**Ministry of Higher Education and Scientific Research**

**University of Kerbala**

**College of Pharmacy**

# *Impact of Genetic Polymorphism of Cytochrome P450 2D6 on Clomiphene Citrate Clinical Response in Iraqi Women with PCOS*

***A Thesis***

***Submitted to the Council of the College of Pharmacy*/ University of Kerbala *as Partial Fulfillment of the Requirements for the Degree of Master of Science in Pharmacology and Toxicology***

***By***

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2025 AD 1446 AH


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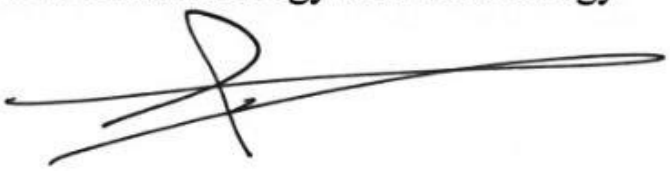

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# *Dedication*

*To all women battling infertility and PCOS……*

*Your strength and hope for motherhood have been the driving force behind this work. May this research bring hope and progress for you and others on the same path.*

*To my beloved parents……*

*Your unwavering support and faith in me are the foundation of all my accomplishments.*

*To my dear sisters….*

*Your encouragement, understanding, and uplifting presence strengthened me throughout this journey.*

*To my amazing friends….*

*Your constant support made every challenge easier to face.*

*And to everyone who offered help along the way….*

*Whether in small or significant ways, your contributions are deeply valued and appreciated.*

***Maryam***

# Acknowledgements

I first bow my head in deepest gratitude to **Allah**, the Almighty, whose grace illuminated my path at every turn. All credit is attributed to His perfection, and to **Allah** alone all praise is due:

My sincere appreciation extends to the esteemed College of Pharmacy, University of Kerbala, an institution that nurtured my academic growth and provided the fertile ground for this research.

I thank the Dean, **Assist. Prof. Dr. Mohammed Ibrahim**, whose leadership and vision fostered an environment of intellectual curiosity and scholarly pursuit.

My sincere thanks also go to my Supervisor, **Prof. Dr. Atheer Majid Rashid AL- juhaishi**, whose dedicated involvement, constructive feedback, and insightful perspectives were invaluable in navigating the complexities of this research.

I also extend my heartfelt gratitude to my Second Supervisor, **Prof. Dr. Hassan Mahmood Abo Al-Maali**, for his support and guidance during my research at several stages

I would like to express my sincere appreciation to **Prof. Dr. Shatha Hussein Kadhim /**Head of the Department of Pharmacology and Toxicology for her nice encouragement and support within the department.

My deep thanks go to **Nadia Sahib Al Sharifi**, as well as all the gynecologists, medical staff, pharmacists, nurses, and laboratory staff at the Obstetrics and Gynecology Hospital in Kerbala. Your collaboration and willingness to assist with data collection were crucial to the success of this study.

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### Chapter One: Introduction

| List of Abbreviations | |
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| **Abbreviation** | **Key** |
| AE-PCOS | Androgen Excess and Polycystic Ovary Syndrome |
| AGA | Androgenetic alopecia |
| AIs | Aromatase Inhibitors |
| AMH | Anti-Mullerian Hormone |
| AR | Androgen Receptor |
| ARMS-PCR | Amplification Refractory Mutation System - Polymerase Chain Reaction |
| BMI | Body Mass Index |
| BPA | Bisphenol A |
| BPS | Bisphenol S |
| CC | Clomiphene Citrate |
| CVD | Cardiovascular Disease |
| CYP450 | Cytochrome P450 |
| DDGIs | Drug–Drug–Gene Interactions |
| DDIs | Drug–Drug Interactions |
| DGIs | Drug–Gene Interactions |
| DHEAS | Dehydroepiandrosterone Sulfate |
| E2 | Estradiol |
| ERs | Estrogen Receptors |
| FPHL | Female Pattern Hair Loss |
| FSH | Follicle-Stimulating Hormone |
| FSHR | Follicle-Stimulating Hormone Receptors |
| GnRH | Gonadotropin-Releasing Hormone |
| HA | hyperandrogenism |
| HPG | hypothalamic-pituitary-gonadal |
| HPO | Hypothalamic-pituitary-ovary |
| HPT | hypothalamic-pituitary-thyroid |
| IM | Intermediate Metabolizers |
| IR | Insulin Resistance |
| IVF | *In Vitro* Fertilization |
| IVM | *In Vitro* Maturation |
| LH | Luteinizing Hormone |

| | |
|---|---|
| LHCGR | Luteinizing Hormone/Choriogonadotropin Receptor |
| LHR | Luteinizing Hormone Receptors |
| LPD | Luteal Phase Deficiency |
| NAFLD | Nonalcoholic Fatty Liver Disease |
| NIH | National Institutes of Health |
| NM | Normal Metabolizers |
| OCPs | Oral Contraceptive Pills |
| OHSS | Ovarian Hyperstimulation Syndrome |
| PCOS | Polycystic Ovary Syndrome |
| PM | Poor metabolizers |
| RFLP-PCR | Restriction fragment length polymorphism – polymerase chain reaction |
| SD | Standard Deviation |
| SERMs | Selective estrogen receptor modulators |
| SHBG | Sex hormone-binding globulin |
| SNP | Single-nucleotide polymorphisms |
| TSH | thyroid-stimulating hormone |
| UM | Ultrarapid Metabolizers |
| WADA | World Anti-Doping Agency |
| WHO | World Health Organization |

# Abstract


## Background

Clomiphene citrate, a selective estrogen receptor modulator (SERM), is commonly prescribed as a first-line agent to induce ovulation in women with infertility secondary to polycystic ovary syndrome (PCOS). It is predominantly metabolized in the liver by cytochrome P450 2D6 (CYP2D6), an enzyme characterized by substantial genetic polymorphism. Variations in the CYP2D6 gene may alter the bioactivation of clomiphene, thereby impacting its pharmacokinetics and therapeutic outcomes.

## Aims

This study aimed to determine the frequencies of two common CYP2D6 genetic variants—rs3892097 and rs1065852 —and to assess the impact of these single-nucleotide polymorphisms (SNPs) on the metabolic processing and clinical efficacy of clomiphene in women diagnosed with polycystic ovary syndrome (PCOS). Additionally, the study aimed to quantify the plasma concentrations of clomiphene and its active metabolite.

## Patients and Methods

A cohort study of 80 women with clinically diagnosed PCOS was recruited from the Gynecology and Obstetrics Hospital in Kerbala. Participants received 100 mg of clomiphene citrate daily for five consecutive days, beginning on day two of their menstrual cycle. Blood samples were collected at cycle day 2 before treatment and cycle day 12 after treatment for CYP2D6 genotyping, hormonal profiling (including LH, FSH, and estradiol), and quantification of clomiphene and its active metabolites via plasma analysis. As well as transvaginal ultrasound assessments were conducted during the

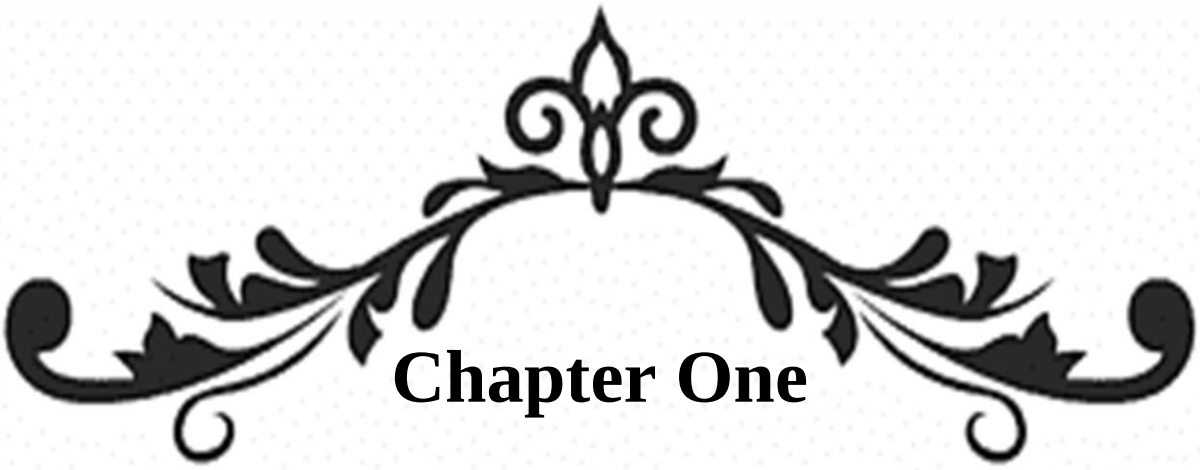
same cycle days by specialist doctors to evaluate ovarian follicular development and endometrial thickness.

**Results**

The study analyzed two CYP2D6 polymorphisms among women with PCOS-associated infertility. The genotype frequencies for CYP2D6*4 (GG, AG, AA) were 66.3%, 30.0%, and 3.8%, respectively, and for CYP2D6*10 (CC, CT, TT) were 66.3%, 27.5%, and 6.3% respectively. Individuals harboring the CYP2D6*4 and CYP2D6*10 variants were associated with significantly lower plasma concentration of the active metabolite of clomiphene and high levels of parent drug, indicating impaired metabolic activity. However, no significant differences ($P > 0.05$) were observed in hormonal profiles or ultrasound parameters among different genotypes.

**Conclusion**

CYP2D6*4 and CYP2D6*10 polymorphisms significantly affected the metabolic conversion of clomiphene to the active metabolite. However, they were not associated with differences in hormonal levels or ultrasound parameters. These findings suggest that while CYP2D6 genotyping may be useful for understanding individual pharmacokinetic profiles, its predictive value for clomiphene treatment outcomes in PCOS remains limited.



# Chapter One

# Introduction

# 1. Introduction

## 1.1. Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is a common and heterogeneous disorder that affects around (5 - 12) %of women of reproductive age. It is defined by clinical and/or biochemical excess androgen in the body, ovulatory failure, and polycystic ovaries (Reddy et al., 2011, Orio and Palomba, 2014). Obesity, diabetes, infertility, and miscarriage are more common in women with this syndrome of persistent anovulation and hyperandrogenism. It is often linked to compensatory hyperinsulinemia and insulin resistance (IR)(Rouzi and Ardawi, 2006). While PCOS may occur at any age, starting at menarche, most cases are reported in women between the ages of 20 and 30 (Bremer, 2010). Typically, problems that drastically lower a patient's quality of life (such as hair loss, alopecia, acne, and issues connected to infertility) are detected only after they have progressed (Azziz et al., 2004).

### 1.1.1. Epidemiology: Incidence & Prevalence

According to estimations of the World Health Organization (WHO), 116 million women worldwide (3.4%) suffered from PCOS in 2012 (Bharathi et al., 2017). In the Middle East and North Africa, there were 6,647,566 prevalent cases of PCOS in 2019. This is a 37.9% rise since 1990. In 2019, there were 236,312 incidents of PCOS, a 33.7% rise since 1990 (Motlagh Asghari et al., 2022).

According to research done in Spain, Greece, and the USA, the prevalence of PCOS is typically considered to be between 4% and 8% of all females at reproductive age. Among Chinese women who are of reproductive age, PCOS prevalence is 5.6% (Li et al., 2013). In the meantime, Iran does not have a high PCOS prevalence rate, although it does seem that PCOS symptoms are

becoming more severe (Jalilian et al., 2015). There is a 3.7% to 22.5% prevalence of PCOS in India (Ganie et al., 2019).

The prevalence of PCOS has increased over time, with estimates for 2020 indicating a range of 6% to 10% globally (Rao et al., 2020). And a recent study stated that the prevalence reached 15.1% (Suturina et al., 2025). The prevalence of PCOS among Iraqi women in Al-Hilla City was estimated to be almost 33%, higher than the global average (Mousa and Al Joborae, 2020).

### 1.1.2. Etiology of Polycystic Ovary Syndrome

#### 1.1.2.1. Androgen Excess

Androgen excess is considered a primary factor contributing to the development of PCOS symptoms. Hyperandrogenism is a result of both the adrenal glands and the ovaries producing too much androgen. In women, clinical hyperandrogenism manifests as hirsutism, acne, androgenic alopecia, and elevated testosterone levels (Aversa et al., 2020, Ashraf et al., 2019). There are three potential explanations for androgen hypersecretion. These include a pituitary Luteinizing Hormone (Olson and Ristau, 2025) hypersecretion leading to excessive thecal stimulation, an intrinsic functional thecal malfunction, or hyperinsulinemia resulting from insulin resistance (Cadagan et al., 2016).

#### 1.1.2.2. Insulin Resistance

IR and compensatory hyperinsulinemia (HI) are present in 65–95% of women with PCOS (Cassar et al., 2016). Insulin resistance is present in most cases, with compensatory hyperinsulinemia contributing to hyperandrogenism via stimulation of ovarian androgen secretion and

inhibition of hepatic sex hormone-binding globulin production (Goodarzi et al., 2011).

### 1.1.2.3. Obesity

Although obesity is one of the most prevalent characteristics of PCOS, it also acts as a separate risk factor for a number of other disease states that have been linked to PCOS (Bates and Legro, 2013). There are linkages between PCOS and the rising rate of obesity. Being overweight or obese is one of the leading chronic diseases in the world because obesity-related insulin resistance makes PCOS symptoms worse overall (Teede et al., 2010). Higher levels of insulin in the blood are commonly linked to obesity, which in turn increases the production of androgens in the ovaries. These androgens are aromatized to estrogen by the excess adipose tissue, which has a negative feedback on the hypothalamic-pituitary-ovary (HPO) axis and gonadotropin production (Cena et al., 2020).

### 1.1.2.4. Gonadotropin Abnormalities

The primary cause of gonadotropin abnormalities is a malfunction in gonadotropin production, which results in an imbalance in the levels of follicle-stimulating hormone and luteinizing hormone (Longobardi et al., 2024), raising the ratio of LH to FSH (Ibrahim et al., 2020). Elevated LH stimulates theca cells in the ovaries to produce excess androgens, which contribute to anovulation and hyperandrogenism seen in PCOS (Coutinho and Kauffman, 2019). LH plays a critical role in the female menstrual cycle by triggering ovulation and stimulating the corpus luteum to produce progesterone. However, persistent elevation of LH in PCOS leads to premature luteinization and disrupted follicular development. This may result in the early maturation of oocytes, impairing their quality and reducing

fertilization potential (Homburg et al., 1988, Wang and Li, 2023). Increased LH pulse frequency is a key pathophysiological feature in PCOS and is linked to hyperandrogenism and menstrual irregularities (Kumar and Sait, 2011).

#### 1.1.2.5. Genetic Factors

While the genetic etiology of PCOS is not yet fully understood, it can occasionally manifest in familial clusters. Many genes are proposed as potential causes of PCOS (Ben-Shlomo, 2003). Genome-wide association studies (GWAS) have identified over 240 candidate genes implicated in PCOS pathogenesis, many of which are involved in hormonal regulation, insulin signaling, and ovarian function (Ajmal et al., 2019). Prominent gene variants include those encoding the androgen receptor (AR), luteinizing hormone/choriogonadotropin receptor (LHCGR), follicle-stimulating hormone receptor (FSHR), and insulin receptor (INSR). These genes contribute to key phenotypic features such as hyperandrogenism, insulin resistance, and anovulation(Shi et al., 2012).

#### 1.1.2.6. Environmental Factors

Potential environmental risk during the prenatal and postnatal periods has been examined, given the early manifestation of PCOS, which typically occurs around adolescence. Evidence suggests that environmental factors may be important in the decline of reproductive health, and that some environmental factors, such as environmental pollutants, diet and nutrition, socioeconomic position, and geography are crucial in this decline (Merkin et al., 2016). Agents in the environment can disturb hormones. The pathophysiology of PCOS may involve elevated levels of bisphenol A (BPA) and bisphenol S (BPS) that mimic estrogen and interfere with hormonal homeostasis. Prenatal exposure to these compounds has been linked to altered

glucose metabolism, insulin resistance, and features resembling PCOS in animal models (Alonso-Magdalena et al., 2006).

### 1.1.3.Pathophysiology of Polycystic Ovary Syndrome

It is thought that IR with compensatory hyperinsulinemia and hyperandrogenemia are the cornerstones of PCOS pathogenesis because IR is so prevalent in the condition. The precise underlying pathogenic processes of PCOS are not fully understood (De Leo et al., 2016, Nestler, 1997). The primary modulator of protein, fat, and glucose metabolism is insulin. Insulin functions as a gonadotropin co-factor. It specifically affects ovarian function by increasing the effect of LH on theca cells, which helps the ovaries secrete androstenedione, an androgen (Diamanti-Kandarakis and Dunaif, 2012). When insulin resistance develops, the elevated insulin plasma levels cause the ovaries to be overstimulated, which leads to an excess of androgens being produced (Genazzani and Genazzani, 2023). A decrease in follicle-stimulating hormone results in anovulatory ovaries, polycystic ovaries, and increased luteinizing hormones, which raise the amount of androgen in the blood (Layacha and Biswas, 2023). The metabolic complications of PCOS like dyslipidemia are exacerbated by obesity and the sedentary lifestyle, poor diet, and overeating that go along with it. (Azziz, 2016).

