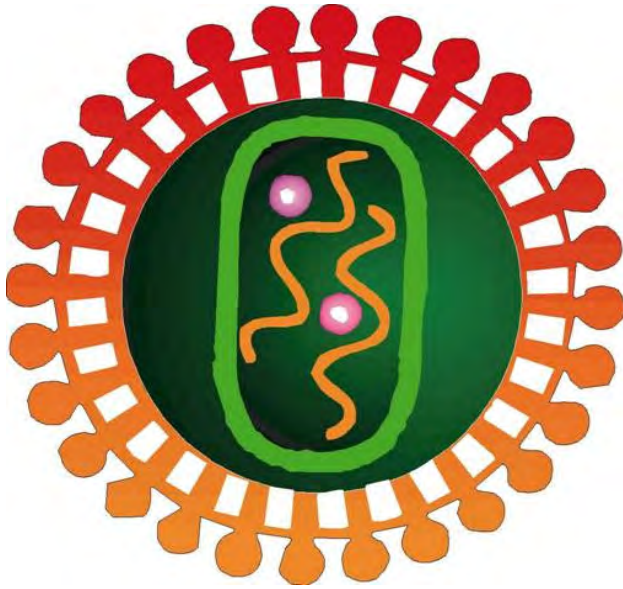


**ADDIS ABABA UNIVERSITY**  
**SCHOOL of GRADUATE STUDIES**



**Impact of Hepatitis C (HCV) Co-infection on HIV patients before and after HAART: An Immuno-haematological and Clinical Chemistry observation**

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<b>TABLE OF CONTENTS</b>	<b>PAGE</b>
ACKNOWLEDGEMENTS.....	i
TABLE OF CONTENTS.....	ii
LIST OF TABLES.....	iv
LIST OF FIGURES .....	v
LIST OF ABBREVIATIONS.....	vi
ABSTRACT.....	ix
<b>CHAPTER 1 INTRODUCTION.....</b>	<b>1</b>
1.1 Literature Review.....	7
1.1.1 Historical Background .....	7
1.1.2 HCV Virion Structure and Genetic Organization.....	7
1.1.3 Route of Transmission.....	10
1.1.4 Life Cycle and viral multiplication.....	11
1.1.5 Disease Out Come.....	12
1.1.6 Clinical Sign and Symptoms.....	13
1.1.7 Pathogenesis and Immune Response.....	14
1.1.8 Laboratory Diagnosis.....	22
1.1.9 Treatment.....	25
1.1.10 Prevention.....	28
1.2 Significance of the Study.....	29
1.3 Limitations of the Study.....	30
1.4 Objectives of the Study.....	32
<b>CHAPTER 2 MATERIALS AND METHODS.....</b>	<b>33</b>

2.1 Study Area.....	33
2.2 Study Design and Period.....	33
2.3 Study Population.....	34
2.4 Sample Size and Sampling Technique.....	34
2.5 Laboratory Analysis.....	35
2.5.1 Specimen Collection and Handling.....	35
2.5.2 Anti-HCV and HBV Antibodies Detection.....	35
2.5.3 CD4 <sup>+</sup> and CD8 <sup>+</sup> T-lymphocytes quantification.....	38
2.5.4 Haematological tests Analysis.....	39
2.5.5 Measurement of liver enzyme levels.....	40
2.6 Data Analysis.....	42
2.7 Ethical Clearance.....	42
<b>CHAPTER 3 RESULT.....</b>	<b>43</b>
3.1 Study Subjects.....	43
3.2 HCV/HIV Prevalence.....	43
3.3 Annual Changes on Immunological and clinical chemistry of the study groups over a period of four years .....	45
3.4 Quarterly changes on Immunohaematological and clinical chemistry tests of the fourth year.....	47
<b>CHAPTER 4 DISCUSSION.....</b>	<b>49</b>
4.1 Conclusions.....	56
4.2 Recommendations.....	57
REFERENCES .....	59

**LIST OF TABLES**

**PAGE**

**1.** Mean annual changes of immunological and clinical chemistry values in each group of HIV patients at Yekatit-12 and Zenbaba general Hospitals, Addis Ababa, Sep. 2006 – Nov. 2010.....46

**2.** Quarterly mean changes of Immunohaematological and liver enzyme levels of patients at Yekatit-12 and Zenbaba general Hospitals, Addis Ababa, Nov. 2009 – Nov. 2010.....48

**LIST OF FIGURES**

**PAGE**

**1.1** Hepatitis C virus (HCV) structure, 1999.....7

**1.2** The HCV genome and expressed polyprotein, 2004.....9

    A) Shows the genome of HCV.....9

    B) Shows the locations of the proteins.....9

**1.3** A phylogenetic tree demonstrating sequence diversity in different strain of hepatitis C virus, 2005 .....10

**1.4** Natural history of HCV, 2002.....12

**2.1** Flavicheck-HCV test device application, interpretation and control system, 2004....37

**3.1** Age-sex distribution of study subjects in each group at Yekatit-12 and Zenbaba General Hospitals, Addis Ababa, Sep. 2006- Nov. 2010.....44

    A) Sex distribution of the study subjects in the different patient grouping.....44

    B) Sex distribution of HCV co-infected study subjects with age interval.....44

## LIST OF ABBREVIATIONS/ACRONYMS

AIDS	Acquired immunodeficiency syndrome
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
ART	Anti-retroviral treatment
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BD	Becton Dickinson
CDC	Center for Disease Control
CD	Cluster of designation / differentiation /
CTLs	Cytotoxic T-lymphocytes
DC	Dendritic cells
DNA	Deoxyribonucleic acid
E	Envelope
EDTA	Ethylene diaminetetraacetic acid
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbent assay
ER	Endoplasmic reticulum
FACS	Fluorescent activated cell sorter
FasL	Fatty acid ligand
FDA	Food and drug administration authority
FITC	Fluorescein isothiocyanate

GGTP	Gamma-glutamyl transpeptidase
GOT	Glutamate oxaloacetate transaminase
GPT	Glutamate pyruvate transaminase
HAART	Highly active anti-retroviral therapy
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HepG2	Hepatocyte group2 cells
HIV	Human immunodeficiency virus
Huh	Human Hepatocyte
HVR	Hyper variable regions
IDU	Intravenous drug use
Ig	Immunoglobulin
IFN	Interferon
IL	Interleukin
JFH	Japan fulminant hepatitis
LDL	Low density lipoprotein
MHC	Major Histocompatibility complex
MoAbs	Monoclonal antibodies
NANB	Non-A Non-B
NK	Natural killer cells
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
NS	Non-structural

ORF	Open reading frame
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PEG-INF-1	Pegylated interferon alpha
PCP	Pneumocystis carinii pneumonia
PE	Phycoerythrin
PI	Protease inhibitor
PLT	Platelet
RBC	Red blood cell
RdRp	RNA dependent-RNA polymerase
RIBA	Recombinant immunoblot assay
RNA	Ribonucleic acid
RT-PCR	Real time PCR
SVR	Sustained virological response
TLC	Total lymphocyte count
TLRs	Toll like receptors
UNAIDS	United Nations office of AIDS
USA	United States of America
UTRs	Untranslated regions
VCT	Voluntary counseling and testing
WBC	White blood cell
WHO	World Health Organization

## **ABSTRACT**

*Hepatitis C virus (HCV) is an enveloped single stranded RNA virus belonging to the genus Hepacivirus within the family Flaviviridae. It causes an acute or chronic necroinflammatory disease of liver. Due to shared risk factors of transmission, co-infection with HIV is common. The aim of this study was to assess the impact of HCV co-infection on immunohaematological and clinical chemistry parameters in HIV patients before and after HAART. 387 HIV infected patients at Yekatit-12 and Zenbaba General Hospitals were screened for HCV infection and 25 of them were found HCV/HIV co-infected. From those 25 co-infected patients, 9 of them were pre-ART and the rest 16 were under HAART patients. All those 25 HCV/HIV co-infected patients were observed for 4 years for their annual and quarterly changes in immunohaematological and clinical chemistry parameters. For this study, leftover blood samples collected for immunohaematological and clinical chemistry tests were used. Flavichck-HCV two-site sandwich, 4<sup>th</sup> generation EIA was used for the detection of total antibodies specific to HCV in serum or plasma. CD4<sup>+</sup> and CD8<sup>+</sup> T-cells were counted using FACS counter (Becton Dickinson), haematological tests were done using Celldyn-1800 haemoanalyzer and liver enzymes were measured using Humastar80 chemistry analyzer. From the result, HCV/HIV co-infection prevalence was 6.5% (7.3% for males and 5.5% for females) and from the 25 co-infected, more males were co-infected than females (60% Vs 40%). The percentage increments in CD4<sup>+</sup> and CD8<sup>+</sup> T-cells of co-infected patients were lower than HIV mono-infected patients (131% Vs 432%) and (14% Vs 31%) respectively. It is highly associated with increased liver enzymes in both pre-ART and under HAART co-infected patients. In conclusion, if confirmed in large studies, it is advisable for HIV patients to screen HCV before initiation of HAART in order to minimize HAART associated liver damage and for better management of HIV in HIV/HCV co-infected individuals.*

**Key words/phrases:** *Immunohaematological, Pre-ART, HCV/HIV co-infection, HAART, CD4<sup>+</sup>, CD8<sup>+</sup>, GOT, GPT, ALP*

## Chapter One: Introduction

Hepatitis C virus (HCV) is one of the five hepatitis virus (A-E) which infect human liver. It is an enveloped single-stranded positive sense RNA virus belonging to the genus Hepacivirus within the family *Flaviviridae* (Su, et al., 2002). It is 9.6 kb and measures about 50 nm in diameter, with a positive-sense genome (Grimm and Kay, 2006). It has six major genotypic groups of variants (1-6) and hundreds of quasispecies showing variation in their molecular composition. Different variants are found in different geographical regions of the world. The genotype 1, 2 and 3 have a worldwide distribution. Genotype 4 appears to be prevalent in North Africa and the Middle East while genotypes 5 and 6 are confined to South Africa and Asia respectively. In the USA and Western Europe genotype 1a and 1b are most common, followed by genotype 2 and 3 (Baye and Yohannes, 2008; Sillanpää, et al., 2009).

Worldwide there are approximately 170 million (3%) people infected with hepatitis C virus. About 2% of those cases found in the developed world (Rodríguez, *et al.*, 1999). The highest prevalence (28%) was recorded from Egypt. In U.S.A about 4.1 million (2.4%) people infected with HCV (Blackard, *et al.*, 2008; Kindrick, 2002). It is also responsible for an estimated 300,000 deaths/year. More than 75% of the deaths due to HCV infection are recorded in the developed countries. For example, according to Center for Disease Control and Prevention (CDC) about 12,000 people die annually in the United States. The mortality, however, is expected to triple in the next two decades mainly because of increasing sharing of infected needles whilst injecting drug use and homosexuality coupling with no 100% effective drugs (Balasubramanian, et al., 2008; Cox, et al., 2005).

The prevalence of HCV in Ethiopia is 0.9% for the total population and 1.4% for healthy adult Ethiopian blood donors. On the other hand, the prevalence of anti-HCV among Ethiopian patients with chronic hepatitis, cirrhosis of the liver and hepatocellular carcinoma (HCC) was found to be 21%, 36% and 46% respectively (Baye and Yohannes, 2008).

At present, the exact mechanism of HCV pathogenesis is not well understood. However, there are reports which indicate host reactions against HCV proteins have roles during its pathogenesis. Other reports indicate that inflammation participates in the pathogenesis of HCV infection by enhancing the production of IL-8 (Helbig, et al., 2009). In general, the information about the pathogenesis of HCV is contradictory and not conclusive, primarily because only very recently productive HCV culture system developed and lack of small animal models (Heller, et al., 2005; Sookoian, 2003). Although the exact mechanism of pathogenesis is not defined, however, the virus selectively multiplies in the liver cells. It also extends to extrahepatic replication in the lymph nodes, pancreas and, to a lesser extent, spleen, adrenal gland, bone marrow and thyroid tissue of patients co-infected with HIV (Bertoletti and Ferrari, 2003).

Hepatitis C virus infection is distinguished from other hepatitis virus infections by its low incidence of an acute phase and the higher rate of chronicity. The acute is characterized by rapid viral replication and intense inflammation while the chronic phase is characterized by persistent or intermittent viremia with normal or slightly increased alanine aminotransferase (ALT) level. Due to increased rate of chronicity, only 15-45% of acutely infected individuals spontaneously (without drug) clear hepatitis C infection. However, the rest 55-85% of acute infections become chronic (Al-Sherbiny, et al., 2005; Nascimbeni, et al., 2003).

Cirrhosis, a long-term effect which is characterized by diffuse fibrotic disruption of liver tissue architecture and the liver cancer which is also the long-term outcome of HCV are the major diseases due to HCV infection. Acquisition of infection in about 80% of the infected individuals is asymptomatic (Maier and George, 2002). However, symptomatic patients (20%) develop some constitutional symptoms like; weakness, fatigue, headache, nausea, vague abdominal pain, loss of appetite and jaundice (Blackard, et al., 2008; Koziel, 2002). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGTP) and alkaline phosphatase may also be affected (Brass, et al., 2006).

HIV/AIDS is a number one priority global health problem. According to the latest estimate by UNAIDS in 2009, globally there were 33.3 million infected people of whom were, 15.9 women, 2.5 children, 2.6 newly infected. This was in addition to 1.8 deaths and 16.6 Orphans. Sub-Saharan Africa harbored 68% of the infected, 92% of the children, 72% of the deaths and nearly 90% of the orphans. Ethiopia with about 1.1 million (2.1%) infected persons stands the 11<sup>th</sup> country on the list of HIV prevalence ranking. It is still one of the major diseases threatening the productive and reproductive age groups of the societies (UNAIDS, 2009).

