

Impact of the type of Human Leucocyte
Antigen – A Allele on the Outcome of
Hepatitis C Virus Infection in the Egyptian
Population

Thesis

Submitted for Partial Fulfillment
of MD in Clinical and Chemical
Pathology

By

Nancy Samir Wahba Basta

MB BCh, MSc. Clinical & Chemical Pathology

Supervised By

Prof./ Aisha Yassin Abdel-Ghaffar

Professor of Clinical and Chemical Pathology

Faculty of Medicine – Ain Shams University

Prof./ Nahla Mohamed Zakaria Yousef

Professor of Clinical and Chemical Pathology

Faculty of Medicine – Ain Shams University

Doctor/ Amal Ahmed Abbas

Assistant Professor of Clinical and Chemical Pathology

Faculty of Medicine - Ain Shams University

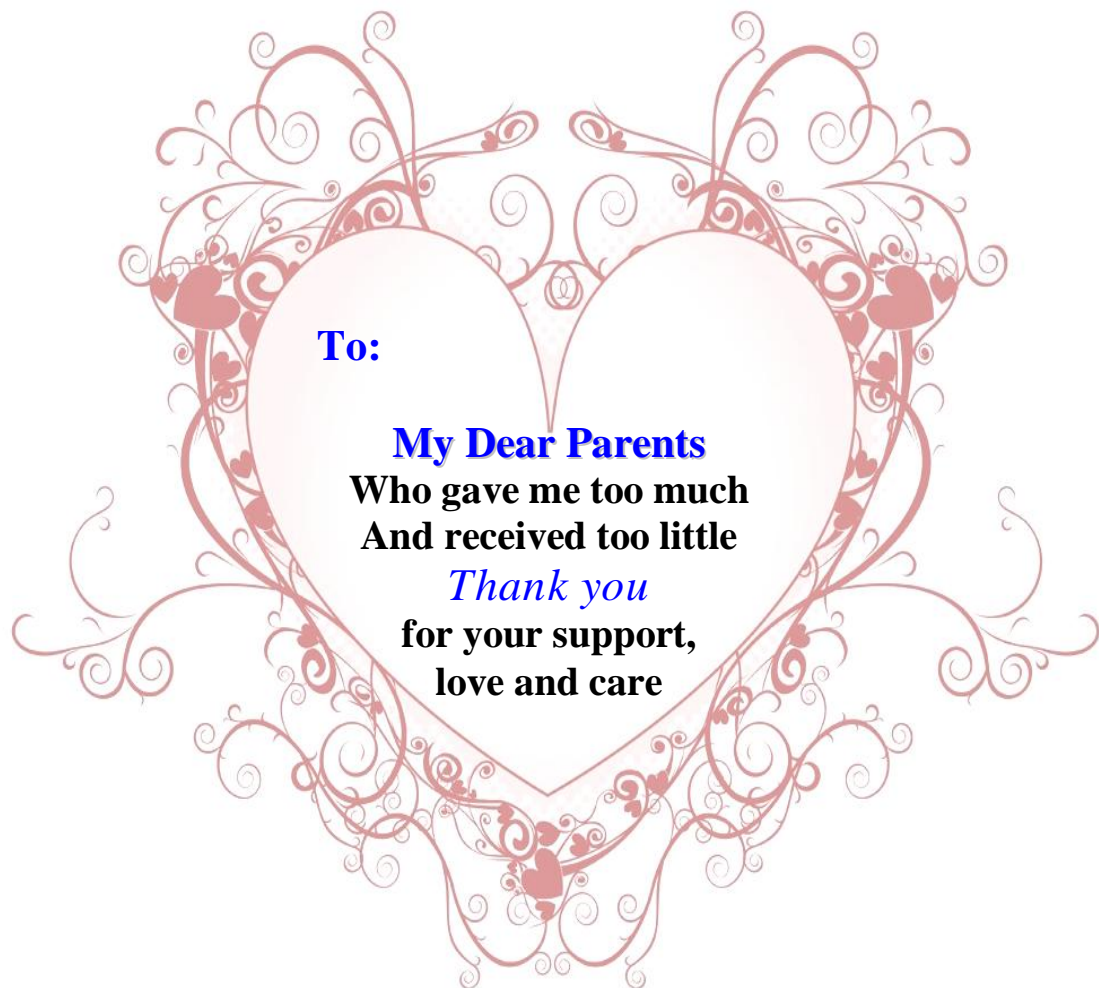
Doctor/ Dina El-Sayed El-Shennawy

Assistant Professor of Clinical and Chemical Pathology

Faculty of Medicine - Ain Shams University

Faculty of Medicine - Ain Shams University

2013



To:

