# Effect of Narrow Band-Ultraviolet B on Circulating T-regulatory cells in Atopic Dermatitis

Thesis

Submitted for the partial fulfillment of Master Degree in Dermatology, Venereology and Androlology

## By Eman Yassin Mahmoud MB. BCh., 2006

Under the supervision of

#### Prof. Dr. May Hussein El Samahy

Professor of Dermatology, Venereology, and Andrology Faculty of Medicine, Ain Shams University

#### Dr. Enas Attia Saad El Din Attia

Assistant Professor of Dermatology, Venereology and Andrology
Faculty of Medicine, Ain Shams University

#### Dr. Abeer Attia Saad El Din Attia

Assistant Professor of Clinical Pathology Faculty of Medicine, Ain Shams University

> Faculty of Medicine Ain Shams University 2014



Acknowledgments

Thanks to Allah first and foremost. I feel always indebted to Allah; the most kind and the most merciful.

I would like to express my gratefulness and respect to **Professor Dr. May El Samahy,** Professor of Dermatology, Venereology, and Andrology, Faculty of Medicine, Ain Shams University, for her moral and scientific support and for giving me the honor of working under her supervision and valuable guidance.

Special thanks and deepest gratitude go to Dr. Enas Attia Saad El-Din Attia, Assistant Professor of Dermatology, Venereology, and Andrology, Faculty of Medicine, Ain Shams University, for her constructive and instructive comments, valuable suggestions, and for her sincerely scientific and moral help.

I would like also to express my great thanks to Dr. Abeer Attia Saad El-Din Attia, Assistant Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her valuable help in the laboratory assay and results interpretation part of this work.

Words cannot describe my gratefulness and gratitude to my family who provided me with every mean of support throughout my life.

Eman Yassin



Title	Page
List of Abbreviations	vi
List of Figures	X
List of Tables	xiii
Introduction	1
Aim of the work	3
Chapter 1 Atopic Dermatitis	5
1.1 Incidence and Prevalence	5
1.2 Clinical Features of AD	7
1.2.1 Clinical Patterns	7
1.2.1.1 Infantile phase	7
1.2.1.2 Childhood phase	8
1.2.1.3 Adulthood phase	9
1.2.2 Clinical Stages	9
1.2.3 Atopic march	9
1.2.4 Other clinical manifestations	10
1.2.4.1 Xerosis	10
1.2.4.2 Keratosis pilaris	11
1.2.4.3 Pityriasis alba	12
1.2.4.4 Eyelid dermatitis	12
1.2.4.5 Dennie-Morgan lines	12
1.2.4.6 Allergic shiners	13
1.2.4.7 Periorbital milia	13
1.2.4.8 Palmar and plantar hyperlinearity	13
1.2.4.9 Atopic keratoconjunctivitis	14
1.2.4.10 Anterior capsular cataracts	14
1.2.4.11 Keratoconus	14
1.2.4.12 Contact urticaria	14
1.2.4.13 Secondary skin infections	14
1.2.4.14 Altered vascular reactivity	15
1.2.4.15 Psychosocial problems	15
1.2.5 Diagnosis of AD	16
1.2.5.1 Diagnostic criteria	16
1.2.5.2 Non- essential features of AD	17
1.2.5.3 Measuring the severity of AD	21

i

Title	Page
1.2.5.3.1 SCORAD (Scoring Atopic Dermatitis)	21
1.2.5.3.2 Nottingham index calculation	23
1.2.5.3.3 The Leicester Score	25
1.3 Diagnostic Tests	26
1.3.1 IgE measurement	26
1.3.2 Skin tests	27
1.3.3 Skin biopsy	27
1.4 Differential Diagnosis	28
1.5 Treatment	29
1.5.1 Hospital Care	30
1.5.2 Moisturization in AD	30
1.5.3 Topical steroids in AD	30
1.5.4 Phototherapy and photochemotherapy	31
1.5.5 Immunomodulators in AD	31
1.5.6 Systemic immunosuppressives	32
1.5.7 Antihistaminics	32
1.5.8 Other treatments in AD	33
1.5.8.1 Probiotics	33
1.5.8.2 Antimicrobials	33
1.5.9 Non-medical efforts in AD	34
1.5.10 Consultations	35
1.6 Prognosis	35
<b>Chapter 2 Pathogenesis of Atopic Dermatitis with</b>	37
Emphasis of Regulatory T cells Role	
2.1 Genetic aspect	37
2.2 Skin barrier dysfunction	39
2.3 Environmental aspects	41
2.3.1 Food Allergens	41
2.3.2 Aeroallergens	42
2.3.2.1 House Dust Mite	42
2.3.2.2 Pollens	43
2.3.2.3 Molds	43
2.3.2.4 Animal Dander and Cockroach Allergens	44
2.3.3 Climate	44
2.3.4 Chemical Irritants	44
2.3.5 Acidic foods	45

