly to signals,

or inducer cytokines, expressed by tissue-resident cells (Vivier, Artis et al. 2018). Based on the signature cytokines they produce, their phenotype, and their developmental pathways, ILCs are divided into three major groups: ILC1s, ILC2s, and ILC3s.

Two additional immune cell types, NK cells and lymphoid tissue inducer (LTi) cells, are generally included in the ILC family because their phenotypic, developmental and functional properties overlap considerably with those of ILC1s and ILC3s, respectively (Panda and Colonna 2019). ILC1s, ILC2s, and ILC3s mirror CD4+ T helper (Th)1, Th2, and Th17 cells, respectively, in terms of function, whereas natural killer (NK) cells mirror the functions of CD8+ cytotoxic T cells. ILCs and T cells play key roles in orchestrating the most appropriate immune response to the threat faced by the individual. ILC1s and Th1 cells react to intracellular pathogens, such as viruses, and to tumors; ILC2s and Th2 cells respond to large extracellular parasites and allergens; and ILC3s and Th17 cells combat extracellular microbes, such as bacteria and fungi (Vivier, Artis et al. 2018).

Natural killer (NK) cells are members of the innate lymphoid cell family, They are potent effectors of the innate immune system and form the first line of defense against diseases, including malignancies (Becker, Suck et al. 2016). the majority of NK cells in peripheral blood are CD56+CD16+. Increased density of the surface antigen CD56 and decreased CD16 expression make CD56+CD16- uterine and decidual NK cells phenotypically distinct from that of peripheral NK cells (Kofod, Lindhard et al. 2018).

An increased amount of CD16+ uNK cells in infertile women is also associated with deficiency of vascular endothelial growth factor (VEGF),

normally necessary for vascular formation in the placenta. In the inflammatory process, cells in the immune system such as natural killer (NK) cells and macrophages are recruited to the sites of the inflammation. Most of the uterine NK (uNK) cells have a CD56bright CD16- phenotype, are related to the synthesis of cytokines and have limited cytotoxic activity (Elpidio, de Alencar et al. 2018). Currently, uterine NK cells are considered to be endometrial markers for PCOS.

Some transcription factors, such as retinoic-acid-receptor-related orphan nuclear receptor (ROR) γt, STAT3, and interferon regulatory factor (IRF) 4 are expressed by Th17 cells (Jafarpour, Pashangzadeh et al. 2020). ILC3s and Lymphoid Tissue-inducer-like cells (LTi-like) are defined by their expression of RORγt (Huhn, Zhao et al. 2021). With high androgen and reduced progesterone, PCOS patients have decreased CXCL10, IL-15, IL-18, and IL-12A levels, which play important roles in maternal-fetal tolerance and maintenance in pregnancy, suggesting that impairment in recruiting NK cells in PCOS patients may lead to a cytokine disorder. The receptivity of the endometrium is a precondition for a successful pregnancy. Thus, NK cells might explain infertility associated with PCOS, besides the main manifestations of follicular dysplasia and ovulation disorder (Hu, Pang et al. 2020).

### 1.3.6.4. T Helper (Th) Cells

As the main component of lymphocytes, T cells have various biological functions, which are mainly involved in the cellular immune response of the body. They can kill target cells directly or through the release of lymphatic factor to enhance and expand the immune effect (Li, Peng et al. 2019). CD4+

T helper (Th) cells are central orchestrators of immune responses. They provide help to CD8+ T cells and B cells and produce cytokines that activate or modulate innate immune cells, stromal cells and epithelial cells (Stadhouders, Lubberts et al. 2018).

In 1986 Mossmann, Coffman and colleagues first reported that upon antigenic stimulation naive CD4+ T cells can differentiate into two functionally distinct subsets: Th1 or Th2 effector cells. Distinct T cell subsets secrete various cytokines that play different roles in a complex regulatory pathway (Azad, Keshtgar et al. 2017). CD4+ T helper cells can be subdivided into different subsets, including Th17 cells, which produce IL-17A, and IL-17F, Th1 cells, which produce IFN-γ, IL-2, and TNF-α, Th2 cells, which secrete IL-4, IL-5, and IL-13, and Regulatory T cells, which express Foxp3, IL-10, and TGF-β (Li, Peng et al. 2019).

As IL-13 levels in the follicular fluid of PCOS patients were found to be significantly lower than those of women with regular ovulation, whereas the concentration of IL-12 increased significantly which could induce a shift from Th2 to Th1 cells (Hu, Pang et al. 2020). Recent studies have reported that fine-tuned balance and coordination among different subsets of T helper cells contributed to fertility and the success of pregnancy (Azad, Keshtgar et al. 2017).

Activated CD4+ T cells are triggered to differentiate into effector Th cells, guided by specific co-stimulatory signals and the cytokine milieu (Stadhouders, Lubberts et al. 2018). Tumor necrosis factor alpha, a Th1-inducing factor, was increased in follicular fluid (FF) of PCOS patients. as well as the significantly elevated production of Th1 cytokines in FF

lymphocytes .These results demonstrated that Th1 type immunity was predominant in local immune regulation in PCOS (Gong, Shi et al. 2018).

Estrogen was shown to augment the secretion of inflammatory cytokines such as TNFα, IL-6, and interferon-gamma (IFNγ) in Th1 lymphocytes, whereas the progesterone spike in the luteal phase decreased these levels (Gong, Shi et al. 2018). IL-6 may inhibit TNFα production and also effectively drive angiogenesis, thus promoting the formation of blood vessels and increases the concentration of the follicle-stimulating hormone (Daan, Koster et al. 2016). Expansion of peripheral blood (PB) cytotoxic CD4+CD28 null T lymphocytes, and higher PB Th17 cells in women with PCOS imply immune dysregulation in PCOS and suggest a correlation between endocrine and immune function (Moulana 2019).

Due to the accumulation of numerous follicles with no ovulation, patients with PCOS show a high level of estrogen without progesterone resistance. IL-6 can also stimulate the expression of the key transcription factors of Th17 cells by activating STAT3 and the expression of RORα and RORγ, thereby promoting the differentiation of Th0 cells to Th17 cells (Gamble, Purgato et al. 2019) (Moulana 2019).

It is confirmed that a significant difference in the Th17/Th2 ratio, with a bias toward Th17, is common among patients with PCOS (Nasri, Doroudchi et al. 2018). Thus, the accumulation of Th1 and Th17 cells leads to immune overaction, which implies that PCOS might have an autoimmune origin (Hu, Pang et al. 2020).

### 1.3.6.5. Cytotoxic T (Tc) Cells

Tc cells are the main effector cells of the cellular immune system. They initiate cytotoxic processes in order to eliminate infected or malignantly transformed cells. Cytokine secretion, cytotoxic agent release, and direct cell-cell contact all contribute to these effects (Van Herck, Weyler et al. 2019). CD4+CD28null cytotoxic T cells have a proinflammatory function, producing high levels of IFN-γ, TNF-α, IL-2, and cellular enzymes, which can lead to the loss of CD28 on the cell surface in states of chronic inflammation and infection (Moulana 2019). Changes in lymphocyte subgroups have been linked to hormone levels (Gamble, Purgato et al. 2019).

According to recent research, CD4+CD28null cells are only associated with the general PCOS status, not with hyperinsulinemia, high- sensitivity C-reactive protein (hsCRP) levels, obesity, or androgen levels, and have high proinflammatory and tissue-damaging properties (Bañuls, Rovira-Llopis et al. 2017). All of this suggests that PCOS is linked to a decrease in immune response

### 1.3.6.6. Regulatory T Cells (T regs)

T regs are classified into two categories: induced regulatory T cells (iTregs), which are produced by the peripheral lymphoid tissues and naturally occurring regulatory T cells (nTregs), which are produced by the thymus gland. CD4+CD25+CD127-/lowFoxp3+ Tregs make up about 1–2% of total CD4+ T cells in the human peripheral blood, and they aid in the prevention of autoimmune diseases by inhibiting the proliferation of effective T cells and the production of cytokines (Walecki, Eisel et al. 2015).

In healthy states, Tregs play an important role in immune tolerance, and their dysregulation is linked to the development of autoimmune diseases.

Indispensable constituent of the normal immune system for the maintenance of immunological self-tolerance and homeostasis (Sakaguchi, Mikami et al. 2020). FoxP3+ natural Tregs have been shown to play key roles in a much broader spectrum of diseases and biological conditions, e.g., fetal-maternal tolerance, immunometabolic diseases (such as obesity and atherosclerosis), degenerative diseases with immunological/inflammatory elements, and tissue regeneration (Dombrowski, O'hagan et al. 2017) (Ito, Komai et al. 2019).

The number of Tregs in the peripheral blood of patients with PCOS was found to be lower than that of healthy (Krishna, Joseph et al. 2015). Thus, the Th17/Tregs ratio will rise, causing a chronic inflammatory state in the ovary and whole of the body. Hyperestrogenism, hyperandrogenism, and hypoprogesterone are all important factors in the dynamic change of T regs in PCOS (Hu, Pang et al. 2020).

## 1.3.7. Heat Shock Protein (HSP90B1) and their Role in PCOS

### 1.3.7.1. Heat Shock Proteins

Heat shock proteins (HSPs) are a family of stress-inducible proteins, the expression and activity of which are highly regulated by the extracellular microenvironment. HSPs are coupled with 100 client proteins to modulate downstream target gene expression and activity by affecting protein folding and degradation. Their expression levels significantly affect cell survival, apoptosis and proliferation, and circadian clock gene expression under physiological and pathological conditions (Schneider, Linka et al. 2014).

Multiple HSP90 isoforms have been identified, including cytosolic HSP90AA1, HSP90AB1 and endoplasmic reticulum (ER)-localized

HSP90B1(Coskunpinar, Akkaya et al. 2014). The HSP90 isoforms share common biological functions in cell survival and proliferation. It has been reported that high expression levels of HSP90B1, also known as gp96 and Grp94, are correlated with cancer cell survival and epithelial ovarian cancer (Block, Maurer et al. 2015).

Many different groups have discovered that GRP94, as a cell protein, is strongly induced by glucose starvation. It is also a major calcium-binding protein in the ER and the most abundant ER-resident protein (Kim, Cho et al. 2021) and GRP94 mainly serves as an ER-resident molecular chaperone that physically interacts with and directs the folding and assembly of several secreted and membrane client proteins Eventually, it participates in regulating various functions, including cell growth, adhesion, and immunity as shown in Figure (1.4).

HSP90B1 protein is involved in the suppression of cell apoptosis and autophagy, as demonstrated by a recent study showing that HSP90B1 inhibitor promoted cell apoptosis and autophagy (Sun, Xiao et al. 2015). Suppression of HSP90B1 expression can greatly reduce cell survival and biological function. For example, reduced expression of HSP90B1 induced slower oocyte growth and greatly decreased the thickness of the zona pellucida (Li, Mo et al. 2016). In HSP90B1-deficient macrophages in mice, an elevated bacterial burden and increased inflammation are observed. These debilitating effects are caused by the disrupted trafficking and function of Toll-like receptors (TLRs) and integrins in the affected cells (106) (Anas, de Vos et al. 2016). Thus, the suppression of HSP expression and activity by HSP inhibitors has potential applicability in anti-tumor therapy and the prevention

of excess inflammation-induced tissue damage (Park, Shim et al. 2015) (Tukaj, Zillikens et al. 2015).

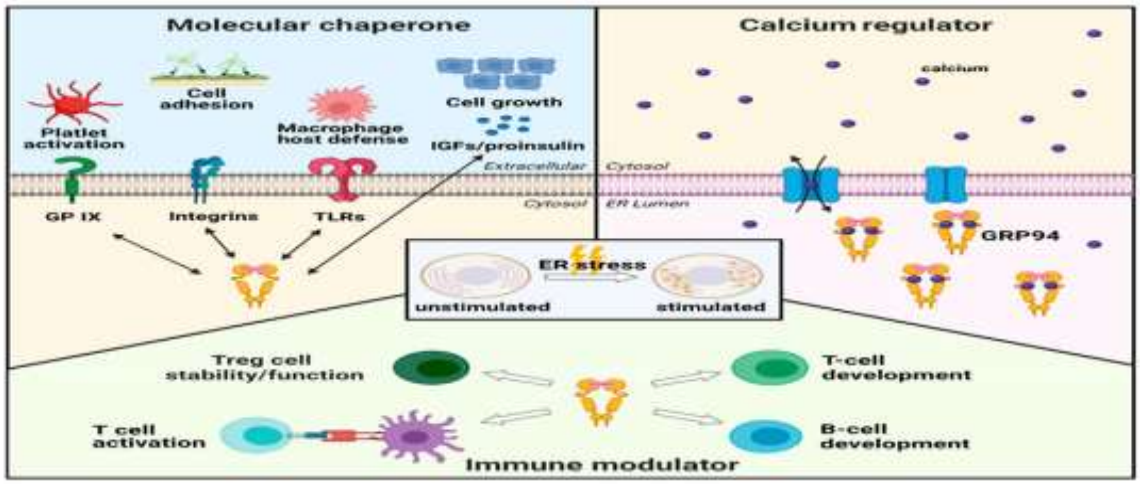


**Figure (1.4):** Schematic representation of the physiological roles of GRP94. GRP94 is a molecular chaperone upregulated by ER stress. Under stress conditions, GRP94 accelerates its function as a molecular chaperone. Client proteins of GRP94 include toll-like receptors (TLRs), glycoprotein (GP) IX subunit, insulin-like growth factors (IGFs), proinsulin, and integrins. These interactions are closely associated with direct function in macrophage host defense, activation of platelets in blood clotting, cell growth, and cell adhesion. Calcium regulation is another physiological function of GRP94, one of the major calcium-binding proteins in the ER regulating calcium homeostasis. Lastly, GRP94 is a multifunctional immune modulator. As demonstrated, GRP94 is essential in early T- and B-cell development while also regulating Treg cell stability and immunosuppressive function. GRP94 activates T-cells through peptide binding (Kim, Cho et al. 2021).

### 1.3.7.2. The signaling mechanism of HSP90B1 and Its role in inflammatory diseases

HSP90B1 is a stress-inducible chaperone protein. Its expression levels significantly affect cell proliferation and survival, cell cycle progression and apoptosis, as well as, other features of malignant cells, such as invasion, tumor angiogenesis and metastasis. In addition, to its known functions, our current study suggested the role for HSP90B1 in the promotion of cell proliferation in the pathogenesis of PCOS. Evidence has indicated that the dysfunction of

granulosa cells affects folliculogenesis in patients with PCOS. Because HSP90B1 is a dominant isoform in the HSP90 family and is critically involved in disease development (Sun, Xiao et al. 2015).

The molecular chaperone HSP90 is expressed at a high-level during stress response, which promotes the stability of functional proteins and has a certain protective effect on cells (Deng, Sun et al. 2017). Production of HSPs is triggered by different types of cellular stress, such as inflammation, pathogens, thermal stress and oxidants. HSP90 consists mainly of two isoforms: the inducible HSP90α and constitutively expressed HSP90β (Prodromou 2016).