### 1.1.4. Clinical Symptoms of Polycystic Ovary Syndrome

The clinical signs and symptoms of PCOS include acne, hirsutism, and androgenic alopecia, which are manifestations of clinical hyperandrogenism (Jalilian et al., 2015). Both androgen excess and the unique reaction of the pilosebaceous unit to androgens are linked to hirsutism in PCOS (Spritzer et al., 2022). Because hyperandrogenism increases sebum production, which

contributes to the pathophysiology of adult acne, it is more likely for acne to develop in in women with PCOS (Carmina et al., 2022).

Although there are few research looking at the effects of female pattern hair loss (FPHL) among PCOS patients, it has been observed that FPHL is more prevalent and related with PCOS. The pathogenesis of FPHL remains unclear despite its association with hyperandrogenism, as the condition can coexist with normal biochemical androgen indicators (Jiang et al., 2022).

### 1.1.5. Diagnosis of Polycystic Ovary Syndrome

There are three sets of criteria dependent on the identification of PCOS, including the National Institutes of Health (NIH) criteria (1992), Rotterdam criteria (2003), and Androgen Excess Society criteria (2006) (Rao et al., 2020).

PCOS was first defined as a hyperandrogenic disorder of exclusion with an ovarian etiology and/or consequences in 1990 by the National Institute of Child Health and Human Development Conference. This recommendation included both clinical/biochemical hyperandrogenism and chronic anovulation as necessary for the diagnosis and the exclusion of other known disorders (Zawadeski and Dunaif, 1992).

The European Society of Human Reproduction and Embryology and the American Society for Reproductive Medicine then sponsored a consensus workshop group that suggested that at least two of the three criteria—oligo-anovulation, clinical/biochemical hyperandrogenism, and the appearance of polycystic ovary on ultrasonography—be required (Rotterdam, 2004).

Most recently, It was recommended by the Androgen Excess and PCOS (AE-PCOS) Society that ovulatory disruption, such as oligo-anovulation or

PCO, be required in addition to clinical or biochemical hyperandrogenism as a prerequisite for diagnosis (Azziz et al., 2006).

The classification of Rotterdam is the most used, but with varying frequency depending on the country and medical specialties (Dewailly, 2016).

### 1.1.6. Screening Test

In PCOS, Small follicle growth is enhanced, but this is followed by a growth arrest that results in the characteristic polycystic morphology (Ibáñez et al., 2017). The evaluation of ovarian morphology can now be done non-invasively with the help of ultrasound. Pelvic ultrasonography reveals the existence of polycystic ovaries when more than 20 follicles are measuring 2 to 9 mm and an ovarian number of more than 10 (Kriedt et al., 2019).

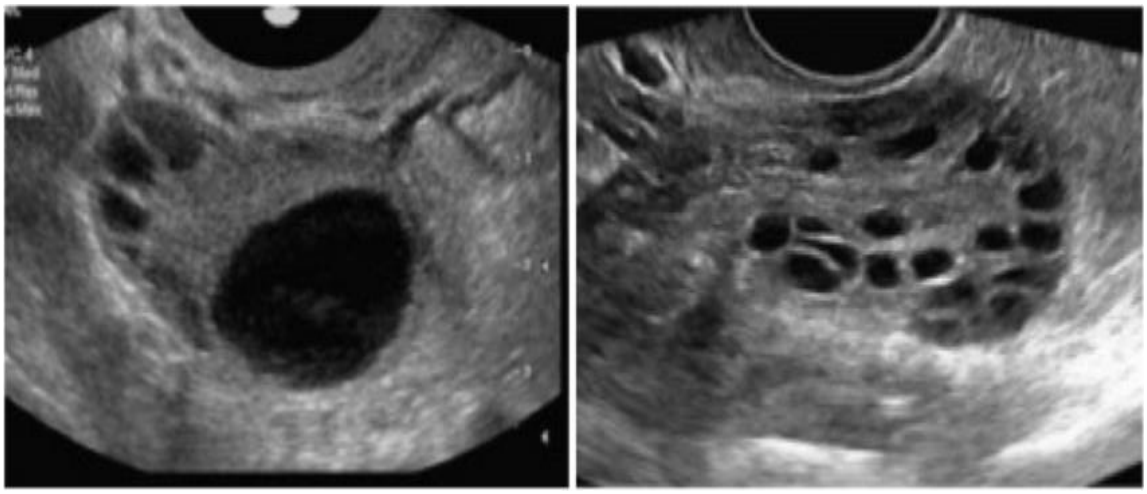


**Figure (1 – 1): ultrasound image of a normal ovary(the left) and a PCOS ovary (the right) (Shanmugavadivel et al., 2024).**

### 1.1.7. Laboratory Assessment

As a surrogate marker for ovarian morphology, anti-Mullerian hormone (AMH) is a polypeptide released by granulosa cells of the preantral and small

antral ovarian follicles. PCOS women may have much greater levels of AMH (Christ and Cedars, 2023).

Excess androgen secretion is a key clinical feature of PCOS. Approximately 50–75% of women with PCOS exhibit elevated levels of total or free testosterone and dehydroepiandrosterone sulfate (DHEAS) (Xu and Qiao, 2022, Huang et al., 2010).

Fasting insulin concentration is widely regarded as a simple and practical marker for assessing IR, particularly in clinical settings. Numerous studies have demonstrated that elevated fasting insulin levels, even in individuals with normal glucose tolerance, are strongly associated with IR and may predict the future development of type 2 diabetes. Among women with PCOS, fasting insulin is frequently recommended as a convenient, office-based tool for identifying insulin resistance (Amisi, 2022).

In addition, gonadotropin release in PCOS was observed to have much higher LH secretions compared to normal FSH abundance (Xu and Qiao, 2022). As a result of the raised LH/FSH ratio, ovulation does not occur in polycystic ovary disease patients (Saadia, 2020). Despite remaining within acceptable ranges, serum FSH concentrations are typically lower in PCOS-afflicted women than in those with healthy ovaries (Homburg and Crawford, 2014).

Abnormal levels of thyroid-stimulating hormone (TSH) can significantly impact both female metabolism and reproductive system function. In women with PCOS, endocrine hormone imbalances—particularly disruptions in the hypothalamic-pituitary-gonadal (HPG) axis—can also influence the hypothalamic-pituitary-thyroid (HPT) axis, leading to alterations in thyroid regulation. Consequently, studies have reported that serum TSH levels in

PCOS patients are often higher than those in the general female population. TSH is considered the most sensitive and widely used biomarker for evaluating thyroid gland function and detecting both overt and subclinical thyroid disorders(Jo et al., 2023).

Sex hormone-binding globulin (SHBG)—a plasma glycoprotein that binds to estrogens and androgens, thereby regulating their bioavailability and activity—is commonly used as an indirect marker of hyperandrogenism in PCOS. Reduced serum SHBG levels are considered a reliable biomarker of metabolic dysfunction in these patients and have been strongly associated with IR, hyperandrogenism (HA), and disturbances in glucose and lipid metabolism(Xing et al., 2022).

### 1.1.8. Complication

#### 1.1.8.1. Infertility

PCOS is one of the most common causes of anovulatory infertility in women. Many PCOS-affected women have elevated levels of insulin, testosterone, and luteinizing hormone. They also have decreased levels of follicle-stimulating hormone (Hussain et al., 2025), which can lead to amenorrhea or oligomenorrhea (Zehravi et al., 2021).

#### 1.1.8.2. Gestational complication

A growing amount of data indicates that PCOS may increase the likelihood of unfavorable pregnancy outcomes. These include gestational diabetes, preterm delivery, low birth weight, hospitalization to the newborn critical care unit, hypertensive disease/preeclampsia, and an increased risk of cesarean delivery (Schneider et al., 2019). PCOS women are 10 times more likely to acquire gestational diabetes and up to 5 times more likely to have

insulin-related problems, such as spontaneous miscarriage, due to the high frequency of insulin resistance in PCOS (Randeva et al., 2012).

#### 1.1.8.3. Cardio-metabolic Abnormalities

Insulin resistance plays a major role in the pathophysiology of PCOS, increasing the risk of cardiovascular disease (CVD) in women by causing several cardio-metabolic abnormalities like dyslipidemia, hypertension, glucose intolerance, diabetes, and metabolic syndrome (Osibogun et al., 2020). Women diagnosed with PCOS exhibit reduced levels of high-density lipoprotein cholesterol and elevated levels of triglycerides and low-density lipoprotein cholesterol (Guan et al., 2022).

Women with PCOS appear to have a higher risk of hypertension, at least in their later years after conception. Premenopausal PCOS women have a greater estimated prevalence of hypertension (9–25.7%), primarily systolic, than the overall population (Anagnostis et al., 2018).

#### 1.1.8.4. Gynecological Malignancies

Women with PCOS may be more susceptible to endometrial cancer, an estrogen-sensitive condition, as a result of their protracted anovulation, which is exacerbated by exposure to endogenous or exogenous estrogen that is not counteracted concurrently by a progestogen (Harris and Terry, 2016).

#### 1.1.8.5. Nonalcoholic Fatty Liver Disease

Nonalcoholic fatty liver disease (NAFLD) is one of the most prevalent fo rms of chronic liver disease in the Western world (Spremović Rađenović et al., 2022). It has been stated that patients with PCOS have a higher prevalence of NAFLD. Insulin resistance and obesity are thought to be the primary causes of NAFLD (Vassilatou, 2014).

#### 1.1.8.6. Mental Health Disorders

The scientific community is paying greater attention to the psychological effects of PCOS. Compared to the general population, women with PCOS had greater rates of anxiety, depression, and affective disorders (Dokras, 2012). These patients have higher rates of stressful life events, which can lead to psychological problems. Contributing factors may include physical manifestations such as hirsutism and obesity, reproductive issues like infertility, and psychological stressors, including low self-esteem and negative body image (Bazarganipour et al., 2013).

### 1.1.9. Management

The goals of PCOS management include weight reduction, improvement of insulin sensitivity, treatment of acne and hirsutism, restoration of regular menstruation, and prevention of associated complications (Baban et al., 2018). Women with PCOS should undergo a comprehensive lipid profile assessment. Lifestyle modifications, including diet and exercise, are considered the first-line treatment, particularly for those with dyslipidemia (Kim and Choi, 2013). There are many other different treatment options, but the fundamental therapeutic concepts are based on ovarian stimulation with FSH, a drop in insulin concentrations, and a decrease in LH levels (Homburg, 2003).

#### 1.1.9.1. Nonpharmacological Treatment

##### 1.1.9.1.1. Diet and Nutrition

Weight loss achieved through dietary interventions in overweight or obese women with PCOS reduces total and abdominal fat mass, thereby improving ovulatory function and, in many cases, restoring regular menstrual cycles and fertility (Gambineri et al., 2002). Additionally, losing weight raises levels of sex hormone-binding protein and decreases androgen release; these effects are

at least partially mediated by improving insulin resistance and lowering blood insulin concentrations (Escobar Morreale, 2008). Even in the absence of noticeable weight loss, effective nutrition and exercise programs enhance reproductive health, endocrine characteristics, and cardiometabolic risk profile. It is recommended that calories be consumed in multiple meals throughout the day, with minimal consumption of snacks and beverages (Farshchi et al., 2007).

#### 1.1.9.1.2. Physical Activity

Physical activity is a key component in the primary management of PCOS. Studies suggest that vigorous aerobic exercise can improve body composition, cardiorespiratory fitness, and insulin resistance. Additionally, reducing sedentary behavior and incorporating strength training exercises twice weekly are recommended strategies (Cowan et al., 2023).

### 1.1.9.2. Pharmacological Therapy

#### 1.1.9.2.1. Oral Contraceptive Pills

For women with PCOS, oral contraceptive pills (OCPs) have been the first-line medication for the concurrent management of monthly irregularity, acne, and hirsutism (Shah and Patil, 2018). Women with metabolic syndrome, hypertension, obesity, cerebro-cardiovascular disease, breast or endometrial cancer, and those with a history of venous thromboembolism need to be prescribed these medications cautiously. Persistent amenorrhea in PCOS following withdrawal is a relatively common side effect related to patients with previous oligo-amenorrhea, which is caused by a prolonged block of the hypothalamus (Armanini et al., 2016).

#### 1.1.9.2.2. Antiandrogens

Symptoms of PCOS that are associated with hyperandrogenism may be decreased with anti-androgens and OCPs (Alesi et al., 2023). Spironolactone is a synthetic 17-lactone steroid that has a modest affinity for both progesterone and androgen receptors and functions as a non-selective antagonist of mineralocorticoid receptors. Spironolactone also had superior effects on hirsutism, menstrual cycle problems, and hormonal disturbances (Alpañés et al., 2017).

#### 1.1.9.2.3. Antiestrogens

##### 1.1.9.2.3.1. Selective Estrogen Receptor Modulators

Selective estrogen receptor modulators (SERMs) exert partial agonist and antagonist effects according to the tissue estrogen receptor content and estrogen availability level. The hypothalamus pituitary unit is negatively impacted by circulating estrogen, and selective estrogen receptor modulators work by preventing this influence. The three SERMs that are frequently used in the management of PCOS women are tamoxifen, raloxifene, and clomiphene citrate (An, 2016). Since its debut into clinical practice in the 1960s, clomiphene citrate has been used extensively in the treatment of infertility (Aghassa et al., 2007, Greenblatt et al., 1961).

##### 1.1.9.2.3.2. Aromatase Inhibitors

In 2001, aromatase inhibitors (AIs), which were previously utilized to treat breast cancer in postmenopausal women, were first suggested as novel ovulation-inducing medications for anovulatory women who did not respond well to CC (Misso et al., 2012). Anastrozole and letrozole are nonsteroid competitive inhibitors of aromatase (Kharb et al., 2020).

#### 1.1.9.2.4. Insulin Sensitizers

Metformin is the most common insulin sensitizer that has been investigated as a potential treatment for women with PCOS who have increased insulin resistance by increasing the sensitivity of insulin receptors in peripheral cells (Palomba et al., 2013).

## 1.2. Clomiphene Citrate

Clomiphene citrate (CC) is a nonsteroidal ovarian stimulant that was approved for medical uses in the United States and the WHO in 1967 and most frequently recommended medicine to induce ovulation. When treating normogonadotrophic oligo/amenorrhoeic infertility, which is primarily linked to polycystic ovaries, this medicine is the first choice (Nasseri and Ledger, 2001, Sha et al., 2024). Clomiphene is thought to be a selective estrogen receptor modulator (SERM), meaning that it can attach to ERs and change how estrogen functions (Kurosawa et al., 2010).

An uneven mix of two geometric isomers as their citrate salts is present in every CC tablet. These E- and Z-forms, often known as Z- and E-clomiphene, make up approximately 38% and 62% of the entire medication, respectively (Ghobadi et al., 2009b) seen in Figure (1 - 2).

SERM are receptor ligands that, depending on the cell and tissue context, display either agonistic or antagonistic bio-character (Goldstein et al., 2000). The medicine functions as either an estrogen receptor agonist or an antagonist depending on the isomer, the target tissue, the type of receptor, and the concentration (Euler et al., 2022). E-clomiphene is antiestrogenic centrally and estrogenic peripherally and Z-clomiphene is the other way round (estrogenic centrally and antiestrogenic peripherally) (Nagori, 2021).