People with HIV infection, because of the weakened immune system, become highly vulnerable to 'opportunistic infections'. These are infections of viral, bacterial, fungal and protozoal origin that normally are controlled by the immune system but take advantage of the weakness in the immune defense in HIV patients and cause health problems at different stages of the infections. For example almost every one with HIV, tests positive to Cytomegalovirus but develops disease only when the CD4 count drops below 50 cells/ $\mu$ l, a sign of serious damage in immune system. In early HIV disease, people can develop tuberculosis, malaria, bacterial pneumonia, herpes

zoster, hepatitis, staphylococcal skin infections and septicaemia. When the immune system is very weak due to advanced HIV disease or AIDS, opportunistic infections such as *Pneumocystis carinii* pneumonia (PCP), toxoplasmosis and cryptococcosis develop (Gatti, et al., 2007).

With the advent of HIV infection, HCV co-infection has become a major health concern in both developed and developing countries. Co-infection is common because they share the same route of transmission (Anderson, et al., 2004; Sulkowski, 2002). At the moment about 14 million (40%) HIV patients co-infected with HCV worldwide are left for the challenges of co-infection (Blackard and Sherman, 2008). Advanced countries like U.S.A (30%), France (24.3%) and Spain (33%) have almost the same prevalence of HCV infection among HIV patients. In Africa the information is patchy and scarce and the percentage of co-infection reported is relatively low: Nigeria (8.2%), Morocco (19.8%) and Ethiopia (8.6%) (Chung, 2004; Larsen, *et al.*, 2008; Workenesh, et al., 2002). In all cases, demographically HIV/HCV co-infection is higher in males over 40 years of age and positively biased towards blacks and Latinos (Carbonero, et al., 2007; Sugimoto, et al., 2003).

The introduction of highly active anti-viral therapies (HAART) in the mid-1990s has reduced the morbidity and mortality due to HIV dramatically by reducing the viral replication as measured by lower plasma levels of HIV RNA and increases in CD4<sup>+</sup> T lymphocyte count and thereby delays disease progression even in advanced cases (Marina and Vincent, 2003). However, it is still a problem in patients who have not been tested and/ or treated. Furthermore, HAART by its own has a side effect on the liver. And more important liver disease that appears in individuals co-infected and is under HAART has become a cause to significant number of deaths. In this respect Hepatitis C Virus (HCV) co-infection is labeled amongst those that are in direct cause,

on one hand it synergizes in exacerbation of hepatotoxicity, rapid progression of chronic hepatitis, liver fibrosis, aggressive cirrhosis, bleeding and hepatocellular carcinoma and on the other affects the treatment outcomes of the administration of HAART (Gatti, et al., 2007; Koziel, 2002). Although it either increases drug related hepatotoxicity or interferes in selection of specific agents that negatively affect the liver, however, the precise mechanism it uses to increase the incidence of hepatotoxicity in infected patients is not clearly known as yet. (Blackard and Sherman, 2008; Rehermann and Nascimben, 2005). In addition, although there are contrasting view suggesting HAART to play in the control of HCV replication, recent literatures show that nucleoside analogs (lamivudine and zidovudine) and HIV protease inhibitor (Nelfinavir) are indicated in the control of HCV replication (Highleyman, 2009).

Furthermore, recent studies show that HCV is more important in the process of causing liver disease. First, it is associated with the liver diseases of three-quarters of HIV patients that are admitted and/or die in hospitals. Second, the number of patients who are admitted because of liver failure is high (9-16%) in co-infected patients. Third, end-stage liver disease cases are more frequent (45%) in HCV co-infected individuals (Dhoot, 2003; Marina and Vincent, 2003). Fourth, the interval between acquisition of HCV infection and development of cirrhosis was shorter in HIV co-infected individuals (Verma, 2006; Maier and George, 2002). Indeed, within 10-15 years of initial HCV infection, 15-25% of HIV co-infected patients develop cirrhosis compared with 2-6% of HIV-negative patients (Chung, 2004). Making the situation even worse, the severe hepatotoxicity effect results in 10% co-infected patients discontinue the treatment of HAART (Sulkowski, 2002; DeSimone, et al. 2000).

HIV infection breaks the vigorous and broad cellular immune response, which could clear HCV infection early in primary infection and HAART induced side effects are influencing the treatment outcomes of HCV drugs. The decrease in the rate of drug response is among others due to high genetic diversity of HCV during HIV co-infection and selection of genotypes that are resistant to current standard drugs like 1 and 4 as evidenced by their higher prevalence in HIV/HCV co-infected patients than in HCV alone or immuno-suppression as a result of impairment that might reduce the effectiveness of response to HCV (Helbig, et al., 2009). Thus, the management of chronic hepatitis C in HIV/HCV co-infected patients is a challenge because of the unpredictable consequences of anti-HIV and HCV drug interactions. i.e. optimal approach of treatment is not yet established (Chung, 2004).

### **Hypothesis**

HIV is known to impair T-helper type 1 immune response which in turn alters the response of immune cells to HCV. The decline in cell mediated immunity associated with progressive HIV infection permits higher HCV replication and consequently, greater infection and injury to hepatocytes which leads to more rapid progression to HCV-related liver diseases. Having this background we hypothesized that, HCV co-infection impairs the recovery of immune cells and associated with increased liver enzymes in HIV infected patients before and after HAART.

Therefore, the aim of the present work is to provide a base line data on the immunohaematological and liver enzyme changes due to HCV co-infection in HIV infected patients before and after initiation of HAART. The outcome of the research is expected to improve the treatment, care and management of HIV disease in HIV/HCV co-infected patients.

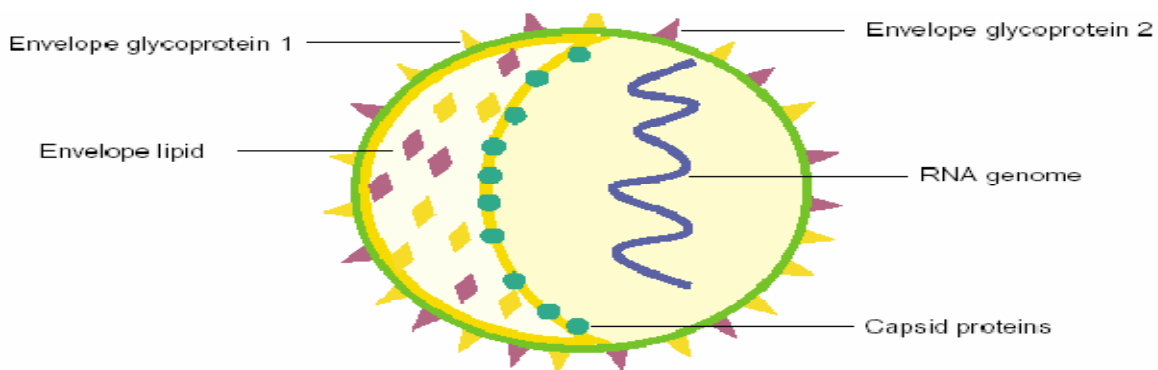
## 1.1 Literature Review

### 1.1.1 Historical background

Although acute viral hepatitis described over 2,000 years ago, it is only within the past three decades that the different types of hepatitis have been identified and the biology, epidemiology, pathology, immunology, genetics and clinical feature described. The original description of the existence of a non-A and non-B form of viral hepatitis was made in 1974 and 1975 (Dziora, et al., 2005). After 14 years in 1989, researchers identified the etiologic agent of non-A, non- B hepatitis using advanced molecular techniques and named it HCV (Tellinghuisen, et al., 2007).

### 1.1.2 HCV Virion structure and Genetic organization

At present there are five kinds of liver infecting hepatitis virus denoted A-E. Among these virus hepatitis C virus is the main virus which infect and damage the liver (Balasubramanian, et al., 2008). HCV is an enveloped single-stranded RNA virus belonging to the genus Hepacivirus within the family *Flaviviridae* (Su, et al., 2002). The virus is 9.6 kb (Heller, et al., 2005), and it measures about 50 nm in diameter, with a positive-sense genome (Grimm and Kay, 2006).



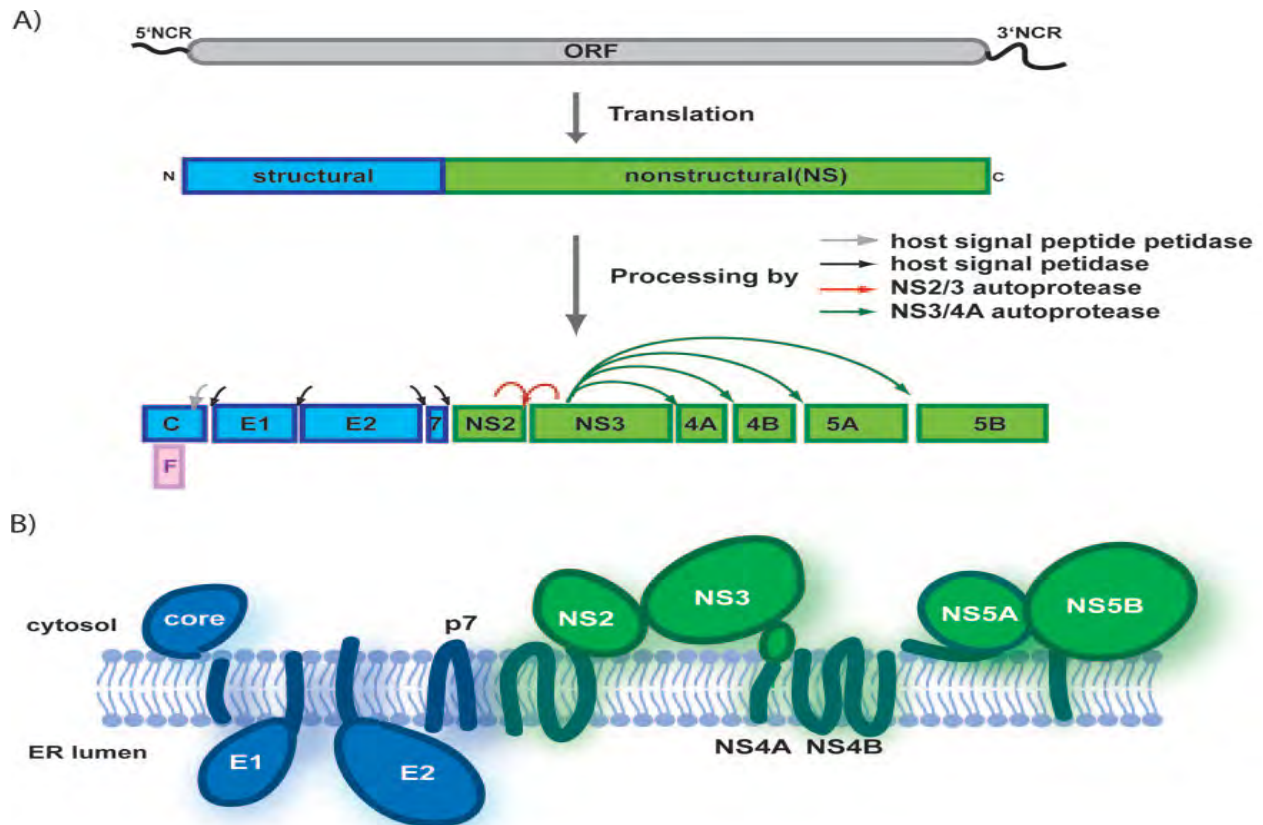
**Figure 1.1:** Hepatitis C virus (HCV) structure (Rodríguez, et al., 1999)

HCV genome has a length of approximately 9,600 nucleotides consisting of a single open reading frame (ORF) which is flanked at 5' and 3' by untranslated regions (UTRS). Both the 5' and 3' UTRS are required for RNA translation and replication (Heller, et al., 2005; Sookoian, 2003). This single ORF encodes a precursor polyprotein of approximately 3,000 amino acids, which is cleaved into different proteins by a host signal peptidase in the structural region and the HCV encoded protease in the non-structural (NS) region (Kuroki, et al., 2009). Cleavage of polyprotein yields 10 mature structural, non-structural HCV and the p7 proteins (Sookoian, 2003). The structural proteins participate in the assembly of viral particles of the new offspring, while the non-structural proteins are involved in viral replication and processing of polyprotein (Murillo, et al., 2004).

The structural region contains the core protein and two envelope proteins E1 and E2 (Pawlotsky and Gish, 2006). The core protein (C) has a highly conserved sequence of amino acids and is the main component of the nucleocapsid. E2 has two regions called hypervariable regions 1 and 2 (HVR1 and HVR2) that show extreme sequence variability. E2 contains the binding site for CD81, the putative HCV receptor or co-receptor (Murillo, et al., 2004). The region between the structural and non-structural genes encodes for an integral membrane cation channel protein p7 which is essential for virus production (Sillanpää, et al., 2009).