The Hsps have been involved in various functions including chaperone activity, protein folding, apoptosis, autophagy and immunity. Generally, Hsps were studied for their role in protecting cells from high temperature and stress conditions. Moreover, several roles for Hsps in the immune system were determined including intracellular roles in antigen presentation and expression of innate receptors, and extracellular roles in tumor immunosurveillance and autoimmunity (Milani, Basirnejad et al. 2019). Some of these constitutively-expressed heat shock polypeptides are involved in protein folding and translocation of organelles across cellular membranes, prompting many authors to label them “molecular chaperones” (Jee 2016) (Shiber and Ravid 2014).

In general, overexpression of Hsp27, Hsp70, Hsp60 or Hsp90 suppressed apoptosis and prevented caspase activation in different cellular models following a variety of cellular stressors (Figure (1.5C)) (Milani, Basirnejad et al. 2019). Hsp90 could enhance the survival pathway regulated by Akt and

BAD phosphorylation, inactivate the proapoptotic proteins Bax and Bak in the mitochondrial membrane and reduce the intrinsic apoptotic pathway by activation of procaspase-3 (Butler, Ferraldeschi et al. 2015).

HSP90B1 inhibitor is able to degrade HSP90B1 client proteins and increase cytochrome c release, activation of caspase-3, and expression of rapamycin complex 1 (mTOR) and LC-3 (Sun, Xiao et al. 2015) (Gong, The Gong et al. 2015) (Zhang, Shen et al. 2015). These responses enable cellular protection against protein denaturation and possible degradation of misfolded proteins, which may, in turn, result in protein aggregation and cancer (Ikwegbue, Masamba et al. 2017).

HSPs increased resistance to cell death induced by various conditions. For instance, HspA1, HspB1, HspB5 (αβ-crystallin), Hsp90 and Hsp105 were also implicated to inhibit and regulate apoptosis. Indeed, the Hsps blocked both the intrinsic and the extrinsic apoptotic pathways through the interaction with main proteins at upstream of mitochondria, at the mitochondrial level and at the postmitochondrial level (Kennedy, Jäger et al. 2014).

Although the involvement of HSPB1 activity in cancer growth has been well described in recent years, the role of HSP90B1 in the pathogenesis of PCOS has not been well investigated. Recent studies in animal models indicated that adrenocorticotropic hormone or cold stress-induced PCOS were associated with increases in HSP90B1, glucocorticoid receptor and androgen receptor expression in ovarian tissues, indicating the possible function of the up regulated genes in the pathogenesis of PCOS (Sun, Xiao et al. 2015). In addition, HSP90B1 protein is involved in the suppression of cell apoptosis

and autophagy, as demonstrated by a recent study showing that HSP90B1 inhibitor promoted cell apoptosis and autophagy (Sun, Xiao et al. 2015).

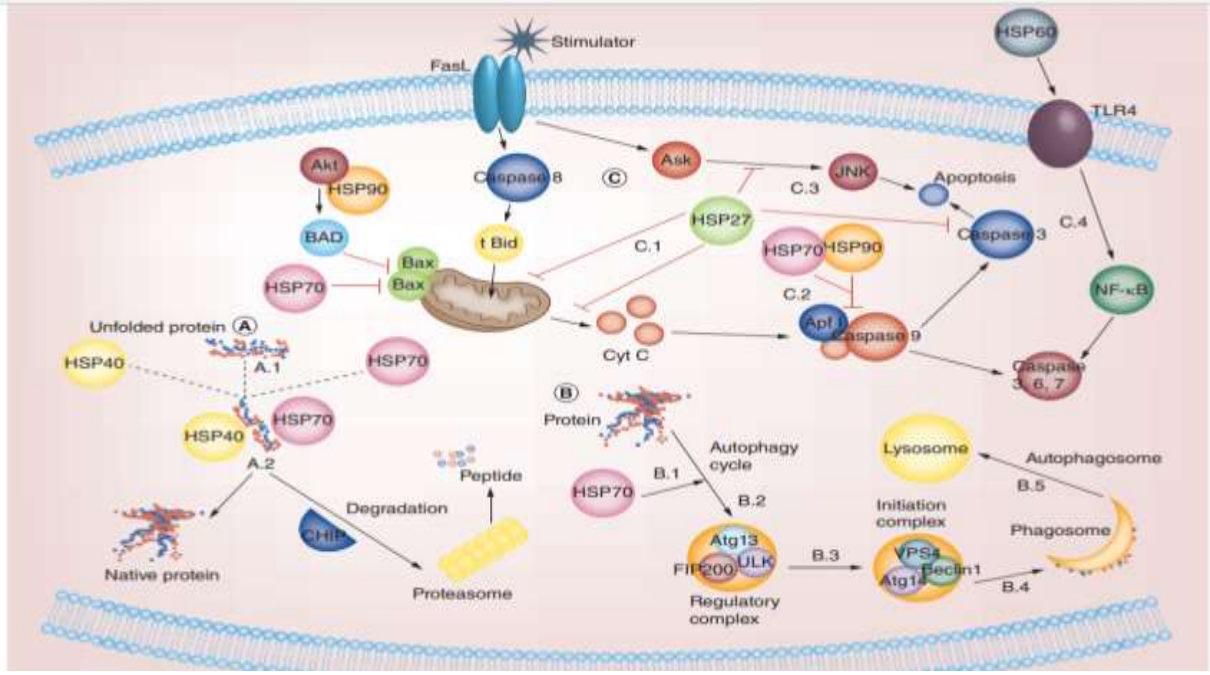


**Figure (1.5):** Biochemical functions of heat-shock proteins. (A) Chaperone activity: accumulated misfolded proteins bind to HSPs for the maintenance of protein hemostasis (A.1). For instance, Hsp70 interacts with Hsp40 for converting misfolded proteins to native form and/or these chaperones guide misfolded proteins to proteasome for degradation into small peptides (A.2). (B) Autophagy pathway: At first, Hsp70 binds to the protein and autophagy cycle begins (B.1). The regulatory complex contains ULK1, Atg13 and FIP200 activated by Hsps (B.2). Then, the initiation complex composed of Beclin1, VPS4 and Atg14 are stabilized by Hsps (B.3). After that, phagosome is formed by collecting phagosomal markers (B.4). Finally, the completed autophagosome fuses with a lysosome (B.5). (C) Apoptosis pathway: The proapoptotic signals through FasL can be modulated by Hsps at the mitochondrial and postmitochondrial levels. Hsp27 and Hsp70 can inhibit the release of proapoptotic proteins from mitochondria through suppression of active truncated Bid (tBid) and Bax, respectively (C.1). Sequestering cytosolic cytochrome C by Hsp27, Hsp70 and Hsp90 from apoptosis protease-activating factor (Apaf-1) resulting in the inhibition of apoptosome formation and thus prevention of apoptosis activation (C.2). Hsp27 can also suppress the apoptosis through the inactivation of apoptosis-signal-regulating kinase (Ask) and caspase 3 (C.3). Also, interaction of Hsp60 and TLR4 can mediate NF-κB signaling pathway resulting in the activation of caspase 3, 6, 7 and DNase (C.4). HSP: Heat-shock protein (Milani, Basirnejad et al. 2019).

## 1.3.8. B-cell Lymphoma 2 (BCL-2) and their Role in PCOS

### 1.3.8.1. B-cell Lymphoma 2 (BCL-2)

The Bcl-2 protein family are the central gatekeepers of the intrinsic or mitochondrial apoptotic response. The family is comprised of structurally-related proteins with opposing functions that either promote or inhibit apoptosis by interacting with one another (Kale, Osterlund et al. 2018). The Bcl-2 family is typically classified into three groups, including pro-apoptotic initiators, pro-apoptotic effectors, and anti-apoptotic proteins (Figure (1.6A)).

The apoptotic- promoting effects from the pro-apoptotic initiators and effectors are countered by their direct interaction with the anti-apoptotic family members. It is this delicate and dynamic balance between the pro- and anti-apoptotic Bcl-2 family members that governs whether a B cell undergoes apoptosis or survives (Adams, Clark-Garvey et al. 2019). All anti-apoptotic family members and a subset of pro-apoptotic members are multi-domain proteins, sharing sequence homology within three to four BH domains. A subset of pro-apoptotic Bcl-2 family members known as BH-3 only proteins only contain the BH-3 domain, which is known as the minimal death domain that is required for binding the multi-domain Bcl-2 family members (Adams, Clark-Garvey et al. 2019).

Regulation of apoptotic signaling in the ovary is achieved by the Fas system and Bcl2 family, including Bcl2 (anti-apoptotic) and Bax (pro-apoptotic) proteins. The increased expression of the Bax death susceptibility gene coincides with the induction of apoptosis in granulosa cells during atresia in vivo and in vitro (Figueroa, Motta et al. 2015).

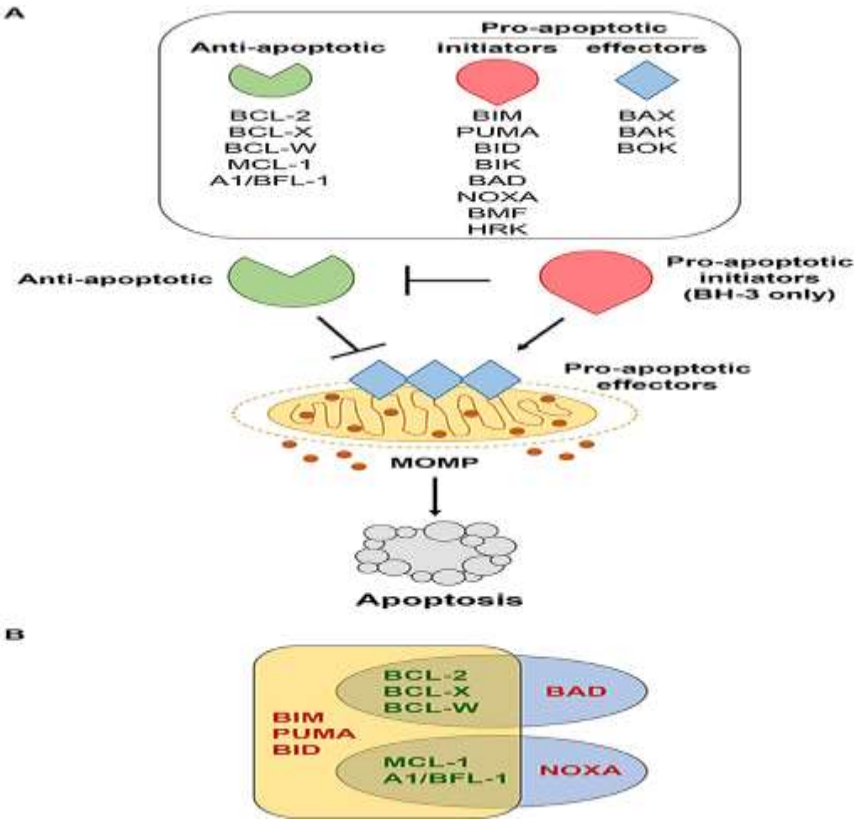


**Figure (1.6):** Bcl-2 family members regulate apoptosis. (A) Various cellular stressors induce apoptosis through the intrinsic, mitochondrial pathway, which is regulated by the Bcl-2 family of proteins. These stress signals activate pro-apoptotic BH-3 only initiators (red), which inhibit the anti-apoptotic proteins (green). This, in turn, allows the pro-apoptotic effectors (blue) to be activated. Activation of the effector proteins results in their oligomerization and subsequent mitochondrial outer membrane permeabilization (MOMP), enabling the release of apoptotic factors that initiate the caspase cascade and final stages of cellular destruction. (B) Pro-apoptotic BH-3 only proteins bind to anti-apoptotic Bcl-2 family members with different affinities. BIM, PUMA, and BID bind strongly to all anti-apoptotic Bcl-2 proteins, whereas BAD binds preferentially to BCL-2, BCL-X, and BCL-W, and NOXA binds preferentially to MCL-1 and A1/BFL-1 (Adams, Clark-Garvey et al. 2019).

Mitochondrial membrane permeabilization (Figure (1.6A)) (Adams, Clark-Garvey et al. 2019). The BH-3 only group of pro-apoptotic Bcl-2 proteins consists of Bim (BCL2L11), Puma/BBC3, Bad (Bcl-2/Bcl-x-associated death promoter), Bid (BH-3 interacting-domain death agonist), Bik (Bcl-2-interacting killer), Noxa/PMAIP1, Bmf (Bcl-2-modifying factor), and

Hrk (Harakiri) [Figure (1.6A)], and are essential for initiating the apoptotic cascade. While the BH-3 only proteins can initiate apoptotic signaling by binding directly to the anti-apoptotic Bcl-2 proteins, thereby freeing up Bax and Bak to undergo homo-dimerization, some have been reported to bind directly to and activate Bax and Bak (Adams, Clark-Garvey et al. 2019). evidence continues to reveal that elevated expression of anti-apoptotic Bcl-2 family members (Bcl-2, Bcl-x, Bcl-w, Mcl-1, A1/Bfl-1) is one of the major contributing factors to B cell lymphomagenesis (Adams, Cory et al. 2018).

The loss of mitochondrial membrane potential (MMP) is regulated by members of the BCL2 protein family. This consists of 18 proteins that share BCL2 homology (BH) domains, and can be categorised into anti-apoptotic, pro-apoptotic multi-domain and pro-apoptotic BH3-only proteins (Figure (1.7)) (Vogler, Walter et al. 2017).

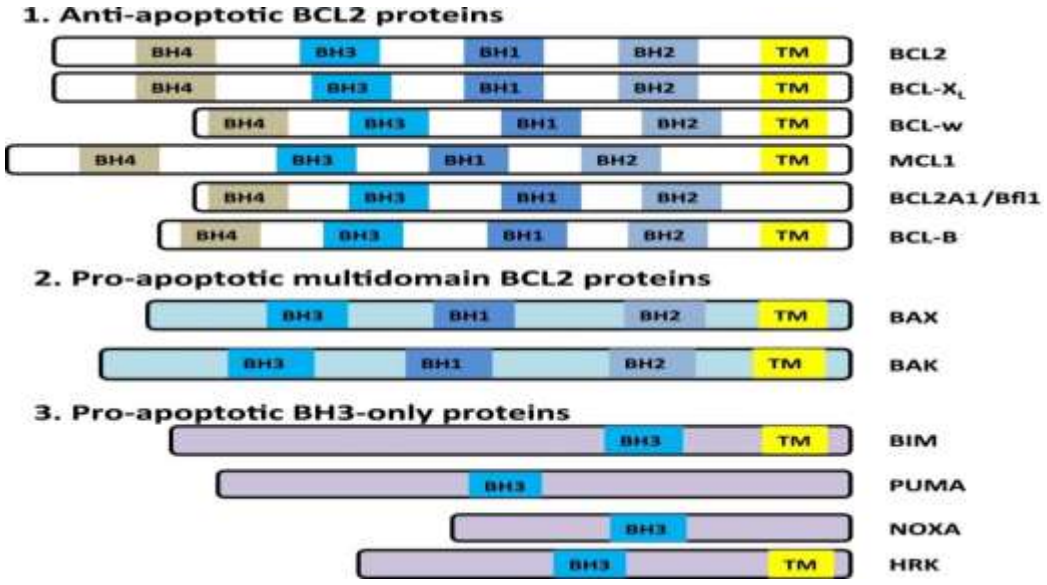


**Figure (1.7):** The BCL2 proteins. Schematic representation of selected BCL2 proteins with their BH-domains and if applicable, transmembrane domain (TM) (Vogler, Walter et al. 2017).

