# ESTIMATION OF SERUM PROLACTIN LEVELS AND EXPRESSION OF ITS RECEPTORS IN ALOPECIA AREATA

THESIS SUBMITTED FOR THE PARTIAL FULFILLMENT OF THE MASTER DEGREE IN DERMATOLOGY, ANDROLOGY& STDs

BY

#### RIMA KASSEM KAHHAL

(M.B., B.Ch)

SUPERVISED BY

#### SAMAR MOHAMED RAGAIE EL-TAHLAWI, MD

PROFESSOR OF DERMATOLOGY

FACULTY OF MEDICINE, CAIRO UNIVERSITY

#### NERMINE HAMDI EL EISHI, MD

PROFESSOR OF DERMATOLOGY

FACULTY OF MEDICINE, CAIRO UNIVERSITY

#### **OLFAT GAMIL SHAKER, MD**

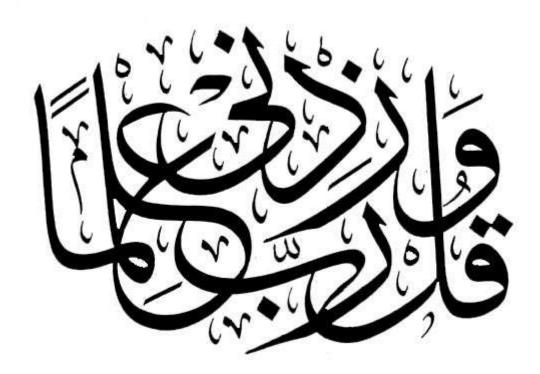
PROFESSOR OF MEDICAL BIOCHEMISTRY

AND MOLECULAR BIOLOGY

FACULTY OF MEDICINE, CAIRO UNIVERSITY

2015

# 



صَّال وَاللهُ العَظَمِينَ،

#### **ACKNOWLEDGEMENT**

First and foremost, I feel always deeply indebted to *ALLAH*, the Most Gracious and the Most Merciful.

I would like to express my deepest gratitude and cardinal appreciation to *Prof. Dr. Samar Mohamed Ragaie El-Tahlawi*, Professor of Dermatology, Faculty of Medicine, Cairo University, who kindly supervised and motivated the performance of this work, for her kind guidance and constant encouragement throughout this work.

I am greatly honoured to express my deep thanks and gratitude to *Prof. Dr. Nermine Hamdi El-Eishi*, Professor of Dermatology, Faculty of Medicine, Cairo University, for her continuous support and guidance, valuable suggestions, expert advice and generous help which have greatly helped me to complete this work.

I am greatly honoured to express my deep thankfulness to *Prof. Dr. Olfat Gamil Shaker*, Professor of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, for devoting part of her precious time to help me in the preparation of this work.

Also my profound gratitude to *Dr. Rehab Hegazi*, Assistant Professor of Dermatology, Faculty of Medicine, Cairo University, for her kind supervision, great support and guidance; it was great honour to work under her supervision.

I am very grateful to *Dr. Ghada Mohamed El-Hanafi*, Assistant Professor of Dermatology, Faculty of Medicine, Cairo University, for her precious time to help me finish this work.

I also want to thank all my *Family Members* (my mother, my brother, my sister, and my two kids, Yousef and Lamis), for their patience and support, and a special and sincere thanks to *My Dearest Husband* (Mohamed), who encourages and supports me through all my steps.

Last but not least, I would like to dedicate this work to the *Soul of My Dad*, may he rest in peace, and may he always be happy and satisfied with my work...

#### **ABSTRACT**

**Background:** Prolactin is one of the major hormonal signals that are immediately upregulated upon psychoemotional and physical stress. It was found that high prolactin levels are associated with many autoimmune diseases. The relation between prolactin levels and prolactin receptor levels and alopecia areata (a tissue-specific autoimmune disease of hair follicles) represents an attractive area of research.

**Purpose:** To measure serum levels of prolactin and to detect the expression of its receptors in alopecia areata patients in an attempt to verify their possible roles in the pathogenesis of this disease.