NS2, NS3, NS4A, NS4B, NS5A and NS5B (Sillanpää, et al., 2009) are non-structural proteins. These non-structural proteins have been assigned function as protease (in the case of NS2, NS3 and NS4A), helicase (in the case of NS3), and RNA-dependant RNA polymerase (NS5B) (Murillo *et al.*, 2004). NS2 is a cysteine protease responsible for an autoproteolytic NS2–NS3 cleavage. NS3 is a multifunctional protein with both serine protease and RNA helicase/ NTPase activities and NS4A is as an essential co-factor for NS3 protease functions. NS5A is a

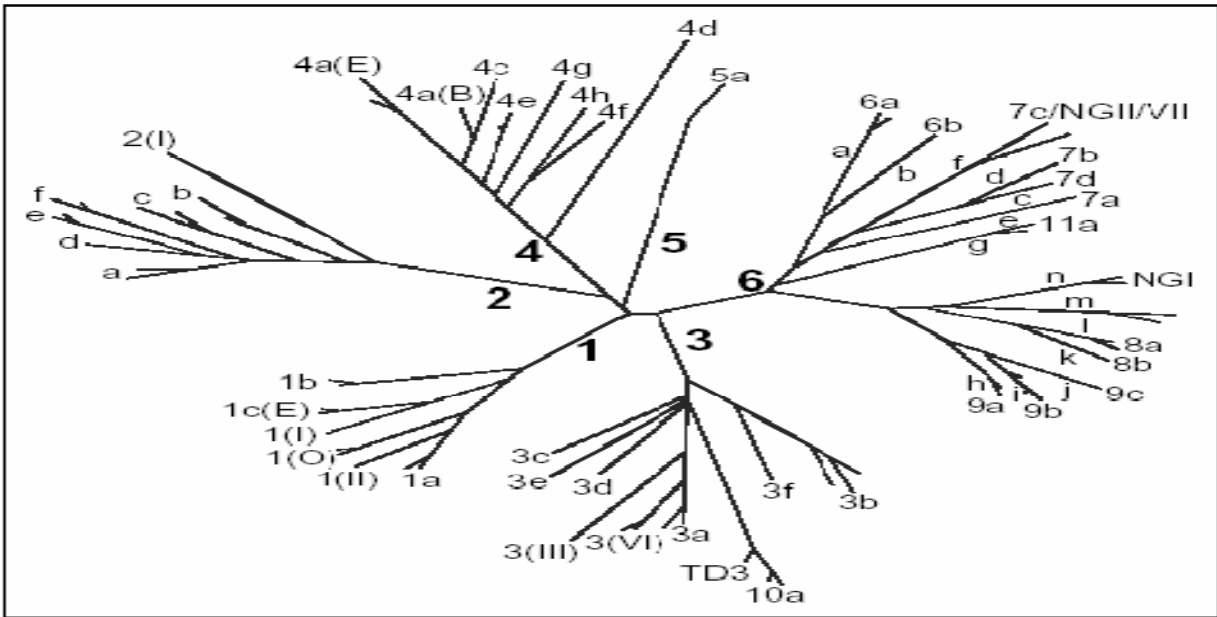
phosphoprotein which takes part in virus particle formation. The NS5B protein encodes for an RNA-dependent RNA polymerase (RdRp), which is the central catalytic enzyme of the HCV replicase (Sillanpää, et al., 2009).



**Figure 1.2:** The HCV genome and expressed polyprotein (Murillo *et al.*, 2004). A) Shows the genome of HCV, B) shows the locations of the proteins.

Six groups of HCV have been identified on the basis of molecular relatedness. The most commonly used classification system lists these groups as HCV genotype 1, 2, 3, etc. The amino acid sequences of the major HCV genotypes differ approximately by 31-34% from each other (Grimm and Kay, 2006). The genotype 1, 2, and 3 appears to have a worldwide distribution and

their relative prevalence varies from one geographic area to another. HCV genotype 4 appears to be prevalent in North Africa and the Middle East, and genotype 5 and 6 seems to be confined to South Africa and Asia respectively. In the USA and Western Europe genotype 1a and 1b are most common, followed by genotype 2 and 3 (Sillanpää, et al., 2009).



**Figure 1.3:** A phylogenetic tree demonstrating sequence diversity in different strain of hepatitis C virus (Dziora, et al., 2005)

### 1.1.3 Route of transmission

Hepatitis C virus is primarily a blood-borne or parentally transmitted infection (Carbonero, et al., 2007; Jarlais, et al., 2005). Most studies conducted mainly in industrialized countries have shown that the main mode of HCV transmission is parenteral route and the intravenous drug use. Injection drug use alone accounts up to 60% of transmissions in developed world. Sexual, maternal-fetal and nosocomial transmissions are also common. Vehicles and routes of parenteral

transmissions include contaminated blood and blood products, contaminated instruments, occupational and nosocomial exposure (e.g. needle stick injuries) and injection drug use (Cox, et al., 2005).

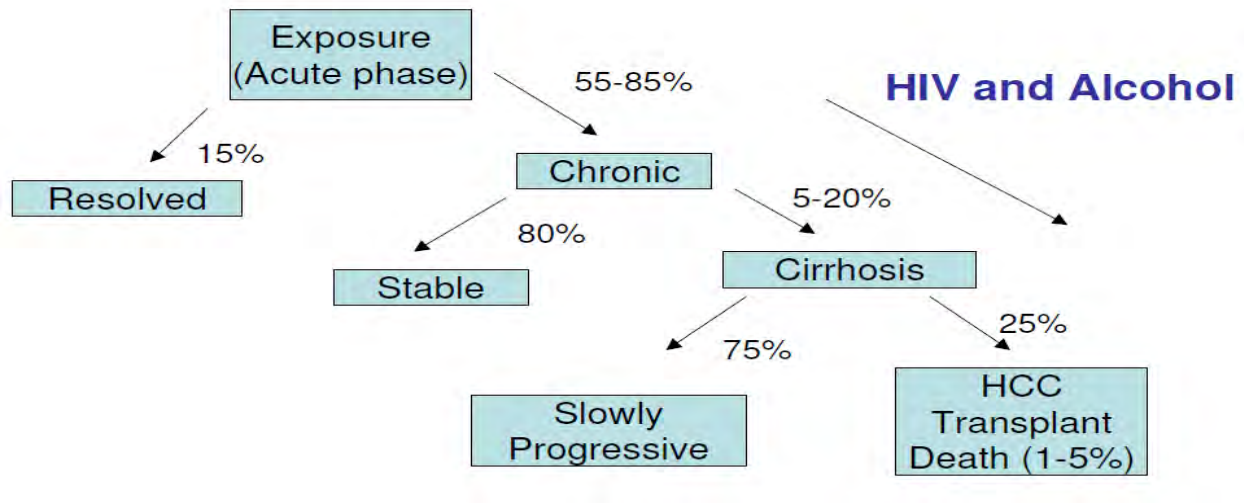
#### **1.1.4 Life Cycle and viral multiplication**

Hepatitis C virus mainly infects and multiplies in hepatocytes. However, earlier studies of HCV infection suggests that only small number of hepatocytes become infected, but more recent estimates suggest that 50% or more harbor the virus (Blackard and Sherman, 2008; Rehermann and Nascimben, 2005). Little is known about details of the HCV replication cycle because of lack of small animal model and it is very recently efficient cell culture system developed (Heller, et al., 2005; Sookoian, 2003). HCV reaches the liver binding with low density lipoprotein (LDL) in the blood (Murillo, et al., 2004). It binds to one or more cellular receptors organized as a receptor complex (Interaction of E2 with CD81 and scavenger receptor class B type 1) (Barth, et al., 2008; Randall, et al., 2007). After endocytosis, uncoating takes place in the cytoplasm and viral genome translation occurs in association with the rough endoplasmic reticulum (ER) leading to the production of a precursor polyprotein (Sookoian, 2003).

HCV has an extremely high replication rate. Approximately  $10^{10}$ – $10^{12}$  virions are produced per day in an infected person (Uebelhoer, et al., 2008). Replication occurs through an RNA dependent RNA polymerase that lack “proof reading” function which results in the rapid evolution of diverse but related quasispecies within an infected person (Radziewicz, et al., 2008). Progeny virions are assembled from cytoplasmic vesicles formed by budding through intracellular membranes. Finally, mature virions are released into the extracellular milieu by exocytosis (Radziewicz, et al., 2008; Tellinghuisen, et al., 2007).

### 1.1.5 Disease out come

Hepatitis C virus distinguishes itself from other viral hepatotropic infections by the low incidence of an acute phase and the high incidence of progression to chronicity. Initial infection (acute) with hepatitis C virus is characterized by rapid viral replication and intense inflammation.



**Figure 1.4:** Natural history of HCV. (Kindrick, 2002)

As shown in fig. 1.4, approximately 55-85% of acute HCV infections become chronic, with persistent or intermittent HCV viremia and slightly increased ALT level. Persistent or fluctuating serum ALT elevation in chronically infected patients is indicative of active liver inflammation. The increase in chronicity is due to enhanced expansion of CD4<sup>+</sup> CD25<sup>+</sup> (Regulatory) T cells that are able to suppress CD8<sup>+</sup> T-cell responses to different viral antigens (Kindrick, 2002; Koziel, 2002).

Occult HCV is another type of chronic infection which has been identified recently in a group of patients who have abnormal liver function tests and histological damage. Occult HCV infection

is characterized by the presence of HCV RNA in the liver in patients who consistently test negative for antibodies to HCV and HCV RNA in serum. Compared with chronic hepatitis C, occult HCV infection seems to be a less aggressive form of the disease caused by the HCV (Quiroga, et al., 2006).

#### **1.1.5.1 Cirrhosis**

Cirrhosis is a diffuse fibrotic disruption of liver tissue architecture. Approximately 5-20% of chronic patients asymptotically progress to cirrhosis after 20-30 years of infection. Those with cirrhosis or end-stage liver disease could develop potentially fatal complications such as bleeding esophageal varices, ascites, encephalopathy, hepatocellular carcinoma and liver failure. These conditions are common causes of morbidity and mortality in HCV/HIV co-infected patients (Carbonero, et al., 2001; Highleyman, 2007).

#### **1.1.5.2 Hepatocellular carcinoma (HCC)**

Hepatocellular carcinoma is the cancer of liver. It is the long-term outcome of HCV infection which evolves in approximately 5-25% of patients after 20 years of infection. Chronic inflammation and the sustained pressure of cellular regeneration may play important roles during carcinogenesis. Once cirrhosis develops, the rate of hepatocellular carcinoma increases to approximately 1-4% per year (WHO, 2003).

#### **1.1.6 Clinical Sign and Symptoms**

HCV infection causes an acute or chronic necroinflammatory disease of the liver. Acquisition of infection in about 80% of the cases is asymptomatic (Maier and George, 2002). After an

incubation period of about 6 weeks, 25-35% of patients develop some constitutional symptoms like; weakness, fatigue, headache, nausea, vague abdominal pain, loss of appetite and jaundice (Blackard, et al., 2008; Koziel, 2002).

Hepatitis C is also distinguished from other viral hepatic conditions by serological determinations. Liver enzymes characteristically affected by HCV infection include serum alanine transesferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGTP), and alkaline phosphatase. The increase in serum ALT levels occurs 8–14 weeks after HCV infection (Rehermann and Nascimben, 2005; Koziel, 2002).

### **1.1.7 Pathogenesis and Immune Response**

#### **1.1.7.1 Pathogenesis**

Hepatitis C virus has been known to infect only humans and chimpanzees (Bertoletti and Ferrari, 2003; Dhoot, 2003). Even though, the exact mechanism of HCV pathogenesis is not well understood, there are reports which indicate host reactions against HCV proteins have roles during its pathogenesis. Although the exact mechanism of pathogenesis is not defined, inflammation, NK cells, DC cells and toll-like receptors (TLRs) are indicated to play in the immunopathogenesis during HCV infection.

First of all, the normal uninfected liver maintains a largely tolerogenic environment and contains a large number of intrahepatic T-cells (Rehermann and Nascimben, 2005). In the liver, hepatocytes express low levels of major histocompatibility complex (MHC) and virtually no immune co-stimulatory molecules (such as CD80/B7.1, CD86/B7.2, and CD40). These conditions ensure that T-cells “ignore” antigens expressed by the parenchymal cells (Sun, et al., 2005). To mount an efficient immune response, co-stimulatory molecules on antigen-presenting

cells need to engage their ligands on T-cells, and this interaction provides a crucial signal permitting the activation and differentiation of T-cells into effector cells. At present, it is not completely clear how this tolerogenic environment, which is in part mediated by liver-specific antigen-presenting cells such as liver sinusoidal endothelial cells and Kupffer cells, changes to an inflammatory environment (Rehermann and Nascimben, 2005). In human HCV infection, high levels of MHC class II and co-stimulatory molecules are expressed on the activated Kupffer cells and hepatocytes, and their levels are also correlated with the extent of intrahepatic inflammation and elevation of serum ALT a biochemical marker of liver injury (Sun, et al., 2005). In addition, it is also not clear how the pre-existing T-cell population in the liver contributes to the adaptive immune response in viral hepatitis, whether T-cell priming occurs exclusively in draining lymph nodes or whether hepatocytes can prime T-cells under inflammatory conditions (Rehermann and Nascimben, 2005).

Recent studies show that inflammation plays an important role during the pathogenesis of HCV infection in the liver. It depends upon the rapid expression of chemokines which play an important role in inflammation. Pro-inflammatory cytokine IL-8 production is reported to be enhanced when HepG2 cells and primary hepatocytes are stimulated with HCV-E2 (Helbig, et al., 2009; Chung, 2004). Moreover, IL-8 expression is found to be positively regulated by a protein tyrosine phosphatase. The enhanced activities of non-receptor mediated (binding of HCV-E2 protein to the cell surface independent of direct viral infection) tyrosine kinases and serine kinases observed during HCV-E2 stimulation may also induce the downstream phosphorylation of transcriptional factors causing upregulation of fatty acid synthase-L (FasL). Altered mitochondrial membrane potential followed by cytochrome C release and caspase-3 activation, appears to be the mode of HCV-E2 induced Fas-mediated cell death in hepatic cells

via an ‘innocent bystander’ mechanism and persistent inflammation (Kanda, et al., 2007; Lindenbach and Rice, 2005). A similar induction of these signaling pathways by HCV envelope proteins also recently reported in human endothelial cells (WHO, 2003).