### 1.3.8.2. Role B-cell Lymphoma 2 (BCL-2) in Inflammation

Apoptosis is a natural event responsible for ovarian functions. There are many hormonal and intracellular factors coordinating life and cell death during the development and maintenance of ovarian activity (Chuffa, Lupi Júnior et al. 2016). In experimental PCOs, the involvement of Bcl-2, an anti-apoptotic member, seems to be important during the transformation of growing follicles into cystic follicles (Bas, Abramovich et al. 2011). One of the main regulatory proteins is members of the Bcl-2 family of proteins. They can be divided into those having either an antiapoptotic (e.g. Bcl-2, Bcl-W, Bcl-xL) or proapoptotic (Bax, Bad, Bim, Bcl-xS, Bod, Bok/Mtd) function. Bcl-2 protein locates in endoplasm omentum and mitochondrial membranes and it can prevent cell apoptosis by inhibiting the release of apoptosis inducing factors (Chi, Zhang et al. 2018).

B-cell lymphoma-2 (BCL-2) is involved in regulating the apoptotic process. In particular, BCL-2 prevents apoptosis (intrinsic pathway) by inhibiting the release/activation of caspase-9, thus decreasing mitochondrial permeability and inhibiting apoptosis. BCL-2-associated X (BAX) is a pro-apoptotic protein that can increase outer mitochondrial membrane permeability and stimulate the release of apoptotic molecules, including cytochrome c and DIABLO, thus promoting the apoptotic process. Considering the potential role of apoptosis in PCOS.

## 1.3.9. Bcl-2 associated X protein (Bax) and their Role in PCOS

### 1.3.9.1. Bcl-2 associated X protein (Bax)

Bax was the first Bcl-2 homologue gene to be identified acting as an apoptosis executer. Bax protein is expressed in various tissues, as multiple alternative splice variants, normally localized in the cytosol or loosely attached to the mitochondria. The best characterized isoform is the 21 kDa Baxα which contains three BH domains and membrane anchor domains, allowing for insertion in the mitochondria upon apoptosis stimulation (D'Orsi, Mateyka et al. 2017).

The pro-apoptotic Bax-like subfamily, including Bax (Bcl-2 associated-x) and Bak (BH3 homologous agonist killer), and potentially Bok (Bcl-2 related ovarian killer). These three proteins contain three conserved BH domains (BH1, BH2 and BH3). Activated and oligomerized Bax and Bak form pores within the outer mitochondrial membrane that allow for the release of pro-apoptotic factors from the intermembrane space into the cytosol, a process called mitochondrial outer membrane permeabilization (MOMP) (D'Orsi, Mateyka et al. 2017).

Whereas overexpression of Bax and Bak alone was able to trigger mitochondrial fission, Therefore, an increase in Bax and Bak at the MOM, or changes in the ratio of pro-apoptotic Bax and Bak versus anti-apoptotic Bcl-2 family members may influence not only cell death signaling, but also mitochondrial morphology, with increased Bax and Bak signaling causing mitochondrial fragmentation or inhibition of mitochondrial fusion.

### 1.3.9.2. Role Bcl-2 associated X protein (Bax) in inflammation

Bcl-2-associated X protein (Bax) was the first identified pro-apoptotic member of the Bcl-2 protein family. It is activated by various stimuli in a p53-dependent manner and plays key roles in cell survival by mediating the programmed cell death process. Importantly, cell death or cell survival in response to an apoptotic stimulus may ultimately be controlled by the relative ratio of Bax/Bcl-2. Previous studies showed that the process of apoptotic cell death was accelerated by Bax overexpression and that the apoptotic repressor activity of Bcl-2 was countered by Bcl-2 homo dimerization in vivo (Guo, Lv et al. 2020).

Demonstrated by immunohistochemistry that the expression of Bcl-2 and BAX are mainly localized in granulosa cells of antral follicles. Also, they proposed that BAX expression was increased in these follicles of PCOs. It is important to consider that a balance between pro- and anti-apoptotic components exists, and an overexpression of pro-apoptotic molecules (e.g. BAX) may lead the cells to apoptosis (Chuffa, Lupi Júnior et al. 2016).

## 1.3.10. Symptoms of PCOS

### 1.3.10.1. Irregular menstrual cycles

The majority of women with PCOS have an abnormal menstrual cycle and the most frequent pattern is infrequent menstruation associated with anovulation. According to Rotterdam criteria, PCOS is characterized by the presence of at least two of the classic three features which are menstrual irregularity, hyperandrogenism, and enlarged “polycystic” ovaries in pelvic ultrasonography (Yurtdaş and Akdevelioğlu 2020). when ovulation does not

occur, the uterine lining (called the endometrium) does not shed and regrow uniformly as it does during a normal menstrual cycle. Instead, the endometrium becomes thicker and may shed irregularly, which can result in heavy and/or prolonged bleeding. Irregular or absent menstrual periods can increase a woman's risk of endometrial overgrowth (called endometrial hyperplasia) or even endometrial cancer.

Longer menstrual cycle length and irregular cycles have been associated with higher androgen and lower sex hormone binding globulin levels (SHBG), and this altered hormonal environment may increase the risk of specific histologic subtypes of ovarian cancer (Harris, Titus et al. 2017). As for menstrual irregularities, females may present with oligomenorrhea, secondary amenorrhea, or dysfunctional uterine bleeding (Foster and Al-Zubeidi 2018). Oligomenorrhea is defined as fewer than eight cycles per year after 2 years of menarche. Secondary amenorrhea is defined as absence of a menstrual cycles for 90 days after a woman has had regular periods for at least 6 months. Abnormal uterine bleeding (AUB) is defined as excessive menstrual flow, cycles occurring more frequently than every 21 days or less frequently than every 45 days, and intermenstrual or breakthrough bleeding.

### 1.3.10.2. Weight gain

Obesity and metabolic abnormalities linked to insulin resistance are common among women with PCOS (Kent, Dodson et al. 2018). About 80% of the PCOS women gain weight (Kataoka, Tassone et al. 2017). Although it is well known that obesity exacerbates PCOS symptoms and increases the risks for type 2 diabetes mellitus and cardiovascular diseases, the results of previous studies have been inconsistent regarding the association between weight,

weight gain, and prevalence of PCOS during reproductive life (Ollila, Piltonen et al. 2016).

### 1.3.10.3. Hirsutism

Is defined as the occurrence of terminal hair in a masculine on the face and/or body. It is one of the main characteristics of hyperandrogenism in PCOS. The incidence of hirsutism in PCOS women ranges between 60 and 80% (Mara Spritzer, Rocha Barone et al. 2016) (Keen, Shah et al. 2017). Hirsutism is the most consistent and reliable symptom used for evaluating clinical hyperandrogenism. hair is scored in nine parts of the body, which include the upper lip, chin, chest, upper and lower back, upper and lower abdomen, and upper and lower limbs (Ashraf, Nabi et al. 2019). The amount and distribution of hair growth is determined by the androgens, particularly testosterone. Hirsutism in PCOS women is attributed to increased circulatory levels of free testosterone and more active form of testosterone, i.e., dihydrotestosterone, formed by the activity of 5α reductase on testosterone in the pilosebaceous gland (Ismael, Alattabi et al. 2021).

### 1.3.10.4. Acne in adults

Is regarded as a sign of hyperandrogenism and is included as an equivalent of hyperandrogenism in the diagnostic criteria of most PCOS guidelines (Teede, Misso et al. 2018). It is not surprising that PCOS is associated with acne due to the role of increased levels of androgens in determining acne (Carmina and Research 2020).

### 1.3.10.5. Skin modification

Insulin resistance and high insulin levels can cause thick, velvety skin patches that are darker in color than normal skin tone. Acanthosis nigricans is a skin condition that causes creases in the neck, groin, and breasts . Insulin resistance can also be indicated by skin tags (very small skin growths) in your armpits or neck (Napolitano, Megna et al. 2015).

## 1.3.11. Diagnosis of PCOS

### 1.3.11.1. History

The approach to the evaluation of a girl with signs and symptoms suggestive of PCOS begins with a thorough history, including detailed family history (Witchel, Oberfield et al. 2019).

### 1.3.11.2. Clinical examination

Two out of three criteria (hyperandrogenism, ovulatory dysfunction, and PCOM) are needed to diagnose PCOS and each case has to be classified into a specific phenotype which are listed in Tables (1.1) and (1.2) (Gainder and Sharma 2019).

**Table (1.1): Adult Diagnostic Criteria (Rotterdam)**

| Phenotype 1 (Classic PCOS) | Phenotype 2 (Essential NIH Criteria) | Phenotype 3 (Ovulatory PCOS) | Phenotype 4 (Nonhyperandrogenic PCOS) |
|---|---|---|---|
| a. Clinical and/or biochemical | a. Clinical and/or biochemical | a. Clinical and/or biochemical | a. Evidence of oligo-anovulation |

| | | | |
|---|---|---|---|
| evidence of hyperandrogenism | evidence of hyperandrogenism | evidence of hyperandrogenism | |
| b. Evidence of oligo- anovulation | b. Evidence of oligo-anovulation | b. Ultrasonographic evidence of a polycystic ovary | b. Ultrasonographic evidence of a polycystic ovary |
| c. Ultrasonographic evidence of a polycystic ovary | - | - | - |

### 1.3.11.3. Blood Tests

**a.** Anti-Mullerian hormone (AMH) are often elevated in women with PCOS and 17-hydroxyprogesterone (Rudnicka, Kunicki et al. 2021).

**b.** Free and total testosterone higher in women with PCOS (Cooney, Dokras et al. 2018).

**c.** Dehydroepiandrosterone Hormone (DHEAS ) may be higher-than-normal level and sex hormone binding globulin (SHBG) may be lower than normal (Witchel, Oberfield et al. 2019).

**d.** Serum Follicle-stimulating hormone ( FSH) will be normal or low with PCOS and serum Luteinizing hormone will be elevated (Trent and Gordon 2020).

**e.** liver function (aspartate aminotransferase, alanine aminotransferase) testing is warranted in obese and have profound chemical metabolic abnormalities

may present with nonalcoholic fatty liver disease (Ibáñez, Oberfield et al. 2017).

### 1.3.11.4. The Imaging of Ovaries

The ultrasound report should include ovarian volumes, follicle counts and any other relevant information, like the presence of a dominant follicle or corpus luteum cyst. it is done with the right tools and it is important in the assessment of patients with suspected PCOS. This is quite common to find polycystic ovaries during routine ultrasounds, but it is important to be aware of the requirements for making a PCOS diagnosis, especially if patients are being assessed for other syndromes that may signal the presence of this condition (Kadri, Izhar et al. 2021).

### 1.3.11.5. Adrenal and Pelvic Imaging

May be considered that depending on the clinical information, physical examination, and initial laboratory data (Witchel, Oberfield et al. 2019).

## 1.3.12. Treatment of PCOS

### 1.3.12.1. Lifestyle

According to the Androgen Excess Society and the European Society of Endocrinology, PCOS therapy should be based on lifestyle changes, including increased physical activity and dietary modifications concerning the intake of SFA, low Glycaemic Index (GI) foods and weight reduction if necessary (Bykowska-Derda, Czlapka-Matyasik et al. 2021). as well as the recent international evidence base guideline on PCOS (Teede, Misso et al. 2018). improving insulin sensitivity and reducing cardiovascular risk. Lifestyle

modifications, including a calorie restricted diet and/or physical activity, has proven effective in altering the disease course of PCOS (Cooney, Dokras et al. 2018).

Evidence-based guidelines recommend that all women with PCOS should be routinely screened for anxiety and depression symptoms at diagnosis and referred for psychological therapy if required (Ee, Pirotta et al. 2021).

### 1.3.12.2. Medications

**a.** COCP (oestrogen and progestin preparations), Vitamin D and probiotic cosupplementation (Rothenberg, Beverley et al. 2018).

**b.** Combined COCP, metformin and Antiandrogens (Peña, Witchel et al. 2020).

**c.** Oral Contraceptive Pills, Spironolactone, Cyproterone Acetate, Flutamide, Cosmetic/Local Therapy, Infertility Treatment (Shermin, Noor et al. 2019).

**d.** cyproterone acetate, flutamide and finasteride (Trent and Gordon 2020).

**e.** Omega-3 fatty acid supplementation (Xia, Wang et al. 2021).

### 1.3.12.3. Tropical therapy

Can be augmented by topical and mechanical treatments for hirsutism (eg, electrolysis, laser therapy, plucking, waxing, shaving, and bleaching to achieve the desired cosmetic result) and topical therapies for acne (Bednarska, Siejka et al. 2017).

### 1.3.12.4. Surgical therapy

Ovarian drilling by laparoscope (Ismael, Alattabi et al. 2021).

## 1.3.13. Complication of PCOS

**a.** Infertility (Bachelot 2016).

**b.** Metabolic Syndrome or Syndrome X (Chandrasekaran, Sagili et al. 2018).

**c.** Mood disorders (Borghi, Leone et al. 2018).

**d.** Pregnancy complications (Fougner, Vanky et al. 2021).

**e.** Obstructive sleep apnoea (OSA) (Tahrani and Research 2017).

**f.** Endometrial Cancer (Ding, Chen et al. 2018).

**g.** Prediabetes & type 2 diabetes (Teede, Misso et al. 2018).

# Chapter Two
# Materials
# and
# Methods

# 2. Study design, Materials and Methods

## 2.1. Study design

The type of the study is case-control study. A total of 130 women who consists of 66 patients and 64 controls who were attending in Karbala gynecological and obstetric teaching hospital, Iraq. were carried out for the period September 2021 to June 2022.

### 2.1.1. Subjects

The study included 130 subjects who consists of two groups: The first group is the patients group, which consists of 66 patients with PCOS who their age ranged from (15-39) years, they were chosen randomly and were all diagnosed with PCOS by a gynecologist, who attended a private gynecological clinic in karbala city and Karbala gynecological and obstetric teaching hospital, they were diagnosed by specialized gynecologists according to The Rotterdam Consensus (2003) (Eshre and Reproduction 2004), which requires the presence of at least two of the following characteristics: polycystic ovaries on ultrasound scan , menstrual irregularities and hyperandrogenism.

The second group is the control group, who consists of 64 females with age ranged from (14-45) years, who were apparently healthy without clinical features with PCOS according to the diagnoses by specialized gynecologists according to The Rotterdam Consensus (2003) (Eshre and Reproduction 2004). normal testosterone, a regular cycle, and normal ovulation are eligible.

## 2.1.2. Inclusion Criteria and Exclusion criteria

**Inclusion Criteria:** patients with polycystic ovarian syndrome diagnostic for the following criteria of PCOS according to the Rotterdam Consensus (2003) (Eshre and Reproduction 2004):

1. Oligo- and/or anovulation.

2. Clinical and/or biochemical signs of hyperandrogenism.

3. Polycystic ovaries (presence of 12 or more follicles in each ovary measuring 2 ± 9 mm in diameter and/or increased ovarian volume).

**Exclusion criteria:**

Every female with autoimmune, inflammatory (such as inflammatory bowel disease) and infectious diseases were excluded.

