



# **Evaluation of Natural Killer Cells in HCV Patients undergoing Hemodialysis**

*Thesis*

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## Evaluation of Natural Killer Cells in HCV Patients undergoing Hemodialysis

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### Abstract:

**Background:** Hepatitis C virus (HCV) is a hepatotropic virus and one of the major causes of liver disease and a potential cause of substantial morbidity and mortality worldwide. Patients on hemodialysis are at high risk for HCV, with frequency of infection several times higher than that in non-uremic patients. Natural killer (NK) cells are best appreciated for innate defense against viral infections and in tumor cell surveillance. **Objective:** To study the percentage of peripheral blood natural killer cells among chronic hepatitis C patients undergoing hemodialysis compared to chronic HCV patients with normal kidney functions by flow cytometry immunophenotyping. **Methodology:** Frequency of CD3<sup>+</sup> CD56<sup>+</sup> cells was assessed in two distinct groups. Group I: 35 chronic HCV patients with end stage renal disease and undergoing hemodialysis, group II: 35 age and sex matched chronic HCV patients with no kidney disease. **Results:** No significant difference was observed between HCV hemodialysis patients with ESRD and HCV patients with normal kidney function regarding the frequency of both NK cells (CD3<sup>+</sup> CD56<sup>+</sup>) and NKT cells (CD3<sup>+</sup> CD56<sup>+</sup>). However, group I had significantly higher percentage of CD3<sup>+</sup> cells than group II. **Conclusion:** no difference was found in this study between HCV patients on hemodialysis and HCV with normal kidney function regarding NK cell frequency. However, assessment of NK cell function in future studies might reveal differences.

Key words: HCV, NK

## LIST OF ABBREVIATIONS

Abb.	Full Term
<b>ADCC</b> ---	: antibody-dependent cellular cytotoxicity
<b>ALT</b> -----	: alanine aminotransferase
<b>AST</b> -----	: aspartate aminotransferase
<b>BAT3</b> ----	: B associated transcript 3
<b>BID</b> -----	: BH3-interacting domain
<b>BUN</b> -----	: blood urea nitrogen
<b>CD</b> -----	: cluster of differentiation
<b>cDNA</b> ----	: complementary DNA copy
<b>CHILPs</b> -	: common helper innate lymphoid progenitors
<b>CILPs</b> ----	: common innate lymphoid progenitors
<b>CLDN</b> ---	: claudin
<b>CLPs</b> -----	: common lymphoid progenitors
<b>DC</b> -----	: dendritic cells
<b>EDTA</b> ----	: ethylenediamine tetra-acetic acid
<b>EIA</b> -----	: enzyme immunoassay
<b>ESRD</b> ----	: end-stage renal disease
<b>FasL</b> -----	: Fas ligand
<b>Fs</b> -----	: forward scatter
<b>GAGs</b> ---	: glycos-aminoglycans
<b>GM-CSF</b>	: granulocyte/monocyte colony-stimulating factor
<b>GzmA</b> ----	: granzyme A
<b>GzmB</b> ----	: granzyme B
<b>HCV</b> -----	: Hepatitis C virus
<b>HD</b> -----	: hemodialysis
<b>HLA</b> -----	: human leukocyte antigen