My Dear Parents

**Who gave me too much
And received too little**

Thank you

**for your support,
love and care**

Acknowledgement

*First and foremost, Thanks are given to **God**, the source of all knowledge, by whose abundant aid this work has come to fruition.*

*It has been a great honor to proceed this work under the supervision of **Professor/ Aisha Yassin Abdel-Ghaffar**, Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University. I will never forget her unlimited help, kind encouragement and wise guidance. To her, words of praise are not sufficient and I am really greatly indebted to her.*

*I would like also to express my sincere gratitude and appreciation to **Professor/ Nahla Mohamed Zakaria Yousef**, Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for her enthusiastic guidance, unique effort, valuable advice and generous help throughout this work.*

*I pay my profound thanks to **Assistant Professor/ Amal Ahmed Abbas**, Assistant professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for her enlightening supervision and guidance with valuable comments.*

*Special thanks to **Assistant Professor / Dina El-Sayed El-Shennawy**, Assistant professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for her useful assistance and helpful supervision throughout this work.*

*Last but not least my sincere thanks and appreciation to **Dr. Dina Ali & Dr. Waleed Abdelhady** for their cooperation and profound help in this study.*

*This thesis was supported by the **STDF** grant number 457.*

List of Contents

Title	Page No.
List of Tables.....	i
List of Figures.....	iii
List of Abbreviations	v
Introduction	1
Aim of the Work	3
Review of Literature	
Chpater (1): Hepatitis C Virus.....	4
I- Structure:.....	4
II- Genome:.....	4
III- HCV genotypes:	14
IV- Life cycle	16
V- Immune response	22
VI- Immunosuppression and immunoevasion in HCV infection	36
VII- HCV epidemiology:	42
VIII- HCV transmission:	42
IX - Course of HCV infection	47
X- Treatment for HCV infection	54
XI- The Need for prophylactic and therapeutic HCV vaccines	57
Chapter (2): Human Leukocyte Antigens (HLA)	65
I- Introduction:.....	65
II- HLA class I.....	66
III- HLA class II	70
IV- Inheritance of HLA.....	73
V- HLA polymorphism.....	75
VI- HLA system nomenclature.....	77

List of Contents (Cont...)

Title	Page No.
VII- Association of HLA alleles with diseases	83
VIII- HLA typing:	87
Chapter (3): HLA Alleles in HCV Infection	113
Subjects and Methods.....	118
Results	150
Discussion.....	168
Summary and Conclusion.....	182
Recommendations	185
References	186
Arabic Summary	

List of Tables

Table No.	Title	Page No.
Table (1):	Extrahepatic manifestations of chronic hepatitis C infection	53
Table (2):	Broad antigens and splits	81
Table (3):	Molecular HLA typing techniques.	93
Table (4):	Comparison of HLA typing methods: DNA-based and serologic	111
Table (5):	Results of AST, ALT and HCV-RNA PCR determination in HCV chronic patients.	151
Table (6):	Statistical comparison between the frequencies of HLA-A alleles in healthy control group (130 individuals) with total 260 HLA-A alleles (2 alleles/individual) and in HCV patients (82 patients) with total 164 HLA-A alleles (2 alleles/patient).	153
Table (7):	Statistical comparison between the frequencies of HLA-A alleles in healthy control group (130 individuals) with total 260 HLA-A alleles (2 alleles/individual) and in +ve P.I. HCWs group (22 individuals) with total 44 HLA-A alleles (2 alleles/individual).	157
Table (8):	Statistical comparison between the frequencies of HLA-A alleles in +ve P.I. HCWs group (22 individuals) with total 44 HLA-A alleles (2 alleles/individual) and in -ve P.I. HCWs group (18 individuals) with total 36 HLA-A alleles (2 alleles/individual).	160

List of Tables (Cont...)