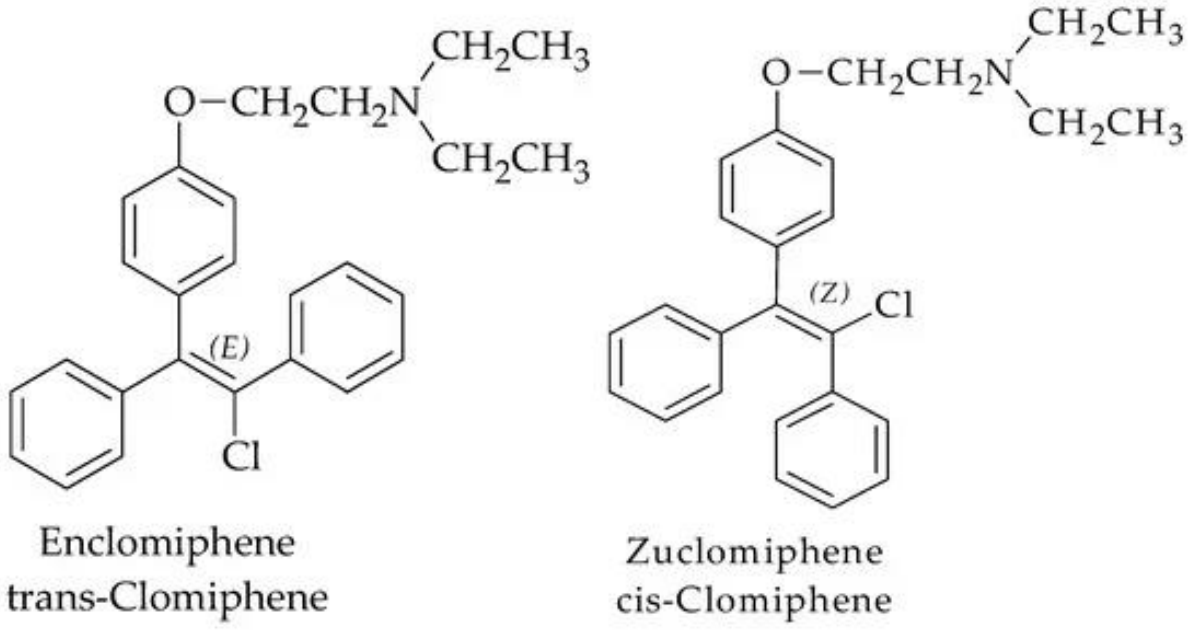



**Figure (1 - 2) : Enclomiphene and Zuclomiphene as trans and cis isomers.(Girase et al., 2023)**

### 1.2.1. Pharmacodynamics

The anterior pituitary can release FSH when clomiphene is administered, and this is frequently sufficient to restart the sequence of events that leads to ovulation (Homburg, 2005).

At the hypothalamus level, CC selectively modulates estrogen receptors to provide anti-estrogenic effects. GnRH's pulsatile release is improved by Clomiphene. As a result, the anterior pituitary gland will secrete more endogenous gonadotropins, particularly FSH, which will promote cyclic follicular growth by inhibiting the negative feedback of estradiol on the hypothalamus. Follicle growth and ovulation are consequently triggered by the release of LH and FSH (Jayasena and Franks, 2014).

### 1.2.2. Pharmacokinetic

Clomiphene citrate is efficiently absorbed from the gastrointestinal tract when administered orally with bioavailability exceeding 90% (Rostami-Hodjegan et al., 2004). Clomiphene is a prodrug being activated through the cytochrome P450 pathway into 4-hydroxyclomifene and 4-hydroxy-N-desmethylclomifene. After CC is administered, the E-clomiphene level rises quickly before falling quickly to an undetectable concentration. After therapy, z-clomiphene remains in the body for more than a month and exerts a cumulative effect over subsequent treatments (Gupta and Khanna, 2018). The high affinity for plasma proteins, entry into the enterohepatic cycle, and storage in adipose tissues prolong the half-life of CC clearance (5 days). It has an onset of action of 5 to 10 days following the course of treatment (Kim et al., 2018). Through hepatic transformation, CC is metabolized and eliminated through the stool (Kahyaoğlu et al., 2017).

### 1.2.3. Medical Uses of Clomiphene Citrate

#### 1.2.3.1. Ovulation Induction

Clomiphene citrate is one of the main medications used to stimulate ovulation in PCOS treatment. It has been demonstrated that in about 30% of cases, clomiphene citrate can lead to pregnancy (Al-Thuwaynee and Swadi, 2023).

#### 1.2.3.2. Luteal Phase Deficiency

Low follicular phase FSH levels are linked to short luteal phase, which results in luteal phase defect (LPD). In the follicular phase, Clomiphene will boost FSH and LH, create healthy follicles, and fix any abnormalities in the luteal phase. For LPD, CC is the recommended medication because of its brief luteal phase (Medicine, 2012).

### 1.2.3.3. Unexplained Infertility

Clomiphene citrate is used to induce ovulation in women with unexplained infertility, aiming to enhance the chances of conception by stimulating the development of a dominant follicle (Hughes et al., 2010).

### 1.2.3.4. Off-Labeling

Treatment for male hypogonadism with clomiphene can be used off-label. For certain patients with male factor infertility who also report hypogonadal symptoms, CC is thought to be a beneficial treatment (Herzog et al., 2020). CC raises testosterone and may raise semen parameters, while some men may not show any increase in testosterone (Patel et al., 2015). Because it increases serum levels of gonadotropin and testosterone, clomiphene citrate is also known to be abused by healthy athletes seeking to boost their performance (Miller et al., 2018). CC is another well-known doping agent that is used to lessen the negative effects of anabolic steroid usage. As a result, it is now outlawed by the World Anti-Doping Agency (WADA) (Euler et al., 2023).

## 1.2.4. Adverse Effects

In general, clomiphene citrate is well tolerated. While some adverse effects are somewhat prevalent, they are rarely severe or prolonged enough to affect the outcome of the subsequent cycle of treatment or the standard 5-day course of treatment (Rotterdam, 2004). Patients should be informed about potential mood-related side effects, such as irritability and mood swings, associated with clomiphene citrate therapy (Choi et al., 2005). Moreover, the ovarian hyper-stimulation syndrome (OHSS) and multiple gestations can complicate the use of clomiphene citrate, increasing the risk of premature birth and the related expenditures and morbidity of the newborn (Diamond et al., 2015). Since OHSS is an iatrogenic side effect of treatment for infertility.

Ovarian cystic expansion and a fluid shift from the intravascular to the interstitial space, resulting from ovarian neoangiogenesis and enhanced capillary permeability, are the hallmarks of this condition (Kumar et al., 2011).

### 1.2.5. Clomiphene Interaction

Cytochrome P450 (CYP) 2D6 and CYP3A4 are the main enzymes that eliminate clomiphene and its metabolites; exposure to clomiphene may be impacted by CYP2D6 polymorphisms and concurrent use of CYP inhibitors (Kovar et al., 2022). Drug-gene interactions (DGIs), drug-drug interactions (DDIs), and drug-drug-gene interactions (DDGIs) may occur when using clomiphene treatment. As Table (1 – 1) shows examples of drugs affect each enzyme(Hanke et al., 2018, Fuhr et al., 2021).

**Table (1 – 1): Drugs that induce/inhibit CYP2D6 and CYP3A4 enzymes**

| | |
|---|---|
| CYP2D6 enzyme inducers | Dexamethasone |
| CYP2D6 enzyme inhibitors | Fluoxetine |
| CYP3A4 enzyme inducers | Carbamazepine |
| CYP3A4 enzyme inhibitors | Clarithromycin |

### 1.2.6. Metabolism of Clomiphene

Phase I metabolic reactions involve the metabolism of clomiphene, primarily through CYP2D6 and CYP3A4 enzymes, which play a major role, and to a lesser extent, with CYP3A5, CYP2B6, CYP2C9, and CYP2C19 isoforms (Mazzarino et al., 2013). The primary metabolites of E-clomiphene are CYP2D6, while CYP3A4 and CYP3A5 are responsible for Z-clomiphene metabolism (Robin et al., 2021) as shown in Figure (1 - 3).

CYP2D6 breaks down clomiphene by hydroxylating one of the phenyl rings at the para position, producing 4-hydroxyclomiphene as a metabolite.

Although it also undergoes N-demethylation but N-desethylclomiphene is not as active as the parent drug. 4-hydroxy metabolite of N-desethylclomiphene is also active and is thought to have a 50-fold higher contribution than the parent drug (Obach, 2013).

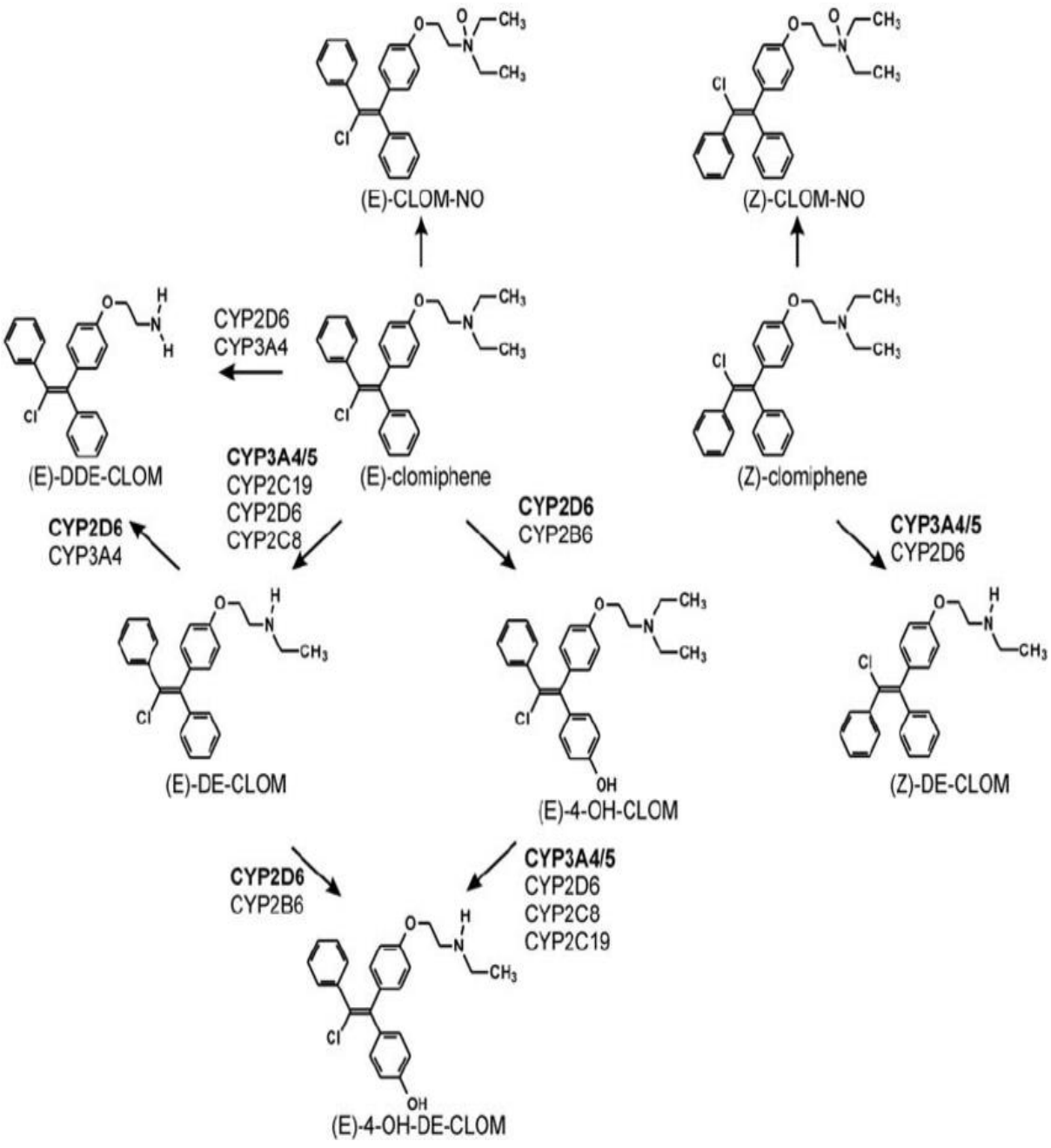



**Figure (1 - 3) : Human metabolism of (E)- and (Z)-clomiphene (Mürdter et al., 2012)**

## 1.3. Cytochrome P450

The first-pass metabolism of drugs and foreign substances in the body is carried out by a broad family of heme-containing monooxygenase enzymes known as CYPs (Schneider and Clark, 2013).

### 1.3.1. Cytochrome P450 2D6

The location of the CYP2D6 enzyme's gene is on chromosome 22 (22q13.2) (Taylor et al., 2020) comprises nine exons and encodes a protein that localizes to the endoplasmic reticulum. It is predominantly expressed in the liver, with additional expression in the brain, intestinal tissue, and lymphoid cells. The CYP2D6 gene exhibits a high degree of polymorphism (Dutheil et al., 2009). CYP2D6 is a crucial enzyme in drug metabolism, responsible for the oxidative metabolism of approximately 20–25% of commonly prescribed medications, despite constituting only about 2–4% of hepatic CYP content (Zanger et al., 2004, Wendt et al., 2018). Antidepressants, antiarrhythmics, beta-blockers, and opioid analgesics are typical substrates of CYP2D6. Hepatic CYP2D6 expression varies significantly among individuals; some possess nonfunctional alleles resulting in the absence of active enzyme (Tirona and Kim, 2017).

CYP2D6 phenotypes include poor (PM), intermediate (IM), normal (NM, previously termed 'extensive'), and ultrarapid metabolizers (UM), classifications that help predict the efficiency of drug metabolism (Koopmans et al., 2021). Standard dosages of the majority of drugs metabolized by CYP2D6 should normally be tolerated and well-tolerated by normal metabolizers who have at least one fully functional CYP2D6 allele as well as intermediate metabolizers who are carriers of two reduced-function alleles or one reduced-function and one null allele (Gaedigk et al., 2019, Haslemo et al.,

2019). Those who have two nonfunctioning alleles and are unable to bioactivate or metabolize medicines via the CYP2D6 pathway are considered poor metabolizers. Ultrarapid metabolizers, on the other hand, are individuals who possess two or more copies of a functional allele on a single chromosome, together with at least one higher function allele, making them on the extreme end of the spectrum. Depending on the specific substrate involved, these two metabolizer groups are most likely to have dose-related adverse effects or therapy failure (Gaedigk et al., 2017).

### 1.3.2. Effect of Cytochrome P450 2D6 Polymorphisms on the Therapeutic Response of Clomiphene

Clomiphene Citrate exhibits inter-individual variability in treatment response. 73% of cases achieve ovulation with dosages ranging from 50mg to 150mg (Homburg, 2005). The success of clomiphene treatment relies heavily on the activity of the highly polymorphic CYP2D6 enzyme, which is responsible for bio-activation of E-clomiphene to the active metabolite (E)-4-OH-clomiphene which has the highest inhibitory affinity towards the estrogen receptor (Kovar et al., 2022).

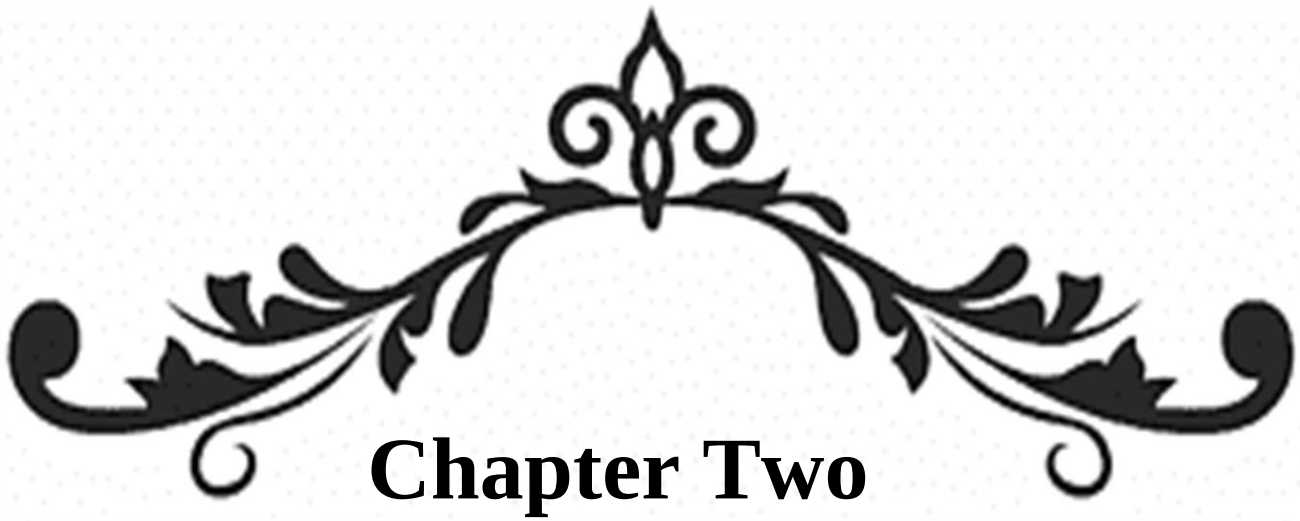
CYP 2D6*4 G1934A is a single-nucleotide polymorphism (SNP) located at the splice junction between intron 3 and exon 4. The presence of this variant disrupts the normal mRNA splicing process. This disruption leads to a frameshift mutation, which alters the reading frame of the downstream coding sequence and ultimately results in the non-functional CYP2D6 protein. The G1934A substitution is recognized as a primary genetic defect responsible for poor metabolizer (PM) status in the CYP2D6 locus and may require adjusted dosages for optimal response. In approximately 5-10% of the Caucasian population detect a homozygous genotype (Levkovich et al., 2011). In

Germany study, since CYP2D6-dependent pathways are the primary mechanism for the formation of (E)-4-OH-clomiphene, PM exhibited the highest exposure for the parent drug and the lowest area under the curve (AUC) for the active metabolite (Kovar et al., 2022) as well as PM German subjects who lacking functional CYP2D6 showed equal AUCs for both Clomiphene isomers indicating a defective metabolism (Mürdter et al., 2012).

