"Impact of Some Genetic and Biochemical Factors on Insulin Resistance in Polycystic Ovary Syndrome"

A thesis submittedfor the partial fulfillment of Doctor of Philosophy Degree in Pharmaceutical Sciences (Biochemistry)

by:

Rania Shafik Salah El-Din Abd-El-Razek

Assistant Lecturer of Biochemistry,Faculty of Pharmacy, Ain Shams University

Master Degree in Pharmaceutical Sciences (Biochemistry), Ain Shams University, 2008

Under supervision of:

Dr. Hala Osman El-Mesallamy

Professor of Biochemistry
Vice Dean for Graduate Studies and Research
Faculty of Pharmacy
Ain Shams University

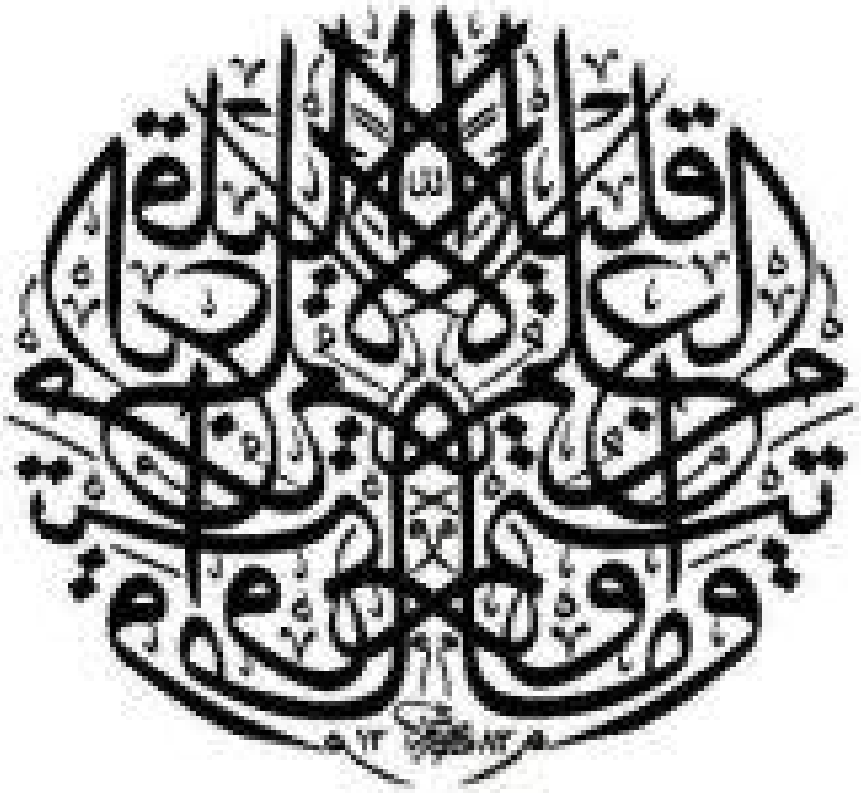
Dr. Tamer Ahmed El-Refaie

Lecturer of Obstetrics and Gynecology
Obstetrics and Gynecology Department
Faculty of Medicine
Ain Shams University

Biochemistry Department
Faculty of Pharmacy
Ain Shams University

(2013)

بسم الله الرحمن الرحيم



"وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا"

صدق الله العظيم

سورة الإسراء الآية رقم "٨٥"

LIST OF ABBREVIATIONS

ABBREVIATION	MEANING
ABI	Applied Biosystems International
ACE	Angiotensin converting enzyme
ACTH	Adrenocorticotropic hormone
Ang	Angiotensin
ASRM	American Society of Reproductive Medicine
AT_1R	Angiotensin II type 1 receptor
AT_2R	Angiotensin II type 2 receptor
BMI	Body mass index
BP	Blood pressure
CD40	Cluster of differentiation 40
CD40L	CD40 ligand
CI	Confidence interval
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
ESHRE	European Society of Human Reproduction and Embryology
FBG	Fasting blood glucose
FFAs	Free fatty acids
FI	Fasting insulin

FSH	Follicle stimulating hormone
g	Gravity
GIR	Glucose to insulin ratio
GnRH	Gonadotropin releasing hormone
HC	Hip circumference
HDL-C	High density lipoprotein-cholesterol
HOMA-IR	Homeostasis model assessment of insulin resistance
hs-CRP	High sensitivity C-reactive protein
HWE	Hardy-Weinberg Equilibrium
IGFBP-1	Insulin like growth factor binding protein-1
IGT	Impaired glucose tolerance
ILs	Interleukins
IQR	Inquartile range
IR	Insulin resistance
IRS	Insulin receptor substrate
LDL-C	Low density lipoprotein-cholesterol
LH	Luteinizing hormone
MGB	Minor groove binder
NAPD	Nicotinamide adenine dinucleotide phosphate
NCEP	National Cholesterol Education Program
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PCOS	Polycystic ovary syndrome
PCR	Polymerase chain reaction
PI-3K	Phosphatidylinositide-3 kinase

QUICKI	Quantitative insulin sensitivity check index
r	Pearson´s correlation coefficient
r_s	Spearman´s correlation coefficient
RAS	Renin angiotensin system
ROS	Reactive oxygen species
rs number	Reference single nucleotide polymorphism number
SBP	Systolic blood pressure
sCD40L	Soluble CD40 ligand
S.D.	Standard deviation
SHBG	Sex hormone binding globulin
SNP	Single nucleotide polymorphism
TAG	Triacylglycerol
TC	Total cholesterol
T2DM	Type 2 diabetes mellitus
TNF-α	Tumor necrosis factor-alpha
TT	Total testosterone
TVU	Transvaginal ultrasonography
UTR	Untranslated region
WC	Waist circumference
WHO	World health organization
WHR	Waist to hip ratio

LIST OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

- First and foremost, I thank ALLAH, Who, without HIS help,this work would never be accomplished and may this work add to our good deeds to gain HIS kind mercifulness and forgiveness.