## 2.1.3. The subjects data

The information about each case was recorded on a questionnaire form that included the subjects data to be collected from patients with ethical considerations, The form included the patient's name, age, marital status, fertility, menstrual cycle, hirsutism, finding of ultra sound, residence, family history, medical history and phone number.

## 2.1.4. Ethical Issue

The approval of the consent protocol was done by the medical ethics committee of the Faculty of Medicine/Karbala University. The Research Ethics Committee of karbala Health Department approved the project proposal and sampling method according to the department's administrative.

This project also received the permission from the director of Karbala gynecological and obstetric teaching hospital. So to get blood sample, verbal consent will be taken from patients and controls prior to sample collection, women will be informed that we would use their blood for research purposes.

### 2.1.5. Sample Collection

Blood samples were collected by venipuncture from 130 subjects (66 patients and 64 controls), five millimeters of venous were drawing by disposable syringe under sterilization technique and putting in gel tube then, allowed to clot; after that serum was separated by centrifugation 1500 rpm for 5 minute. The serum has been collected in Eppendorf tubes then stored at -20c to be used for ELISA test to determine the concentration of HSP90B1, Bcl-2 and Bax.

## 2.2. Materials

### 2.2.1. Instruments and Equipments

The main instruments and equipment's used in this study were listed in table (2.1)

**Table (2.1) : Instruments and Equipment's**

| Instruments and Equipment's | Company |
|---|---|
| Disposable syringes 5ml | Medi/ China |
| Gel tube | Plastilab/Lebanon |
| Centrifuge | Hettich/ Germany |
| Eppendorf tube | Germany |

| | |
|---|---|
| Gloves | TGR/ Malaysia |
| Micropipette (10-100 micron) Micropipette (100-1000 micron) | Human/ Germany |
| Multi-channel (50-250 micron) pipette | Slamed/ Germany |
| yellow micropipette tips | Plastilab/ Lebanon |
| Refrigerator | Concord/ Lebanon |
| Plastic rack | China |
| Incubator | Nuve/ Turkey |
| ELISA automated washer | Biotek/ USA |
| ELISA reader and printer | Biotek/ USA |

## 2.2.2. Materials (Kits) used in the study

Three different kits had been used in this study (Note: for each marker we used kit 96 wells and 48 wells kit) as shown in (table 2.2).

**Table (2.2) List of chemicals and Kits used**

| **Kits** | **Company** |
|---|---|
| Human Heat Shock Protein 90kDa Beta 1, (HSP90b1) | BT LAB/China Lot: 202201006 |
| Human B-cell leukemia/lymphoma 2, (Bcl-2) | BT LAB/ China Lot: 202201006 |
| Human Bcl-2 associated X protein, (Bax) | BT LAB/China Lot: 202201006 |

**Table (2.3): kit component of HSP90b1, Bcl-2 and Bax**

| components | Quantity (96T/48T) | Storage |
|---|---|---|
| Standard solution (32ng/ml) /(800U/ml) /(96ng/ml) | 0.5 ml | 2-8ºC |
| Pre-coated ELISA plate | 12x8 / 12x4 well strips | 2-8ºC |
| Standard diluent | 3ml | 2-8ºC |
| Streptavidin-HRP | 6 / 3 ml | 2-8ºC |
| Stop solution | 6 / 3 ml | 2-8ºC |
| Substrate solution A | 6 / 3 ml | 2-8ºC |
| Substrate solution B | 6 / 3 ml | 2-8ºC (sensitive to light) |
| Wash buffer concentrate (25x) | 20 ml | 2-8ºC |
| Biotinylated Human HSP90b1/ Bcl-2/ Bax antibody | 1 ml | 2-8ºC |
| User instruction | 1 copy | |
| Plate sealer | 2 pieces | |
| Zipper bag | 1 piece | |

## 2.3. Methods

## 2.3.1. The Assay Principle of Human Heat Shock Protein 90kDa Beta 1 (HSP90b1) kit, Human B-cell leukemia/lymphoma 2 (Bcl-2) kit and Human Bcl-2 associated X protein (Bax) kit

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with (Human HSP90b1 antibody for HSP90b1, human Bcl-2 antibody for Bcl-2 and human Bax antibody for Bax). (HSP90b1, Bcl-2 and Bax) present in the sample is added and binds to antibodies coated on the wells. And then biotinylated (Human HSP90b1 Antibody, human Bcl-2 antibody and human Bax antibody) is added and binds to (HSP90b1, Bcl-2 and Bax) in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated (HSP90b1 antibody, Bcl-2 antibody and Bax antibody). After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of (Human HSP90b1, human Bcl-2 and human Bax). The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

## 2.3.2. Reagent Preparation

## 2.3.2.1. Reagent Preparation of HSP90b1

1. All reagents should be brought to room temperature before use.
2. **Standard** Reconstitute the 120µl of the standard (32ng/ml) with 120µl of standard diluent to generate a 16ng/ml standard stock solution. Allowed the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepared duplicate standard points by serially diluting the standard stock solution (16ng/ml) 1:2 with standard diluent to produce

8ng/ml, 4ng/ml, 2ng/ml and1ng/ml solutions. Standard diluent served as the zero standard(0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows (table 2.4):

**Table (2.4): Dilution of standard solutions of HSP90b1**

| | | |
|---|---|---|
| 16 ng/ml | Standard No.5 | 120µl Original Standard + 120µl Standard Diluent |
| 8 ng/ml | Standard No.4 | 120µl Standard No.5 + 120µl Standard Diluent |
| 4 ng/ml | Standard No.3 | 120µl Standard No.4 + 120µl Standard Diluent |
| 2 ng/ml | Standard No.2 | 120µl Standard No.3 + 120µl Standard Diluent |
| 1 ng/ml | Standard No.1 | 120µl Standard No.2 + 120µl Standard Diluent |

## 2.3.2.2. Reagent Preparation of Bcl-2

1. All reagents should be brought to room temperature before use.

2. Standard Reconstitute the 120µl of the standard (800U/ml) with 120µl of standard diluent to generate a 400U/ml standard stock solution. Allowed the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepared duplicate standard points by serially diluting the standard stock solution (400U/ml) 1:2 with standard diluent to produce 200U/ml, 100U/ml, 50U/ml and 25U/ml solutions. Standard diluent served as the zero standard (0 U/ml). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows (table 2.5):

**Table (2.5): Dilution of standard solutions of Bcl-2**

| | | |
|---|---|---|
| 400 U/ml | Standard No.5 | 120µl Original Standard + 120µl Standard Diluent |
| 200 U/ml | Standard No.4 | 120µl Standard No.5 + 120µl Standard Diluent |
| 100 U/ml | Standard No.3 | 120µl Standard No.4 + 120µl Standard Diluent |
| 50 U/ml | Standard No.2 | 120µl Standard No.3 + 120µl Standard Diluent |
| 25 U/ml | Standard No.1 | 120µl Standard No.2 + 120µl Standard Diluent |

## 2.3.2.3. Reagent Preparation of Bax

1. All reagents should be brought to room temperature before use.

2. Standard Reconstitute the 120µl of the standard (96ng/ml) with 120µl of standard diluent to generate a 48ng/ml standard stock solution. Allowed the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepared duplicate standard points by serially diluting the standard stock solution (48ng/ml) 1:2 with standard diluent to produce 24ng/ml, 12ng/ml, 6ng/ml and 3ng/ml solutions. Standard diluent served as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows (table 2.6):

**Table (2.6): Dilution of standard solutions of Bax**

| | | |
|---|---|---|
| 48 ng/ml | Standard No.5 | 120µl Original Standard + 120µl Standard Diluent |
| 24 ng/ml | Standard No.4 | 120µl Standard No.5 + 120µl Standard Diluent |
| 12 ng/ml | Standard No.3 | 120µl Standard No.4 + 120µl Standard Diluent |
| 6 ng/ml | Standard No.2 | 120µl Standard No.3 + 120µl Standard Diluent |
| 3 ng/ml | Standard No.1 | 120µl Standard No.2 + 120µl Standard Diluent |

## 2.3.3. Wash Buffer for (HSP90b1, Bcl-2 and Bax)

Diluted 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mixed gently until the crystals have completely dissolved.

## 2.3.4. Assay procedure of (HSP90b1, Bcl-2 and Bax)

**1.** All reagents were prepared, standard solutions and samples as instructed. all reagents were brought to room temperature before use. The assay was performed at room temperature.

**2.** The number of strips required for the assay must be Determined. The strips were Inserted in the frames for use. The unused strips would be stored at 2-8°C.

**3.** 50µl standard was Added to standard well. Note: biotinylated antibody should not be added to standard well because the standard solution contains biotinylated antibody.

**4.** 40µl sample was Added to sample wells and then added 10µl anti-HSP90b1 antibody to sample wells, Then added 50µl streptavidin-HRP to sample wells and standard wells ( Not blank control well ). Mixed well. The plate was covered with a sealer and Incubated 60 minutes at 37°C.

**5.** The sealer was removed and washed the plate 5 times with wash buffer. Soaked wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirated or decant each well and wash 5 times with wash buffer. The plate was blotted onto paper towels or other absorbent material.

**6.** 50µl substrate solution A was Added to each well and then 50µl substrate solution B was added to each well. Incubated plate covered with a new sealer for 10 minutes at 37°C in the dark.

**7.** 50µl Stop Solution was Added to each well, The blue color would be changed into yellow immediately.

**8.** Determined the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

### 2.3.5. Calculation of Result for (HSP90b1, Bcl-2 and Bax)

Constructed a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and drew a best fit curve through the points on the graph. These calculations could be best performed with computer-based curve-fitting software and the best fit line could be determined by regression analysis.

## 2.4. Waste Disposal

Remnants of procedure which is involve syringes, gloves, tubes, biological materials, chemicals and micro plates consider as a biohazards material dispose according to protocol followed by Al-Hussein medical city lab-disposal approach.

## 2.5. Statistical Analysis

Statistical analyses were performed using SPSS statistical package for Social Sciences (version 20.0 for windows, SPSS, Chicago, IL, USA).

Quantitative data are represented as mean, standard deviation and range. To test differences between the patient and control groups Student's t-test was used.

Normal distributed data are presented as mean ± SD. Median and IQR (Inter Quartile Range) were used to describe HSP-90B1, Bcl-2 and Bax as their statistical distribution was non-normal (Kolmogorov-Smirnov test). Mann Whitney test was used to study the difference between the two groups.

Qualitative data are represented as count and percentage. Chi-square test was used to test the relation of qualitative data.

P value of $<0.05$ was considered statistically significant.

Sensitivity and specificity test were measured using ROC test (Receiver Operating Characteristic). P value of $<0.05$ was considered statistically significant.

# Chapter Three
# Results

# 3. Results

## 3.1. Demographic Characteristics of the Studied Subjects

One hundred and thirty cases were included in this study, 66 patients with PCOS (50.8%) and 64 control (49.2%) were selected. Demographic characteristics of the subjects are presented in table (3.1).

Regarding age, The study found out that the number of patients who their age ranged from (15-39) with (mean±SD) (32.4±8.5), while the ages of controls ranged from (14-45) with (mean±SD) value (25.8±6.4). There was significantly difference between patients and control (p=0.005).

The number of married patients was 54 (81.8%) and unmarried patients were 12 (18.2%) while in the control group, the number of married women was 60 (93.8%) while the unmarried women were 4 (6.2%) There was a significant relation between patients with PCOS and controls and marital status (P=0.038).

According to infertility, the number of infertile patients was 30 (45.5%) and the number of fertile women with one child reached 18 (27.3%), fertile woman with two children was 10 (15.2%) and the number of fertile women with more than two children reached 8 (12.1%), The control group showed that the number of infertile women was 7 (10.9%) and the fertile women with one child was 6 (9.4%), the number of fertile women with two children reached 7 (10.9%) and the fertile women with more than two children was 44 (68.8%). There was a significant difference between PCOS patients and controls (P=0.005).

The number of patients with PCOS with a regular Menstruation was 11 (16.7%) and the patients that were suffering from irregular menstruation were 55 (83.3%), In the control group, The number of women with a regular menstruation

was 36 (56.2%) while the number of women that had irregular menstruation reached 28 (43.8%) There was a significant difference between PCOS patients and controls (P=0.005).

In the patient group with PCOS, women which had Hirsutism were 37 (56.1%) and the women that did not have hirsutism were 29 (43.9%), While in the control group, The women with hirsutism were 0 (0.00%) and the women which had not hirsutism were 64 (100%). There was a significant difference between PCOS patients and controls (P=0.005).

Some Patients with PCOS had a family history of PCOS, their number was 7 (10.6%) and the patients that did not have a history of PCOS were 59 (89.4%). In the control group, The number of women with a family history of PCOS was 4 (6.2%) while the number of women that did not have a history of PCOS reached 60 (93.8%). There was no significant difference between PCOS patients and controls (P= 0.531).

There was a significant relation between the group studied and marital status, fertility, menstruation and hirsutism, but the family history was not significant. Age was significantly different between patients and control. Table (3.1) displays the means of the patient's demographic characteristics and the controls.

**Table (3.1): Demographic characteristics of the studied subjects**

| | | Group | | | | P value |
|---|---|---|---|---|---|---|
| | | Patients | | Control | | |
| | | **Mean±SD** | | **Mean±SD** | | |
| Age | | 32.4±8.5 | | 25.8±6.4 | | **0.005*** |
| | | **Count** | **%** | **Count** | **%** | |
| Marital status | Yes | 54 | 81.8% | 60 | 93.8% | **0.038*** |
| | No | 12 | 18.2% | 4 | 6.2% | |
| Fertility | None | 30 | 45.5% | 7 | 10.9% | **0.005*** |
| | One child | 18 | 27.3% | 6 | 9.4% | |
| | Two children | 10 | 15.2% | 7 | 10.9% | |
| | >2 children | 8 | 12.1% | 44 | 68.8% | |
| Menstruation | Regular | 11 | 16.7% | 36 | 56.2% | **0.005*** |
| | Irregular | 55 | 83.3% | 28 | 43.8% | |
| Hirsutism | Yes | 37 | 56.1% | 0 | 0.0% | **0.005*** |
| | No | 29 | 43.9% | 64 | 100.0% | |
| Family history | Yes | 7 | 10.6% | 4 | 6.2% | 0.531 |
| | No | 59 | 89.4% | 60 | 93.8% | |

*p value is significant (P<0.05), Data are (mean±SD) for quantitative variables and number (%) for categorical variables.

## 3.2. Statistical Analysis of Serum Levels of HSP90B1, Bcl-2 and Bax between Patients with PCOS and Control Groups

Statistical analysis shows the correlation of the biomarkers HSP90b1, Bcl-2 and Bax with polycystic ovarian syndrome (PCOS) patients and control of the studied group. HSP-90B1 and Bax were found significant (P<0.05) in that correlation While Bcl-2 was not significant (P>0.05). mean, SD, median, IQR, minimum and maximum values were summarized in the table (3.2).

Serum level of HSP90B1 in patients with PCOS reached a (mean±SD) (5.41 ± 2.40) and median (5.1) with IQR (1.4) , and the (mean±SD) in control group was (4.47 ± 0.83) and the median (4.4) with IQR (1.1). there was a significant difference between patients and control (p=0.005*).

