

Role of Estrogen and Matrix Metalloproteinase–1 in Skin Aging and Photoaging

Thesis

**Submitted for partial fulfillment of
Master degree in Dermatology, Venereology
and Anderology**

By
Gehan Fetouh El Mowafy
(M.B, B.CH.)

Under Supervision of

Professor Dr. Hanan Mohamed El Kahky
Professor of Dermatology, Venerology and Andrology
Faculty of Medicine, Ain Shams University

Professor Dr. Sahar Saad El Din Zaki
Professor of Pathology
Faculty of Medicine, Ain Shams University

Dr. Enas Attia Saad El Din Attia
Lecturer of Dermatology, Venerology and Andrology
Faculty of Medicine, Ain Shams University

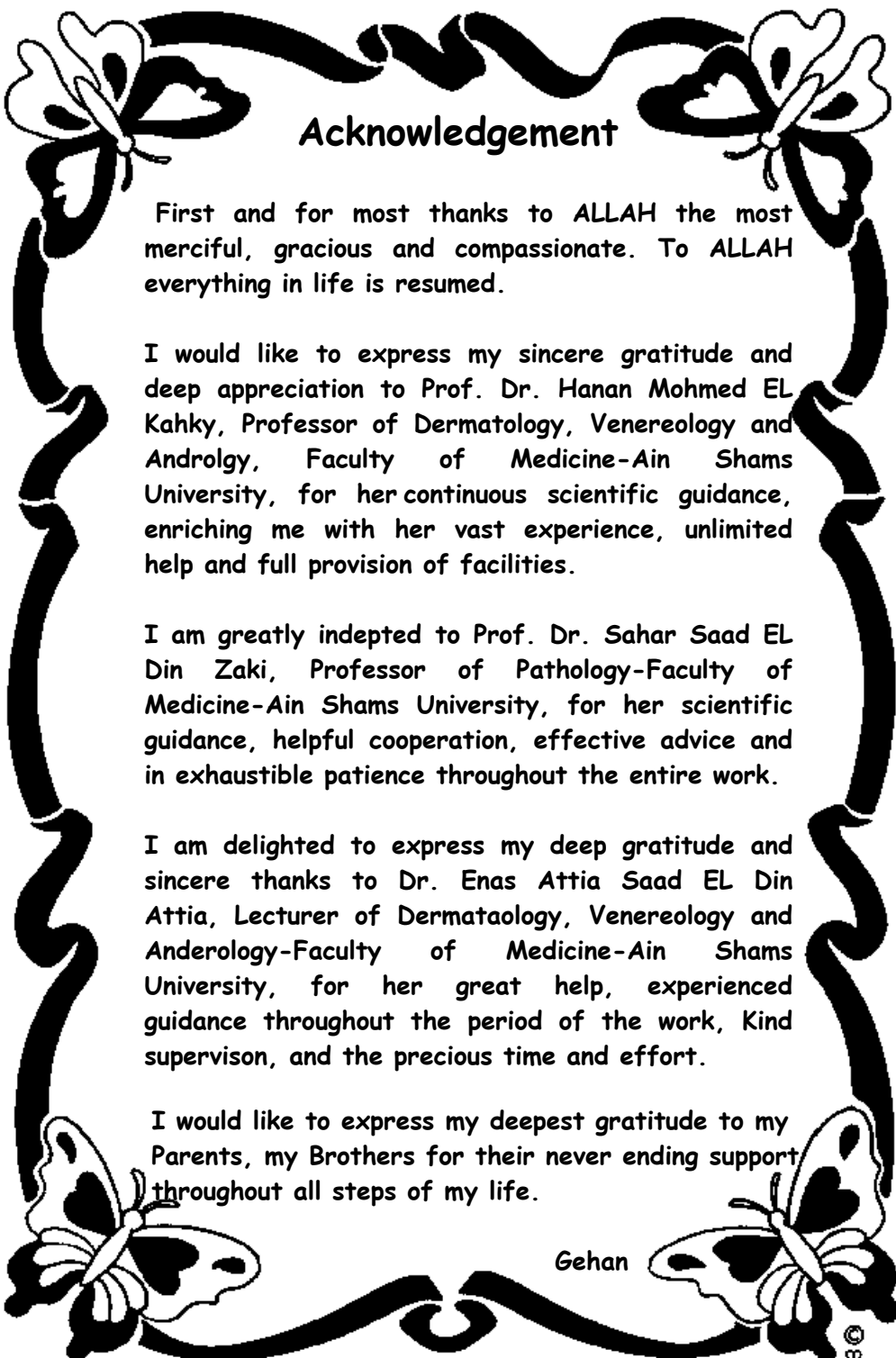
**Faculty of Medicine
Ain Shams University**

