

Single Nucleotide Polymorphisms of Toll-Like Receptor 7 in patients with Chronic Hepatitis C Versus Chronic Hepatitis C with Hepatocellular Carcinoma

Thesis

Submitted for Partial Fulfillment of M.D Degree in
Medical Microbiology and Immunology

By

Yara Said Abdel Ghany Elsedawy

M.B.,B.Ch

Master of Medical Microbiology and Immunology- Ain Shams University

Under supervision of

Prof. Abeer Abd EL-Fattah El-Sayed

Professor of Medical Microbiology and Immunology
Faculty of Medicine-Ain Shams University

Prof. Soha Abdel-Rahman El- Hady

Professor of Medical Microbiology and Immunology
Faculty of Medicine-Ain Shams University

Dr. Mona Adel Salah Khattab

Lecturer of Medical Microbiology and Immunology
Faculty of Medicine-Ain Shams University

Dr. Ashraf Mohamed Elbreedy

Assistant professor of Tropical Medicine
Faculty of Medicine-Ain Shams University

Dr. Zeinab Mohamed Hefny

Lecturer of Tropical Medicine
Faculty of Medicine-Ain Shams University

**Faculty of Medicine
Ain Shams University**

2016

Abstract

Background: After discovery of the hepatitis C virus (HCV) more than 20 years ago, HCV infection has become a global problem that requires active interventions for prevention and control. There is a significant association between chronic hepatitis C infection and the development of cirrhosis and hepatocellular carcinoma worldwide. Epidemiological studies estimate that 130-170 million persons or 2-3% of the world population are infected with HCV. Egypt has the greatest burden of HCV infection in the Middle East, where 15% of the population are infected with HCV where HCV genotype-4 (HCV-4) represents 90% of infected cases.

Aims: The aim of this study is to determine the association between TLR7 (rs179008 gene) polymorphism and (Chronic hepatitis C infection with and without Hepatocellular Carcinoma).

Methodology: The present study was conducted on 59 patients suffering from HCV and HCC and 21 sex and age matched healthy controls. Among the 59 patients, 33 were men and 26 women. Their ages ranged from 28 to 67 years.

Results: This study was conducted on 59 patients diagnosed as chronic HCV infection (**Group 1**). It divided into 29 patients with Chronic hepatitis C virus infection not associated with hepatocellular carcinoma, 17 males and 12 females (**Group 1a**). Thirty patients with Chronic hepatitis C virus infection associated with hepatocellular carcinoma 16 males and 14 females (**Group 1b**). Twenty one age and sex matched apparently healthy volunteers with no history of liver disease as a control group (**Group 2**). Eleven females and 10 males.

Conclusions: This study shows that (AT) genotype is significantly higher in female HCC group compared to control group. These patients could then be subjected to a more careful or earlier routine screening for HCC.

Recommendations: Considering detection of TLR7 gene polymorphism as a screening marker to detect patients with an increased risk to develop HCV and HCC.

Keywords: Toll-Like Receptor 7, Hepatitis C Versus, Chronic Hepatocellular Carcinoma, IFN-a

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم العظيم

صدق الله العظيم

سورة البقرة الآية: ٣٢



ACKNOWLEDGEMENT

*First of all, thanks to **Allah** whose magnificent help was the main factor in completing this work.*

I am greatly honored to express my deep gratitude to **Dr. Abeer Abd EL-Fattah El-Sayed**, Professor of Microbiology & Immunology Faculty of Medicine, Ain Shams University. She gave me much of her time, experience, meticulous advice, kind support & mastery teaching, without her care this work could never be within hands.

I wish to thank **Dr. Soha Abdel-Rahman El- Hady**, Professor of Microbiology & Immunology Faculty of Medicine for her active participation & kind supervision, great and effective help, tremendous assistance and valuable criticism.

I am sincerely grateful to **Dr. Mona Adel Salah Khattab**, lecturer of Microbiology & Immunology Faculty of Medicine, Ain Shams University, for her great help and assistance.

I am sincerely grateful to **Dr. Ashraf Mohamed Elbreedy**, Assistant Professor of Tropical Medicine Faculty of Medicine, Ain Shams University, for his great help and assistance.

