



Ministry of Higher Education and
Scientific Research
University of Kerbala
College of Pharmacy



***Impact of Genetic Polymorphism of Cytochrome
P4503A4 on the Clomiphene Citrate Clinical
Response in a Sample of Iraqi Women with
Polycystic Ovary Syndrome***

A Thesis

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

By

Nadia Sahib Abd Ali Al-Sharefi

B.Sc. in Pharmacy (Baghdad College of Medical Sciences, 2008)

Supervised by

Prof. Dr. Atheer Majid

Asst. Prof. Dr. Hameedah Hadi

Rashid Al-Juhiashi

Abdel Wahid

PhD. Pharmacology

OB-GYN Board Certification

2025 AD

1446 AH

بسم الله الرحمن الرحيم

وَيَرَى الَّذِينَ أُوتُوا
الْعِلْمَ الَّذِي أَنْزَلَ إِلَيْكَ
مِنْ رَبِّكَ هُوَ الْحَقُّ
وَيَهْدِي إِلَى صِرَاطٍ
الْعَزِيزِ الْحَمِيدِ

صدق الله العلي العظيم

سورة سبأ: آية (٦)



Supervisors' Certification

We hereby certify that the thesis entitled "Impact of Genetic Polymorphism of Cytochrome P4503A4 on the Clomiphene Citrate Clinical Response in a Sample of Iraqi Women with Polycystic Ovary Syndrome " was prepared by Nadia Sahib Al-Sharefi under our supervision in the Department of Pharmacology and Toxicology, College of Pharmacy, University of Kerbala, as a partial fulfillment of the requirements for the degree of Master of Science in Pharmacology and Toxicology

Supervisor

Professor

Dr. Atheer Majid Rashid

College of Pharmacy

University of Kerbala

Date: / / 2025

Supervisor

Assist. Professor

Dr.Hameedah Hadi

College of Medicine

Warith Al anbiyaa University

Date: / / 2025

In the view of the available recommendation, we forward the present thesis for debate by the examining committee.

Prof. Dr.Shatha Hussein Kadhim

Chairman of Pharmacology and Toxicology Department

University of Kerbala / College of Pharmacy

Committee Certification

We, the examining committee, certify that we have read the thesis entitled "Impact of Genetic Polymorphism of Cytochrome P4503A4 on the Clomiphene Citrate Clinical Response in a Sample of Iraqi Women with Polycystic Ovary Syndrome ", and have examined the student (**Nadia Saheb Abd-Ali**) on its contents. We find it adequate and acceptable as a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Pharmacology and Toxicology.

Thesis Committee Names:



Assist. Prof. Dr. Mohammed Ibrahim Rasool

Chairman



Assist. Prof. Dr. Asaad H. Alzaidy

Member



Assist. Prof. Zahraa Abed Al kareem

Member



Prof. Dr. Atheer Majid Rashid

Al-Juhiashi

Supervisors



Assist. Prof. Dr. Hameedah

Hadi

Approved by

College of Pharmacy / University of Kerbala

As a thesis for the degree of
Masters in Pharmacology and Toxicology

A handwritten signature in green ink, consisting of a large loop at the top and a horizontal stroke below it.

Asst.Professor

Dr. Mohammed Ibrahim Rasool

Dean

College of Pharmacy / University of Kerbala

Seal

Higher Studies Registration

College of Pharmacy / University of Kerbala

Dedication

To the finest father in the world, the one whose name I proudly bear, and who has stood by me in achieving my life's ambitions.

To the embodiment of love and compassion, my mother, whose prayers are the foundation of my success and whose unwavering presence is central to my life.

To my life partner, my husband, whose steadfast support, boundless generosity, and immense sacrifices have enabled me to fulfill my dreams and earn my degree.

To my beloved children, who have brought laughter to my days, dried my tears, held me close, and cheered me on every step of the way.

To my sisters, who have been my anchors as I pursued my dreams. Together, let's continue to soar.

Nadia 2025

Acknowledgements

I would first like to express my deepest gratitude to **Allah** for granting me the health, strength, and ability to complete this study.

I am grateful to **Asst. Prof. Dr. Mohammed Ibrahim Rasool**, Dean of the College of Pharmacy, University of Kerbala, for his kind assistance. Additionally, I would like to express my sincere thanks to **Prof. Dr. Shatha Hussein Kadhim**, Head of the Department of Pharmacology and Toxicology, University of Kerbala, for her invaluable support.

I am sincerely thankful to **Prof. Dr. Atheer Majid Rashid Al-Juhaishi**, Assistant Professor and my supervisor, for her unwavering support, vast expertise, and endless patience throughout this research.

I also extend my heartfelt appreciation to my second supervisor, **Asst. Prof. Dr. Hameedah Hadi**, for his support and encouragement during various stages of this study.

My deep appreciation goes to Prof. Dr. Mazin Hamid Ouda Al-Rubaiee, College of Pharmacy, University of Kerbala, for his kind encouragement and invaluable assistance.

I am truly grateful to my colleague, Maryam Al-Har, and to all the staff working in the infertility consultation clinic, the consulting pharmacy, and the blood-drawing unit at the Obstetrics and Gynecology Hospital / Karbala Health Department for their cooperation and support during the research period.

I also extend my heartfelt thanks to all the patients who participated in this study, as well as to everyone who contributed in any way to the completion of this thesis.

Nadia

List Of Contents		
Contents		Page
Quranic verse		I
Supervisors ' Certification		II
Committee Certification		III
Approval		IV
Dedication		V
Acknowledgements		VI
List of Contents		VIII
List of Tables		XIII
List of Figures		XIV
List of Abbreviations		XVI
Abstract		XX
Chapter One: Introduction		
No	Title	Page
1	Introduction	1
1.1	Polycystic Ovarian Syndrome	1
1.1.1	Epidemiology: Incidence and Prevalence	2
1.1.2	Etiology and Pathophysiology	3
1.1.2.1	Genetic and Epigenetic Mechanisms	3
1.1.2.2	Environmental Toxicants	4
1.1.2.3	Physical and Emotional Stress	5
1.1.2.4	Diet	6
1.1.2.5	Insulin Resistance	6
1.1.2.6	Hyperandrogenism	6
1.1.2.7	Inflammation	7
1.1.2.8	Oxidative Stress	7
1.1.3	Diagnosis Criteria	7
1.1.3.1	Clinical Symptoms	8
1.1.3.1.1	Acnevulgaris	8
1.1.3.1.2	Hirsutism	9
1.1.3.1.3	Alopecia	9
1.1.3.1.4	Acanthosis Nigricans	9

1.1.3.1.5	Irregular Menses and Infertility	9
1.1.3.2	Hormonal levels	10
1.1.3.3	Imaging	10
1.1.4	Types of PCOS according to the reasons	11
1.1.4.1	Insulin-resistant PCOS	11
1.1.4.2	Post Pill PCOS	11
1.1.4.3	Inflammatory PCOS	11
1.1.4.4	Hidden PCOS	11
1.1.4.5	Adrenal Gland PCOS	11
1.1.5	Managements	12
1.1.5.1	Lifestyle Modification and Non-Pharmacological Approaches	12
1.1.5.2	Pharmacological Treatments	14
1.1.5.2.1	Oral Contraceptives and Anti-Androgens	14
1.1.5.2.2	Insulin Sensitizers	15
1.1.5.2.3	Ovulation Inducers	16
1.1.5.2.4	Emerging Therapeutics	17
1.1.5.2.4.1	Statins	17
1.1.5.2.4.2	Glucagon-Like Peptide-1(GLP-1) Agonist	17
1.1.5.2.4.3	Inositol	17
1.1.5.2.4.4	Interleukin (IL)-22 Therapy	18
1.1.5.2.4.5	Probiotics and Prebiotics	18
1.2	Clomiphene Citrate	19
1.2.1	Pharmacodynamics	19
1.2.2	Pharmacokinetics	20
1.2.3	Medical uses	22
1.2.3.1	Anovulation	22
1.2.3.2	Luteal Phase Deficiency	22
1.2.3.3	Off-label Use to Treat Male Hypogonadism	23
1.2.4	Adverse Effects	23
1.2.5	Clomiphene Interactions	23
1.3	Genetic Polymorphism of <i>Cytochromes P4503A4 gene</i>	24
1.3.1	<i>CYP3A4*18</i>	25
1.3.2	<i>CYP3A4*22</i>	25
1.4	Aims of the Study	27
Chapter Tow :Patients, Materials, and Methods		

No	Title	Page
2	Patients ,Materials , and Methods	28
2.1	Patients	28
2.1.1	Patients Criteria	28
2.1.1.1	Inclusion Criteria	28
2.1.1.2	Exclusion Criteria	28
2.1.2	Ethical and Scientific Approval	29
2.2	Materials	30
2.2.1	Instruments, Equipment, and Their Suppliers	30
2.2.2	Chemicals and Kits and Their Producing Companies	31
2.3	Methods	32
2.3.1	Study Design	32
2.3.2	Blood Collection	33
2.3.3	Determination of Body Mass Index	33
2.3.4	Hormonal Assay Methods	33
2.3.4.1	Measurement of Serum Anti-Mullerian Hormone (AMH) Level	33
2.3.4.2	Measurement of Serum Prolactin Level	34
2.3.4.3	Measurement of Serum Estradiol Level	35
2.3.4.4	Measurement of Serum Follicles Stimulating Hormone Level	36
2.3.4.5	Measurement of Seum Luteinizing Hormone Level	37
2.3.5	Quantification of Zuclomiphene and Z-desethylclomiphene	38
2.3.6	Genetic Analysis	39
2.3.6.1	Extraction of Genomic DNA from Blood Sample	39
2.3.6.2	Quantification of DNA Using a Spectrophotometric Approach.	40
2.3.6.3	Polymerase Chain Reaction	40
2.3.6.4	Primers Design	42
2.3.6.5	Enzyme digested product in (RFLP-PCR)	43
2.3.6.6	PCR optimization conditions	43
2.3.6.6.1	Optimization of Condition for <i>CYP3A4*22 (G>A) (rs35599367)</i> Genetic Polymorphism	43
2.3.6.6.2	Optimization of Conditions for <i>CYP4*18 (T>C) (rs28371759)</i> Genetic Polymorphism	44
2.3.6.7	PCR Running and Working Solution	44

2.3.6.7.1	RFLP- PCR Running and Working Solution	44
2.3.6.7.2	T- ARMS-PCR Running and Working Solution	45
2.3.6.8	Agarose Gel Electrophoresis	46
2.4	Statistical Analysis	47
Chapter Three : Results		
No	Title	Page
3	Results	48
3.1	Demographic Data and Clinical Symptoms in Infertile Women with PCOS	48
3.2	Hormonal Level and Ultrasound Analysis in Infertile Women with PCOS Before and After Clomiphene Treatment	50
3.2.1	Hormonal Status	50
3.2.2	Follicle size and Endometrial Thickness	50
3.3	Genetic Analysis	51
3.3.1	Genotyping and Allele Frequency Analysis of <i>CYP3A4*18 (T>C) (rs28371759)</i> in Infertile Women with PCOS	51
3.3.2	Genotyping and Allele Frequency Analysis of <i>CYP3A4*22 (G>A) (rs3559967)</i> in Infertile Women with PCOS	54
3.3.3	Frequency of <i>CYP3A4*18 (T>C) (rs28371759)</i> Genotypes Based on Demographic Data and Clinical Symptoms in Infertile Women with PCOS	57
3.3.4	Frequency of <i>CYP3A4*22 (G>A) (rs3559967)</i> Genotypes Based on Demographic Data and Clinical Symptoms in Women with PCOS	58
3.3.5	Levels of Reproductive Hormones and Ultrasound Findings in Infertile PCOS Women Based on <i>CYP3A4*18 (T>C) (rs28371759)</i> Genotypes	60
3.3.6	Levels of Reproductive Hormones and Ultrasound Findings in Infertile PCOS Women Based on <i>CYP3A4*22 (G>A) (rs3559967)</i> Genotypes	61
3.3.7	Analysis of the Drug and its Metabolite	62
3.3.7.1	Effect of <i>CYP3A4*18 (T>C) (rs28371759)</i> Genotypes on the Plasma Concentration of Clomiphene and its Metabolite	62

3.3.7.2	Effect of <i>CYP3A4*22 (G>A) (rs3559967)</i> Genotypes on the Plasma Concentration of Clomiphene Citrate and its Metabolite	64
Chapter Four: Discussion		
No	Title	Page
4	Discussion	65
4.1	Demographic Data and Clinical Symptoms in Infertile Women With PCOS	65
4.2	Hormonal and Ultrasound Analysis of Infertile PCOS Patients Before and After Treatment	67
4.3	Genetic Analysis	68
4.3.1	Frequency of <i>CYP3A4*18(T>C) (rs28371759)</i> Genotypes	68
4.3.2	Frequency of <i>CYP3A4*22(G>A) (rs3559967)</i> Genotypes	69
4.3.3	The Frequency of <i>CYP3A4*18(T>C) (rs28371759)</i> Genotypes Based on Demographic Data and Clinical Symptoms in Infertile Women with PCOS	70
4.3.4	The Frequency of <i>CYP3A4*22 (G>A) (rs3559967)</i> Genotypes Based on Demographic Data and Clinical Symptoms in Infertile Women With PCOS	71
4.3.5	Levels of Reproductive Hormones and Ultrasound Findings in Infertile Women With PCOS Based on <i>CYP3A4*18 (T>C) (rs28371759)</i> Genotypes	71
4.3.6	Levels of Reproductive Hormones and Ultrasound Findings in Infertile Women With PCOS Based on <i>CYP3A4*22 (G>A) (rs3559967)</i> Genotypes	72
4.3.7	The Effect of <i>CYP3A4*18 (T>C) (rs28371759)</i> Genotypes on the Plasma Concentration of Clomiphene Citrate and its Metabolite	74
4.3.8	The Effect of <i>CYP3A4*22 (G>A) (rs3559967)</i> Genotypes on the Plasma Concentration of Clomiphene Citrate and its Metabolite	76
4.4	Limitations	81
Conclusions & Recommendations		
Conclusions		82
Recommendations		83

References	
References and Appendices	84

List of Tables		
No	Title	Page
1-1	Diagnostic Criteria for Polycystic Ovary Syndrome.	8
2-1	Instruments and Their Manufactures	30
2-2	Chemicals and Kits and Their Producing Companies.	31
2-3	Primers Sequences and Expected Product Size of <i>CYP3A4*22 (G>A) (rs35599367)</i> Genetic Polymorphism.	42
2-4	Primers Sequences and Expected Product Size for <i>CYP3A4*18 (T>C)</i> Genetic Polymorphism.	43
2-5	PCR Thermal Program for <i>CYP3A4*18 (T>C) (rs28371759)</i> .	45
2-6	PCR Thermocycler Program for <i>CYP3A4*22 (G>A) (rs35599367)</i> .	46
3-1	Demographic Data and Clinical Symptoms of Infertile Women with PCOS	49
3-2	Reproductive Hormone Levels, Follicle Size, and Endometrial Thickness Before and After Clomiphene Treatment	51
3-3	Hardy-Weinberg Equilibrium for <i>(rs28371759)</i> in patients	53
3-4	Hardy-Weinberg Equilibrium for <i>(rs3559967)</i> in patients	56
3-5	Frequency of <i>CYP3A4*18 (T>C) (rs28371759)</i> Genotypes Based on Demographic Data and Clinical Symptoms in Infertile Women with PCOS	58
3-6	Frequency of <i>CYP3A4*22 (G>A) (rs3559967)</i> Genotypes Based on Demographic Data and Clinical Symptoms in Infertile Women with PCOS	59
3-7	Levels of Reproductive Hormones and Ultrasound Findings in Infertile PCOS Women Based on <i>CYP3A4*18 (T>C) (rs28371759)</i> Genotypes	61
3-8	Levels of Reproductive Hormones and Ultrasound Findings in Infertile PCOS Women Based on <i>CYP3A4*22 (G>A) (rs3559967)</i> Genotypes	62

3-9	Effect of <i>CYP3A4*18 (T>C) (rs28371759)</i> Genotypes on the Plasma Concentration of Clomiphene Citrate and its Metabolite	63
3-10	Effect of <i>CYP3A4*22 (G>A) (rs3559967)</i> Genotypes on the Plasma Concentration of Clomiphene Citrate and its Metabolite	64

List Of Figures		
No	Title	Page
1-1	Ovary with PCOS	1
1-2	Pharmacodynamics of Clomiphene Citrate	20
1-3	Human Metabolism of (E)- and (Z)-Clomiphene	21
3-1	Genotyping of <i>CYP3A4*18(T>C) (rs28371759)</i> gene using RFLP-PCR on 2% agarose gel stained with ethidium bromide and viewed under UV light	52
3-2	Frequency of <i>CYP3A4*18 (T>C) (rs28371759)</i> Genotypes Among All Studied Females (N = 80)	53
3-3	Bar Chart Comparing Observed vs.Expected Genotype Distribution for (<i>rs28371759</i>)	54
3-4	Genotyping of <i>CYP3A4*22 (G>A) (rs3559967)</i> using T-ARMS-PCR on 2% agarose gel stained with ethidium bromide and viewed under UV light	55
3-5	The Frequency of <i>CYP3A4*22 (G>A) (rs3559967)</i> genotypes among all the studied females (N=80)	55
3-6	Bar Chart Comparing Observed vs.Expected Genotype Distribution for (<i>rs3559967</i>)	56

List of Abbreviations	
A	Adenine
AES	Androgen Excess Society
AGEs	Advanced glycation end products
AIs	Aromatase inhibitors
AMH	Anti-mullerian hormone
AR	Androgen receptor
BMI	Body Mass Index
bp	Base pair
BPA	Bisphenol A
C	Cytosine
CAM	Complementary and Alternative Medicine
CC	Clomiphene citrate
CL- series	Chemiluminescence series
CRP	C-reactive protein
CVD	cardiovascular disease
CYP p450	Cytochrome p 450
CYP2B6	Cytochrome P450 family 2 Subfamily B Member 6
CYP2D6	Cytochrome P450 Family 2 Subfamily D Member6
CYP3A4	Cytochrome P450 Family 3 Subfamily A Member4
CYP3A43	Cytochrome P450 family 3 subfamily A member 43
CYP3A5	Cytochrome P450 Family 3 Subfamily A Member5
DCI	d-chiro-inositol
DDGIs	drug-drug-gene interactions
ddH ₂ O	Double Distilled Water
DDIs	drug-drug interactions
DGIs	Drug-gene interactions
DNA	Deoxyribonucleic acid
EDCs	endocrine-disrupting chemicals
EDTA	Ethylene Diamine Tetraacetic Acid
EPHX1	epoxide hydrolase 1
ER	estrogen receptor
ESHRE/ASRM	European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine

ESHRE/ASRM	European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine
FDA	Food and Drug Administration
FOXO	forkhead box O
FPHL	Female pattern hair loss
FSH	Follicle-stimulating hormone
G	Guanine
GWAS	Genome-wide association studies
GIP	Gastric inhibitory polypeptide
GLP-1	Glucagon-Like Peptide-1
GLUT-4	Glucose transporter Type 4
Gm	Grame
GnRh	Gonadotropin-releasing hormone
Gs	Gas chromatography
GSB buffer	Genomic DNA binding Solution Buffer
HA	Hyperandrogenism
HIV	Human Immunodeficiency Virus
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
HPA	hypothalamic-pituitary-gonadal axis
I-F	Inner Forward
IGF-1	Insulin-like growth factors 1
IGF1R	Insulin-like growth factor 1 receptor
IL	Interleukin
IMs	intermediate metabolizers
IQR	inter quartail range
IR	Insulin resistance
I-R	Inner Revers
Kg/m ²	kilograms per square meter
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LH	Luteinizing Hormone
LHCGR	Luteinizing Hormone /choriogonadotropin receptor
MAF	minor allele frequency
MgCl ₂	magnesium chloride
MI	myoinositol
Mm	Millimeters
mRNA	Messenger Ribonucleic acid

MTHFR	methylenetetrahydrofolate reductase polymorphisms
NAFLD/NASH	nonalcoholic fatty liver disease/ nonalcoholic steatohepatitis
NC	Nitrocellulose
NF- κ B	nuclear factor-kappa B
ng/dl	Nanograms per decilitre
NIH/NICHD	National Institutes of Health/National Institute of Child Health and Human Disease
NMs	normal metabolizers
OCP	oral contraceptive tablets
O-F	Outer Forward
OHSS	ovarian hyperstimulation syndrome
O-R	Outer Revers
OS	Oxidative stress
OSA	Obstructive sleep apnea
PCOS	Polycystic ovarian syndrome
PCR	Polymerase Chain Reaction
Pg/ml	Picogramme per millilitre
p-gp	P-glycoprotein
pmol/ μ L	picomol/microliter
PMs	poor metabolizers
PON1	polymorphic paraoxonase
PPAR- γ	peroxisome proliferator-activated receptor gamma
Ppm	Parts per million
PRL	Prolactin
PXR	Pregnane X receptor
RAGE	receptor for advanced glycation end products
RFLP-PCR	Restriction fragment length polymorphism
RLUS	relative light units
RPM	revolutions per minute
STK11	serine/threonine kinase 11
SERM	selective estrogen receptor modulator
SFAs	saturated fatty acids
SHBG	sex hormone-binding globulin
SNP	single nucleotide polymorphism
SPSS	Statistical package for social sciences
T	Tyrosine

T-ARMS-PCR	Tetra amplification refractory mutation system
TBE buffer	tris borate- EDTA
TE buffer	Tris-EDTA
TNF- α	Tumor necrosis factor-alpha
TSH	Thyroid-stimulating hormone
TZDs	Thiazolidinediones
UCP	Up-Converting Phosphor
UM	ultra-rapid metabolizers
UPT	Up-converting Phosphor Technology
UPT	Up-converting Phosphor Technology
UV	Ultraviolet
V	Voltage
v/cm	Volt per centimeter
Xg	Times gravity
Z-form	Zuclomiphene
μ M	Micro meter
10 cc	Cubic centimeters
μ L	Microliter

Abstract

Background

Polycystic ovary syndrome (PCOS) is a common hormonal disorder affecting women of reproductive age. Clomiphene Citrate is often prescribed to induce ovulation in these patients. The cytochrome P450 enzymes, particularly *CYP3A4*, play a crucial role in the metabolism of many drugs. Genetic variations in this enzyme can lead to differences in drug metabolism. Among the most common coding variants are *CYP3A4*18* (*rs28371759*) and *CYP3A4*22* (*rs35599367*), which are particularly relevant to the metabolism of Clomiphene Citrate.

Aims of the study

The study aimed to detect *CYP3A4*22* *G>A* (*rs35599367*) and *CYP3A4*18* (*rs28371759*) genetic polymorphisms among Iraqi women with polycystic ovarian syndrome and to investigate the influence of these gene variants on the efficacy of clomiphene citrate.

Patients and methods

This cohort study was conducted at the Obstetrics and Gynecology Teaching Hospital in Karbala Governorate between December 2023 and June 2024. A total of 80 Iraqi women diagnosed with PCOS, aged 20–40 years, who were receiving Clomiphene Citrate (50 mg orally, twice daily) for at least two cycles, were included. Blood samples were collected for genotyping and for measuring reproductive hormone levels, including estradiol, Anti-Mullerian Hormone, Luteinizing Hormone, Follicle-Stimulating Hormone,

and prolactin. Plasma concentrations of Zuclomiphene and its metabolites were also analyzed by using Liquid chromatography-tandem mass spectrometry. The Tetra-Amplification Refractory Mutation System (T-ARMS-PCR) was used to detect *CYP3A4*22 G>A* (*rs35599367*), while Restriction Fragment Length Polymorphism PCR (RFLP-PCR) was employed to detect *CYP3A4*18 T>C* (*rs28371759*) genetic polymorphism.

Results

The findings revealed variations in the genetic distribution of *CYP3A4*22 G>A* (*rs35599367*) and *CYP3A4*18 T>C* (*rs28371759*). For *rs35599367 G>A*, the wild-type homozygous (GG) genotype was the most common (67.5%), followed by the heterozygous (GA) genotype (25%), and the mutant homozygous (AA) genotype (7.5%).

For *rs28371759 C>T*, the wild-type homozygous (TT) genotype was the most frequent (83.8%), while the heterozygous (TC) and mutant homozygous (CC) genotypes were less common, with frequencies of 11.25% and 5.0%, respectively.

Significant differences were observed across *CYP3A4*18* genotypes in FSH ($P = 0.03$), estrogen ($P = 0.008$), zuclomiphene levels ($P = 0.044$), and the metabolite/prodrug ratio ($P = 0.002$). Similarly, significant differences were found across *CYP3A4*22* genotypes in LH ($P = 0.026$), endometrial thickness ($P = 0.048$), and the metabolite/prodrug ratio ($P = 0.000003$).

Conclusion

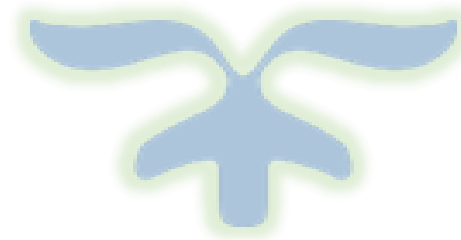
The present study concluded that there is a correlation between genetic variations in the *CYP3A4*22* and *CYP3A4*18* genes and the variability in

response to clomiphene therapy among the study participants with polycystic ovary syndrome (PCOS). This variability is attributed to changes in certain hormone levels and ultrasound findings among different genotypes. Genetic patterns of *CYP3A4*22* (*rs35599367*) and *CYP3A4*18* (*rs28371759*) also affected the metabolic ratio of clomiphene.



