“ESTIMATION OF FOXP3 LEVEL IN THE BLOOD OF VITILIGO PATIENTS”.

Thesis

Submitted for fulfillment of the

Master degree (M.Sc) in
Dermatology, Andrology & S.T.DS

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2011

Acknowledgements

First and foremost thanks to ***"God"*** *for granting me the power to proceed and finish this work.*

I would like to express my endless gratitude and appreciation to ***Dr. Mostafa Abou El Ela*** *Professor of Dermatology, Faculty of medicine, Cairo University, for giving me the honor of working under his supervision and for his generous cooperation, great help and encouragement throughout this work and always.*

I would like to acknowledge my profound gratitude to ***Dr. Rehab Aly Abdel Salam Hegazy*** *Lecturer of Dermatology, Faculty of medicine, Cairo University, for her effort in guiding and giving me instructions throughout this work in order to come in this form.*

I would like also to express my deep thanks to ***Dr. Laila Ahmed Rashed*** *Professor of Biochemistry, Faculty of medicine, Cairo University, for her effort, support and encouragement.*

I would like to thank ***Dr. Marwa Mohamed Fawzy*** *Assistant Professor of Dermatology, Faculty of medicine, Cairo University, for her support and encouragement to finish this work &* ***Dr. Mostafa Mohamed*** *Assistant Lecturer of Biochemistry, Faculty of medicine, Cairo University, for the statistical analysis of this work.*

Finally I am grateful to my ***Mother, Husband, Daughter*** *and* ***all my family*** *for baring me and for their support and encouragement.*

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List of abbreviations

ADCC	*Antibody-dependent cellular cytotoxicity*
AFMK	*N(1)-acetyl-N(2)-formyl-5-methoxykynuramine. An antioxidant metabolite of melatonin.*
APCs	*Antigen presenting cells*
6-BH4	*Co-factor (6R)-5,6,7,8 tetrahydrobiopterine*
C3	*Complement 3*
CAT	*Catalase enzyme*
CCL20	*Class Chemokine (C-C motif) ligand 20*
CCL22	*Class Chemokine ligand 22*
CCR6	*Class Chemokine receptor 6*
CGRP	*Calcitonin gene related peptide*
CLA	*Cutaneous lymphocyte- association antigen*
CTL	*Cutaneous T lymphocytes*
CTLA-4	*Cytotoxic T lymphocyte-associated antigen 4*
DC	*Dendritic cells*
DN	*Double negative regulatory T cells (TCR+CD3+CD4-CD8-)*
EAE	*Experimental autoimmune encephalitis*
ETC	*Electron transport chain*
FOXP3	*Forkhead box protein 3*
GATA3	*Trans-acting T-cell-specific transcription factor GATA-3 is*

	a protein that in humans is encoded by the GATA3 gene
GITR	*Glucocorticoid-induced tumor necrosis factor receptor family-related protein*
GTP	*Guanosine triphosphate*
HLA	*Human leucocytic antigen*
H2O2	*Hydrogen peroxide*
ICAM-1	*Intracellular adhesion molecule- 1*
IDO	*Indoleamine 2,3-dioxygenase*
IFN γ	*Interferon gamma*
IKP	*Isomorphic Koebner phenomenon*
IL	*Interleukin*
IPEX	*X-linked, immuno-dysregulation, polyendocrinopathy, enteropathy syndrome*
iTregs	*Inducible Treg population*
KDa	*Kilo Dalton*
LAG3	*Lymphocyte activation gene-3*
LAP	*Latency associated peptide*
LC	*Langerhans cells*
MALT	*Mucosa-associated lymphoid tissue*
MAO	*Mono amine oxidase enzyme.*
MCHR1	*Melanin-concentrating hormone receptor 1*
MHC	*Major histocompatibility complex*
NK	*Natural killer cells*

nTregs	*Naturally occurring Foxp3+ Treg population*
4aOH-BH4	*4ahydroxy- BH4 dehydratase*
PBMCs	*Peripheral blood mononuclear cells*
Pmel17	*Melanosomal matrix protein gp100*
RA	*Rheumatoid arthritis*
Rab38	*Ras-related protein Rab-38 is a protein that in humans is encoded by the RAB38 gene*
RORγt	*The orphan nuclear receptor, retinoic acid-related orphan receptor gamma t*
ROS	*Reactive oxygen species*
SMS	*skin melatoninergic system*
SOX 10	*The melanocyte transcriptor factor*
STAT	*Signal transduction and activator of transcription*
SV	*Segmental vitiligo*
4-TBP	*4-tertiary butylphenol*
TCR	*T cell receptor*
TGFβ	*Transforming growth factor beta*
Th	*T helper cells*
Thp	*T helper cell precursors*
TLRs	*Toll like receptors*
Tol-DC	*Tolerogenic dendritic cells*
Tr1	*Type 1 regulatory T cells*

T regs	*T regulatory cells*
TYRP	*Tyrosinase related protein*
UVR	*Ultra violet rays*

Abstract

Background: Vitiligo is a common depigmenting disorder which may have devastating psychological and social consequences. The exact cause is still unknown but autoimmunity is highly implicated in the pathogenesis. T regulatory cells are among the cells that control autoimmunity.