I am sincerely grateful to **Dr. Zeinab Mohamed Hefny**, Lecturer of Tropical Medicine, Faculty of Medicine, Ain Shams University, for her great help and assistance

Yara Said Abdel Ghany Elsedawy

Contents

Subjects	Page
List of abbreviations.....	II
List of Figures.....	V
List of Tables.....	VI
• Introduction	1
• Aim of the Study	4
• Review of Literature	
♦ Chapter (1): Hepatitis C Virus	5
♦ Chapter (2): Hepatocellular carcinoma	27
♦ Chapter (3): Toll Like Receptor	43
• Patients and Methods	59
• Results	69
• Discussion	82
• Summary	97
• Conclusion	101
• Recommendation	102
• References	103
• Arabic Summary	

List of Abbreviations

Abbrev.	Meaning
AASLD	Association for the Study of Liver Diseases
AFB1	Aflatoxin B1
AFP	alpha-fetoprotein
AIH	Autoimmune hepatitis
ALT	Alanine transaminase
APCs	antigen presenting cells
AST	Aspartate transaminase
cDC	Conventional dendritic cell
CDK	cyclin-dependent kinase
CT	Computed tomography
CTLs	cytotoxic T lymphocytes
CIA	Chemiluminescent immunoassays
DAAs	Directly acting antiviral agents
DCs	dendritic cells
DEAE	Diethylaminoethyl
DM	Diabetes mellitus
EIA	Enzyme immunoassays
FDA	Food and Drug Administration
Gln	Glutamine
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus

List of Abbreviations

HLA	Human leucocyte antigen
HUH7	Human hematoma cell line
IFN	Interferon
IL	Interleukin
IRF	Interferon Regulatory factor
ITIM	immunoreceptor tyrosine–based inhibition motif
KC	Kuffer Cells
KIRs	Killer Inhibitory Receptors
LDL	Low-density lipoprotein
Leu	Leucine
LRR	Leucine rich repeat
LSECs	Liver sinusoidal endothelial cells
MAPK	Mitogen-activated protein kinases
MyD88	Myeloid differentiation factor 88
MHC	Major histocompatibility complex
MRI	Magnetic resonance imaging
NAFLD	Non-alcoholic fatty liver disease
NF	Nuclear factor
NK	Natural killer
NS	Non Structural Protein
PAMPs	Pathogen associated molecular patterns
PBMCs	Peripheral Blood Monocytes
PCR	Polymerase chain reaction
PD1	Programmed cell death protein 1
PDCs	plasmacytoid dendritic cells

List of Abbreviations

PDL1	Programmed cell death protein 1 ligand
PEG-IFN	Pegylated-interferon
PKR	protein kinase R
PRRs	pattern recognition receptors
PT	Prothrombin time
PTT	Partial Prothrombin time
Rb	retinoblastoma
RFA	radiofrequency ablation
RIG	retinoic acid-inducible gene
RNA	Ribonucleic acid
RNI	Reactive nitrogen intermediates
ROI	Reactive oxygen intermediates
RPM	Run per minute
SARM	Sterile-alpha and Armadillo motif containing protein
ssRNA	Single stranded Ribonucleic acid
SNP	single nucleotide polymorphisms
SR-B1	scavenger receptor class B type 1
SVR	sustained virologic response
TACE	Transarterial chemoe-mbolization
TAP1	transporter associated with antigen processing 1
TGF-β	Transforming Growth Factor Beta
TH	T Helper
TIRAP	TIR domain containg adaptor protein
TIR-domain	Toll/interleukin-1 receptor domain

List of Abbreviations

TLR	Toll Like Receptor
TNF	Tumor Necrosis Factor
TRAM	TRIF-related adaptor molecule
TRIF	TIR-domain-containing adaptor-inducing beta interferon
WHO	World Health Organization

List of Figures

<u>No.</u>	<u>Figure</u>	<u>Page</u>
<u>1</u>	Toll Like receptor signaling.	47
<u>2</u>	Comparison between HCV male cases and male controls regarding SNP.	73
<u>3</u>	Comparison between HCC male cases and male controls regarding SNP.	74
<u>4</u>	Comparison between HCV female cases and female controls regarding SNP.	75
<u>5</u>	Comparison between HCC female cases and female controls regarding SNP.	76
<u>6</u>	Comparison between HCV male cases and HCC male cases regarding SNP.	77
<u>7</u>	Comparison between HCV female cases and HCC female cases regarding SNP.	78
<u>8</u>	Relation between AFP and SNP among HCV male cases.	79
<u>9</u>	Relation between AFP and SNP among HCV female cases.	80