Chapter One

Introduction



1. Introduction

1.1. Polycystic Ovarian Syndrome

Polycystic ovarian syndrome (PCOS), also known as hyperandrogenic anovulation (HA) or Stein–Leventhal syndrome, is a common endocrine disorder affecting women of reproductive age. This chronic and heterogeneous condition can lead to menstrual irregularities, hirsutism, acne, infertility, and weight gain. It is characterized by at least one ovary measuring more than 10 cc in volume and containing ten or more small cysts, each ranging in size from 2 to 9 mm (Choudhary et al., 2019).

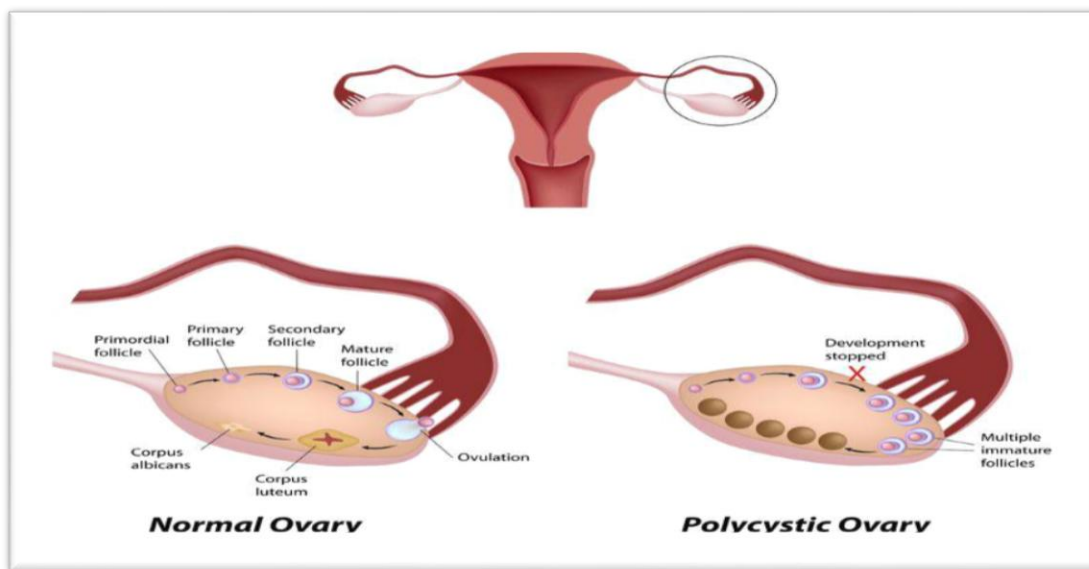


Figure (1-1): Ovary with PCOS (Dambala et al., 2019).

Polycystic Ovary Syndrome (PCOS) is often underdiagnosed. Diagnosis typically requires multiple consultations with different healthcare providers and can take over a year. This delay can be frustrating for patients and may hinder the timely adoption of lifestyle changes aimed at improving well-being

and quality of life. PCOS is associated with various morbidities, including infertility, metabolic syndrome, and obesity, as well as comorbidities such as type 2 diabetes mellitus, depression, obstructive sleep apnea (OSA), increased cardiovascular risk, endometrial cancer, and nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (NAFLD/NASH) (Rasquin et al., 2017).

1.1.1. Epidemiology: Incidence and Prevalence

Polycystic ovary syndrome (PCOS) affects approximately 8–13% of women of childbearing age, with an estimated 70% of cases worldwide remaining undiagnosed (Islam et al., 2022). Infertility has increased alarmingly among couples, particularly in Iraq, compared to previous years. The infertility rate among all couples is approximately 12%. The prevalence of PCOS is estimated to be around 35% among all women and 45% among married women, with a decline observed as age increases (Mousa and Al Joborae, 2020).

Research conducted in Baghdad indicated that PCOS was associated with around 29% of infertility cases (Hanaa Rahman et al., 2015). At Tikrit Teaching Hospital, a study found that 73.3% of infertility cases were due to PCOS (Eleawi et al., 2015). A case-control study involving patients from the infertility unit at Babylon Teaching Hospital and a private clinic found that 33.0% of infertile women had PCOS (Mousa, 2019). Additionally, a prospective cross-sectional study conducted at Azadi Teaching Hospital (Family Planning Center) in Kirkuk found that 35% of infertile women had PCOS (Ibrahim et al., 2024). Random effects modeling has been used to assess the incidence of PCOS across different ethnicities. According to the 2003 Rotterdam criteria, approximately 15% of women in Western populations may

be affected by PCOS. A study involving 15,000 Chinese women reported a prevalence of 5.6%. Women from Southern China (5.6%), Thailand (5.7%), Sri Lanka (6.3%), and Iran (14.3%) exhibit varying prevalence rates (Huang and Yong, 2016). Most women with polycystic ovary syndrome (PCOS) have insulin resistance. Among them, 10% have diabetes before they reach 40 (Tabassum et al., 2013). The prevalence of PCOS also varies by body weight category: 8.2% in underweight individuals, 9.8% in those with normal weight, 9.9% in overweight individuals, and 9.0% in those with obesity (Yildiz et al., 2008).

1.1.2. Etiology and Pathophysiology

The exact etiology and pathophysiology of PCOS remain unclear; however, substantial evidence indicates that it is a multifactorial disorder influenced by factors such as genetics, obesity, diet, and environmental pollutants. These contributors may lead to excessive androgen production, disrupted folliculogenesis, insulin resistance, and chronic low-grade inflammation, which together increase the risk of metabolic syndrome. Determining the precise causative factors is challenging because of their complex and interrelated mechanisms (Siddiqui et al., 2022).

1.1.2.1. Genetic and Epigenetic Mechanisms

Recent research shows that PCOS has a strong genetic component, with heritability estimated at around 80% (Bhandari et al., 2020). Genome-wide association studies (GWAS) have identified nearly 19 genetic loci located within or near genes linked to PCOS, including several single nucleotide polymorphisms (SNPs). Among these are SNPs in the serine/threonine kinase 11 (STK11) and forkhead box O (FOXO) genes. Additionally, studies in

PCOS patients who experienced acute hyperglycemic episodes suggest that polymorphisms in the insulin receptor substrate 1 (IRS1) gene may play a role. Since IRS1 is a key mediator in insulin signaling and essential for normal cellular function, genetic variations in this gene can disrupt its activity, acting as competitive inhibitors of the insulin receptor. Consequently, IRS1 mutations may contribute both to the development of PCOS and to increased susceptibility to insulin resistance (Zahraa et al.,2021).

Epigenetic modifications include heritable alterations in gene expression, shown as elevated Luteinizing Hormone (LH) activity in women with PCOS. This may result from follicular development abnormalities and Hyperandrogenism (HA) concerns, as the Luteinizing Hormone/choriogonadotropin receptor (LHCGR) undergoes hypomethylation, leading to enhanced gene expression and sensitivity (Sadeghi et al., 2022).

The overexpression of LHCGR, epoxide hydrolase 1, and peroxisome proliferator-activated receptor gamma (PPAR- γ) were discovered to be linked to these hypomethylated locations. Epoxide hydrolase 1 (EPHX1) overproduction lowers the testosterone-to-estradiol ratio, which in turn contributes to polycystic ovary syndrome (PCOS). Patients with polycystic ovary syndrome who also have hypermethylation of histone deacetylase 3, nuclear co-repressor 1, and PPAR- γ are found to have HA (Soni et al., 2018).

1.1.2.2. Environmental Toxicants

Various environmental factors disturb the body's normal hormone synthesis, secretion, transport, binding, action, or elimination, particularly within the neurohormonal system, which includes endocrine-disrupting

chemicals (EDCs). EDCs are synthetic versions of steroid hormones that are present in common household items. The synthetic chemical bisphenol A (BPA) influences metabolism via oogenesis, estrogen receptors, and testosterone catabolism. Extended use of EDCs may increase the risk of polycystic ovary syndrome (PCOS) (Meier, 2018).

Excess androgen accumulation, granulosa cell hyperplasia, and interstitial theca-like cell effects are some pathways through which bisphenol A (BPA) affects these cells. It also affects testosterone and sex hormone-binding globulin (SHBG) levels.

Inflammation and oxidative stress result from the action of pro-inflammatory molecules called advanced glycation end products (AGEs) that bind to RAGE receptors. These have an effect also on the general health of PCOS patients (Meier, 2018).

1.1.2.3. Physical and Emotional Stress

Other factors like stress has a major impact in PCOS, affecting quality of life and mental health. It is known to result in hypertrophy and hyperplasia of adipocytes, adipokine release, and activation of stromal fat immune cells. It alters levels of hormones such as anti-mullerian hormone (AMH) and reproductive hormone. It also disturb the oxidant-antioxidant balance and activates the hypothalamic-pituitary-adrenal axis with an increased release of cortisol, the latter in turn promotes insulin secretion and the accumulation of visceral fat (Patel, 2018).

1.1.2.4. Diet

Nutritional factors, such as saturated fatty acids (SFAs) that induce inflammation and diminish insulin sensitivity, contribute to polycystic ovary syndrome (PCOS). Vitamin D deficiency may exacerbate PCOS or associated comorbidities by inducing insulin resistance, inflammation, and downregulation of the AMH promoter (Qureshi et al., 2016).

1.1.2.5. Insulin Resistance

Insulin resistance (IR) is a tissue-selective condition in PCOS women. It affects ovarian growth, hormone secretion, and steroidogenesis. Insulin stimulates CYP450c17 and CYP17A1 enzymes, promoting androgen production and testosterone production (Wu et al., 2019).

Hyperinsulinemia reduces hepatic SHBG and increases free testosterone levels. It also hinders the hepatic manufacturing of IGF-1 binding protein. This leads to higher androgen production in thecal cells. Additionally, IGF-1 upregulates fasters' granulosa cells apoptosis and prevents folliculogenesis. Both hyperandrogenic disorder and hyperinsulinemia stop follicle growth, causing irregular menstrual cycles, non-ovulation infertility, and non-developed ovum accumulation (Barbosa et al., 2016).

1.1.2.6. Hyperandrogenism

Hyperandrogenism (HA) may involve elevated free testosterone concentrations due to decreased hormone-binding proteins such as sex hormone-binding globulin (SHBG). As a result, testosterone can be converted into estrone in adipose tissue. This transformation affects ovum development and increases the LH-to-FSH ratio, leading to ovulatory dysfunction (Xing et al., 2022).

1.1.2.7. Inflammation

Inflammatory biomarkers, such as elevated white blood cell count and C-reactive protein (CRP), are associated with PCOS and hyperandrogenism (HA). Pro-inflammatory cytokines like TNF- α can exacerbate insulin resistance by inhibiting insulin signaling, thereby affecting follicular development. Thus, inflammation plays a critical role in ovum maturation and ovulation (Mukherjee et al., 2023).

1.1.2.8. Oxidative Stress

Oxidative stress (OS) is a pathological condition characterized by an imbalance between the excessive production of free radicals and the body's antioxidant defenses. It disrupts signaling pathways, cell growth, differentiation, and ovarian function. In patients with PCOS, elevated OS activates nuclear factor-kappa B (NF- κ B), contributing to inflammation and obesity (Dabravolski et al., 2021).

1.1.3. Diagnosis Criteria

Despite the possibility that up to 70% of women with PCOS go misdiagnosed, an accurate diagnosis is frequently hampered by the condition's apparent resemblance to numerous other disorders (Islam et al., 2022).

Numerous sets of PCOS diagnostic criteria have been presented, as Table (1-1) illustrates. However, the most often applied is the Rotterdam criteria (Fahs et al., 2023).

Table (1-1): Diagnostic Criteria for Polycystic Ovary Syndrome

The National Institutes of Health/National Institute of Child Health and Human Disease 1990 (NIH/NICHD) (Legro et al., 2006) .	Requires the following conditions to be met in tandem: 1. Hyperandrogenism (clinical or biochemical). 2. Dysfunction of the ovaries
The European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (Rotterdam /2003 (ESHRE/ASRM)) (Bloom et al., 2006).	Requires the fulfillment of a minimum of two requirements: 1. Clinical and/or biochemical Hyperandrogenism 2. Dysfunction of the ovaries 3. Ovarian polycystic morphology
Androgen Excess Society (AES/2006) (Bloom et al., 2006).	Requires either: 1. The presence of biochemical or clinical hyperandrogenism or dysfunctional ovulation 2. The morphology of polycystic ovary
Androgen Excess and PCOS Society/2009 (Yildiz et al., 2008)	Prerequisites for concurrent presence include: 1. Clinical or biochemical Hyperandrogenism 2. Dysfunctional ovarian morphology, including polycystic ovarian morphology and/or ovulatory failure.

1.1.3.1. Clinical Symptoms

Women with PCOS may pursue dermatological consultations and may present with acne, hirsutism, alopecia, acanthosis nigricans, skin tags, or hyperpigmentation associated with obesity. Irregular menstrual periods and primary infertility are the main concerns for women with PCOS (Abusailik et al., 2021).

1.1.3.1.1. Acne vulgaris

PCOS women experience inflammatory acne that is minimally responsive to conventional treatment. Acne lesions are usually found on the face, chest, shoulders, and back and may have a hormonal basis (Mukerjee, 2020).

1.1.3.1.2. Hirsutism

In regions of heightened androgen sensitivity, androgens promote hair growth, augment its width, and enhance its pigmentation. Thin, coarse, terminal hair in androgen-dependent areas serves as a sign of a hyperandrogenic condition, presenting both aesthetic and practical challenges (Mukerjee, 2020).

1.1.3.1.3. Alopecia

Female pattern hair loss (FPHL) in PCOS may be androgenic or due to hyperandrogenism. Early-onset FPHL is more likely to develop hyperandrogenism, converting terminal to vellus hair and making the scalp look bald (Sakpuwadol et al., 2023).

1.1.3.1.4. Acanthosis Nigricans

Thick, velvety skin in friction-prone areas such as the neck, axillae, and groin is indicative of insulin resistance. This skin thickening is caused by activation of epidermal tyrosine kinase growth factor signaling pathways, which promote insulin-like growth factor 1 receptor (IGF-1R) activity. Skin tags in these areas are also common in individuals with obesity (Khairnar, 2023).

1.1.3.1.5. Irregular Menses and Infertility

PCOS involves chronic menstrual irregularities, reduced fertility, and estrogen-induced endometrial hyperplasia, increasing the risk of endometrial cancers (Johnson et al., 2023).

1.1.3.2. Hormonal levels

Some hormones play roles in regulating and maintaining menstrual cycle, ovulation, and fertility including FSH and LH. The normal levels of LH and FSH are in the range of 4-8 during reproductive age in women, and the “LH: FSH ratio is 1. However, in women with PCOS”, this ratio may be as high as 2:1 or even 3:1. FSH levels may even be normal in PCOS, but LH levels may be elevated to 10-20 (Yang and Chen, 2024).

Another hormone that has an important role is testosterone. It presents reproductive-age women with a normal total testosterone level is 15-70 ng/dl. In contrast, the normal free testosterone level is 0.7-3.6 pg/ml. Elevated levels of both total and free testosterone are commonly seen in women with PCOS (Salimon and Olaniyan, 2024).

One of the most sensitive indicators of ovarian reserve is anti-müllerian hormone (AMH). A peptide produced by follicle granulosa cells, PCOS is associated with elevated levels of this hormone. According to some research, polycystic ovary syndrome (PCOS) may be diagnosed with an anti-Müllerian hormone (AMH) level over 3.8-5 ng/mL (Tsukui et al., 2022).

1.1.3.3. Imaging

According to the Rotterdam Consensus (2003) and Androgen Excess & PCOS Society (2006), PCOS diagnosis requires ovarian sonography. This criterion requires ≥ 12 follicles with a diameter of 2-9 mm and/or ovarian volume ≥ 10 cm (Mansour et al., 2022).

1.1.4. Types of PCOS according to the reasons

1.1.4.1. Insulin-resistant PCOS

Most polycystic ovary syndromes (PCOS) are insulin-resistant. Light meals, air pollution, smoking, and trans fat all contribute to polycystic ovary syndrome. Insulin resistance makes ovulation less likely and increases testosterone production by the ovaries (Shukla , 2020).

1.1.4.2. Post Pill PCOS

As far as PCOS types go, the second most common is pill-induced PCOS. It arises as a result of ovulation-restricting birth control pills. Ovulation often resumes after a few days of using the pill, and these side effects are typically temporary (Devi et al., 2021) .

1.1.4.3. Inflammatory PCOS

Inflammation prevents ovulation, imbalances hormones, and produces androgens in PCOS. Stress, environmental pollutants, and gluten trigger inflammation (Fabozzi et al., 2022).

1.1.4.4. Hidden PCOS

Hidden PCOS is a simpler version of PCOS that takes three to four months to resolve after addressing the source. It commonly results from certain conditions, including high prolactin levels, thyroid disorder, iodine deficiency, vegetarian women who are suffering from zinc deficiency, and synthetic sweeteners (MacKinnon, 2023).

1.1.4.5. Adrenal Gland PCOS

In this type of PCOS, the adrenal glands are the main source of excess androgen, rather than the ovaries, which is the case in other forms of PCOS.

A key factor is often an abnormal, hyper-responsive stress response, leading to excessive release of adrenal hormones. Elevated levels of DHEA-S (dehydroepiandrosterone sulfate) (Boucher et al.,2024).

1.1.5. Managements

The treatment strategy and therapeutic selection are contingent upon the patient's priorities and problems, including fertility, menstrual irregularities, weight loss, or hyperandrogenic symptoms. A personalized approach is essential for the best outcomes since there is no universal therapy for PCOS (Sadeghi et al., 2022).

1.1.5.1. Lifestyle Modification and Non-Pharmacological Approaches

PCOS women acquire weight due to high androgenic hormone levels, resulting in an apple-shaped figure. Diet and calorie restriction help lose weight, restore menstruation, and lower free testosterone. For obese ladies, a normal BMI is optimal (Sadeghi et al., 2022).

Women must follow a certain diet to succeed. The ideal diet is rich in fiber and low in carbs and saturated fats. Low-glycemic foods include broccoli, raw carrots, lentils, soy, and whole-grain bread. White rice, cakes, cookies, fries, and fruits are high-glycemic foods not recommended for prevention (Shermin et al., 2019).

Physical activity and exercise are essential for losing weight and increasing insulin sensitivity. The American Heart Association suggests 75–150 minutes per week of intense or moderate activity. In PCOS-afflicted women, exercise can trigger ovulation again by regulating the hypothalamic-pituitary-gonadal axis and regaining HPA control (Ono et al.,2025).

Pharmacological treatments for PCOS are currently only moderately effective, and some of the patients remain untreated. However, complementary and alternative medicine might be beneficial in the treatment of the disease, an argument now proposed according to the recent data analysis (Robin et al., 2021).

More than 70% of patients with PCOS employ CAM, that can be classified into three main types of therapy: nutritional, psychological, physical, or others that is using all mentioned components. Complementary and alternative medicine Therapies including traditional Chinese medicine, immunotherapy, diet modification, psychotherapy, spa, yoga, Tai Chi, and oxygen therapies have been found to have a positive impact on the severity and complications associated with PCOS (Muralidharan et al., 2023).

Acupuncture, a traditional Chinese medicine with a history exceeding 3000 years, alleviates the symptoms of PCOS by stimulating somatic afferent nerves, modulating nervous system activity, and influencing endocrine processes. It boosts the creation of β -endorphins, which have an impact on hormone secretion, ovulation, and the menstrual cycle and may even help the menstrual cycle return (Sadeghi et al., 2022).

Myoinositol (MI), d-chiro-inositol (DCI), berberine, omega-3, folic acid, resveratrol, α -lipoic acid, vitamin D, and other supplement items may help cure polycystic ovary syndrome (PCOS) in some women. Fertility, insulin sensitivity, and lipid profiles are all enhanced by these supplements. Folic acid and berberine may help fight obesity and insulin resistance (Pkhaldze et al., 2023, Su et al., 2023).

1.1.5.2. Pharmacological Treatments

PCOS treatments must be personalized based on symptoms, including hyperandrogenic symptoms, ovulation regulation, menstruation regulation, and cardiometabolic complications (Trent and Gordon, 2020). Diet and lifestyle changes are crucial, with pharmacological and non-pharmacological interventions. Combining lifestyle changes with medications leads to better metabolic benefits (Setji and Brown, 2007). Treatment goals for PCOS include maintaining normal endometrium, targeting androgens, reducing insulin resistance, and correcting anovulation. Therapy options include symptomatic control, fertility management, and long-term complications (Goodman et al., 2015).

1.1.5.2.1. Oral Contraceptives and Anti-Androgens

The long-term treatment of women with PCOS frequently involves the use of oral contraceptive tablets (OCP). They treat hirsutism and acne while addressing hyperandrogenism and irregular menstrual cycles. It usually takes 6 months to complete the regimen, and the optimum option is low-dose OCP with neutral or anti-androgenic progestins (Shah and Patil, 2018)

To control hyperandrogenism and treat PCOS, anti-androgens such as spironolactone, Cyproterone acetate (combined birth control pills), flutamide, and finasteride suppress androgen levels. They interfere with the activity of testosterone and have an impact on ovarian, pituitary, and hypothalamic steroidogenesis. In women with polycystic ovary syndrome (PCOS), the competitive antagonist flutamide reduces acne and hirsutism. At the same time, third-generation combination oral contraceptives improve metabolic phenotype lipid and adipokine profiles (Mukerjee, 2020).

Patients with PCOS who take flutamide medicine have more regular ovulation and menstrual cycles. In contrast, those who take steroidal AR blockers have less hirsutism and acne. Finasteride treats hirsutism and lessens the effects of hyperandrogenic hormones. Anti-androgenic drugs can help with several PCOS characteristics, but thorough screening is essential to find risk factors (Messinis , 2023).

1.1.5.2.2. Insulin Sensitizers

The pathophysiology of polycystic ovary syndrome includes problems in insulin production and function; hyperinsulinemia and insulin resistance impact ovarian function. Elevated insulin levels not only inhibit follicular growth but also induce polycystic ovarian morphology and stimulate androgen output. Type 2 diabetes mellitus and cardiovascular disease (CVD) are chronic conditions to which individuals with insulin resistance are more susceptible. A successful treatment of insulin resistance necessitates a therapeutic strategy that incorporates both pharmacological intervention and lifestyle modifications (Shermin et al., 2019).

Metformin and thiazolidinediones (TZDs) are insulin sensitizers that improve menstrual cycle and ovulation by reducing insulin resistance. They enhance insulin sensitivity, decrease glucose production, and boost absorption. Women taking metformin see a noticeable drop in BMI. However, only those taking metformin also see a drop in testosterone levels (Mukerjee, 2020).

In people with PCOS who were overweight or obese, metformin resulted in a significant decrease in body weight, independent of dietary or activity modifications. It has been shown to improve dyslipidemia by either changing

the liver's free fatty acid metabolism or somewhat lowering hyperinsulinemia. By improving insulin sensitivity and lowering testosterone levels, TZDs like pioglitazone and rosiglitazone lower insulin levels. According to a meta-analysis, metformin and TZD work better together to improve lipid metabolism, insulin resistance, and testosterone levels (Zaki et al., 2023)

1.1.5.2.3. Ovulation Inducers

For those with PCOS requiring reproductive therapy, ovulation induction is a very effective choice. In PCOS, the formation of antral follicles ceases, and decreased FSH levels are associated with anovulation. The overproduction of LH, androgens, and insulin may influence this process by enhancing steroidogenesis while impeding follicular growth. The main ovulation-inducing agent is clomiphene citrate, which inhibits estrogen signaling and increases FSH availability (Yildiz et al., 2008).

Ovulation and mono-follicular development can both be achieved with low-dose gonadotropin treatment. Aromatase inhibitors (AIs), used as either primary or secondary treatments, such as letrozole and anastrozole, are recommended for ovulation induction. These agents are thought to stimulate ovulation by inhibiting the conversion of testosterone to estrogen in the brain, peripheral tissues, and ovarian follicles. This reduction in estrogen levels leads to an increase in GnRH pulse frequency, followed by elevated FSH secretion, which stimulates ovarian follicle growth and subsequently induces ovulation (Khan and Farid, 2024).

1.1.5.2.4. Emerging Therapeutics

1.1.5.2.4.1. Statins

Statins (atorvastatin, pravastatin, rosuvastatin, fluvastatin, and simvastatin) can treat polycystic ovary syndrome (PCOS) by lowering sex steroid production, improving dyslipidemia, lowering inflammation, and increasing ovarian androgen production. By blocking the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase, they halt the formation of cholesterol. It seems that atorvastatin enhances the effects of metformin since statins perform much better than the placebo on homeostatic model assessment for insulin resistance (HOMA-IR), free androgen index, total testosterone, and serum hematocrit (SHBG) (Allahbadia and Merchant, 2011).

1.1.5.2.4.2. Glucagon-Like Peptide-1 (GLP-1) Agonist

Incretin hormones such as gastric inhibitory polypeptide (GIP) and glucagon-like protein 1 (GLP-1) stimulate the release of insulin in response to changes in blood glucose levels. Treating polycystic ovary syndrome (PCOS) by concentrating on this mechanism may be helpful. The process by which GLP-1 agonists increase insulin sensitivity is multi-faceted and involves inflammation, oxidative stress, lipid metabolism, and GLUT-4 expression. Women with polycystic ovary syndrome who are overweight may get relief from metabolic risk by using dual GLP-1/GIP receptor agonists (Szczesnowicz et al., 2023).