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<b>ICAM-1</b>	- :	inter-cellular adhesion molecule- 1
<b>IFN</b>	----- :	interferon
<b>IL</b>	----- :	interleukin
<b>ILC</b>	----- :	innate lymphoid cell
<b>ILCP</b>	----- :	innate lymphoid cell precursors
<b>ITAM</b>	---- :	immunoreceptor tyrosine-based activating motifs
<b>ITIM</b>	----- :	immunoreceptor tyrosine-based inhibitory motifs
<b>KIRs</b>	----- :	killer cell immunoglobulin-like receptors
<b>LDL-R</b>	-- :	LDL-receptor
<b>LFA</b>	----- :	Lymphocyte function associated antigen
<b>LIRs</b>	----- :	leukocyte Ig like receptors
<b>LTi</b>	----- :	lymphoid tissue-inducer
<b>LTiPs</b>	----- :	lymphoid tissue inducer progenitors
<b>mDCs</b>	----- :	myeloid DCs
<b>MHC</b>	----- :	major histocompatibility complex
<b>MICA/B</b>	- :	major histocompatibility complex class I-related chain A/B
<b>NADPH</b>	- :	Nicotinamide Adenine Dinucleotide Phosphate
<b>NCR</b>	----- :	natural cytotoxicity receptor
<b>NK</b>	----- :	natural killer
<b>NKP</b>	----- :	NK cell precursors
<b>NS proteins</b>	:	non-structural proteins
<b>NS</b>	----- :	non-significant
<b>PBMCs</b>	-- :	peripheral blood mononuclear cells
<b>PCR</b>	----- :	polymerase chain reaction
<b>pDCs</b>	----- :	plasmacytoid DCs
<b>PFN</b>	----- :	perforin
<b>RIBA</b>	----- :	recombinant immunoblot assay

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**RNA -----** : ribonucleic acid  
**S -----** : Significant  
**SD -----** : standard deviation  
**SPSS -----** : statistical package for social science  
**SR-B I ---** : scavenger receptor class B type I  
**Ss -----** : side scatter  
**TAP -----** : transporter associated with antigen  
**TGF-B 1-** : transforming growth factor- B 1  
**TNF -----** : tumor necrosis factor  
**TRAIL---** : tumor necrosis factor-related apoptosis-inducing ligand  
**TRAIL-R** : TNF-related apoptosis-inducing ligand-receptor  
**UTR -----** : untranslated regions  
**VCAM---** : vascular adhesion molecules

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# INTRODUCTION

Hepatitis C is an infectious disease caused by the hepatitis C virus (HCV) that primarily affects the liver (*Lavanchy, 2011*). HCV spreads primarily by blood-to-blood contact associated with intravenous drug use, poorly sterilized medical equipment, needle stick injuries in healthcare and transfusions. (*Maheshwari and Thuluvath, 2009*).

Chronic kidney disease is a progressive loss in renal function over a period of months or years. All individuals with a glomerular filtration rate of less than 15/ml/min/1.73 m<sup>2</sup> for 3 months are classified as having end-stage renal disease (ESRD) (*Levin et al., 2008*).

In Egypt, the prevalence of dialysis patients is presumed to be increasing and the main causes of ESRD in Egypt, other than diabetic nephropathy, include hypertensive kidney disease, chronic glomerulonephritis, unknown etiology, chronic pyelonephritis, schistosomal obstructive uropathy, and schistosomal nephropathy (*Afifi et al., 2004*).

Patients on hemodialysis are at high risk for HCV, with frequency of infection several times higher than that in non-uremic patients. The spread of HCV in hemodialysis units is

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declining, but the prevalence of HCV in hemodialysis patients remains high (*Selcuk et al., 2006*).

The knowledge about the immunological aspects of the chronic hepatitis C especially in ESRD patients on regular hemodialysis needs further study (*Poordad et al ., 2004*).

The immunophenotyping of peripheral blood has been employed in studies focusing on the pathogenesis of chronic hepatitis C. The study of natural killer (NK) cells, a type of cytotoxic lymphocyte critical to the innate immune system, in hepatitis C pathogenesis has been the focus of several studies (*Larrubia et al .,2007*).

## **AIM OF THE WORK**

The aim of this work is to study the percentage of peripheral blood natural killer cells among chronic hepatitis C patients undergoing hemodialysis compared to chronic HCV patients with normal kidney functions by flowcytometry immunophenotyping.

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# HEPATITIS C VIRUS

Hepatitis C virus is a hepatotropic virus and one of the major causes of liver disease and a potential cause of substantial morbidity and mortality worldwide. Moreover, it is estimated that >184 million people have been infected with HCV (on the basis of positive anti-HCV antibody results), representing >2.8% of the world population (*Thrift et al., 2017*).