As CYP2D6*10 (rs1065852) represent intermediate metabolizers is a cytosine-to-thymine substitution (C>T) at nucleotide 100 in the CYP2D6*10 allele, resulting in a modification of proline-to-serine at codon 34 and it is associated with reduced metabolism of various CYP2D6 substrates. it is the most prevalent variant in East Asian ethnicities, including Japanese, Korean, and Chinese (Bagheri et al., 2015). In a Korean study, the CYP2D6*10 allele was identified as the most prevalent variant (Ji et al., 2016). Furthermore, Iranian women who had allele *10 showed some clomiphene therapy resistance if compared to women who did not have the allele (Afsharian et al., 2013).

## 1.4. The aims of the study

1. To investigate the distribution of genetic polymorphisms of phase I metabolizing enzyme CYP2D6, especially the allele CYP2D6*4 (1934G > A (rs3892097)) and CYP2D6*10 (100 C>T (rs1065852)) among Iraqi poly cystic ovary syndrome women.
2. To evaluate the effects of these CYP2D6 genetic polymorphisms the clinical response to clomiphene citrate treatment in Iraqi PCOS.
3. to measure plasma concentration of E-clomiphene and its metabolite.



# Chapter Two

## Patients, Materials, and Methods

# 2. Patients, Materials, and Methods

## 2.1. Patients

This study included 80 women aged 20 to 35 years, diagnosed with PCOS based on the Rotterdam criteria (Rotterdam, 2004). The patients were recruited from the infertility outpatient clinic of the Gynecology and Obstetrics Hospital in Kerbala between November 2023 to April 2024.

### 2.1.1. Patient Criteria

#### 2.1.1.1. Inclusion Criteria

Women aged 20 to 35 years who had been unable to conceive naturally for over one year, diagnosed with PCOS based on the Rotterdam criteria (presence of at least two of the following: oligo/anovulation, clinical or biochemical hyperandrogenism, and polycystic ovaries on ultrasound), and were planning to receive clomiphene citrate at a dose of 50 mg twice daily (total 100 mg/day) via oral administration for at least two treatment cycles.

#### 2.1.1.2. Exclusion Criteria

Patients were excluded from this study if they had started clomiphene citrate therapy simultaneously with adjuvant therapy, such as insulin-sensitizing drugs (like metformin), lipid-lowering agents (like atorvastatin), or women taking drugs that affect the activity of the CYP2D6 enzyme (inducers or inhibitors). Any patients who had problems with the thyroid gland, hyperprolactinemia or severe systemic illness.

### 2.1.2. Ethical and Scientific Approval

The research proposal underwent ethical and scientific review by the Scientific and Ethical Committee of the College of Pharmacy, Kerbala University, and received subsequent approval. Additionally, the study was approved by the Karbala Health Department, Ministry of Health, Iraq, under approval number 2023180. All participants provided written informed consent following a detailed explanation of the study objectives and completed a specifically designed questionnaire before enrollment.

## 2.2. Materials

### 2.2.1. Chemicals and Kits

The chemicals and biochemical/genetic kits used in this study, along with their manufacturers and countries of origin, are listed in Table (2 – 1).

**Table (2 – 1): Chemicals and kits used with their Manufacturer and Origin.**

| Types | Materials | Company/origin |
|---|---|---|
| Chemicals | Agarose | Bio Basic/Canada |
| | Absolute Ethanol | Scharlau/ German |
| | Ethidium Bromide | Intron/Korea |
| | Deionized sterile D.W | Bioneer/Korea |
| | TBE buffer | Bioneer - Korea |
| | BstNI | BioLabs/USA |
| | Vaseline oil | Liofilchem/Italy |
| Biochemical Kits | AMH kit | Roche/Germany |
| | Estradiol kit | Roche/Germany |

| | | |
|---|---|---|
| | Prolactin kit | Roche/Germany |
| | LH | Roche/Germany |
| | FSH | Roche/Germany |
| Genetic Kits (Primers) | gSYNC™ DNA Extraction Kit | Geniod/Taiwan |
| | DNA ladder marker | Bioneer/Korea |
| | Master Mix | BioLabs/ USA |
| | Primers | Macrogen/Korea |

### 2.2.2. Instruments

Many instruments used in the genetic and biochemical analysis of samples in this study with manufacturers and countries of origin, are listed in Table (2 – 2).

**Table (2 – 2): Instruments used with their Manufacturers and Origin**.

| Instruments | Manufacture and Origin |
|---|---|
| Centrifuge | Hettic/Germany |
| Cobas e 411 Analyzer | Roche/Germany |
| Digital timer | BIOBASE/China |
| Electrophoresis apparatus | Techne me/England |
| Freezer (−20 °C) | Concord /Lebanon |
| Gel & Clot Activator Blood Collection Tubes | AFCO/ Jordan |
| High-speed Centrifuge | Thermo Scientific™/USA |
| Hood | LabTech /Korea |
| $K_3$ EDTA tube | Nipigon Health Corp/Canada |
| LC-MS/MS | AB Sciex/USA |
| Micropipettes | SLAMED/UK |
| Nanodrop | Thermo Scientific™/USA |

| | |
|---|---|
| Refrigerator | Concord /Lebanon |
| Sensitive balance | DENVER/Germany |
| Thermos cycler (PCR apparatus) | Thermo Scientific™/USA |
| UV-trans illuminator | Syngene /England |
| Vortex | HumanTwist/Germany |
| Drying Oven | BIOBASE/China |
| Digital camera | Canon/England |

## 2.3. Methods

### 2.3.1. Study Design

A prospective cohort study was conducted from November 2023 to April 2024. The participants were enrolled at the onset of their menstrual cycle (day two) before drug initiation. Follow-up assessments were performed on cycle day 12 of at least two consecutive menstrual cycles post-drug administration. The data were obtained from the medical records of consenting females and the patients themselves. These include age, weight, date of first menarche, irregularity of menses, family history of any diseases, smoking, dose of CC, number of children (if present) and abortion if occurred as well as each patient was also asked whether she had used any medications known to interfere with clomiphene citrate metabolism to make sure that all the potentially interacting drugs were captured in the database. A qualified radiologist performed transvaginal ultrasound on the $2^{nd}$ day and $12^{th}$ day of the treatment cycle for each patient to evaluate follicular size and endometrial thickness.

### 2.3.2. Blood Collection

Blood samples were taken from eligible females who had signed informed consent. About 5 ml of venous blood was drawn from all females who

contributed to this study before taking CC on the 2nd day of their menstrual cycle. The blood sample was divided into two aliquots: 2 mL for genetic analysis (in EDTA tubes) and 3 mL for hormonal analysis (in clot activator tubes), and serum was obtained after centrifugation of blood at 5000 rpm for 10 minutes and used for hormonal assessment. During follow-up, another 5 ml of venous blood was drowned from all females on the 12th day of their menstrual cycle and divided into two parts as follows 2ml of blood was placed in a gel tube for measuring the drug and its metabolite's concentration after centrifuging and send to laboratory as it stored in freezing condition and 3ml was placed in gel tube, and serum was obtained after centrifugation of blood at 5000 rpm for 10 minutes for hormonal assessment.

## 2.3.3. Hormonal Assessment

### 2.3.3.1. Measurement of Serum Follicles Stimulating Hormone Level

A novel immunometric assay based on the sandwich principle is presented for the rapid quantification (18 minutes) of FSH levels. This assay leverages a pair of strategically designed monoclonal antibodies including biotinylated anti-FSH (monoclonal) which exhibits high affinity for a specific epitope formed by both subunits of the FSH molecule and ruthenium complex-conjugated anti-FSH (monoclonal) which specifically targets a distinct epitope located on the FSH beta subunit. The assay follows a two-step incubation process. In the first step, the sample is incubated with both antibodies, allowing them to form a specific "sandwich" complex with any FSH present. The second step involves the introduction of streptavidin-coated microparticles. These microparticles capture the biotinylated antibody, effectively immobilizing the entire FSH-antibody complex on a solid phase. Following a washing step to remove unbound substances, a voltage is applied

to the electrode, inducing a chemiluminescent reaction within the ruthenium complex. The intensity of this light emission is then quantified using a photomultiplier. Finally, the measured light signal is compared to a pre-established calibration curve, enabling the accurate determination of FSH concentration in the sample(Van Casteren et al., 2000).

### 2.3.3.2. Measurement of Serum Luteinizing Hormone Level

The Elecsys LH assay employs a sandwich principle and leverages a highly specific approach for rapid LH measurement (18 minutes). It utilizes two precisely designed monoclonal antibodies, each targeting unique regions (conformational epitopes) of the human LH molecule. One antibody, labeled with biotin, recognizes an epitope formed by both LH subunits, while the other, tagged with a ruthenium complex, binds specifically to an epitope on the beta subunit. This dual targeting strategy minimizes interference from other hormones, resulting in high assay specificity. In the first step, a sample containing LH is incubated with these antibodies, allowing them to bind to the LH molecule, forming a sandwich complex. The second step involves introducing streptavidin-coated microparticles. These particles capture the biotinylated antibody, effectively immobilizing the entire LH-antibody complex on a solid surface. Following a wash step to remove unbound materials, an electrical stimulus triggers a light-emitting reaction (chemiluminescence) within the complex. The intensity of this light emission is measured and compared to a pre-defined calibration curve, enabling accurate quantification of LH levels in the sample. This technique provides a specific and rapid method for LH detection (Kronenberg, 2007).

### 2.3.3.3. Measurement of Serum Estradiol Level

The CL-series E2 assay leverages a competitive binding immunoassay methodology for the quantitative determination of estradiol concentration. The initial incubation step combines the patient sample containing estradiol with paramagnetic microparticles coated with goat anti-rabbit IgG. Additionally, a sample treatment solution is included in this step, likely to optimize sample compatibility within the assay. Before use, a thorough visual inspection of the reagent bottle is recommended to ensure complete resuspension of the microparticles. If settling is observed, gentle inversion of the vial is necessary until complete resuspension is achieved. In cases where resuspension is not possible, discarding the reagent and contacting the supplier for assistance is advised. Notably, the assay protocol requires a minimum sample volume of 35 µL per test, excluding any dead volume associated with the sample container. For situations involving multiple tests from a single sample, users should consult the instrument's operation manual for specific minimum sample volume requirements(Mohammadzadeh and Amberg, 2023).

### 2.3.3.4. Measurement of Prolactin Level in serum

The CL-series prolactin (PRL) assay utilizes a two-step "sandwich" approach to measure prolactin levels. In the first step, a sample is combined with magnetic particles coated with one antibody specific to prolactin (anti-PRL) and another enzyme-labeled antibody (anti-PRL-alkaline phosphatase conjugate). Any prolactin in the sample binds to both antibodies, forming a sandwich complex. The magnetic particles are then isolated, washing away unbound substances. In the second step, a substrate solution is introduced. This substrate is broken down by the enzyme (alkaline phosphatase) attached

to the second antibody, only in the presence of the prolactin-containing sandwich complex. The resulting light emission is measured and correlates directly to the amount of prolactin present. A calibration curve allows researchers to convert this light signal into a precise prolactin concentration (Chung, 2016).

### 2.3.3.5. Measurement of Serum Anti-Müllerian Hormone

The diagnostic assay used for detecting AMH utilizes a novel combination of Up-converting Phosphor Technology (UPT) with a double-antibody sandwich immunochromatographic format. The test cassette's nitrocellulose membrane (NC membrane) incorporates two distinct zones: the reaction zone (T line) coated with capture antibodies specific to the target analyte (AMH) and the control zone (C line) coated with goat anti-mouse antibodies. Upon introduction of the diluted sample into the designated cavity, capillary action propels the liquid across the membrane. As the sample migrates, AMH molecules within the sample first interact with AMH antibodies conjugated to UCP nanoparticles. These antigen-antibody complexes are subsequently captured by the immobilized AMH antibodies on the T line, forming a sandwich-like structure. Unbound UCP nanoparticles continue their migration and bind to the goat anti-mouse antibodies on the C line, establishing a secondary antibody-antibody-UCP complex. Notably, UCP particles possess the unique property of emitting visible light when stimulated by an infrared source. The emitted light intensity at both the T and C lines is measured concurrently. The ratio between the T-line and C-line emission intensities (T/C) exhibits a direct correlation with the concentration of AMH in the sample. This ratio is then automatically calculated by the UPT system

referencing a pre-programmed calibration curve. Finally, the calculated AMH concentration is displayed on the instrument's screen (Iliodromiti et al., 2015).

### 2.3.5. Measurement of E-Clomiphene and Its Metabolite Concentrations

The standard samples were prepared by dissolving them in a solution of 5% acetic acid and acetonitrile. For every standard, a solution containing 10 ppm was prepared and subsequently introduced into the LC/MS/MS apparatus. To estimate the value in the samples, the intensity was measured. The procedures used were gel filtration and protein precipitation with a 5% acetic acid solution. In this phase, 400 µL of acetic acid was added to 200 µL of the lysed blood sample, that were placed into 1.5-milliliter microcentrifuge tubes. The samples were centrifuged for 10 minutes at 10,000 rpm after the proteins had been denatured. The supernatant was then injected into the LC/MS/MS apparatus (Ganchev et al., 2011).

The analysis made use of a SCIEX 4500 QTrap LC/MS/MS apparatus. An 80:20 ratio of acetonitrile to water was utilized as the mobile phase. To detect the presence of unidentified metabolites, the device was injected for one minute and scanned from mass 360 to 450. 500°C was chosen as the interface gas temperature. Using serial dilutions of standard samples, the standard curve was created based on the peak area ratio and the concentration of metabolites to quantitatively assess the data. Next, each peak associated with the metabolites E-clomiphene and E-4-hydroxyclomiphene was measured for peak area using SCIEX 4500 QTrap system software, and the results were plotted on the appropriate standard curve. Each metabolite peak's concentration was ascertained in this manner (Ganchev et al., 2011).

### 2.3.6. Molecular Analysis

#### 2.3.6.1. DNA Extraction from Blood Sample

The DNA extraction was conducted at the College of Pharmacy / University of Kerbela / Laboratory of Molecular Biology. Genomic DNA was extracted from a blood sample as stated by the protocol (gSYNC™ DNA Extraction Kit) for the blood genomic DNA extraction kit. The following method is suitable for DNA extraction from whole blood.

1. 200 µl of whole blood was transferred to a 1.5 ml microcentrifuge tube. After that, 20 µl of Proteinase K was added and mixed by pipetting. At 60ºC, the microcentrifuge tubes were incubated for 5 minutes.
2. After that, 200 µl of GSB Buffer was added and mixed by shaking vigorously by vortex, incubated at 60ºC for 5 minutes. The tube was inverted every 2 minutes, using a digital timer to track intervals.
3. 200 µl of absolute ethanol was added to the sample lysate and mixed right away for ten seconds by vigorous shaking.
4. The Genomic Spin (GS) column was placed in a 2 ml collection tube. The entire mixture was poured into the GS column; thereafter, it was centrifuged at 14–16,000 x g for one minute.
5. When the centrifuge had finished, the 2 ml collection tube containing flow-through was discarded and then transferred to the GS column to a new 2 ml collection Tube.
6. 400 µl of W1 Buffer was added to the GS column and centrifuged at 14-16,000 x g for 30 seconds, then the flow-through was discarded. Afterward, the GS column was put back in the 2 mL collection tube.
7. Absolute ethanol was added to the wash buffer and 600 µl of the formed solution was added to the GS column. Afterward, it was centrifuged at 14-16,000 x g for 30 seconds then discarded the flow-through.

8. The GS column was put back in the 2 ml collection tube and centrifuged for 3 minutes at 14-16,000 x g to dry the column matrix. The dried GS column was transferred to a clean 1.5 ml microcentrifuge tube.
9. Elution buffer was heated in the oven and 100 µl of pre-heated elution buffer was added to the center of the column matrix. It was allowed to stand for at least 3 minutes to allow the elution buffer to be completely absorbed and then centrifuged at 14-16,000 x g for 30 seconds to elute the purified DNA.

### 2.3.6.2. Quantitation of DNA

The NanoDrop spectrophotometer (Nanodrop) is used to determine the concentration and purity of the isolated DNA. After blanking, 1 µL of each sample was loaded into the Nanodrop micro detector, and the DNA concentration and A260/A280 ratio were recorded from the device(García-Alegría et al., 2020).

### 2.3.6.3. Polymerase Chain Reaction

The process known as polymerase chain reaction (PCR) involves replicating specific DNA regions using the enzyme DNA polymerase, allowing targeted DNA fragments to be amplified from one molecule to millions of copies (Joshi and Deshpande, 2010); A PCR reaction requires basic components including DNA template, contains the section of the DNA fragment that needs to be amplified, two primers that are complementary to the target DNA which determine the beginning and end of the region to be amplified; the intended DNA sequences will be amplified between two primers that bind to opposite sides of the DNA, Taq polymerase that copies the region to be amplified, Nucleotides which the DNA-Polymerase uses to

make new DNA and buffer, which gives the enzyme an appropriate chemical environment (Rahman et al., 2013).

