



بسم الله الرحمن الرحيم

∞∞∞∞

تم رفع هذه الرسالة بواسطة / حسام الدين محمد مغربي

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى

مسئولية عن محتوى هذه الرسالة.

ملاحظات : لا يوجد





Evaluation of Granulysin Level in Active Vitiligo

Thesis

Submitted for Partial Fulfillment of Master Degree
in **Dermatology, Venereology and Andrology**

By

Asmaa Harith Mustafa Alkhateeb

M.B.B.CH

Faculty of Medicine -Tikrit University-Iraq

Under Supervision of

Dr. Ghada Fathy Mohammed

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

Dr. Rania Mahmoud Elhusseiny

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

*Faculty of Medicine
Ain Shams University*

2022

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالَ

لَسْبَّانِكَ لَا أَعْلَمُ لَنَا
إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ
الْعَلِيمُ الْعَظِيمُ

صدقة الله العظيم

سورة البقرة الآية: ٣٢

Acknowledgments

*First and foremost, I feel always indebted to **Allah** the Most Beneficent and Merciful.*

*I'd like to express my respectful thanks and profound gratitude to **Dr. Ghada Fathy Mohammed**, Professor of Dermatology, Venereology and Andrology Faculty of Medicine- Ain Shams University for her keen guidance, kind supervision, valuable advice, and continuous encouragement, which made possible the completion of this work.*

*I am also delighted to express my deepest gratitude and thanks to **Dr. Rania Mahmoud Elhusseiny**, Assist. Professor of Dermatology, Venereology and Andrology Faculty of Medicine - Ain Shams University, for her kind care, continuous supervision, valuable instructions, constant help, and great assistance throughout this work.*

*Thanks to all **staff members** of the dermatology, Venereology, and Andrology Department, Ain Shams University for their support.*

*Last but not least, **my Family**, I do not know how I can ever thank you for all that you have borne with me, no words can ever fulfill what you deserve...*

Asmaa Harith Mustafa Alkhateeb

List of Contents

Title	Page No.
List of Abbreviations.....	i
List of Tables.....	iii
List of Figures.....	v
Introduction.....	1
Aim of the Work.....	4
Review of Literature	
Chapter 1: Vitiligo.....	5
Classification of vitiligo.....	5
Epidemiology.....	5
Etiology and pathogenesis.....	6
Disease burden.....	16
Differential diagnosis.....	19
Assessment of vitiligo.....	21
Vitiligo scoring systems.....	24
Dermoscopy in vitiligo.....	28
Histopathology of Vitiligo.....	30
Treatment of vitiligo.....	32
Chapter 2: Granulysin.....	43
Granulysin Enzyme.....	43
Granulysin Structure.....	43
Function and mechanism of action.....	44
Granulysin level.....	45
Role of Granulysin in different diseases.....	45
Patients and Methods.....	53
Results.....	62
Discussion.....	84
Conclusion.....	89
Recommendations.....	90
Summary.....	91
References.....	94
Arabic Summary	

List of Abbreviations

Abb.	Full term
AA	Alopecia areata
AChE	Acetylcholinesterase
AMPs	Antimicrobial peptides
APS	Autoimmune polyglandular syndromes
ATP	Adenosine triphosphate
AUC	Area under the curve
CLM	Confocal laser microscopy
CTLs	Cytotoxic T lymphocytes
DAMP	Damage-associated molecular pattern
DHPR	Dihydropyridine reductase
DOPA	3,4-dioxyphenylalanin
ELISA	Enzyme-Linked Immunosorbent Assay
GNLY	Granulysin
gp100	Glycoprotein 100
H ₂ O ₂	Hydrogen peroxide
HLA-A2	Human leukocyte antigen
HS	Highly significant
ICAM-1	Intercellular Adhesion Molecule 1
IFN- γ	Interferon- γ
IFN	Interferon- α
IL10	Interleukin10
IL-8	Interleukin 8
IQR	Inter-quartile range
M.tb.	Mycobacterium tuberculosis
MART-1	Melanoma antigen recognized by T cells 1
MCP	Monocyte chemoattractant protein -1
MHC II	Major histocompatibility complex class II
MIP	Macrophage inflammatory protein -1a
MV	Mixed vitiligo
NADH	Nicotinamide adenine dinucleotide
NB-UVB	Narrow-band UVB
NK	Natural killer
NPV	Negative predictive value