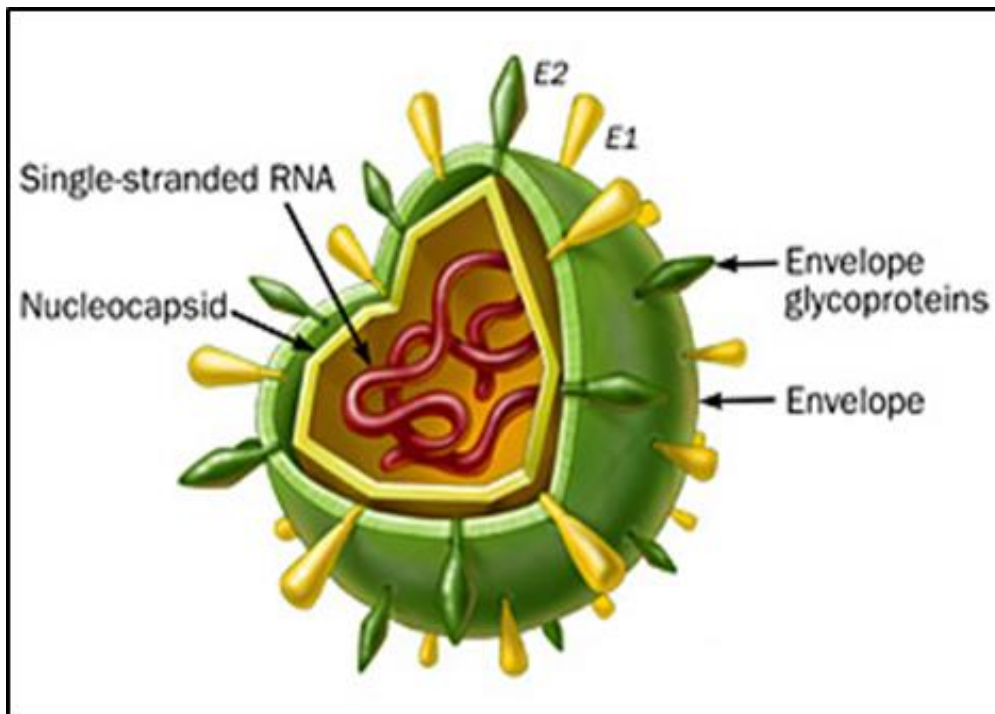
Chronic HCV is one of the major causes of advanced liver disease, including cirrhosis, hepatocellular carcinoma, and related complications. In the United States, approximately one-third of cirrhosis cases and one-fourth of hepatocellular carcinoma cases are due to chronic HCV infection (*Armstrong et al., 2000*).

## A. Structure of HCV:

Hepatitis C virus is a member of the Hepacivirus genus (of the family Flaviviridae). Other members of this family include viruses that cause yellow fever, dengue, Japanese encephalitis and tickborne encephalitis (*Rogo et al., 2011*).

HCV is an enveloped, small (55-65nm in size), positive sense, single stranded ribonucleic acid (RNA) virus. The HCV particle consists of a core of genetic material “RNA”, surrounded by an icosahedral protective shell of protein, and further encapsulated in a lipid envelope of cellular origin. Two viral envelope

glycoproteins, E1 and E2, are embedded in the lipid envelope (*Budkowska, 2017*) (Fig.1).



**Figure (1):** Structure of Hepatitis C virus

([https://microbewiki.kenyon.edu/index.php/modern\\_treatments\\_for\\_hepatitis\\_c\\_virus](https://microbewiki.kenyon.edu/index.php/modern_treatments_for_hepatitis_c_virus))

The genome of HCV is an open reading frame of 9600 nucleotide bases that has at its 5' and 3' ends untranslated regions (UTR) that are not translated into proteins but are important to translation and replication of the viral RNA. The 5' UTR has a ribosome binding site that starts the translation of a 3011 amino acid containing protein that is later cut by cellular and viral proteases into 10 active structural and non-structural smaller proteins (*Rogo et al., 2011*) (Fig. 2).