2010

بسم الله الرحمن الرحيم



صدق الله العظيم
سورة طه الآية رقم 14



Acknowledgement

First and for most thanks to ALLAH the most merciful, gracious and compassionate. To ALLAH everything in life is resumed.

I would like to express my sincere gratitude and deep appreciation to Prof. Dr. Hanan Mohamed EL Kahky, Professor of Dermatology, Venereology and Andrology, Faculty of Medicine-Ain Shams University, for her continuous scientific guidance, enriching me with her vast experience, unlimited help and full provision of facilities.

I am greatly indebted to Prof. Dr. Sahar Saad EL Din Zaki, Professor of Pathology-Faculty of Medicine-Ain Shams University, for her scientific guidance, helpful cooperation, effective advice and in exhaustible patience throughout the entire work.

I am delighted to express my deep gratitude and sincere thanks to Dr. Enas Attia Saad EL Din Attia, Lecturer of Dermatology, Venereology and Andrology-Faculty of Medicine-Ain Shams University, for her great help, experienced guidance throughout the period of the work, Kind supervision, and the precious time and effort.

I would like to express my deepest gratitude to my Parents, my Brothers for their never ending support throughout all steps of my life.

Gehan



Contents

	Page
List of abbreviations	I
List of figures	II
List of tables	V
Introduction and Aim of the work	1
Review of literature:	
Chapter 1: Skin aging and photoaging	5
I. Intrinsic aging	5
▪ Factors affecting intrinsic aging	5
▪ Pathomachanisms of endogenous skin aging ...	6
▪ Clinical picture of intrinsically aged skin	9
▪ Histologic characteristics of endogenously aged skin	12
II. Extrinsic aging	15
▪ Factors affecting extrinsic skin aging	15
▪ Mechanism of skin photoaging	16
▪ Clinical picture of photoaged skin	20
▪ Histologic characteristics of exogenously aged skin	24
III. Similarities and dissimilarities between chronological aging and photoaging.....	28
IV. Age associated decline in skin functions.	31
Chapter II Role of estrogen in skin aging and photoaging	34
▪ Synthesis, transport and metabolism of estrogen	34
▪ Regulation of estrogen synthesis	39
▪ Age related estrogen levels	41
▪ Mechanism of estrogen action in the skin	42
▪ Biology of estrogens in skin	45

▪ Hormone replacement therapy and topical estrogen treatment	51
▪ Effects of phytoestrogenic SERMs on skin aging	56
▪ Effects of selective ER modulators on skin aging	57
Chapter III: Role of MMP-1 in skin aging and photoaging	59
▪ Classification of MMPs	59
▪ Domain structure of MMPs.....	60
▪ Biological and pathological roles of MMPS	61
▪ Regulation of MMPs activation and proteolytic activity	62
▪ Matrix metalloproteinase-1	64
▪ Synthesis and activation of MMP-1	64
▪ Structure of MMP-1	64
▪ Regulation of MMP-1	66
▪ Biological function (substrate specificity)	67
▪ Biological and pathological role of MMP-1 in skin aging and photoaging	68
▪ Estrogen and MMP-1 in skin aging and photoaging	72
Subjects and Methods	75
Results	79
Discussion	107
Summary and conclusion	114
References	117
Arabic summary	