Title	Page
2.3.6 Sweating	45
2.3.7 Physical Irritants	46
2.3.7.1 Synthetic fabrics	46
2.3.7.2 Scratching	46
2.3.8 Atopic personality	46
2.3.9 Occupation	47
2.3.10 Hormones	47
2.3.11 Microorganisms	47
2.2.11.1 Bacterial infections	47
2.2.11.2 Fungal infections	48
2.2.11.3 Viral infections	50
2.4 Acquired immunity dysfunction	50
2.4.1 Humoral immunity abnormalities	51
2.4.1.1 IgE and IgE receptors	51
2.4.1.2 IgA deficiency	53
2.4.2 Cytokines and chemokines	53
2.4.3 Cell mediated immunity abnormalities and key	55
effector cells in AD skin	
2.4.3.1 Effector T cells	55
2.4.3.2 Regulatory T cells	55
2.4.3.3 Tregs in AD	60
2.4.3.4 Clinical and Therapeutic Consequences of	62
T regulators	
2.4.3.5 T-suppressor cells and natural killer cells	64
(NK-cells)	
2.4.3.6 CD4 / CD8 ratio	64
2.4.3.7 Biphasic role of Th cells in AD	65
2.4.3.8 APCs	66
2.4.3.9 Monocytes/Macrophages	67
2.4.3.10 Keratinocytes	67
2.4.3.11 Eosinophils	69
2.4.3.12 Mast cells	70
2.4.3.13 Endothelial Cells	71
2.4.4 Disturbance of sweating	72
2.4.5 Neuropeptides (NPs)	74
2.4.6 Vascular abnormalities	75

Title	Page
2.4.7 Phosphodiesterase type 4 (PDE4)	75
Chapter 3 NB-UVB in Atopic Dermatitis	77
3.1 NB-UVB Phototherapy Unit	80
3.2 NB-UVB dosing schedule	81
3.3 Dermatological Indications of NB-UVB	83
3.4 Adverse effects of NB-UVB	83
3.4.1 Acute effects	83
3.4.1.1 Erythema	83
3.4.1.2 Blistering	84
3.4.1.3 Pruritus	84
3.4.1.4 Infection	84
3.4.1.5 Ocular complication	84
3.4.2 Chronic effects	85
3.4.2.1 Photoaging	85
3.4.2.2 Epidermal hyperplasia	85
3.4.2.3 Carcinogenesis	85
3.5 Advantages of NB-UVB Phototherapy	86
3.6 Disadvantages of NB-UVB phototherapy	87
3.7 Mechanism of Action of NB-UVB	88
3.7.1 Molecular aspects of NB-UVB irradiation	88
3.7.1.1 Urocanic acid isomerization	88
3.7.1.2 DNA damage	88
3.7.2 Cellular aspects of NB-UVB irradiation	89
3.7.2.1 NB-UVB induced apoptosis of T cells	89
3.7.2.2 NB-UVB induced suppression of APCs	89
3.7.2.3 NB-UVB regulation of NK cells	90
3.7.2.4 NB-UVB regulation of cytokine	90
production	
3.3.2.5 NB-UVB influence on melanocytes	91
3.8 NB-UVB in Atopic Dermatitis	92
3.8.1 Modulation of cell mediated immune response	93
3.8.2 Suppression of Staphylococcus aureus	94
3.8.3 Modulation of cytokine mediators	95
3.8.4 Effect of NB-UVB on regulatory T cells	95
Subjects & Methods	97
Subjects	97
- Patients	97

Title	Page
Exclusion criteria	97
- Controls	98
Methods	98
A] Full history taking	98
B] Clinical examination	99
C] Severity score calculation	99
D] Blood sample collection for assessment of	100
circulating Tregs by flow cytometry	
E] NB-UVB phototherapy	103
Data analysis	106
Results	107
Discussion	135
Summary	141
Conclusion	145
Recommendation	147
References	149
Arabic Summary	-