- No words can repay or express my thanks and gratitude toDR. HALA OSMAN EL-MESALLAMY, Professor of Biochemistry, Vice Dean for Graduate Studies and Research, Faculty of Pharmacy, Ain Shams University, for her generous supervision, keen interest and precious time she offered methroughout this study. I really consider myself fortunate that I worked under her generous supervision.

- I would like also to express my gratefulness and appreciation forDR.TAMER AHMED EL-REFAIE, Lecturer of Obstetrics and Gynecology, Faculty of Medicine, Ain Shams University, for his invaluable efforts, continuous support and kind guidance throughout this work. I hope him every success in his life and work.

- I am greatly thankful to all members of Ain Shams University Maternity Hospital, for their friendly cooperation. My thanksalso extendto all staff members of Biochemistry Department, Faculty of Pharmacy, Ain Shams University, who supported and helped me to complete this work.

- Never to be forgotten or repaid back; the priceless help, support and understanding of my FAMILY during the tedious period of this work.

Rania Shafik Salah El-Din

1. Introduction and Aim of The Work

Polycystic ovary syndrome (PCOS) is the most frequent feminine endocrinopathy affecting up to 10 % of reproductively aged women ***(Fauser et al., 2012)***. The syndrome is recognized as a heterogeneous constellation of clinical and/or biochemical features including; androgen excess, ovulatory dysfunction and polycystic ovaries ***(Goodarzi et al., 2011)***. Despite speculations regarding the underlying pathogenetic mechanism, PCOS is currently accepted to be multifactorial in origin, where environmental factors are acting in a genetic background, resulting in a broad spectrum of reproductive and metabolic defects as well ***(Allahbadiaa and Merchantb, 2011)***.

Two-thirds of women with PCOS experience metabolic anomalies, chiefly, insulin resistance (IR) and compensatory hyperinsulinemia ***(DeUgarte et al., 2005)***. These anomalies, in turn, not only aggravate hyperandrogenism and chronic anovulation encountered in PCOS, but also put those affected women at substantial risk of developing glucose intolerance ***(Salley et al., 2007)***, type 2 diabetes mellitus (T2DM) ***(Galazis et al., 2011)*** and eventually cardiovascular disease (CVD) in later years ***(Schmidt et al., 2011)***.

Insulin resistance is increasingly recognized as a chronic, low-grade, inflammatory state and several mechanisms had been proposed to explain their inter-relation. These include enhanced expression of high sensitivity C-reactive protein (hs-CRP) that may occur by counteracting the physiologic effect of insulin on hepatic acute phase protein synthesis as a result of decreased insulin sensitivity ***(Haffner, 2003; González, 2012)***.

Furthermore, the cluster of differentiation 40 (CD40)/CD40 ligand (CD40L) signaling pathway, consisting of a membrane receptor CD40 and

its ligand, have been implicated in the crosstalk between leukocytes and adipocytes, providing another link between inflammation and IR ***(Poggi et al., 2009)***. The CD40L is cleaved and circulates as soluble CD40L (sCD40L), an inflammatory marker whose levels as well as those of hs-CRP have been reported to be elevated among PCOS women, a finding suggestive of the association of these inflammatory factors with the pathogenesis of the syndrome ***(Oktem et al., 2009; Escobar-Morreale et al., 2011)***.

In addition to a well-documented role in regulating blood pressure (BP) and cardiovascular physiology ***(Yang et al., 2011)***, there is ample evidence implicating the renin-angiotensin system (RAS) in ovulation, steroidogenesis, as well as in the formation of corpus luteum through complex interactions with other systems ***(Gonçalves et al., 2012)***. Interestingly, this system has also been reported to play a central role in the regulation of insulin signaling in the vasculature and thus in the modulation of insulin sensitivity ***(Kalupahana and Moustaid-Moussa, 2012)***. Due to these roles, the RAS could be considered a relevant target for research in reproductive endocrinology, including in PCOS research.

Angiotensin II (Ang II), the effector substance of the RAS, interacts with 2 distinct subtypes of receptors. Among these 2 subtypes, the predominant molecular effects of Ang II are mediated via the Ang II type 1 receptor (AT_1R) subtype ***(Fyhrquist and Saijionmaa, 2008)***. A single nucleotide polymorphism (SNP) in the 3′ untranslated region (UTR) of the *AT_1R* gene, which consists of an A to C nucleotide transversion at position 1166 *($AT_1R/A1166C$)*, has been the most extensively studied polymorphism of this gene ***(Abboud et al., 2010; Zhang et al., 2011)***.

Although sited in the UTR, it has been suggested that this polymorphism might alter IR by enhancing the responsiveness to Ang II