The (mean±SD) of Bcl-2 was (111.45 ± 35.09) in the patients with PCOS, the median was (109.9) and IQR was (47.5), While in control group the (mean±SD) was (118.14± 101.13) and the median was (105.4) with IQR (46.7). Bcl-2 was found not to have relation to PCOS, there was no significant difference between patients and control (p=0.696).

While Bax was found with a (mean±SD) value (17.27 ± 14.02), median (14.4) and IQR (8.7) in patients with PCOS. The control group was found with a (mean±SD) (18.64 ± 14.96) and the median was (17.6) while IQR was (8.2). there was a significant difference between patients and control group (p=0.049*).

When observing the means of the measured biomarkers, the Bcl-2 had the highest mean value of the patients with PCOS. While the HSP90B1 had the lowest.

**Table (3.2): Serum level of HSP90B1, Bcl-2 and Bax comparison according to patients with PCOS and control subjects**

| | Group | | | | | | P valve |
|---|---|---|---|---|---|---|---|
| | Patients | | | Control | | | |
| | Mean ± SD | Med | IQR | Mean ± SD | Med | IQR | |
| HSP-90B1 ng/ml | 5.41 ± 2.40 | 5.1 | 1.4 | 4.47 ± 0.83 | 4.4 | 1.1 | **0.005*** |
| Bcl-2 U/ml | 111.45 ± 35.09 | 109.9 | 47.5 | 118.14± 101.13 | 105.4 | 46.7 | 0.696 |
| Bax ng/ml | 17.27 ± 14.02 | 14.4 | 8.7 | 18.64 ± 14.96 | 17.6 | 8.2 | **0.049*** |

*p value is significant (P<0.05), SD= standard deviation, Min=Minimum, Max=Maximum, IQR=Inter Quartile Range, HSP90B1=Heat Shock protein 90B1, Bcl-2= B-cell lymphoma 2, Bax= Bcl-2 associated X protein

## 3.3. Serum Level of HSP90B1 Comparison According to Different Patient's Characteristics

Heat shock protien (HSP90b1) was correlated to patients characteristics. Hirsutism had significant effect on HSP90B1 (p=0.003*). While the Marital status, fertility, menstruation and family history had no effect on HSP90B1 (P>0.05) as shown in table (3.3).

HSP90B1 was significantly different only between hirsute and non-hirsute patients (P<0.05).

**Table (3.3): Serum level of HSP90B1 comparison according to different patients characteristics**

| HSP90B1 ng/ml | | Mean ± SD | Median | IQR | P value |
|---|---|---|---|---|---|
| Marital | Yes | 4.47 ± 0.84 | 4.40 | 1.16 | 0.894 |
| | No | 4.47 ± 0.83 | 4.42 | 0.94 | |
| Fertility | None | 4.37 ± 0.74 | 4.30 | 1.05 | 0.199 |
| | One child | 4.52 ± 0.90 | 4.80 | 1.20 | |
| | Two children | 4.18 ± 0.60 | 4.36 | 0.76 | |
| | >2 children | 5.09 ± 1.03 | 4.56 | 4.41 | |
| Menstruation | Regular | 4.24 ± 0.72 | 3.98 | 1.20 | 0.225 |
| | Irregular | 4.51 ± 0.85 | 4.48 | 1.02 | |
| Hirsutism | Yes | 4.22 ± 0.69 | 4.26 | 0.74 | **0.003*** |
| | No | 4.78 ± 0.90 | 4.80 | 0.85 | |
| Family history | Yes | 4.83 ± 1.17 | 4.55 | 1.30 | 0.343 |
| | No | 4.42 ± 0.78 | 4.39 | 1.11 | |

*p value is significant (P<0.05), SD= standard deviation, IQR=Inter Quartile Range, HSP90B1=Heat Shock protein 90B1

## 3.4. Serum Level of Bcl-2 Comparison According to Different Patient's characteristics

Bcl-2 was found to be significantly different only between patients with family history (P=0.024*), while the Marital status, fertility, menstruation and hirsutism had no effect on Bcl-2 (P>0.05). Means, SD, Median and IQR were summarized in the table (3.4).

**Table (3.4): Serum level of Bcl-2 comparison according to different patients characteristics**

| Bcl-2 U/ml | Mean±SD | Median | IQR | P value |
|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| Marital | Yes | 19.02±16.31 | 111.83 | 45.80 | 0.572 |
| | No | 16.90±6.07 | 98.14 | 56.10 | |
| Fertility | None | 15.70±6.20 | 101.73 | 41.03 | 0.773 |
| | One child | 23.95±27.00 | 120.57 | 53.42 | |
| | Two children | 16.73±4.49 | 115.50 | 39.80 | |
| | >2 children | 20.09±2.55 | 110.23 | 46.89 | |
| Menstruation | Regular | 16.57±5.36 | 109.96 | 45.83 | 0.576 |
| | Irregular | 19.05±16.22 | 113.62 | 49.76 | |
| Hirsutism | Yes | 19.98±19.31 | 110.03 | 36.37 | 0.995 |
| | No | 16.93±5.90 | 106.51 | 51.03 | |
| Family history | Yes | 20.38±5.03 | 145.94 | 47.98 | **0.024*** |
| | No | 18.43±15.75 | 108.22 | 37.38 | |

*p value is significant (P<0.05), SD= standard deviation, IQR=Inter Quartile Range, Bcl-2= B-cell lymphoma 2

## 3.5. Serum Level of Bax Comparison According to Different Patients Characteristics

The difference of Bax according to different patient characteristics were statically studied in table (3.5). All of the characteristics had no significant difference on Bax (P>0.05). Means, SD, Median and IQR were summarized in the table.

**Table (3.5): Serum level of Bax comparison according to different patients characteristics**

| Bax ng/ml | | Mean±SD | Median | IQR | P value |
|---|---|---|---|---|---|
| Marital | Yes | 17.94±7.79 | 17.94 | 7.79 | 0.606 |

| | | | | | |
|---|---|---|---|---|---|
| | No | 16.40±7.42 | 16.40 | 7.42 | |
| Fertility | None | 15.96±7.53 | 15.96 | 7.53 | 0.147 |
| | One child | 21.11±14.01 | 21.11 | 14.01 | |
| | Two children | 17.59±4.86 | 17.59 | 4.86 | |
| | >2 children | 19.81±3.73 | 19.81 | 3.73 | |
| Menstruation | Regular | 16.36±9.28 | 16.36 | 9.28 | 0.612 |
| | Irregular | 17.72±8.54 | 17.72 | 8.54 | |
| Hirsutism | Yes | 17.42±8.54 | 17.42 | 8.54 | 0.993 |
| | No | 17.74±6.64 | 17.74 | 6.64 | |
| Family history | Yes | 19.95±10.17 | 19.95 | 10.17 | 0.239 |
| | No | 17.53±9.92 | 17.53 | 9.92 | |

* SD= standard deviation, IQR=Inter Quartile Range, Bax= Bcl-2 associated X protein

## 3.6. Correlation Test between Serum Levels of HSP 90B1, Bcl-2 and Bax According to Patients with PCOS

The correlation was studied between HSP90B1, Bcl-2 and Bax in 66 patients with PCOS as shown in the table (3.6).

None of the mentioned biomarkers was found to be of significance in that correlation (P>0.05) all were shown in the figures (3.1), (3.2) and (3.3).

personal correlation, p value and the number of patients were summarized in the table.

**Table (3.6): correlation test between serum level of HSP 90B1, Bcl-2 and Bax according to patients with PCOS**

| | | HSP 90B1 | Bcl-2 |
|---|---|---|---|
| Bcl-2 | Pearson Correlation | 0.089 | |

| | | | |
|---|---|---|---|
| | P value | 0.476 | |
| | N | 66 | |
| Bax | Pearson Correlation | 0.187 | 0.093 |
| | P value | 0.134 | 0.455 |
| | N | 66 | 66 |

*****N=number of patients, HSP90B1=Heat Shock protein 90B1, Bcl-2= B-cell lymphoma 2, Bax= Bcl-2 associated X protein

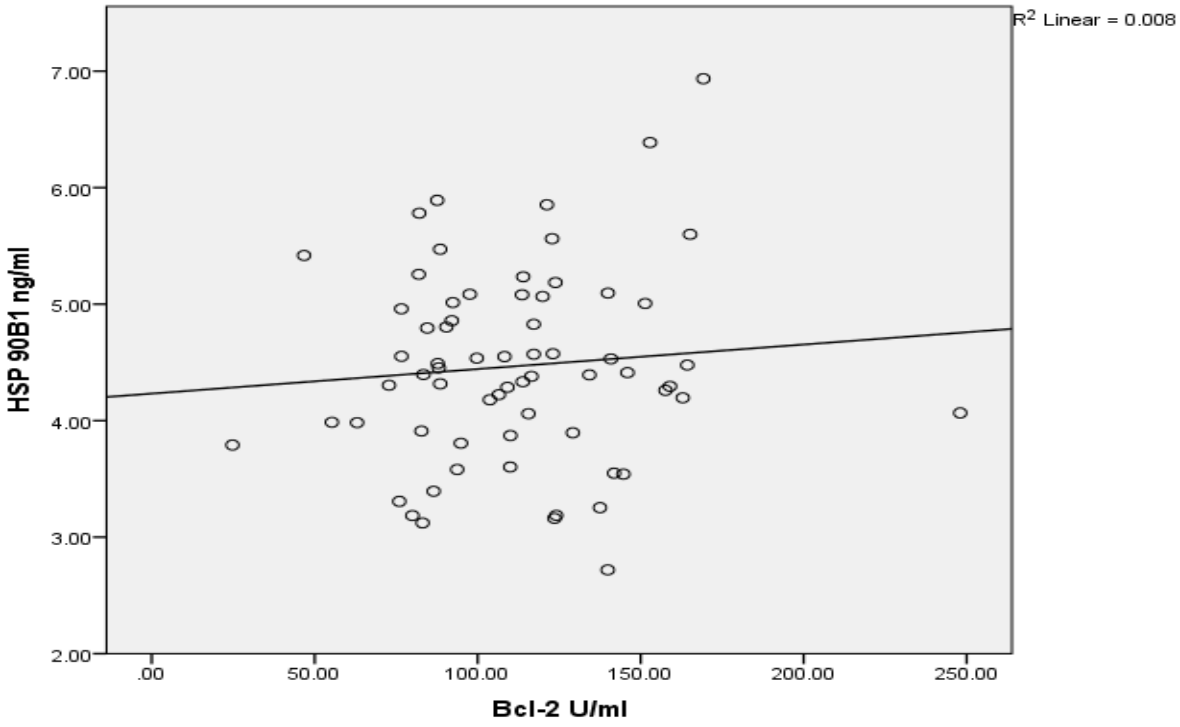


**Figure (3.1): the correlation between HSP90B1 and Bcl-2.**

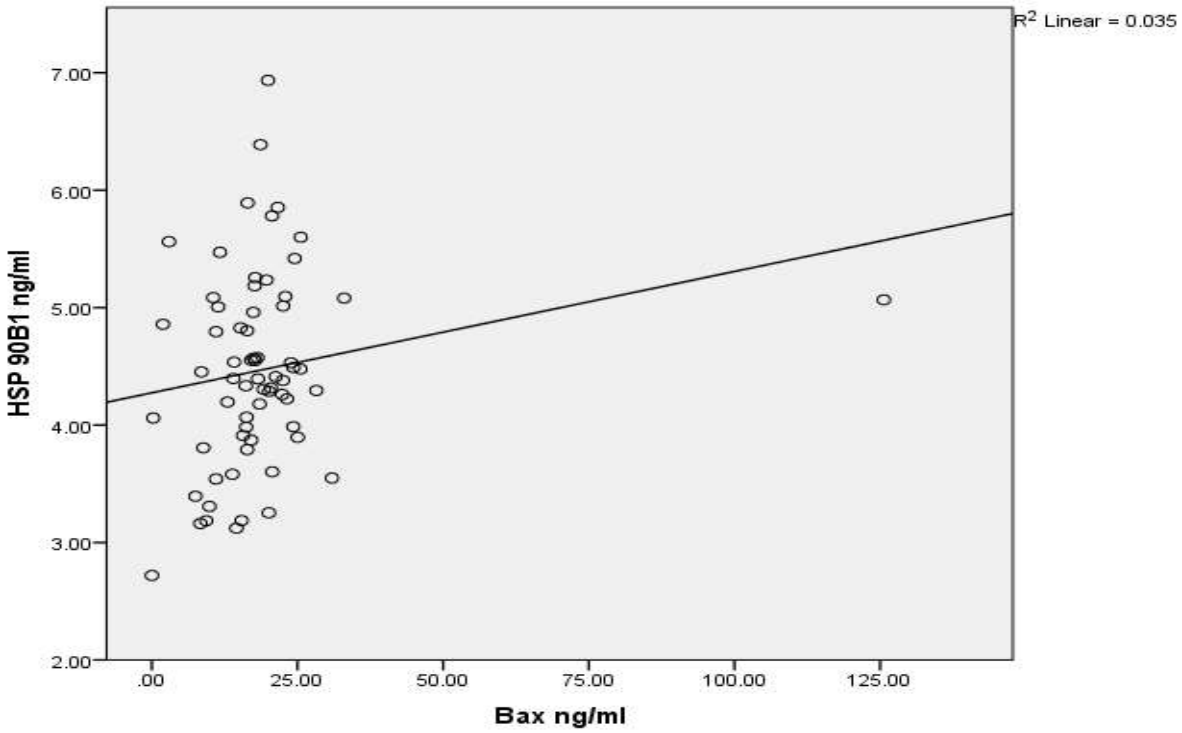


**Figure (3.2): the correlation between HSP90B1 and Bax.**

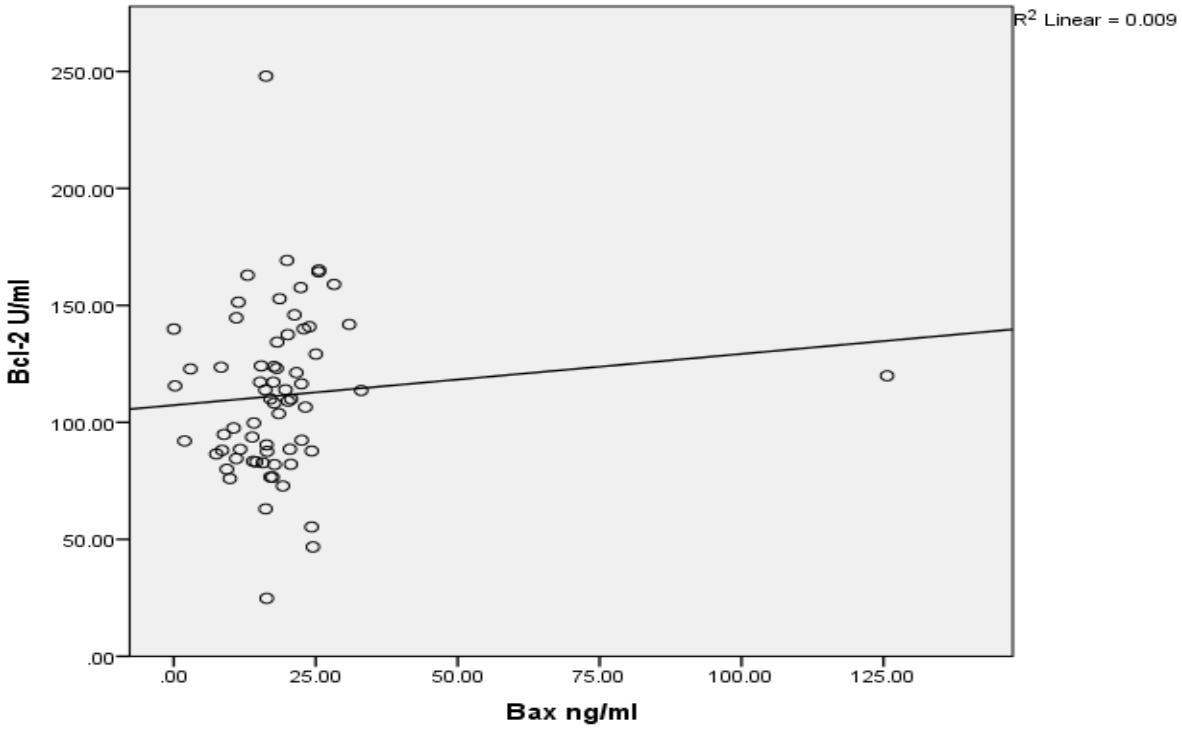


**Figure (3.3): the correlation between Bcl-2 and Bax.**

## 3.7. ROC

using ROC analysis were studied in table (3.7). results showed a non-significant area under the curve of 30.7% for HSP-90B1, 52.0% for Bcl-2 and 60.0% for Bax.

