

**TOLL- LIKE RECEPTOR 2  
EXPRESSION IN PATIENTS WITH  
CHRONIC HEPATITIS C INFECTION**

*Thesis*

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*Presented by*

**Safaa Mohamed Abdel-Rahman**

M.B, B. Ch, M. Sc.

*Under supervision of*

**Prof. Tahany Ahmed Abdel-R aouf**

Professor of Medical Microbiology and  
Immunology

Faculty of Medicine-Ain Shams University

**Prof. Sanaa Mohamed Ibrahim Zaki**

Professor of Medical Microbiology and  
Immunology

Faculty of Medicine-Ain Shams University

**Dr. Shereen Bendary- EL Sayed**

Ass. Professor of Medical Microbiology and  
Immunology

Faculty of Medicine-Ain Shams University

**Dr. Amany Ahmed Ibrahim**

Ass. Professor of Tropical Medicine  
Faculty of Medicine-Ain Shams University

Faculty of Medicine  
Ain Shams University

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## LIST OF ABBREVIATIONS

<b>μl</b>	micro liter
<b>ALT</b>	Alanine Aminotransferase
<b>AP-1</b>	Activating protein-1
<b>ARF</b>	Alternative reading frame
<b>AST</b>	Aspartate Aminotransferase
<b>Bambi</b>	Bone morphogenetic protein and activin membrane bound inhibitor
<b>BLPs</b>	Bacterial lipopeptides
<b>CBC</b>	Complete blood count
<b>CD</b>	Cluster of differentiation
<b>CpG</b>	Cytosine-phosphate-guanine
<b>DC</b>	Dendritic cells
<b>DD</b>	Death domain
<b>DNA</b>	Deoxyribonucleic acid
<b>dsRNA</b>	Double-stranded RNA
<b>E</b>	Envelope glycoproteins
<b>ECM</b>	Extracellular matrix
<b>EDTA</b>	Ethylene diamine tetra acetic acid
<b>EGF</b>	Epidermal growth factor
<b>EIA</b>	Enzyme immunoassay
<b>ER</b>	Endoplasmic reticulum
<b>ERK</b>	Extracellular signal regulated kinase
<b>EVR</b>	Early virologic response
<b>FITC</b>	Flourescin isothiocyanate
<b>fl</b>	Femtoliter,
<b>g / dl</b>	Gram/ deciliter
<b>HCC</b>	Hepatocellular carcinoma
<b>HCV</b>	Hepatitis C virus
<b>HCV-G4</b>	HCV- genotype 4
<b>Hb</b>	Hemoglobin
<b>HGF</b>	Human growth factor
<b>HIV</b>	Human immunodeficiency virus
<b>HRP</b>	Horseradish peroxidase
<b>HSCs</b>	Hepatic stellate cells
<b>HSP</b>	Heat shock protein
<b>HVR</b>	Hypervariable region
<b>ICAM-1</b>	Intercellular cell adhesion molecule-1
<b>IFN</b>	Interferon

<b>IKK</b>	I $\kappa$ B kinase
<b>IL-1R</b>	IL-1 receptor
<b>IL</b>	Interleukin
<b>IP</b>	Interferon- inducible protein
<b>IPS-1</b>	Interferon- $\beta$ promoter stimulator 1
<b>IRAK</b>	IL-1 receptor-associated kinase
<b>IRES</b>	Internal ribosomal entry site
<b>IRF</b>	Interferon regulatory factor
<b>IRG</b>	Immunoresponsive gene
<b>IU/L</b>	International unit/ liter
<b>JNK</b>	Jun N-terminal kinase
<b>KDa</b>	Kilo dalton
<b>LBP</b>	LPS binding protein
<b>LPS</b>	Lipopolysaccharide
<b>m DCs</b>	Myeloid denderitic cells
<b>MAbs</b>	Monocolonal antibodies
<b>Mal</b>	MyD88 adaptor-like molecule
<b>MAP</b>	Mitogen-activated protein
<b>MCH</b>	Mean corpuscular hemoglobin
<b>MCV</b>	Mean corpuscular volume
<b>MD-2</b>	Myeloid differentiation protein-2
<b>MFI</b>	Mean fluorescence intensity
<b>mg/dl</b>	Milligrams/deciliter
<b>ml</b>	Milliliter
<b>MyD88</b>	Myeloid differentiation factor 88
<b>NF-<math>\kappa</math>B</b>	Nuclear transcription factor- $\kappa$ B
<b>NK</b>	Natural killer cells
<b>nm</b>	nanometer
<b>NS</b>	Non- structural protein
<b>NTPase</b>	Nucleoside triphosphatase
<b>ORF</b>	Open reading frame
<b>P7</b>	Polyprotein 7
<b>PAMP</b>	Pathogen- associated molecular pattern
<b>PBMC</b>	Peripheral blood mononuclear cells
<b>PBS</b>	Phosphate buffer saline
<b>PCR</b>	Polymerase chain reaction
<b>pDCs</b>	plasmacytoid denderitic cells
<b>Peginterferon</b>	pegylated interferon
<b>PG</b>	Peptidoglycan