Table No.	Title	Page No.
Table (9):	Statistical comparison between the frequencies of HLA-A alleles in +ve P.I HCWs (22 individuals) with total 44 HLA-A alleles (2 alleles/individual) and in chronic HCV patients (82 patients) with total 164 HLA-A alleles (2 alleles/individual).....	162
Table (10):	Statistical comparison of the frequencies of HLA-A alleles in HCV chronic patients with low level of viraemia (L) (31 patients) with total 62 HLA-A alleles (2 alleles/patient) and those with moderate or high level of viraemia (MH) (51 patients) with total 102 HLA-A alleles (2 alleles/patient).....	164
Table (11):	Statistical comparison between the frequencies of HLA-A alleles in HCV infected patients with normal ALT (43 patients) with a total of 86 HLA-A alleles (2 alleles/patient) and those with elevated ALT (39 patients) with a total of 78 HLA-A alleles (2 alleles/patient).....	166

List of Figures

Fig. No.	Title	Page No.
Fig. (1):	Structure of Hepatitis C Virus	4
Fig. (2):	Organization of the hepatitis C virus genome	6
Fig. (3):	Geographic distribution of HCV genotypes.....	15
Fig. (4):	HCV life cycle.....	17
Fig. (5):	Innate and Adaptive immune responses in HCV infection.....	24
Fig. (6):	Immune suppressive mechanisms in HCV infection.....	37
Fig. (7):	Course of acute, resolving hepatitis C	48
Fig. (8):	Course of acute hepatitis C that evolves into chronic infection	50
Fig. (9):	Natural history of HCV infection.....	52
Fig. (10):	Map of the human MHC.....	66
Fig. (11):	Structure of class I MHC molecule.....	67
Fig. (12):	Antigen presentation by MHC class I.....	69
Fig. (13):	Structure of class II MHC molecule	71
Fig. (14):	Antigen presentation by MHC class II	72
Fig. (15):	Segregation of haplotypes in family	74
Fig. (16):	Increasing number of HLA alleles from 1987 to July 2012.....	76
Fig. (17):	HLA nomenclature.....	79
Fig. (18):	Complement mediated microlymphocytotoxicity technique.....	89
Fig. (19):	Reverse SSOP technique	95
Fig. (20):	HLA Genotyping using SSOP in combination with Luminex Technology	97
Fig. (21):	Typing for HLA class II by sequence- specific priming (SSP) technique	99
Fig. (22):	DNA sequencing	102
Fig. (23):	Heteroduplex technique	106
Fig. (24):	Dilution of CFSE with cell division	122

List of Figures (Cont...)

Fig. No.	Title	Page No.
Fig. (25):	Flowcytometry histogram showing HCV specific peptides- stimulated cell proliferation assay by CFSE for a HCW.....	128
Fig. (26):	Principle of INNO-LiPA line probe assay for HLA-A typing.....	134
Fig. (27):	Location of the marker line (Prussian blue line on Strip 1 and turquoise line on Strip 2), the conjugate control line (conj. control), the HLA-A Update control line (HLA-A control), and the 44 probe lines on the INNO-LiPA HLA-A Update Strips.....	145
Fig. (28):	A photo of HLA-A typing strips showing the positive bands.	147
Fig. (29):	A. HLA-A*32 allele frequency % in relation to other alleles in chronic HCV patients and healthy control group; B. HLA-A*92 allele frequency % in relation to other alleles in HCV patients and healthy control group.	155
Fig. (30):	A. HLA-A*32 allele frequency % in relation to other alleles in +ve P.I. HCWs and healthy control group; B. HLA-A*33 allele frequency % in relation to other alleles in +ve P.I. HCWs and healthy control group.....	159
Fig. (31):	Percent bar figure for comparison of HLA-A*01, -A*11, -A*26, -A*31 and -A*69 alleles frequency % in HCV chronic patients with low viraemia and moderate to high viraemia.	165
Fig. (32):	Percent bar figure for comparison of HLA-A*30 and -A*31 alleles frequency % in HCV chronic patients with normal ALT level and those with elevated ALT level.	167

