



***Impact of Human Leukocyte antigen (HLA)
typing on the outcome of Hepatitis C Virus
(HCV) infection in Egyptian patients***

Thesis submitted for M.Sc. Degree
as a partial fulfilment for requirements of the master in Science
(Microbiology)

By

Mai Mohamed El-Sayed Lotfy

(B.Sc. Microbiology Department, Ain Shams University 2007)

Supervisors

**Prof. Dr. Ahmed Barakat
Barakat**

Professor of Virology,
Microbiology Department,
Faculty of Science,
Ain Shams University.

**Prof. Dr. Abd El-Rahman N.
Zekri**

Professor of Virology and Immunology,
Cancer Biology Department,
National Cancer Institute,
Cairo University.

Prof. Dr. Ashraf Omar Abd El-Azeez

Professor of Tropical Medicine,
Faculty of Medicine,
Cairo University.

Microbiology Department,
Faculty of Science,
Ain Shams University.

2016



Approval sheet

Title of Thesis: “Impact of Human Leukocyte antigen (HLA) typing on the outcome of Hepatitis C Virus (HCV) infection in Egyptian patients.”

Degree: M.SC. In Microbiology

Name of students: Mai Mohamed El-Sayed Lotfy

This Thesis for M.SC degree has been approved by:

Supervisors

Approved

Prof. Dr. Ahmed Barakat Barakat

Virology, Microbiology Department, Faculty of Science,
Ain Shams University.

Prof. Dr. Abd El-Rahman N. Zekri

Virology and Immunology, Cancer Biology Department,
National Cancer Institute, Cairo University.

Prof. Dr. Ashraf Omar Abd El-Azeez

Tropical Medicine, Faculty of Medicine, Cairo University.

Examination committee:

Prof. Dr. Ahmed Barakat Barakat

Prof. of Virology, Microbiology
Department, Faculty of Science, Ain
Shams University.

Prof. Dr. Abd El-Rahman N. Zekri

Prof. of Virology and Immunology,
Cancer Biology Department, National
Cancer Institute, Cairo University.

Prof. Dr. Nabila Anwer El Sheikh

Prof. of Immunology, Faculty of
Medicine for Girls, Al Azhar University.

Prof. Dr. Mohamed Ahmed Aly

Prof. of Virology, National Research
Center.

Date of examination / /

Approval date / /

Faculty Council approved / /

University Council approved / /

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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

صدق الله العظيم

{ البقرة آيه: 32 }

Declaration

I certify that the thesis titled "Impact of Human leukocyte antigen (HLA) Typing on the Outcome of Hepatitis C Virus (HCV) Infection in Egyptian patients" is my own work. The work has not been presented elsewhere for assessment. Where material has been used from other sources it has been properly acknowledged / referred.

Signed

Mai Mohamed El-Sayed Lotfy

LIST OF CONTENTS

<i>Acknowledgment</i>	
<i>Abstract</i>	I
<i>List of abbreviations</i>	III
<i>List of figures</i>	VII
<i>List of tables</i>	VIII
<i>1. Chapter I: Introduction and aim of work</i>	1
<i>2. Chapter II: Review of literature</i>	8
<i>Part I: Hepatitis C virus</i>	
2.1 Discovery of Hepatitis C virus (HCV)	8
2.2 HCV Structure and genomic organization	9
2.2.1 HCV structure	9
2.2.2 HCV genome organization	11
2.3 Genotypes and worldwide distribution	13
2.3.1 HCV genotypes	13
2.3.2 HCV genotypes distribution	14
2.4 Transmission of HCV infection	15
2.5 HCV Epidemiology	17
2.5.1 HCV global prevalence	18
2.5.2 HCV prevalence in Egypt	19
2.6 Natural History of HCV infection	21
2.6.1 Hepatic manifestations of HCV infection	22
2.6.1.1 Acute hepatitis C	22
2.6.1.2 Chronic hepatitis C	23
2.6.1.3 Hepatocellular carcinoma	24
2.6.2 Extra hepatic manifestations of HCV infection	26
2.7 Serological and Molecular diagnosis of HCV infection	27
2.7.1 Serological diagnosis of HCV infection	27
2.7.1.1 HCV antibody assays (EIAs)	28
2.7.2. Molecular diagnosis of HCV infection	29
2.7.2.1 Qualitative tests	30

2.7.2.2 Quantitative tests -----	30
2.8 Treatment of Hepatitis C (antiviral therapies) -----	31

Part II: Human leukocyte Antigen (HLA)

2.9 Definition -----	36
2.10 History -----	36
2.11 Genomic map of HLA genes -----	38
2.11.1 HLA class I -----	39
2.11.2 HLA class II -----	40
2.11.3 HLA class III -----	41
2.12 HLA Nomenclature -----	42
2.13 HLA Structure -----	45
2.13.1 HLA class I structure -----	45
2.13.2 HLA class II structure -----	46
2.14 HLA-peptide interaction -----	48
2.14.1 HLA class I-peptide interaction -----	49
2.14.2 HLA class II-peptide interaction -----	49
2.15 HLA Polymorphism -----	50
2.16 HLA Function -----	51
2.16.1 HLA class I antigen processing and presentation -----	52
2.16.2 HLA class II antigen processing and presentation -----	53

Part III: Role of HLA in innate and adaptive cellular immune response versus HCV infection.