The interactions between HCV and the innate immune system play a critical role in the immunopathogenesis of HCV disease. Natural killer (NK) cells eliminate virus-infected hepatocytes through direct lysis (perforin/granzyme) or indirect (secretion of anti-viral cytokines) mechanisms. In addition, NK cells play an essential role in recruiting virus-specific T-cells and exerting anti-viral immunity in the liver (Wang, et al., 2008). *In vitro* studies show that the E2 protein of HCV can inhibit NK cell function directly. Because NK cell activation contributes to dendritic cell (DC) maturation, it is possible to speculate that this inhibitory effect may influence indirectly the function of DCs. Thus, the ability of DCs to pick up HCV antigens and migration to draining lymph nodes or to induce proper T-cell activation is negatively influenced (Bertoletti and Ferrari, 2003; Barnes, et al., 2002).

The innate immune response involving toll-like receptors (TLRs) has been also shown to play an important role in HCV pathogenesis. Toll-like receptors (TLRs) function as pattern recognition receptors for the detection of and response to microbial ligands. TLR activation leads to the transcription of inflammatory and anti-inflammatory cytokine genes leading to TLR-mediated innate immune responses and inflammation (Dolganiuc, et al., 2007; Machida, et al., 2006).

Furthermore, the mechanisms for hepatic damage during HCV infection are poorly defined due to lack of small animal models (Heller, et al., 2005; Sookoian, 2003). Several mechanisms are proposed to elucidate the pathophysiology of liver disease during HCV infection (Balasubramanian, et al., 2008). Some researchers hypothesized that the damage of liver during

HCV infection might be due to cellular immune responses against the virus, specifically involving CD8<sup>+</sup> T-cells which activate hepatic stellate cells leading to liver inflammation and fibrosis (Vali, et al., 2008). Others have hypothesized that damage of liver during HCV infection might be due to the responses of infected and uninfected liver cells against the viral proteins during infection (Mizukoshi, et al., 2008; Mohsen and Easterbrook, 2003). Structural and non-structural proteins shown to modulate host immune responses, participate in the process of pathogenesis and carcinogenesis (Balasubramanian, et al., 2008). Therefore, as yet, there is no a conclusive data that indicate the liver damage associated with HCV infection to be the result of a direct cytopathic effect or the host immune-mediated cytolytic response (WHO, 2003).

#### **1.1.7.2 Immune Response**

The role of host immune system in both defense against HCV infection and in the pathogenesis of HCV has been a subject of interest and controversy over the past several years (Capa, et al., 2007). The integrated activation of both the cellular and humoral arms of the adaptive immune response is important to control HCV infection. The different components of the adaptive immune system are so interconnected that the failure of one of them clearly affects the expansion and protective efficacy of the others. Lack of CD4<sup>+</sup> T-cell help can impair CD8<sup>+</sup> T-cell activity and antibody production, whereas the inability to mount a virus-specific CD8<sup>+</sup> T-cell response results in a level of circulating virus that cannot be cleared by antibodies alone (Bertoletti and Ferrari, 2003).

Current evidence suggests that both CD8<sup>+</sup> and CD4<sup>+</sup> T-cells play significant roles in animal models and limited human studies of HCV infection (Levin, 2005). During primary HCV infection of chimpanzees, virus-specific antibody responses are detectable 8 to 12 weeks after

infection. HCV-specific T-cells are detected in the blood of infected patients and chimpanzees 5–9 weeks after infection and in the liver of chimpanzees 6–12 weeks after infection (Fan, et al., 2007; Rehermann and Nascimben, 2005). Unfortunately, only about 15% of HCV infected individuals spontaneously (without drug) clear hepatitis C infection (Al-Sherbiny, et al., 2005; Nascimbeni, et al., 2003). Furthermore, these cellular responses appear to correspond in time with the resolution of infection (Machida, et al., 2008; Radziejewicz, et al., 2008). In addition, the immunological determinants that orchestrate the spontaneous clearance of HCV and the resolution of disease are also incompletely understood. However, vigorous intrahepatic CD4<sup>+</sup> T-cell responses to individual non-structural and structural (core and E2) proteins, specific CD8<sup>+</sup> T-cell cytolytic action, and high level local gamma-interferon production are believed to be important (Blackard, et al., 2008; Tedaldi, et al., 2005).

It is well documented that a strong and sustained antiviral T-cell response is associated with a self-limited course after acute infection. In contrast, chronic HCV infection is characterized by attenuated CD4<sup>+</sup> and CD8<sup>+</sup> HCV-specific T-cell responses. The causes of attenuation of immune responses to HCV during chronic infection are poorly understood (Capa, et al., 2007). There have been many reasons proposed as to why the immune system fails in the face of chronic HCV infection, including early T-cell exhaustion particularly of the CD4<sup>+</sup> T-helper subset, DC dysfunction (loss of antigen presenting function), high viral load, action of regulatory T-cells, defects in T-cell differentiation, impairment of effector cells, and cytotoxic T lymphocyte (CTL) viral epitope escape (Barnes, et al., 2008; Uebelhoer, et al., 2008; Averill, et al., 2007). Moreover, HCV is capable of escaping from immunological control through mutation of its E2 hyper variable region with subsequent evolution into diverse quasispecies. This region may

represent major target of cell-mediated and humoral immune mechanisms (Alatrakchi, et al., 2007; Barnes, et al., 2002).

Recovery from acute HCV infection requires elimination of HCV-infected cells directed by virus-specific cytotoxic CD8<sup>+</sup> T-cells. The functions of those virus-specific CD8<sup>+</sup> T-cells are further dependent on sufficient support from virus-specific T-helper cells (Bertoletti and Ferrari, 2003). In addition, those HCV-specific cellular immune responses are markers not only of previous exposure to and recovery from HCV but also of ongoing occult HCV infection (Quiroga, et al., 2006). Moreover, recently those virus-specific T-cells have been detected in the blood of HCV-seronegative healthy persons frequently exposed to HCV and in patients lacking humoral responses (Pawlotsky and Gish, 2006). Furthermore, the detection of HCV-specific T-cells in individuals in whom HCV RNA can persist in the liver despite the absence of viremia indicates that HCV replication is also prolonged in the face of virus-specific CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses (Quiroga, et al., 2006).

Recovery from acute infection didn't always associated with (but not necessarily related to) the development of specific antibodies directed against HCV (Al-Sherbiny, et al., 2005; Nascimbeni, et al., 2003). Individuals who develop chronic infection also develop HCV specific humoral responses (Rehermann and Nascimben, 2005). With respect to acute HCV infection, humoral immunity can assist in the direct neutralization of cell-free virions. However, the role of antibodies in long-term antiviral T-cell memory in humans is not clearly known, although it plays a critical role in murine models (Semmo, et al., 2006). The fact that HCV infection persists in the face of antibody response indicates that, in the chronically infected patient, antibody is insufficient to clear the infection (Sookoian, 2003).

Recent studies have shown that HIV infection might exert a direct cytopathic effect on liver cells (Blackard and Sherman, 2008). Although, pathways of viral entry differ, both viruses can be targeted to the same host cells via binding to shared surface molecules and the envelope proteins of the either virus cooperatively induce hepatocyte apoptosis via an ‘‘innocent bystander ‘‘ mechanisms (Chung, 2004). Moreover, HIV infection seems to facilitate HCV infection of extra hepatic cells (Blackard and Sherman, 2008; Chung, 2004). Although such a mechanism might contribute to higher blood HCV viral loads and exaggerated liver damage in HIV positive patients most epidemiological data suggest that accelerated progression of liver disease is linked to the loss of CD4<sup>+</sup> T-cells (Katie, et al., 2004). Level of CD4<sup>+</sup> T-cells immunosuppression has emerged as one of the most important determinants of progression to liver fibrosis, and patients with CD4<sup>+</sup> T-cell counts less than 500 cells x 10<sup>6</sup>/L are 3.2 times more likely to have advanced liver fibrosis on liver biopsy. The HIV/HCV co-infected patients also had a 1.2 relative hazard of AIDS progression or death within two years, compared to HIV mono-infected patients (Chung, 2004). Furthermore, studies have shown that the prevalence of extensive fibrosis and severe necroinflammation were higher in co-infected patients (60% and 54%) than in control patients (47% and 30%) (Chung, 2004; Marina and Vincent, 2003). In addition, the magnitudes of CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses were in parallel and correlated inversely with the extent of liver HCV infection. In co-infected persons, the cellular immune response against HCV is much strong than the immune response towards HIV (Alatrakchi, et al., 2007).

Nonetheless, it does seem paradoxical that immune reconstitution improves rather than worsens liver injury in HCV infection, as the injury is largely immune mediated via HCV-specific CD4<sup>+</sup> T-cells. However immune-mediated injury may be more applicable to immunocompetent

patients, and in those with immunosuppression a direct cytopathic effect of HCV may be possible. In HIV/HCV co-infection there is evidence (rarely) of such a cytopathic injury, producing the fatal condition fibrosing cholestatic hepatitis. This is associated with high HCV viral load, which leads to accumulation of viral proteins in the hepatic endoplasmic reticulum resulting in cell death (Verma, 2006).

HIV infection might influence the HCV-specific T-cell response by means other than simply the general failure of the immune system in more advanced stages of HIV infection (Mohsen and Easterbrook, 2003). One explanation for the low frequency of HCV-specific T-cell in peripheral blood might be compartmentalization of these cells to the liver as the main site of infection. Indeed, there is some evidence that HCV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T-cells enriched in the liver (Alatrakchi, et al., 2007). As compartmentalization alone is unlikely to explain the difference in the response against HIV and HCV, other mechanisms must contribute to the relative lack of cellular immune response against HCV compared to the response against HIV. It is specifically surprising that the HCV-specific CD8<sup>+</sup> T-cells response is so weak despite such high levels of viral replication (Caetano, et al., 2008), where as HIV-specific CD8<sup>+</sup> T-cells are easily detected even in the absence of detectable viruses (Bensch, et al., 2007). In chronic hepatitis C, immune-mediated mechanisms may still be able to control HCV infection partially in much the same way as cellular immune response can hold HIV infection in check for some time (WHO, 2003).

Furthermore, HCV-specific CD8<sup>+</sup> T-cell responses in peripheral blood mononuclear cells (PBMCs) from mono-infected individuals are generally weak (Mizukoshi, et al., 2008). Although, peripheral HCV-specific CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses are somewhat weaker in HCV/HIV co-infected individuals, similar frequencies of intrahepatic HCV-specific responses

appear to be obtained in HCV versus HCV/HIV co-infection. However, HIV-specific CD8<sup>+</sup> T-cell responses in PBMCs from HIV mono-infected individuals are about one log higher than ex-vivo HCV-specific responses in HCV mono-infection. In addition, impairment in cellular immune responses to HCV compared to HIV has been shown in HCV/HIV co-infection (Vali, et al., 2008).

### **1.1.8 Laboratory Diagnosis**

Since the discovery of the hepatitis C virus in 1989, the field of HCV diagnosis has advanced rapidly. Conventional methods fail to isolate the virus in cell culture or visualize it by electron microscope. Two major types of tests are available for the laboratory diagnosis of HCV infection: detection of antibody to various HCV antigens and molecular methods to detect and quantitate the nucleic acid of the virus (Mohsen and Easterbrook, 2003; Novick, 2000).

The first generation HCV-antibody test approved by FDA became commercially available in 1990 and was widely used. First, a screening enzyme immunoassay (EIA) is performed with repeat positive results confirmed by recombinant immunoblotting assay (RIBA) or other supplemental antibody test (Kindrick, 2002).

The 2<sup>nd</sup> generation EIA detects IgG anti-HCV antibody and overall, EIA-2 testing allows detection of anti-HCV in about 95% of individuals with HCV as confirmed by highly sensitive molecular tests such as the PCR and false-positive results are uncommon. False-positive reactions with EIA-2 are primarily limited to low-risk populations such as blood donors. Whereas, false-negative tests are seen most commonly among immunosuppressed individuals such as patients co-infected with HIV (Mohsen and Easterbrook, 2003).

The 3<sup>rd</sup> generation EIA-3 adds an additional antigen from the non-structural region (NS5), core and structural regions (Forbi, et al., 2007). EIA-3 appears to have increased sensitivity in the high prevalence setting and slightly better specificity in the blood donor population (Mohsen and Easterbrook, 2003).

A new 4<sup>th</sup> generation test has recently been developed to detect all anti-HCV antibody isotypes to all major HCV genotypes. The test employs a genotype cross-reactive recombinant peptide derived from the core, NS3, NS4 and NS5 regions of multiple HCV genotypes. It utilizes the principle of immunochromatography, a unique two-site immunoassay on a nitrocellulose membrane. The test has a higher sensitivity and specificity over the earlier immunoassay methods (Dziora, et al., 2005).

Antibody tests are unable to identify subjects in the early stage of infection, in what is known as the diagnostic windows period, during which specific antibodies has not yet been produced, but the virus is present in the plasma, sometimes in large quantities. It should be also noted that HCV antibodies might be lost during the course of HIV infection as a result of the underlying immunosuppression (Maier and George, 2002).

The detection of HCV-RNA by reverse transcriptase PCR has been essential for the diagnosis of HCV infection (Drexler, et al., 2009). HCV RNA detection by real-time RT-PCR is now standardized, reliable and reproducible, and offers a broad dynamic quantitation range. The principal role of HCV RNA testing is in the tailoring, selecting and monitoring of antiviral therapy (WHO, 2003). The sensitivity of PCR for HCV RNA detection may vary according to the choice of primers and the handling of pre-extraction samples. However, PCR testing for the virus is not widely available, is expensive and of limited reliability (WHO, 2003).

