



# **EVALUATION OF SERUM INTERLEUKEN-10 & INTERFERON- $\gamma$ IN PATIENTS WITH MULTIPLE WARTS AFTER TREATMENT WITH BCG VACCINE**

*Thesis*

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

*By*

**Shaimaa Awad Mosaad Taha**  
(M.B;B.Ch.)

*Supervised by*

**Prof. Dr. Nader Fouad Ragab**

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

**Prof. Dr. Mahmoud Abdel-Rahim  
Abdallah**

Professor of Dermatology, Venereology and Andrology  
Faculty of Medicine - Ain shams University

**Dr. Dina Al Sayed El Shennawy**

Assistant Professor of clinical Pathology  
Faculty of Medicine - Ain shams University

Faculty of Medicine  
Ain Shams University  
2017

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا  
إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ  
الْعَلِيمُ الْحَكِيمُ

صَدَقَ اللَّهُ الْعَظِيمُ



سورة البقرة آية (٣٢)

# Acknowledgement

First, all thanks to **ALLAH** for blessing this work until it has reached its end, as a little part of his help throughout life.

I would like to express my gratitude and special thanks to **Prof. Nader Fouad Ragab**, *Professor of Dermatology, Venereology and Andrology, Faculty of Medicine, Ain Shams University*. I am greatly indebted for his great help, constant encouragement, close supervision and care throughout carrying out this work until it came to light. Without his ideas, efforts, guidance and cooperation, this work would not be possible. It is great honor to work under his supervision, words cannot express his great efforts.

I would like to express my great gratitude and profound appreciation to **Prof. Mahmoud Abdel-Rahim Abdallah**, *Professor of Dermatology, Venereology and Andrology, Faculty of Medicine, Ain Shams University*, for his remarkable suggestions and close supervision, which were behind the fruitful outcome of the practical part of this work.

I am very much indebted to **Dr. Dina Al-Sayed El-Shennawy**, *Assistant Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University* for her continuous encouragement, support and endless advice.

Great thanks to all staff members of the Faculty of Medicine, Ain Shams University for their support, I want also to express my gratitude towards our kind patients who let us hope to complete this work.

*Shaimaa Taha*

## List of Contents

Title	Page
▪ <b>List of Abbreviations</b> .....	II
▪ <b>List of Tables</b> .....	V
▪ <b>List of Figures</b> .....	VII
▪ <b>Introduction</b> .....	1
▪ <b>Aim of the Work</b> .....	4
▪ <b>Review of Literature</b>	
- Human Papilloma Virus .....	5
- Immune Responses to Human Papilloma Virus .....	16
- Treatment of Human Papilloma Virus infections.....	48
- Bacille Calmette-Guérin (BCG) Vaccine .....	78
▪ <b>Patients and Methods</b> .....	95
▪ <b>Results</b> .....	107
▪ <b>Illustrative cases</b> .....	130
▪ <b>Discussion</b> .....	137
▪ <b>Summary</b> .....	151
▪ <b>Conclusions and Recommendations</b> .....	156
▪ <b>References</b> .....	157
▪ <b>Arabic Summary</b> .....	--

## List of Abbreviations

<b>5-FU</b>	.....	5-Fluorouracil
<b>AP1</b>	.....	Activator Protein 1
<b>APCs</b>	.....	Antigen-Presenting Cells
<b>BCG MRDJ</b>	.....	BCG Moreau Rio De Janeiro
<b>BCG</b>	.....	Bacillus Calmette-Guérin
<b>BMPs</b>	.....	Bone Morphogenic Proteins
<b>CIN</b>	.....	Cervical Intraepithelial Neoplasia
<b>CMI</b>	.....	Cell Mediated Immunity
<b>CPG</b>	.....	Cytokine Phosphate Guarine
<b>CREB</b>	.....	CAMP Response Element binding protein
<b>CSIF</b>	.....	Cytokine Synthesis Inhibitory Factor
<b>CTL</b>	.....	Cytotoxic T Lymphocytes
<b>DC</b>	.....	Dendritic Cells
<b>DCP</b>	.....	Diphencyprone
<b>DNCB</b>	.....	Dinitrochlorobenzene
<b>DPCP</b>	.....	Diphenylcyclopropenone
<b>DTH</b>	.....	Delayed Type Hypersensitivity
<b>DUSP1</b>	.....	Dual Specifity phosphatase 1
<b>E</b>	.....	Early Viral Gene
<b>ECM</b>	.....	Extracellular Matrix
<b>EGWs</b>	.....	External Genital Warts
<b>ERK</b>	.....	Extracellular Signal Regulated Kinases
<b>FC</b>	.....	Fragment, Crystallizable
<b>FOXP3</b>	.....	Factor Forkhead Box P3
<b>GM-CSF</b>	.....	Granulocyte Monocyte Colony Stimulating Factor