Three steps are involved in a repeating series of cycles that amplify the target sequence:

1. Denaturation: Denaturation was performed at 95°C, which is the highest temperature the enzyme can withstand for 30 or more cycles without being damaged. Involves breaking down double-stranded DNA into single strands.
2. Annealing: This stage takes place between 55 and 65°C, and it starts the reaction by binding the two short oligonucleotide sequences to the ends of the DNA template strands.
3. Extension: This step requires primer extension to create a new strand complementary to the template, and it takes place in the presence of DNA Taq polymerase between 72 and 74°C. (Ehtisham et al., 2016)

There are several polymerase chain reaction (PCR) techniques that differ in principle, this study uses two techniques:

1. Amplification Refractory Mutation System – polymerase chain reactions (ARMS-PCR): The amplification refractory mutation system (ARMS), also known as allele-specific PCR, is a very straightforward technique that uses sequence-specific PCR primers to enable test DNA amplification only when the target allele is present in the sample. It is useful for detecting known point mutations or small deletions by using allele-specific primers (Yang et al., 2018). Using a set of allele-specific PCR primers, the ARMS PCR approach amplifies mutated alleles while leaving the wild-type allele unaffected. The PCR produces two amplicons: a larger fragment serving

as an internal control and a shorter, allele-specific fragment indicating the presence of the target mutation (Shankarnarayan et al., 2020).

2. Restriction Fragment Length Polymorphism (RFLP-PCR): It is a frequently used technology to examine small yet specific differences in a double-stranded DNA sequence. It is predicated on the specificity of restriction endonucleases, which cleave DNA at specific locations after identifying a set of nucleotides known as restriction sites. A specific RFLP pattern emerges on electrophoretic separation of digested DNA, which is indicative of a certain DNA sequence and has varying lengths of cleavage fragments (Mittal et al., 2013).

#### 2.3.6.3.1. Primer Design

Primer sequences specific to CYP2D6*4 (rs3892097) were adopted from previously validated studies by (Sudjaroen et al., 2020) and the expected results are that wild-type (GG) patients have 104 and 230 bp fragments, heterozygous (GA) patients have 104, 230, and 334 bp fragments, and homozygous (AA) patients have only 334 bp fragments, as shown in Tables (2 – 3).

**Table (2 – 3): Primers sequences of CYP2D6*4 (rs3892097*)* genetic polymorphism, enzyme, and product size.**

| SNPs | Primer sequence (5 ’->3 ’) | Enzyme | Product size | Reference |
|---|---|---|---|---|
| Forward primer | GCT TCG CCA ACC ACT CCG | BstNI | 334 bp<br>230 bp<br>104 bp | (Sudjaroen et al., 2020) |
| Reverse primer | AAA TCC TGC TCT TCC GAG GC | | | |

While primer sequences specific to CYP2D6*10 (rs1065852) were adopted from (Hinrichs et al., 2007). The 505bp represents the internal control, 351bp is an indication of a wildtype allele, and 192bp is an indication of a mutant allele as presented in Table (2 – 4).

**Table (2 – 4): Primers sequences of CYP2D6*10 (rs1065852) genetic polymorphism with product size.**

| SNPs | Primer sequence (5 '->3 ') | Product size | Reference |
|---|---|---|---|
| 2D6*10 F out | GGG GCA AGA ACC TCT GGA GC | 505 bp | (Hinrichs et al., 2007) |
| 2D6*10 R out | CTG GTC CAG CCT GTG GTT TC | | |
| 2D6*10 R WT | AGT GGC AGG GGG CCT GGA GG | 351 bp | |
| 2D6*10 F*10 | ACG CTG GGC TGC ACG CTT CT | 192 bp | |

F out= outer forward primer, R out= outer reverse primer, R WT= reverse wildtype primer, & F*10= Mutant primer

Lyophilized primers were reconstituted in a specific volume of nuclease-free, sterile deionized water (D.W.) to obtain a primary concentration of 100 pmol/µL (as a stock solution) as shown in Tables (2 – 5) and (2 – 6).

**Table (2 – 5): The volume of nuclease-free water added to each primer of CYP2D6*4 (rs3892097) to obtain 100 pmol/µL.**

| Primers of CYP2D6*4 1934G>A | Volume of Deionized sterile D.W (µL) |
|---|---|
| Forward | 300 |
| Reverse | 300 |

**Table (2 – 6): The volume of nuclease-free water added to each primer of CYP2D6*10 (rs1065852) to obtain 100 pmol/µL.**

| Primers of CYP2D6*10 100C>T | Volume of Deionized sterile D.W (µL) |
|---|---|
| 2D6*10 F out | 300 |
| 2D6*10 R out | 300 |
| 2D6*10 R WT | 300 |
| 2D6*10 F*10 | 300 |

F out= outer forward primer, R out= outer reverse primer, R WT= reverse wildtype primer, & F*10= Mutant primer

For the working solution, 10µL of stock solution was diluted with 90 µl of Deionized sterile D.W to obtain a final working concentration of 10 pmol/µL, and the primer was kept at -20 °C until further use.

### 2.3.6.3.2. PCR Optimization Conditions

Optimization of the polymerase chain reaction was attained after several trials.

#### 2.3.6.3.2.1. Optimization of *CYP2D6*4 (rs3892097)* Genetic Polymorphism Conditions.

*CYP2D6*4 1934G>A (rs3892097)* of PCR conditions was prepared by using different annealing temperatures (55°C, 58°C, 60 °C, 63°C, 66°C). The optimal annealing temperature for both wild-type and mutant primers was determined to be 60°C.

#### 2.3.6.3.2.2. Optimization of *CYP2D6*10 (rs1065852)* Genetic Polymorphism Conditions.

*CYP2D6*10 100C>T (rs1065852)* of PCR conditions was prepared by using different annealing temperatures (55°C, 58°C, 60 °C, 63°C, 66°C) for each wild and mutant type primer and the best annealing temperature was 63°C.

### 2.3.6.3.3. Running and Working Solution of PCR

#### 2.3.6.3.3.1. RFLP-PCR Running and Working Solution for CYP2D6*4 (rs3892097) Polymorphism

*CYP2D6*4 1934G > A (rs3892097)* RFLP-PCR working solution was prepared as follows:

1. 1 µl of 10 pmol/µL from forward primer, 1 µl of 10 poml/µL from reverse primer, 8 µl of OneTaq Quick-load 2X Master Mix, and 2 µl of extracted DNA were added in a PCR tube.
2. The final volume was adjusted to 20 µL with sterile deionized water.
3. The thermal program for *CYP2D6*4 1934G > A (rs3892097)* ***is*** presented in Table (2 – 7).

**Table (2 – 7): the thermal program for CYP2D6*4 (rs3892097) polymorphism**

| Steps | Temperature (°C) | Minute: second | Cycles |
|---|---|---|---|
| Initial denaturation | 95 | 5:00 | 35 |
| Denaturation | 95 | 0:30 | |
| Annealing | 60 | 0:30 | |
| Extension | 72 | 0:30 | |
| Final extension | 72 | 5:00 | |

After PCR products were obtained, 10 µL of the product was added to a new tube with 2 µL of BstNI enzyme, 1.5 µL of the NEBuffer r3.1, and 1.5 µL deionized distilled water, and incubated at 60 °C overnight after adding a droplet of mineral oil (commonly used to prevent evaporation during incubation).

#### 2.3.6.3.3.2. ARMS-PCR Running and Working Solution for CYP2D6*10 (rs1065852)

ARMS-PCR working solution was prepared as follows:

1. 1 µl of 10 poml/µL from the outer forward and 1 µl of 10 poml/µL from the outer reverse primer were added to two separate PCR tubes, 1 µL of the inner forward primer was added to one tube (wild-type detection), and 1 µL of the inner reverse primer was added to the other (mutant detection).
2. 8 µL of OneTaq Quick-load 2X Master Mix and 2µL of extracted DNA were added to each PCR tube.
3. The volume was completed to 20 µL with 7 µL deionized distilled water.
4. The thermal program for *CYP2D6*10 100 C>T (rs1065852)* ***is*** demonstrated in table (2 – 8).

**Table (2 – 8): the thermal program for CYP2D6*10 (rs1065852) polymorphism**

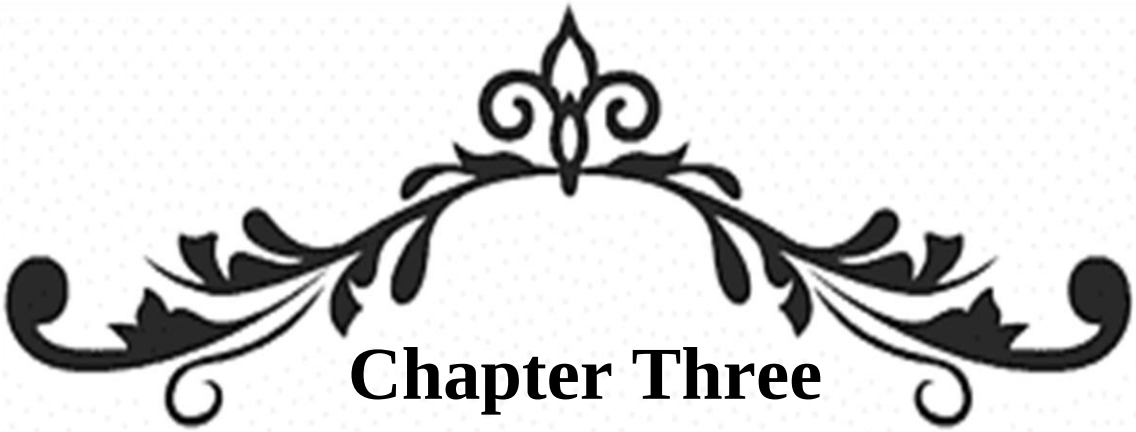
| Steps | Temperature (°C) | Minute: second | Cycles |
|---|---|---|---|
| Initial denaturation | 95 | 5:00 | 35 |
| Denaturation | 95 | 0:30 | |
| Annealing | 63 | 0:30 | |
| Extension | 72 | 1:00 | |
| Final extension | 72 | 10:00 | |

### 2.3.6.3.4. Agarose Gel Electrophoresis

1. Agarose gel 2% was prepared by dissolving 2 g of agarose powder in 100 mL of 1X TBE buffer by heating in a microwave oven for 1 minute.
2. The mixture was allowed to cool slightly before adding ethidium bromide (2μL), and then the solution was poured into the apparatus.
3. The comb was fixed on one end of the tray to make holes where the samples were to be loaded.
4. After the agarose solution had been poured, it was left to solidify at 25°C.
5. The comb was removed lightly from the tray.
6. The tray was placed into the electrophoresis chamber, and the chamber was filled with 1X TBE buffer.
7. One of the wells of agarose gel was loaded with 5μl of 1000bp DNA ladder, while the others were loaded with 5 μl of each PCR product.
8. Electrophoresis was conducted at 95 V, corresponding to an electric field strength of approximately 5 V/cm for a 20 cm distance between electrodes, until appropriate separation was achieved.
9. At the end of the run, an ultraviolet trans-illuminator was used for the band's detection.
10. The gel was photographed using a digital camera (canon EOS 80D)

## 2.4. Statistical Analysis

Statistical analysis is performed using the Statistical Package for Social Sciences (SPSS 26). Descriptive statistics for the numerical data were presented as the means ± standard deviations (SD), and the non-numerical data were numbers and %. The Shapiro–Wilk test was used to assess the normality of continuous variables. If $p > 0.05$, the data are considered normally distributed; if $p \leq 0.05$, the data significantly deviate from normality(Mohd Razali and Yap, 2011). For non-normally distributed numerical data, non-parametric tests, such as the Wilcoxon Signed-Rank test (for paired comparisons) and the Kruskal–Wallis test (for multiple group comparisons), were employed. Categorical variables were compared using the Chi-square ($\chi^2$) test. A p-value < 0.05 was considered statistically significant (Habibzadeh, 2024).



# Chapter Three

# Results

# 3. Results

## 3.1. Demographic Data of Infertile Women with Polycystic Ovary Syndrome

Descriptive statistics for demographic variables, including mean age, Menarche, BMI, and marriage duration, were 26.6 ± 5.37, 12.35 ± 1.31, 27.37 ± 4.63, and 5.36 ± 4.21, respectively, as presented in Table (3 – 1).

**Table (3 – 1): Demographic characteristics (n=80) of Infertile Women with PCOS (data presented as mean ± SD)**

| Variables | Mean ± SD |
|---|---|
| Age (y) | 26.6 ± 5.37 |
| Menarche (y) | 12.35 ± 1.31 |
| BMI ($Kg/m^2$) | 27.37 ± 4.63 |
| Marriage Duration (y) | 5.36 ± 4.21 |

**BMI: body mass index**

Among the PCOS patients studied, the majority had completed primary school education, accounting for 51 women (63.7%), followed by 16 women (20%) with no formal education. Secondary school education was reported in 9 women (11.3%), while only 4 women (5%) had attended college.

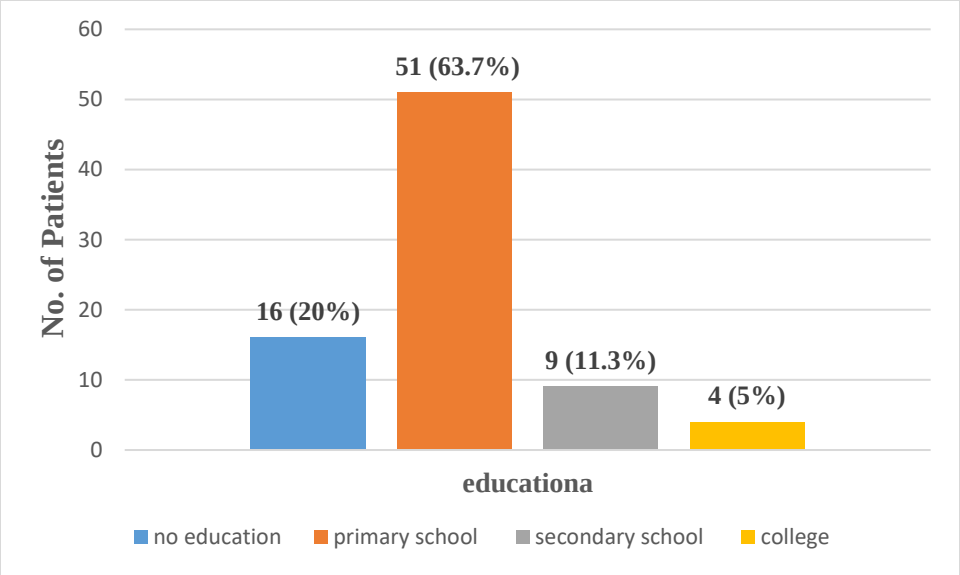



**Figure (3 – 1): frequencies of educational level among the studied women**

## 3.2. Prevalence of Polycystic Ovary Syndrome-Related Symptoms

This study examined the prevalence of several common symptoms in a group of infertile women with PCOS. Among the participants, 40 (50.0%) exhibited hirsutism. Alopecia was reported by 26 women (32.5%), and 49 (61.3%) experienced menstrual irregularities. Only 7 participants (8.8%) had a history of miscarriage. These data are visualized in Figure (3 – 2).

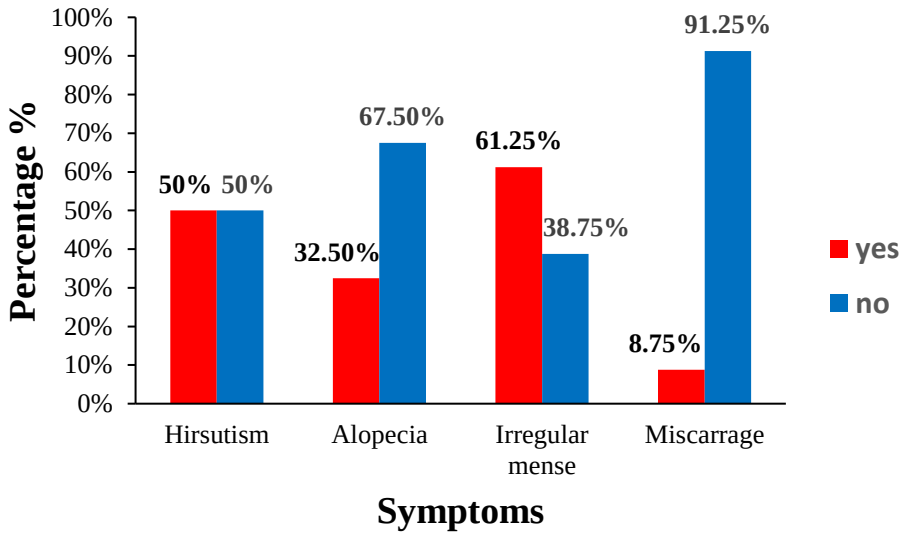



**Figure (3 – 2): Prevalence of PCOS-related symptoms**

### 3.3. Levels of Hormones and Ultrasound Findings

Baseline hormone levels were measured before treatment. The mean Anti-Müllerian hormone (AMH) level was 3.25 ± 1.75 ng/ml, and the mean prolactin level was 16.38 ± 7.69, as shown in Table (3 – 2).