HSP90B1 area under the curve was 30.7% (AUC) (P=0.000 non-significant) for indicating a patient at a cut-off value of 4.793, the sensitivity was 34.8% and specificity was 39.1%.

Bcl-2 area under the curve was 52.0% (AUC) (P=0.696 non-significant) for indicating a patient at a cut-off value of 109.99, the sensitivity was 50.0% and specificity was 56.2%.

Bax area under the curve was 60.0% (AUC) (P=0.050 non-significant) for indicating a patient at a cut-off value of 16.05, the sensitivity was 65.2% and specificity was 67.2%.

**Table (3.7): ROC test results of HSP90B1, Bcl-2 and Bax**

| Area Under the Curve | | | | | |
|---|---|---|---|---|---|
| Test Result Variable(s) | Area | Std. Error | P value | Asymptotic 95% Confidence Interval | |
| | | | | Lower Bound | Upper Bound |
| HSP 90B1 | 0.307 | 0.047 | 0.000 | 0.216 | 0.399 |
| Bcl-2 | 0.520 | 0.051 | 0.696 | 0.420 | 0.620 |
| Bax | 0.600 | 0.051 | 0.050 | 0.499 | 0.701 |

* HSP90B1=Heat Shock protein 90B1, Bcl-2= B-cell lymphoma 2, Bax= Bcl-2 associated X protein

Sensitivity measures the proportion of true positives that are correctly identified and specificity measures the proportion of true negatives.

Below figures (3.4), (3.5) and (3.6) that shows sensitivity and specificity of HSP90B1, Bcl-2 and Bax respectively.

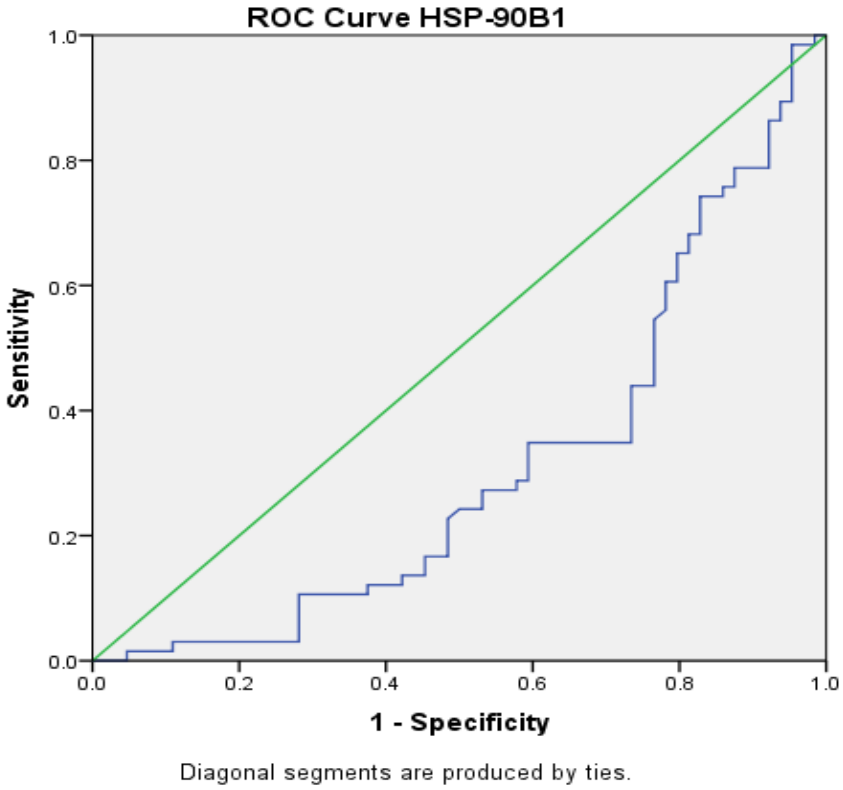


**Figure (3.4): Sensitivity and specificity of HSP90B1.**

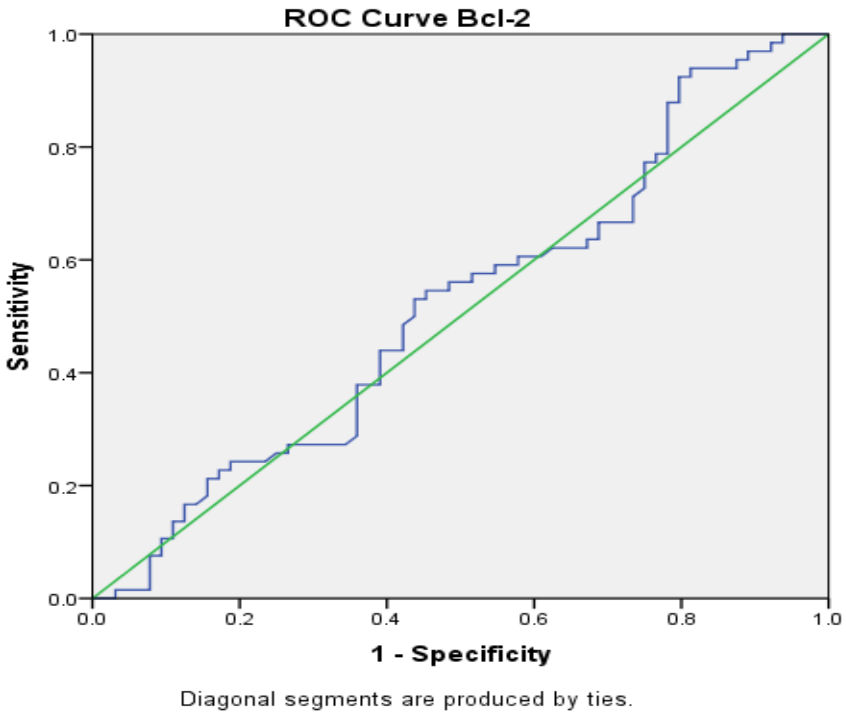


**Figure (3.5): Sensitivity and specificity of Bcl-2.**

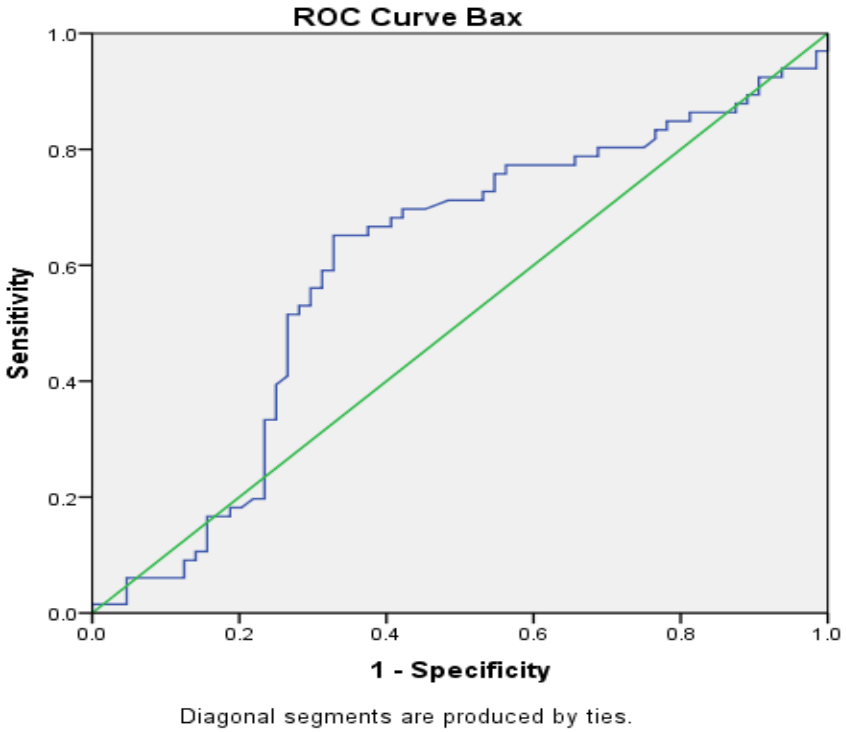


**Figure (3.6): Sensitivity and specificity of Bax.**

# Chapter Four

# Discussion

# 4. Discussion

Polycystic ovarian syndrome (PCOS) is the most common endocrinopathy that affects 5–10% women of reproductive age. The characteristics of PCOS are hyperandrogenemia, polycystic ovaries, and/or ovulation dysfunction (Gong, Luo et al. 2020).

Despite extensive studies about the pathogenesis of PCOS, the cause is not determined yet. In recent years, several causative hypotheses have been proposed for PCOS, such as insulin resistance, chronic inflammation, oxidative stress, family history, and genetics (Fitzgerald, DiVasta et al. 2018; Šimková, VÍTKŮ et al. 2020; Tosatti, Alves et al. 2021).

## 4.1. Demographic Data of PCOS Patients and Controls

### 4.1.1. Age Distribution

The results obtained in the present study found that there was a significant difference in age between patients with PCOS and control. Present study agreed with the study by Bazarganipour, Ziaei et al., (2014), women with PCOS were included in the study which indicates that PCOS is a young age disease. In line with our study, the results of a study by Hong, Hong et al., (2020), women with PCOS and control, the women with PCOS were younger than the control group. the current results also agreed with Curtis, Karki, et al., (2018), who concluded that there was a highly significant difference ($P< 0.001$) in mean age between healthy individuals and patients with PCOS. Age was significantly related to the incidence of PCOS (Shan, Cai et al. 2015).

In contrast to the present results, a study by Alwan and Al-Heety (2021), showed non-significant difference in mean age between PCOS patients and

control group ($p > 0.05$). Also, our results disagreed with a study done by Deswal, Nanda et al., (2019), which did not find any difference in age between patients with PCOS and control. A case group was composed of females with a definite diagnosis of PCOS and the control group was comprised of females, There were no significant differences between the two groups regarding the age (Basirat, Faramarzi et al. 2019).

Furthermore, Fatima, Amin et al., (2019), showed that there was no significant difference in age between PCOS patients and controls. As well as there was no significant variation in age across the groups of patients with PCOS and control ($P > 0.05$) (Ameen, Sulaiman et al. 2021). ) On a study done by Ali, AL-Jedda, et al., (2021), who concluded that non-statistically significant difference in age between the PCOS and control groups. The lack of a significant difference between groups indicates neutrality in the results because age is related to immunity, hormone factors, hormone secretion, and other factors.

### 4.1.2. Marrital State

The findings of this study indicate that there was a significant difference between the two groups in terms of marital status. In the study of Tabassum et al., (2021), as in the present study, there was a significance difference between PCOS group and control group according to marital status. Moreover, similar results were found in the study by Shahrokhi, Naeini et al., (2020), PCOS cases and healthy control, results showed a significant difference between the two groups in terms of marital status. another study by Shan, Cai, et al., (2015), also found a significant difference in marital status between patients with PCOS and control.

The results of the present study disagreed with other studies, In the study by Panjeshahin, Salehi-Abargouei et al., (2020), PCOS cases and healthy control, their study found non significance difference in the term of marital state between patients with PCOS and control group. Moreover, In the study by Mehrabadi, Sadatmahalleh et al., (2020), there was a no significant relation in the marital status between PCOS patients and control. Also, Mirza, Shafique, et al., (2014), found no significant difference in distribution of married individuals between cases and controls.

### 4.1.3. Fertility

Infertility is defined as "a disease of the reproductive system characterized by failure to achieve clinical pregnancy after 12 months or more of regular unprotected sexual intercourse" . When a woman is unable to ever bear a child, it is classified as primary infertility. Secondary infertility occurs when a woman is unable to bear a child, following either a previous pregnancy or a previous ability to carry a pregnancy to a live birth (Dumbala, Bhargav et al. 2020).

PCOS women are at increased risk for infertility owing primarily to anovulation (Lentscher, Slocum et al. 2021). The prevalence of infertility in PCOS women varies between 70 and 80% (Melo, Ferriani et al. 2015). In this community-based cohort of women, infertility and the use of fertility hormone treatment were significantly higher in women reporting PCOS (Joham, Teede et al. 2015).

In this study, there was a significant difference in infertility between PCOS patients and control (P=0.005). Similar to our findings, Joshi, Yonzon et al., (2017), also showed that patients group were married, single, complained of infertility. Moreover, in PCOS group, only few women were fertile (≥ 1 children),

while in the control group, more women were fertile (≥ 1 children) (Badri-Fariman, Naeini et al. 2021). According to the current study's findings, PCOS patients have higher levels of primary infertility than the control group, which is consistent with other observation performed previously (Al-Quraishy, Al-Tu'ma et al. 2022).

A study by Angin, Yoldemir et al., (2019), against current study found that the rate of fertile women was higher than infertile women in the PCOS group, There were no differences in fertility between the PCOS and non-PCOS group (Karsten, Wekker et al. 2021). The incidence of infertility is higher in the present study due to the higher prevalence of infertility in present times.

### 4.1.4. Menstruation

The current study found that PCOS patients had high levels of irregular menstruation, There was a significant difference in irregular mensturation between patients with PCOS and control. Most PCOS patients experience the onset of irregular menstruation since adolescence and Endocrine dyscrasia along any part of the hypothalamic-pituitary-gonadal axis may lead to irregular menstruation and anovulation (Du, Xu et al. 2012). It is also confirmed by the present study that PCOS is closely related to irregular menstruation.

A study by Nazeer, Lone et al., (2021), agreed with present study, ovaries with multiple cysts and irregular menstrual cycle were significantly higher in PCOS women than in non-PCOS women (P-value < 0.05). another study showed that the women with PCOS had significantly higher irregular menstruation ratio in comparison with the control group (Bykowska-Derda, Czlapka-Matyasik et al. 2021). Demographic parameters was taken and revealed obvious increase in irregular menstruation pattern (Al-Quraishy, Al-Tu'ma et al. 2022).