1.1.5.2.4.3. Inositol

There are nine isomeric forms of the carbocyclic sugar inositol, including myo-inositol (MI) and d-chiro-inositol (DCI). It controls insulin, TSH, and FSH, among other hormones. Errors in this mechanism can cause insulin

resistance. Organs have different inositol isomers, which cause insulin resistance and hyperinsulinemia in PCOS (Qureshi et al., 2016).

1.1.5.2.4.4. Interleukin (IL)-22 Therapy

Intestinal immune cells secrete the cytokine IL-22, which is critical for both host defense and inflammation. It plays a role in tissue integrity, anti-microbial and mucosal defense systems processes as well as in the generation of mucosal barrier function while it participates in wound healing. Infertility in PCOS patients may be reduced by IL-22, as well as insulin sensitivity and ovarian function. The use of exogenous IL-22 might have therapeutic advantages (Zhang et al., 2023).

1.1.5.2.4.5. Probiotics and Prebiotics

Fermented foods often include beneficial microbes called probiotics, which may reduce inflammation, kill bacteria, and even act as an antioxidant. In particular, they improve the regulation of immunological responses, intestinal flora, and metabolic variables. Studies have shown that using probiotic supplements may improve polycystic ovary syndrome (PCOS) metabolic profile, reduce body mass index (BMI), and manage inflammatory and hormonal indicators (Qureshi et al., 2016).

1.2. Clomiphene Citrate

Enclomiphene and Zuclomiphene, the E- and Z-forms of Clomiphene Citrate (CC), make up 62% and 38% of the tablet's pharmacological content, respectively (Robin, 2021). It is the first-line PCOS treatment, widely available, affordable, well-tolerated, safe and effective (Legro, 2017). Only over 50% of women who begin Clomiphene Citrate ovulation induction will conceive after six cycles, even though 60–85% of these women will ovulate (Moolenaar et al., 2014).

1.2.1. Pharmacodynamics

Clomiphene citrate is a non-steroidal oral ovulation inducer that functions as a selective estrogen receptor modulator (SERM). It mimics estrogen by binding to estrogen receptors (ERs) throughout the reproductive system. However, with prolonged use, ER levels may decrease. Clomiphene interacts with estrogen receptors in several organs, including ER α in the hypothalamus, ER α and ER β in the pituitary, ER β in the ovary, and ER α in the endometrium, vagina, and cervix (Gupta and Khanna, 2018). When ER levels are low, the body thinks it is getting too much estrogen. This leads to more pulsed GnRH production, gonadotropin release, and ovarian follicular activity. Treatment with CC makes the GnRH pulses happen more often. However, there may also be direct action at the pituitary level. During treatment, the amounts of LH and FSH rise and fall. This causes a mid-cycle LH surge and ovulation (Al-Suhaimi et al., 2022). Clomiphene Citrate can make a woman ovulate more than once, which makes it more likely that she will have twins, as shown in Figure (1-2) (Moore et al., 2022) .

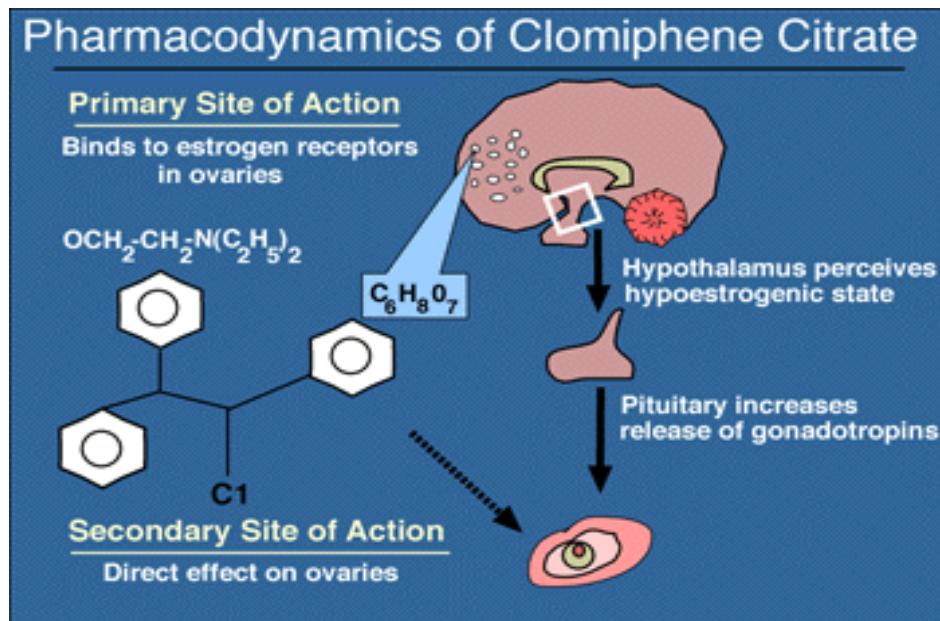


Figure (1-2): Pharmacodynamics of Clomiphene Citrate (Gupta and Khanna, 2018).

1.2.2. Pharmacokinetics

The drug is easily absorbed orally in humans and is primarily excreted in the feces. Approximately 85% of the dose is eliminated after 6 days, with traces remaining in circulation for longer. For CC, two geometric isomers of clomiphene, (E)- and (Z)-, are presented as an uneven mixture. Zuclomiphene stays in the body for over a month after treatment, suggesting stereospecific enterohepatic recycling or sequestering. Some active drugs may remain in the body during early pregnancy in women on clomiphene citrate therapy (Gupta and Khanna, 2018). Zuclomiphene is the (Z)-stereoisomer of clomiphene, while enclomiphene is the (E)-stereoisomer, whereas Zuclomiphene is described as mildly estrogenic. Enclomiphene is described as antiestrogenic (Lioudakis, 2015). Zuclomiphene is also about five times more potent than Enclomiphene in inducing ovulation (Feh et al., 2024), (Fischer and Ganellin, 2010). “E-clomiphene is mainly metabolized by cytochrome *P2D6*

(CYP2D6), and Z-clomiphenes by CYP3A4 and CYP3A5 (Robin et al., 2021). In 2012, the studies in pooled liver microsomes to describe 9 metabolites of CC. Two of them were very active in vitro in antagonism of estrogen receptors: E-4-hydroxy-N-desethylclomiphenes and E-4-hydroxy clomiphenes, both derived from E-clomiphenes and the metabolic pathway mediated by CYP3A4 and CYP3A5 for the synthesis of Z-desethylclomiphenes as shown in Figure (1-3) (Robin et al., 2021), (Mürdter et al., 2012).

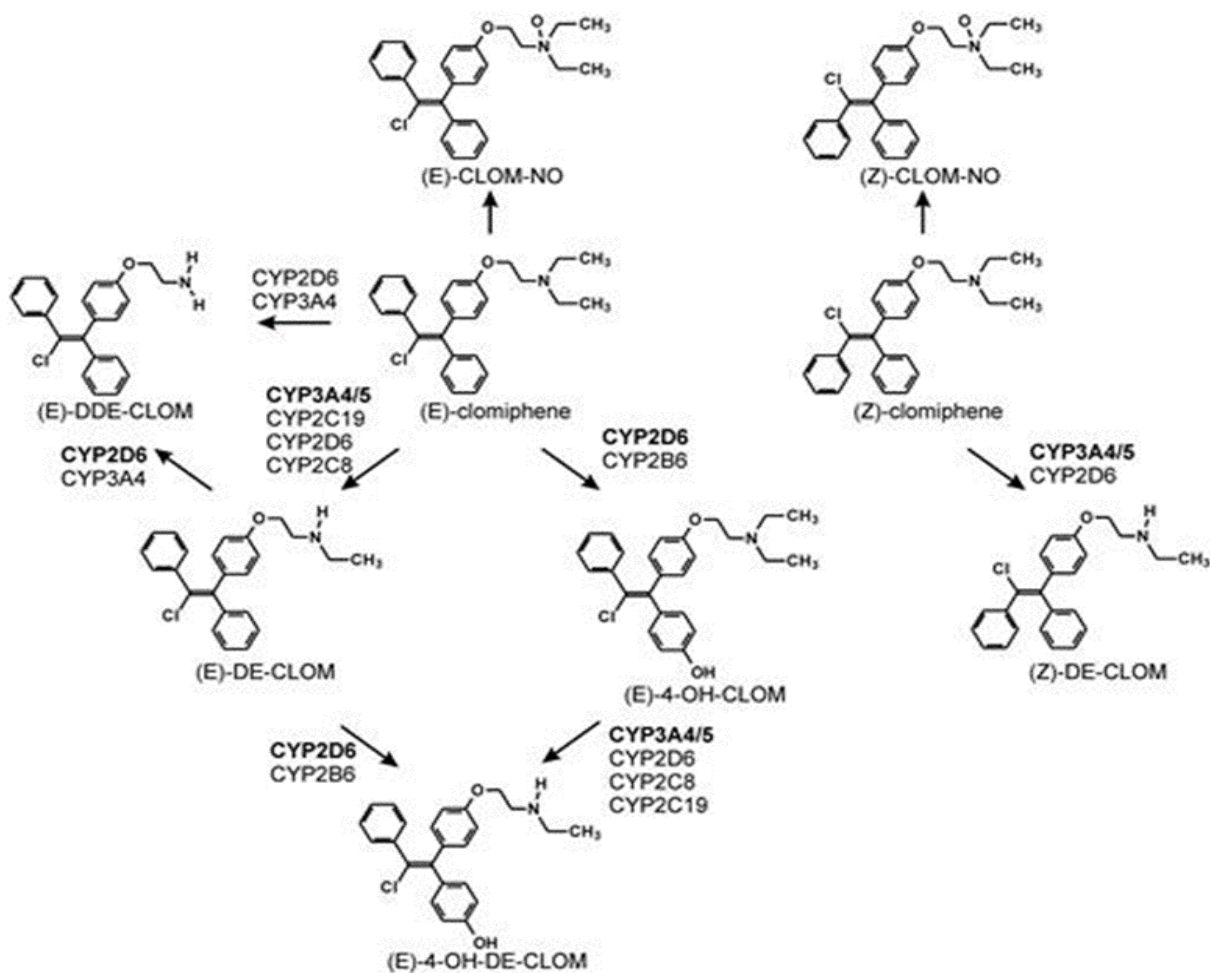


Figure (1-3): Human Metabolism of (E)- and (Z)-Clomiphenes (Robin et al., 2021) .

1.2.3. Medical Uses

1.2.3.1. Anovulation

Clomiphene Citrate is an FDA-approved drug that is used to treat impotence caused by insufficient or irregular ovulation (Malik et al., 2015). It can be used alongside other therapies, including flying needle therapy, or on patients with secondary amenorrhea, amenorrhea-galactorrhea syndrome, psychogenic amenorrhea, and amenorrhea-post-oral contraceptive amenorrhea, as well as those with polycystic ovarian syndrome (PCOS), benefit from clomiphene medication. According to patients are not permitted to have ovarian cysts, liver problems, or vaginal. Clomiphene Citrate is still the drug of choice for inducing ovulation in anovulatory women with PCOS (Adeleye and Zamah, 2023). During the follicular phase, a standard CC strategy for ovulation induction entails a starting dose of 50 mg/day for five days. If ovulation does not occur after progesterone-induced withdrawal bleeding, the dose is raised by 50 mg in the following cycle (Wachs and Chang , 2007).

1.2.3.2. Luteal Phase Deficiency

Luteal phase deficiency is a contentious condition that is probably best understood as a mild type of ovulatory dysfunction in which ovulation takes place. However, the corpus luteum does not produce enough progesterone in sufficient quantities or for long enough to enable implantation or the typical course of early pregnancy (Miller and Soules, 2009). The functionality of the corpus luteum, which originates from an ovulatory follicle, is influenced by the quality of preovulatory follicle growth. Consequently, Clomiphene Citrate is a viable option for addressing luteal phase deficiency (Miura et al., 2015). A possible explanation for the increased progesterone levels after CC

treatment compared to spontaneous cycles is the stimulation of preovulatory follicle and corpus luteum growth and hormone production from many corpus luteum (Cable and Grider, 2020).

1.2.3.3. Off-label Use to Treat Male Hypogonadism

Hypogonadism in adult men (testosterone deficiency) encompasses a clinical and biochemical state characterized by a reduction of testosterone levels with negative effects on multiple organ function and general quality of life (Lunenfeld et al., 2015). In males with hypogonadism, the off-label use of clomiphene citrate is widespread in Europe. It is safer and simpler to use than several other drugs, including testosterone and gonadotropin analogs, and has been proven effective in some indications (Ide et al., 2020).

1.2.4. Adverse Effects

Some women taking CC medicine may have a problem called ovarian hyperstimulation syndrome (OHSS), especially after the first treatment. Symptoms of OHSS, which could be life-threatening, include bloating, abdominal pain, emesis, diarrhea, nausea, rapid weight gain, urinating very little or not at all, thoracic pain, tachycardia, and dyspnea. Clomiphene Citrate frequently causes headache, mastalgia, facial flushing (specifically warmth, redness or tingling), hemorrhage or spot (Nasseri and Ledger, 2001).

1.2.5. Clomiphene Interactions

Interactions between drugs, such as those that inhibit *CYP2D6* or stimulate *CYP3A4*, may affect the amount of (E)-Clom and its metabolites that reach the bloodstream. Factors such as *CYP2D6* and *CYP3A4* metabolism are crucial for the biotransformation of the active metabolites of Clomiphene. Consequently, Clomiphene treatment is not immune to drug-gene interactions

(DGIs), drug-drug interactions (DDIs), and drug-drug-gene interactions (Kovar et al., 2022). In long-term epilepsy medication, the anticonvulsant carbamazepine is often utilized as a substrate and inducer of cytochrome *P450 3A4 and CYP2B6* (Keri and Nilson, 2001).

Many substrates of cytochrome *P450 3A4* are also substrates of P-glycoprotein (P-gp). Presently known to be common *CYP3A4* inhibitors are macrolide antibiotics (e.g., erythromycin and clarithromycin), anti-HIV drugs (e.g., ritonavir and delavirdine), antidepressants (e.g., fluoxetine and fluvoxamine), calcium channel blockers (e.g., verapamil and diltiazem), steroids and modulators thereof (e.g., gestodene and mifepristone), and a number of herbal and nutritional supplements (Rodríguez et al., 2024). Many of these drugs also serve as mechanism-based inhibitors of *CYP3A4*, inhibition that involves binding to the enzyme, generation of reactive metabolites, and irreversible inactivation of the enzyme. Some drugs such as phenytoin, rifampin, and ritonavir have been reported to induce *CYP3A4* (Dreshaj et al., 2024).

The orphan nuclear receptor PXR is necessary for *CYP3A4* induction. Depending upon the patient, the drug and the enzyme, it may lead to undesired and long-lasting drug-drug interactions and fatal toxicity for the drug activating *CYP3A4* (Qiu et al., 2024).

1.3. Genetic Polymorphism of Cytochromes *P450 3A4* gene

CYP enzymes do 75–80% of phase I drug metabolism. Due to genetic differences in CYP enzymes, ultra-rapid (UMs), normal (NMs), intermediate (IMs), and poor (PMs) metabolizers have different effects on drug metabolism. However, these categories only show how an enzyme works in

one individual. Age, sex, illness, co-medication, food, and smoking may affect CYP activity in addition to inheritance (Thomas, 2020).

CYP3A4, *CYP3A5*, *CYP3A7*, and *CYP3A43* are the four isoforms that make up the human CYP3A subfamily. There are 13 exons in each of the four *CYP3A* enzymes, and they are highly structurally similar (between 71.5% and 84.1%) (Saiz-Rodríguez et al., 2020).

The *CYP3A4* enzyme metabolizes more than half of all prescribed drugs. Its activity (including induced or inhibited activity) is believed to be a key factor in determining interindividual variability in drug disposition, subpar therapeutic efficacy, and adverse drug response (Wang et al., 2023).

1.3.1. *CYP3A4*18*

Asian people are most likely to have *CYP3A4*18* (1% Chinese, 1.3% Japanese, and 2.1% Malaysian) (Karimova & Asadov et al., 2023). As the most prevalent allelic variant of *CYP3A4*, the increased-function SNP *CYP3A4*18* (878T>C) at exon 10 was reported. At position 878, a nucleotide shift from tyrosine (T) to cytosine (C) causes an amino acid change from leucine to proline at codon 293 (Leu293Pro)(Asadov et al., 2023). Because the donor may have extensive metabolism and express *CYP3A4*18*, liver transplant patients may need a substantial dosage of tacrolimus (Yu et al., 2018).

1.3.2. *CYP3A4*22*

The reduced-function single nucleotide polymorphism (SNP), *CYP3A4*22*, is one of the most often investigated reduced-function SNPs in *CYP3A4*. *CYP3A4*22* has a low *CYP3A4* enzyme activity and decreased mRNA expression (Saiz-Rodríguez et al., 2020).

An allelic expression imbalance method was used to characterize the *CYP3A4**22 intron 6 variation (*G>A*; *rs35599367*) (Wang et al., 2011). It explained 12% of the individual variation in *CYP3A4* enzyme activity. Individuals of European minor allele frequency (MAF) 5% and mixed American MAF 2.6% origin are more likely to carry the *CYP3A4**22 allele than those of African (MAF, <0.1%) or Asian (MAF, <0.6%) descent. This variation was also linked to lower enzymatic activity in vivo. It was demonstrated to reduce the amount of mRNA and protein produced (Henderson, 2019). Comparing the *CYP3A4**22 allele to the homozygous wild-type showed a tendency towards a better simvastatin lipid-lowering response, as seen by the higher reduction in both total cholesterol and low-density lipoprotein levels (Elens et al., 2013).

1.4. Aim of the Study

- 1- To find out how the metabolizing enzyme *CYP3A4*18 (T>C)* (*rs28371759*) and *CYP3A4*22 (G>A)* (*rs3559967*) genes are distributed genetically among PCOS women in Iraq.
- 2- To assess how *CYP3A4*18 (T>C)* (*rs28371759*) and *CYP3A4*22 (G>A)* (*rs3559967*) genetic polymorphisms affect Clomiphene Citrate response in term of hormone levels, follicle size, and endomaterial thickness.
- 3- Determine differences in Clomiphene Citrate metabolism across genotypes by measuring metabolite/prodrug ratio.



Chapter Two

Patients, Materials, and Methods



2. Patients, Materials, and Methods

2.1. Patients

This study comprised 80 Iraqi women diagnosed with polycystic ovarian syndrome, according to the Rotterdam criteria established by the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) in 2003 (Geisthövel, 2003). The participants were women aged between 20 and 40 years and receiving clomiphene citrate at dose(50mg/twice daily)for two cycle. They were recruited from the infertility department at the Obstetrics and Gynecology Teaching Hospital in Iraq's Karbala Governorate as well as from various private clinics.

2.1.1. Patients Criteria

2.1.1.1. Inclusion Criteria

Patients newly diagnosed with PCOS and experiencing infertility underwent a treatment regimen involving Clomiphene Citrate at a dose of 50mg, taken twice per day, for at least 2 cycles.

2.1.1.2. Exclusion Criteria

Alongside polycystic ovarian syndrome (PCOS), women with certain medical conditions—such as androgen-secreting tumors, hyperprolactinemia, thyroid dysfunction, blocked fallopian tubes, uterine fibroids, polyps, Cushing’s syndrome, liver disease, smoking, and other related disorders may not be suitable candidates for clomiphene citrate therapy. Furthermore, individuals who conceive while taking other drugs or supplements should exercise caution, as these may interact with clomiphene citrate.

2.1.2. Ethical and Scientific Approval

1. The Ethical and Scientific Committee at the College of Pharmacy, University of Kerbala, approved the research request after a thorough review.
2. The Karbala Health Department, Ministry of Health in Iraq, also approved the study on September 25, 2023 (Project No. 146).
3. After a thorough explanation of the study's aims and justification, each participant received a properly signed informed consent form. After completing a written informed consent form, all participants were formally enrolled in the research and asked to complete a carefully crafted questionnaire.

2.2. Materials

2.2.1. Instruments, Equipment, and Their suppliers

A comprehensive list of all instruments used in this inquiry, together with their corresponding manufacturers, are given in Table (2-1).

Table (2-1): Instruments and Their Manufacturers

Instruments	Manufacture and Origin
Cobas e 411 Analyzer	Roche/Germany
UPT-2800	Hotgen/India
Digital timer	BIOBASE/China
Electrophoresis apparatus	Techinme /England
Freezer (-20 °C)	Concord /Lebanon
Gel & Clot Activator Blood Collection Tubes	AFCO/ Jordan
Centrifuge	Hettich/Germany
Hood	LabTech /Korea
EDTA tube	Nipigon Health Corp/Canada
SCIEX 4500 QTrap LC-MS/MS	AB Sciex/USA
Micropipettes	SLAMMED/USA
NanoDrop	Thermo Scientific™/USA
Refrigerator	Concord /Lebanon

Sensitive balance	DENVER/Germany
Thermos cycler (PCR apparatus)	Thermo Scientific™/USA
UV-trans illuminator	Syngene /England
Vortex mixture	Human Twist/Germany
Drying Oven	BIOBASE/China
Digital camera	Canon/England

2.2.2. Chemicals and Kits and Their Producing Companies

Table (2-2) includes a list of all the compounds and kits that were utilized in this investigation, along with the generating business and country.

Table (2-2): Chemicals and Kits and Their Producing Companies

Types	Materials	Company/origin
Chemicals	Agarose	Condalab/China
	Clomid 50mg	Sanofi/France
	Ethanol	SDI/ Iraq
	Ethidium Bromide	Promega/USA
	Deionized sterile D. W.	Bioneer/Korea
	TBE buffer	G-Biosciences –USA
Hormonal Kits	AMH kit	Hotgen/Germany
	Estradiol kit	Roche/Germany
	Prolactin kit	Roche/Germany

	LH	Roche/Germany
	FSH	Roche/Germany
Kits for Genetic Study	DNA Extraction Kit	Geneaid/Taiwan
	DNA ladder marker	Bioneer/Korea
	MSP1 restriction enzyme	BioLab/England
	Primers	Macrogen/Korea

2.3. Methods

2.3.1. Study Design

The Cohort study was done on 80 Iraqi women who had been diagnosed with PCOS. The recruitment of patients took place from December 2023 to June 2024. Participants were recruited at the beginning of their menstrual cycle (day two) before starting the medication. Subsequent evaluations were conducted on the twelfth day of at least two consecutive menstrual cycles after the administration of the medication. Patient's medical records and those of consenting females were the sources of the data. The collected information including age, weight, academic achievement, date of first menarche, irregularity of menstrual cycles, family history of PCOS or other diseases, smoking status, number of children (if applicable), history of abortion, and any drug usage that could potentially affect the metabolism of CC. A professional doctor performed ultrasound examinations on each patient to measure the size of their follicles and the thickness of their endometrium on the 2nd and 12th day of their menstrual cycle.

2.3.2. Blood Collection

The patient's 5 ml blood sample, which was taken on the second day of her menstrual cycle before starting CC therapy, was split into two sections. The first portion of the sample was put in an EDTA tube so that DNA could be extracted. The second portion was kept in a gel tube and then centrifuged at around 5,000 rpm for about 10 minutes so that hormonal testing could be performed. On day 12 of the second menstrual cycle, or following the administration of CC medication, a second blood sample (5 ml) was taken from the same patient. Additionally, the blood sample was split into two portions: the first portion was used for hormonal testing. The second portion was stored in an EDTA tube to determine the concentration of Zuclophene and its metabolite Z-desethylclomiphene in the plasma.

2.3.3. Determination of Body Mass Index

A person's BMI was calculated as weight divided by height. The body mass index (BMI) formula is weight in kilograms divided by height in meters squared. A body mass index (BMI) in the 18.5-24.9 range is deemed to be at a normal weight. If it is between 25 and 30, will be overweight. If the value is greater than 30 then it is judged to be obese (Chooi et al., 2019).

1.1.4. Hormonal Assay Methods

2.3.4.1. Measurement of Serum Anti-Müllerian Hormone (AMH) Level

The kit employs a combination of up-converting phosphor technology (UPT) with double antibody sandwich immunochromatography. The reaction zone (T band) on the nitrocellulose (NC) membrane of the test cassette was coated with AMH antibody, while the control zone (C band) was coated with

goat anti-mouse antibody. When the diluted sample was added to the sample cavity on the test cassette, the capillary effect caused the fluid to flow to the other end. During the migration, AMH in the sample first bound to the AMH antibody coated on the up-converting phosphor (UCP) nanoparticles, then bound to the AMH antibody immobilized on the T line of the test cassette, forming antibody-antigen-antibody-UCP complex, and the rest UCP nanoparticles flew forward and bound to the goat anti-mouse antibody on the C line, forming secondary antibody-antibody-UCP complex. UCP particles emitted visible light when excited by an infrared source. The intensity of the emission from the UCP particles at the T line and C line were measured simultaneously and the ratio T/C of the emission intensity was proportional to the AMH concentration in the sample. AMH concentration was automatically calculated by reference to a calibration curve stored in the UPT system and displayed on the screen of the instrument (Deadmond, et al. 2022).

2.3.4.2. Measurement of Serum Prolactin Level

A two-site sandwich test, the CL-series prolactin (PRL) assay measures prolactin levels. The initial stage involved adding a sample to a reaction vessel together with paramagnetic microparticles coated with mouse monoclonal anti-PRL and prolactin antibodies, as well as an alkaline phosphatase conjugate. The PRL formed a sandwich with the anti-PRL antibody-coated microparticle and alkaline phosphatase-labeled conjugate after incubation. The microparticle was magnetically captured while washing away loose material. The substrate solution was added to the reaction vessel in step two. An immunocomplex of alkaline phosphatase and mouse anti-PRL antibody aided the process. Chemiluminescence was measured in relative light units using a built-in photomultiplier. The reaction's RLUs were proportionate to

the sample's PRL concentration. Prolactin levels were measured using a calibration curve (Glezer, et al. 2024).