Objective: Is to estimate the level of Foxp3 which serves as the dedicated mediator of the genetic program governing Tregs development and function which has questionable immunomodulatory function in vitiligo, aiming to verify the possible role of them in the pathogenesis of such disease.

Methods: The present case controlled cross sectional study included 40 vitiligo patients and 40 age and sex matched healthy controls. Presence of any systemic or dermatological disease affecting the immune system as well as receiving any systemic or topical immunomodulatory treatments for 3 months prior to the study precluded participation. After history taking & clinical examination, a 5 ml blood sample was retrieved from each patient and control to determine the level of Foxp3 gene expression by quantitative real time PCR.

Results: The mean level of Foxp3 was found to be significantly lower in the vitiligo patients (0.39±0.29) in comparison to the healthy control subjects (1.48±0.50) ($p<0.0001$). The non-significant ($p>0.05$) least levels were reported among the segmental type, as well as in those with a progressive disease, and in those with a positive family history. Concerning the relationship between Foxp3 and stress a significantly lower value was reported with presence of stress ($p< 0.05$) and a negative correlation was observed between Foxp3 and the presence of stress as a precipitating factor ($r = -0.45$, $p = 0.004$).

Conclusion: The current study demonstrates the diminishing T reg cells to be another important player sharing in the complex pathogenesis of vitiligo. It has been shown to be associated with a less favorable prognosis of the disease.

Key word: p3-vitiligo-Tr1-SOX 10-Tol_DC

Introduction

Vitiligo is a common depigmenting disorder which may have devastating psychological and social consequences and is characterized by the presence of circumscribed white macules in the skin due to the destruction of melanocytes in the epidermis***(Sandoval-Cruz et al,. 2011).***

The cause of vitiligo appears to be a combination of genetic effects in both the immune system and the melanocyte itself with a precipitating factor instigating their interaction and resulting in the melanocyte destruction ***(Boissy and Nordlund, 2011).***

The autoimmune pathogenesis of the disease has become a rapidly evolving field of research. Detection of circulating melanocyte antibodies in human and animal models implicates a possible role of humoral immunity. Histological and immunohistochemical studies in perilesional skin suggest the involvement of cellular immunity in vitiligo. Recently, T-cell analyses in peripheral blood further support this hypothesis ***(Ongenae et al., 2003).***

Effector T helper (Th) cells derive from progenitor naïve CD4+ T cells, after maturational process induced by antigenic stimulation. Their commitment depends on complex interactions with antigen presenting cells in a permissive milieu, including antigenic type and load, costimulatory molecules and cytokine signaling. Committed CD4+ T cells may differentiate into Th1, Th2, TH17 phenotypes (the effector Th cell triade), with distinct cytokine products and biological functions, or evolve into the inducible regulatory T (Treg) lineage, with immunomodulatory functions ***(Fietta and Delsante, 2009).***

INTRODUCTION & AIM OF WORK

Alterations in cellular immunity, including CD4(+) T and CD8(+) T lymphocytes, have been proposed in the pathogenesis of vitiligo. There is also a proposed role for cytokines in the depigmentation observed in vitiligo. Along with the multiple factors that have been implicated in the pathogenesis of vitiligo, reduced serum transforming growth factor-beta levels, has also been suggested to contribute to enhanced cellular immunity. This may facilitate the occurrence of vitiligo by leading to diminished maturation of regulatory T cells, followed by impaired inhibition of inflammation ***(Basak et al., 2009).***

Abnormality of CD4+CD25+Treg cells can be observed in patients with progressive vitiligo. Decrease amount of CD4+ CD25+ Treg cells and decrease expression of Foxp3 impair the suppressive activity of CD4+ CD25+ Treg cells which may be a factor in pathogenesis of vitiligo ***(Ting-huil et al., 2009)***.

Forkhead box P3 (FoxP3) maintains a pattern of gene expression necessary for T reg cell function ***(Isomura et al., 2010)*** and regulates T (reg) development. It has been proven that epigenetic modification agents that can induce Foxp3 expression promote the conversion of naïve T cells to T regs ***(Moon et al., 2009).*** Thereby it can be deduced that Foxp3 is immunomodulatory marker for the T regs ***(Lee et al., 2010).***

So measuring Foxp3 level might help to know the role of T regulatory cells in the pathogenesis of vitiligo.

Aim of work:

The aim of this study was to estimate level of Foxp3 which serves as the dedicated mediator of the genetic program governing Tregs development and function which has questionable immunomodulatory function in vitiligo, aiming to verify the possible role of them in the pathogenesis of such disease.