Furthermore, a study by Asdaq and Yasmin, (2020) showed that there was a significant difference in menstrual cycle between PCOS patients and control. Among the PCOS students, students had irregular menstrual cycle, whereas only few had menstrual irregularity among the non-PCOS group (Chatterjee and Bandyopadhyay 2020). Also, a study by Shan, Cai, et al., (2015), reported that irregular menstruation was significantly related to the incidence of PCOS.

Also Basirat, Faramarzi et al., (2019), revealed that the frequency of irregular menstruation was significantly higher in females with PCOS than the ones without PCOS ($P<0.001$). prevalence of menstrual irregularity was found higher in PCOS girls as compared to non PCOS girls, This shows significant correlation between menstrual irregularity and PCOS (Desai, Tiwari et al. 2018).

### 4.1.5. Hirsutism

The present study showed that there was a significant difference between PCOS patients and controls ($P=0.005$). current results agreed with Al-Quraishy, Al-Tu'ma, et al., (2022), who concluded that hirsutism values was obviously and significantly increased in PCOS women compared to control group. in other studies such as Asdaq and Yasmin, (2020), showed Significantly high proportion of PCOS cases were found with hirsutism when compared to control group.

Moreover, Chatterjee and Bandyopadhyay, (2020), showed that participants diagnosed with PCOS had hirsutism, whereas the occurrence of the same was much lower in the non-PCOS group. Moreover, a study by Bakry, Al Gayed et al., (2020), agreed with the current study in that PCOS cases showed significant increase in hirsutism score compared with controls. Also, Women with PCOS had increased hirsutism scores compared to controls (Kazemi, Jarrett et al. 2020).

Furthermore, Ilagan, Paz-Pacheco et al., (2019), revealed a higher proportion of hirsute women was observed in the PCOS group versus the non-PCOS group.

### 4.1.6. Family History

This study showed a negative relationship between family history and PCOS. There was no significant difference between PCOS patients and control. The results of the present study was agreed with other studies which observed that cases and control did not differ significantly regarding the history of PCOS. there was no significant difference in family history of PCOS among cases and controls (Mirza, Shafique et al. 2014). Positive family history of PCOS is seldom elicited as 2-3 decades back PCOS was seldom documented (Jungari, Nair et al. 2020). The results of family history of PCOS done by Shan, Cai, et al., (2015), was reported to be significantly related to the incidence of PCOS.

In contrast to the present study, A study by Munawar Lone, Babar et al., (2021), the literature revealed that family history of PCOS was significantly higher among patients with PCOS vs. control, also another study by Altamimi, Alqahtani et al., (2020), showed that all the patients included in the study had a diagnosis of PCOS, and most of them had a positive family history of PCOS. Also, there was a highly significant difference ($P< 0.001$) in the distribution of patients with PCOS to that of control subjects according to family history. Family history of diabetes, notably an inherited metabolic disorder, also poses a significantly high risk for PCOS (Alzamily, Obaid et al. 2021).

Nominally positive family histories of PCOS are considered a risk factor for PCOS expansion in women. There is a piece of evidence showing that a family history of T2D, as a reflection of genetic risk, is associated with an increased risk of the progression of T2D in PCOS women, with T2D and obesity-associated

genes and genetic polymorphisms being connected to hyperandrogenism, which has been associated with the PCOS phenotype, suggesting an important genetic background (Lerchbaum, Schwetz et al. 2014). This might be argued that as history of PCOS is strongly related to the incidence of PCOS, however, persons who don't need to have PCOS always have a history of PCOS. Although family history is an important risk factor, environmental triggers are also playing a role, e.g. diet, and exercise (Alzamily, Obaid et al. 2021).

## 4.2. Serum Levels of HSP90B1, Bcl-2 and Bax between Patients with PCOS and Control Groups

This study focused on the HSP90B1, Bcl-2 and Bax and measured them in comparison with patients with PCOS and control cases, where only HSP90B1 and Bax were found to be of significance in that correlation.

**HSP90B1:** Heat-shock protein 90kDa beta1 (HSP90B1), a stress-inducible molecular chaperone that is a member of the heat-shock protein (HSP) 90 family, is also known as GRP94 and GP96 (Wang and Wang 2022). Through the maintenance of the endoplasmic reticulum (ER) stress sensors, the preservation of the ER protein folding capability, and the repression of ER-associated proapoptotic machinery, HSP90B1 plays an essential part in regulating the delicate balance between the survival and death of cancer cells (Kim, Cho et al. 2021). In present study, we identified a significant relation between HSP90B1 and patients with PCOS compared to control cases.

Li, Mo et al., (2016), identified a significant increase in the expression of HSP90B1 in the ovarian tissues from PCOS patients. Another study done by Li, Zhang, et al., (2016), agreed with the current study in that HSP90B1 had significantly increased expressions ($P<0.01$) in the ovarian tissues of women with

PCOS, The findings of their study imply that HSP90B1 may have a role in promoting cell proliferation in PCOS pathogenesis. Furthermore, Hoter and Naim, (2019), had found the majority of HSPs show increased expression in ovarian cancer tissues. These results are consistent with the previous findings in which HSP90B1 was found to be highly involved in ovarian cell survival.

Disagreed with the present study, Fachim, Iqbal et al., (2021), had found that heat shock protein 90 Beta family member 1 (HSP90B1) proteins that correlated with inflammatory markers in relation to fold change at specific time points, was found with a fell down levels.

**Bcl-2:** is a protein that supports cell survival through antiapoptotic activity (Alawsi, Alshammari et al. 2019). Additionally it was described in some parts of the endoplasmatic reticulum and nuclear envelope. Expressed widely during embryonic development, in the adult it is confined to long-lived cells (e.g. stem cell populations, resting B lymphocytes, and peripheral neurons).The biochemical function remains largely unknown, although bcl-2 oncoprotein is known to inhibit programmed cell death (Al-Temimi 2011). Due to the inhibitor of apoptosis gene Bcl-2 can affect the apoptosis by affecting intracellular information conduction, many scholars believe it is the key of regulating factor in cell apoptosis. A number of hypotheses about the pathogenesis of PCOS have been proposed. However, the etiology and pathology of PCOS still has not been clarified clearly (Chi, Zhang et al. 2018).

No studies have been performed recently on the effects of Bcl-2 on apoptosis of ovarian cells in PCOS. The present study revealed no significant association between Bcl-2 and PCOS. Moreover, Wiweko, Beelonie et al., (2018), their study had found no differences in Bcl-2 levels between the patients with PCOS and the

control groups. Another study involved fifty infertile couples, the patients were divided into four groups: control, unexplained infertility, polycystic ovary syndrome (PCOS) and poor ovarian response (POR), Cumulus cells were isolated individually from 437 oocytes obtained. A significant and negative correlation was found between Bax and Bcl-2 expressions of the cumulus cells of poor-quality embryos (Yaka, Çil et al. 2022). which supports our study results.

**Bax:** is a protein acts as an antagonist of Bcl-2 and is a related protein homologue of Bcl-2. Studies have shown that Bax expression in tissues and organs is more widespread than that of Bcl-2 in mice, and it is also expressed in the male testes and female ovaries (Sun, Lin et al. 2012). The pathway of promoting apoptosis by Bax protein may be related to the anti-apoptotic protein Bcl-2, which can cause Bax to lose its pro-apoptotic effect. When there is excess of Bax that cannot be fully integrated over time, it leads to apoptosis.

No studies have been performed recently on the effects of Bax on apoptosis of ovarian cells in PCOS. The present study revealed a significant difference in Bax serum levels between PCOS patients and controls. Level of BAX proteins in goat ovaries showed that the relative expression of BAX was greater in atretic than in healthy follicles ($p < 0.05$) (Zhang, Wan et al. 2015).

Disagreed with the present study, Wiweko, Beelonie et al., (2018), had found no differences in Bax levels between the patients with PCOS compared to the control groups. Furthermore, a negative correlation was found between Bax expressions of the cumulus cells of poor-quality embryos (Yaka, Çil et al. 2022).

These results indicate the importance of these biomarkers and their role in the disease pathogenesis and the possibility of using such markers for it’s diagnosis.

## 4.3. Serum Level of HSP90B1 Comparison According to Different Patient's Characteristics

The present study had found that (HSP90B1) was significantly different only between hirsute and non-hirsute patients. Polycystic ovary syndrome (PCOS) is the most common cause of hirsutism, accounting for more than 70% of cases (Matheson and Bain 2019). The upregulated proteins were identified as heat shock protein 90 (HSP90B1) in PCOS (Li, Zhang et al. 2016).

Many studies were agreed with the present research, Nada and Al-Wazzan, (2021), found that the percentage of hirsutism in the group of patient women with PCOS was high, reaching 68%. similar results were found by Bode, Seehusen et al., (2012), when investigating PCOS is the leading cause of hirsutism, with 72 to 82 % of cases.

## 4.4. Serum Level of Bcl-2 Comparison According to Different Patient's Characteristics

The protein Bim (also known as Bcl-2-related ovarian death gene, Bod) was originally identified as a Bcl-2-interacting protein. Similar to other members of the Bcl-2 family (Sun, Yu et al. 2001), Bim isoforms show tissue-specific expression patterns. There were distinct temporal and spatial roles for Bcl-2 specific Bim isoforms during apoptosis (Miao, Chen et al. 2007).

However, Wang, Wu, et al., (2012), found that only the BimEL protein was detected in apoptotic granulosa cells by Western blot in their experiment. Based on their observation that porcine BimEL expression levels in granulosa cells varied with the health status of follicles. Apoptosis can be induced via either the

extrinsic or death receptors mediated pathway or the intrinsic or mitochondrial pathway.

The intrinsic pathway is triggered by cellular stress, such as growth factor deprivation, chemotherapeutic agents, radiation, hypoxia and oncogene activation.This pathway is regulated by the members of the Bcl-2 family proteins, which are characterized by the presence of specific regions of homology called Bcl-2 homology (BH) domains (Basu and therapeutics 2021).

Bcl-2 have a genetic role in apoptosis. In this study, family history had a significant effect on Bcl-2 levels in patients with PCOS, which was consistent with previous studies. Also further studies are required to estimate such possibilities.

## 4.5. Serum Level of Bax Comparison According to Different Patient's Characteristics

The current study has found that Bax was not significantly different between all of the studied characteristics of the patients with PCOS. A study by Alaee, Mirani et al., (2022), involved thirty-two female rats were randomly divided into four groups: (1) Control, (2) PCOS, (3) PCOS+5 mg/kg thymoquinone and (4) PCOS+10 mg/kg thymoquinone, they found that Gene expression of Bax ($p < 0.05$) as an apoptotic activator and Bax/Bcl2 ratio ($p < 0.01$) as an apoptotic marker were significantly upregulated in the ovaries of the PCOS group when compared to the control group, They were markedly decreased in the PCOS groups which received thymoquinone in comparison to the PCOS group ($p < 0.01$ and $p < 0.05$ for Bax and Bax/Bcl2 ratio, respectively).

During folliculogenesis, oocytes receive important signaling and nutrition only through communication with the cumulus cells around them. Through this communication, oocytes and cumulus cells complete folliculogenesis, oocyte maturation, and ovulation (Chermuła, Brązert et al. 2019). Therefore, microcommunication plays a critical role in oocyte maturation. (Liu, Kong et al. 2020). Bax expression and the Bax/Bcl-2 ratio are high in immature oocyte cumulus cells has shown us that the apoptotic process may begin when the cumulus–oocyte connection exists (Yaka, Çil et al. 2022).

The expressions of pro-apoptotic genes: including Bax were significantly reduced in PCOS women (Das, Djahanbakhch et al. 2008). which supports the current study results. moreover, Further studies are required to estimate such possibilities.

## 4.6. Correlation Test between Serum Levels of HSP 90B1, Bcl-2 and Bax According to Patients with PCOS

The current result did not find any correlation between HSP90B1, Bcl-2 and Bax. HSPs having anti-apoptotic properties and drug resistance characteristics, overexpression of HSPs in variant malignancies has been correlated to cell survival, tumor progression and metastasis as well as poor prognosis.Therefore, most studies consider HSPs not only as diagnostic/prognostic markers but also as ideal therapeutic targets for cancer therapy (Chatterjee and Burns 2017) (Narayanankutty, Narayanankutty et al. 2019) (Milani, Basirnejad et al. 2019).

Proteomic studies in women with PCOS have demonstrated two-fold increase in HSP90B1 levels suggesting a role in promoting cell survival and suppression of apoptosis (Hoter and Naim 2019). As a conclusion, the aberrant expression of

HSPs in ovarian cysts is suggested to counteract apoptosis and delay regression of cystic follicles (Velazquez, Alfaro et al. 2010) (Velázquez, Alfaro et al. 2011).

A study by Wiweko, Beelonie et al., (2018), has found no differences in the Bax/Bcl-2 ratio between the PCOS and the control groups ($p = 0.31$). another study found that the Bax/Bcl-2 ratio was significantly lower in cumulus cells of mature oocytes, A significant and negative correlation was found between Bax and Bcl-2 expressions of the cumulus cells of poor-quality embryos (Yaka, Çil et al. 2022). These studies are correspondent with what was found in our investigations. Further studies are required to estimate such possibilities.

A second Cross-sectional study women with PCOS and eight normal-cycling non-PCOS controls indexes (BCL2/BAX) in the endometrium of the PCOS group were significantly higher (Giordano, Giordano et al. 2022). Further studies are required to estimate such possibilities. To our knowledge, this is the first study to investigate serum HSP90B1, Bcl-2 and Bax levels in PCOS women in Iraqi population.

# Conclusion
# And
# Recommendation

## Conclusion

1- The HSP90B1 and Bax were found a significant association with PCOS, and Bcl-2 was not significant according to PCOS.

2- The HSP90B1 levels was found to be significantly different in patients with PCOS between hirsute and non-hirsute patients.

3- The Bcl-2 levels was found to be significantly different in patients with PCOS according to the family history.

## Recommendations

1-Study these types of HSP90B1, Bcl-2 and Bax in a larger sample

size of patients with PCOS.

2- Studying the genes Polymorphisms of HSP90B1, Bcl-2 and Bax in Women with PCOS.

3- Studying the role of other immunological markers in PCOS patients like cleaved caspase-3 (CASP3), Fas cell surface death receptor (FAS) and FAS ligand (FASLG).