2.3.4.3. Measurement of Serum Estradiol Level

The elecsys estradiol III assay is a competitive binding Immunoenzymatically assay to determine the level of estradiol and employs a competitive test principle using two monoclonal antibodies specifically directed against 17 β -estradiol. Endogenous estradiol in the sample was released by mesterolone and was view to compete with the added estradiol derivative labeled with a ruthenium complex for binding sites on the biotinylated antibody. In this test a sample was incubated with two biotinylated antibodies specific for estradiol, immunocomplexes were created, their quantity was related to the concentration of analyte in the sample . In 2nd incubation, after the addition of streptavidin-coated microparticles and an estradiol derivative labelled with a ruthenium complex, the still-vacant sites of the biotinylated antibodies became occupied, with the formation of an antibody-hapten complex. The entire complex had been bounded to the solid phase via interaction of biotin and streptavidin. The reaction mixture was aspirated into the measuring cell, where the microparticles were magnetically captured onto the surface of the electrode. Unbound substances were then removed with ProCell/ProCell M. Application of a voltage to the electrode then induced chemiluminescent emission which was measured by a photomultiplier. Results had been determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode (Wang et al., 2016).

2.3.4.4. Measurement of Serum Follicles Stimulating Hormone Level

The present investigation introduces a new immunometric test that uses the sandwich concept to quickly measure FSH levels in just 18 minutes. This test utilized a carefully designed pair of monoclonal antibodies. One of these antibodies, biotinylated anti-FSH (monoclonal), had a strong attraction to a specific part of the FSH molecule formed by both subunits. The other antibody, ruthenium complex-conjugated anti-FSH (monoclonal), specifically targeted a different part of the FSH molecule located on the beta subunit. The assay involved a two-step incubation method. During the initial stage, the sample was subjected to incubation with both antibodies, facilitating the formation of a distinct "sandwich" complex with any follicle-stimulating hormone (FSH) that may be present. In the second step, streptavidin-coated microparticles were introduced. The microparticles efficiently trapped the biotinylated antibody, therefore immobilizing the whole FSH-antibody complex on a solid surface. After performing a washing procedure to eliminate substances that were not bound, an electric potential was given to the electrode, causing a chemiluminescent reaction to occur in the ruthenium complex. The magnitude of this light emission was subsequently measured using a photomultiplier. Ultimately, the detected light signal was contrasted with a pre-existing calibration curve, allowing for the precise calculation of the FSH concentration in the sample (Lima et al., 2024).

2.3.4.5. Measurement of Serum Luteinizing Hormone Level

The Elecsys LH assay utilizes a sandwich principle and employs a highly specific method to quickly assess LH levels (in just 18 minutes). The method employed two monoclonal antibodies that had been meticulously developed to specifically target distinct areas (conformational epitopes) of the human LH molecule. One antibody, labelled with biotin, identified a specific region created by both LH subunits, while the other antibody, tagged with a ruthenium complex, selectively attached to a specific region on the beta subunit. By employing a dual targeting technique, the interference from other hormones was minimized, leading to a high level of specificity in the experiment. During the initial stage, an LH-containing sample was subjected to incubation with certain antibodies, facilitating their attachment to the LH molecule and resulting in the formation of a sandwich complex. The next stage entailed the introduction of microparticles coated with streptavidin. The particles captured the biotinylated antibody, anchoring the entire LH-antibody complex on the surface. After a washing step to remove any non-bonded material, an electrical stimulation triggered a chemiluminescent reaction of the complex and resulted in light emission from the complex. The intensity of this light emission was measured and compared to a predetermined calibration curve, wherein LH levels in the sample were quantitated. This method provided an accurate and efficient method for the measurement of LH (Kreisman et al.,2022)

2.3.5. Quantification of Zuclomiphene and Z-desethylclomiphene

The standard samples were made by dissolving (Zuclomiphene and Z-desethylclomiphene) in acetonitrile and 5% acetic acid solution. A 10 ppm standard solution was injected into the LC/MS/MS (Liquid chromatography-tandem mass spectrometry) device (Maizels and Budde, 2004). The intensity was detected to get the samples. All samples were subjected to centrifugation at 4000–10,000 rpm. The sample was finally subjected to gel filtration and protein precipitation with addition of 5% acetic acid solution. For that, 200 microliters of the gently lysed blood were placed in 1.5 ml microcentrifuge tubes and acetic acid was added (400 ml). The suspension of proteins was denatured, samples were centrifuged for 10 minutes at 10,000 rpm and the supernatants were used to inject the LC/MS/MS machine.

In this study, a SCIEX 4500 QTrap LC-MS/MS device was used. For the mobile phase, a mixture of acetonitrile and water was used in a ratio of 80:20. The injection duration into the device was one minute, and it was scanned from mass 360 to 450 to determine the presence of unknown metabolites. The interface gas temperature was set at 500°C (Dengler, 2019).

To analyze the data quantitatively, after determining the relevant peaks, the standard curve was drawn based on the peak area ratio and the concentration of metabolites, using serial dilutions of standard samples and ImageJ software . Then, using ImageJ software, the peak area of each of the peaks related to Z-clomiphene and Z-desethylclomiphene metabolites was determined and placed on the corresponding standard curve. In this way, the concentration of each metabolite peak was determined.

2.3.6. Genetic Analysis

2.3.6.1 Extraction of Genomic DNA from Blood Sample

Genomic DNA was extracted from whole blood using the gSYNC complete DNA extraction kit from the Company. The following method is used for DNA extraction from whole-blood

1. To prepare the blood sample, 200 μ l of whole blood was transferred to a 1.5 ml microcentrifuge tube. After that 20 μ l of Proteinase K was added and then mixed by pipetting. At 60°C the microcentrifuge tubes were incubated for 5 minutes.
2. To cell lysis, 200 μ l of GSB buffer was added to the mixture and well-blended to create a lysis solution. The mixture was left to cool at room temperature for two minutes. To ensure full lysis, the resultant lysate was incubated for 5 minutes at 60°C and mixed every 2 times by inverting the tube. If the lysis is flawless, the lysate's red color turns dark green.
3. For DNA binding, the lysate was combined with 200 μ l of absolute ethanol using a pulse vortex; any leftover drops were then removed from the cover by centrifuging the tube. The mixture was gently moved, being cautious not to wet the rim, to the Gas chromatography (GS) column, and centrifuged for one minute at 16,000 xg. After removing the filtrate, the GS column was placed in a fresh 2 ml collection tube.
4. After that, the GS column was filled with 400 μ l of W1 buffer and the mixture was centrifuged for one minute at 16,000 xg. The through-flow was abandoned. The GS column was backed in the 2ml collection tube

and a 600 μ l of wash buffer was added to the GS column, then centrifuged at 16,000 rpm for one minute. After removing the flow-through and replacing the column in a fresh 2 ml collection tube, the GS column was dried by centrifuging the mixture for an additional 3 minutes at 16000 xg.

5. For elution processing, the dried GS Column was transferred to a clean 1.5 ml microcentrifuge tube and a 100 μ l of pre-heated elution buffer TE buffer was added into the center of the column matrix. It was let stand for 3 minutes to allow elution buffer TE to be completely absorbed and centrifuged at 16000xg for 1 minute to elute purified DNA which was kept for a long time at -20 °C.

2.3.6.2. Quantification of DNA Using a Spectrophotometric Approach

The purity and concentration of the extracted DNA were assessed using the nano-spectrophotometric method, namely Nanodrop. The tiny detector was developed. After removing any debris, a volume of 1 μ L of the samples was subsequently placed onto the Nanodrop instrument. The microdetector was used to measure the concentration and A260/A280 ratio of DNA (García-Alegría et al., 2020).

2.3.6.3. Polymerase Chain Reaction

The polymerase chain reaction (PCR) is a process that involves the replication of a specific DNA region using the enzyme DNA polymerase, which enables the amplification of a desired DNA fragment from a single molecule to several million copies. Several components are required for the PCR process, including two primers that are complementary to the target

DNA. One primer binds to each side of the target region, and the DNA sequence between them is amplified (Kadri, 2019).

There are three main steps for any PCR that are cycled about 25-35 times, which include:

1. Denaturation occurred at 94-98°C and requires decoding of double-strand DNA into single strands.
2. Annealing was done at 55-65°C, the reaction begins by stiffening the pair of short oligonucleotide sequences to the ends of the strands of the DNA template.
3. Extension was run at 72-74°C, it needs extension of the primers to form a new strand complementary to the template table and this happens in the presence of DNA Taq polymerase (Coral, 2017).

There are several PCR techniques that differ in principle, this study uses two techniques:

1. Tetra-Primer Amplification refractory mutation system PCR (T-ARMS-PCR) is referred to the mutation detection method based on specific PCR primers also called amplification of specific alleles, in which a specific set of primers; two forward primers (forward wild and forward mutant type), and two reverse primers are complementary to the DNA template which include the region to be amplified (Afzal et al., 2018).
2. Restriction fragment length polymorphism (RFLP-PCR) 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 (Marwal and Gaur, 2020).

2.3.6.4. Primers Design

*CYP3A4*22 (G>A) (rs3559967)* (Abed et al., 2022) and *CYP3A4*18 (T>C) (rs28371759)* (Maddin et al., 2016) were the primers used in PCR. The primer sequences used for the analysis of amplification of *CYP3A4*22* and *CYP3A4*18* gene for SNP identification are listed in Table (2-3) and Table (2-4) respectively.

Table (2-3): Primers Sequences and Expected Product Size of *CYP3A4*22 (G>A) (rs35599367)* Genetic Polymorphism

SNPs	Primer sequence (5' -> 3')	Product size	Reference
O-F	AGGGGTCTTGTGGATTGTTGA	474 bp	(Abed et al., 2022)
O-R	CACCTGTCTTGAGCCCCTTAG		
I-F allele G	GATGCAGCTGGCCCTACG	215 bp	
I-R allele A	AGTGTCTCCATCACACCCAGT	297 bp	
O-F: Outer Forward, O-R: Outer Revers, I-F: Inner Forward, I-R: inner Revers			

Table (2-4): Primers Sequences and Expected Product Size for CYP3A4*18 (T>C) Genetic Polymorphism"

SNPs	Primer sequence(5'→3')	Enzyme	Product size	Reference
Forward primer	CACATCAGAATGAAACCACC	MSP1	450 bp	(Maddin et al., 2016)
Reverse primer	CAAGAGCCTTCCTACATAGAGT			

2.3.6.5. Enzyme digested product in (RFLP-PCR)

PCR products were digested by Msp1 restriction endonucleases enzyme for *CYP3A4*18 (T>C)* (RFLP-PCR). The anticipated DNA fragments(bp) after digestion by enzyme MSP1 endonuclease enzyme were homozygous wild-type allele (TT) that we observed a band that was not digested (450bp), heterozygous allele(TC) which was detected as three bands 450bp, 282bp, and 168bp and homozygous variant allele(C) that we observed the two bands 282bp and 168bp (Maddin et al., 2016).

2.3.6.6. PCR optimization conditions

Optimization of PCR was attained after several trials.

2.3.6.6.1. Optimization of Conditions for *CYP3A4*22 (G>A) (rs35599367)* Genetic Polymorphism

*CYP3A4*22 (G>A) (rs35599367)* optimization of PCR conditions was prepared by using a different volume of primers (1µL, 0.5 µL), different annealing temperatures (55°C, 60°C, 63°C), and different volumes of DNA

sample (3 µL, 5 µL). The best results of this SNP were obtained by adding 0.5 µl outer forward primer, 0.5 µl outer reverse primer, 1 µl inner reverse primer, 1 µl inner forward primer, 5 µl DNA sample, and 12 µl nuclease-free water at 55°C as annealing temperatures.

2.3.6.6.2. Optimization of Conditions for *CYP3A4*18 (T>C) (rs28371759)* Genetic Polymorphism

*CYP4*18 (T>C) (rs28371759)* optimization of PCR conditions was prepared by using a different volume of primers (1 µL, 2 µL), different volumes of DNA sample (2 µL, 5 µL), and different annealing temperatures (55 °C, 58°C, 60°C, 63°C, 66°C). The best results of this SNP were obtained by adding 1 µM reverse primer, 1 µM forward primer, 7.5 µl volume of dd H₂O, 0.5 µM magnesium chloride (MgCl₂), 8 µl master mix, and 2 µl of DNA sample at annealing temperatures of 63°C followed by 35 cycles at 95 °C for 5 mins, 63 °C for 30 s, 72 °C for 30 s and a final extension step at 72 °C for 5 min. The 450-bp PCR products were electrophoresed on a 2% agarose gel at 45V for 1 hr.

2.3.6.7. PCR Running and Working Solution

2.3.6.7.1. RFLP- PCR Running and Working Solution

After the PCR was amplified, 5 µl of the 450-bp PCR products were treated with 8 µl of ddH₂O, 1.5 µl of NE buffer, and 0.5 µl of Msp1 restriction enzyme overnight at 37 °C. After digestion, PCR products were electrophoresed on a 2% agarose gel at 45 V for 1 hour. Table (2-5) displays the thermal program for *CYP3A4*18 (T>C) (rs28371759)*.

Table (2-5): PCR Thermal Program for CYP3A4*18 (T>C) (rs28371759)

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

2.3.6.7.2. T-ARMS-PCR Running and Working Solution

*G>A CYP3A4*22 (rs35599367)* to prepare the T-ARMS-PCR working solution, combine 5µL of extracted DNA with 0.5µL of 10 pmol/µL outer and outer reverse primers, 1µL of 10 pmol/µL inner and inner reverse primers, and 0.5µL of each outer and inner primer in the PCR premix tube. After adding 12 µL of nuclease-free water, the volume reached 20 µL. Table (2-6) shows the *CYP3A4*22 (G>A) (rs35599367)* thermal program.

Table (2-6): PCR Thermocycler Program for CYP3A4*22 (G>A) (rs35599367)

Steps	Temperature (°C)	Minute: second	Cycles
Initial denaturation	95 °C	3:00	35
Denaturation	55°C	0:30	
Annealing	55°C	0:30	
Extension	72°C	0:55	
Final extension	72°C	5:00	

2.3.6.8. Agarose Gel Electrophoresis

1. Add 0.3 gm of agarose powder to a beaker and mix until dissolved to make 1.5% glycerine gel.
2. Next, 2 mL of 10X Tris-EDTA buffer (tris borate) and 18 mL distilled water were added.
3. The mixture was microwaved to dissolve agarose.
4. Once the solution cooled, 2 µL of Ethidium Bromide was added.
5. The comb was attached to one tray end to create sample loading holes.
6. The agarose solution was allowed to congeal at 25 °C after it was put into the test tube.
7. The comb was carefully removed from the tray and put in the device chamber. 1X Tris-Borate-EDTA (TBE) buffer filled the chamber.

8. A 5 µl DNA ladder was added to one of the agarose gel wells.
9. Each PCR product was 5 µl poured into the other holes.
10. Through the perforations, glycerol and bromophenol were introduced to the PCR products.
11. For a 10 cm separation between the cathode and anode, the electrophoresis apparatus's voltage was set at 45 volts to produce an electrical field of 5 v/cm.
12. Upon completion of the run, the bands were detected using a UV trans-illuminator.
13. A digital camera, namely a Canon EOS 80 D, was used to capture images of the gel .

2.4. Statistical Analysis

The Statistical Package for the Social Sciences (SPSS 26) was used to conduct the statistical analysis. Descriptive statistics for numerical data were presented as median and interquartile range (Median (IQR)), while non-numerical data were described using numbers and percentages.

Study characteristics before and after therapy during menstruation were compared using the Wilcoxon Signed-Rank Test. The Shapiro-Wilk test was employed to assess the normality of the data distribution. For numerical data that were not normally distributed, nonparametric tests such as the Kruskal-Wallis test were used. The Chi-square test was applied to analyze non-numerical data . A P-value of less than 0.05 was considered statistically significant.



Chapter Three

Results



3. Results

3.1. Demographic Data and Clinical Symptoms in Infertile Women with PCOS

The demographic data of PCOS patients were assessed in this study. The median age, puberty onset, and body mass index (BMI) were 26.0 (9.250), 12.0 (1.0), and 27.055(5.207), respectively. Regarding education, 51 patients (63.75%) had elementary education, surpassing the 4 patients (5%) with university education. Furthermore, 16 patients (20%) had no formal education, whilst 9 patients (11.25%) had attained secondary education. A substantial proportion of women were childless, including 56 people, or 70%. Merely 2.5% of women had several children, while 22 cases (27.5%) had a single kid as presented in Table (3-1).

In this study, 73 women (91.25%) had no history of miscarriage, while 7 women (8.75%) had undergone miscarriages. Alopecia (hair loss) is a common sign of PCOS. In this cohort study, 54 patients (67.50%) did not suffer from alopecia. In contrast, 26 individuals (32.50%) did. Another common symptom, irregular menstrual cycles, affected 49 patients (61.25%), whereas 31 patients (38.75%) reported regular menstrual cycles. Hirsutism (excess hair growth) is also a key symptom of PCOS. In this study, 40 patients (50%) exhibited hirsutism. In contrast, the other half did not, suggesting varying levels of androgen excess among the patients as shown in Table (3-1).

Table (3-1): Demographic Data and Clinical Symptoms of Infertile Women with PCOS

Parameters		Median (IQR) or No (%)
Age (y)		26.0 (9.250)
Puberty(y)		12.0 (1.0)
BMI(Kg/m ²)		27.055 (5.207)
Educational levels	No Education	16 (20%)
	Primary Education	51 (63.75%)
	Secondary Education	9 (11.25%)
	University Education	4 (5%)
Number of children	No Child	56 (70%)
	One Child	22 (27.5%)
	More than One Child	2 (2.5%)
Abortion status	No	73 (91.25%)
	Yes	7 (8.75%)
Alopecia	No	54 (67.50%)
	Yes	26 (32.50%)
Menstrual regulation	Regular	31 (38.75%)
	Irregular	49 (61.25%)
Hirsutism	No	40 (50%)
	Yes	40 (50%)

y: years, BMI: Body mass index, Data Presented as Median (IQR) and Number (%)

3.2. Hormonal Levels and Ultrasound Analysis in Infertile Women with PCOS Before and After Clomiphene Treatment

3.2.1. Hormonal Status

The estradiol (E2) level in infertile women with PCOS was significantly increased on day 12 after clomiphene treatment (75.7 (80.63)) compared to day 2 before treatment (35.6 (29.30)). LH levels and the LH/FSH ratio were also significantly higher on day 12 after treatment compared to day 2 before treatment. However, there was no significant difference in FSH levels between the two time points, as shown in Table (3-2).

3.2.2. Follicle Size and Endometrial Thickness

The follicle size and endometrial thickness were significantly increased after clomiphene treatment day 12 (16 (8.75)), (8.0 (3.0)) in comparison to day 2 before starting treatment (6.0 (2.0)), (4.5 (1.5)) respectively, as presented in Table (3-2).

Table (3-2): Reproductive Hormone Levels, Follicle Size, and Endometrial Thickness Before and After Clomiphene Treatment

Hormone level	Clomiphene treatment		P-value
	Before CC	After CC	
E2 (pg/ml)	35.6 (29.30)	75.7 (80.63)	0.001*
FSH (mIU/ml)	6.0 (4.0)	5.8 (2.65)	0.127
LH (mIU/ml)	7.9 (5.76)	15.0 (7.48)	0.001*
LH/FSH	1.3 (1.27)	2.3 (2.00)	0.001*
Follicle sizes (mm)	6.0 (2.0)	16 (8.75)	0.001*
Endometrial thickness (mm)	4.5 (1.5)	8.0 (3.0)	0.001*
* : indicates a significant effect , p-values were derived from the Wilcoxon Signed-Rank Test, Significance: $p \leq 0.05$, non-significant : $p > 0.05$,E2: estradiol , FSH: follicle-stimulating hormone, LH: luteinizing hormone, data Presented as Median (IQR)			

3.3. Genetic Analysis

3.3.1. Genotyping and Allele Frequency Analysis of *CYP3A4*18* (T>C) (rs28371759) in Infertile Women with PCOS

The results of *CYP3A4*18* (T>C) (rs28371759) genotyping were presented as clear bands with molecular sizes of 450 bp, 282 bp, and 168 bp. The homozygous wild-type allele (TT) was identified by the presence of an undigested band at 450 bp. The heterozygous genotype (TC) was confirmed by the presence of three fragments at 450 bp, 282 bp, and 168 bp. The homozygous variant allele (CC) was identified by the presence of two

fragments at 282 bp and 168 bp, as shown in Figure (3-1). The wild-type genotype (TT) was detected in approximately 83.75% of women with PCOS, while the mutant genotype (CC) and the heterozygous genotype (TC) were detected in approximately 5% and 11.25% of cases, respectively, as presented in Figure (3-2).

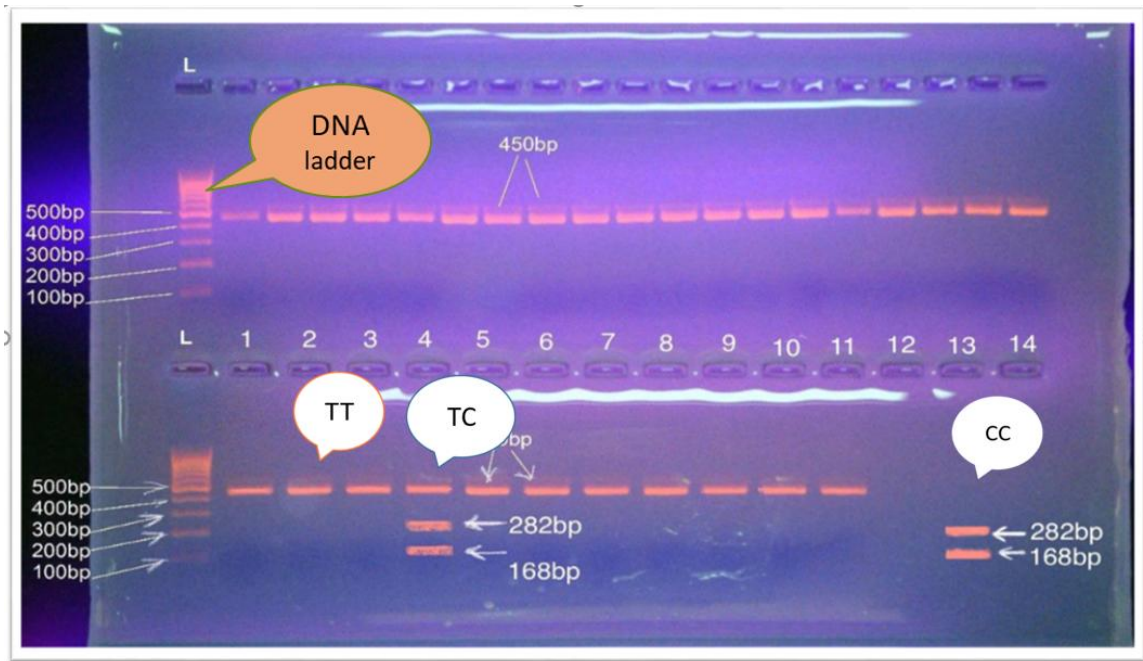


Figure (3 -1): Genotyping of *CYP3A4*18* (T>C) (rs28371759) gene using RFLP-PCR on a 2% agarose gel stained with ethidium bromide and viewed under UV light (Lane L: DNA ladder (100–500 bp) used as a molecular size reference, Homozygous wild-type allele (TT): identified by the presence of an undigested band (450 bp), Heterozygous allele (TC): confirmed by the presence of three fragments at 450, 282, and 168 bp, Homozygous variant allele (CC): identified by the presence of two fragments at 282 and 168 bp)

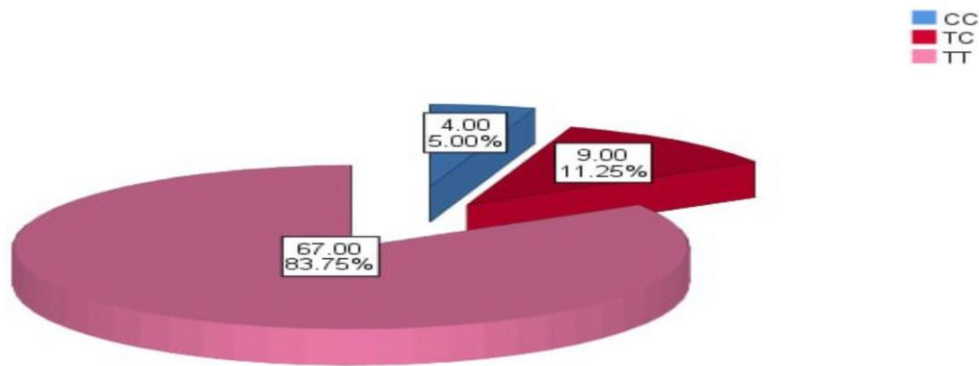


Figure (3-2): Frequency of *CYP3A4*18* (T>C) (*rs28371759*) Genotypes Among All Studied Females (N = 80)

Table (3-3): Hardy–Weinberg Equilibrium for (*rs28371759*) in patients.