List of Abbreviations

AP-1	Activator protein-1
APCs	Antigen presenting cells
ATP	Adenosine triphosphate
BCC	Basal cell carcinoma
bFGF	Basic fibroblast growth factor
Cdk	Cyclin dependent kinases
CyR61	Cysteine rich growth regulatory factor
DEJ	Dermo-epidermal junction
DHEA	Dehydroepiandrostrone
ECM	Extracellular matrix
EGF	Epidermal growth factor
ER	Estrogen receptor
FSH	Follicle stimulating hormone
GAGs	Glycosaminoglycans
GM-CSF	Granulocyte macrophage colony stimulating factor
H&E	Hematoxylin and Eosin
HRT	Hormone replacement therapy
HSD	Hydroxysteroid dehydrogenase
IGF	Insulin-like growth factor
IGFBP	Insulin-growth factor binding protein
IL	Interleukin
LCs	Langerhans cells
LDL	Low density lipoproteins
LH	Luteinizing hormone
MED	Minimal erythema dose
MIF	Macrophage migration inhibitory factor
MMPs	Matrix metalloproteinases
mt DNA	Mitochondrial DNA
MT-MMPs	Membrane type MMPs
NF- κ B	Nuclear factor κ B
PDGF	Platelet derived growth factor
ROS	Reactive oxygen species
SCC	Squamous cell carcinoma
SERMs	Selective ER modulators
TGF	Transforming growth factor
TIMPs	Tissue inhibitors of matrix metalloproteinases
TNF α	Tumour necrosis factor α
UV	Ultraviolet
VEGF	Vascular endothelial growth factor

List of Figures

Figure		Page
Fig.1	Expression wrinkles.	10
Fig.2	Gravitaional wrinkles.	11
Fig.3	Model depicting solar UV irradiation damage to skin connective tissue.	17
Fig.4	Elastotic wrinkles.	22
Fig.5	Atrophic response to phtodamage.	22
Fig.6	Solar lentigines.	23
Fig.7	Principal pathway of estrogen biosynthesis in ovary.	37
Fig.8	Ovarian synthesis, transport, and metabolism of estrogens.	39
Fig.9	Feedback control of anterior pituitary and ovary.	40
Fig.10	Diagrammatic representation of the effect of estrogen hormone levels on cutaneous wound healing.	47
Fig.11	Structural classification of human MMPs based on their domain organization.	60
Fig 12	Classification of MMPs according to their domain structure.	61
Fig.13	The three-dimensional structure of human MMP-1.	65
Fig.14	Anti-skin aging effect of topical 17- β estradiol in aged human skin in vivo.	74
Fig. 15	Thickened epidermis, hypermelanosis and solar elastosis in sun-exposed premenopausal skin.	80
Fig. 16	Hypermelanosis, fragmented collagen and solar elastosis in sun-exposed premenopausal skin.	81
Fig.17	Thickened epidermis, hypermelanosis and solar elastosis in sun-exposed premenopausal skin.	82
Fig. 18	Fragmented collagen in sun-exposed premenopausal skin.	83
Fig. 19	Thin epidermis, hypomelanosis and flat dermoepidermal junction in sun-protected postmenopausal skin.	84
Fig. 20	Strong positive nuclear staining of epidermal keratinocytes, dermal fibroblast and hair follicle in sun-exposed premenopausal skin.	85