Liver biopsy allows determining the necro-inflammatory activity (grading) and the degree of fibrosis (staging) as well as to recognize or exclude co-existing liver pathology (such as alcoholic liver disease, iron overload). Different combinations of blood tests, transient elastography or magnetic resonance imaging are currently being explored to predict liver fibrosis (Levin, 2005).

#### **1.1.8.1 Cells Culture System**

Very recently, the first productive HCV cell culture system was described. It is based on the transfection of genomic HCV RNAs of the genotype 2a isolate Japan fulminant hepatitis (JFH-1) into Huh-7 cells. Virus particles released from transfected cells are infectious in naive Huh-7 cells and can establish productive infection in vivo (Zhu, et al., 2006; Rehermann and Nascimben, 2005). Besides the JFH-1 wild type, two groups have described the generation of chimeric HCV genomes allowing the production of higher-titer virus stocks. Most efficient is Jc1, a chimera in which the JFH-1 region encoding core up to the first transmembrane segment of NS2 is replaced by the analogous region of the genotype 2a isolate J6 (Schaller, et al., 2007; Guha, et al., 2005).

#### **1.1.8.2 Animal Models**

The lack of a robust small animal model for HCV (due to narrow host range) has hindered the study of pathogenesis, discovery and development of novel drug treatments for HCV infections (Elmowalid, et al., 2007; Brass, et al., 2006). Over the past few years, several small animal models such as the HCV-Trimera and chimeric scid-albumin urokinase (Alb/uPA) Hepatech mouse models have been developed (Guha, et al., 2005; Murillo, et al., 2004). The model utilizes a mouse-adapted replicon-containing Huh-7 human hepatoma cell line expressing a luciferase

reporter linked to the HCV subgenome. The replicon used in this model expresses the HCV non-structural proteins that comprised the replisome and is transfected into human hepatoma Huh-7 cells (Murillo, et al., 2004).

The chimpanzee, *Pan troglodytes*, is the only animal model that has been infected with HCV, and is capable of developing persistent infections, even in the presence of humoral and cellular immune response. This animal model presents clinical and histopathological manifestations that resemble those that appear in humans. However, because of ethical reasons and high costs, only a few studies have been performed in these animals (Guha, et al., 2005; Murillo, et al., 2004). In chimpanzees infected with HCV, expansion of a multispecific and sustained HCV-specific CD8<sup>+</sup> T-cell-mediated response was observed that cleared the virus, but not in that developed a chronic infection. Thus, the chimpanzee represents a valuable model for HCV infection (Nascimbeni, et al., 2003).

### **1.1.9 Treatment**

There is intense interest in the developing therapeutic regimens that are capable of inhibiting HCV replication and improving the natural history of the disease (Drexler, et al., 2009; Manns, et al., 2007). The goal of HCV therapy is actual clearance of the virus and limit potential interference with other drugs (Antonucci, et al., 2005; Sun, et al., 2005).

Current standard therapy of chronic hepatitis C consists of pegylated interferon- $\alpha$  (PEG-IFN- $\alpha$ ), administered once weekly by subcutaneous injection, combined with ribavirin, which is taken orally on a daily basis (Rodríguez, et al., 1999). Interferons, a heterogeneous group of cytokines that are expressed in the response to viral infections, induce the expression of multiple genes that have antiviral or antiproliferative activity or both (Manns, et al., 2007). Ribavirin is a nucleoside

analogue drug that inhibits the replication of many different viruses. Its specific mechanism of antiviral activity against HCV is not well known (Rodríguez, et al., 1999).

Standard treatment duration is 48 weeks for HCV genotype 1 and 24 weeks for genotypes 2 and 3 (Maier and George, 2002). With this treatment, 40–50% of genotype 1 and about 80% of genotype 2- and 3-infected patients achieve a sustained virological response (SVR) (Manns, et al., 2007). It is now well established that the genotype 1 and 4 have the lowest rate of treatment success, while genotype 2 and 3 have the highest. Hence, determining the HCV genotype and viral load has become increasingly important in guiding the duration of combination therapy with interferon and ribavirin. As a result, HCV genotype determination has become the mainstay of both diagnosis and management of patients with HCV (Lusk, 2006).

Overall, about 50% of patients with chronic hepatitis C can be cured with the current treatment (Tellinghuisen, et al., 2007; Rodríguez, et al., 1999). For these patients, ongoing efforts are aimed at tailoring treatment to the individual needs in order to improve tolerability. For the other patients and for the important proportion of patients who cannot tolerate current treatment, there is an urgent need to develop more effective and better tolerated therapies (Sookoian, 2003, Kindrick, 2002).

### **Treatment of Hepatitis C in HIV Co-infected Patients**

The management of chronic hepatitis C in HIV/HCV co-infected patients has become an important challenge, since possible interactions among anti HIV drugs and interferon or ribavirin have to be taken into account (Chung, 2004). For instance, ribavirin may interact with those

selected nucleoside reverse transcriptase inhibitors (AZT, ddI, d4T) and reduce their anti-HIV activity (Kindrick, 2002; Rodríguez, et al., 1999). If AZT is given concomitant with ribavirin, there is an increased incidence of anemia, leukocytopenia and thrombocytopenia and it should be carefully monitored. However, Tenofovir appears safe when administered concomitantly with ribavirin in HIV/HCV co-infected patients. Among the NNRTIs, Nevirapine frequently causes elevation in transaminases, while Efavirenz is least likely to cause liver toxicity (Maier and George, 2002). Furthermore, the protease inhibitor Indinavir can cause severe hyperbilirubinemia in patients with HCV co-infection. Nelfinavir and Saquinavir are the safest among protease inhibitors in patients with compromised liver function (Maier and George, 2002).

There are a number of contra-indications, and adverse effects (including flu-like symptoms, depression, leukocytopenia, anaemia, fatigue aches and pain, diarrhea, hair loss, nausea and sleep and eating disturbances) which are sometimes serious and frequent (Chung, 2004; Kindrick, 2002). Anaemia, caused by ribavirin is of particular concern for people living with HIV (Lusk, 2006; Maier and George, 2002). Ribavirin can also cause birth defects (Levin, 2005). The main cause of neutropenia and thrombocytopenia during anti-HCV therapy is partially due to the direct myelotoxic action of IFN on bone marrow progenitors (Gatti, et al., 2007; Arizcorreta, et al., 2006).

Although, hepatotoxicity from HAART is the main aspect of HIV/HCV co-infection that complicates the management of patients (Maier and George, 2002), the optimal approach to treatment of HIV/HCV co-infected patients is still unknown (Dhoot, 2003). Treatment of HIV

first or concurrently with HCV therapy may improve the clinical acceleration of HCV morbidity and mortality but this strategy awaits further clinical trial (Maier and George, 2002). If possible, HCV should be treated before HIV; reasons include the increased hepatotoxicity of HAART with concurrent hepatitis C, possibly impaired immune reconstitution resulting from hepatitis C, better compliance, and finally prevention of drug interactions (Marina and Vincent, 2003; Maier and George, 2002). Finally, side effect management including prescribing additional drugs for depression, anaemia, or other effects of medication are key practices to treatment adherence (Lusk, 2006).

#### **1.1.10 Prevention**

The lack of a preventive vaccine, coupled with common unresponsiveness to treatment and co-infection with HIV, has made HCV a major threat to public health (Guha, et al., 2005). Extensive genetic variation between different strains and genotypes, the virus ability to modify envelope and other proteins rapidly in the faces of immunological pressure, incomplete understanding of the pathogenesis and immunogenesis of HCV infection, the likely need to develop a vaccine that stimulates both humoral and cellular immunity against HCV, and the inability to culture the virus easily are challenges to develop HCV vaccine (Elmowalid, et al., 2007). However, extensive studies of a recombinant vaccine in chimpanzees showed encouraging results. Based on the viral envelope proteins E1/E2, it protected more than 80% of the animals from developing chronic infection following the experimental challenge with either homologous or heterologous HCV-1a viral strains. A T-cell vaccine eliciting broad cellular responses to HCV-1b non-structural proteins 3, 4 and 5, was also shown to exhibit prophylactic activity in chimpanzees after heterologous HCV-1a challenge (Manns, et al., 2007; Su, et al., 2002). In the absence of a

vaccine, all precautions to prevent infection by other means must be taken. Thus, the key to reducing the incidence of HCV infection is decreasing exposure to contaminated blood (WHO, 2003).

## **1.2 Significance of the Study**

Even though, there are more than 1.1 million people are living with HIV/AIDS in Ethiopia, the impacts of HCV infection on immunohaematological parameters and liver enzyme levels of these victims was not been well documented. On the other hand, hepatitis C virus is now been viewed as an opportunistic infection in HIV infected persons. In developed world for instance in U.S.A. and Europe, HCV is responsible for 70% of liver diseases in HIV/HCV co-infected patients. So that HCV-related liver diseases have become major causes of death and hospital admission. Moreover, hepatitis C virus infection is known to be a risk factor for anti retroviral /HAART/ related hepatotoxicity. i. e. the presence of HCV increases the frequency of hepatotoxicity with antiretroviral therapy. Despite these, many infected individuals in Ethiopia are taking anti-retroviral drugs. Moreover, recent literatures also show that, both nucleoside analogs (lamivudine and zidovudine) and HIV protease inhibitor (PI) (Nelfinavir) have an impact on the replication of HCV. To ensure the optimal clinical managements of HIV patients, it is important to know the impacts HCV infection on immunohaematological parameters and liver enzyme levels. However, there is no such study that indicates the impacts of HCV infection on immunohaematological parameters and liver enzyme levels in Ethiopia. Considering the relatively low number (n= 387) of HIV patients screened for HCV, therefore, the aim of the present study was to provide baseline information on impacts of HCV infection on

immunohaematological parameters and liver enzyme levels for effective treatment strategies and better care of co-infected patients.

### **1.3 Limitations of the Study**

- ▶ Selection of the study subjects on voluntary basis introduces the possibility of selection bias this in turn affecting representativeness.
- ▶ Due to small number of study participants, it is difficult to generalize the findings of the present study to the population.
- ▶ When human serum samples are tested for anti-HCV antibodies using different commercial rapid test devices, discrepant results often occur. Usually the so-called confirmatory test RIBA is commonly used to evaluate positive rapid test devices results. Due to unavailability of RIBA kit, no confirmatory test in this study was carried out so that some false-positive results may be expected.
- ▶ Antibody test devices cannot determine whether or not someone is a carrier or if disease has run its course and the antigen is no longer present. However, there is no antigen test that is able to detect both antibody bound and free antigen. The Flavicheck-HCV test device which is used in this study detects total antibodies to all major genotypes of HCV. Therefore, the presence of viraemia in HCV-antibody positive samples was not confirmed using antigen test.
- ▶ Due to unavailability of viral load measurement, the amount of HIV RNA in the blood of patients was not quantitated.

- ▶ This study was also limited by the unavailability of important data regarding HIV and HCV genotype, alcohol use and viral load which were not routinely and reliably available for most patients.
  
- ▶ There is no any data that shows for how long time these patients lived infected with both virus and with which virus they infected first and the duration of HCV infection and initiation of HAART.
  
- ▶ Even though most studies about the impacts of HCV infection on HIV disease progression in HCV/HIV co-infected patients have been conducted in developed countries, little is known in Africa. Therefore, the present study was deficient about local information.

## **1.4 Objectives of the Study**

### **1.4.1 General Objective**

- ▶ To assess the impact of HCV infection on immunohaematological parameters and liver enzyme levels in HCV/HIV co-infected patients before and after initiation of HAART.

### **1.4.2 Specific Objectives**

- ▶ To determine the changes in CD4<sup>+</sup> and CD8<sup>+</sup> T-cells count.
- ▶ To determine the changes in the white blood cell and total lymphocyte count
- ▶ To determine the changes in liver enzyme levels.
- ▶ To determine HCV and HBV status of the study patients.

## **Chapter Two: Materials and Methods**

### **2.1 Study area**

The study was conducted at ART clinics of Yekatit-12 (Bethesaida) and Zenbaba General Hospitals, Addis Ababa; both are referral hospitals with a separate ART clinic.

### **2.2 Study design and period**

A hospital-based, observational, cohort study was conducted on leftover blood samples collected for the purpose of clinical investigations from ART follow-up and VCT clinics at Yekatit-12 and Zenbaba General Hospitals. All eligible patients who came for VCT and those who have regular follow up were included in the screening test. Leftover blood samples collected from volunteer patients from Sep 1-30, 2006 were screened for HCV. HIV patients those are co-infected with HCV and hepatitis B virus (HBV) were excluded from study. The control groups were also screened for HBV infection. Only those patients who reconfirm the consent and those willing to come every three month for further follow up were selected for the study.

Selected patients were put into four groups for follow up: i) 16 patients only with HIV infection and on HAART (Group 1), ii) 16 HCV/HIV co-infected patients receiving HAART (Group 2), iii) 9 HIV positive pre-ART patients without HCV infection (Group 3) and iv) 9 HIV/HCV co-infected pre-ART patients (Group 4). The pre-ART patients, though naïve for HAART, were under follow up to start their treatment. For this study, immunohaematological and clinical chemistry tests were done. All the above mentioned tests were only done for selected patient after the approval of proposal annually for three years and every three month for one year during the detailed follow up of the fourth year. The study subjects were also screened for HCV and HBV each year for a total of four times. The study groups were prospectively followed for a total

of four years from Sep 2006 to Nov 2010 in order to determine the impact of HCV infection on immunohaematological and clinical chemistry parameters.

### **2.3 Study population**

The study population comprised of HIV patients on follow-up and VCT attendants at Yekatit-12 and Zenbaba General Hospitals between 2006 and 2010. For the present study, all clients above 18 years old those who are HIV/HCV co-infected and selected HIV mono-infected patients were included in the study.