List of Abbreviations

Abb.	Full term
<i>7meG</i>	<i>7-methylguanosine</i>
<i>aa</i>	<i>Amino acid</i>
<i>AIDS</i>	<i>Acquired immunodeficiency syndrome</i>
<i>ALT</i>	<i>Alanine transaminase</i>
<i>APCs</i>	<i>Antigen presenting cells</i>
<i>ARF</i>	<i>Alternate reading frame</i>
<i>ATPase</i>	<i>Adenosine Triphosphatase</i>
<i>BLyS</i>	<i>B-lymphocyte stimulator</i>
<i>bp</i>	<i>Base pairs</i>
<i>CD</i>	<i>Cluster of differentiation</i>
<i>CDC</i>	<i>Complement dependant microlymphocytotoxicity</i>
<i>cDNA</i>	<i>Complementary DNA</i>
<i>CLDN</i>	<i>Claudin</i>
<i>CREG</i>	<i>Cross Reacting Group</i>
<i>CTLs</i>	<i>Cytotoxic T-lymphocytes</i>
<i>DAA</i>	<i>Direct antiviral agents</i>
<i>dATP</i>	<i>Deoxyadenosine triphosphate</i>
<i>DC</i>	<i>Dendritic cell</i>
<i>dCTP</i>	<i>Deoxycytidine triphosphate</i>
<i>ddATP</i>	<i>Dideoxyadenosine triphosphate</i>
<i>ddCTP</i>	<i>Deoxycytidine triphosphate</i>
<i>ddGTP</i>	<i>Dideoxyguanosine triphosphate</i>
<i>ddTTP</i>	<i>Dideoxythymidine triphosphate</i>
<i>DGGE</i>	<i>Denaturing gradient gel electrophoresis</i>
<i>dGTP</i>	<i>Deoxyguanosine triphosphate</i>
<i>DNA</i>	<i>Deoxyribonucleic acid</i>
<i>dsRNA</i>	<i>Double stranded RNA</i>
<i>dTTP</i>	<i>Deoxythymidine triphosphate</i>
<i>E</i>	<i>Envelope</i>
<i>EHMs</i>	<i>Extrahepatic manifestations</i>
<i>eIF2</i>	<i>Eukaryotic Initiation Factor 2</i>

<i>ER</i>	<i>Endoplasmic reticulum</i>
<i>ERAP</i>	<i>ER-resident aminopeptidase</i>
<i>F</i>	<i>Frameshift</i>
<i>Foxp3</i>	<i>Forkhead box P3</i>
<i>GAGs</i>	<i>Glycosaminoglycans</i>
<i>GN</i>	<i>Glomerulonephritis</i>
<i>GWAS</i>	<i>Genome-wide association studies</i>
<i>H⁺</i>	<i>Hydrogen ion</i>
<i>HBV</i>	<i>Hepatitis B virus</i>
<i>HCC</i>	<i>Hepatocellular carcinoma</i>
<i>HCV</i>	<i>Hepatitis C virus</i>
<i>HCV-LPs</i>	<i>HCV-like particles</i>
<i>HDL</i>	<i>High density lipoprotein</i>
<i>HIV</i>	<i>Human immunodeficiency virus</i>
<i>HLA</i>	<i>Human leukocyte antigens</i>
<i>HPV</i>	<i>Human papilloma virus</i>
<i>hsp</i>	<i>Heat shock protein</i>
<i>HVR</i>	<i>Hypervariable regions</i>
<i>IDDM</i>	<i>Insulin dependant diabetes mellitus</i>
<i>IDU</i>	<i>Injection drug use</i>
<i>IFN</i>	<i>Interferon</i>
<i>Igs</i>	<i>Immunoglobulins</i>
<i>IL</i>	<i>Interleukin</i>
<i>IL-2Rα</i>	<i>IL-2 receptor α</i>
<i>IP-10</i>	<i>INFγ-inducible protein 10</i>
<i>IRES</i>	<i>Internal ribosome entry side</i>
<i>IRF3</i>	<i>IFN regulatory factor 3</i>
<i>ISDR</i>	<i>IFN-α sensitivity-determining region</i>
<i>ITIM</i>	<i>Immunoreceptor tyrosine-based inhibitory motif</i>
<i>IU/L</i>	<i>International unit/Litre</i>
<i>IU/ml</i>	<i>International unit/millilitre</i>
<i>JAK</i>	<i>Janus kinase</i>
<i>kb</i>	<i>kilobase</i>
<i>kDa</i>	<i>kilodalton</i>
<i>KIR</i>	<i>Killer immunoglobulin-like receptor</i>
<i>LD</i>	<i>Linkage disequilibrium</i>
<i>LDL</i>	<i>Low density lipoprotein</i>
<i>LDL-R</i>	<i>LDL-receptor</i>