<b>Pg</b>	Picograms
<b>PH</b>	Partial hepatectomy
<b>poly(I:C)</b>	Polyinosinic-polycytidylic acid
<b>RBC</b>	Red blood cell
<b>RdRp</b>	RNA-dependent RNA polymerase
<b>RIG- I</b>	RNA helicase retinoic acid-inducible gene I
<b>RNA</b>	Ribonucleic acid
<b>RT-PCR</b>	Reverse transcription polymerase chain reaction
<b>SRB-I</b>	Human scavenger receptor class B type I
<b>ssRNA</b>	Single-stranded RNA
<b>STAT-C</b>	Specifically targeted antiviral therapy drugs for HCV
<b>SVR</b>	Sustained virologic response
<b>TAK</b>	transforming growth factor activated kinase
<b>TBK</b>	TRAF family member-associated nuclear factor kB activator-binding Kinase
<b>TGF -B</b>	tumor growth factor-B
<b>TICAM</b>	TIR domain-containing adaptor molecule
<b>TIR</b>	Toll/interleukin-1 receptor
<b>TIRAP</b>	TIR domain containing adaptor protein
<b>TLR</b>	Toll-like receptor
<b>TMB</b>	Tetramethylbenzidine
<b>TNF</b>	Tumor necrosis factor
<b>TNF-R</b>	Tumor necrosis factor receptor
<b>TRAF</b>	TNF receptor associated factor
<b>TRAM</b>	TRIF-related adaptor molecule
<b>Treg</b>	Regulatory T cells
<b>TRIF</b>	TIR-domain-containing adapter-inducing interferon-Beta
<b>Ubc</b>	Ubiquitin-conjugating enzymes
<b>VCAM-1</b>	Vascular cell adhesion molecule 1
<b>WBC</b>	White blood cells

## **I-Introduction**

Although hepatitis C virus (HCV) only accounts for a minority of cases of clinical acute hepatitis, it is a major cause of chronic liver disease and liver transplantation in both developed and developing countries (**WHO, 2007**). The global prevalence of HCV is 3% with 170 million persons infected worldwide. Egypt has the highest prevalence of hepatitis C in the world, reaching 13% of the population, equating to an estimated 10 million anti-HCV-positive persons (**Kamal, 2008**).

While 20–50% of patients with acute hepatitis achieve spontaneous resolution, between 50% and 80% of individuals develop chronic infection, Approximately 20% of chronic hepatitis C patients can be expected to develop cirrhosis; of these, 6% will decompensate to end-stage liver disease and an additional 4% will develop hepatocellular carcinoma (HCC) (**Thomas and Seeff, 2005**).

The discovery and characterization of Toll-like receptor (TLR) family have increased our understanding of how the innate immune system recognizes pathogens by recognition of their pathogen- associated molecular pattern (PAMP) and how its activation lead to appropriate and effective immune response . Among TLRs, TLR2 recognizes a wide range of PAMPS including peptidoglycan (PG) and lipoteichoic acids which are cell wall components of Gram-positive bacteria and lipoproteins and lipopeptides from cell walls of Gram-negative bacteria (**Takeda et al, 2003**).

Liver injury is associated with a chronic inflammatory response and production of proinflammatory cytokines as tumor necrosis factor-  $\alpha$  (TNF-  $\alpha$ ), which plays a central role in promoting inflammatory response, fibrogenesis and cirrhosis (**Parola and Robino, 2001**).

Intestinal bacterial overgrowth and increased bacterial translocation of gut flora from the intestinal lumen contributes to the proinflammatory state in cirrhotic patients even in the absence of overt infection (**Hanck et al., 2001**).

A significant correlation between circulating bacterial components (PG and endotoxins) and proinflammatory cytokine levels has not been shown in cirrhotic patients raising the possibility that TLRs especially TLR2 may play a role in the pro-inflammatory cytokines production while they responding to different components of translocated intestinal bacterial flora (**Almeida et al., 2006**).

*In vitro* study done by **Dolganuic et al. (2004)** showed that HCV core protein and non- structural protein 3 (NS3) can activate human peripheral monocytes and trigger increased TNF-  $\alpha$  production via TLR2.

The role of TLR2 in increasing the circulating TNF-  $\alpha$  in patients with liver cirrhosis complicating chronic HCV infection need to be further studied to increase our understanding of the mechanisms by which TLR2 promote the hepatic inflammation and fibrosis to propose new designed therapies for this serious health problem and its accompanying complications.

## **II-Aim of the study**

### **This study aims to:**

- Determine the expression of TLR2 on peripheral blood monocytes of patients with liver cirrhosis complicating chronic hepatitis C infection and compare it to normal individuals.
- Measure serum levels of TNF-  $\alpha$  in those patients and compare them to TNF-  $\alpha$  serum levels of normal individuals.
- Correlate between TLR2 expression and serum TNF-  $\alpha$  level.

This may help in understanding the molecular mechanisms of liver cirrhosis complicating chronic HCV infection, aiming to use TLR2 as possible target for future trials in treating such devastating health problem.