### **2.4 Sample size and sampling technique**

Leftover blood samples collected for clinical investigations from ART follow-up and VCT clinics at Yekatit-12 and Zenbaba General Hospitals from Sep 1-30, 2006 were included in the study. Hence, a convenient, non-probability sampling technique was employed and a total of 50-60 study individuals were planned for follow up at the beginning of the project. To get the planned number of study subjects, from Sep 1-30, 2006 a total of 387 HIV positive patients were screened for HCV. At end of screening, we got 25 HCV/HIV co-infected patients and two patients co-infected with HIV, HCV and HBV were excluded from the study. All 25 patients those who are HIV/HCV co-infected and 25 HIV mono-infected patients were enrolled in this observational follow-up study.

## **2.5 Laboratory Analysis**

### **2.5.1 Specimen collection and handling**

Blood samples were collected for serological, immunohaematological and clinical chemistry investigations from VCT attendants, follow-up patients including those who were on HAART and pre-ART patients by professional laboratory personnel. For the present research, we used leftover of these blood samples. For immunohaematological tests whole blood samples were used. Serum or plasma was used for the screening of HCV and HBV and serum for measurement of liver enzyme levels. Only clear non-hemolyzed specimens were used. All the necessary precautions were taken in handling serum or plasma samples, as these were capable of transmitting etiological agents.

### **2.5.2 Anti-HCV and HBV antibodies detection**

The diagnosis and identification of HCV infection is based principally on the detection of antibodies to recombinant HCV peptides. Different researchers have employed different immunoassay procedures for detection of antibodies. For present study, a commercial fourth generation two-site sandwich immunoassay for total anti-HCV antibody test device (Qualpro Diagnostics, 88/89, phase IIC, Verma Industrial Estate, Verna, Goa-403 722, India, 2004) was employed to test total anti-HCV antibodies. The results were interpreted according to the manufacturer's instruction. Each initially reactive HCV samples were re-tested using the same test device by another qualified laboratory technologist to increase the reliability and validity of the test method. Three samples which showed discrepant results were rejected.

The Flavicheck-HCV is a rapid, qualitative, fourth generation two-site sandwich immunoassay for the detection of total antibodies specific to HCV in serum or plasma. It uses a multi-epitope

recombinant peptide antigen that is broadly cross-reactive to all major HCV genotypes. It detects total anti-HCV antibodies ensuring detection of all antibody isotypes IgG, IgM, IgA, IgE and IgD. The test utilizes the principle of immunochromatography, a unique two-site immunoassay on a nitrocellulose membrane. The conjugate pad contains two components- a multi-epitope HCV recombinant peptide antigen conjugated to colloidal gold and rabbit IgG conjugated to colloidal gold. As the test specimen flows through the membrane test assembly the HCV recombinant peptide antigen-colloidal gold conjugate complexes with the anti-HCV antibodies in the specimen and travels on the membrane due to capillary action along with the rabbit IgG-colloidal gold conjugate. This complex moves further on the membrane to the test region (T) where it is immobilized by another multi-epitope HCV recombinant peptide antigen coated on the membrane leading to formation of a pink to pink-purple coloured band which indicates a positive test result. The absence of this coloured band in the test region indicates a negative test result. The unreacted conjugate and unbound complex, if any, along with rabbit IgG gold conjugate move further on the membrane and are subsequently immobilized by the goat anti-rabbit antibodies coated on the membrane at the control region (C), forming a pink to pink-purple coloured band. This control band acts as a procedural control and serves to validate the results (Qualpro Diagnostics, 88/89, phase IIC, Verma Industrial Estate, Verna, Goa-403 722, India, 2004).

### **Test procedure**

The test procedure was following the manufacturer's protocol. The test device was allowed to stand to room temperature. Using dropper, exactly two drops (50 $\mu$ ) of specimen (Serum or plasma) was dispensed into the port "A". Then, three drops of sample running buffer were added into the port "B". A time of 15 minutes was allowed until the pink line (s) appears on the test

device. The results were interpreted at the end of 15 minutes. A diagrammatic presentation of the interpretation of the test device is shown in Figure below.

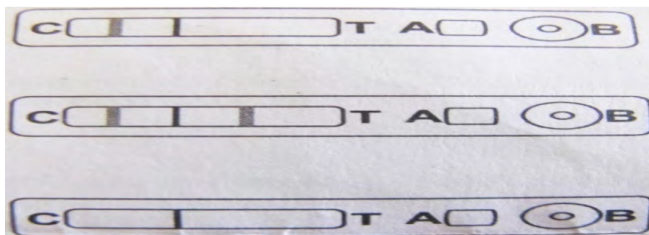
### **Interpretation of results**

**NEGATIVE:** One pink line appears on the control region (C). No apparent pink line appears in the test region (T).

**POSITIVE:** Two distinct pink lines appear. One line should be in the control region (C) and another line should be in the test region (T).

**NOTE:** The intensity of the pink color in the test line region (T) will vary depending on the concentration of anti-HCV antibodies present in the specimen. Therefore, any shade of pink in the test region (T) should be considered positive.

**INVALID:** Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure.



**Fig 2.1** Flavicheck-HCV test device application, interpretation and control system (Qualpro Diagnostics, 88/89, phase IIC, Verma Industrial Estate, Verna, Goa-403 722, India, 2004).

The Flavicheck-HCV two-site sandwich immunoassay test device has sensitivity of 100% and relative specificity 99.7% (Qualpro Diagnostics, 88/89, phase IIC, Verma Industrial Estate, Verna, Goa-403 722, India, 2004). However, the test limitation includes the following:

- Neither the quantitative value nor the rate of anti-HCV antibody concentration can be determined by this qualitative test.
- The test cannot detect extremely low concentrations of anti-HCV antibodies in specimens.
- A negative result at any time does not preclude the possibility of hepatitis C infection.

The Flavicheck-HCV two-site sandwich immunoassay test device sensitivity and specificity was tested by the company in comparison with Enzyme immunoassay techniques and resulted with the indicated sensitivity and specificity.

**Note:** Except the recombinant peptide inserted the principle, procedure and interpretation of HBV are the same with that of HCV. The HCV and HBV kit were manufactured from the same company.

### **2.5.3 CD4<sup>+</sup> and CD8<sup>+</sup> T-lymphocytes quantification**

A standard 3-color flowcytometry using Fluorescent Activated Cell Sorter (FACS) count (Becton Dickinson) were used to enumerate the absolute values of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells for each sample according to manufacturer's instructions. FACS is a machine that measures cell size, granularity and fluorescence due to bound fluorescent antibodies as single cells pass in a stream past photodetectors. The analysis of single cells in this way is known as flowcytometry. Cells to be analyzed by flowcytometry are first labeled with a specific fluorescent dye conjugated to a particular monoclonal antibody to detect surface molecules. The suspension containing the mixture of labeled cells is then forced through a narrow aperture so that the cells are lined up in a single file. Then, as each cell passes through a laser beam, it scatters the laser light. In addition, the fluorescent dye on any antibodies bound to the cells is excited by the laser beam and will fluoresce. The scattered light and the fluorescence are then detected by

photomultiplier tubes that send the information to a computer for data collection. The amount of fluorescence on a cell indicates the level of expression of the cell surface molecules to which the fluorescent antibodies are indicated. The scattered lights also provide information about the size and granularity of each cell. The vibrations in the cell stream cause it to disperse into droplets. The falling droplets are next given an electrical charge in proportion to the amount of fluorescence they have emitted. Droplets containing charged cells are then deflected from the main stream of droplets as they pass between a pair of oppositely charged plates, so that positively charged droplets are attracted to a negatively charged plate and vice versa. The amount and direction of deflection depend on the type and intensity of charge on each cell (BD Biosciences, San Jose, CA 95131-1807, USA).

### **Test procedure**

The immunophenotyping was performed on whole blood. EDTA blood (50 µl) was incubated with combination of PE conjugated CD4<sup>+</sup> or CD8<sup>+</sup> MoAbs and FITC conjugated CD3<sup>+</sup> MoAbs for 60 minutes at room temperature in the dark. Then 50 µl of fixative (FACSfixative; Becton Dickinson) was added to each tube and incubated at room temperature for 30 minutes in the dark. At each step the preparation was well mixed. Controls (Zero, low, medium and high) were also done with the sample test to increase the reliability of the test result. Then, the FACS count machine detects each cell type depending on the cell size and granularity to provide a quantitative data and fluorescence (BD Biosciences, San Jose, CA 95131-1807, USA).

### **2.5.4 Haematological tests Analysis**

Celldyn-1800 (haemoanalyzer) was used to count WBC, absolute lymphocytes and differential counts. The machine uses the impedance method to count the cells. It counts cells depending on

their granularity and size by detecting and measuring changes in electrical impedance when a particle in a conductive liquid passes through a small aperture. Each cell passes through the aperture which is found in between the external and internal electrodes which has a constant DC current flow. The phenomenon causes some change in the impedance of the conductive blood cell suspension. These changes are recorded as increases in the voltage between the electrodes. The number of pulses correlates to the number of particles (blood cells). The intensity of each pulse is proportional to the volume of that particle (WBC, RBC, PLT). Pulses are counted only in channels (in terms of femtoliters, fl) which are between the preset lower and upper discriminators. After counting and sorting, the computer system calculates the amount of each cell and displays it on the screen (Abbott Laboratories, Abbott Park, IL 60064, USA).

### **Test procedure**

Anti-coagulated whole blood sample (30 $\mu$ l) was aspirated by the sampling needle and 4ml of diluents were added into the mixing chamber. From here, 25 $\mu$ l of primary dilution were aspirated and then added with 5 $\mu$ l of diluents into the RBC chamber (RBC dilution). The primary dilution remaining in the mixing chamber flows via the tubing into the WBC chamber where the necessary amount of reagent was added (WBC dilution). Then the machine counts and sorts each cell as mentioned above. Controls (low, medium and high) were always run before sample test done.

### **2.5.6 Measurement of liver enzyme levels**

Chemistry analyzers measure the concentration of analytes in blood or other bodily fluids based on specific chemical reactions by photometry. Applications vary from clinical diagnostic, drug abuse monitoring to forensic testing, etc. In this study, Humastar80 (chemistry analyzer) was

used to measure the liver enzyme levels (serum GPT, GOT and ALP). Principle of operation is based on the fact that substances of clinical interest selectively absorb or emit energy (light) at different wavelengths. The analyzer has an optical device that measures light absorption at various wavelengths in a given liquid sample. In general, when a colored solution is illuminated with a monochromatic light (light of a single wavelength), its absorbance is proportional to the concentration of the colored solution. There is a computer system which can calculate the amount of light absorbed and /or emitted and relates to the amount analyte we want to measure. Then, it displays the result in numbers on the computer screen. Using this principle, it is possible measure any kind of analyte we are interested on (Human GmbH.65205 wiesbaden, Germany).

### **Procedure**

The instrument operates under software control. The analyzer was programmed to perform analysis and calculation according to an optimized protocol. In the instrument, the cuvettes were arranged in a circle (rotor), and this was a slowly rotated in step at a fixed time interval (cycle time). Access to the cuvette was possible only at these intervals for sample or reagent application and reading if needed. Enough amounts of non-hemolyzed serum samples, reagents and standards were added to the appropriate cuvettes. Then, the aspirator aspirated the required amounts of sample, reagent and standard in to a separate mixing and reaction cuvette. When the rotor starts to spin, the contents of these compartments were mixed simultaneously. After the samples and reagents were mixed, then the required analyte in the mixture was measured by the spectrophotometer. Quality control done on daily basis labeled with normal and pathological.

## **2.6 Data analysis**

Data from the research was analyzed using computer soft ware. The mean, percent and ratio were used to compare the results of samples among and within groups. No statistical package and tools used primarily because there were no any data that shows for how long time these patients lived infected with both virus and with which virus they infected first and the duration of HCV infection and initiation of HAART.

## **2.7 Ethical Clearance**

This study was conducted at ART clinics of Yekatit-12 and Zenbaba General Hospitals on leftover blood samples that were collected for the purpose of CD4<sup>+</sup> and CD8<sup>+</sup> counts, chemistry tests and for other clinical investigations from follow-up patients including those who are on anti-retroviral treatment (HAART) and pre-ART patients. The study was ethically cleared from AAU, biology department ethics committee. The study was conducted after obtaining a letter informing the hospital administrators about the objective of the study from the Biology Department Ethics Committee of the AAU. The consent form has information about the researcher including his address. The aims and benefits of the study were explained to the study subjects. Participation was based on voluntary basis and those who were agreed to participate in the study sign consent form. All laboratory tests were done free of charge and those whose serum sample became reactive for HCV and HBV were notified to their respective doctors or nurses for any possible further measures to be taken. Confidentiality of the information about patients was maintained throughout the study time and thereafter.

## Chapter Three: Result

### 3.1 Study Subjects

From a total of 387 (182 female and 205 male) known HIV patients who visited Yekatit-12 and Zenbaba hospitals in Addis Ababa during the month of September 2006, 50 eligible patients, 25 HCV+ve and 25 HCV-ve, were enrolled for follow up study. Based on their HCV infection and HIV treatment status, these patients were put into four groups. Group 1: ART+veHCV-ve; group 2: ART+veHCV+ve; group 3: Pre-ARTHCV-ve and group 4: Pre-ARTHCV+ve. Though the total number of male and female patients taken was 25 each, their gender ratio does not much in all the groups: Groups 1 and 4 had more or less equal number of males and females; whereas in group 2 the number of males is almost double that of females and group 3, 8 times more females. i. e. as shown in fig. 3.1a, group 1 with 7 (43.75%) females and 9 (56.25%) males making male to female ratio about 4:3 and group 4, with 4 (44.44%) females and 5 (55.56%) males had about 1:1 ratio where as group 2, had male to female ratio of 2:1 { 6 (37.5%) females and 10 (62.5%) males} and group 3, had the highest female to male ratio, 8:1, {8 (88.89%) females only one (11.11%) male}.