List of Abbreviations *cont...*

Abb.	Full term
NS	Non-significant
NSV	Non-segmental vitiligo
OM, IM	Outer and inner membranes
PAMP	Pathogen-associated molecular pattern
PASI	Psoriasis Area Severity Index
PPV	Positive predictive value
PRI	Potential Repigmentation Index
PRR	Pattern recognition receptor
PsA	Psoriatic arthritis
PUVA	Psoralen and UV-A
RANTES	Regulated upon activation T cell expressed and secreted
RCT	Randomized controlled trial
ROC	Receiver operating characteristic curve
ROS	Reactive oxygen species
S	Significant
SAPLIP	Saposin-like protein
SD	Standard deviation
sIL-2R	Soluble interleukin-2 receptor sIL-2R
SJS	Stevens-Johnson syndrome
SPSS	Statistical Package for Social Science
Σ (sigma)	Summation
SV	Segmental vitiligo
TEN	Toxic epidermal necrolysis
TIM	Topical immunomodulatory
TNF- α	Tumor necrosis factor- α
TREGs	Regulatory T cells
TRP1	Tyrosine-related protein 1
UV	Ultraviolet
VASI	Vitiligo Area Scoring Index
VETF	Vitiligo European Task Force
VETI	Vitiligo Extent Tensity Index
VIDA	Vitiligo disease activity score

List of Tables

Table No.	Title	Page No.
Table 1:	Classification of vitiligo	16
Table 2:	Differential diagnosis of vitiligo	20
Table 3:	A comparison of the quality of subjective vitiligo assessment methods used to measure skin pigmentation	22
Table 4:	A comparison of the quality of semi-objective and objective vitiligo assessment methods used to measure skin pigmentation	23
Table 5:	Management of vitiligo	33
Table 6:	VIDA score	58
Table 7:	Demographic and clinical characteristics of the whole vitiligo patients group (active and stable vitiligo).	64
Table 8:	Demographic data of the control subjects.....	65
Table 9:	Comparison between vitiligo patients and controls regarding age and sex.	66
Table 10:	Comparison between stable and active patients regarding family history, disease duration, age of onset, type of distribution, VIDA, and VES scores.	69
Table 11:	Measurement of granulysin level in vitiligo patients.	71
Table 12:	Comparison between granulysin level in stable and active vitiligo patients.....	72
Table 13:	Measurement of granulysin level in control subjects.....	73

List of Tables *cont...*

Table No.	Title	Page No.
Table 14:	Comparison between the control group and patient groups regarding granulysin level.	74
Table 15:	Grading of granulysin expression among the whole vitiligo patients.	75
Table 16:	Comparison of grading of granulysin expression in controls, stable and active vitiligo patients.....	76
Table 17:	Correlation of granulysin level with patient's age, disease duration, age of onset, VIDA, and VES scores of the whole vitiligo patients (stable and active vitiligo).	78
Table 18:	Relation of granulysin level with sex, type of vitiligo, and family history in the whole vitiligo patients (stable and active vitiligo).....	79
Table 19:	Correlation of granulysin level with patient's age, disease duration, age of onset, VIDA, and VES scores of stable vitiligo patients.	80
Table 20:	Relation of granulysin level with sex, type of distribution, and family history among stable vitiligo patients.	81
Table 21:	Correlation of granulysin level with patient's age, disease duration, age of onset, VIDA, and VES scores of active vitiligo patients.....	82
Table 22:	Relation of granulysin level with sex, type of vitiligo, and family history among active vitiligo patients.	83

List of Figures

Fig. No.	Title	Page No.
Figure 1:	Vitiligo pathogenesis.....	11
Figure 2:	Vitiligo Global Issues Consensus Conference classification	12
Figure 3:	Non-segmental Vitiligo.....	13
Figure 4:	Typical segmental vitiligo of the face with eyelash and eyebrow whitening.....	14
Figure 5:	Determinants of quality of life in vitiligo	18
Figure 6:	Rule of nines	25
Figure 7:	Dermoscopy of various stages of vitiligo.....	29
Figure 8:	Vitiliginous skin (A) houses only two panmelanoma positive cells (red) in contrast to the normal number of melanocytes in unaffected skin (B).....	30
Figure 9:	A single c-kit positive cell (red) is present in the basal layer of vitiliginous skin (A) as opposed to the normal number seen in unaffected skin (B).....	31
Figure 10:	Punch grafting.....	40
Figure 11:	Application of melanocyte cell suspension following dermabrasion.....	41
Figure 12:	Therapeutic algorithm of vitiligo	42
Figure 13:	Model of bacterial death by granzymes and GNLV	45
Figure 14:	Mechanism of tumor lysis by GNLV.....	47
Figure 15:	Wood's lamp examination of vitiligo patient's knee at dermatology outpatient clinic of Ain Shams University hospital.....	56