## List of Abbreviations

<b>GSK</b> .....	Glycogen Synthase Kinase
<b>HPVs</b> .....	Human Papillomaviruses
<b>HSCs</b> .....	Hematopoietic Stem Cells
<b>HSIL</b> .....	High Grade Squamous Intraepithelial Lesion
<b>HSPGs</b> .....	Heparan Sulfate Proteoglycans
<b>IL-10</b> .....	Interleukin-10
<b>ISGs</b> .....	Interferon Stimulated Genes
<b>JAK</b> .....	Janus Activated Kinase
<b>L</b> .....	Late Viral Gene
<b>LC</b> .....	Langerhans cell
<b>LCR</b> .....	Long Control Region
<b>LSIL</b> .....	Low Grade Squamous Intraepithelial Lesion
<b>MAPK</b> .....	Mitogen Activated Protein Kinase
<b>MCP</b> .....	Monocyte Chemotactic Protein
<b>MHC</b> .....	Major Histocompatibility Complex
<b>MIP</b> .....	Macrophage Inflammatory Protein
<b>NF-KB</b> .....	Nuclear Factor KB
<b>NK</b> .....	Natural Killer
<b>PCR</b> .....	Polymerase Chain Reaction
<b>PI3K</b> .....	Phosphoinositide 3 Kinase
<b>PPD</b> .....	Purified Protein Derivative
<b>PRPS</b> .....	Pattern Recognition Receptors
<b>SADBE</b> .....	Squaric Acid Dibutylester
<b>STAT3</b> .....	Signal Transducer and Activator of Transcription 3
<b>TB</b> .....	Tuberculosis

## List of Abbreviations

<b>TGF</b>	.....	Transforming Growth Factor
<b>TLR</b>	.....	Toll-Like Receptor
<b>TNF</b>	.....	Tumor Necrosis Factor
<b>TPL2</b>	.....	Tumor Progression Locus 2
<b>TPO</b>	.....	Thrombopoietin
<b>TST</b>	.....	Tuberculin Skin Test
<b>UNICEF</b>	.....	United Nations Children's Fund
<b>VLP</b>	.....	Virus Like Particles
<b>WHIM syndrome</b>	....	Warts, Hypogamma globulinemia, Immunodeficiency and Myelokathexis
<b>WHO</b>	.....	World Health Organization

## List of Tables

Table N.	Title	Page
<b>Table (1):</b>	Common HPV genotypes .....	7
<b>Table (2):</b>	The HPV proteins and functions .....	10
<b>Table (3):</b>	Disease associations with selected human papillomavirus types .....	13
<b>Table (4):</b>	The most common immune-therapeutic methods for the treatment of warts .....	49
<b>Table (5):</b>	Demographic data of studied patients.....	107
<b>Table (6):</b>	Patients included in the study; demographic data, response to treatment and side effects .....	109
<b>Table (7):</b>	Characters of warts .....	111
<b>Table (8):</b>	Interleukin-10 and IFN- $\gamma$ levels among patients and controls before BCG vaccination .....	112
<b>Table (9):</b>	Interleukin-10 and IFN- $\gamma$ levels among patients and controls after BCG vaccination .....	114
<b>Table (10):</b>	Interleukin-10 and IFN- $\gamma$ levels in patients before and after BCG vaccination.....	116
<b>Table (11):</b>	Response and side effects of BCG vaccination.....	119
<b>Table (12):</b>	Correlative study between response of BCG vaccination and other parameters.....	120



## List of Tables (Continue)

Table N.	Title	Page
<b>Table (13):</b>	Correlative study between IL-10 after BCG vaccination and other parameters.....	122
<b>Table (14):</b>	Correlative study between INF- $\gamma$ after BCG vaccination and other parameters.....	123
<b>Table (15):</b>	Interleukin-10 and IFN- $\gamma$ levels in relation to response.....	124
<b>Table (16):</b>	Interleukin-10 and IFN- $\gamma$ levels in relation to number of warts .....	126
<b>Table (17):</b>	Criteria of warts in relation to response .....	129

## List of Figures

<b>Figure N.</b>	<b>Title</b>	<b>Page</b>
<b>Fig. (1):</b>	Schematic representation of human papillomavirus (HPV) showing icosahedral symmetry .....	9
<b>Fig. (2):</b>	The infection and maturation cycle of HPV in keratinocytes .....	12
<b>Fig. (3):</b>	An abbreviated version of the cytokine network highlighting the molecular communication between different cell types in the immune system .....	19
<b>Fig. (4):</b>	HPV reaches the basement membrane of the cervix through microtrauma .....	37
<b>Fig. (5):</b>	Family history in studied cases .....	108
<b>Fig. (6):</b>	Comparison between IL-10 level in patients before BCG vaccination and control groups.....	113
<b>Fig. (7):</b>	Comparison between IFN- $\gamma$ level in patients before BCG vaccination and control groups.....	113
<b>Fig. (8):</b>	Comparison between IL-10 level in patients after BCG vaccination and control groups.....	114
<b>Fig. (9):</b>	Comparison between IFN- $\gamma$ level in patients after BCG vaccination and control groups.....	115