**Patients and methods:** This study recruited 30 patients with alopecia areata and 20 age-and sex-matched healthy control subjects. Levels of serum prolactin and prolactin receptors were estimated by PCR technique. The correlations between serum prolactin levels as well as the prolactin receptor levels and age, sex, stress exposure, duration of disease and severity of alopecia areata were all assessed.

**Results:** When comparing between prolactin receptor level in AA patients and controls, it was found that it was significantly higher in patients (mean $\pm$ SD = 49.90  $\pm$  18.29) than in controls (mean $\pm$ SD = 37.07  $\pm$  19.30) (P=0.02); but no significant difference in the serum level of prolactin was detected in patients (mean $\pm$ SD = 9.44  $\pm$  4.90) in comparison with controls (mean $\pm$ SD = 8.67  $\pm$  4.07) (P=0.56). In addition, correlating prolactin receptors in the patients to their SALT score showed a statistically significant positive correlation i.e. the greater the SALT score is, the higher the level of the prolactin receptors expressed in the lesion (P=0.05). On the

other hand, no statistically significant correlations were found between the

prolactin receptors and the age of the patient, or the disease duration. Also, no

statistically significant correlations were found between the serum prolactin levels

and the age of the patient or the SALT score. Moreover, both serum prolactin

levels and prolactin receptors were not found to be significantly affected by

patients' sex, family history, or presence of associated disorders.

**Conclusion:** Higher levels of prolactin receptors are associated with AA. Moreover,

the prolactin receptor expression is positively related to the disease severity through

a significant positive correlation between prolactin receptors and the SALT score in

patients with AA.

**Key words:** Alopecia areata, autoimmune, serum prolactin, prolactin receptors

### **CONTENTS**

Title	Page
-------	------

<b>♦</b>	Table of abbreviations	1
<b>♦</b>	Table of figures	4
<b>♦</b>	List of tables	6
<b>♦</b>	Introduction and Aim of the work	7
<b>♦</b>	Review of Literature:	
<b>♦</b>	Chapter 1: Alopecia Areata	11
	Definition	11
	Epidemiology	11
	Genetics	12
	Pathogenesis	14
	Clinical Picture	26
	Histopathology	29
	Management	33
<b>♦</b>	Chapter 2: Prolactin	40
	Prolactin Chemistry and Molecular Biology	40
	Circadian Rhythm of Prolactin Secretion	42
	Extrapituitary prolactin	42
	Prolactin expression and its regulation	43
	Prolactin receptors	48
	Human Prolactin Receptor Antagonists	50
	Biological Actions of Prolactin	51

Chapter 3: Prolactin and Autoimmune diseases	56
Prolactin and Systemic Lupus Erythematosis	57
Prolactin and Psoriasis Vulgaris	59
Prolactin and Systemic Sclerosis	59
Prolactin and Sjogren's Syndrome	60
Prolactin and Behçet's Disease	61
Prolactin and Alopecia Areata	61
Patients and Methods	67
Results	75
Discussion	102
Conclusion and Recommendations	107
Summary	108
References	110
Arabic Summary	146
	Prolactin and Systemic Lupus Erythematosis  Prolactin and Psoriasis Vulgaris  Prolactin and Systemic Sclerosis  Prolactin and Sjogren's Syndrome  Prolactin and Behçet's Disease  Prolactin and Alopecia Areata  Patients and Methods  Results  Discussion  Conclusion and Recommendations  Summary  References