Fig. 21	Strong positive nuclear staining of epidermal keratinocytes in sun-exposed premenopausal skin.	86
Fig. 22	Moderate positive nuclear staining of epidermal keratinocytes and dermal fibroblast in sun-protected premenopausal skin.	86
Fig. 23	Moderate positive nuclear staining of epidermal keratinocytes in sun-exposed postmenopausal skin.	87
Fig. 24	Weak positive nuclear staining of epidermal keratinocytes in sun-protected postmenopausal skin.	88
Fig. 25	Negative staining of epidermal keratinocytes in sun-protected postmenopausal skin.	88
Fig. 26	Moderate positive cytoplasmic staining of epidermal keratinocytes and dermal matrix in sun-exposed premenopausal skin.	90
Fig. 27	Weak positive cytoplasmic staining of epidermal keratinocytes and dermal matrix in sun-protected premenopausal skin.	90
Fig. 28	Negative staining of epidermal keratinocytes and dermal matrix in sun-protected premenopausal skin.	91
Fig. 29	Strong positive cytoplasmic staining of epidermal keratinocytes and dermal collagen in sun-exposed postmenopausal skin.	92
Fig. 30	ER β expression in the skin of premenopausal and postmenopausal women.	95
Fig. 31	Premenopausal woman with type I Glogau photoaging classification.	96
Fig. 32	Premenopausal woman with type II Glogau photoaging classification.	97
Fig. 33	Premenopausal woman with type II Glogau photoaging classification.	98
Fig. 34	The expression of MMP-1 in the skin of premenopausal and postmenopausal women.	101
Fig. 35	Postmenopausal woman with type III Glogau photoaging classification.	102
Fig. 36	Postmenopausal woman with type III Glogau photoaging classification.	103
Fig. 37	Positive correlation between the expression of ER β and the level of collagen in the skin of premenopausal and postmenopausal women.	104

Fig. 38	Negative correlation between the expression of MMP-1 and the level of collagen in the skin of premenopausal and postmenopausal women.	105
Fig. 39	Negative correlation between the expression of ER β and MMP-1 in sun-exposed skin of premenopausal and postmenopausal women.	105
Fig. 40	Negative correlation between the expression of ER β and MMP-1 in sun-protected skin of premenopausal and postmenopausal women.	106

List of tables

Table		Page
Table 1	Characteristics of different skin phototypes.	21
Table 2	Glogau's photoaging classification.	22
Table 3	The histologic abnormalities underlying photoaged phenotype.	25
Table 4	Classification of photoaging of premenopausal and postmenopausal groups according to Glogou's photoaging classification.	80
Table 5	H&E skin changes of group I (A)	81
Table 6	H&E findings of group II (A).	83
Table 7	H&E skin changes of group II (B).	84
Table 8	The results of ER β immunostaining in the skin of premenopausal and postmenopausal women.	89
Table 9	The results of MMP-1 immunostaining in the skin of premenopausal and postmenopausal women.	92
Table 10	Comparison between ER β expression in sun-exposed versus sun-protected skin of premenopausal and postmenopausal women.	93
Table 11	Comparison between the expression of ER β in both sun-exposed and sun-protected skin of premenopausal versus postmenopausal women.	94
Table 12	Comparison between the expression of MMP-1 in sun-exposed versus sun-protected skin of premenopausal and postmenopausal women.	99
Table 13	Comparison between the expression of MMP-1 in both sun-exposed and sun-protected skin of premenopausal versus postmenopausal women.	100

Protocol

**Role of Estrogen and Matrix
Metalloproteinase-1 in Skin Aging
and Photoaging**

Protocol for Thesis
**Submitted for partial fulfillment of
Master degree in Dermatology, Venereology and
Anderology
By**

**Gehan Fetouh El Mowafy
(M.B, B.CH.)**

Under Supervision of

**Professor Dr. Hanan Mohamed El Kahky
Professor of Dermatology, Venerology and Andrology
Faculty of Medicine, Ain Shams University**

**Professor Dr. Sahar Saad El Din Ahmed Zaki
Professor of Pathology
Faculty of Medicine, Ain Shams University**

**Dr. Enas Attia Saad El Din Attia
Lecturer of Dermatology , Venerology and Andrology
Faculty of Medicine, Ain Shams University**

**Faculty of Medicine
Ain Shams University
2008**

Role of Estrogen and Matrix Metalloproteinase–1 in Skin Aging and Photoaging

Introduction

Human skin, like all other body organs undergoes chronological aging. In addition, unlike other organs, skin is exposed to ultraviolet (UV) irradiation and, therefore, undergoes aging as a consequence of sun exposures (photoaging) (*Fisher et al., 2002*).