Variable			Frequency	Percent	Alleles		Hardy-Weinbery equilibrium χ^2 test
Genotypes	TT	Observed	67	83.75%	T	C	
	Wild	expected	64	79.95%			
	TC	Observed	9	11.25%			
	Hetero	expected	15	18.96%	89.38%	10.62%	$\chi^2 = 13.2944$ p < 0.001(S)
	CC	Observed	4	5%			
	mutant	expected	1	1.09%			

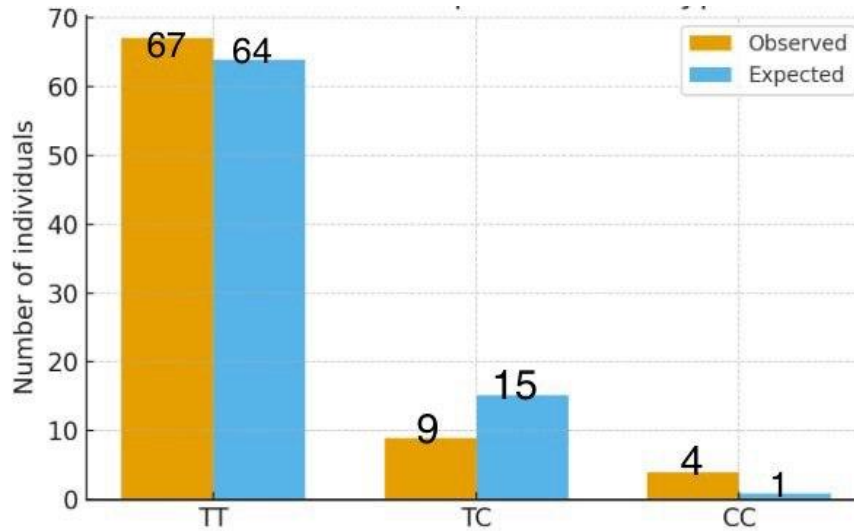


Figure (3-3) :Bar Chart Comparing Observed vs. Expected Genotype Distribution for (rs28371759).

3.3.2. Genotyping and Allele Frequency Analysis of *CYP3A422 (G>A) (rs3559967) in Infertile Women with PCOS**

The results of *CYP3A4**22 (G>A) (rs3559967) genotyping were presented as clear bands with molecular sizes of 474 bp, 215 bp, and 297 bp. The homozygous wild-type allele (GG) was identified by the presence of two fragments at 474 bp and 215 bp. The heterozygous allele (GA) was confirmed by the presence of three fragments at 474 bp, 297 bp, and 215 bp. The homozygous mutant allele (AA) was identified by the presence of two fragments at 474 bp and 297 bp, as shown in Figure (3-4). The wild-type (GG) genotype was detected in approximately 67.5% of PCOS women. The mutant type (AA) and heterozygous type (GA) were detected in about 7.5% and 25% of cases, respectively, as presented in Figure (3-5).

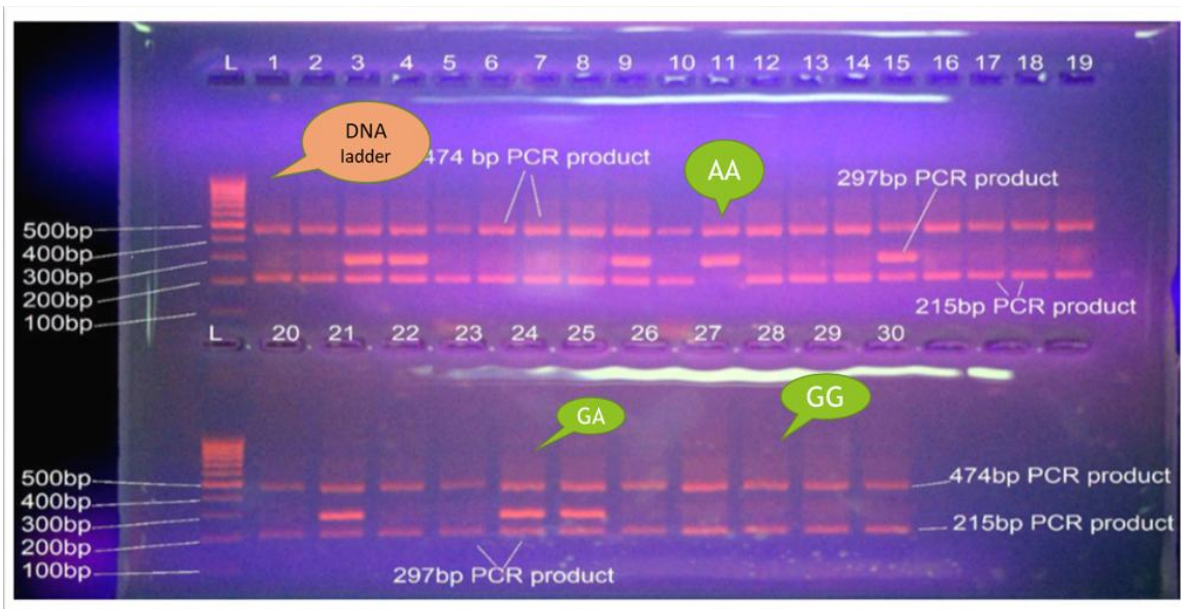


Figure (3-4): Genotyping of *CYP3A422 (*G>A*) (*rs3559967*) using T-ARMS-PCR on a 2% agarose gel stained with ethidium bromide and viewed under UV light**(Lane L: DNA ladder (100–500 bp) used as a molecular size reference, Homozygous wild-type allele (GG): identified by the presence of two fragments at 474 bp and 215 bp, Heterozygous allele (GA): confirmed by the presence of three fragments at 474 bp, 297 bp, and 215 bp, Homozygous variant allele (AA): identified by the presence of two fragments at 474 bp and 297 bp)

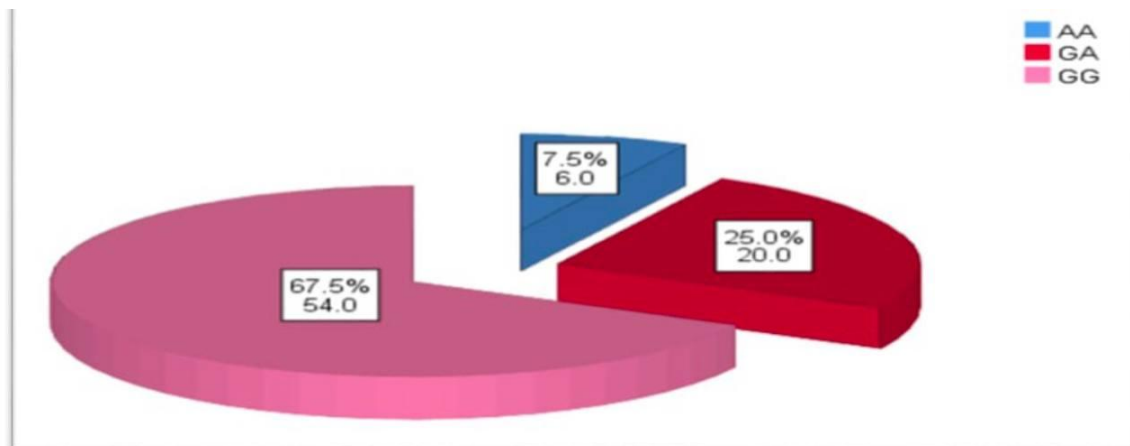
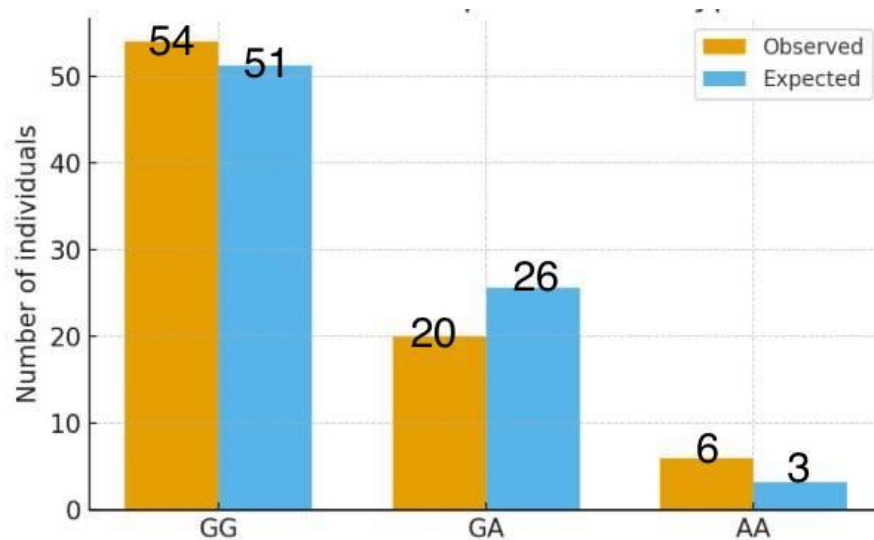


Figure (3-5) The Frequency of *CYP3A422 (*G>A*) (*rs3559967*) genotypes among all the studied females (N=80)**

Table (3-4): Hardy–Weinberg Equilibrium for (rs3559967) in Patients

Genotypes	Variable		Frequency	Percent	Alleles		Hardy-Weinberg equilibrium χ^2 test
	GG Wild	Observed expected			G	A	
	GA Hetero	Observed expected	20 26	25.0% 32.0%	128 80.0%	32 20.0%	$\chi^2 = 3.30$ $p \approx 0.07 \rightarrow$ not significant
	AA mutant	Observed expected	6 3	7.5% 4.0%			

**Figure (3-6) : Bar Chart Comparing Observed vs. Expected Genotype Distribution for (rs3559967)**

3.3.3. Frequency of *CYP3A4*18* (T>C) (rs28371759) Genotypes Based on Demographic Data and Clinical Symptoms in Infertile Women with PCOS

The demographic data of infertile women with PCOS, including age, onset of puberty, BMI, length of marriage, and number of abortions, showed no significant differences among the different genotypes of the *CYP3A4*18* gene ($p > 0.05$). Similarly, PCOS symptoms such as menstrual irregularity, hirsutism, and alopecia also showed no significant differences among the different genotypes of the *CYP3A4*18* gene ($p > 0.05$), as presented in Table (3-5).

Table (3-5): Frequency of *CYP3A4*18* (T>C) (*rs28371759*) Genotypes Based on Demographic Data and Clinical Symptoms in Infertile Women with PCOS

Demographic variable		CYP3A4*18 Genotypes			P– Value
		CC (4)	TC (9)	TT (67)	
Age (y)		27.0 (15)	26.0 (12.0)	26.0 (11.5)	0.965
Puberty (y)		11.5 (1.2)	13.0 (1.5)	13.0 (2.0)	0.400
BMI (Kg/m ²)		25.9(12.7)	26.2 (5.1)	26.2 (3.0)	0.597
Marriage Duration (y)		4.0 (12.3)	5.0 (3.0)	4.0 (4.0)	0.983
Abortion	No (73)	4	8	61	0.799
	Yes (7)	0	1	6	
Menstrual Regularity	Regular (31)	1	3	27	0.780
	Irregular t(49)	3	6	40	
Hirsutism	No (40)	3	3	34	0.356
	Yes(40)	1	6	33	
Alopecia	No (54)	1	6	47	0.173
	Yes (26)	3	3	20	
p-value for numerical data derived from Kruskal–Wallis test, p-value for non-numerical data derived from Chi-square test, Significance: $p \leq 0.05$, Non-significant: $p > 0.05$, BMI: Body Mass Index, data presented as median (IQR) and numbers (%), y: year					

3.3.4. Frequency of *CYP3A4*22* (G>A) (*rs3559967*) Genotypes Based on Demographic Data and Clinical Symptoms in Infertile Women with PCOS

There were no significant differences among the various *CYP3A4*22* genotypes in relation to the demographic characteristics of women with PCOS,

including age, onset of puberty, BMI, length of marriage, and number of abortions. Similarly, there were no significant differences among the genotypes regarding PCOS symptoms such as menstrual regularity, hirsutism, and alopecia ($p > 0.05$), as shown in Table (3-6).

Table (3-6): Frequency of *CYP3A4*22 (G>A) (rs3559967)* Genotypes Based on Demographic Data and Clinical Symptoms in Infertile Women with PCOS

Demographic variable		CYP3A4*22Genotypes			P – value
		GG (54)	GA (20)	AA (6)	
Age (y)		26.5 (10.0)	26.5(10.0)	26.5 (10.0)	0.772
Puberty (y)		12.0 (1.0)	12.5 (2.0)	12.0 (1.8)	0.922
BMI (Kg/m ²)		27.2 (5.5)	26.9 (5.0)	26.6 (5.4)	0.916
Marriage Duration (y)		4.0 (3.0)	5.0 (5.8)	3.5 (3.8)	0.466
Menstrual regularity	Rrgular(31)	22	7	2	0.868
	Irregular(49)	32	13	4	
Abortion	No (73)	50	17	6	0.433
	Yes (7)	4	3	0	
Hirsutism	No (40)	29	9	2	0.559
	Yes (40)	25	11	4	
Alopecia	No (54)	39	10	5	0.134
	Yes (26)	15	10	1	
p-value for numerical data derived from Kruskal–Wallis test, p-value for non-numerical data derived from Chi-square test, Significance: p < 0.05, Non-significant: p > 0.05, BMI: Body Mass Index, data presented as median (IQR) and numbers (%), y: year					

3.3.5. Levels of Reproductive Hormones and Ultrasound Findings in Infertile PCOS Women Based on *CYP3A4*18 (T>C) (rs28371759)* Genotypes

The FSH level in infertile PCOS women who received clomiphene and carried the wild-type (TT) genotype of the *CYP3A4*18 (rs28371759)* gene was significantly higher compared to those with the TC and CC genotypes ($p = 0.005$), as shown in Table (3-7).

The E2 levels in infertile PCOS women who received clomiphene and carried the mutant (CC) genotype were significantly elevated compared to those with the wild-type (TT) or heterozygous (TC) genotypes ($p = 0.008$), as shown in Table (3-7).

However, there were no significant differences in LH levels or the LH/FSH ratio among PCOS women with different *CYP3A4*18* genotypes who received clomiphene ($p > 0.05$), as presented in Table (3-7).

Additionally, no significant differences were observed in follicle size or endometrial thickness among women with different *CYP3A4*18* genotypes who received clomiphene ($p > 0.05$), as shown in Table (3-7).

Table (3-7): Levels of Reproductive Hormones and Ultrasound Findings in Infertile PCOS Women Based on *CYP3A4*18* (T>C) (*rs28371759*) Genotypes

Parameters	<i>CYP3A4*18</i> Genotypes			P-value
	TT (67)	CT (9)	CC (4)	
FSH (mIU /ml)	6.98 (2.95)	4.5 (1.46)	3.94 (2.35)	0.005*
LH (mIU /ml)	15.0 (7.3)	14.9(11.48)	12.0 (5.63)	0.465
LH/FSH Ratio	2.29 (2.03)	1.9 (2.48)	3.98 (2.35)	0.339
E2 (pg/ml)	71.5 (76.5)	79.8 (55.8)	247.85(50.2)	0.008*
Follicle sizes(mm)	17 (9)	14 (8)	17 (8)	0.313
Endometrial thickness (mm)	8 (3)	9 (3.5)	8 (2.25)	0.888
*: Significant effect , P-values were derived from Kruskal Wallis test , Significant: $p \leq 0.05$, Non-significant: $p > 0.05$, FSH :follicle-stimulating hormone, LH : luteinizing hormone, E2:estradiol, data expressed as median (IQR)				

3.3.6. Levels of Reproductive Hormones and Ultrasound Findings in Infertile PCOS Women Based on *CYP3A4*22* (G>A) (*rs3559967*) Genotypes

Infertile PCOS women who received clomiphene and carried the wild-type (GG) genotype of *CYP3A4*22* exhibited significantly elevated LH levels compared to those with the heterozygous (GA) and mutant (AA) genotypes ($P = 0.026$), as shown in Table (3-8).

There were no significant differences in FSH or estrogen (E2) levels among the different *CYP3A4*22* genotypes, as presented in Table (3-8).

Additionally, no significant differences were observed in follicle size among the GA, GG, and AA genotypes ($P = 0.916$).

However, endometrial thickness was significantly increased in PCOS women with the AA genotype compared to those with the GG and GA genotypes ($p = 0.048$), as shown in Table (3-8).

Table (3-8): Levels of Reproductive Hormones and Ultrasound Findings in Infertile PCOS Women Based on *CYP3A4*22* (G>A) (*rs3559967*) Genotypes

Parameters	<i>CYP3A4*22</i> Genotypes			P – value
	GG (54)	GA(20)	AA(6)	
FSH (mIU /ml)	5.89 (2.98)	5.37 (3.1)	4.81 (2.71)	0.278
LH (mIU /ml)	15.3 (6.77)	12.8 (6.35)	7.78 (7.24)	0.026*
LH/FSH Ratio	2.32 (1.94)	2.77 (2.52)	1.74 (2.61)	0.598
E2 (pg/ml)	77.4 (82.85)	65.2 (85.55)	78.5 (57.63)	0.994
Follicles sizes (mm)	16 (10)	16 (8.25)	17.5 (7)	0.916
Endometrial thickness (mm)	8 (1.5)	8 (2)	10 (1.5)	0.048*
*: Significant effect , P-values were derived from Kruskal Wallis test , Significant: $p \leq 0.05$, Non-significant: $p > 0.05$, FSH :follicle-stimulating hormone, LH : luteinizing hormone, E2:estradiol, data expressed as median (IQR)				

3.3.7. Analysis of the Drug and its Metabolite

3.3.7.1. Effect of *CYP3A4*18* (T>C) (*rs28371759*) Genotypes on the Plasma Concentration of Clomiphene and its Metabolite

The plasma concentration of Zuclomiphene was significantly higher in PCOS women with the wild-type (TT) and mutant-type (CC) genotypes

compared to those with the heterozygous (TC) genotype ($p = 0.044$), as shown in Table (3-9). PCOS women with the mutant (CC) genotype exhibited the highest metabolite/prodrug ratio compared to those with the wild-type (TT) and heterozygous (TC) genotypes ($p = 0.002$), as presented in Table (3-9).

However, there were no significant differences in the plasma concentration of the clomiphene metabolite (Z-desethyl clomiphene) among PCOS women with different *CYP3A4*18* genotypes ($p > 0.05$), as shown in Table (3-9).

Table (3-9): Effect of *CYP3A4*18* (T>C) (*rs28371759*) Genotypes on the Plasma Concentration of Clomiphene Citrate and its Metabolite

Parameters	Alleles of <i>CYP3A4*18</i> Genotypes			P- value
	TT (67)	TC (9)	CC (4)	
Zuclomiphene(ng/ml)	109.2 (44.3)	59.4 (86.3)	106.5 (79.1)	0.044*
Z-Desethyl clomiphene(ng/ml)	62.4 (17.0)	40.0 (58.1)	68.3 (68.7)	0.404
Metabolite/Prodrug Ratio	0.6 (0.2)	0.7 (0.2)	0.8 (0.4)	0.002*
*: Significant effect , P-values were derived from Kruskal-Wallis test, Significant: $p \leq 0.05$, Non-significant: $p > 0.05$, data expressed as median (IQR),Metabolite/Prodrug Ratio : Z-Desethylclomiphene/ Zuclomiphene				

3.3.7.2. Effect of *CYP3A4*22 (G>A) (rs3559967)* Genotypes on the Plasma Concentration of Clomiphene Citrate and its Metabolite

The plasma concentrations of Zuclophene and Z-desethyl clomiphene in PCOS women who received clomiphene citrate did not differ significantly among the different *CYP3A4*22* genotypes ($p > 0.05$), as presented in Table (3-10). However, the metabolite/prodrug ratio was significantly elevated in infertile PCOS women with the wild-type (GG) genotype compared to those with the heterozygous (GA) or mutant-type (AA) genotypes ($p = 0.000003$), as shown in Table (3-10).

Table (3-10): Effect of *CYP3A4*22 (G>A) (rs3559967)* Genotypes on the Plasma Concentration of Clomiphene Citrate and its Metabolite

Parameters	Alleles of <i>CYP3A4*22</i> Genotypes			P-value
	GG (54)	GA (20)	AA (6)	
Zuclophene(ng/ml)	106.6 (37.0)	105.9 (49.9)	118.4 (141.5)	0.680
Z-Desethylclomiphene(ng/ml)	66.3 (18.1)	56.3 (31.9)	55.8 (20.8)	0.180
Metabolite/Prodrug Ratio	0.65 (0.1)	0.52 (0.19)	0.29 (0.07)	0.000003*
*: Significant effect , P-values were derived from Kruskal Wallis test , Significant: $p \leq 0.05$, Non-significant: $p > 0.05$, data expressed as median (IQR) ,Metabolite/Prodrug Ratio : Z-Desethylclomiphene/ Zuclophene				



Chapter Four

Discussion



4. Discussion:

Clomiphene citrate, a selective estrogen receptor modulator, has long been considered the first-line treatment for PCOS-related subfertility due to its ability to induce ovulation. However, patient responses to ovulation induction vary for several reasons (Sha et al., 2024) and approximately 15–40% of patients develop resistance to CC. This failure is often unpredictable and may occur without an identifiable cause (Thabet et al., 2024). Individual responses to CC therapy differ, and currently, there is no reliable method to predict ovulation induction failure. Predictive criteria for CC resistance remain poorly defined, with inconsistent findings across studies. The variability in pharmacological outcomes is largely attributed to the complex interplay of genetic, clinical, biochemical, and environmental factors (Robin et al., 2021).

This study examined explicitly the *CYP3A4*18* and *CYP3A4*22* genotypes, which are implicated in CC metabolism, to investigate how interindividual variability affects the response and clinical outcomes of Iraqi women with PCOS for the first time.

4.1. Demographic Data and Clinical Symptoms in Infertile Women with PCOS

According to Table (3-1) the infertile PCOS individuals had an average age of 26 years, with a mean age of puberty onset at 12, which often affects women during puberty. Previous studies indicate that the age range of women exhibiting reproductive disorders, specifically PCOS is notably similar (Stener-Victorin et al., 2024). Globally, earlier onset of puberty

correlates with an increased risk of metabolic problems and hyperandrogenism (Rosenfield, 2015).

The majority of participants were overweight; individuals with obesity are at an increased risk of reproductive disorders, including PCOS and irregular menstruation. Women with metabolic and reproductive dysfunctions tend to exhibit similar BMI distributions (Escobar-Morreale, 2018). The association between irregular menstrual cycles and elevated body mass index underscores the importance of lifestyle modifications (Wang et al., 2024). Ashkar, et al. (2020) also revealed similar findings that show the vast majority of his study cases suffering from obesity and high BMI (Ashkar et al., 2020).

A majority of the infertile women in this study had attended primary education. "Previous studies have suggested that lower educational level is associated with reduced health literacy and PCOS management skills. Women with less education might experience less access to health care, which may make symptom control difficult (Haddad-Filho et al., 2023). These results underscore the importance of tailored educational programs for the knowledge and management of PCOS in women with lower education level.

The majority of the subjects did not have a history of abortion. Previous studies have observed analogous results in settings with stigmatized and restricted abortion (Sedgh et al., 2016). It demonstrated that some participants experienced alopecic changes. These results are consistent with other findings that prevalence of alopecia in women with hyperandrogenism and PCOS (Carmina and Lobo, 2019 ,Ashkar et al., 2020).

Irregular menstrual cycles and being childless were common among participants. This is consistent with previous studies that confirmed higher

infertility frequency and menstrual disorders in PCOS subjects (Mgheer et al., 2023, Stachowska et al., 2024).

4.2. Hormonal and Ultrasound Analysis of Infertile PCOS Patients Before and After Treatment

The results of this study demonstrated that the median values of E2 were significantly increased following clomiphene citrate administration, which is considered to be an effective stimulation of follicular development, given that estradiol is secreted by developing follicles (Zhang et al., 2015). Lack of a significant day 2-to-day 12 FSH change in polycystic women may suggest an abnormal pattern that could adversely impact ovulation and fertility outcomes. Clomiphene acts mainly at the level of hypothalamus and pituitary, rather than increasing serum FSH directly. The lack of a significant difference in FSH level can be attributed to the mechanism of action of FSH (Al-Obaidi et al., 2019). The present research revealed that circulating LH concentrations were significantly higher in PCOS women during clomiphene citrate treatment, which enhances hypothalamic-pituitary negative feedback necessary for ovulation (Fourie, 2016). LH to FSH ratio raised to a great extent indicates LH dominance in the periovulatory period. The release of LH induces ovulation, which in turn allows the maturation and release of the dominant follicle. This hormonal response is the basic mechanism of clomiphene's ability to reverse the symptoms and signs of poor ovarian reserve (Al Shaikhly, 2021).

In the current study, in marked contrast, CC had a significantly larger follicle size indicative of sufficient ovarian follicle stimulation that is important for successful ovulation. As a selective estrogen receptor modulator

(SERM), CC inhibits the estrogen receptors in the hypothalamus and enhances the release of gonadotropins (FSH and LH) by the pituitary, resulting in the growth and development of the follicles. These findings are confirmed by several studies demonstrating that CC significantly increased the dominant follicle size and number, resulting in higher ovulation rates and improved reproductive outcomes in anovulatory or PCOS women (Rachmawati et al., 2023). Clomiphene Citrate also significantly increased ET, which indicates improved endometrial receptivity. While CC's primary role is to induce ovulation, its effects on the endometrium can be variable. Elevated estrogen levels may promote endometrial proliferation, thus enhancing thickness to levels favorable for implantation. Studies show that adequate follicular development during ovulation induction is associated with increased endometrial thickness, thereby improving uterine receptivity despite potential anti-estrogenic effects (Cetin et al., 2021, Lu et al., 2024). These findings are summarized in Table (3-2).