## TABLE OF ABBREVIATIONS

Abbreviation	Meaning
AA	alopecia areata
AT	alopecia totalis
AU	alopecia universalis
APS-1	autoimmune polyglandular syndrome -1
APECED	autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy
HLA	human leukocyte antigen
MHC	major histocompatibility complex
CD8	cluster of differentiation 8
CD4	cluster of differentiation 4
MICA	major histocompatibility complex class I chain-related protein
NK cells	natural killer cells
AIRE	autoimmune regulator
IL	interleukin
MIF	macrophage migration inhibitory factor
HF	hair follicle
IP	immune privilege
ORS	outer root sheath
TGF-β	transforming growth factor beta
ACTH	adrenocorticotropic hormone
α MSH	alpha melanocyte-stimulating hormone
T <sub>h</sub> 17	T helper 17
IFN-γ	interferon- gamma
TGF-β	tumor growth factor beta
T-reg	T-regulatory
AR	activating receptor
IR KIR	inhibitory receptor, killer immunoglobulin receptor
MIG	monokine induced by Interferon- gamma
mRNA	messanger ribonucleic acid
IP- 10	interferon inducible protein-10
Th1	T-helper 1
IL-1α	interleukin-1 alpha
IL-1 β	interleukin-1 beta
TNF-α	tumor necrosis factor alpha
BAFF	B cell-activating factor of the TNF family
HPA	hypothalamic-pituitary-adrenal

NGF	nerve growth factor
CGRP	calcitonin gene-related peptide
TR	thioredoxin reductase
GCR	glucocorticoid receptor
PUVA	psoralen plus ultraviolet light A
CsA	cyclosporine A
PRL	prolactin
kDa	kilo Dalton
Cys	cysteine
GH	growth hormone
hPRL	human prolactin
REM	rapid eye movement
PIT-1	pituitary-specific transcription factor
TIDA	tuberoinfundibular dopaminergic system
Ach	acetylcholine
ANG	angiotensin
ANP	atrial natriuretic peptide
BOM	bombesin-like peptide
CCK	cholecystokinin
CT	calcitonin
DA	dopamine
EGF	epidermal growth factor
Est	estrogen
FGF	fibroblast growth factor
GABA	Gamma aminobutyric acid
GAL	galanin
Н	histamine
5-HT	serotonin
IGF	insulin-like growth factor
L	lactotrophs
NO	nitric oxide
NorA	norepinephrine
NPY	neuropeptide Y
NT	neurotensin
PACAP	pituitary adenylate cyclase-activating peptide
PC	pituitary cell
SST	somatostatin
TGF-b	transforming growth factor-b
TRH	thyrotropin-releasing factor
TSH	thyroid-stimulating hormone

[	
VIP	vasoactive intestinal peptide
MPHD	multiple pituitary hormone deficiency
MRI	magnetic resonance imaging
IgG	immunoglobulin G
PRLR	prolactin receptors
Ab	antibody
VEGF	vascular endothelial growth factor
DC	dendritic cells
GM-CSF	granulocyte macrophage colony stimulating factor
HO-1	hemeoxygenase-1
HPRL	hyperprolactinemia
SLE	systemic lupus erythematosis
SSc	systemic sclerosis
SS	sjogren's syndrome
SLEDAI	systemic lupus erythematosus disease activity index
PRL-IgG	prolactin- immunoglobulin G
dsDNA	double stranded deoxyribo nucleic acid
PAPS	primary anti-phospholipid syndrome
LARC	liver activation regulated chemokine
BD	Behçet's disease
SP	substance P
Derma REC	Dermatology Research Ethical Committee
OPC	outpatient clinic
SALT	severity of alopecia tool
PCR	polymerase chain reaction
SPSS	statistical Package for the Social Science
SD	standard deviation
ROC	receiver operator characteristic
AUC	area under the curve
у	years
N	number
M	male
F	female
r	Pearson correlation
Fig	figure
RANTES	regulated on activation normal T expressed and secreted