**Table (3 – 2): The basal levels of Anti-Müllerian hormone and Prolactin (data presented as mean ± SD)**

| Variables | Mean ± SD |
|---|---|
| AMH (ng/ml) | 3.25 ± 1.75 |
| Prolactin (ng/ml) | 16.38 ± 7.69 |

**AMH: Anti-Müllerian hormone**

The E2 level in infertile women with PCOS was significantly increased after treatment (97.72 ± 73.01) compared to before treatment (40.59 ± 26.21). The LH level and the LH/FSH ratio were also significantly increased after

treatment compared with before. There was no significant difference in FSH levels between before and after treatment, as demonstrated in Table (3 – 3).

**Table (3 – 3): The level of reproductive hormones and ultrasound changes before and after treatment (data presented as mean ± SD)**

| Parameter | Clomiphene treatment | | *P*-value ❖ |
|---|---|---|---|
| | 2ND | 12TH | |
| E2 (pg/ml) | 40.59 ± 26.21 | 97.72 ± 73.01 | 0.001 |
| FSH (mIU /ml) | 6.99 ± 2.89 | 6.58 ± 4.75 | 0.127 |
| LH (mIU /ml) | 9.06 ± 7.74 | 14.7 ± 7.74 | < 0.001 |
| LH/FSH ratio | 1.61 ± 1.27 | 2.6 ± 1.41 | 0.001 |
| Follicle size (mm) | 5.53 ± 1.45 | 16.63 ± 5.17 | 0.001 |
| ET (mm) | 4.66 ± 0.92 | 8.3 ± 1.83 | 0.001 |

❖ **p-vale derived from Wilcoxon Signed-Rank Test, E2: Estradiol, FSH: Follicle-Stimulating Hormone, LH: Luteinizing Hormone, LH/FSH ratio: Luteinizing Hormone to Follicle-Stimulating Hormone Ratio, ET: Endometrial Thickness**

## 3.4. Genetic Analysis

### 3.4.1. Frequency of CYP2D6*4 (rs3892097) in Infertile Women with Polycystic Ovary Syndrome

The PCR amplification of the CYP2D6*4 1934G>A (rs3892097) SNP yielded distinct bands of 334bp, 230bp, and 104bp. A single band 334bp indicates a homozygous wild-type (GG). Meanwhile, the heterozygous (AG) genotype is indicated by the presence of three distinct bands: 334 bp, 230 bp, and 104 bp. The homozygous variant (AA) is represented by two bands, 230bp and 104bp, as shown in Figures (3 – 3).

The predominant CYP2D6*4 genotype among infertile women was the allele (GG), accounting for 53 (66.3%). Heterozygous (AG) and homozygous

variant (AA) alleles were observed in 24 (30%) and 3 (3.8%) of PCO women, respectively, as shown in Figure (3 – 4).

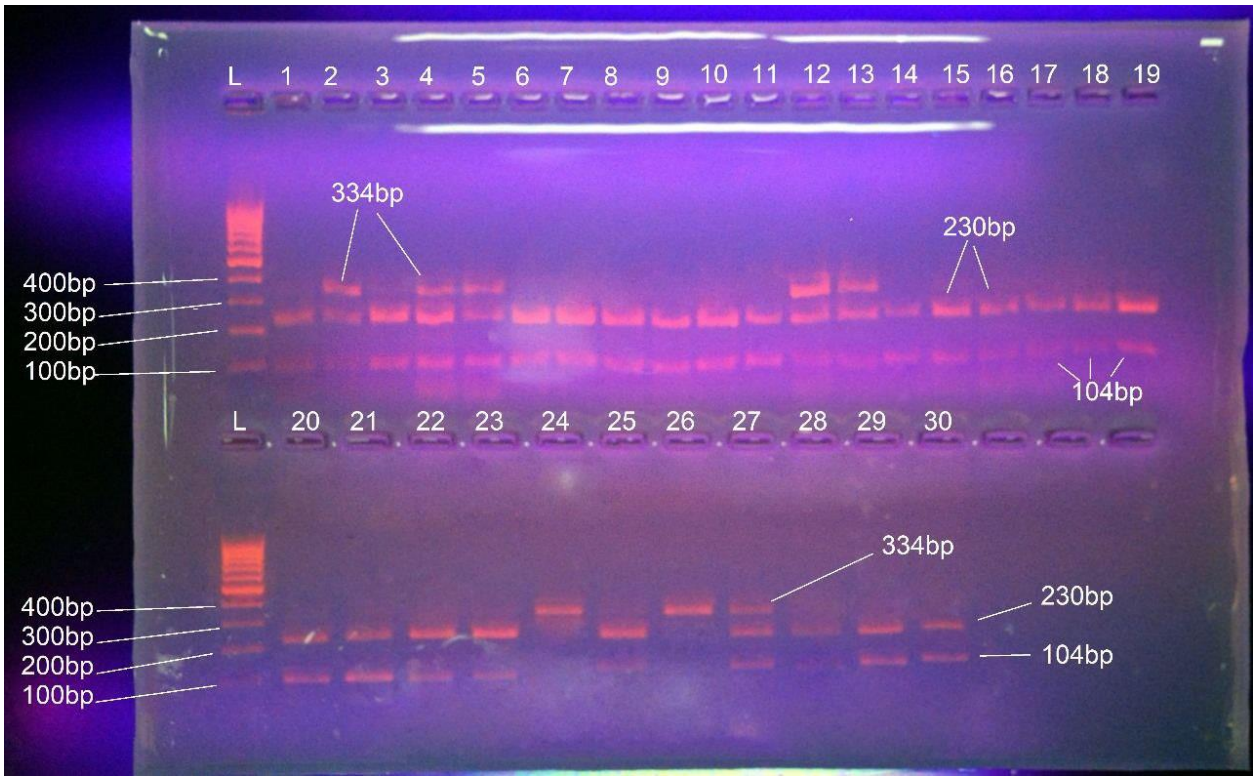



**Figure (3 – 3): Genotyping of CYP2D6*4 polymorphism. lane L: DNA ladder; lanes 2,4,5,12,13 and 27 GA genotype; lanes 24 and 26 AA genotype; other lanes GG genotype.**

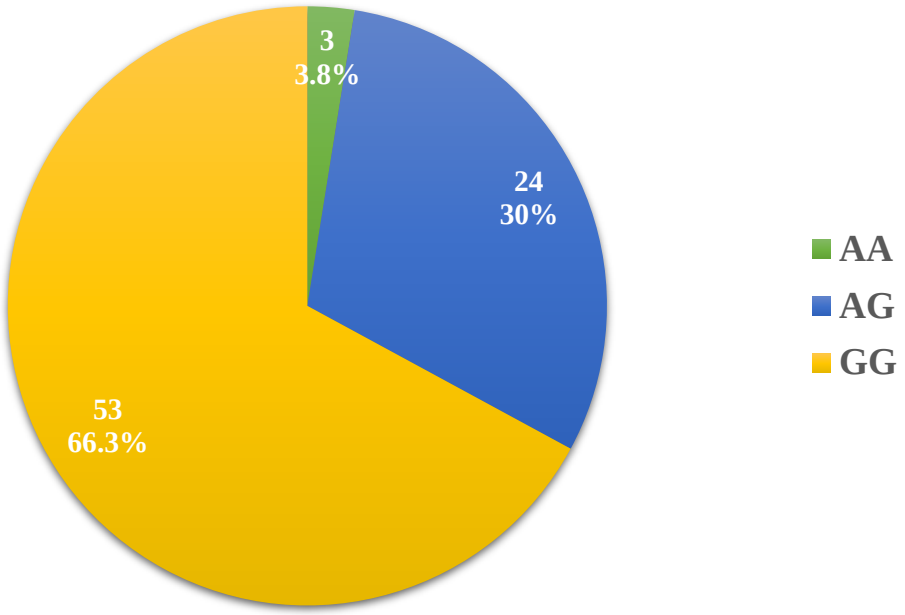



**Figure (3 – 4): The frequency of CYP2D6*4 genotypes of patients. The wild-type homozygous genotype (GG) was predominant, while the heterozygous genotype (AG) and the homozygous mutant genotype (AA) were less frequent.**

### 3.4.2. Frequency of CYP2D6*10 (rs1065852) in PCOS Infertile Women

The results of the amplification of the SNP of CYP2D6*10 100C>T (rs1065852) are shown as clear bands of 505bp, 351bp, and 192bp. 505bp serves as internal control, 351bp represents the wild-type allele, and 192bp represents the mutant allele as in Figure (3 – 5). The homozygous wild-type (CC) genotype was observed in 53 individuals (66.3%), the heterozygous (CT) genotype in 22 individuals (27.5%), and the homozygous mutant (TT) genotype in 5 individuals (6.3%), as depicted in Figure (3 – 6).

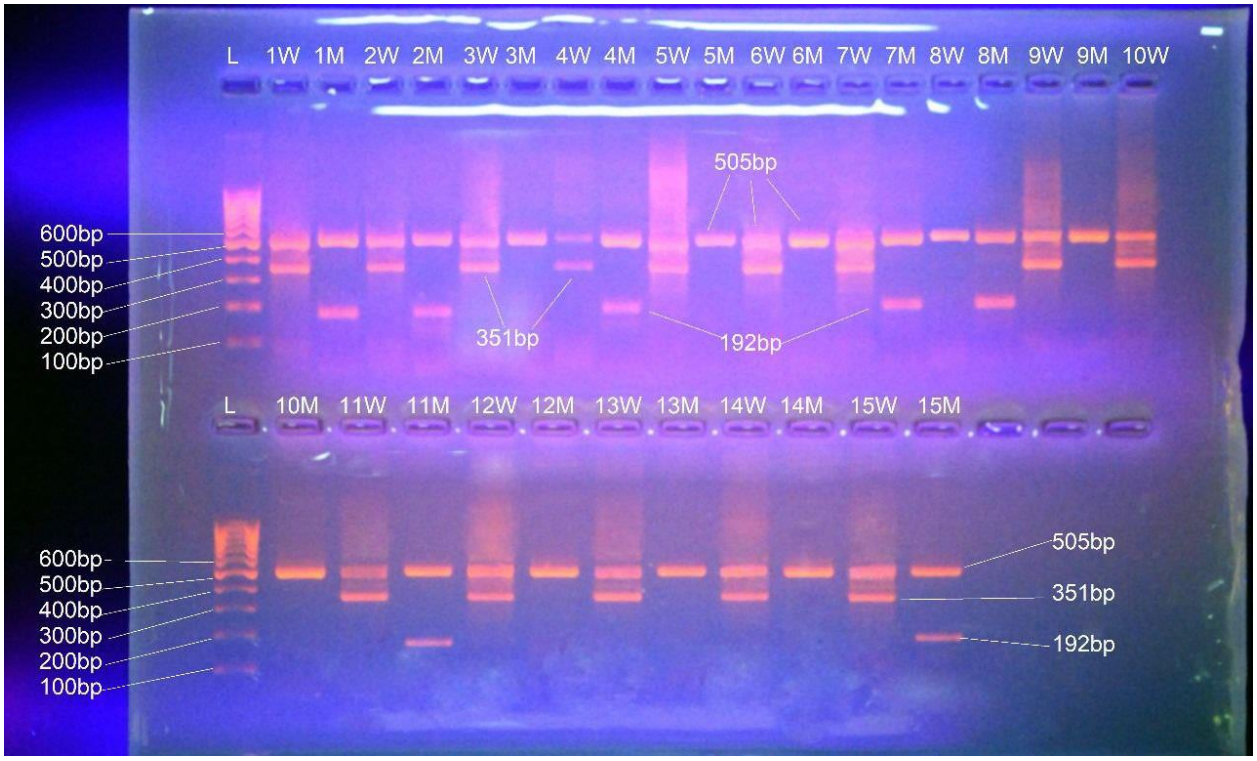



**Figure (3 – 5): Genotyping of CYP2D6*10 polymorphism. lanes L: DNA ladder; lanes marked with "W" for wild type primer; lanes marked "M" for mutant primers. samples 3,5,9,10,12,13 and 14 Wildtype genotype; samples 1,2,4,7,11 and 15 heterozygous genotype; sample 8 mutant genotype**

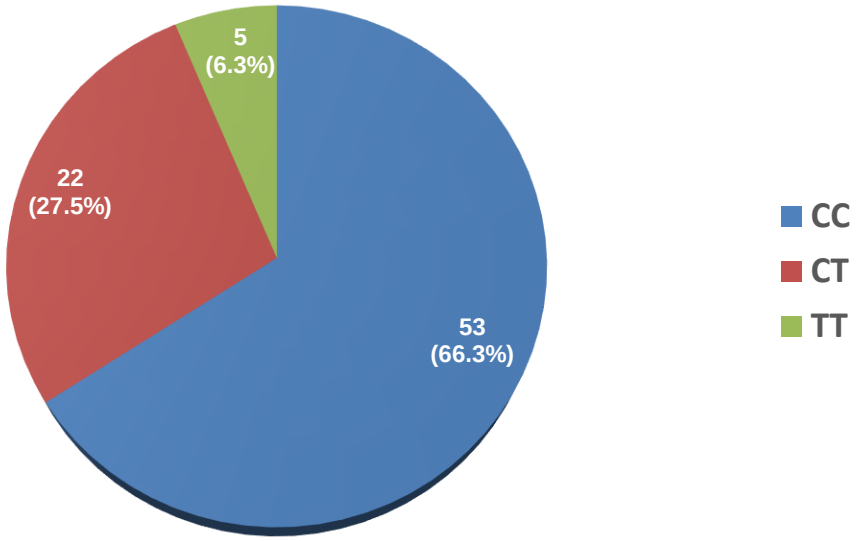



**Figure (3 – 6): The frequency of the CYP2D6*10 Genotype of patients. The CC genotype was most prevalent, followed by CT and TT genotypes.**

### 3.4.2. Demographic Data and PCOS Symptoms of Infertile Women Based on CYP2D6*4 (rs3892097) Genotypes

The demographic data of infertile women with PCOS, like age, menarche, BMI, duration of marriage, and history of abortions, did not significantly vary across different CYP2D6*4 gene alleles ($p > 0.05$). Similarly, the PCOS symptoms, including menstrual irregularities, hirsutism, and alopecia, showed no significant association with the different CYP2D6*4 gene alleles ($p > 0.05$), as detailed in Table (3 – 4).

**Table (3 – 4): Demographic data and PCOS symptoms of infertile women based on CYP2D6*4 genotypes (data presented as mean ± SD and numbers (%))**

| Variables | | CYP2D6*4 genotypes | | | *P* – value |
|---|---|---|---|---|---|
| | | GG (n=53) | AG (n=24) | AA (n=3) | |
| Age (y) | | 26.79 ± 5.39 | 26.95 ± 5.52 | 22.66 ± 1.15 | 0.587 |
| BMI ($Kg/m^2$) | | 27.79 ± 4.56 | 26.71 ± 4.97 | 27.05 ± 1.16 | 0.770 |
| Menarche (y) | | 12.5 ± 1.29 | 12.26 ± 1.21 | 11.33 ± 0.57 | 0.124 |
| Duration of Marriage (y) | | 5.35 ± 4.69 | 5.39 ± 3.17 | 6 ± 3.46 | 0.489 |
| Hirsutism | No (n=40) | 29(54.7%) | 10 (41.66%) | 1 (33.3%) | 0.479 |
| | Yes (n=40) | 24 (45.3%) | 14 (58.33%) | 2 (66.7%) | |
| Alopecia | No (n=54) | 34 (64.1%) | 17 (70.83%) | 3 (100%) | 0.399 |
| | Yes (n=26) | 19 (35.8%) | 7 (29.1%) | 0 (0%) | |
| Irregular menses | Yes (n=49) | 35 (66.1%) | 14 (58.33%) | 0 (0%) | 0.069 |
| | No (n=31) | 18 (33.9%) | 10 (41.66%) | 3 (100%) | |
| Abortion | No (n=73) | 48 (90.5%) | 22 (91.6%) | 3 (100%) | 0.85 |
| | Yes (n=7) | 5 (9.5%) | 2 (8.3%) | 0 (0%) | |

**BMI: body mass index**

### 3.4.3. Demographic Data and PCOS Symptoms of Infertile Women Based on CYP2D6*10 (rs1065852) Genotypes

Among infertile women with PCOS, different CYP2D6*10 genotypes did not significantly influence demographic characteristics ($P > 0.05$) such as age, menarche, BMI, marriage duration, and abortion rate. Furthermore, no notable differences in the prevalence of PCOS symptoms, including menstrual regularity, hirsutism, and alopecia, were observed across these genetic variations, as presented in Table (3 – 5).