4.3. Genetic Analysis

4.3.1. Frequency of *CYP3A4*18(T>C)* (rs28371759) Genotypes

In this study, the wild TT genotype is the predominant variant, representing 83.75% of the sample population, the mutant CC genotype constitute 5 %, and the heterozygous TC genotype may exert an intermediate influence at a rate of 11.25%, as presented in Figure (3-2). The seldom CC genotype may indicate a recessive or uncommon trait. Pharmaceutical research on drug metabolism and treatment responses frequently investigates the incidence of the *CYP3A4*18* polymorphism (TT, TC, and CC genotypes) across various populations. This polymorphism influences *CYP3A4*-metabolized medicines, such as imatinib and rivaroxaban, in Malaysian and

Chinese populations at distinct frequencies. In Malaysian patients with chronic myeloid leukemia, the *CYP3A4**18 variant allele (C) was identified in 5% to 15% of cases, with heterozygous (TC) and homozygous mutant (CC) genotypes exhibiting differential responses to imatinib. Caucasians exhibit a reduced prevalence of this variant allele compared to Asians (Maddin et al., 2016), and an approachable study in Iraq found that the most common variety, accounting for 87.6% of the sample population, is the TT genotype (Ali et al., 2024).

4.3.2. Frequency of *CYP3A4**22(G>A) (rs3559967) Genotypes

This study examined the *CYP3A4**22 genotype distribution and indicated a prevalent frequency of the wild GG genotype (67.5%), succeeded by heterozygous GA (25.0%) and mutant AA (7.5%) genotypes, as shown in Figure (3-3). These frequencies provide insight into the genetic diversity within our sample population. The GG genotype is purportedly the most prevalent among women with PCOS. The *CYP3A4**22 polymorphism reduced the expression and activity of *CYP3A4* protein in the human liver (Okubo et al., 2013). A study at King Abdulaziz University Hospital in Jeddah, Saudi Arabia, investigated the impact of *CYP3A4**22 and *CYP3A5**3 polymorphisms on hypercholesterolemic individuals that revealed the association between these genotypes and the necessary dose changes of statins due to interindividual variability in drug metabolism. None of the subjects possessed a homozygous *CYP3A4**22 mutant genotype. Simultaneously, 12 individuals (4.4%) exhibited a heterozygous genotype, while 262 (95.6%) displayed a wild-type homozygous genotype (Elalem et al., 2022). The homozygous genotype of (rs35599367) was exceedingly uncommon in the Gordian population, occurring in only 2% of cases (Al-Eitan et al., 2019). As

found, the occurrence of the homozygous mutant (AA) genotype in Caucasian females was 5%-7% (Sim et al., 2018, Hertz et al., 2017) whereas it was sub-1 % in African and Asian females (Holmberg et al., 2019).

4.3.3. The Frequency of *CYP3A4*18(T>C)* (rs28371759) Genotypes Based on Demographic Data and Clinical Symptoms in Infertile Women With PCOS

This study found that PCOS symptoms, such as hirsutism, alopecia, monthly irregularity and also demographic data which include, age, BMI, puberty, and abortion history were all unaffected by *CYP3A4*18* genotypes, as presented in Table(3-5). Prior research on the pharmacokinetic effects of *CYP3A4*18* revealed no associations with age, BMI, or puberty. This aligns with the findings of the study (Asadov et al., 2023, Li et al., 2014). The investigation of this study revealed no discernible link between the *CYP3A4*18* polymorphism and irregular menstruation and hirsutism. Prior research suggests that hormonal imbalances, especially increased androgens, and insulin resistance are more significantly associated with irregular menstruation in PCOS than individual *CYP3A4* polymorphisms (Sadeghi et al., 2022). Genes that relate to the androgen synthesis, like *CYP17A1*, *CYP11A1*, are more prominently associated with menses disruption in women with PCOS particularly in the hyperandrogenic phenotype (Ye et al., 2021, Abdelmonem et al., 2024). Abortion history was not associated with *CYP3A4*18*, which is consistent with other research showing that other features, including insulin resistance, chronic inflammation, and endometrial dysfunction may influence the course of PCOS or of related disorders (Zarek, 2024).

4.3.4. The Frequency of *CYP3A4*22 (G>A) (rs3559967)* Genotypes Based on Demographic Data and Clinical Symptoms in Infertile Women With PCOS

In this study *CYP3A4*22* genotypes did not appear to play a role in demographic characteristics (age, BMI, puberty, and abortion history) or PCOS symptoms (hirsutism, alopecia, and irregular menstrual cycle), as shown in Table (3-6). The impact of *CYP3A4* polymorphisms on drug metabolism was also studied in each population and further supported the concept that variability in genes can influence pharmacokinetic parameters, which are not always correlated with demographic characteristics of patients, such as age and body mass index (Rahman et al., 2021). Furthermore, a meta-analysis that revealed no discernible demographic variations among different genotype groups supported these findings. However, carriers of the *CYP3A4*22* variation did show different drug metabolism patterns. This is consistent with earlier studies that showed genetic variants do not always correspond with demographic characteristics, even if they can influence drug metabolism (Zhou and Lauschke, 2022, Erzurumluoglu et al., 2018).

4.3.5. Levels of Reproductive Hormones and Ultrasound Findings in Infertile Women With PCOS Based on *CYP3A4*18 (T>C) (rs28371759)* Genotypes

In this study, the FSH levels in polycystic women with wild (TT) alleles of *CYP3A4*18* and who had taken clomiphene citrate were significantly higher in comparison to those with heterozygous (TC) and mutant (CC) genotypes. In contrast, no significant differences are observed in LH levels or the LH/FSH ratio, as shown in Table (3-7). Significant differences in estrogen levels are noted, with the CC genotype (a mutant variant associated with

increased enzyme activity) showing a notably higher median estrogen concentration than other genotypes. Previous research indicates that clomiphene citrate inhibits the pituitary gland and hypothalamus, thereby stimulating estrogen synthesis (Sharma and Balasundaram, 2021). This could be consistent with these findings that the highest amounts of estrogen are found in the (CC) group that has the mutation that boosts the enzyme activity.

Regarding follicular and endometrial parameters, no statistically significant differences are observed between genotypes. Prior research has often linked variations in follicular size and endometrial thickness to hormone treatment regimens rather than genetic factors (Palatnik et al., 2012). Although limited, some studies have explored the genetic basis of these outcomes. Trevisan, et al. (2019) reported that variations in the FSH receptor gene can influence follicle growth (Trevisan et al., 2019). while Wu et al. (2024) found that polymorphisms in the estrogen receptor gene may affect endometrial thickness through altered estrogen signaling pathways, potentially impacting uterine receptivity (Wu et al., 2024).

4.3.6. Levels of Reproductive Hormones and Ultrasound findings in Infertile Women With PCOS Based on *CYP3A4*22 (G>A) (rs3559967)* Genotypes

Significant variations in LH levels were observed among *CYP3A4*22* genotypes in this study. The GG genotype (wild type) exhibited the highest LH levels, while the AA genotype (a mutant variant that reduces enzyme activity) showed the lowest, as shown in Table (3-8). A previous study found that patients who reacted to clomiphene citrate had significantly higher serum levels of LH (Arshad et al., 2024). This partially aligns with the current findings, where reduced LH levels were observed in individuals with the

enzyme-impairing mutation. These results are consistent with other studies examining how *CYP3A4* polymorphisms affect hormone regulation and drug metabolism. For instance, Sanchez, et al. (2017) demonstrated that carriers of the *CYP3A4*22* allele had notably different drug metabolism profiles, which could be associated with hormonal abnormalities due to alterations in metabolic pathways (Sanchez Spitman et al., 2017). Additionally, Mulder's et al. (2021) revealed different pharmacokinetic responses in drugs in *CYP3A4*22* carriers, indicating a wider effect of this allele on physiological processes beyond drug metabolism (Mulder et al., 2021).

No significant variations were found in FSH and estrogen, Elalem, et al. (2022) reported no significant correlation between *CYP3A4*22* genotypes and estradiol levels in breast cancer patients, suggesting that the impact of this polymorphism may vary depending on population characteristics and the hormonal parameters evaluated (Elalem et al., 2022).

LH and FSH receptor gene variants as risk factors for androgenic pillar in assisted reproductive technology. The objective of this study was to assess how genetic dissimilarities, especially *CYP3A4*22*, influence LH and FSH responses during ovarian stimulation, an important piece of knowledge regarding hormone oscillations (Alviggi et al., 2023).

Endometrial thickness was significantly different according to *CYP3A4*22* genotypes, with the AA genotype having the thickest endometrium. In the past, the development or evolution of endometrial thickness had been linked with polymorphisms in hormone receptor genes especially estrogen receptors (Yuan et al., 2012). Another analysis also explored genetic basis of endometrial thickness, and provided additional

evidence for current findings related to *CYP3A4*22* and uterine lining changes (Craciunas et al., 2019).

The mean intra-follicular diameters were similar in ranges with little diversity between the genotypes, with the exception of the *CYP3A4*22*, indicating that the allelic variant may not affect follicular growth greatly. This is consistent with the overall concept of follicular dynamics, where genetic factors, in particular the *CYP3A4* gene, are more important in drug metabolism than in reproductive physiology proper. Although *CYP3A4*22* has been reported to modify the pharmacokinetics of various drugs, its influence on follicular development and ovarian response is still unknown (Mulder et al., 2021). This illustrates a common theme where reproductive genetics is affected by pharmacogenetics based on a subset of genetic factors. Follicular development is influenced by several factors including genetic background and hormone regulation. It is proposed by some studies that the genetic polymorphisms, in addition to rendering no direct effect on follicular development, have influence on drug metabolism or other aspects of physiologica (Zheng et al., 2023, Kahraman et al., 2017).

4.3.7. The Effect of *CYP3A4*18* (T>C) (rs28371759) Genotypes on the Plasma Concentration of Clomiphene Citrate and its Metabolite

PCOS patients who were treated with clomiphene citrate and were mutant homozygous (CC) in this study had a greater metabolite/prodrug ratio than either (TT) or (TC) genotypes. Zuclomiphene is, however, elevated in those with the TT genotype but not the CT genotype, as presented in Table (3-9). These results illustrate the role of genetic polymorphisms in enzyme activity and drug metabolism. Several studies have shown that genetic polymorphisms of *CYP3A4*, especially *CYP3A4*18*, have a great effect on

drug pharmacokinetics and pharmacodynamics. Carriers of the *CYP3A4*18* allele have unique metabolic phenotypes for multiple substrates such as sex hormones and other drugs (Zhou et al., 2019, Kang et al., 2009).

The influence of *CYP3A4*18B* and *CYP3A53* polymorphisms on the pharmacokinetics of the CYP3A-metabolized immunosuppressive cyclosporine in healthy Chinese subjects was investigated in a study conducted in 2015. The results also showed that the *CYP3A4*18B* variant was related to higher metabolic capability, as C max of cyclosporine was significantly lower in male carriers than in the non-carriers. This study highlights the effect of *CYP3A4*18B* on cyclosporine in vivo kinetics and the prevalence of gender differences and gender-related genetic variability in drug metabolism (Tao et al., 2015).

CYP3A4 genetic polymorphisms particularly *CYP3A4*18* are the predominant factor that impacts on interindividual repaglinide pharmacokinetic variability, according to a 2010 Malaysian study (Ruzilawati and Gan, 2010).

In 2023, a study conducted in Azerbaijan revealed that *CYP3A4*18* was significantly associated with the response of individuals with chronic myeloid leukemia to imatinib treatment. In contrast, the more common *CYP3A5*3* variant showed no such association (Asadov et al., 2023).

A systematic review and meta-analysis conducted in 2021 in Iran demonstrated that the pharmacokinetics of Calcineurin inhibitors (CNIs) are affected by the genetic variations of *CYP3A4* among renal transplant recipients (Salehi et al., 2021).

The impact of *CYP3A4*, *CYP3A5* and *ABCB1* polymorphisms on the intravenous tacrolimus dose requirements and adverse drug reactions in adult allogeneic hematopoietic stem cell recipients was investigated in a study 2019. The results showed that these polymorphisms played clinically relevant roles in drug treatment monitoring; there was an association between the specific *CYP3A4/ABCB1* polymorphisms and high tacrolimus blood concentration and the incidence of adverse effects was increased (Hamadeh et al., 2019).

Pfizer's laboratory wing has replied that fesoterodine should not be taken in conjunction with strong *CYP3A4* inducers (Rodríguez et al., 2024).

Translating to differential efficacy and side effects with the use of clomiphene citrate (Robin et al., 2021). In view of the large amount of genetic variation in individual responses observed, this study highlights the need for pharmacogenetic analysis to customise treatment for PCOS patients treated with clomiphene citrate (Ruzilawati et al., 2007).

4.3.8. The Effect of *CYP3A422 (*G>A*) (*rs3559967*) Genotypes on the Plasma Concentration of Clomiphene Citrate and its Metabolite**

This study demonstrated that the metabolite/prodrug ratio was significantly higher in PCOS women with the wild (GG) genotype compared to those with the mutant alleles that decrease enzyme activity heterozygous (GA) or mutant (AA) genotypes and the metabolite/prodrug ratio was significantly higher in PCOS women with the heterozygous (GA) genotype compared to those with mutant (AA) genotype, as shown in Table (3-10), which suggests that people with the mutant alleles (AA) genotype may have a different metabolism than GG and GA carriers. There were no significant

differences between genotypes when comparing the concentrations of Zuclomiphene and its metabolite, Z-desethylclomiphene, separately. However, there was a significant difference in the metabolite-to-prodrug ratio across the different genotypes, reflecting the impact of these genotypes on enzyme activity and drug metabolism. Moreover, calculating the ratio of the metabolite Z-desethylclomiphene to its parent compound, Zuclomiphene, provides a more accurate assessment of *CYP3A4* enzyme activity in drug metabolism than measuring the concentrations of either compound alone.

This approach is advantageous in reducing interindividual variability. Drug concentrations can vary between individuals due to differences in absorption, distribution, and elimination processes. Measuring the metabolite-to-parent drug ratio helps normalize these variations, as both compounds are subject to the same physiological factors. This normalization offers a clearer understanding of *CYP3A4*'s role in the metabolic pathway, minimizing the influence of external variables.

In a study from 2018, the influence of genetic polymorphisms of *CYP2D6*, *CYP3A4*, *CYP3A5*, and *ABCB1* on aripiprazole's pharmacokinetic and safety profile was investigated in healthy volunteers. The investigation also examined *CYP3A4* polymorphisms, although the primary focus was *CYP2D6*. These results showed that aripiprazole metabolism and adverse drug responses could be influenced by the genetic polymorphisms of these enzymes, and indicated the significant role of *CYP3A4* in drug metabolism. (Belmonte et al., 2018).

There are few studies on the role of the *CYP3A4**22 allele and its impact on clomiphene citrate metabolism. But in 2021, a research was conducted that demonstrated the influence of *CYP2D6*, *CYP3A4*, and *CYP3A5*

polymorphisms on the clinical response of anovulatory PCOS women to clomiphene citrate. The discrepancy of these enzymes might play a role in the variation of the treatment outcomes (Robin et al., 2021).

One such study from 2014 investigated the influence of the *CYP3A4**22 genotype on the pharmacokinetics of tacrolimus in stable renal allograft recipients. Carriers of the *CYP3A4**22 allele had 36.8% lower tacrolimus clearance than non-carriers, which led to a 50% reduction in the tacrolimus dose required to attaining therapeutic drug concentrations. Such results demonstrate the importance of adjusting tacrolimus dose according to *CYP3A4**22 genotype in order to circumvent drug toxicity (De Jonge et al., 2015).

*CYP3A4**22 and *CYP3A5* polymorphisms on clinical outcome in patients treated with ticagrelor for acute myocardial infarction with ST-elevation (STEMI) were evaluated. The thrombotic and bleeding rates in carriers and non-carriers of *CYP3A4**22 did not show statistical significance in the study. The small number of *CYP3A4**22 carriers, however, indicates that more extensive research is required before firm conclusions can be made (Azzahhafi et al., 2022).

One further study evaluated the association between paclitaxel-induced neurotoxicity and the *CYP3A4**22 genotype. Individuals carrying the *CYP3A4**22 genotype had a higher probability of developing neurotoxic side effects as a result of greater systemic exposure to paclitaxel. This suggests that *CYP3A4**22 genotyping could contribute to regulation and prediction of paclitaxel toxicity (De Graan et al., 2013).

A complete analysis of the potential for adding *CYP3A4*22* genotyping in clinical routine has been described in 2021. The review noted that while the impact of the *CYP3A4*22* variant on drug clearance are significant in some drugs like tacrolimus and some statins, toxicity and clinical outcomes studies are needed to establish the feasibility of dosage based on genotype (Mulder et al., 2021).

Tamoxifen and tacrolimus are examples of drugs metabolized by *CYP3A4* that have previously been associated with reduced enzymatic activity of the *CYP3A4*22* variant (Sanchez Spitman et al., 2017, Mulder et al., 2021). The metabolite/prodrug ratios findings are reinforced by some studies that found *CYP3A4*22* to be linked with altered metabolic ratios in other drug contexts. (Kreutz et al., 2013, Mulder et al., 2021).

The association between *CYP3A4*22* allele and tacrolimus pharmacokinetics in kidney transplantation patients was assessed by a recently published meta-analysis (Kim et al., 2023). Compared with the noncarrier group, patients with the *CYP3A4*22* allele required fewer daily doses to achieve therapeutic concentrations, and markedly higher dose-adjusted tacrolimus trough concentrations were observed in patients carrying the *CYP3A4*22* allele. Based on these findings, genotyping *CYP3A4*22* might be applied to individualise tacrolimus dosing for optimisation of efficacy and minimisation of toxicity (Li et al., 2024).

Study in oncology investigated kinase inhibitor dosage in cancer patients with the *CYP3A4*22* allele based on genotype. Pharmacokinetic investigations at steady state showed that the modified dosing produced drug exposure levels equivalent to those in patients without the allele getting conventional doses, and patients with this allele obtained dose reductions of

20–33%. This method demonstrates how *CYP3A4*22* genotyping can be used to tailor cancer treatment to increase effectiveness and decrease side effects (Van Eerden et al., 2023).

Clinical annotations from the PharmGKB database suggest that simvastatin plasma concentrations may be higher in patients with the *CYP3A4*22/22* diplotype than in those with the *CYP3A41/1* diplotype. This implies that *CYP3A4*22* carriers have a decreased metabolism of simvastatin, which may raise the likelihood of dose-related side effects including myopathy. As a result, some patients may require dose modifications (Sharp et al., 2020).

*CYP3A4*22* allele is differently distributed among populations. It is more common in individuals of European ancestry (at a minor allele frequency (MAF) of about 5%) and less common in populations of African or Asian ancestry. This difference underscores how important population-specific research is when considering genotype-based dosing regimens(Pratt et al., 2023).

4.4. Limitations

Sample Size: The restricted sample size may hinder the study's capacity to discern subtle differences among genotypes. Larger cohorts are required to corroborate these findings and identify subtle effects.

Lack of Mechanistic Insights: The research identifies associations between genotypes and physiological results but does not explore the molecular mechanisms that underpin these associations. Further investigation is necessary to elucidate the specific genes and pathways affected by the *CYP3A4*18* and *CYP3A4*22* genotypes.

Ethnic and demographic diversity: In terms of race and place of origin, the study might not be diverse enough. Consequently, the findings' applicability to larger populations is limited. Groups may have different genetic influences. Future studies should therefore aim to include a wider variety of people.

Single genetic locus: By focusing on *CYP3A4*18* and *CYP3A4*22* genotypes, one may ignore the possible polygenic nature of factors such as hormone levels and drug metabolism. The observed results are probably due to a combination of environmental factors (such as diet and concurrent drugs) and a number of genetic factors.



Conclusions & Recommendations



Conclusions

From the present study, the following points can be concluded:

1. The wild homozygous genotype (TT) of *CYP3A4*18* was detected in a higher percentage (83.75%), followed by the heterozygous TC (11.25%), and the mutant CC genotype group had the lowest percentage 5%.
2. The wild homozygous genotype (GG) of *CYP3A4*22* was detected in 67.5%, followed by the GA genotype 25%, and the lowest percentage was in people who had the AA genotype 7.5%.
3. The genetic polymorphism of *CYP3A4*18* may change the effectiveness of Clomiphene Citrate in Karbala polycystic patients. There was a significant change in Clomiphene metabolic ratio ($p=0.002$), Estrogen level ($p=0.008$), and FSH level ($p=0.005$) among different *CYP3A4*18* genotypes. Endometrial thickness and follicle size are not significantly impacted by genetics.
4. The genetic polymorphism of *CYP3A4*22* may influence the clinical outcomes of Clomiphene Citrate in anovulatory PCOS patients. There was a significant change in clomiphene metabolic ratio ($p=0.000003$), LH level ($p=0.026$) and endometrial thickness ($p=0.048$) among different *CYP3A4*22* genotypes.
5. The *CYP3A4*18* and *CYP3A4*22* genotypes do not significantly differ among PCOS individuals in terms of age, BMI, number of abortions, regular menstrual cycles, hirsutism, alopecia, and length of marriage.

Recommendations

1. Further studies are necessary to evaluate the impact of other genetic variants associated with the efficacy and responsibility of Clomiphene Citrate response, like the *CYP3A5* gene, *CYP2D6* gene, estrogen receptor genes, and androgen receptor gene
2. A larger-scale study including more polycystic patients from different Iraqi cities is required.
3. In the clinical setting, it is recommended that genetic tests should be developed to predict the efficacy and a person's response to clomiphene.