#### List of Abbreviations

## **List of Abbreviations**

<..... Less than
> ..... More than
% Percentage

**α.....** Alpha **β.....** Beta

γ..... Gamma

(AA) arachidonic acid(AD). Atopic dermatitis

(APC) antigen-presenting cell

(BB-UVB) broad band-UVB(C. albican) Candida albicans

(c-AMP) cyclic adenosine monophosphate(CCL27) Chemokine (C-C motif) ligand 27

(cis-UCA) cis-urocanic acid

(CTLA-4) ... Cytotoxic T lymphocyte antigen-4

(DCs) dendritic cells

(DLNs) draining lymph nodes

(EDC) epidermal differentiation complex

(EDTA) ethylene diamine tetra acetate

(EFA) essential fatty acid

(ETAF) epidermal cell derived thymocyte activation factor

(FcεRIβ) β-subunit of the high-affinity IgE receptor

(FoxP3) forkhead box P3

(GM-CSF) granulocyte-macrophage-colony stimulating factor

(GVHD) graft-versus-host disease

(HDM) house dust mites

#### List of Abbreviations

inflammatory dendritic epidermal cells (IDECs) (IFN) interferon (IgE) immunoglobulin (IL) interleukin inducible protein 10 (IP-10)(IPEX syndrome) immune dysregulation, polyendocrinopathy, enteropathy, and X-linked syndrome (KCs) keratinocytes (LCs) Langerhans cells (LEKTI) lymphoepithelial Kazal-type-related inhibitor Lymphocyte function-associated antigen 1 (LFA-1) leukotreine B4 (LTB4) (MCC1) Mast cell chymase 1 mixed epidermal cell lymphocyte reaction (MECLR) (MED) minimum erythema dose major histocompatibility complex (MHC) (MSH) melanocyte stimulating hormone (MTX) methotrexate Narrow band-UVB (NB-UVB) (NPs) Neuropeptides probability p (PAR-2) protease-activated receptor-2 (PBS) phosphate buffer saline (pDCs) plasmacytoid DCs phosphodiesterase enzyme (PDE) (PDE4) Phosphodiesterase type 4 (PG) prostaglandin (PUVA) psoralen plus UV-A

#### List of Abbreviations

(RANTES) regulated on activation, normal T cell expressed and

secreted)

(ROS) reactive oxygen species(S. aureus) Staphylococcus aureus

(SASSAD) Six-Area, Six-Sign Atopic Dermatitis

(SCORAD) Scoring Atopic Dermatitis

(SD) Standard deviation

(SEB) staphylococcus enterotoxin B

(SPINK5) serine protease inhibitor Kazal-type 5

(TCR) T- cell receptor

(TGF) transforming growth factor

(Th) T-helper

(TIM-1) T-cell immunoglobulin domain and mucin domain 1

(TNF) tumour necrosis factor

(TRAIL) TNF-related apoptosis-inducing ligand

(Tregs) Regulatory T cells

(TSLP) thymic stromai lymphopoietin

(UCA) urocanic acid(UV) Ultraviolet

(UVR) ultraviolet rays

# **List of Figures**

Figure	Page
Figure (1): Infantile atopic dermatitis.	8
Figure (2): Childhood atopic dermatitis.	8
Figure (3): Surface area measurement using tick boxes.	24
Figure (4): Histologic features of 'Acute' atopic dermatitis.	28
Figure (5): Immune response regulation mechanisms.	56
Figure (6): Regulatory homeostasis balance.	58
Figure (7): Possible mechanisms of Tregs-mediated suppression.	59
Figure (8): Immune effect or mechanisms in atopic dermatitis.	66
Figure (9): Mechanisms of eosinophil recruitment and activation in atopic dermatitis.	71
<b>Figure (10):</b> The role of mast cells in allergic diseases goes beyond the early phase reaction (EPR).	73
Figure (11): Sequential steps of leukocyte extravasation and the various tethering and adhesion molecules, chemokines, and receptors involved. Its site in text is not identified and may be wrong	74
Figure (12): Electromagnetic spectrum.	78
<b>Figure (13):</b> Depth of skin penetration of UV, visible and infrared radiations.	79
<b>Figure (14):</b> The biophysical interaction of UVR and visible radiation with skin.	80