# **TABLE OF FIGURES**

Figures	
Figure 1: Normal hair cycle	
Figure 2: Hair cycle in AA	16
Figure 3: Activating and inhibitory receptors for hair growth in normal	20
individuals Vs AA patients	20
Figure 4: Proposed pathogenesis of AA	26
Figure 5: Patchy alopecia areata in the right frontotemporal area with	28
eyebrow involvement	20
Figure 6: Alopecia totalis with a 100% loss of scalp hair	28
Figure 7: Ophiasis pattern of alopecia areata	28
Figure 8: Close view showing multiple exclamation mark hairs and	20
regrowing white hairs	28
Figure 9: Classic peribulbar "swarm of bees" inflammation in AA,	31
some eosinophils are present within the infiltrate	31
Figure 10: Abnormal hair shaft formation (trichomalacia)	32
Figure 11: Catagen transformation in subacute AA	32
Figure 12: Marked miniaturization of HF: a feature of long-standing AA	
Figure 13: Nanogen follicle is very typical of AA	33
Figure 14: Treatment protocol for AA	
Figure 15: Structure of prolactin	34 41
Figure 16: Pituitary PRL regulation	
Figure 17: Simplified overview of the main PRLR signaling pathways	44 49
Figure 18: Visual aid (Olsen/Canfield) for estimating percentage scalp	77
hair loss, "x" score	
Figure 19: Comparison between serum prolactin level of patients and	0.2
controls included in the study (measured by ng/ml).	82
Figure 20: Comparison between prolactin receptor level of patients and	83
controls included in the study (measured by Pg/gm).	
Figure 21: ROC curve for the level of prolactin receptors	
Figure 22: Correlation between serum prolactin level and prolactin	0.6
receptors in the patients.	86
Figure 23: Correlation between serum prolactin level and prolactin	07
receptors in the controls.	87
Figure 24: Correlation of serum prolactin level with age of the patients	90

Figure 25: Correlation of prolactin receptors with the age of the patients	91
<b>Figure 26:</b> Correlation of serum prolactin level with duration of the disease.	92
<b>Figure 27:</b> Correlation of the prolactin receptors with the duration of the disease	
<b>Figure 28:</b> Correlation of the serum prolactin level with SALT score	94
<b>Figure 29:</b> Correlation of the prolactin receptors with SALT score.	95
<b>Figure 30:</b> Relation of serum prolactin levels with sex in the patients	97
<b>Figure 31:</b> Relation of serum prolactin levels with sex in the controls	97
<b>Figure 32:</b> Relation of prolactin receptors with sex in the patients group	
<b>Figure 33:</b> Relation of prolactin receptors with sex in the controls group	
<b>Figure 34:</b> Relation of serum prolactin levels with family history in the patients group	
<b>Figure 35:</b> Relation of prolactin receptors with family history in the patients group	
<b>Figure 36:</b> Relation of serum prolactin levels with presence of associated disorders in the patients group	
<b>Figure 37:</b> Relation of prolactin receptors with presence of associated disorders in the patients group	

# LIST OF TABLES

Tables	Page
Table 1: Classical and non-classical functions of PRL	52
Table 2: PRL as an immunomodulatory cytokine	54
<b>Table 3:</b> Real-time PCR primer sequences and PCR product size for GAPDH, PRLR	71
Table 4: Raw data of the patients	76
Table 5: Summary of the clinical and demographic data of the patients	78
Table 6: Raw data of the controls	79
Table 7: Comparison between the studied groups as regard sex and age	80
<b>Table 8:</b> Summary of the serum prolactin levels and prolactin receptors in the patients and control groups	81
<b>Table 9:</b> Comparison between serum prolactin level (ng/ml) in the patients and the controls	82
<b>Table 10:</b> Comparison between prolactin receptors in the patients and the controls	83
<b>Table 11:</b> Correlation between serum prolactin level and prolactin receptors in the patients and controls	85
<b>Table 12:</b> Correlation of serum prolactin level with age, duration of lesion and SALT score	88
<b>Table 13:</b> Correlation of prolactin receptors with the age, duration of the disease and SALT Score	89
<b>Table 14:</b> Relation of serum prolactin levels and prolactin receptors with sex in the patients and control groups	96
<b>Table 15:</b> Relation of serum prolactin levels and prolactin receptors with presence of family history	99
<b>Table 16:</b> Relation of serum prolactin levels and prolactin receptors with presence of associated disorders	100



# Introduction and Aim of Work