References



References

- Abdelmonem, B.H., Abdelaal, N.M., Anwer, E.K., Rashwan, A.A., Hussein, M.A., Ahmed, Y.F., et al. (2024)." Decoding the role of CYP450 enzymes in metabolism and disease: A comprehensive review ." *Journal of Biochemistry*, 12:1467.
- Abed, S.N., Sahib, A.S. & Kadum, M.A.A. (2022)." Genetic polymorphic impact of metabolizing enzyme (CYP3A4 and UGT1A4 genes) on anastrozole response in Iraqi breast cancer women." *Annals of Clinical and Medical Case Reports*, 8(7): 1–6.
- Abusailik, M. A., Muhanna, A. M., Almuhsen, A. A., Alhasanat, A. M., Alshamaseen, A. M., Mustafa, S. M. B. et al .(2021)." Cutaneous manifestation of polycystic ovary syndrome." *Dermatology Reports*, 13(2).
- Adeleye, A.J. & Zamah, A.M. (2023)." Reproductive endocrinology and infertility, an issue of Obstetrics and Gynecology Clinics, E-book. Elsevier Health Sciences." *Obstetrics and Gynecology Clinics of North America*, 50(1).
- Afzal, M., Rahim, A., Naveed, A. K., Ahmed, S., & Kiyani, M. M. (2018). "Development of cost-effective Tetra-primer Amplification Refractory Mutation System (T-ARMS) PCR for the detection of miR-146a gene rs2910164 C/G polymorphism in breast cancer." *Biochemistry and Molecular Biology Journal* , 4(01).
- Al-Eitan, L. N., Rababa'h, D. M., Alghamdi, M. A. & Khasawneh, R. H. J. (2019)." Association of CYP gene polymorphisms with breast cancer risk and prognostic factors in the Jordanian population." *BMC Medical Genetic*,20:1-12.
- Al-Suhaimi, E. A., Khan, F. A. & Homeida, A. (2022)." Regulation of male and female reproductive functions." *Emerging Concepts in Endocrine Structure and Functions*. Springer.
- Al-Obaidi, M.T., Ali, Z.H., Al-Saadi, W.I., Al-Wasiti, E.A. & Al-Aubaidy, H.J. (2019)." Impact of letrozole versus clomiphene citrate on endometrial receptivity in Iraqi women with polycystic ovarian syndrome." *Journal of Clinical Pharmacy and Therapeutics* ,44: 618-622.
- Al Shaikhly, R. (2021). "The Effect of Genetic Polymorphism on Therapeutic Response of Metformin in Women with Polycystic Ovarian Syndrome in Iraqi Population." *Lebanese American University*.
- Ali, S. M., Abdulhussein, I. F. & Kazom, S. N. (2024). "Comparison of Letrozole vs Clomiphene Citrate for induction of ovulation in

- polycystic ovarian syndromeAn Iraqi study." *Journal of Medical Sciences*, 30:23-39.
- Allahbadia, G. N. & Merchant, R. (2011)." Polycystic ovary syndrome and impact on health. "*Middle East Fertility Society Journal*, 16(1): 19-37.
- Alviggi, C., Longobardi, S., Papaleo, E., Santi, D., Alfano, S., Vanni, V. S., et al. (2023)." Genetic Variants of Gonadotropins and Their Receptors Could Influence Controlled Ovarian Stimulation: IVF Data from a Prospective Multicenter Study." *Genes*, 14 (6):1269.
- Arshad, A., Rehman, K. U., Butt, H. I., Bashir, S., Umar, F., Yaseen, Z., et al. (2024)." Effect of Clomiphene Citrate on Fertility Hormones in Women with Polycystic Ovarian Syndrome." *International Journal of Human Health Research* ,4(1): 1-13.
- Asadov, C., Karimova, N., Hasanova, A., Bayramov, B., Shirinova, A. & Alimirzoyeva, Z. (2023)." Association of CYP3A5* 3, CYP3A4* 18 & CYP2B6* 6 polymorphisms with imatinib treatment outcome in Azerbaijani chronic myeloid leukaemia patients." *Indian Journal of Medical Research*, 158(2): 151-160.
- Ashkar, F., Rezaei, S., Salahshornezhad, S., Vahid, F., Gholamalizadeh, M., Dahka, S. M. , et al. (2020)." The Role of medicinal herbs in treatment of insulin resistance in patients with Polycystic Ovary Syndrome: A literature review." *Biomolecular Concepts*,11(1): 57-75.
- Azzahhafi, J., Bergmeijer, T. O., van den Broek, W. W., Chan Pin Yin, D. R., Rayhi, S., Peper, J., et al. (2022)." Effects of CYP3A4* 22 and CYP3A5 on clinical outcome in patients treated with ticagrelor for ST-segment elevation myocardial infarction: POPular genetics sub-study." *Frontiers in Pharmacology*,13: 1032995.
- Barbosa, G., de Sá, L. B. P. C., Rocha, D. R. T. W. & Arbex, A. K. (2016)." Polycystic ovary syndrome (PCOS) and fertility." *Open Journal of Endocrine and Metabolic Diseases* , 6(1): 58-65.
- Belmonte, C., Ochoa, D., Román, M., Saiz-Rodríguez, M., Wojnicz, A., Gómez-Sánchez, C. I., et al. (2018)." Influence of CYP 2D6, CYP 3A4, CYP 3A5 and ABCB 1 polymorphisms on pharmacokinetics and safety of aripiprazole in healthy volunteers." *Basic & Clinical Pharmacology & Toxicology*,122(6): 596-605.
- Bhandari, R., Aatre, R.D. and Kanthi, Y., (2020)." Diagnostic approach and management of genetic aortopathies". *Vascular Medicine*, 25(1), pp.63–77.
- Bloom, M.S., Schisterman, E.F. & Hediger, M.L. (2006)." Selecting controls is not selecting “normals”: design and analysis issues for studying the

- etiology of polycystic ovary syndrome." *Fertility and Sterility*, 86(1): 1-12.
- Boucher, H., Robin, G., Ribière, L., Martin, C., Espiard, S. and Catteau-Jonard, S., (2024). Is it useful to measure DHEAS levels in PCOS?. *Annales d'Endocrinologie*, 85(2), pp.95–99.
- Cable, J. K. & Grider, M. H.(2020). "Physiology, progesterone.",*StatPearls*. [online] Treasure Island (FL): StatPearls Publishing.
- Carmina, E., & Lobo, R. A. (2019). "Evaluation of hormonal status,Yen & Jaffe's Reproductive Endocrinology: Physiology, Pathophysiology, and Clinical Management" *8th ed. Philadelphia: Elsevier* :887-915.
- Cetin, E., Siblini, H. & Al-Hendy, A. (2021). "What is the Mechanism of Poor Endometrial Proliferation in Patients With Unexplained Infertility After Clomiphene Citrate Treatment?" *The Journal of Clinical Endocrinology & Metabolism* ,106(11): e4776-e4777.
- Chooi, Y. ,Ding C1, Magkos F. (2019)." The epidemiology of obesity. *Metabolism.Clinical and Experimental*, 92:6-10.
- Choudhary, K., Singh, R., Garg, A., Verma, N., Purohit, A. & Deora, D. J. I. J. B. S. (2019). "An updated overview of polycystic ovary syndrome." *International Journal of Biomedical Science*, 7(3): 1-13.
- Coral, L. (2017)."High-resolution nucleic acid analysis with a DNA nanotechnology approach." *PhD thesis. Università degli Studi di Trieste*.
- Craciunas, L., Gallos, I., Chu, J., Bourne, T., Quenby, S., Brosens, J. J.,et al. (2019)." Conventional and modern markers of endometrial receptivity: a systematic review and meta-analysis." *Human Reproduction Update*,25(2): 202-223.
- Dabravolski, S. A., Nikiforov, N. G., Eid, A. H., Nedosugova, L. V., Starodubova, A. V., Popkova, T. V., et al. (2021)." Mitochondrial dysfunction and chronic inflammation in polycystic ovary syndrome." *International Journal of Molecular Sciences*, 22(8): 3923.
- Dambala, K., Paschou, S. A., Michopoulos, A., Siasos, G., Goulis, D. G., Vavilis, D. , et al. (2019)." Biomarkers of endothelial dysfunction in women with polycystic ovary syndrome." *Angiology*, 70(9): 797-801.
- Deadmond, A., Koch, C.A. & Parry, J.P. (2022)." Ovarian reserve testing". *Endotext* [online].
- De Graan, A.J. M., Elens, L., Sprowl, J. A., Sparreboom, A., Friberg, L. E., Van Der Holt, B., et al.(2013)." CYP3A4* 22 genotype and systemic exposure affect paclitaxel-induced neurotoxicity." *Clinical Cancer Research*,19(12): 3316-3324.

- De Jonge, H., Elens, L., De Loor, H., Van Schaik, R. & Kuypers, D. J. T. p. j.(2015). "The CYP3A4* 22 C> T single nucleotide polymorphism is associated with reduced midazolam and tacrolimus clearance in stable renal allograft recipients." *Therapeutic Drug Monitoring*, 37(1):144-152.
- Dengler, M. (2019). "Trapped: Developing an Unbiased Approach to Identify Epoxide Hydrolase Substrates in Vivo." (*Doctoral dissertation, ETH Zurich*).
- Devi, O. J., Pushpari, C., Manaswini, K., Anjum, S. N. & Lakshmi, V. B. (2021)." PCOS: a common but underdiagnosed condition in reproductive women." *World Journal of Pharmaceutical Research* ,10(7): 598-612.
- Dreshaj, A., Dreshaj, A., Sinanaj, D., Morina, E., Dehari, S. & Sciences, M. (2024)." Main consequences of enzymatic induction and inhibition during the interaction of drugs and the role of CYP3A4, CYP3A45 enzymes." *Current Issues in Pharmacy and Medical Sciences*, 37(1): 1-6.
- Elalem, E.G., Jelani, M., Khedr, A., Ahmad, A., Alaama, T.Y., Alaama, M. N., et al.(2022). "Association of cytochromes P450 3A4*22 and 3A5*3 genotypes and polymorphism with response to simvastatin in hypercholesterolemia patients." *PloS One*, 17 :e0260824.
- Eleawi, H.R., Abdul-Karim, E.T. & AL-Salihi, A.R. (2015)." Study of occurrence of polycystic ovarian syndrome among infertile women. *Iraqi Postgrad." Medical Journal*,14(3): 329-336.
- Elens, L., Van Gelder, T., Hesselink, D.A., Haufroid, V. & Van Schaik, R.H. (2013). "CYP3A4* 22: promising newly identified CYP3A4 variant allele for personalizing pharmacotherapy." *Pharmacogenomics.*, 14(1): 47-62.
- Erzurumluoglu, A.M., Baird, D., Richardson, T.G., Timpson, N.J. & Rodriguez, S. J. G. (2018)." Using Y-chromosomal haplogroups in genetic association studies and suggested implications." *Scientific Reports*, 9: 45.
- Escobar-Morreale, H.F. (2018)." Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment." *Nature Reviews Endocrinology* ,14:270-284.
- Fabozzi, G., Verdone, G., Allori, M., Cimadomo, D., Tatone, C., Stuppia, L., et al. (2022)." Personalized nutrition in the management of female infertility: new insights on chronic low-grade inflammation." *Nutrients*. 14(9): 1918.

- Fahs, D., Salloum, D., Nasrallah, M. & Ghazeeri, G. (2023)." Polycystic Ovary Syndrome: pathophysiology and controversies in diagnosis." *Diagnostics*,13(9): 1559.
- Feh, M. K. M., Patel, P. & Wadhwa, R. (2024)." Clomiphene." *StatPearls [Internet]*. StatPearls Publishing.
- Fischer, J. & Ganellin, C. R. (2010)." Analogue-based drug discovery." *Chemistry International*, 32(4): 12-15.
- Fourie, C., (2016)." Effects of clomiphene citrate on the expression of kisspeptin, dynorphin A and neurokinin B in female Sprague-Dawley rats". *Master's thesis. University of Pretoria, South Africa*.
- García-Alegría, A.M., Anduro-Corona, I., Pérez-Martínez, C.J., Guadalupe Corella-Madueño, M.A., Rascón-Durán, M.L., & Astiazaran-Garcia, H. (2020). "Quantification of DNA through the NanoDrop spectrophotometer: methodological validation using standard reference material and Sprague Dawley rat and human DNA." *International Journal of Analytical Chemistry* ,2020(1): 8896738.
- Geisthövel, F. (2003)." A comment on the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine consensus of the polycystic ovarian syndrome." *Reproductive Biomedicine Online*, 7(6): 602-605.
- Glezer,A. (2024)." Hyperprolactinemia in women: diagnostic approach." *Archives of Endocrinology and Metabolism*,46, p.e-FPS04.
- Goodman, N.F., Cobin, R.H., Futterweit, W., Glueck, J.S., Legro, R.S. & Carmina,E. (2015)." American Association of Clinical Endocrinologists, American College of Endocrinology, and Androgen Excess and PCOS Society disease state clinical review: guide to the best practices in the evaluation and treatment of polycystic ovary syndrome-part 2" *Endocrine Practice*. 21(11): 1415-1426.
- Gupta, M.C. & Khanna,J. (2018)." Clomiphene citrate: the changing landscape. " *International Journal of Basic & Clinical Pharmacology*, 7(8): 1437-1443.
- Haddad-Filho, H., Tosatti, J.A., Vale, F.M., Gomes, K.B. & Reis, F. M. (2023)." Updates in diagnosing polycystic ovary syndrome-related infertility." *European Review for Medical and Pharmacological Sciences* ,23: 123-132.
- Hamadeh, I.S., Zhang,Q., Steuerwald,N., Hamilton,A., Druhan,L.J., McSwain, M., et al. (2019)." Effect of CYP3A4, CYP3A5, and ABCB1 polymorphisms on intravenous tacrolimus exposure and adverse events in adult allogeneic stem cell transplant patients." *Bone Marrow Transplant*.,25: 656-663.

- Hanaa Rahman, E., Enas Talib, A. K. & Anam Rasheed, A.(2015)." Study of occurrence of polycystic ovarian syndrome among infertile women." *Iraqi Postgraduate Medical Journal*,14(3):336-329.
- Henderson, L.M. (2019)." Impact of Warfarin Pharmacogene Variation on Drug Metabolism and Pharmacological Response in Alaska Native and American Indian Populations." *University of Washington, Ph.D. Dissertation*.
- Hertz, D.L., Kidwell,K.M., Seewald, N. J., Gersch, C. L., Desta, Z., Flockhart, D.A., et al. (2017)". Polymorphisms in drug-metabolizing enzymes and steady-state exemestane concentration in postmenopausal patients with breast cancer." *The Pharmacogenomics Journal*,17: 521-527.
- Holmberg,M.T., Tornio,A., Paile-Hyvärinen,M., Tarkiainen,E.K., Neuvonen, M., Neuvonen, P. J., et al. (2019)." CYP3A4* 22 impairs the elimination of ticagrelor, but has no significant effect on the bioactivation of clopidogrel or prasugrel." *Clinical Pharmacology & Therapeutics*, 105: 448-457.
- Huang, Z. & Yong, E.-L. (2016)." Ethnic differences: Is there an Asian phenotype for polycystic ovarian syndrome?" *Best Practice & Research Clinical Obstetrics & Gynaecology*, 37:46-55.
- Ibrahim, R. O., Khorsheed, T. H., Bahram, K. A. (2024)." Investigating Infertility Causes, Obesity Rates, and Mental Status of Infertile Females Attending the Infertility Center at Azadi Teaching Hospital in Kirkuk City, Iraq." *Journal of Advanced Institute of Medical Sciences*, 26(1): 29-38.
- Ide, V., Vanderschueren, D. & Antonio, L. (2020)." Treatment of men with central hypogonadism: alternatives for testosterone replacement therapy." *International Journal of Molecular Sciences*, 22: 21.
- Islam, H., Masud, J., Islam, Y. N. & Haque, F. K. M. (2022)." An update on polycystic ovary syndrome: A review of the current state of knowledge in diagnosis, genetic etiology, and emerging treatment options." *Journal of Women's Health*, 18: 1745-5065.
- Johnson, J.E., Daley, D., Tarta, C. & Stanciu, P. I. (2023)." Risk of endometrial cancer in patients with polycystic ovarian syndrome: A meta-analysis. " *Journal of Oncology Letters*,(4):25, 1-9.
- Kadri, K. (2019). "Polymerase chain reaction (PCR): Principle and applications." *Synth. Biology - New Interdisciplinary Science*: 1-17.
- Kahraman, S., Cetinkaya, C. P., Cetinkaya, M., Yelke, H., Colakoglu, Y. K., Aygun,M. et al. (2017). "The effect of follicle size and homogeneity of follicular development on the morphokinetics of human embryos." *Journal of Assisted Reproduction and Genetics*, 34: 895-903.

- Kang, Y.S., Park, S.Y., Yim,C.H., Kwak,H.S., Gajendrarao,P., Krishnamoorthy, N., et al. (2009)." The CYP3A4*18 genotype in the cytochrome P450 3A4 gene, a rapid metabolizer of sex steroids, is associated with low bone mineral density." *Clinical Pharmacology & Therapeutics*, 85: 312-8.
- Kar, P. & Cummings, M. (2005)." Polycystic ovary syndrome. Wiley Online Library." *Pract. Diabetes International* ,22(8):420-415.
- Karimova, N., Asadov, C., Hasanova, A., Bayramov, B., Shirinova, A., & Alimirzoyeva, Z. (2023). "CYP3A5* 3, CYP3A4* 18 and CYP2B6* 6 genotypes and chronic myeloid leukemia development in Azerbaijan." *Advances in Biology and Earth Sciences*, 8(2).
- Keri, R. A. & Nilson, J. H. (2001)." Glycoprotein Hormones." *Endocrine System in Animals*,1(4):261-275.
- Khairnar, R. & Khairnar, .(2023) ."A Clinical, endocrinal and radiological profile of cases with polycystic ovary syndrome in tertiary care centre." *Indian Journal of Obstetrics and Gynaecology*, 1(1):2342-2454.
- Khan, U.I. & Farid,R. (2024)." Polycystic ovary syndrome phenotypes. " in *Polycystic Ovary Syndrome*. Elsevier.p:6-10.
- Kim, J. S., Shim, S., Yee, J., Choi, K. H., & Gwak, H. S. (2023). "Effects of CYP3A4* 22 polymorphism on trough concentration of tacrolimus in kidney transplantation": a systematic review and meta-analysis. *Frontiers in Pharmacology*, 14, 1201083
- Kovar, C., Kovar, L., Rüdesheim, S., Selzer, D., Ganchev, B., Kröner, P., et al.(2022)." Prediction of Drug–Drug–Gene Interaction Scenarios of (E)-Clomiphene and Its Metabolites Using Physiologically Based Pharmacokinetic Modeling." *Pharmaceutics*, 14: 2604.
- Kreisman MJ, McCosh RB, Breen KM.(2022)." A Modified Ultra-Sensitive ELISA for Measurement of LH in Mice." *Endocrinology*.163(9): bqac109.
- Kreutz, R.P., Owens, J., Jin,Y., Nystrom, P., Desta, Z., Kreutz, Y., et al. (2013)." Cytochrome P450 3A4*22, PPAR- α , and ARNT polymorphisms and clopidogrel response." *Clinical Pharmacology: Advances and Applications*, 5:185-92.
- Legro, R.S. (2017)." Evaluation and treatment of polycystic ovary syndrome." *MDText.com Inc*. Bookshelf ID: NBK278874.
- Legro, R.S., Myers, E.R., Barnhart, H.X., Carson, S.A., Diamond, M. P., Carr, B. R., et al.(2006). "The Pregnancy in Polycystic Ovary Syndrome study: baseline characteristics of the randomized cohort including racial effects. " *Fertility and sterility*, 86(4): 914-933.

- Li, C.-J., Li, L., Lin, L., Jiang, H.-X., Zhong, Z.-Y., Li, W.-M., et al. (2014). "Impact of the CYP3A5, CYP3A4, COMT, IL-10 and POR genetic polymorphisms on tacrolimus metabolism in Chinese renal transplant recipients." *PLoS ONE*, 9: e86206.
- Li, Z., Wang, X., Li, D., Cheng, S., Li, Z., Guo, H., et al. (2024). "Effects of CYP3A4* 22 and POR* 28 variations on the pharmacokinetics of tacrolimus in renal transplant recipients: a meta-analysis of 18 observational studies." *Bioscience and Nanotechnology*, 25: 48.
- Lima, E. R., Sevilhano, T., Caramori, T., Oliveira, J. E., Suzuki, M. F., Torati, L. S., et al. (2024). "Physical Chemical, Immunological and Biological Characterization of Arapaima 2 gigas Follicle-Stimulating Hormone (ag-FSH) synthesized in HEK293 Cells." *Preprints*: 2024052027.
- Lioudakis, E. (2015). "Current trends in the development of steroidal hormones modulators." in: *Proceedings of the Scientific Conference; Hradec Králové, Czech Republic*.
- Lu, Y., Cherouveim, P., Jiang, V., Dimitriadis, I., James, K. E., Bormann, C. & Souter, I. J. F. i. E. (2024). "The impact of clomiphene citrate on the endometrium in comparison to gonadotropins in intrauterine insemination cycles: is it thinner and does it matter?" *Frontiers in Endocrinology*.15:1414481.
- Lunenfeld, B., Mskhalaya, G., Zitzmann, M., Arver, S., Kalinchenko, S., Tishova, Y., & Morgentaler, A. (2015). "Recommendations on the diagnosis, treatment and monitoring of hypogonadism in men." *The Aging Male*, 18(1), 5-15.
- MacKinnon, M. (2023). "The hormone type cookbook: nourishing recipes for balancing the 7 different hormone types-recipes for healthy cycles, PMS, PCOS, fertility, and menopause." *Fair Winds Press*.
- Maddin, N., Husin, A., Gan, S. H., Aziz, B. A., & Ankathil, R. (2016). "Impact of CYP3A4* 18 and CYP3A5* 3 polymorphisms on imatinib mesylate response among chronic myeloid leukemia patients in Malaysia." *Oncology Therapy*, 4: 303-314.
- Maizels, M., & Budde, W. L. (2004). "A LC/MS method for the determination of cyanobacteria toxins in water." *Analytical Chemistry*, 76(5): 1342-1351.
- Malik, S., Verma, S., Jain, K., Talwar, P., Dhorepatil, B., Devi, G., et al. (2015). "Good clinical practice recommendations on management of infertility in patients from India with polycystic ovary syndrome." *Fertility Science and Research*, 2(2):107-132.
- Mansour, A., Hashemi Taheri, A. P., Moradi, B., Mohajeri-Tehrani, M. R., Qorbani, M., Ghorbani Pashakolaei, S., et al. (2022). "Ovarian volume,

- not follicle count, is independently associated with androgens in patients with polycystic ovary syndrome." *BMC Endocrine Disorder*, 22(1): 298.
- Marwal,A., & Gaur, R.K. (2020). "Molecular markers: tool for genetic analysis. "In *Animal biotechnology* , pp: 353-372. Academic Press
- Meier, R.K. (2018)." Polycystic ovary syndrome. "*Nursing Clinics of North American* , 53(3), 407-420.
- Messinis, J.T.O.C. (2023)." Contraception in women with polycystic ovary syndrome. "*In: Sexual & Health Research*. Chapter 33: 204.
- Mgheer, T.H., Abdulmahdi, B. S. & Mohammed, L. A. J. (2023)." Bio-Chemical study and preparation of some new mixed ligand complexes with novel (azo) ligand derived from 5-methyl imidazole and 1, 10-phenanthroline compounds." *Journal of Pharmaceutical Negative Results*, 7135-7146.
- Miller, P., & Soules, M. (2009)." Luteal Phase Deficiency: pathophysiology, diagnosis, and treatment." *Global Library of Women's Medicine*,1756-2228.
- Miura, R., Haneda, S. & Matsui, M. (2015)." Ovulation of the preovulatory follicle originating from the first-wave dominant follicle leads to formation of an active corpus luteum." *Journal of Reproduction and Development*, 61(4): 317-23.
- Moolenaar, L. M., Nahuis, M. J., Hompes, P. G., Van Der Veen, F., & Mol, B. W. J. (2014). "Cost-effectiveness of treatment strategies in women with PCOS who do not conceive after six cycles of clomiphene citrate." *Reproductive Biomedicine Online*, 28(5): 606-613.
- Moore, V., Rumbold, A., Fernandez, R., McElroy, H., Moore, L., Giles, L., et al. (2022)." Dispensing of clomiphene citrate to treat infertility: medication supplied and population prevalence of assisted pregnancies and multiple births. "*Fertility and Sterility*, 117(1):202-212.
- Mousa, B. A. & Al Joborae, S. F. F. M.(2020)." Analysis of Patients with PCOS According to Demographic Factors & Hormonal Assayin Babylon Government in Iraq." *Medico Legal Update*,20(2),473-478.
- Mousa, B.A. (2019). "The Prevalence of PCOS in Infertile Women According to Clinical Features and its Associated Hormonal Changes in Al-Hilla City, Iraq." *Indian Journal of Public Health Research & Development*,10(10):2607-2611.
- Mukerjee, N. (2020)." Polycystic Ovary Syndrome (PCOS) Symptoms, Causes & Treatments-A Review." *International Journal of Scientific Research*, 9(7): 1949-1957.

- Mukherjee,A.G., Wanjari,U.R., Kannampuzha,S., Murali,R., Namachivayam, A., Ganesan, R., et al.(2023)." The Implication of Mechanistic Approaches and the Role of the Microbiome in Polycystic Ovary Syndrome (PCOS)": A Review. *Metabolites*, 13(1):129.
- Mulder, T. A. M., van Eerden, R. A. G., de With, M., Elens, L., Hesselink, D. A., Matic, M., et al.(2021)." CYP3A4(*)22 Genotyping in Clinical Practice: Ready for Implementation? " *Frontiers in genetics*, 12: 711943.
- Muralidharan, C., Ambika, A.S.S., Vyawahare, R.S. and Darmode, S.A.,(2023)." The effectiveness of a structured exercise program and progressive muscle relaxation technique on physical and emotional disturbance among college girls with PCOS. " *Journal of Biomedical Research Reports*, 2 :45-53.
- Mürdter, T. E., Kerb,R., Turpeinen,M., Schroth,W., Ganchev,B., Böhmer,G.M., et al.(2012). "Genetic polymorphism of cytochrome P450 2D6 determines oestrogen receptor activity of the major infertility drug clomiphene via its active metabolites." *Human Molecular Genetics*, 21(5): 1145-1154.
- Nasseri, S. & Ledger, W. L. J. H. F. (2001)." Clomiphene citrate in the twenty-first century." *Human Fertility*, 4(3): 145-151.
- O'Donnell, R., Warner, P., Lee, R., Walker, J., Bath, L., Kelnar, C., et al.(2012)." Physiological sex steroid replacement in premature ovarian failure: randomized crossover trial of effect on uterine volume, endometrial thickness and blood flow, compared with a standard regimen." *Human Reproduction*, 27: 1130-1138.
- Okubo, M., Murayama, N., Shimizu, M., Shimada, T., Guengerich, F. P. & Yamazaki, H. J. T. J. o. t. s. (2013)." The CYP3A4 intron 6 C> T polymorphism (CYP3A4* 22) is associated with reduced CYP3A4 protein level and function in human liver microsomes." *Journal of Toxicological Sciences*, 38: 349-354.
- Palatnik, A., Strawn, E., Szabo,A., & Robb, P. (2012). "What is the optimal follicular size before triggering ovulation in intrauterine insemination cycles with clomiphene citrate or letrozole? An analysis of 988 cycles. " *Fertility and Sterility*, 97(5): 1089–1094.e3.
- Patel, S. (2018)." Polycystic ovary syndrome (PCOS), an inflammatory, systemic, lifestyle endocrinopathy." *Journal of Steroid Biochemistry and Molecular Biology*, , 182: 27-36.
- Pkhaladze, L., Unfer, V. & Dewailly, D. (2023)." Use of myo-inositol in the treatment of PCOS symptoms in adolescents." *A Clinical Guide to Inositols*. Elsevier.(pp. 151-165). Academic Press.

