# Study of Innate Immunity and Natural Killer T Cells (NK T) in hepatitis C Virus (HCV) Infection

Thesis

*Submitted for Partial Fulfillment in Master Degree in Internal Medicine*

By

***Naglaa Taher Gad***

*M.B., B.Ch*

*Faculty of Medicine, AL-Azhar University*

Under Supervision of

**Prof. Dr. Fawzia Hassan Abo Ali**

Professor of Internal Medicine, Allergy and Clinical Immunology
Faculty of Medicine, Ain Shams University

**Prof. Dr. Iman Hussein Shehata**

Assistant Professor of Microbiology
Faculty of Medicine, Ain Shams University

**Dr. Mohamed Nazmy Farres**

Assistant Professor of Internal Medicine, Allergy and Clinical Immunology, Faculty of Medicine, Ain Shams University

Faculty of Medicine
Ain Shams University
2011

# دراسة المناعة الذاتية وخلايا القتل الطبيعية التائيه فى عدوي التهاب الكبد الفيروسى (سى)

رساله
توطئة للحصول على درجة الماجستير فى الباطنة العامة


مقدمة من
الطبيبة / نجلاء طاهر جاد
بكالوريوس الطب والجراحه – جامعة الازهر


**تحت إشراف**


الأستاذة الدكتورة/ فوزية حسن ابو على
أستاذ أمراض الباطنة العامة والحساسية والمناعة الاكلينيكيه
كلية الطب - جامعة عين شمس

الأستاذة الدكتورة/ إيمان حسين شحاتة
أستاذ مساعد علم الكائنات الدقيقة
كلية الطب - جامعة عين شمس

الدكتور/ محمد نظمى فارس
أستاذ مساعد أمراض الباطنة العامة والحساسية والمناعة الاكلينيكيه
كلية الطب - جامعة عين شمس



كلـــية الطـــب - جامعة عين شمس
2011

# Summary

The current work was done in order to study the crucial role of Natural Killer cells and Natural Killer T cells in pathogenesis of HCV infection comparing to healthy control.

The present study was conducted on 30 non-diabetic adult patients with chronic HCV infection, who were recruited from the Internal Medicine and Hepatology outpatient clinics at Ain Shams University Hospital. They included 18 males and 12 females. The age of the patients ranged from 27 to 52 years. All patients had been previously diagnosed with chronic hepatitis due to HCV infection, and were seropositive for HCV antibodies, and positive for HCV-RNA by PCR. Chronic hepatitis was diagnosed by the presence of elevated ALT levels for at least the past 6 months, in addition to liver biopsy, which was performed for all patients as part of their routine workup at the clinic.

For all included cases full medical history was taken, clinical examination was done together with (liver functions, kidney functions, Hb%, and fasting blood sugar and 2 hour post prandil and Triglycerides).

The result of the present study showed that there is a decrease in NK cells & NK T cells in HCV patient (CD3-& CD56+) NK and (CD3+&CD56+) NKT and subsets in peripheral blood Compared to healthy control,

# List of Contents


| Title | Page No. |
|---|---|

***Introduction*** ..... ***1***

***Aim of the Work*** ..... ***3***

***Review of Literature***

- *Hepatitis C Virus (HCV)* ..... *4*
- *Innate Immunity* ..... *43*
- *Natural Killer Cells* ..... *54*
- *Natural Killer Cells and HCV Infection* ..... *63*

***Patients and Methods*** ..... ***75***

***Results*** ..... ***79***

***Discussion*** ..... ***99***

***Summary*** ..... ***105***

***Conclusion*** ..... ***106***

***Recommendations*** ..... ***107***

***References*** ..... ***108***

***Arabic Summary***

# List of Tables


| Table No. | Title | Page No. |
|---|---|---|
| **Table (1):** | HCV proteins and their functions in the viral life cycle | 20 |
| **Table (2):** | Importance of different exposures to HCV transmission patterns in low, moderate and high prevalence areas worldwide | 22 |
| **Table (3):** | Sex distribution among cases and controls. | 79 |
| **Table (4):** | Age distribution among cases and controls. | 80 |
| **Table (5):** | Comparison between cases and controls as regards liver function test ALT | 81 |
| **Table (6):** | Comparison between cases and controls as regards liver function test AST. | 82 |
| **Table (7):** | Comparison between cases and controls as regards liver function test total bilirubin | 83 |
| **Table (8):** | Comparison between cases and controls as regards liver function test Albumin | 84 |
| **Table (9):** | Comparison between cases and controls as regards lipid profile Triglyceride. | 85 |
| **Table (10):** | Comparison between cases and controls as regards lipid profile Cholesterol. | 86 |
| **Table (11):** | Comparison between cases and controls as regards platelet count. | 87 |
| **Table (12):** | Comparison between cases and controls as regards hemoglobin level. | 88 |
| **Table (13):** | Comparison between cases and controls as regards WBCS count | 89 |
| **Table (14):** | Comparison between cases and controls as regards kidney function test serum creatinine | 90 |