List of Figures *cont...*

Fig. No.	Title	Page No.
Figure 16:	Scoring sheet Vitiligo Extent Score (VES).	57
Figure 17:	Insulin syringe, 3-way valve, 10 ml syringe, and 50 ml syringe.	59
Figure 18:	Steps of collection the suction fluid of the skin blister.	59
Figure 19:	Eppendorf tube.	60
Figure 20:	Comparison between the studied groups regarding age.	67
Figure 21:	Comparison between the studied groups regarding sex.	67
Figure 22:	Comparison between stable and active vitiligo patients regarding VIDA score	70
Figure 23:	Comparison between the stable and active vitiligo patients regarding granulysin level.	72
Figure 24:	Comparison between the control group and patient groups regarding granulysin level.	74
Figure 25:	Receiver operating characteristic curve (ROC) for granulysin level to differentiate between patients and controls.	77

ABSTRACT

Background: Vitiligo is an acquired, a progressive, multifactorial, depigmenting disorder characterized by the appearance of circumscribed white macules in the skin due to chronic, progressive loss of functional melanocytes in the epidermis.

Aim of the work: We aimed to evaluate GNLY- levels in stable and active vitiligo patients in comparison to healthy controls.

Patients and Methods: A case-control study which was carried out on 66 subjects: 22 patients with active vitiligo, 22 with stable vitiligo, and 22 age and sex-matched controls. All patients were recruited from the Dermatology Outpatient Clinic of Ain Shams University hospitals during the period from February 2021 to May 2021.

Results: We found a statistically significant difference between vitiligo patients and controls regarding GNLY levels with higher GNLY-levels among cases as compared to control. There were no statistically significant relations between GNLY-levels and gender or age of patients, family history, distribution or duration of vitiligo as well as activity or severity of vitiligo disease. This suggests that GNLY has an important role in vitiligo pathogenesis regardless of the activity, severity, and different disease characteristics. To the best of our knowledge, our study was the first study that measured GNLY-level in vitiligo patients and compare it with controls. Also, compared GNLY level in stable vitiligo versus active vitiligo.

Conclusion: Our findings supported the possible role of GNLY in the pathogenesis of vitiligo. However, measurement of GNLY level can not be used as a discriminating marker between stable and active vitiligo.

Keywords: Granulysin Level, Vitiligo

INTRODUCTION

Vitiligo is an acquired cutaneous disorder of pigmentation of unknown etiology. It is characterized by milky white depigmented macules and patches, often symmetric, whose size increase during the time, relating to the loss of functioning melanocytes. The prevalence ranges from less than 0.1% to more than 2% worldwide, with the disease can appear at any time, and it significantly impairs the patients' quality of life (*Lotti & D'Erme, 2014*). Vitiligo susceptibility is not believed to be sex-linked, but 6–38% of patients have family members with the disease indicating a hereditary factor (*Rezaei et al., 2007*).

The exact etiology of vitiligo is unknown. It is believed that vitiligo is a polygenic trait and that a convergence theory combining elements of different etiological theories across a spectrum of expression is the most accurate etiology (*Garg et al., 2010*).

Research into the pathogenesis of vitiligo suggests that oxidative stress may be the initial event in the destruction of melanocytes. Indeed, melanocytes from patients with vitiligo were found to be more susceptible to oxidative stress than those from unaffected individuals and are more difficult to culture ex-vivo than those from healthy controls (*Bergqvist & Ezzedine, 2020*).

Many studies have indicated a role for both cellular and humoral immunity in the pathogenesis of vitiligo. Furthermore, Lots of evidence have confirmed that Cytokines that have a crucial function in the development, differentiation, and regulation of immune cells, may play a role in the pathogenesis of vitiligo (*Singh et al., 2019*).

Granulysin(GNLY) is a cytolytic and proinflammatory peptide that belongs to a family of saposin-like, lipid-binding antimicrobial peptides (AMPs), and co-localizes in the granular compartments of human cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, along with granzymes and perforin (*Elgarhy et al., 2015*).

GNLY expression has been studied in some inflammatory skin diseases, including lichen planus (LP), atopic eczema (AE), and Alopecia areata (AA)(*Pivarcsi et al., 2004; Ono et al., 2014*).

GNLY is a chemoattractant for T lymphocytes, monocytes, and other inflammatory cells. It activates the expression of several cytokines, including, IL-10, IL-1, IL-6, and interferon (IFN)- α (*Krensky & Clayberger, 2009*).

On the other hand, *Moretti et al. (2002)* reported a significantly higher expression of pro-inflammatory cytokines with an inhibitory effect on pigmentation, such as IL-6 and TNF- α , in lesional and perilesional skins in vitiligo patients.

To date, no study has addressed the expression of GNLY in vitiligo and assessed its possible role in disease kinetics before. Thus, our study evaluated the expression of GNLY in perilesional skin of vitiligo patients compared with healthy controls to investigate the possible role of GNLY in the pathogenesis of vitiligo.