<i>LKM</i>	<i>Liver-kidney microsomal</i>
<i>LTA</i>	<i>Lymphotoxin alpha</i>
<i>LTB</i>	<i>Lymphotoxin beta</i>
<i>Mbp</i>	<i>Mega basepair</i>
<i>MHC</i>	<i>Major histocompatibility complex</i>
<i>MICA</i>	<i>Major histocompatibility complex class I-related chain A</i>
<i>miRNA</i>	<i>MicroRNA</i>
<i>MLC</i>	<i>Mixed lymphocyte culture</i>
<i>mRNA</i>	<i>Messenger RNA</i>
<i>MS</i>	<i>Multiple sclerosis</i>
<i>NF-κB</i>	<i>Nuclear factor kappa B</i>
<i>NHL</i>	<i>Non-Hodgkin's lymphoma</i>
<i>NK</i>	<i>Natural killer</i>
<i>NKT</i>	<i>Natural killer T cell</i>
<i>NS</i>	<i>Non Structural</i>
<i>NTPase</i>	<i>Nucleoside triphosphatase</i>
<i>OCLN</i>	<i>Occludin</i>
<i>ORF</i>	<i>Open reading frame</i>
<i>PBMCs</i>	<i>Peripheral blood mononuclear cells</i>
<i>PCR</i>	<i>Polymerase chain reaction</i>
<i>PD-1</i>	<i>Programmed cell death protein 1</i>
<i>PD-L1</i>	<i>PD-1 ligand</i>
<i>PKR</i>	<i>Protein kinase RNA-activated</i>
<i>RA</i>	<i>Rheumatoid arthritis</i>
<i>RC</i>	<i>Replication complex</i>
<i>RdRp</i>	<i>RNA-dependent RNA polymerase</i>
<i>RF</i>	<i>Rheumatoid factor</i>
<i>RFLP</i>	<i>Restriction fragment length polymorphism</i>
<i>RIG-I</i>	<i>Retinoic acid-inducible gene I</i>
<i>RNA</i>	<i>Ribonucleic acid</i>
<i>ROS</i>	<i>Reactive oxygen species</i>
<i>SL</i>	<i>Stem-loop</i>
<i>SLE</i>	<i>Systemic lupus erythematosus</i>
<i>SNPs</i>	<i>Single nucleotide polymorphisms</i>
<i>SR-BI</i>	<i>Scavenger receptor class B type I</i>
<i>SSCP</i>	<i>Single strand conformation polymorphism</i>
<i>SSOP</i>	<i>Sequence specific oligonucleotide probe/probing</i>

<i>SSP</i>	<i>Sequence specific primer/priming</i>
<i>STAT</i>	<i>Signal transducer and activator of transcription</i>
<i>SVR</i>	<i>Sustained virologic response</i>
<i>TAP</i>	<i>Transporter protein</i>
<i>Taq</i>	<i>Thermus aquaticus</i>
<i>TCRs</i>	<i>T-cell receptor</i>
<i>TGF</i>	<i>Transforming growth factor</i>
<i>TGGE</i>	<i>Temperature gradient gel electrophoresis</i>
<i>Th</i>	<i>T helper</i>
<i>TLR3</i>	<i>Toll like receptor 3</i>
<i>TNF-α</i>	<i>Tumor necrosis factor alpha</i>
<i>Tregs</i>	<i>Regulatory T cells</i>
<i>U/UC</i>	<i>Polyuridine polypyrimidine</i>
<i>UTR</i>	<i>Untranslated region</i>
<i>VLDL</i>	<i>Very low density lipoprotein</i>
<i>WHO</i>	<i>World Health Organization</i>
α	<i>Alpha</i>
β	<i>Beta</i>
λ	<i>Delta</i>

INTRODUCTION

Hepatitis C virus (HCV) was identified and cloned in 1989. It is estimated that more than 170 million persons are infected with HCV world-wide (3% of population) and as many as 3 million individuals are newly infected each year. In 20–30% of infections the virus is cleared spontaneously; however, in the majority of patients the virus persists. The mechanism by which some individuals spontaneously resolve infection, while others become chronically infected is not clearly understood (**Edwards et al., 2012**). Both virus-related factors such as viral heterogeneity and replicative activity and the host determinants such as lack of efficient immune responses are involved in the pathogenesis of chronic hepatitis (**Tripathy et al., 2009**).

The most striking features of HCV are its propensity to persist in a large proportion of infected individuals and the broad spectrum of liver disease that result from infection. Currently, there is no available vaccine to prevent HCV infection (**Obeid, 2011**). Studies in humans and animal models of HCV infection have demonstrated that HCV elicits innate immune responses early after infection. However, the virus can persist in the face of the innate immune response. Indeed, viral clearance occurs only in the presence of antiviral CD4⁺ and CD8⁺ T cell responses (**Dustin and Rice, 2007**).

A successful T cell response requires the presentation of viral peptides bound to HLA molecules on the surface of