# List of Tables **(Cont...)**

| Table No. | Title | Page No. |
|---|---|---|
| **Table (15):** | Comparison between cases and controls as regards Fasting blood sugar | 91 |
| **Table (16):** | Comparison between cases and controls as regards the frequency of peripheral Natural Killer cells. | 92 |
| **Table (17):** | Comparison between cases and controls as regards Natural Killer T cells (NKT). | 93 |
| **Table (18):** | Correlation between Natural Killer cells and other parameter (laboratory criteria) among cases | 94 |
| **Table (19):** | Correlation between Natural Killer T cells and other parameter (laboratory criteria) among cases. | 95 |
| **Table (20):** | Sensitivity, specificity, between cases and controls as regards NK cell percentages. | 95 |
| **Table (21):** | Sensitivity, specificity between cases and controls as regards Natural Killer T cell percentages | 97 |

# List of Figures


| Fig. No. | Title | Page No. |
|---|---|---|
| **Fig. (1):** | Kinetics and possible partial cleavage products of HCV polyprotein processing. | 8 |
| **Fig. (2):** | Organization of Flaviviridae genomes. | 11 |
| **Fig. (3):** | HCV viral particle produced in a tissue culture system from a cloned viral genome. | 18 |
| **Fig. (4):** | HCV genome organization (top) and polyprotein processing (bottom). | 19 |
| **Fig. (5):** | Hypothetical HCV replication cycle. | 32 |
| **Fig. (6):** | Triggering the host response to HCV infection. | 45 |
| **Fig. (7):** | Signaling the host response to HCV infection. | 46 |
| **Fig. (8):** | a) The innate immune induction pathway through RLR activation. (b) The RLR family members. | 50 |
| **Fig. (9):** | Domains of the HCV genome featuring the 3′ NTR. | 53 |
| **Fig. (10):** | Modulation of innate immunity during hepatitis C virus (HCV) infection. | 65 |
| **Fig. (11):** | CD16+ CD8 T cells are expanded in patients with chronic HCV infection. | 71 |
| **Fig. (12):** | Sex distribution among cases and controls. | 79 |
| **Fig. (13):** | Age distribution among cases and controls. | 80 |
| **Fig. (14):** | ALT level among cases and controls. | 81 |
| **Fig. (15):** | AST level among cases and controls. | 82 |
| **Fig. (16):** | Total bilirubin level among cases and controls. | 83 |
| **Fig. (17):** | Albumin level among cases and controls. | 84 |
| **Fig. (18):** | Triglyceride level among cases and controls. | 85 |


# List of Figures **(Cont...)**

| Fig. No. | Title | Page No. |
|---|---|---|
| **Fig. (19):** | Cholesterol level among cases and controls. | 86 |
| **Fig. (20):** | Platelet count among cases and controls. | 87 |
| **Fig. (21):** | Hemoglobin level among cases and controls. | 88 |
| **Fig. (22):** | WBCS count among cases and controls. | 89 |
| **Fig. (23):** | Creatinine level among cases and controls. | 90 |
| **Fig. (24):** | Fasting blood sugar levels among cases and controls. | 91 |
| **Fig. (25):** | Natural killer cells among cases and controls. | 92 |
| **Fig. (26):** | NKT cells among cases and controls. | 93 |
| **Fig. (27):** | ROC curve for NK cell percentages to determine the optimum cut-off levels for diagnosis of chronic hepatitis C. | 96 |
| **Fig. (28):** | Natural Killer cells (NK) show sensitivity of 56.7% and specificity of 85.0% at a cut –off of<=5.5% | 96 |
| **Fig. (29):** | ROC curve for NKT cell percentages to determine the optimum cut-off levels for diagnosis of chronic hepatitis C | 97 |
| **Fig. (30):** | Natural Killer T cells show Sensitivity 30%, specificity 100%, at a cut-off of <=3.1% | 98 |
| **Fig. (31):** | ROC curve for NK and NKT cell percentages to determine the optimum cut-off levels for diagnosis of chronic hepatitis C. | 98 |