2.17 Innate cellular immune response to HCV infection -----	55
2.18 Bridging innate and adaptive immunity -----	59
2.19 Adaptive cellular immune responses in HCV infection -----	60
2.20 Evasion of the Immune System by HCV -----	64
3. Chapter III: Patients and methods -----	68
3.1. Inclusion criteria of the participants -----	68
3.2. Exclusion criteria of the participants -----	68
3.3. Assigned groups of the included patients -----	69
3.4. The laboratory investigations of the selected patients -----	71

LIST OF CONTENTS

3.5. Other investigations of the selected patients -----	72
3.6. Sample collection and preparation -----	72
3.6.1 Sample collection -----	72
3.6.2 Sample preparation -----	73
3.6.2.1 Viral RNA extraction -----	73
3.6.2.2 Genomic DNA extraction -----	73
3.7 HLA typing by SSO for the selected patients -----	73
3.7.1 Principle -----	73
3.7.2 Materials -----	74
3.7.2.1 Reagents -----	74
3.7.2.2 Other materials -----	75
3.7.2.3 Instrument requirements -----	76
3.7.3 HLA typing Procedure -----	77
3.8 Statistical analysis -----	83
4. Chapter IV: Results -----	84
4.1. Demographic and clinical data of the studied participant -----	84
4.2. Laboratory data of the studied participants -----	87
4.3. HLA typing analysis -----	93
5. Chapter V: Discussion -----	109
6. Chapter VI: Summary and conclusion -----	118
7. Chapter VII: References -----	125
Arabic summary -----	

Acknowledgment

*First of all, thanks to **GOD** for his grace and mercy, and for giving me the effort to complete this work.*

*Completing a M.Sc. is a marathon event, and I would not have been able to complete this journey without the aid and support of countless people over the past five years. I must first express my gratitude towards my advisor, Professor **Abel-Rahman Zekri**. His leadership, support, attention to detail and hard work have set an example I hope to match some day. Thanks for always being a father before being a teacher.*

*I would additionally like to thank Prof. **Ahmed Barakat** for his support in both the research and especially the revision process that has lead to this document. His knowledge and understanding of the written word has allowed me to fully express the concepts behind this research.*

*I also extend my thanks and appreciation to **Prof. Ashraf Omar**, he gave me much of his time and experience. His valuable comments were the causes to complete this work properly.*

I would like to thank my loved ones, who have supported me throughout entire process, both by keeping me harmonious and helping me putting pieces together. I will be grateful forever for your love.

Finally, I thank my adorable parents for instilling in me confidence, love, support and a drive for pursuing my M.Sc.

Abstract

Abstract:

Hepatitis C (HCV) is the most pressing public health challenge in Egypt; 10% of Egyptians between 15 – 59 years of age had been infected with HCV infection, while 7% are chronic active hepatitis C patients. Although, progresses were made in antiviral therapies; this cannot ensure controlling the virus infection due to many reasons one of which is the lack of HCV vaccine. Achievement of this goal will be fulfilled from a clear understanding of virus–host interactions and protective immunity in HCV infection taking in consideration the ethnic and geographical variations. This preliminary study was designed aiming to find out the frequencies of HLA Class I and II alleles in HCV infected Egyptian patients for assessing the correlations between HLA phenotypes and the consequences of HCV infection.

Two hundreds and eighty two HCV subjects were enrolled in the present study and they were categorized into 7 groups; Group1: Spontaneous clearance (**SC**: n=37), Group 2: Asymptomatic HCV infection (**AS**: n=40), Group 3: Chronically infected HCV patients (**CHCm**: n= 86 with no, mild or moderate fibrosis); Group 4: Chronically infected HCV patients (**CHCa**: n=12 with advanced fibrosis); Group 5: Cirrhotic liver disease either compensated or decompensated (**LC**: n=42), Group 6:

Hepatocellular Carcinoma patients (**HCC**: n=40), Group 7: Healthy Controls (**N**: n=25). HLA alleles were typed by DNA based Typing method “LABType® sequence-specific oligonucleotide (SSO) probes” for HLA-A, HLA-B, HLA-C, HLA-DRB1 and HLA-DQ (A and B) alleles from peripheral blood of all participants.