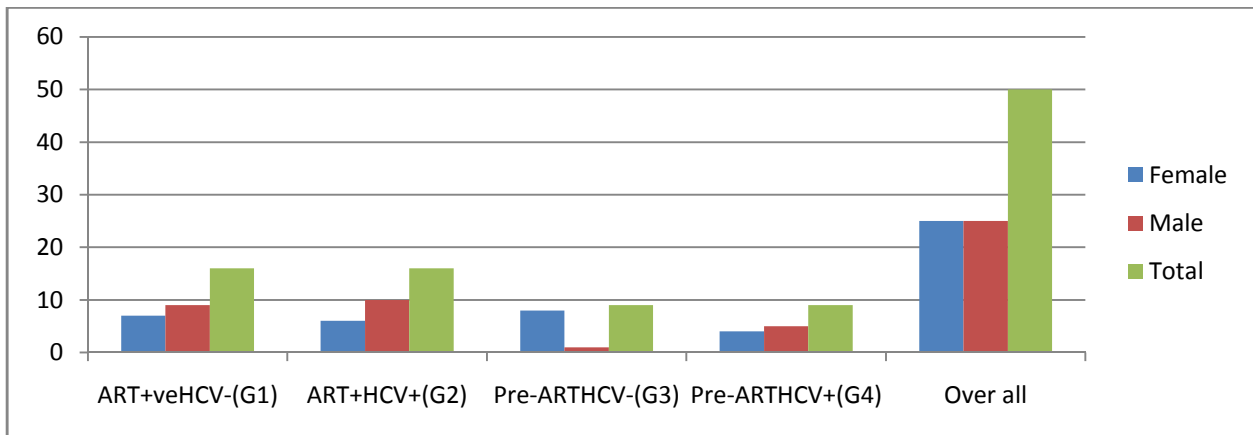
### 3.2 Prevalence of HCV/HIV among the study subjects

The prevalence of HCV among the 387 HIV patients who visited the two hospitals and were screened for the study was 6.5%. Of these, 206 (53.23%) were under ART and 181 (46.77%) pre-ART patients. Relatively more HCV infected patients, (7.8%), were from those under ART and (5.0%) from pre-ART. The age of patients ranged between 20-65 years. The mean age for the patients under investigation was 38.9 years. Females were relatively younger (34.5 years) when compared to males (42.6 years). The mean age of patients in the four groups was

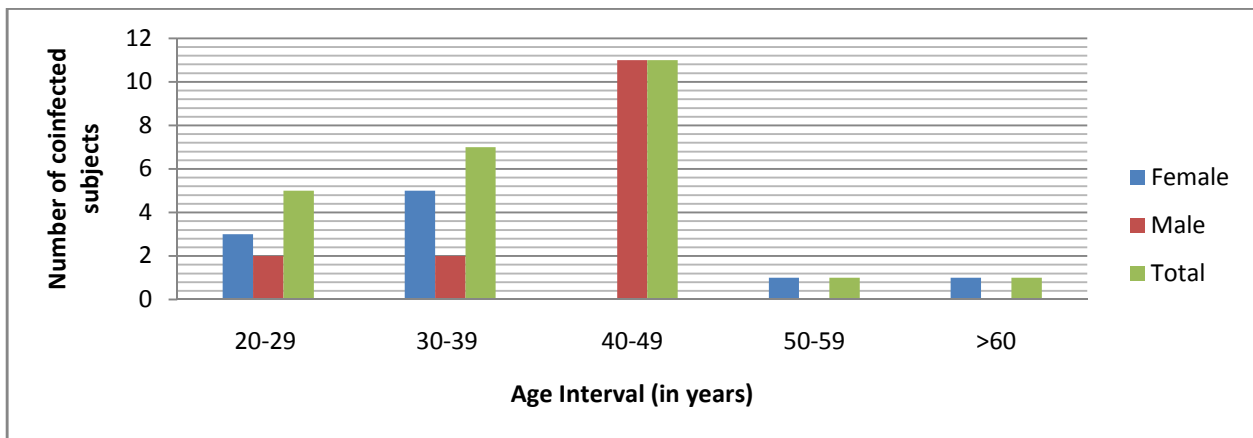
comparable; 41.2, 41.3, 46.2 and 40.2 years respectively. The overall mean age and group mean ages of the study subjects had a minimal difference (38.9 Vs 41.2, 41.3, 46.2 and 40.2). As shown in fig. 3.1b, from 25 HCV/HIV co-infected patients 23 (92%) were between 20-49 years and 18 (72%) between 30-49 years of age. The male to female ratio was different in different age intervals: 1:1 in the 20-29, female dominated (2:1) in the 30-39 and completely male (1:0) in 40-49 age groups. In age groups below 39 years females were more in number. In this study, HCV/HIV co-infection was higher in males (60%) than females (40%). Furthermore, 11(73.33%) of the co-infected males were over the age of 40 years.

**Figure 3.1: Age-sex distribution of study subjects in each group at Yekatit-12 and Zenbaba General Hospitals, Addis Ababa (Sep. 2006- Nov. 2010).**

**a) Sex distribution of the study subjects in the different patient grouping**



**b) Sex distribution of HCV co-infected study subjects with age interval**



### **3.3 Annual Changes on Immunological and clinical chemistry of the study groups over a period of four years**

Table 1 shows the mean changes of each variable across all four groups. The Change in CD4<sup>+</sup> T-cells count of patients under HAART of both HCV co-infected (G2) and non-infected (G1) showed an increase while those of the pre-ART G3 and G4 showed a decline between the baseline and the fourth year. The increase in CD4<sup>+</sup> T-cells in those not co-infected with HCV was relatively more (432%) compared with those co-infected with HCV (131%). On the contrary, the percentage decrease in CD4<sup>+</sup> count in pre-ART patients was greater in those co-infected with HCV (31% > 28%). The percentage increase and decrease in CD4<sup>+</sup> T-cells of the two co-infected groups G2 and G4 was 131% and 31% respectively.

The changes in the CD8<sup>+</sup> T-cells follow the same pattern with that of CD4<sup>+</sup> T-cells change. Both HCV positive and HCV negative patients under HAART showed an increase in CD8<sup>+</sup> while the pre-ART patients showed a decrease. In the HAART groups, the percentage increase in CD8<sup>+</sup> of G1 was better (31%) than the HCV co-infected G2 (14%) whereas in the pre-ART groups the percentage decrease in CD8<sup>+</sup> was higher in the co-infected G4 (52%) in comparison with G3 (35%). Furthermore, the mean CD8<sup>+</sup> T-cells were highly oscillated in G1 and slight change in G2 whereas in the pre-ART groups (G3 and G4), it showed the same pattern of change. In the first three years, generally the pre-ART groups had greater mean CD8<sup>+</sup> T-cells than the ART groups.

Liver enzymes levels generally showed a decline to the normal values from baseline to the fourth year which indicates the improvement of hepatotoxicity. The percentage decline in GPT level followed different pattern regardless of HCV status in the four groups (18, 6, 15 and 81% respectively from G1 to G4). The HAART G1 showed better improvement (18%) in GPT level

than G2 (6%) whereas in the pre-ART groups, the improvement in G4 was much higher (81%) than G3 (15%). The mean liver enzyme levels of G1 and G3 generally fall within the normal range. However, the mean liver enzyme level values of the two co-infected groups G2 and G4 were slightly greater than the normal range. In group 4, the mean GPT and GOT levels were extremely higher than the normal range in the 1<sup>st</sup> year and there after they went down to the normal range. Furthermore, the mean GPT and GOT of G2 at 3<sup>rd</sup> year showed high levels which were at least two fold greater than the normal value.

**Table 1: Mean annual changes of immunological and clinical chemistry values in each group of HIV patients at Yekatit-12 and Zenbaba General Hospitals, Addis Ababa (Sep. 2006- Nov. 2010).**

HCV status	Group	Year	Immunological Variables		Clinical Chemistry Variables		
			CD4 <sup>+</sup> (mean) cells/ $\mu$ l	CD8 <sup>+</sup> (mean) cells/ $\mu$ l	GPT (mean) IU/l	GOT (mean) IU/l	ALP (mean) IU/l
Positive	ART+veHCV+ve (N=16)	1	148	1104	35	32	243
		2	300	1456	43	41	287
		3	399	1430	66	54	296
		4	343	1259	33	36	235
	Pre-ART HCV+ve (N=9)	1	303	1612	246	207	239
		2	312	1461	73	75	263
		3	352	1477	40	41	259
		4	211	772	46	35	207
Negative	ART+veHCV-ve (N=16)	1	80	950	28	37	202
		2	277	686	32	30	216
		3	408	1400	24	35	285
		4	426	1247	23	32	262
	Pre-ART HCV-ve (N=9)	1	435	1676	26	31	245
		2	388	1593	31	32	179
		3	380	1704	27	39	188
		4	314	1092	22	32	175

### **3.4 Quarterly changes on Immunohaematological and clinical chemistry tests of the fourth year**

In attempt to learn the detailed interaction of HCV to HAART as shown in table 2, blood samples were collected in quarterly basis for the fourth year. The mean CD4<sup>+</sup> T-cells count of patients under HAART HCV co-infected group declined by 3% and non-infected G1 increased by 65%. Both of the pre-ART G3 and G4 showed an increase of 17% and 3% respectively. When the two co-infected groups compared, the HAART G2 decreased by 3% in CD4<sup>+</sup> T-cells whereas pre-ART G4 increased by 3%. Furthermore, the mean CD4<sup>+</sup> T-cells changes in every three month showed a fluctuating pattern. The fluctuation was more pronounced in G1 and G3 (the controls) whereas in the co-infected groups (G2 and G4), the changes in mean CD4<sup>+</sup> T-cells was poor. Generally, the G1 and G3 have higher mean CD4<sup>+</sup> T-cells than G2 and G4.

The percentage change in the CD8<sup>+</sup> T-cells follows the same pattern with CD4<sup>+</sup> T-cells change. In the HAART groups, the percentage increase in CD8<sup>+</sup> of G1 was 20% while decreased by 25% in the HCV co-infected G2. In the pre-ART groups, the CD8<sup>+</sup> count was increased by 54% in G3 and decreased by 10% in G4. In addition, the mean change of CD8<sup>+</sup> T-cells during the one year detailed follow up time was highly oscillated in G2 and G3 and marked change in G4 whereas in G1 showed a slight change. Furthermore, the mean CD8<sup>+</sup> T-cells of G4 in each sample were low in comparison with the rest groups.

As shown in table 2, the change in the mean GPT level was almost the same as observed in the four years data. In all samples the mean GPT and GOT of the controls (G1 and G3) were lower than the co-infected groups (G2 and G4). In the co-infected groups it was generally higher than

the normal range while in the control groups it was within the normal value. Liver enzymes levels generally showed a decrease to the normal range. Except G3, all the rest groups showed improvement/decrease in GPT and GOT level. The HAART groups G1 and G2 showed a comparable percentage decrease/improvement of GPT (22% Vs 25%) and GOT (8% Vs 9%) while the pre-ART group G3 increased (4% and 21%) and G4 decreased/improved (69% and 75%). Generally, better improvement of those biochemical enzymes were seen in the co-infected groups. In addition, only the mean GPT level of G4 has showed more than twofold increase over G3. G2 and G4 generally have shown roughly twofold increase in their GPT levels when compared with their respective control groups. GPT and GOT of G2 were lower than that of G4.

**Table 2: Quarterly mean changes of Immunohaematological and liver enzyme levels of patients at Yekatit-12 and Zenbaba General Hospitals, Addis Ababa (Nov. 2009- Nov. 2010).**

Group	Sample	Variables						
		Immunological		Clinical Chemistry			Haematological	
		CD4 <sup>+</sup> (mean) cells/ $\mu$ l	CD8 <sup>+</sup> (mean) cells/ $\mu$ l	GPT (mean) IU/l	GOT (mean) IU/l	ALP (mean) IU/l	WBC (mean) x 10 <sup>3</sup> / $\mu$ l	TLC (mean) x 10 <sup>3</sup> / $\mu$ l
ART+veHCV-ve (n=16)	1 <sup>st</sup>	256	1087	36	36	212	6.72	1.88
	2 <sup>nd</sup>	267	989	29	35	196	7.51	1.38
	3 <sup>rd</sup>	281	1092	32	34	222	6.47	2.57
	4 <sup>th</sup>	395	1247	26	33	233	6.12	2.09
	5 <sup>th</sup>	423	1305	28	33	246	6.47	2.18
ART+veHCV+ve (n=16)	1 <sup>st</sup>	322	1505	52	45	241	5.03	1.78
	2 <sup>nd</sup>	311	1139	66	51	233	5.91	2.29
	3 <sup>rd</sup>	300	882	66	46	256	6.81	2.25
	4 <sup>th</sup>	346	1424	48	39	242	5.70	1.75
	5 <sup>th</sup>	313	1124	39	36	237	6.98	2.01
Pre-ART HCV-ve (n=9)	1 <sup>st</sup>	400	1250	26	28	196	6.70	2.21
	2 <sup>nd</sup>	449	816	31	37	195	5.76	1.99
	3 <sup>rd</sup>	528	2267	25	25	199	7.67	2.77
	4 <sup>th</sup>	481	1232	25	28	213	6.83	2.30
	5 <sup>th</sup>	468	1927	27	34	197	7.09	2.44
Pre-ART HCV+ve (n=9)	1 <sup>st</sup>	227	995	132	143	208	4.38	1.49
	2 <sup>nd</sup>	266	784	46	41	208	5.04	1.79
	3 <sup>rd</sup>	235	568	49	42	207	6.29	2.17
	4 <sup>th</sup>	249	889	38	31	230	4.75	1.88
	5 <sup>th</sup>	220	895	41	36	256	6.93	2.19

## Chapter Four: Discussion

Hepatitis C virus infection is one of the major diseases of mankind and is a serious global public health problem. The effect of HCV co-infection on the recovery of immune cells and liver enzymes in HIV patients before and after HAART remains controversial. However, a number of studies have suggested that the presence of HIV infection accelerates the course of HCV-related liver disease in HCV/HIV co-infected patients. HIV is known to impair T-helper type 1 immune response which in turn alters the response of immune cells to HCV. This permits greater HCV replication and consequently, greater infection and injury to hepatocytes which leads to more rapid progression to HCV-related liver diseases (fibrosis, cirrhosis and hepatocellular carcinoma). (Blackard, et al., 2008; Mohsen and Easterbrook, 2003).