**Table (3 – 5): Demographic data and PCOS symptoms of infertile women based on CYP2D6*10 genotypes (data presented as mean ± SD and numbers (%))**

| Variables | | CYP2D6*10 genotypes | | | *P* – value⁂ |
|---|---|---|---|---|---|
| | | CC (n=53) | CT (n=22) | TT (n=5) | |
| Age (y) | | 26.96 ± 5.57 | 25.63 ± 4.66 | 28.75 ± 6.29 | 0.771 |
| BMI (Kg/m$^2$) | | 27.61 ± 4.94 | 26.94 ± 4.04 | 28.1 ± 2.86 | 0.907 |
| Menarche (y) | | 12.41 ± 1.32 | 12.5 ± 0.96 | 11.5 ± 1.91 | 0.278 |
| Duration of Marriage (y) | | 5.66 ± 4.68 | 4.45 ± 1.81 | 7 ± 5.11 | 0.928 |
| Hirsutism | No (n=40) | 26(49.1%) | 11 (50%) | 3 (60%) | 0.896 |
| | Yes (n=40) | 27 (50.9%) | 11 (50%) | 2 (40%) | |
| Alopecia | No (n=54) | 39 (73.5%) | 11 (50%) | 4 (80%) | 0.115 |
| | Yes (n=26) | 14 (26.5%) | 11 (50%) | 1 (20%) | |
| Irregular menses | Yes (n=49) | 19 (35.8%) | 9 (70.83%) | 3 (60%) | 0.554 |
| | No (n=31) | 34 (64.2%) | 13 (29.1%) | 2 (40%) | |
| Abortion | No (n=73) | 48(90.5%) | 21(95.4%) | 4 (80%) | 0.519 |
| | Yes (n=7) | 5 (9.5%) | 1(4.6%) | 1 (20%) | |

⁂ **p-value derived from Kruskal–Wallis test, BMI: body mass index**

### 3.4.4. Effect of CYP2D6*4 (rs3892097) Genotypes on Reproductive Hormone Levels and Ultrasound Findings in Infertile Women with Polycystic Ovary Syndrome

The plasma levels of FSH, LH, and E2 and LH/FSH ratio of PCOs infertile women who took clomiphene showed no significant difference among CYP2D6*4 1934G>A genotypes (*P* >0.05). Furthermore, the ultrasound findings were also not statistically significant in either follicle size or ET

among the different CYP2D6*4 genotypes (*P*>0.05), as mentioned in Table (3 – 6).

**Table (3 – 6): The Effect of CYP2D6*4 genotyping on reproductive hormone levels and ultrasound findings of the PCOs infertile women (data presented as mean ± SD)**

| Variables | CYP2D6*4 genotypes | | | P-value ❖ |
|---|---|---|---|---|
| | GG (n=53) | AG (n=24) | AA (n=3) | |
| FSH (mIU /ml) | 6.99 ± 5.68 | 5.7 ± 1.9 | 6. 25±1.52 | 0.615 |
| LH (mIU /ml) | 15.71 ± 8.74 | 12.8 ± 5.04 | 13.63 ± 3.6 | 0.319 |
| LH/FSH ratio | 2.67 ± 1.43 | 2.54 ± 1.43 | 2.39 ± 1.32 | 0.597 |
| E2 (pg/ml) | 103.37 ± 75.54 | 90.32 ± 72.87 | 61.36 ± 9.07 | 0.762 |
| Follicle Size (mm) | 17 ± 5.02 | 16.26 ± 5.62 | 12 ± 1 | 0.145 |
| ET (mm) | 8.29 ± 1.97 | 8.45 ± 1.51 | 6.66 ± 1.15 | 0.190 |

**❖ p-vale derived from Kruskal–Wallis test, E2: Estradiol, FSH: Follicle-Stimulating Hormone, LH: Luteinizing Hormone, LH/FSH ratio: Luteinizing Hormone to Follicle-Stimulating Hormone Ratio, ET: Endometrial Thickness**

### 3.4.5. Effect of CYP2D6*10 (rs1065852) Genotypes on Reproductive Hormone Levels and Ultrasound Findings in Infertile Women with Polycystic Ovary Syndrome

The plasma levels of FSH, LH, and E2 and LH/FSH ratio of infertile women with polycystic ovary syndrome who took clomiphene did not show a significant difference among the three genotypes (P>0.05). Moreover, ultrasound parameters such as follicle size and ET exhibited no significant changes across the CYP2D6*10 genotypes, as shown in Tables (3 – 7).

**Table (3 – 7): Effect of CYP2D6*10 genotyping in reproductive hormone levels and ultrasound findings of the PCOS women (data presented as mean ± SD)**

| Variables | CYP2D6*10 genotypes | | | P-value ❖ |
|---|---|---|---|---|
| | CC (n=53) | CT (n=22) | TT (n=5) | |
| FSH (mIU /ml) | 7.08 ± 5.61 | 5.57 ± 2.26 | 5.65 ± 0.93 | 0.313 |
| LH (mIU /ml) | 15.22 ± 8.96 | 14.51 ± 4.5 | 10.5 ± 1.63 | 0.167 |
| LH/FSH ratio | 2.52 ± 1.45 | 2.98 ± 1.36 | 1.92 ± 0.54 | 0.20 |
| E2 (pg/ml) | 101.9 ± 79.78 | 78.51 ± 47 | 153 ± 85.59 | 0.168 |
| Follicle Size (mm) | 16.39 ± 5.17 | 16.95 ± 5.53 | 18 ± 3.65 | 0.786 |
| ET (mm) | 8.33 ± 1.93 | 8.15 ± 1.68 | 8.25 ± 1.7 | 0.703 |

❖ **p-vale derived from Kruskal–Wallis test, E2: Estradiol, FSH: Follicle-Stimulating Hormone, LH: Luteinizing Hormone, LH/FSH ratio: Luteinizing Hormone to Follicle-Stimulating Hormone Ratio, ET: Endometrial Thickness**

### 3.4.6. The Effect of SNPs on The Level of E-Clomiphene and E-4-hydroxyclomiphene

#### 3.4.7.1. The Effect of CYP2D6*4 (rs3892097) Genotypes on the Plasma Concentration of Clomiphene and Its Metabolite

The plasma concentration of clomiphene in infertile women with PCOS shows no significant differences among CYP2D6*4 genotypes (P>0.05). The plasma concentration of clomiphene metabolite (E-4-OH-clomiphene) was significantly lower in PCOs infertile women with the mutant genotype (AA) compared to those with the wild-type (GG) and heterozygous (AG) genotypes (p = 0.036), as shown in Table (3 – 8).

**Table (3 – 8): CYP2D6*4 genotypes' effect on drug metabolism (data present as mean ± SD)**

| Variables | CYP2D6*4 genotypes | | | *P*-value ⁘ |
|---|---|---|---|---|
| | GG (n=53) | AG (n=24) | AA (n=3) | |
| E-Clomiphene (nM) | 3.34 ± 0.34 | 3.38 ± 0.32 | 3.29 ± 0.77 | 0.839 |
| E-4-OH-clomiphene (nM) | 4.26 ± 0.74 | 4.26 ± 0.61 | 3.33 ± 0.2* | 0.036 |
| Drug/metabolite ratio | 0.79 ± 0.15 | 0.84 ± 0.23 | 0.99 ± 0.22 | 0.306 |

⁘ p-vale derived from Kruskal–Wallis test, *: significant effect compared with other genotypes

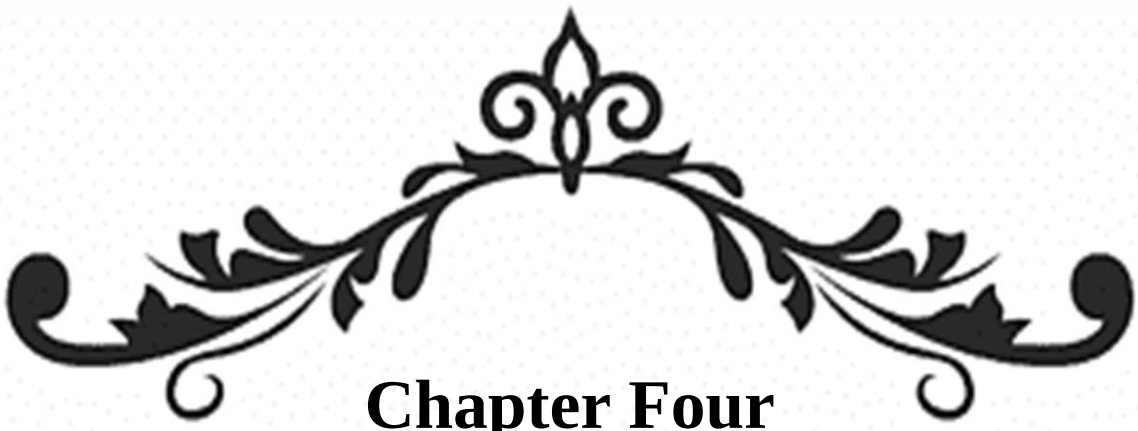
### 3.4.7.2. The Effect of CYP2D6*10 (rs1065852) Genotypes on the Plasma Concentration of E-Clomiphene and Its Metabolite

The plasma concentration of clomiphene in infertile women with PCOS showed a significant increase in the TT genotype compared to CC and CT genotypes at P=0.005. The plasma concentration of clomiphene active metabolite (E-4-OH-clomiphene) in PCOS infertile women after taking clomiphene showed significantly decreased levels in the TT genotype compared to CC and CT genotypes at $P < 0.001$, as shown in Table (3 – 9).

**Table (3 – 9): The CYP2D6*10 Genotype's effect on metabolism (data presented as mean ± SD)**

| Variables | CYP2D6*10 genotype | | | *P* – value⁘ |
|---|---|---|---|---|
| | CC (n=53) | CT (n=22) | TT (n=5) | |
| E-Clomiphene (nM) | 3.3 ± 0.35 | 3.37 ± 0.29 | 3.9 ± 0.14* | 0.005 |
| E-4-OH-clomiphene (nM) | 4.27 ± 0.45 | 4.37 ± 0.42 | 2.9 ± 0.18* | 0.001 |
| Drug/metabolite ratio | 0.78 ± 0.13 | 0.77 ± 0.09 | 1.36 ± 0.05* | 0.001 |

⁘ p-vale derived from Kruskal–Wallis test, *: significant effect compared with other genotypes



# Chapter Four

# Discussion

# 4. Discussion

Clomiphene citrate is a selective estrogen receptor modulator (SERM) commonly used as first-line therapy for infertile women with PCOS. However, approximately 15–40% of these women do not respond to clomiphene treatment. (Wang et al., 2017). Many researchers have tried to identify the etiologies of resistance and found solutions to this problem. One of these problems is that it is a prodrug and requires bioactivation by 4-hydroxylation. In particular, clomiphene action is mediated through estrogen receptor binding of (E)-4-hydroxylated clomiphene metabolites (Mürdter et al., 2012). CYP2D6 is a highly polymorphic enzyme that can metabolize multiple drugs. It is the enzyme that metabolizes clomiphene to its active metabolite (E-4-OH-Clomiphene. This genetic polymorphism significantly changed the pharmacokinetic properties of clomiphene among individuals and made some of these patients slow metabolizers, thus reducing the efficacy of the drugs, and other patients rapid metabolizers; thereafter, enhanced the incidence of toxicity (Kim et al., 2018).

## 4.1. Demographic Data of Infertile Women with Polycystic Ovary Syndrome

This study enrolled young infertile women with PCOS who have been struggling with infertility for a long period. All of them reached puberty around the same age. However, some research indicates that factors like BMI and genetic variants can influence menarche timing in PCOS patients, suggesting that girls with PCOS may experience menarche at a wider age range (Welt and Carmina, 2013, Carroll et al., 2012). Another study reported no significant difference in the average age of menarche was noticed between

women with PCOS and a control group (Khashchenko et al., 2020). This study shows that patients involved are slightly overweight, together with a previous study by (Teede et al., 2013), which established a strong correlation between obesity and PCOS prevalence. Furthermore, another study comparing PCOs and non-PCOs groups revealed that women with PCOs had a significantly higher BMI mean (Neubronner et al., 2021). Obesity can exacerbate PCOs, as research indicates a strong link between the two conditions. Obesity can influence PCOs in various ways, including through hormonal changes and insulin resistance. It's noteworthy that PCOs can make weight loss and maintenance more challenging (Barber et al., 2019).

## 4.2. Polycystic Ovary Syndrome-Related Symptoms

In this study, half of the participants exhibited hirsutism. This prevalent condition affects 5–15% of women and can have a major effect on psychological health (Tewary et al., 2021). Furthermore, studies comparing PCO patients to women in the general population have shown that hirsutism prevalence ranges from 4% to 11% in the general population, and it reaches 70 – 80% in PCO patients (Mara Spritzer et al., 2016). Additionally, another study found that PCOS and a high BMI were correlated with increased hair growth (Neubronner et al., 2021). PCOS involves complications with steroidogenesis, insulin resistance, and the hypothalamic-pituitary axis. It is commonly described as anovulation (oligomenorrhea, infertility, and dysfunctional uterine bleeding) combined with polycystic ovaries and hyperandrogenism (Barbosa et al., 2016).

Androgenetic alopecia (AGA) is the most common cause of hair loss in men and women and is linked to a hormone called dihydrotestosterone (Chen et al., 2022). Nearly only one-third of the participants in this study have

alopecia; likewise, Studies indicate that AGA affects about 22% of women with PCOS(Quinn et al., 2014). Another study stated there was an association between PCOS and androgenetic alopecia, as patients diagnosed with PCOS also presented with AGA (Quinn et al., 2014, Christodoulopoulou et al., 2016).

This study lists a small number of abortions, while earlier studies attributed that women with PCOS may be more susceptible to consequences like spontaneous abortion or stillbirth (Sun et al., 2013). Moreover, broader research shows that PCOS, especially with high BMI and insulin resistance, is associated with a higher risk of spontaneous abortion(Sun et al., 2020)

Lastly, regarding menstrual regularity, this study indicates that more than half of the women had an irregular menstrual cycle. This aligns with studies, as the majority of PCO women experienced irregular menstruation (Hart et al., 2004).

## 4.3. Levels of Hormones and Ultrasound Findings

In this study, prolactin levels were measured for PCOS patients, and they did not show elevated levels (Table 3 – 2). While some studies showed mixed results, some indicated an elevated level of prolactin (Mahboobifard et al., 2022). While another research suggests that hyperprolactinemia is not a defining feature of PCOS and may be present only in a minority of cases (Podvin et al., 2015). At the same time, in this study, serum Anti-Müllerian Hormone (AMH) levels are mildly elevated in the PCOS cohort. Multiple epidemiological data exist, indicating a positive correlation between elevated AMH levels and PCOs, as AMH levels were higher in PCOs in general (Calzada et al., 2019).

In this study, following clomiphene citrate administration, a significant increase in serum LH was observed. The LH surge typically occurs 5–7 days after the final clomiphene dose (Sovino et al., 2002). While this study revealed that FSH did not show a significant increase, Prior research showed that anovulatory women undergoing ovulation induction with 100 mg of clomiphene had lower mid-cycle FSH levels than ovulatory women (Kovalak et al., 2022).

As follows, this study showed a significant increase in the LH/FSH ratio after taking clomiphene. According to studies, the PCOs group had significantly greater LH and lower FSH levels (Calzada et al., 2019). LH levels elevation can invert this ratio to 2-3 (Saadia, 2020).

More importantly, this study showed Estradiol (E2) levels increased significantly post-clomiphene treatment, which is expected due to stimulated follicular development. Although clomiphene exerts anti-estrogenic effects on the hypothalamus, this indirectly promotes increased GnRH, FSH release, and ovarian estradiol synthesis (Sun et al., 2013). A previous studies show a considerable increase in E2 after clomiphene administration (Budinetz et al., 2015).

This study reveals a significant increase in the mean follicle size regarding ultrasound findings (Table 3 – 3). The size of the dominant follicle is crucial for successful fertilization and implantation, especially if the patient is on ovulation induction. A study by Palatnik et al comparing natural cycles to clomiphene-induced cycles has shown that clomiphene cycles result in significantly larger dominant follicles (Palatnik et al., 2012) and their result aligns with a previous study of (Haritha and Rajagopalan, 2003).

Furthermore, this study shows that Endometrial thickness (ET) increased post-clomiphene administration in this study. However, clomiphene's anti-estrogenic action can suppress endometrial estrogen receptors, potentially resulting in a thinner endometrium in some patients (Takasaki et al., 2013). Studies have shown that ET <8 mm may compromise implantation rates. While the potential for clomiphene to cause endometrial thinning should be considered, the study also noted that increased E2 concentrations can counteract this effect (Magaton et al., 2022).