The results of this work implicate that special HLA patterns were found; *HLA-DRB1*03*, *-DRB1*14* antigen may play a role in viral persistence. While, *HLA-C*05*, *-DRB1*11*, *-DQA1*05* and *-DQB1*03* is associated with viral clearance. In conclusion, HLA system is an ideal system in which to explore predictions on the outcome of HCV infections in Egyptians’ gene pool. These data support the critical role of the critical role of some HLA alleles of class I and II that consequently confirm that successful clearance of HCV infection requires strong CD4+ and CD8+ T cell responses. Also, the data generated from our study may help clinicians to predict the treatment outcome for HCV- seropositive individuals in Egypt. Further investigations in this regard with larger number of HCV patients are recommended.

Keywords: HLA, MHC, Hepatitis C virus, Egyptian patients

LIST OF ABBREVIATIONS

AFP:	Alpha Feto Protein
ALT:	Alanine aminotransferase
APC:	Antigen Presenting Cells
AST:	Aspartate aminotransferase
bDNA:	branched DNA Assay
C:	Core protein
CDC:	Centers for Disease Control and Prevention
Cen:	Centromeric
CHC:	Chronic hepatitis C
CLIP:	Class II-associated Invariant Chain Peptide
CTL:	Cytotoxic T Lymphocytes
DAAs:	Directly Acting Antivirals
DCs:	Dendritic Cells
DNA:	Deoxyribonucleic Acid
dsRNA:	double strand RiboNucleic Acid
E:	Envelope Glycoproteins
EIA:	Enzyme Immunoassay
ELISA:	Enzyme linked immunosorbent assay
EM:	Electron Microscopy
EOT:	End of Treatment
ER:	Endoplasmic Reticulum
eRVR:	Extended Rapid Virological Response
ETR:	End of Treatment Response
EVR:	Early Virological Response
FDA:	Food and Drug Administration
GAG:	Glycosaminoglycans
GWAS:	Genome Wide Association Studies

LIST OF ABBREVIATIONS

HAV:	Hepatitis A virus
HCC:	Hepatocellular Carcinoma
HCV:	Hepatitis C virus
HIV:	Human Immunodeficiency Virus
HLA:	Human Leukocyte Antigen
HSPs:	Heat-Shock Proteins
HVR:	Hypervariable Region
IDU:	Injecting Drug Use
IFN:	Interferon
Ii:	Invariant Chain
IL:	Interleukin
INR:	International Normalized Ratio of prothrombin time of blood coagulation
IRES:	Internal Ribosome Entry Site
IRF:	IFN Regulatory Factors
ISGs:	Interferon Stimulated Genes
IU:	International Unit
KB:	Kilobase
KDa:	Kilodalton
KIR:	Killer cell Immunoglobulin like Receptors
LDL:	Low-Density Lipoproteins
LDL-R:	Low-Density Lipoproteins Receptor
LMP:	Low-Molecular-weight Proteins
MAVS:	Mitochondrial Antiviral Signaling
MHC:	Major Histocompatibility Complex
mRNA:	Messenger RNA
NAT:	Nucleic acid Testing

LIST OF ABBREVIATIONS

NHANES:	Health and Nutrition Examination Study
NK:	Natural Killer Cells
NR:	IFN Non Responders
NS:	Non Structural Proteins
NTRs:	Non-translated Regions
OD:	Optical Density
ORF:	Open Reading Frame
PAMPs:	Pathogen Associated Molecular Patterns
PCR:	Polymerase Chain Reaction
PCT:	Porphyria Cutanea Tarda
PEG-IFN α:	Pegylated interferone- α
PKR:	Protein-Kinase R
pMHC:	peptide-MHC Complex
PRRs:	Pattern Recognition Receptors
RBV:	Ribavirin
RdRp:	RNA-dependent RNA polymerase
RGT:	Response Guided Therapy
RIBA:	Recombinant Immunoblot Antibody Assay
RIG-I:	Retinoic-acid-Inducible Gene I
RNA:	Ribonucleic Acid
RT-PCR:	Reverse transcription polymerase chain reaction
RVR:	Rapid Virological Response
SR-BI:	Scavenger Receptor class B type I
SVR:	Sustained Virological Response
TAP:	Transporter Associated with Antigen- Processing
TCR:	T Cell Receptor
Tel:	Telomeric
Th:	T Helper cells

LIST OF ABBREVIATIONS

TLR:	Toll Like Receptors
TMA:	Transcription-Mediated Amplification
TNF:	Tumor Necrosis Factor
Tregs:	Regulatory T cells
UTR:	Untranslated Region
WHO:	World Health Organization