# List of Abbreviation

| ***Abbr.*** | ***Details*** |
|---|---|
| ***Ad*** | *Adenovirus* |
| ***ADCC*** | *Ab-dependant cellular cytotoxicity* |
| ***ALIX*** | *Apoptosis linked gene interacting protein x* |
| ***ASGP-P*** | *Asialoglycoprotein receptors* |
| ***CHMP*** | *Charged multivesicular body protein* |
| ***CMV*** | *Cytomegalo virus* |
| ***DCs*** | *Dendritic cells* |
| ***DC-SIGN*** | *The dendritic cell-specific intracellular adhesion molecule-grabbing nonintergrin* |
| ***DsRNA*** | *double stranded RNA* |
| ***E*** | *Envelope* |
| ***E R*** | *Endoplasmic reticulum* |
| ***ESCRT*** | *Endosomal storing complex required for transport* |
| ***HAART*** | *Highly active anti viral therapy* |
| ***HCC*** | *Hepatocellular carcinoma* |
| ***HCV*** | *Hepatitis c virus* |
| ***HCW*** | *Health care worker* |
| ***HIV*** | *Human immunodeficiency virus* |
| ***HLA-C*** | *Human leucocytic antgin –c* |
| ***HSPG*** | *Heparan sulfate proteoglycans* |
| ***HVR*** | *Hypervariable region* |
| ***ICAM*** | *Intercellular adhesion molecule-* |
| ***IEM*** | *Immuno EM* |
| ***IFN*** | *Interferon* |
| ***IRF*** | *Interferron regulator factor* |
| ***ISGs*** | *Interferon stimulated genes* |
| ***ISRE*** | *Interferon stimulated responce element* |
| ***JFHI*** | *Japanese patient fulminant hepatitis* |
| ***KIR*** | *Killer immunoglobin receptor* |
| ***LDL-R*** | *The low-density lipoprotein receptors* |
| ***LEL*** | *large extracellular loop* |
| ***MHC*** | *Major histocompitability complex* |
| ***MTCT*** | *mother –to- child transmission* |
| ***N K*** | *Natural killer cells* |
| ***N K T*** | *Natural killer T cells* |
| ***NF-kB*** | *Nuclear factor kappa B* |
| ***NKR*** | *Natural killer cell receptors* |
| ***NS*** | *Non-structural protein* |

| | |
|---|---|
| ***NTR*** | *Nontranslated region* |
| ***P*** | *protein no* |
| ***PAMP*** | *Pathogen –associated molecular pattern* |
| ***PAT*** | *Parenteral anti-schistosomiasis treatment* |
| ***PBMCS*** | *Perpheral blood mono nuclear cells* |
| ***PEG-IFN alfa*** | *Pegylated-interferon alfa* |
| ***PRD*** | *Positive regulatory domain* |
| ***PRR*** | *Pattern recognition receptors* |
| ***PRR*** | *Pattern recognition reseptor* |
| ***QS*** | *Quasispecies* |
| ***RBV*** | *Ribavirin* |
| ***RD*** | *Repressor domain* |
| ***RF*** | *Rplicative form* |
| ***RI*** | *Replicative intermediate* |
| ***RLR*** | *Rig-like receptors* |
| ***SEL*** | *Small extracellular loop* |
| ***SFV*** | *Semiliki forest virus* |
| ***SL*** | *Stem –loop structures* |
| ***SR-BI*** | *Scavenger receptor B type* |
| ***STAT*** | *Singl transducer activator of transcription* |
| ***STDS*** | *Sexually transmitted disease* |
| ***SVR*** | *Sustained virological responce* |
| ***TCR*** | *T cell receptors* |
| ***TGF*** | *Transforming growth factor* |
| ***TLR*** | *Toll like receptors* |
| ***TM*** | *Transmembran* |
| ***TNF*** | *Tumor necrosis factor* |
| ***TSG*** | *Tumor susceptibility gene* |
| ***UTR*** | *Untranselated region* |
| ***VAP-A*** | *Vesical-associated membrane protein-associated protein A* |
| ***VAP-B*** | *Vesical-associated membrane protein-associated proteinB* |
| ***VRE*** | *Virus responsive element* |
| ***VSV*** | *Vesicular stomatitis virus* |

# INTRODUCTION

Hepatitis C virus (HCV) has assumed the proportion of a global pandemic. Approximately 170 million people are infected world-wide with this virus. The role of innate immune response in hepatitis C virus (HCV)-related chronic liver disease in controversial and poorly under stood ***(Lauer and Walker, 2001).***

Natural Killer (NK) and Natural killer T (NKT) cells are an important antiviral effector population eliminating virus through direct killing and cytokine production. Like many other viruses, HCV has evolved strategies to evade detection and elimination by NK cell ***(Ali and Fernando, 2004).***

Natural Killer (NK) cells may be impaired in patients with persistence hepatitis C virus (HCV) infection, but studies to date have yielded inconsistent findings due to patient and virus heterogeneity and difficulties obtaining appropriate controls ***(Colucci et al., 2003).***

Altered NK and NKT cell function may contribute to impaired cellular immune responses and chronicity of the disease following HCV infection. Natural Killer (NK) cells play a crucial role in limiting the severity of disease caused by a range of viruses ***(Dolganiuc et al., 2006)***.