## 4.4. Genetic Analysis

### 4.4.1. The Frequency of CYP2D6*4 in Polycystic Ovary Syndrome Women

This study provides valuable insights into the prevalence of the CYP2D6*4 genotype among Iraqi women with PCOS( Table3 - . The results demonstrated a high frequency of the GG genotype, followed by a lower prevalence of the heterozygous GA genotype, and the AA genotype being the least common. In contrast, a previous study on Korean women with PCOS did not identify any poor metabolizers (PMs) or carriers of the CYP2D6 *4 allele (Ji et al., 2016), which aligns with existing evidence that this allele is rare in East Asian populations (Dorji et al., 2019). After all, drawing comparisons with other PCOS populations is challenging due to limited published data from many countries. The prevalence of the CYP2D6 *4 allele varies significantly across the Middle East, with the highest reported frequency in Egypt (up to 28.05%) and the lowest in Saudi Arabia (3.5%). This can be attributed to diverse ethnic groups and methodological differences (Khalaj et al., 2019). According to a study by Ali et al. (2023), the frequency of allele*4 in breast cancer patients was found to be 7.82% (Ali et al., 2023). In contrast,

a study in Peru found that very few people had the AA genotype (0.85%), and a small number had the AG genotype (8.55%) (Alvarado et al., 2021). A study of Iranian female volunteers found a higher prevalence of the AG genotype (48.8%), followed by the GG genotype (46.6%) and a lower frequency of the AA genotype (3.6%). These findings suggest that the CYP2D6 *4 allele may be more prevalent in the Iranian population (Saghafi et al., 2018).

### 4.4.2. The Frequency of CYP2D6*10 in Polycystic Ovary Syndrome Women

This study reveals that the CYP2D6*10 wild-type (CC) genotype was the most prevalent among the studied women, indicating a high prevalence of normal CYP2D6 enzyme activity. Meanwhile, the heterozygous CT and homozygous mutant TT genotypes were less frequent. This contrasts with findings from Korean PCOS populations, where the CYP2D6 *10 allele was reported as the most predominant variant (Ji et al., 2016). Furthermore, a similar study conducted with randomly selected volunteers in Korea also yielded comparable results (Kim et al., 2018). The CYP2D6*10 allele reflects this variant's significantly reduced activity, the most common allele in Asian populations. Although this allele is found in other populations, such as Europeans, Africans, and their descendants, its frequency is lower (Kane, 2021). In comparison, the frequency of the CYP2D6*10 allele varies significantly across Middle Eastern and North African populations. Iran has the highest prevalence of this allele at 20.4%, followed by Jordan and Turkey at 14.8% and 13.14%, respectively (Khalaj et al., 2019). In Iraq, the frequency of this allele is reportedly 13.4%. Notably, Arab populations demonstrate a higher proportion of normal metabolizers (NMs) than Europeans, East Asians, or Americans, with rates of 70.53%, 51.05%, 51.91%, and 63.6%,

respectively (Alali et al., 2022). Another study revealed the frequency of allele*10 in Iraqi breast cancer to be 10.42% (Ali et al., 2023). Similarly, a study conducted on Iranian women revealed that the wild-type genotype was the most predominant, with heterozygous CT observed in 15.1% and the homozygous mutant TT genotype in 24.4% of participants (Bagheri et al., 2015).

### 4.4.3. Demographic data and Polycystic ovary syndrome symptoms of infertility in women based on CYP2D6*4 genotypes

This study analyzed demographic parameters and PCOS symptoms based on CYP2D6*4 1934G>A genotypes. It shows that the means of age, BMI, menarche, and duration of marriage of the three genotypes are very close, Furthermore, hirsutism, alopecia, abortion, and regularity of menses show no significant differences. This suggests that the genotypes have no role in these demographic data. Similarly, other studies also show no role of the enzyme (Ji et al., 2016, Robin et al., 2021).

### 4.4.4. Demographic Data and PCOS symptoms of infertility in women based on CYP2D6*10 genotypes

The demographic characteristics associated with the CYP2D6*10 genotypes reveal no significant differences. Additionally, there are no significant differences in PCO symptoms such as hirsutism, alopecia, abortion rates, or menstrual regularity among these genotypes. Overall, these findings suggest that the CYP2D6*10 genotype does not influence these demographic parameters. This aligns with the Korean study of CYP2D6*10, which also shows no significant differences in demographic data (Kim et al., 2018) as another study shows similar findings (Robin et al., 2021).

### 4.4.5. Effect of CYP2D6*4 Genotypes on Reproductive Hormone Levels and Ultrasound Findings in Women with Polycystic ovary syndrome

In this study, hormone profiles were measured and ultrasounds were performed on the women after 5 days of their last clomiphene dose. CYP2D6*4 genotypes did not appear to substantially impact clinical outcomes or hormonal profiles, as none of the genotypes showed significant differences. Although ultrasound revealed that the wild-type showed the largest mean of follicle size and ET, followed by heterozygous and the homozygous mutant (AA) showed the smallest mean of both but it did not reach statical differences; mainly, clomiphene is used to induce ovulation by binding to estrogen receptors and increasing gonadotropin releasing hormones thus increase releasing of FSH which mainly cause follicle to grow (Girase et al., 2023). This finding aligns with a previous study that explained that the clinical response to clomiphene does not appear to be directly correlated with the women's CYP2D6 genotypes (Robin et al., 2021).

### 4.4.6. Effect of CYP2D6*10 Genotypes on Reproductive Hormone Levels and Ultrasound Findings in Women with Polycystic ovary syndrome

The study showed the patients' reproductive hormone levels after taking clomiphene along with an ultrasound. CYP2D6*10 100C > T polymorphism does not appear to have a significant association with clinical response. These results are in agreement with those of Ji et al. They observed no correlation between the CYP2D6 genotype and the clinical response(Ji et al., 2016). Additionally, this correlates with a previous study done by (Robin et al., 2021). Meanwhile, the Iranian study stated that patients with the wild-type

(CC) genotype showed a better response to the drug, as evidenced by increased follicle diameter (Afsharian et al., 2013).

### 4.4.7. Effect of SNPs on E-clomiphene and E-4-hydroxyclomiphene levels

#### 4.4.7.1. The Effect of CYP2D6*4 Genotypes on The Plasma Concentration of Clomiphene And Its Metabolite

This study showed that patients with two inactive *4 alleles (AA genotype) had significantly lower concentrations of (E)-4-hydroxy-clomiphene after taking the CC drug than other genotypes. However, the study showed no significant difference in the plasma levels of the prodrug among the genotypes after CC administration. This may be attributed to the wild-type genotype (GG) exhibits normal CYP2D6 enzymatic activity; those with the heterozygous genotype (AG) show reduced activity, whereas individuals homozygous for the inactive allele (AA) lack functional enzyme activity, resulting in impaired substrate metabolism and a decreased capacity to metabolize clomiphene citrate (Zihlif et al., 2012, Zayed et al., 2015, Alvarado et al., 2021, El Akil et al., 2022).

This aligns with other studies that revealed that PM women had significantly lower levels of the active metabolite E-4-OH-clom, although they stated higher levels of the parent drug than other metabolic phenotypes (Mürdter et al., 2012). A previous study reported a direct correlation between the amount of CYP2D6 present and the extent of E-clomiphene metabolism (Ghobadi et al., 2008). Several studies have confirmed that PM exhibit markedly reduced levels of (E)-4-hydroxy-clomiphene, with reductions ranging from 8- to 12-fold compared to extensive metabolizers (EM) (Ji et al., 2016). In another study that built pharmacokinetic modeling on female

volunteers, PM were observed to have ten times lower plasma concentrations of the active metabolite (Kovar et al., 2022).

#### 4.4.7.2. The Effect of CYP2D6*10 Genotypes on The Plasma Concentration Of Clomiphene And Its Metabolite

In this study, CYP2D6*10 genotyping revealed that individuals with the TT genotype, which is homozygous for the variant allele, exhibited significantly elevated levels of the parent drug and reduced concentrations of its active metabolite. Individuals carrying the non-functional T-allele of CYP2D6*10 have reduced enzyme activity (Bairova et al., 2019, Kane, 2021). The CYP2D6*10 allele is associated with intermediate metabolizer (IM) status, which may confer an increased risk of suboptimal drug efficacy or adverse effects, particularly for drugs that are prodrugs (Orengo-Mercado et al., 2013).

Despite marked differences in metabolic profiles, the study did not identify any significant variation in clinical outcomes. Supporting this finding, a previous study reported that serum concentrations of the parent drug clomiphene were not significantly correlated with clinical response parameters (Ghobadi et al., 2009a). Similarly, another study identified a correlation between CYP2D6*10 genotypes and plasma concentrations. Plasma levels of the active metabolites were 8 times higher than in the PM women (Mürdter et al., 2012). A previous Korean study reported significant differences in parent drug concentrations between wild-type and mutant genotypes. Their study found a higher concentration of active metabolites in women with CYP2D6 mutations that cause decrease in CYP2D6 activity (Kim et al., 2018).

# Conclusions & Recommendations

## Conclusions

From the present study, conclusions can be given:

1. The majority of Iraqi women with PCOS appear to be normal metabolizers, as the most predominant genotype of CYP2D6*4 (1934G>A; rs3892097) is GG, followed by AG and AA. Similarly, for CYP2D6*10 (100C>T; rs1065852), the CC genotype is the most frequent, followed by CT and TT.
2. The CYP2D6*4 and CYP2D6*10 genotypes show no significant association with hormonal profiles or clinical response to clomiphene citrate in Iraqi women with PCOS.
3. The homozygous mutant genotype (AA) of CYP2D6*4 (1934G>A; rs3892097) was significantly associated with lower plasma concentrations of E-4-OH-clomiphene, compared to heterozygous (AG) and wild-type (GG) genotypes.
4. The homozygous mutant genotype (TT) of CYP2D6*10 (100C>T; rs1065852) was significantly associated with high concentration of parent drug (E-Clomiphene) and a lower concentration of its metabolite (E-4-OH-Clomiphene) suggest reduced metabolic activity in thses individuals.

## Recommendations

1. A larger, multicenter study involving diverse regions in Iraq is recommended to validate these findings and enhance their generalizability across the broader Iraqi population.
2. Further pharmacogenomic research is warranted to investigate additional genetic variants beyond CYP2D6, such as CYP3A4, that may influence clomiphene metabolism and contribute to inter-individual variability in treatment response and safety profiles.
3. Investigating the long-term treatment outcomes associated with CYP2D6 genotypes and other relevant pharmacogenetic polymorphisms during clomiphene therapy. This could include monitoring pregnancy rates, multiple birth rates, and potential side effects.

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Iraqi Ministry of Health
Karbala Health Directorate
Training and Human Development Center
Research Committee

Form number 03/2021
**Decision Number:** 2/10/2023
**Date:**

## Research Committee Decision

The Research Committee of Karbala Health Directorate has examined the research protocol number **2023180** entitled:

**Impact of genetic polymorphism of cytochrome P450 2D6 on clomiphene citrate clinical response in Iraqi PCOs**

Submitted by researchers: **Maryam Hussein Abd Ali** to the Research and Knowledge Management Unit at the Training and Human Development Center of Karbala Health Directorate on 2/10/2023

The Unit has decided to:

*** Accept the above-mentioned research protocol as it meets the standards adopted by the Ministry of Health for the implementation of research, and there is no objection to implementing it in the Directorate's institutions.**

الدكتور
نعيم عبيد الشهداني
**Rapporteur of the committee**

02/10/2023

**Attachments:** None

**Notes:**

· The committee chairperson / committee rapporteur was authorized to sign this decision on behalf of the remaining members of the committee under the rules of procedure of the research committee.

· The Research Committee approval means that the research project submitted to the aforementioned committee has fulfilled the ethical and methodological standards adopted by the Ministry of Health for conducting a research. As for the implementation of the research, it depends on the researcher's adherence to the instructions of the health institution in which the research will be implemented as well as the laws, instructions and recommendations in force that govern the practice of medical and health action in Iraq.

# Questioner

| | | | |
|---|---|---|---|
| Name | | Education level | |
| Number | | Job | |
| Age | | Smoking | |
| Location | | Marriage duration | |
| Menarche | | No. kid (if available) | |
| Weight | | Miscarriage | |
| Height | | Menstrual irregularity | |
| Hirsutism | | Disease | |
| Alopecia | | Another drug | |
| **Cycle day 12** | | **Cycle day 2** | |
| | | AMH: | |
| | | Prolactin: | |
| FSH: | | FSH: | |
| LH: | | LH: | |
| E2: | | E2: | |
| **Ultrasound** | | **Ultrasound** | |
| follicle size | | Follicle size | |
| Endometrial thickness: | | Endometrial thickness: | |

**الخلاصة**

**الاستنتاج :**خلصت هذه الدراسة إلى أن التباين الجيني لـ CYP2D6*4 وCYP2D6*10 لم يؤثر على الاستجابة العلاجية للكلوميفين نظرًا لعدم وجود تغييرات في الملامح الهرمونية ونتائج الموجات فوق الصوتية. يؤثر تحديد النمط الجيني لـ CYP2D6*4 وCYP2D6*10 على استقلاب الكلوميفين

## الخلاصة

**الخلفية:** سترات الكلوميفين هو مُعدّل انتقائي لمستقبلات الإستروجين (SERM)، ويُستخدم على نطاق واسع كعلاج أولي لتحفيز الإباضة لدى النساء المصابات بالعقم المرتبط بمتلازمة تكيس المبايض (PCOS). يخضع هذا الدواء لعملية استقلاب كبدي بشكل رئيسي عن طريق إنزيم السيتوكروم P450 2D6 (CYP2D6)، وهو إنزيم متعدد الأشكال للغاية. قد تؤثر المتغيرات الجينية لإنزيم CYP2D6 على كل من التنشيط الأيضي للكلوميفين وفعاليته العلاجية.

**هدف الدراسة:** هدفت هذه الدراسة إلى الكشف عن التعدد الشكلي الجيني لإنزيم CYP2D6 وتحديد ما إذا كانت الاختلافات الجينية CYP2D6*4 1934G>A (rs3892097) أو CYP2D6*10 (rs1065852) تؤثر على استجابة علاج الكلوميفين في متلازمة تكيس المبايض من خلال تحليل مستويات الهرمونات ونتائج الموجات فوق الصوتية وتركيزات الدواء/المستقلب.

**المرضى والطرق:** شملت هذه الدراسة الأترابية 80 امرأة مصابة بمتلازمة تكيس المبايض، تم اختيارهن عشوائيًا من مستشفى النسائية والتوليد في كربلاء، وتلقين علاجاً بـجرعة 100 ملغ من الكلوميفين يوميًا لمدة خمسة أيام، بدءًا من اليوم الثاني من الحيض. تم جمع عينات الدم للتحليل الجيني وقياس مستويات الهرمونات التناسلية وتحليل تركيزات الكلوميفين ومستقلبه في البلازما. تم تقييم حجم جريب المبيض وسمك بطانة الرحم من خلال فحص بالموجات فوق الصوتية من قبل طبيب متخصص.

**النتائج:** فُحص اختلافان جينيان متميزان في جين CYP2D6 لدى مريضات متلازمة تكيس المبايض المصابات بالعقم. كان توزيع تعدد أشكال CYP2D6*4 (GG وAG وAA) 66.3% و30% و3.8% على التوالي، بينما أظهر تعدد أشكال CYP2D6*10 توزيعًا بنسبة 66.3% CC و27.5% CT و6.3% TT. لم تُظهر المعايير السريرية، مثل مستويات الهرمونات التناسلية ونتائج الموجات فوق الصوتية، دلالة إحصائية عند قيمة $P < 0.05$ لكلا التعددين الشكليين. في الوقت نفسه، بدا أن المرضى الحاملين للنمط الجيني الطافر CYP2D6*4 لديهم مستوى أقل بكثير من المستقلب النشط مقارنةً بالأنماط الجينية الأخرى. علاوة على ذلك، أظهر الأفراد الحاملون للنمط الجيني الطافر CYP2D6*10 انخفاضًا كبيرًا في المستقلب النشط وزيادة في تركيز الدواء الأصلي (قيمة $P < 0.05$)

**جمهورية العراق**

**وزارة التعليم العالي والبحث العلمي**
**جامعة كربلاء**
**كلية الصيدلة**

# تأثير التعدد الجيني لـ CYP450 2D6 على الاستجابة السريرية لسيترات الكلوميفين في النساء العراقيات اللاتي يعانين من متلازمة تكيس المبايض

رسالة مقدمة الى

كلية الصيدلة في جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في

الادوية والسموم

من قبل

## مريم حسين عبد علي الهر

**(بكالوريوس صيدلة/ جامعة كربلاء 2020)**

**بإشراف**

**أ.د. أثير ماجد رشيد الجحيشي**

دكتوراه أدوية وعلاجيات

**أ.د. حسن محمود أبو المعالي**

دكتوراة هندسة وراثية

**2025 ميلادي** **1446 هجري**