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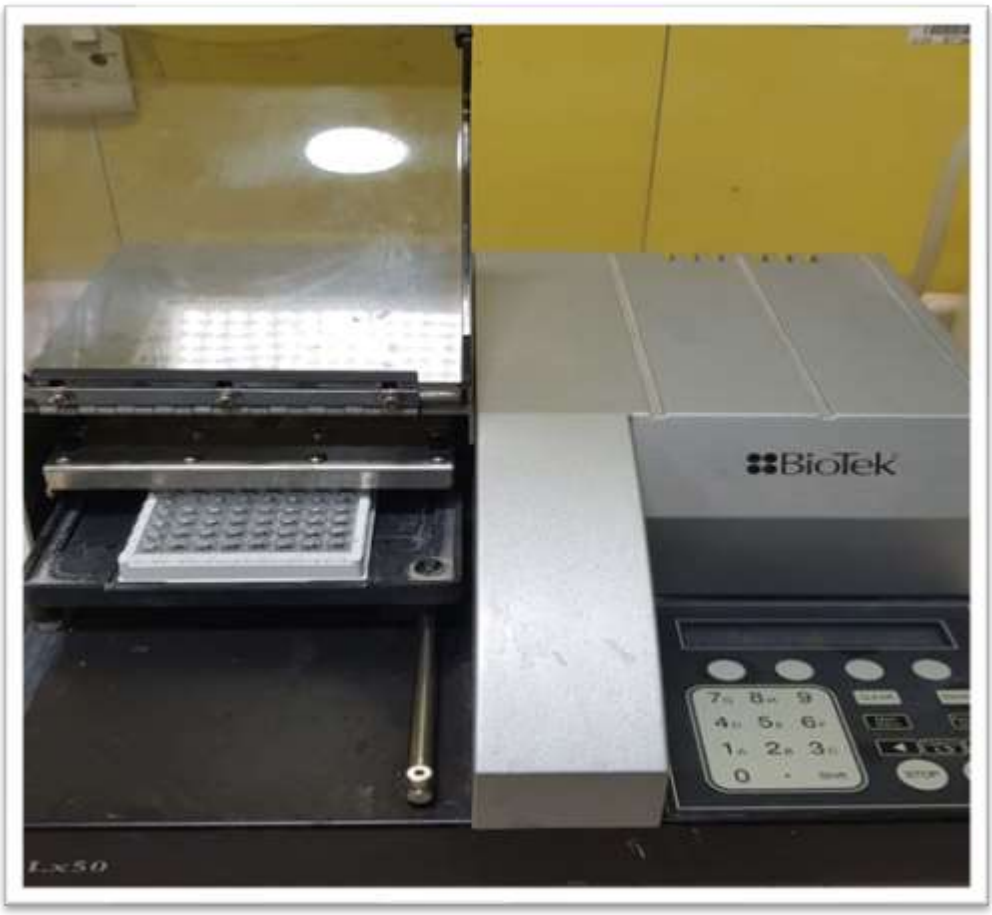
# Appendix

# Appendix 1

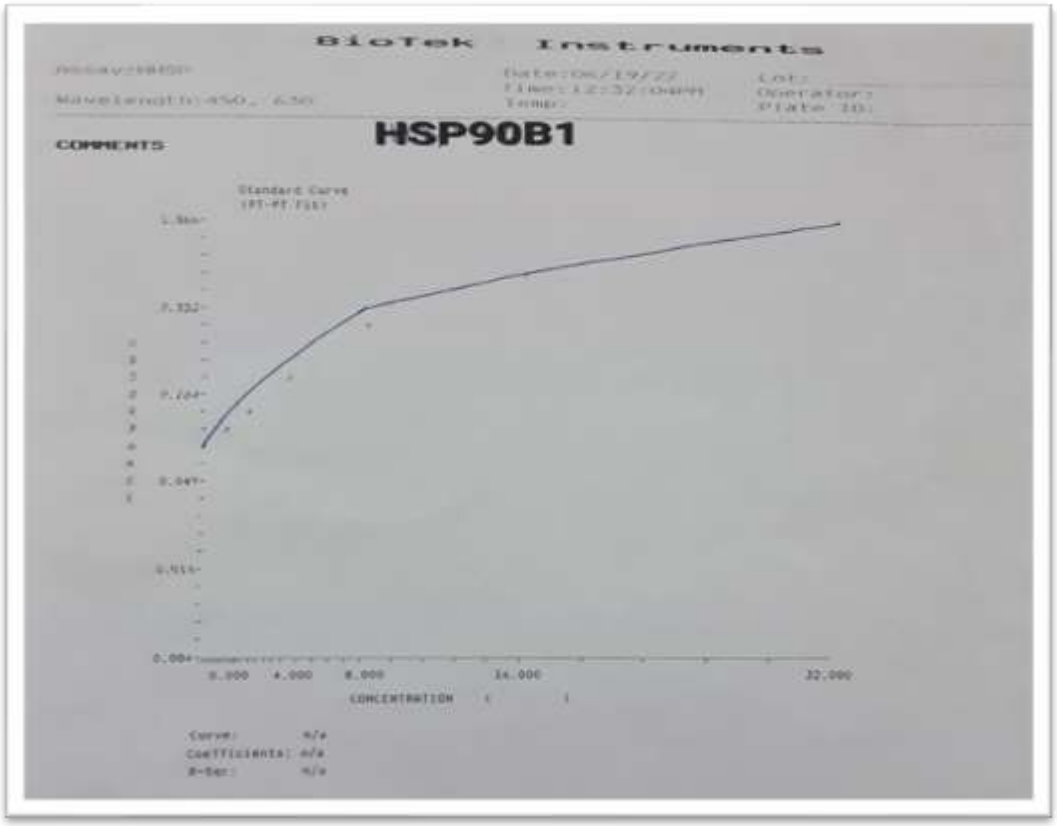
ELISA system with plate

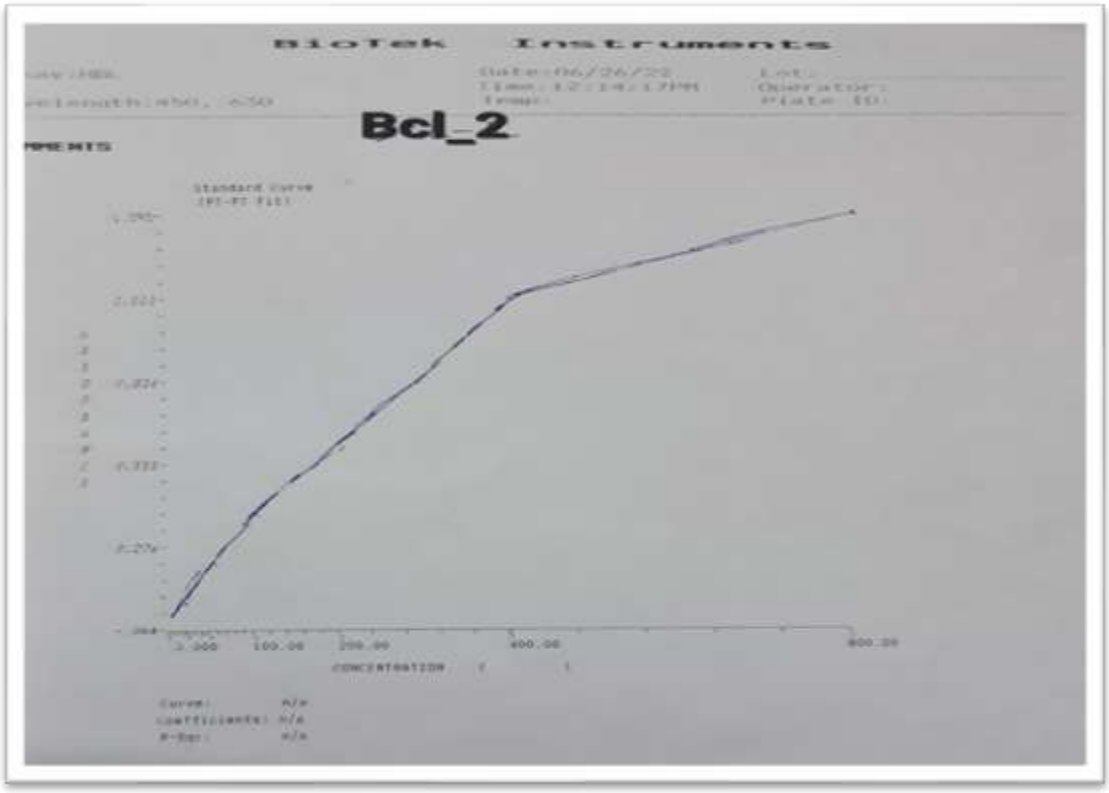

BioTek

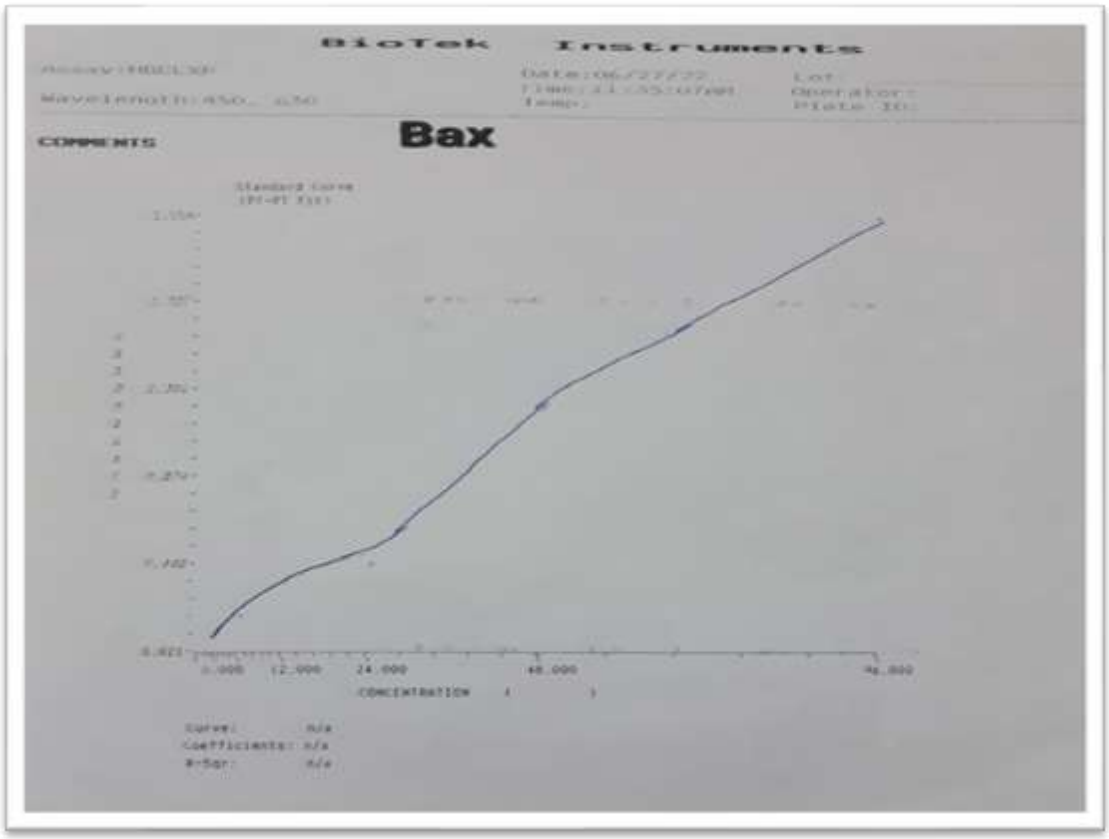

BioTek
Lx50

# Appendix 2

Standard curve




BioTek Instruments
Bcl_2
COMMENTS
Standard Curve
CONCENTRATION


BioTek Instruments
Bax
COMMENTS
Standard Curve
CONCENTRATION

# Appendix 3

The questionnaire sheet of study for Cases: -

Patients ID:

Name:

Age:

Marital status:

Fertility:

Menstrual history:

Hirsutism:

Finding of ultrasound:

Family history:

Medical history:

Residence:

Phone number:

## الخلاصة

متلازمة تكيس المبايض هي واحدة من اكثر حالات الغدد الصماء المزمنة شيوعا التي تؤثر على ما بين 5-10% من النساء في سن الانجاب. حب الشباب, عدم انتظام الدورة الشهرية, نمو الشعر غير الطبيعي في الوجه او السمنة هي ميزات شائعة في مرحلة ما قبل الحيض. متلازمة تكيس المبايض هي اضطراب متعدد العوامل حيث تم ربط عدة عوامل بتطور المتلازمة, والتي تؤدي الى عقم النساء بسبب عدم نضج البيضة, عدم انبات الجنين وعوامل وراثية وبيئية والغدد الصماء هي الأسباب الرئيسية لاختلال الاباضة عند النساء.

لطالما كان التسبب في متلازمة تكيس المبايض مثيرا للجدل لفترة طويلة. ومع ذلك ، تشير الأدلة التي تراكمت على مدى السنوات ال 30 الماضية إلى أن الفسيولوجية المرضية الغير طبيعية السبب الكامن وراء الغالبية العظمى من متلازمة تكيس المبايض هو فرط الأندروجين المفروز من قبل المبيض و مقاومة الأنسولين الموجود في نصف متلازمة تكيس المبايض تؤدي إلى تفاقمها. أحد عوامل الخطر الأكثر شيوعا لتقدم متلازمة تكيس المبايض يشمل التاريخ العائلي لمتلازمة تكيس المبايض.

تلعب الخلايا الحبيبة دورا مهما في نضج البويضات والاخصاب والانبات لاحقا. كشفت العديد من البحوث دورا مهما لHSP90B1, Bcl-2, Baxفي إمراضية متلازمة تكيس المبايض .
زيادة مستوى HSP90B1, Bcl-2وهي بروتينات مضادة للموت المبرمج للخلايا, وانخفاض مستوى Bax ,وهو بروتين محفز للموت المبرمج للخلايا, يلعب دورا مهما في إمراضية متلازمة تكيس المبايض.

HSP90B1 هي بروتينات مستحثة بواسطة الاجهاد. ومستوى التعبير يؤثر بشكل واضح على تكاثر الخلايا وبقاءها, انخفاض دورة حياة الخلية وموت الخلايا المبرمج.
بينما عائلة Bcl-2هي البروتينات الحارسة المركزية للموت المبرمج الداخلي والموت المبرمج الذي يحدث في بيوت الطاقة, Bax هو اول جين متجانس لBcl-2بروتين يعمل وكان كمنفذ للموت المبرمج للخلايا.

تم تصنيف الأشخاص المسجلين في هذه الدراسة إلى مجموعتين من المرضى الذين يعانون من متلازمة تكيس المبايض ومجموعة سيطرة. تم تنفيذ ما مجموعه 66 مريضا و 64 مشاركا في مجموعة السيطرة الذين كانوا يحضرون في مستشفى النسائية والتوليد التعليمي في كربلاء ، العراق ، للفترة من ايلول 2021 إلى حزيران 2022. تم إجراء الاختبارات المختبرية بواسطة التقنيات المصلية ELISA الشطيرية للمرضى, وتم اختبارها لمصل بشري محدد HSP90B1 وBax و Bcl-2.

أظهرت نتيجة الدراسة الحالية أن HSP90B1 و Bax له ارتباط معنوي ($P<0.05$) ,بينما Bcl-2 لم يكن له ارتباط معنوي ($P>0.05$)حسب نشاط المرض.

في الختام يمكن أن تكون HSP90B1 و Bax كمؤشرات حيوية لتقييم التسبب في امراضية متلازمة تكيس المبايض.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء / كلية الطب

فرع الأحياء المجهرية

# تقييم بروتينات Heat Shock Protein 90B1 (HSP 90B1), Bcl-2 Associated X Protein (Bax) و B-cell Lymphoma 2 (BCL-2) وعلاقتها مع إمراضية متلازمة تكيس المبايض

رسالة مقدمة الى

مجلس كلية الطب/ جامعة كربلاء

كجزء من متطلبات نيل شهادة الماجستير في الأحياء المجهرية الطبية

من قبل

اسراء عبد الرسول عباس نفطي

بكالوريوس علوم الحياة, كلية العلوم, جامعة كربلاء (2015).

بأشراف

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