List of Tables

<u>No.</u>	<u>Table</u>	<u>Page</u>
<u>1</u>	Demographic data of the patient groups.	70
<u>2</u>	Clinical data of the patients group (group 1).	71
<u>3</u>	Laboratory findings in the patients group.	72
<u>4</u>	Comparison between TLR7 SNP in HCV male cases and males control.	73
<u>5</u>	Comparison between TLR7 SNP HCC male cases and male controls regarding SNP.	74
<u>6</u>	Comparison between HCV female cases and female controls regarding TLR7 SNP.	75
<u>7</u>	Comparison between HCC female cases and female controls regarding TLR7 SNP.	76
<u>8</u>	Comparison between HCV male cases and HCC male cases regarding TLR7 SNP.	77
<u>9</u>	Comparison between HCV female cases and HCC female cases regarding SNP.	78
<u>10</u>	Relation between AFP and SNP among HCV male cases.	79
<u>11</u>	Relation between AFP and SNP among HCV female cases.	80
<u>12</u>	Relation between AFP and SNP among HCC male cases.	81
<u>13</u>	Relation between AFP and SNP among HCC female cases.	83

Introduction

After discovery of the hepatitis C virus (HCV) more than 20 years ago, HCV infection has become a global problem that requires active interventions for prevention and control. There is a significant association between chronic hepatitis C infection and the development of cirrhosis and hepatocellular carcinoma worldwide (*Wei et al., 2014*). Epidemiological studies estimate that 130-170 million persons or 2-3% of the world population are infected with HCV (*Shepard et al., 2005*). Egypt has the greatest burden of HCV infection in the Middle East, where 15% of the population are infected with HCV where HCV genotype-4 (HCV-4) represents 90% of infected cases (*Mandour et al., 2014*).

As immunity plays a decisive role in host-virus interactions, the outcome of patients with hepatitis virus infection is closely linked to their immune status (*Thio, 2008*). Appropriate sensing and recognition of invasive microbes is an essential task of the immune response that occurs through a variety of pattern recognition receptors, including Toll-like receptors (*Rassa and Ross, 2003*).

Toll-like receptors (TLRs), belonging to a family of pathogen recognition receptors, are an essential part of the innate immune response and can detect conserved

pathogen-associated molecular pattern (PAMPs) of bacteria, parasites, fungi, protozoa components, and viruses (*Takeda et al., 2003*). Stimulation of TLRs by their ligands initiates signaling pathways to produce type I IFN, which is the first line of defense employed by the host to combat hepatitis viruses (*Beutler et al., 2006*).

TLR7 is mainly expressed in the endosome-lysosome membrane of plasmacytoid dendritic cells (pDCs), hepatic natural killer cells, and B lymphocytes. When the phagocytes take up a virus or virus-infected apoptotic cell, phagolysosome will degrade enzymes to release viral RNA, leading to ssRNA release and recognition by TLR7 (*Askar et al., 2010*).

TLR7 has been of great medical importance because its small molecule ligands may serve as immune stimulants by enhancing endogenous IFN- α production and thus, they may complement IFN- α therapy of chronic infection especially in IFN- α -resistant patients (*Horsmans et al., 2005*).

TLR7 plays an important role in defense against HCV-infection, because its engagement leads to production of increased levels of interferon- α (*Heil et al., 2004*). Upon TLR7 stimulation, a complex cascade is formed, starting with myeloid differentiation factor 88 (MyD88) and ending

with the production of IFN- α /IFN-inducible genes and proinflammatory cytokines through the phosphorylation of interferon regulatory factor 7 and the liberation of nuclear factor-Kb (*Seki and Brenner, 2008*).

Hepatitis viruses have developed different evasive strategies to subvert this innate immunity. One of these strategies modulating TLR-mediated signaling pathways to inhibit the production of IFN- α and IFN- β and other inflammatory cytokines (*Miyazaki et al., 2008*).

Down-regulation of TLR7 in hepatocytes has been proposed to be the exclusive mechanism accounting for persistent hepatitis virus infection and hepatocyte transformation (*Chang et al., 2010*). Number of studies suggested associations between single nucleotide polymorphisms (SNP) in TLRs and increased risk of bacterial infections, viral infections, autoimmune disease, and malignancies (*Wei et al., 2014*).

Determination of TLR7 gene polymorphism in Chronic Hepatitis C and HCC among Egyptian patients can help in detecting patients with increased risk to develop HCC and could be a new therapeutic target for preventing the initiation or progression of HCC.

Aim of the Work

The aim of this study is to determine the association between TLR7 (rs179008 gene) polymorphism and (Chronic hepatitis C infection with and without Hepatocellular Carcinoma).