They usually become activated in an early phase of viral infection. Liver is particularly enriched in NK cells, which are activated hetero hepatic viruses such as hepatitis C virus. The

activated NK cells play an essential role in recruiting virus-specific T cells and in inducing antiviral immunity in liver, optimally activated NK cells are important in limiting viral replication in this organ. Paradoxically, NK cell, too act as a double-edged sword and might contribute toward pathogenesis and cause liver damage by killing hepatocytes and by secreting proinflammatory cytokines ***(Crotta et al., 2002).***

They also eliminate virus infected hepatocytes directly by cytolytic mechanisms and indirectly by secreting cytokines, which induce an antiviral state in host cells. Therefore, optimally activated NK cells are important in limiting viral replication in this organ ***(Lucy et al., 2008).***

Further studies are needed to understand the role of these cells in host defense and liver pathology in infections. Recent advances in understanding NK cell biology have opened new avenues for boosting innate and adaptive antiviral immune responses in HCV-infected individual ***(Tseng and Klimpel, 2002).***

# Aim of the Work

The aim of this work is to study the crucial role of Natural Killer cells and Natural Killer T cells in pathogenesis of HCV infection.

# HEPATITIS C VIRUS (HCV)

## Definition:

Hepatitis C virus (HCV) is a member of *Flaviviridae* that contains a 9.6-kb positive-sense RNA genome. Chronic infection with HCV is a major cause of liver cirrhosis and hepatocellular carcinoma ***(Sarbah and Younossi, 2000 and Ariumi et al., 2011)***.

The tropism of HCV is limited to chimpanzees and humans, and the mechanism of HCV infection and replication is not fully understood ***(Yoshida et al., 2011)***.

## Epidemiology:

A total of 170-million people worldwide are infected with HCV, leading to chronic hepatic inflammation, hepatic fibrosis, hepatic cirrhosis and hepatocellular carcinoma ***(Bernini et al., 2011)***.

Hepatitis C virus (HCV) is a major cause of chronic liver disease in both children and adults worldwide ***(Wasley and Alter, 2000)***. Since the advent of universal screening of blood products, mother-to-child transmission (MTCT) has become the major route of HCV infection in children ***(Indolfi and Resti, 2009)***. It is estimated that 10,000–60,000 newborns worldwide are infected with HCV by MTCT each year ***(Yeung et al., 2001 and Babik et al., 2011)***.

The rate of MTCT from HCV-seropositive, HCV RNA-positive women is 4%–6%and transmission occurs almost

exclusively from women who are viremic ***(Indolfi and Resti, 2009)***.

## Prevalence in Egypt

The national prevalence of HCV infection in Egypt was estimated by conducting a nation-wide survey in 1993 to estimate the prevalence of HCV infection among blood donors ***(Morcos et al., 2010)***. 2644 samples were obtained from 24 of 26 governorates, and the prevalence was found to be 24.8%. The prevalence of infection in the different governorates of Egypt was also calculated by ***Fallahian and Najafi (2011)*** by combining two studies.

Seroprevalence of hepatitis C virus in the urban blood donor population was 14.5%, while the seroprevalence was 70.4% in HD patients, 7.7% in health care workers, and 75.6% in thalassemic children. Schistomiasis does not seem to play a role in the seroprevalence of this disease in Egypt ***(Mohamed et al., 1996)***. Moreover, HCV was found in 12.1% of rural primary school children, 18.1% of residents in rural villages, 22.1% of army recruits, 16.4% of children with hepatosplenomegaly, 54.9% of hospitalized multitransfused children, 46.2% of adults on HD, and 47.2% of adults with chronic liver disease or hepatoma ***(Youssef et al., 2009)***.

A consistent increase of seropositivity for HCV antibodies with age was observed, with a peak level of 54.9% in all individuals for the age group 45-49 years. Analysis revealed that age, male sex, marriage, rural residence, living in upper and lower Egypt, injections for bilharziasis and urography were