Clinically, naturally aged skin is smooth, pale and finely wrinkled. In contrast; photoaged skin is coarsely wrinkled and associated with pigmentation and telangiectesia (*Gilchrest, 1989*). Alteration in collagen, which is the major structural component of skin, have been suggested to be the cause of clinical changes, such as skin wrinkling and loss of elasticity, observed in naturally aged and photoaged skin (*Fisher et al., 1997*).

UV irradiation activates growth factors and cytokine receptors on the surface of keratinocytes and fibroblasts. Activated receptors stimulate signal transduction cascades that induce the transcription factor; Activator Protein1 (AP-1), which stimulates transcription of matrix metalloproteinases (MMPs) secreted from keratinocytes and fibroblasts (*Ullrich and Schlessinger, 1990*).

MMPs are a family of zinc dependant endoproteinases that has pivotal role in the dynamic remodeling of extracellular matrix (ECM). MMP-1 is the most abundant MMPs and is produced by fibroblasts. It initiates cleavage of fibrillar collagen at single site within its central triple helix. Once cleaved by MMP-1, collagen can be further degraded by elevated levels of MMP-3 and MMP-9. Thus, MMPs mediate collagen damage in photoaged skin (*Sternlicht and Werb, 2001*).

Estrogen and estrogen like compounds work to reverse the effects of both intrinsic aging and photoaging in the skin, by functioning as both antioxidants and signaling molecules (**Kang et al., 2003**). They mediate their activity by interaction and activation of specific intracellular receptor proteins; Estrogen Receptors (ERs). There are two types of estrogen receptors: ER α and ER β .

ER β were found to show strong expression in the epidermis, dermal fibroblasts, blood vessels, and hair follicles of male and female adult scalp skin, but no specific staining of ER- α was observed (**Thornton et al., 2003; Pelletier and Ren, 2004**).

Estrogens were found to increase the activity of transforming growth factor beta (TGF- β), which increases the production of collagen. On the other hand, they reduce the concentration of reactive oxygen species (ROS). ROS were found to increase signaling pathways that inhibit collagen production and also increase MMPs activity, resulting in a net result of decreased collagen content. Thus, a good part of the changes in collagen, seen in aging, were found to be associated with decreased level of estrogen (**Kang et al., 2003**).

Aim of work

The aim of the work is to study the expression of ER β and MMP-1 in premenopausal and postmenopausal women, both in sun-exposed and sun-protected skin, to find out their role in skin aging and photoaging.

Subjects and Methods.

The study will include 20 female subjects comprising two groups:

- Group I: 10 premenopausal women
- Group II: 10 postmenopausal women

Both groups will be subjected to the following:

- History with emphasis on outdoor activities and smoking.
- Examination

- Skin biopsies: 2 skin biopsies will be taken from each subject, one from sun protected skin and one from sun exposed skin. Each biopsy will be processed and stained by :
- Hematoxylin and Eosin (H&E) for routine histopathological examination.
- Immunoperoxidase technique to study the expression of both ER β and MMP1 by immunohistochemistry.

The study will include:

- _ Introduction and aim of the work.
- _ Review of literature.
- _ Subjects and Methods.
- _ Results.
- _ Discussion .
- _ Summary and Conclusions.
- _ References.
- _ Arabic Summary.

References

- Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S and Voorhees JJ: Pathophysiology of premature skin aging induced by ultraviolet light. N Engl J Med. 1997; 337:1419-1428.
- Fisher GJ, Kang S, Varani J, Wan Y, Datta SC and Voorhees JJ : Mechanisms of photoaging and chronological skin aging. Arch Dermatol. 2002; 138:1462-1470.
- Gilchrest BA: Skin aging and photoaging, an overview. J Am Acad Dermatol. 1989; 21:610-613.
- Kang S, Chung JH and Lee JH : Topical N-acetyl cysteine and genistein prevent ultraviolet light induced signaling that leads to photoaging in human skin in vivo. J Invest Dermatol. 2003; 120(5):835-41.
- Pelletier G and Ren L: Localization of Sex steroid receptors in human skin. Histo Histopathol. 2004; 19:629-638.