## List of Figures (Continue)

Figure N.	Title	Page
<b>Fig. (10):</b>	IL-10 level before and after BCG vaccination among studied patients.....	116
<b>Fig. (11):</b>	IFN- $\gamma$ level before and after BCG vaccination among studied patients.....	117
<b>Fig. (12):</b>	Negative correlation between duration of lesion and response to BCG vaccination .....	121
<b>Fig. (13):</b>	Negative correlation between number of sessions and response to BCG vaccination.....	121
<b>Fig. (14):</b>	Positive correlation between IL-10 level before and after BCG vaccination.....	122
<b>Fig. (15):</b>	Positive correlation between INF $\gamma$ level before and after BCG vaccination.....	123
<b>Fig. (16):</b>	IL-10 level before and after BCG vaccination according to BCG vaccination response .....	125
<b>Fig. (17):</b>	IFN- $\gamma$ level before and after BCG vaccination according to BCG vaccination response .....	125
<b>Fig. (18):</b>	IL-10 level before and after BCG vaccination according to number of warts.....	127
<b>FIG. (19):</b>	IFN- $\gamma$ level before and after BCG vaccination according to number of warts.....	127

## INTRODUCTION

Warts are benign epithelial neoplasms affecting the epithelium of the skin and mucous membranes that result from infection with human papillomavirus (HPV) (*Yazdanfar et al., 2008*).

Common warts affect patients' quality of life by causing adverse psychological effects, also certain types of HPV may induce life-threatening malignancies (*Rijkaart et al., 2012*). HPV 16 has also been strongly associated with various head and neck cancers including head and neck squamous cell carcinoma and oropharyngeal carcinoma of the tonsils (*Rautava et al., 2012*). The incidence of HPV induced oral cancers appears to be increasing (*Gaston and Garry, 2012*).

HPVs are the causative agents of a variety of benign and cancerous lesions of the skin and other epithelial surfaces. At least 189 HPV genotypes have been described (*Gooi et al., 2016*).

Most HPV types are associated with one or a few histopathologically distinct types of lesions and may be restricted to a particular location on the body. HPV types 2, 4, 26, 29 and others are responsible for common warts (verruca vulgaris), which are slightly raised rough surface epithelial proliferations that occur most often on the hands, can also grow elsewhere on the body. Other types of warts include plantar warts (verruca plantaris) that occur most commonly on the soles of the feet (HPV 1 and others), flat warts (verruca plana) usually appearing on the face (HPV

3, 10, 38 and others), butcher's warts of the hands and fingers (HPV 7), oral and genital warts (condyloma acuminata; HPV 6, 11, 16, 18 and many others) (*Gaston and Garry, 2012*).

BCG is a bacterial preparation of a strain called *Bacillus-Calmette-Guerin*. It contains live attenuated *Mycobacterium bovis* that has lost its virulence in humans by being especially cultured in an artificial medium for years and was developed as a vaccine against tuberculosis (*Fine et al., 1999*).

BCG was introduced as a prophylactic agent against tuberculosis (TB), accidentally it has been found that the leprosy incidence has decreased tremendously. The rationale of the use of BCG as a protective vaccine against leprosy rests on the assumption that cross reacting antigens exist between *Mycobacterium leprae* and BCG and that following BCG vaccination, protective immunity against leprosy will be developed (*Zodpey, 2007*).

The BCG also had been used in the treatment of malignant melanoma, transitional cell carcinoma of the bladder, in alopecia areata and recurrent oral aphthosis (*Sharquie and Hayani, 2005*). The percentage of skin disease among BCG vaccinated individuals was significantly lower compared with healthy individual controls and these diseases include psoriasis, fungal infection, cutaneous leishmaniasis, molluscum contagiosum and lichen planus indicating that BCG decreases the frequency of skin diseases (*Sharquie et al., 2008*).

Patients with condyloma accuminata had shown an imbalance of Th1/Th2 cytokines production (*Zuo et al., 2004*). Th2 cells secrete IL-4 and IL-10 (and other cytokines) and help antigen-primed B lymphocytes differentiate into plasma cells and secrete antibodies; the effector molecules of humoral responses. Th1 cells secrete IFN- $\gamma$  and create a milieu in which key cytotoxic effectors, macrophages, natural killer cells and cytotoxic CD8+ T lymphocytes are activated, generating cell mediated immunity (*Rouse and Suvas, 2004*).

A lot of works attempted to prove the efficacy of BCG vaccine as an immunotherapy in warts and other immunomediated diseases whether by topical application (*Metawea et al., 2005*), intradermal administration (*Sharquie et al., 2008*) or intralesionally the study of *Yuan et al. (2007)*.

The mechanism of action of BCG in the treatment of warts, could be explained on the basis of stimulating macrophages, T and B lymphocytes, natural killer cells function that might help in resolution of the viral warts (*Sharquie et al., 2008*).

## **AIM OF THE WORK**

The aim of this work was to evaluate the level of serum IL-10, IFN- $\gamma$  before and after multiple intradermal injections of BCG vaccine and to compare the levels in clinical responders versus non responders to evaluate its possible clinical efficacy in the treatment of warts.