- Pratt, V.M., Cavallari, L.H., Fulmer, M.L., Gaedigk, A., Hachad, H., Ji, Y., et al. (2023). "CYP3A4 and CYP3A5 genotyping recommendations: a joint consensus recommendation of the association for molecular Pathology, clinical pharmacogenetics implementation Consortium, College of American pathologists, Dutch pharmacogenetics working Group of the royal Dutch pharmacists association, European society for pharmacogenomics and personalized therapy, and pharmacogenomics Knowledgebase." *Journal of Molecular Diagnostics*, 25 :619-629.
- Okubo, M., Murayama, N., Shimizu, M., Shimada, T., Guengerich, F.P., & Yamazaki, H. (2013). "CYP3A4 intron 6 C>T polymorphism (CYP3A4*22) is associated with reduced CYP3A4 protein level and function in human liver microsomes." *Journal of Toxicological Sciences*, 38(3) 349–354.
- Qiu, S., Pan, Y., Cui, Y., Li, M., Yue, T., Pu, S., et al. (2024). "HNF4 α improves hepatocyte regeneration by upregulating PXR." *FASEB Journal*, 38(14), e23830.
- Ono, M., Dai, Y., Fujiwara, T., Fujiwara, H., Daikoku, T., Ando, H., et al. (2025) "Influence of lifestyle and the circadian clock on reproduction." *Reproductive Medicine and Biology*, 24(1):e12641.
- Qureshi, S. S., Gupta, J. K., Shah, K. & Upmanyu, N. (2016). "Prevalence and risk factor of polycystic ovarian syndrome." *Asian Journal of Pharmaceutical and Clinical Research*, 9(2):23-25.
- Rachmawati, A., Krisnadi, S. R., Santoso, S. A. & Nugrahani, A. D. J. B. R. N. (2023). "Association between follicle size, endometrial thickness, and types of ovarian stimulation (Clomiphene citrate and Letrozole) with biochemical pregnancy rate in women undergone intrauterine insemination." *Biomedical Research and Therapy*, 16: 286.
- Rahman, K. L., Akhter, Q. S., Rahman, M. S., Rahman, R., Rahman Sami, S., Mukta, F.Y. et al. (2021). "Genetic variations of CYP2R1 (rs10741657) in Bangladeshi adults with low serum 25(OH)D level-A pilot study." *PLOS ONE*, 16: e0260298.
- Rasquin, L.I., Anastasopoulou, C., & Mayrin, J. V. (2017). "Polycystic ovarian disease." *StatPearls* [online]. *Treasure Island* (FL): StatPearls Publishing.
- Robin, C., Hennart, B., Broly, F., Gruchala, P., Robin, G., & Catteau-Jonard, S. (2021). "Could Cytochrome P450 2D6, 3A4 and 3A5 Polymorphisms Explain the Variability in Clinical Response to Clomiphene Citrate of Anovulatory PCOS Women?" *Frontiers in Endocrinology*, 12: 718917.

- Rodríguez, A. T., i Barceló, A. F., González, M. B., Esnal, D. E., Muner, D. S., Martínez, J.A., et al. (2024)." Clinically important pharmacokinetic drug-drug interactions with antibacterial agents." *Revista Española de Quimioterapia* , 37(4): 299.
- Rosenfield, R. L. (2015)." The diagnosis of polycystic ovary syndrome in adolescents." *Pediatrics*,136:1154-1165.
- Ruzilawati, A. & Gan, S. J. (2010)." CYP3A4 genetic polymorphism influences repaglinide's pharmacokinetics." *Pharmacogenetics* ,85: 357-364.
- Ruzilawati, A.B., Suhaimi, A.W. & Gan, S. H. (2007)." Genetic polymorphisms of CYP3A4: CYP3A4*18 allele is found in five healthy Malaysian subjects." *Clinica Chimica Acta* ,383: 158-62.
- Sadeghi, H. M., Adeli, I., Calina, D., Docea, A. O., Mousavi, T., Daniali, M., et al. (2022)." Polycystic Ovary Syndrome: A Comprehensive Review of Pathogenesis, Management, and Drug Repurposing." *International Journal of Molecular Sciences*, 23(2):583.
- Saiz-Rodríguez, M., Almenara, S., Navares-Gómez, M., Ochoa, D., Román, M., Zubiaur, P., et al. (2020)." Effect of the most relevant CYP3A4 and CYP3A5 polymorphisms on the pharmacokinetic parameters of 10 CYP3A substrates." *Biomedicines*, 8(4): 94.
- Sakpuwadol, N., Tejapira, K., Kositkuljorn, C., Pomsoong, C., & Suchonwanit, P. (2023)." Differences in Demographic and Clinical Characteristics Among Subtypes of Female Pattern Hair Loss." *Clinical, Cosmetic and Investigational Dermatology* ,16 :2073-2082.
- Salehi,S., Abedini,F., Shahi,A., Afzali,S., Dashti-Khavidaki,S. & Amirzargar, A. A. J. m. (2021)." The effect of CYP3A4 genetic polymorphisms on the pharmacokinetics of calcineurin inhibitors in renal transplant recipients: a systematic review and meta-analysis protocol." *Journal of Medical Genetics*, 13:21253531.
- Salimon, Y.A., & Olaniyan, M.F. (2024)." Assessment of total and free testosterone, prolactin level among infertile women attending Gynecology Clinic in Ondo State." *Sokoto Journal of Medical Laboratory Science* , 9(2):170-176.
- Sanchez Spitman, A., Moes,D., Gelderblom,H., Dezentje,V., Swen,J. & Guchelaar, H. J. (2017). "Effect of CYP3A4* 22, CYP3A5* 3, and CYP3A combined genotypes on tamoxifen metabolism." *European Journal of Clinical Pharmacology*, 73: 1589-1598.
- Sedgh, G., Bearak, J., Singh, S., Bankole, A., Popinchalk, A., Ganatra, B., et al. (2016). "Abortion incidence between 1990 and 2014: global,

- regional, and subregional levels and trends." *The Lancet*, 388(10041): 258–267.
- Sedgh,G., Singh,S., & Hussain,R. (2014). "Intended and unintended pregnancies worldwide in 2012 and recent trends." *Studies in Family Planning*, 45(3): 301-314.
- Setji,T. L. & Brown, A. J. (2007). "Polycystic ovary syndrome: diagnosis and treatment." *American Journal of Medicine*, 120: 128-132.
- Shah, D., Patil, M.; National PCOS Working Group. (2018)"Consensus Statement on the Use of Oral Contraceptive Pills in Polycystic Ovarian Syndrome Women in India." *Journal of Human Reproductive Sciences* ,11(2):96-118.
- Sharma, S., & Balasundaram, S. (2021)." Clomiphene citrate: A review of its mechanisms and clinical applications in female infertility." *Journal of Human Reproductive Sciences*,14(3): 205–213.
- Sharp, C.N., Linder, M.W. & Valdes, R.Jr. (2020)." Polypharmacy: A healthcare conundrum with a pharmacogenetic solution." *Clinical Research in Cardiology*,57: 161-180.
- Sha Y, Zhu J, Shen F.(2024)." A meta-analysis of efficacy on dexamethasone and clomiphene in the treatment of polycystic ovary syndrome patients." *BMC Women's Health*. 24:3141.
- Shermin, S., Noor, A. & Jahan, S. (2019)." Polycystic ovary syndrome: a brief review with recent updates." *Delta Medical College Journal*, 7(2): 84-99.
- Shukla, G. (2020). "Health is Wealth & Wealth is Pogos Nutrition." *Asian Journal of Applied Science and Technology*, 4(3):215-284.
- Siddiqui, S., Mateen, S., Ahmad, R., & Moin, S. (2022)." A brief insight into the etiology, genetics, and immunology of polycystic ovarian syndrome (PCOS)." *Journal of Assisted Reproduction and Genetics*, 39(11): 2439-2473.
- Sim, S., Bergh, J., Hellström, M., Hatschek, T. & Xie, H. J. P. (2018)." Pharmacogenetic impact of docetaxel on neoadjuvant treatment of breast cancer patients." *Pharmacogenomics* , 19: 1259-1268.
- Soni, A., Singla, S., & Goyal, S. (2018). "Polycystic ovary syndrome: pathogenesis, treatment and secondary associated diseases." *Journal of Drug Delivery and Therapeutics*, 8(5):107-112.
- Stachowska, A., Kicińska, A. M., Kotulak-Chrząszcz, A. & Babińska, A.(2024)." Usefulness of the Sympto-Thermal Method with Standardized Cervical Mucus Assessment (InVivo Method) for Evaluating the Monthly Cycle in Women with Polycystic Ovary Syndrome (PCOS)." *Healthcare*, 12(4):1108.

- Stener-Victorin, E., Teede, H., Norman, R. J., Legro, R., Goodarzi, M. O., Dokras, A., ... & Piltonen, T. T. (2024). "Polycystic ovary syndrome". *Nature Reviews Disease Primers*, 10(1), 27.
- Su, Y. N., Wang, M. J., Yang, J. P., Wu, X. L., Xia, M., Bao, M. H., et al. (2023). "Effects of Yulin Tong Bu formula on modulating gut microbiota and fecal metabolite interactions in mice with polycystic ovary syndrome." *Frontiers in Endocrinology*, 14:1122709.
- Suru Shah, D. S., & Madhuri Patil, M. P. (2018). "Consensus statement on the use of oral contraceptive pills in polycystic ovarian syndrome women in India." *Journal of Human Reproductive Sciences*, 11(2):226.
- Szczesnowicz, A., Szeliga, A., Niwczyk, O., Bala, G., & Meczekalski, B. (2023). "Do GLP-1 analogs have a place in the treatment of PCOS? New insights and promising therapies." *Journal of Clinical Medicine*, 12(18): 5915.
- Tabassum, R., Imtiaz, F., Sharafat, S., & Nusrat, U. (2013). "Prevalence and clinical profile of insulin resistance in young women of polycystic ovary syndrome: A study from Pakistan." *Pakistan Journal of Medical Sciences*, 29(2), 593.
- Tao, X.r., Xia, X.y., Zhang, J., Tong, L.y., Zhang, W., Zhou, X., et al. (2015). "CYP3A4* 18B and CYP3A5* 3 polymorphisms contribute to pharmacokinetic variability of cyclosporine among healthy Chinese subjects." *European Journal of Pharmaceutical Sciences*, 76: 238-244.
- Thabet, M., Abdelhafez, M. S., Elshamy, M. R., Albahlol, I. A., Fayala, E., Wageeh, A., et al. (2024). "Competence of Combined Low Dose of Human Chorionic Gonadotropin (HCG) and Clomiphene Citrate (CC) Versus Continued CC during Ovulation Induction in Women with CC-Resistant Polycystic Ovarian Syndrome: A Randomized Controlled Trial." *Journal of Medicine*, 60: 1300.
- Thomas, R. E. (2020). "Optimising Seniors' Metabolism of Medications and Avoiding Adverse Drug Events Using Data on How Metabolism by Their P450 Enzymes Varies with Ancestry and Drug–Drug and Drug–Drug–Gene Interactions." *Journal of Personalized Medicine*, 10(3):84.
- Trent, M. & Gordon, C. M. (2020). "Diagnosis and management of polycystic ovary syndrome in adolescents." *Pediatrics*, 145(Supplement 2): S210-S218.
- Trevisan, C. M., de Oliveira, R., Christofolini, D. M., Barbosa, C. P., Bianco, B. (2019). "Effects of a polymorphism in the promoter region of the follicle-stimulating hormone subunit beta (FSHB) gene on female reproductive outcomes." *Genetics and Molecular Research*, 23: 39-44.

- Tsukui, Y., Kitahara, Y., Hasegawa, Y., Kobayashi, M., Osuka, S., & Iwase, A. (2022). "Anti-Müllerian hormone levels in the diagnosis of adolescent polycystic ovarian syndrome: a systematic review and meta-analysis." *Endocrine Journal*, 69(8), 897-906.
- Van Casteren, J. I. J., Schoonen, W. G. E. J., & Kloosterboer, H. J. (2000). "Development of time-resolved immunofluorometric assays for rat follicle-stimulating hormone and luteinizing hormone and application on sera of cycling rats." *Biology of Reproduction*, 62(4): 886-894.
- Van Eerden, R. A., IJzerman, N. S., van Meekeren, M., Oomen-de Hoop, E., Guchelaar, N. A., Visser, A. M., et al. (2023). "CYP3A4* 22 genotype-guided dosing of kinase inhibitors in cancer patients." *Clinical Pharmacology*, 62: 1129-1139.
- Van Hooff, M.H., Caanen, M.R., Peters, H.E., Laven, J.S. & Lambalk, C. B. (2025). "Adolescent menstrual cycle pattern, body mass index, endocrine and ovarian ultrasound characteristics of PCOS and future fertility, cardiovascular-, and metabolic health: a 25-year longitudinal follow-up study." *Human Reproduction*, 40: 138-147.
- Wachs, D.S. and Chang, R.J., (2007). "Treatment of infertility in polycystic ovary syndrome: ovulation induction." In: *Polycystic Ovarian Syndrome: Pathogenesis, Evaluation and Treatment. Humana Press (Springer)*, 3:25-37.
- Wang, D., Guo, Y., Wrighton, S. A., Cooke, G. E., & Sadee, W. (2011). "Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs." *Pharmacogenomics Journal*, 11(4), 274-286.
- Wang, F., Zhang, X., Wang, Y., Chen, Y., Lu, H., Meng, X., et al. (2023). "Activation/inactivation of anticancer drugs by CYP3A4: influencing factors for personalized cancer therapy." *Drug Metabolism and Disposition*, 51(5), 543-559.
- Wang, R., Chon, H., Lee, S., Cheng, Z., Hong, S. H., Yoon, Y. H., et al. (2016). "Highly sensitive detection of hormone estradiol E2 using surface-enhanced Raman scattering based immunoassays for the clinical diagnosis of precocious puberty." *ACS Applied Materials & Interfaces*, 8(17): 10665-10672.
- Wang, Z., Jukic, A. M. Z., Baird, D. D., Wilcox, A. J., Li, H., Curry, C. L., et al. (2024). "Irregular Cycles, Ovulatory Disorders, and Cardiometabolic Conditions in a US-Based Digital Cohort." *JAMA Network Open*, 7: e249657.
- Wu, H.M., Tsai, T.C., Liu, S.M., Pai, A. H.Y. & Chen, L.H. (2024). "The Current Understanding of Molecular Mechanisms in Adenomyosis-Associated Infertility and the Treatment Strategy for Assisted

- Reproductive Technology." *International Journal of Molecular Sciences*, 25: 8937.
- Wu, S., Xue, P., Grayson, N., Bland, J. S., & Wolfe, A. (2019). "Bitter taste receptor ligand improves metabolic and reproductive functions in a murine model of PCOS." *Endocrinology*, 160(1), 143-155.
- Xing, C., Zhang, J., Zhao, H., & He, B. (2022). "Effect of sex hormone-binding globulin on polycystic ovary syndrome: mechanisms, manifestations, genetics, and treatment." *International Journal of Women's Health*, 14:91-105.
- Yang, J., & Chen, C. (2024). "Hormonal changes in PCOS." *Journal of Endocrinology*, 261(1):e230342.
- Ye, W., Xie, T., Song, Y., Zhou, L. (2021)." The role of androgen and its related signals in PCOS." *Journal of Clinical Medicine*, 25:1825-1837.
- Yildiz, B. O., Knochenhauer, E. S., & Azziz, R. (2008)." Impact of obesity on the risk for polycystic ovary syndrome. " *Journal of Clinical Endocrinology & Metabolism*, 93(1): 162-168.
- Yildiz, B. O., Woods, K. S., Stanczyk, F., Bartolucci, A., & Azziz, R. (2004). "Stability of adrenocortical steroidogenesis over time in healthy women and women with polycystic ovary syndrome." *Journal of Clinical Endocrinology & Metabolism*, 89(11), 5558-5562.
- Yu, M., Liu, M., Zhang, W., & Ming, Y. (2018). "Pharmacokinetics, pharmacodynamics and pharmacogenetics of tacrolimus in kidney transplantation." *Current Drug Metabolism*, 19(6): 513-522.
- Yuan, R., & Le, A.W. (2012)." A study on the estrogen receptor α gene polymorphism and its expression in thin endometrium of unknown etiology." *Gynecologic and Obstetric Investigation*, 74: 13-20.
- Zahraa, F., Ban, H., Hassan, M. (2021)."Association of Genetic Polymorphism of Insulin Receptor Substrate- 1 with Therapeutic Response of Metformin in Women with Polycystic Ovary Syndrome in Iraq" University of Kerbala College of Pharmacy.
- Zaki, H. A., Iftikhar, H., Shallik, N. A., Shaban, E., Al-Marri, N. D. R., Bashir, I., et al. (2023). "A Systematic Review and Meta-Analysis of Randomized Controlled Trials Comparing the Effects of Biguanides (Metformin) and Thiazolidinediones on Glucose Tolerance and Insulin Sensitivity in Patients With Type II Diabetes Mellitus." *Cureus*, 15(5):e39445.
- Zarek, J. (2024)." Evaluating Treatment of Antiphospholipid Syndrome during Pregnancy with Knowledge Synthesis Methods". University of Toronto (Canada).

- Zhang, W., Xu, S., Zhang, R., Li, Z., Li, N., Zhang, X., et al. (2023)." The TH22-mediated IL-22 deficiency associated with premature ovarian insufficiency." *American Journal of Reproductive Immunology*, 89(4), e13685.
- Zhang, X.J., Liu, S.Y., Fu, W., Sun, X. X.(2015)." The association of serum estradiol level with outcomes of clomiphene citrate/human menopausal gonadotropin ovarian stimulation for in vitro fertilization and embryo transfer." *Reproductive Biology and Endocrinology*, 13: 1-7.
- Zheng, M., Andersen, C.Y., Rasmussen, F. R., Cadenas, J., Christensen, S. T. & Mamsen, L. S. (2023)." Expression of genes and enzymes involved in ovarian steroidogenesis in relation to human follicular development." *Frontiers in Endocrinology*, 14: 1268248.
- Zhou, X.Y., Hu, X.X., Wang, C.C., Lu, X.R., Chen, Z., Liu, Q., et al. (2019)." Enzymatic activities of CYP3A4 allelic variants on quinine 3-hydroxylation in vitro." *Frontiers in Pharmacology*, 10: 591.
- Zhou, Y. & Lauschke, V. M. (2022)." The genetic landscape of major drug metabolizing cytochrome P450 genes—an updated analysis of population-scale sequencing data." *The Pharmacogenomics Journal*, 22, 284-293.

Appendices

Questioner

1. Name -----
 2. Age -----
 3. Wight -----
 4. Height -----
 5. BMI -----
 6. Academic achievement -----
 7. PCOS family history -----
 8. Diagnosis date of PCOS -----
 9. Presence of clinical PCOS symptoms :
 - Acne
 - Hirsutism
 - Alopecia
 - Menstrual disturbance
 - Others
-

Biochemical test (day 3)

1. Follicle-stimulating hormone (FSH)
2. Luteinizing hormone (LH)
3. estradiol (E2)
4. Sonographic

.....

Biochemical test (day 12)

1. Follicle-stimulating hormone (FSH)
2. Luteinizing hormone (LH)
3. estradiol (E2)
4. Sonographic



إلى / جامعة كربلاء / كلية الصيدلة
الموضوع / تسهيل مهمة
قسم التدريب
والتنمية البشرية

تحية طيبة....

كتابكم المرقم د.ع / ١٦٦٣ / ٦ في ٢٠٢٣ / ٩ / ١٨
نود إعلامكم بأنه لا مانع لدينا من تسهيل مهمة طالبة الماجستير / الادوية والسموم
(نادية صاحب عبد علي) لإتجاز بحثها الموسوم:

"Impact of genetic polymorphism of cytochrome P450 3A4 on
clomiphene citrate clinical response in Iraqi woman with PCOs "

في مؤسستنا الصحية / مستشفى النسائية والتوليد التعليمي / وبإشراف الدكتور (موسى
محسن العلق) على ان لا تتحمل دانرتنا اي نفقات مادية مع الاحترام .

الدكتور
نعيم عبيد الله كاشف
تقوى بن خضير
مدير مركز التدريب والتنمية البشرية
٢٠٢٣ / ٩ / ٢٥

نسخة منه الى

- مستشفى النسائية والتوليد التعليمي / لإجراء اللازم مع الاحترام .
- مركز التدريب والتنمية البشرية / شعبة ادارة البحوث والمعرفة مع الاوليات .

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



Form number 146

Decision number: 2023174

Date 25-9-2023

Research committee decision

The Research Committee of Karbala Health Directorate has examined the research protocol number (2023174/Karbala) entitled:

((Impact of genetic polymorphism of cytochrome P450 3A4 on clomiphene citrate clinical response in Iraqi woman with PCOs))

Submitted by researchers: **Nadia Sahib Abd Ali** to the research and Knowledge Management Unit at the Training and Human Development Center of Karbala Health Directorate on 25/09/2023

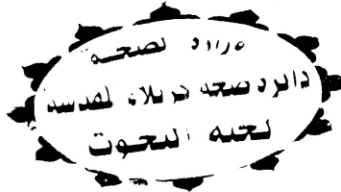
The unit has decided to:

*** 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.**

أندكتورة
تقوى خضر عبد الكريم
اختصاص

Rapporteur of the committee

28/09/2023



Attachment: None

Notes:

- The committee member (Dr. Taqwa Khudhur Abdulkareem)/ committee rapporteur (Dr. Naeem Obaid. Talal) were authorized to sign this decision on behalf of the remaining members of the committee under the rules of procedures 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 researchers 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.

الخلاصة

خلفية الرسالة:

متلازمة تكيس المبايض اضطراب هرموني شائع يصيب النساء في سن الإنجاب. غالباً ما يوصف سترات الكلوميدين لتحفيز الاباضه لدى هؤلاء المريضات. تلعب انزيمات السيتوكروم p450 وخصوصاً CYP3A4 دوراً مهماً في استقلاب العديد من الادويه. يمكن ان تؤدي التباينات الجينية في هذا الأنزيم الى اختلافات في عملية الاستقلاب الدوائي. من بين أكثر الطفرات الشائعة هي CYP3A4*18(rs28371759) و CYP3A4*22(rs35599367) وتعد هذه الطفرات ذات أهمية خاصة في استقلاب كلوميدين سترات.

هدف الدراسة

هدفت الدراسة الى الكشف عن تعدد الأشكال الجينية CYP3A4*22 G>A و CYP3A4*18 C>T لدى النساء العراقيات المصابات بمتلازمة تكيس المبايض, ودراسة تأثير هذه المتغيرات الجينية على فعالية علاج كلوميدين سترات.

المرضى والطرق

أجريت هذه الدراسة الجماعية في مستشفى النسائية والتوليد التعليمي في محافظة كربلاء، العراق، بين ديسمبر ٢٠٢٣ إلى يونيو ٢٠٢٤. شملت الدراسة ٨٠ امرأة عراقية تم تشخيصهن بمتلازمة تكيس المبايض، تتراوح أعمارهن بين ٢٠ و ٤٠ سنة، ويتناولن كلوميدين سترات (٥٠ ملغ عن طريق الفم مرتين يومياً) لدورتين علاجيتين على الأقل. جمعت عينات الدم لتحديد النمط الجيني وقياس مستويات الهرمونات التناسلية بما في ذلك الأسترايول, AMH, LH, FSH, والبرولاكتين تم ايضا تحليل تركيزات الزوكلوميدين ومستقبلاته في البلازما.

تم استخدام تقنية T-ARMS-PCR للكشف عن طفرة CYP3A4*22 G>A (rs35599367)، بينما استخدمت تقنية RFLP-PCR للكشف عن طفرة CYP3A4*18 (T>C).

النتائج

كشفت النتائج عن وجود تباينات في التوزيع الجيني لطفرات G>A CYP3A4*22 و CYP3A4*18 بالنسبة لطفرة G>A rs35599367 , كان النمط الجيني GG (النوع البري المتجانس) هو الأكثر شيوعا (٦٧,٥%), يليه النمط GA (متغاير الزيجوت) بنسبة ٢٥% ثم النمط AA (المتحور المتجانس) بنسبة ٧,٥%. بالنسبة لطفرة rs28371759 C>T فكان النمط TT (النوع البري المتجانس) هو الأكثر تكرارا بنسبة ٨٣,٨%, في حين كانت الأنماط CC,CT أقل شيوعا بنسبة ١١,٣% و ٥,٠% على التوالي. لوحظت فروق ذات دلالة إحصائية بين الأنماط الجينية CYP3A4*18 في مستويات FSH (القيمة الأحصائية = ٠,٠٣), والأستروجين (القيمة الأحصائية = ٠,٠٠٨) ومستويات الزوكلوميفين (القيمة الأحصائية = ٠,٠٤٤) ونسبة المستقلب / الدواء الاولي (القيمة الأحصائية = ٠,٠٠٢). وبالمثل وجدت فروقات ذات دلالة إحصائية بين الأنماط الجينية CYP3A4*22 في هرمون LH (القيمة الأحصائية = ٠,٠٢٦) وسمك بطانة الرحم (القيمة الأحصائية = ٠,٠٤٨), ونسبة المستقلب / الدواء الاولي (القيمة الأحصائية = ٠,٠٠٠٠٠٣) (

الاستنتاج

خلصت الدراسة الى وجود علاقة بين التباينات الجينية في جيني CYP3A4*18 و CYP3A4*22, وتباين الاستجابة لعلاج الكلوميفين سترات لدى النساء العراقيات المصابات بمتلازمة تكيس المبايض. ويعزى هذا التباين الى تغيرات في مستويات هرمونات معينة ونتائج الموجات فوق الصوتية بين الأنماط الجينية المختلفة. كما أثرت الأنماط الجينية (rs35599367) CYP3A4*22 و (rs28371759) CYP3A4*18 أيضا على النسبة الأيضية للكلوميفين.



جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الصيدلة



تأثير تعدد الأشكال الجينية لسيتوكروم P4503A4 على الاستجابة السريرية
للكلوميدين سترات في عينة من النساء العراقيات المصابات بمتلازمة تكيس المبايض
رسالة مقدمة الى

كلية الصيدلة / جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في
علم الادوية و السموم
من قبل

نادية صاحب عبد علي الشريفي

بكالوريوس صيدلة (كلية بغداد للعلوم الطبية, ٢٠٠٨)

بإشراف

الأستاذ المساعد

د. حميدة هادي عبد الواحد

بورد نسائية وتوليد

١٤٤٧ هجري

الأستاذ الدكتور

د. أثير ماجد رشيد الجحيشي

دكتوراه أدوية

٢٠٢٥ ميلادي