# List of Figures

Figure	Page
Figure (15): The gating strategy of Tregs.	102
<b>Figure (16):</b> A: FoxP3 expression on T-reg cells (64.3%). B: FoxP3 expression on T-eff cells (15%).	103
Figure (17): The NB-UVB phototherapy device (Waldmann UV 100L).	104
Figure (18): Gender distribution in patients and controls.	107
Figure (19): Mean age in patients and controls.	108
Figure (20): Patient from group 1 (a): Before NB-UVB therapy. (b): After NB-UVB therapy.	111
Figure (21): Patient from group 1. (a): Before NB-UVB therapy. (b): After NB-UVB therapy.	112
Figure (22): Patient from group 1. (a): Before NB-UVB therapy. (b): After NB-UVB therapy.	113
Figure (23): Patient from group 2. (a) Before NB- UVB therapy (b): After NB-UVB therapy.	114
Figure (24): Patient from group 3. (a): Before NB-UVB therapy. (b): After NB-UVB therapy.	115
<b>Figure (25):</b> Before and after treatment significant lab findings in patients versus controls.	117
<b>Figure (26):</b> Showing flow cytometry: A: patient sample; B normal control sample.	117
<b>Figure (27):</b> Pre-treatment Treg% and Treg/Teff in the three severity grades.	119
Figure (28): Pre-treatment significant laboratory data in moderate disease group versus controls.	121

# List of Figures

Figure	Page
<b>Figure (29):</b> Pre-treatment significant laboratory data in severe disease group versus controls.	122
<b>Figure (30):</b> Severity grades distribution before and after phototherapy.	123
<b>Figure (31):</b> Pre-treatment and post-treatment laboratory data of all patients.	124
<b>Figure (32):</b> Pre-treatment and post-treatment significant laboratory data in group 1 (mild disease).	125
<b>Figure (33):</b> Pre-treatment and post-treatment significant laboratory data in group 2 (moderate disease).	126
<b>Figure (34):</b> Pre-treatment laboratory data in patients with positive versus negative psychological factor.	129
<b>Figure (35):</b> Positive correlation between Treg% and Teff%.	130
<b>Figure (36):</b> Positive correlation between Treg% and Treg/Teff ratio.	131
Figure (37): Positive correlation between severity score improvement and Treg% change	132
<b>Figure (38):</b> Positive correlation between severity score improvement and Treg/Teff ratio change.	132

# **List of Tables**

Table	Page
Table (1): The Hanifin-Rajka criteria for atopic dermatitis.	18
<b>Table (2):</b> United Kingdom working party diagnostic criteria for atopic dermatitis.	19
<b>Table (3):</b> The diagnostic criteria established at the 2003 Consensus Conference on Pediatric Atopic Dermatitis.	20
<b>Table (4):</b> Nottingham Eczema Severity Score results for clinical course, disease intensity and examined extent of disease.	24
Table (5): The final assessment of severity using Nottingham eczema severity scores.	25
Table (6): Differential diagnosis of atopic dermatitis.	29
Table (7): Skin photo types and corresponding MED.	82
<b>Table (8):</b> The ten body zones and the five signs assessed in Leicester score.	100
Table (9): Comparison between patients and controls regarding age and gender.	107
<b>Table (10):</b> Clinical and laboratory data of the studied patients before and after treatment.	110
Table (11): Descriptive statistics for the patients group.	115
Table (12): Descriptive statistics for the control group.	116
Table (13): Comparison between patients and controls regarding to laboratory data.	116

# List of Table

Table	Page
<b>Table (14):</b> Comparison between patients with different grades of severity as regard pre-treatment laboratory findings.	118
<b>Table (15):</b> Results of post-hoc test; assessing the significant difference between the three groups regarding Treg% and Treg/Teff ratio.	118
<b>Table (16):</b> Comparison between patients with mild disease and controls as regard pre-treatment laboratory findings.	120
<b>Table (17):</b> Comparison between patients with moderate disease and controls as regard pre-treatment laboratory findings.	120
<b>Table (18):</b> Comparison between patients with severe disease and controls as regard pre-treatment laboratory findings.	121
<b>Table (19):</b> Comparison between before- and after- therapy clinical severity grades according to Leicester score.	122
Table (20): Comparison between before- and after- treatment laboratory data	123
<b>Table (21):</b> Comparison between before and after lab data in the mild group (group 1).	124
<b>Table (22):</b> Comparison between before and after lab data in the moderate group (group 2).	125
<b>Table (23):</b> Comparison between before and after lab data in the severe group (group 3).	126
<b>Table (24):</b> Comparison between patients with different grades of severity as regard post-treatment laboratory findings.	126