Given the above interaction of the two viruses and their implications on the proper management of the co-infected cases a clear understanding of the clinical histories of each virus in relation to what happens during co-infection is necessary. Therefore, the aim of this thesis was to describe the prevalence of HCV co-infection among HIV patients in two hospitals (Yekatit-12 and Zenbaba General Hospitals) and assess the Haematological, Immunological and Clinical Chemistry results over a four years period. Results show that the prevalence of HCV among the 387 Addis Ababa resident-HIV patients, who visited the two hospitals, Yekatit-12 and Zenbaba during the one month, was 6.5%. This is low in accordance with the previous HIV/HCV co-infection study in Ethiopia by Workinesh, *et al* (2002) which is 8.6%. Compared to Spain, U.S.A and France that have large number of chronically infected people (33%, 30% and 24.3% respectively) and Morocco that experience up to 19.8% co-infection rate it is still low (Larsen, *et al.*, 2008; Chung, 2004).

In Ethiopian setting the epidemiology of HCV is not well described thus little is known about the prevalence. HCV test is not routinely done; most mothers do not know their infection status and therefore are likely to transmit it to their new born. Because of resource limitation and poor access to trained maternity, children are not commonly checked for HCV and those patients infected with HCV are not managed properly. Likewise there is no checking for HIV and ART service for all mothers, hence vertical transmission is likely to continue. The high prevalence of HIV, endemicity of HCV and enormity of co-infection and their adverse effect in pregnant women and expanded use of HAART is the optimal scenario for HCV expansion and severe disease causation. That is to say the above 6.5% prevalence of co-infection if left unchecked is likely to help expansion of both viruses. The expanding access to ART all alone is not sufficient to reduce both infections (Maier and George, 2002).

The co-infection prevalence in patients under HAART (7.8%) was more than Pre-HAART patients (5%). This might be due to the higher numbers of under HAART patients (206) than the pre-ART (181). In this study, from the 25 co-infected patients more males (60%) were co-infected than females (40%). The distribution of HCV/HIV co-infection, in Fig.3.1 shows the direct link of age to HCV prevalence. It starts with 20% in age group 20-29 and grows to nearly 45% in age groups 40-49 years suggesting an association with the age. That probably has to do with sexual transmission or risk behavior to HIV infection and cumulative effect. This is a serious indicator for over 90-95% of co-infected patients develop chronic infection that gives rise to liver cirrhosis (20%) and Hepatocarcinoma in 5-25% (Highleyman, 2007; Brass, et al., 2006).

The reason for higher percentage of male HCV infection, conversely could be that the number of males in the sample population, were also proportionally higher than the females hence does not

justify to saying that it is gender influenced. The interesting observation is that among the HIV/HCV co-infected females were double that of male in age group 30-39 where as in age group 40-49 all were males. It could be that it was common among males or is a more recent introduction that is on the buildup in females. It could as well be a bias reflected due to the relatively small number of sample population considered in this study. A higher prevalence in older age groups could be a reflection due to the chronic nature of the disease or it may be related to hormone and immunity. This finding is in agreement with the works of Carbonero, *et al.* (2001) and Sugimoto, *et al.* (2003) that found HIV/HCV co-infection is higher in males over 40 years of age.

The observation on immunological parameters over 4 years showed that an improvement of CD4<sup>+</sup> (131 Vs 432%) and CD8<sup>+</sup> (14 Vs 31%) counts in both HCV positive and negative patients under HAART, but the change in both CD8<sup>+</sup> and CD4<sup>+</sup>, in those not co-infected with HCV was more than double those of HCV infected. This suggests that although HAART does improve the immune system of HCV co-infected patients its efficiency is relatively compromised by HCV interactions. Detailed follow up of the same subject at 3 months interval for one year similarly revealed the same fact but better amplified. The HCV virus co-infected subjects maintained their CD4<sup>+</sup> and CD8<sup>+</sup> counts while HCV non-infected doubled their CD4<sup>+</sup> and showed a 20% increase in CD8<sup>+</sup> counts (table 1 and 2).

The literature regarding the effect of HAART in decreasing the HCV load in co-infected individuals remains controversial. First it has been postulated that HAART beside HIV viral suppression and immune reconstitution to negatively influence the rate of HCV fibrosis progression, and thus prolong end stage disease. These groups contrary to our finding say

HAART suppresses HIV and increase CD4<sup>+</sup> count response and this is not affected by HCV co-infection (Guo, *et al.*, 2010). And HCV co-infected individuals are not going fast to AIDS. However, more recently researchers have demonstrated that HAART adversely affect HCV outcomes by increasing Hepatitis C viral load, hepatotoxicity and fibrosis progression seem to be dominating. They have also shown increase in mortality and rapid HIV related disease progression in HIV/HCV co-infected people. In fact mortality due to liver failure is implicated to HAART in HCV co-infected patients. Low level of immune activations is correlated with slow HIV progression. HIV non-progressors have higher HIV specific CD8<sup>+</sup> T-cells showing their importance in limiting the viral development (Highleyman, 2008).

One study set to study the mortality attributable to liver failure and the impact of HCV infection on survival of cohort of HIV infected before and after HAART administration showed it decreased mortality due to HIV by 4.5%, but increased mortality due to liver failure by 0.5% i.e increasing the cause of death among HIV infected patients receiving HAART. On the other hand, it claims that HCV has no impact on survival of HIV or is limited to the time before the institution of HAART. But other workers have shown that HIV/HCV co-infected individuals have poor tolerance for HAART as manifested with greater risk of hepatotoxicity (Marina and Vincent, 2003).

Variety of factors is incriminated in influencing these treatment outcomes. From the fact that the presence of high HCV RNA load of genotypes 1 and 4 and a slow decline of the same when treatment starts some say that it selects the drug resistant subtypes. This taking the high rate replication ( $10^{12}$  virions/day), in infected hosts and half life of 3 hours and its exceptionally high mutation rate producing a genetic variety is not impossible (Dziora, *et al.*, 2005). Our result

though does not have data on the influence of viral interaction has shown that the presence of HCV decreases the efficiency of HAART when compare to those that were negative for HCV, hence goes well with the later. The improvement of the immune status with CD4<sup>+</sup> and CD8<sup>+</sup> counts on its own improves survival of patients the minimum by slowing viral load and elimination of infected cells.

Knowledge on the immunological, biochemical and haematological indicators that would enable one to determine the exact status and decide on which viral therapy should be given, at what time during HCV co-infection. It thus gives better insight for the effort and opportunities in improving the HIV/HCV care of HIV patients. At present, the first line drugs against HIV in use include; highly active antiretroviral therapy (HAART) regimens for adults: Lamivudine combined with Zidovudine or Stavudine and Nevirapine or Efavirenz; Second-line regimens consist of combination Abacavir with Tenofovir or Didanosine and one of the three PI: Indinavir, Lopinavir or Saquinavir combined. For pregnant women, HAART is not indicated, short course prophylaxis of Zidovudine, Lamivudine and Nevirapine for the mother and Zidovudine and Nevirapine for baby is recommended (Guideline for treatment of ARV FMOH Ethiopia, 2007; National PMTCT guideline Ethiopia, 2007/2008).

Given the global burden of HCV disease, an understanding of how protective immune responses are generated is critical. Treatment of HCV with peg Interferon- $\alpha$  and Ribavirin co-infected individual is also recommended to individuals with greater than 500 CD4<sup>+</sup> cells/mm<sup>3</sup> blood and a viral load less than 40,000 copies/ ml. Overall, if the treatment of HIV and other viral factors make a difference on the treatment out comes: Certainly increase in CD4<sup>+</sup> and CD8<sup>+</sup> has improved the immune status which is basic for viral control (Manns, et al., 2007).

The enzymes, aspartate aminotransferase (AST), previously known as glutamate oxaloacetate transaminase (GOT), and alanine aminotransferase (ALT), formerly known as glutamate pyruvate transaminase (GPT), are concerned with amino acid metabolism. Large amounts of AST are present in the liver, kidneys, cardiac muscle, and skeletal muscle. Small amounts of the enzyme are present in the brain, pancreas, and lungs. ALT is found principally in the liver with only small amounts being present in other organs. When there is liver cell damage the serum or plasma levels of both enzymes are raised. Measurement of ALT activity is mainly performed to investigate liver disease. Increasingly ALT is being measured to monitor patients receiving antiretroviral drugs associated with hepatotoxicity such as nevirapine and stavudine. While both ALT and AST are raised with hepatocellular injury, ALT is more specific for detecting liver cell damage (Blackard and Sherman, 2008).

In the present study, the liver enzyme levels were much higher in co-infected than HIV mono-infected patients. In supporting the present study; DeSemone and his colleagues (2000) found that increased liver enzyme level often associated with HCV co-infection. The mean GPT and GOT level of G4 at 1<sup>st</sup> year of the four years and 1<sup>st</sup> sample of the fourth year follow up study was at least fivefold greater than that of G2. In the rest years and samples both G2 and G4 had comparatively increased levels of liver enzymes (table 1 and 2). These higher mean levels of liver enzymes were due to few patients that showed high amount of liver enzymes throughout the study period. Otherwise, in the rest patients, the amounts of these enzymes were fall within the normal range. In this study, more than 70% of the co-infected patients showed increased levels of GPT and GOT that agree with the finding of Lawson (2010) in which only 30% of HCV patients have normal GPT levels. However, this finding is in contrary with the work of Levin (2005) who concluded only 8-10% of co-infected patients show increased liver enzymes.

Moreover, Sulkowski and his colleagues (2000) found that hepatitis C has been confirmed to be a risk factor associated with a 3 to 5-fold chance of developing elevated transaminases during HAART, compared to HIV patients without hepatitis C which is compatible with the present study. The present work is incompatible with the study done by Gatti, *et al.* (2007) that showed the synergetic mitochondrial damage by HCV and HAART (especially nucleoside analogs) were responsible for elevation of GPT in HAART groups. This finding also conflicts with the works of Guo, *et al.* (2010) which concluded HCV is independent risk factor for hepatotoxicity with HAART. In addition, the higher enzyme level in the co-infected pre-ART group in this study indicates that the effect of HCV is more pronounced in pre-ART patients than the HAART group. Furthermore, the HIV mono-infected groups, G1 and G3 generally had normal amount enzyme levels throughout the follow up time. In supporting the normal enzyme levels of G1 and G3 in this study Alessandro, *et al.* (2006) found that HAART do not associated with increased liver enzymes.

In areas where Flowcytometric enumeration of CD4<sup>+</sup>/CD8<sup>+</sup> T-cells count is not available, the absolute lymphocyte count is used to initiate HIV treatments. In such situations, a WHO stage II HIV patient with a total lymphocyte count less than 1200/mm<sup>3</sup> is eligible for HAART. In the present study in addition to CD4<sup>+</sup> and CD8<sup>+</sup> count, the total white cell and lymphocyte counts were done for each patient. As shown in table 2, there were no significant differences between the co-infected and HIV mono-infected groups indicating that the influence of HCV on the recovery of total white cell and lymphocyte is minimal. In supporting the present work Sabry, *et al.* (2009) found that HCV infection has minimum effect on haematological parameters. Furthermore, from this result it is also difficult to conclude the exact effect of HCV because those cells can increase against any infection.

## 4.1 Conclusions

The present study has shown that HCV infection has an impact on the recovery of CD4<sup>+</sup> and CD8<sup>+</sup> cells of on HAART patients. The improvement in CD4<sup>+</sup> cell count of under HAART HIV/HCV co-infected subjects were lower than the HIV mono-infected subjects and the mono-infected patients responded to HAART better than the co-infected patients. Moreover, HCV has a significant association with higher liver enzyme level than CD4<sup>+</sup> in HIV/HCV co-infected patients.

The effects of HCV in HIV/HCV co-infected individuals must be better understood, such that safe and effective treatment and preventive measures may be employed. Although HIV/HCV co-infected patients were less likely than those infected only with HIV to receive HAART, their CD4<sup>+</sup> cell count recovery with treatment was good. This suggests that HIV/HCV co-infected patients may be treated successfully for HIV infection (table 1 and 2).

Because of the lower prevalence rate of HIV/HCV co-infection reported from previous few local studies, the disease is given less attention and seems forgotten at various levels of health delivery institutions so that the significance of the problem has been underestimated. Therefore, it is advisable to make HCV-antibody screening for every HIV infected individual prior to initiation of anti-retroviral therapy (HAART). This will in turn influence their clinical management as well as outcome.

The present study has several strengths. Although other tests are crucial in clinical medicine however, CD4<sup>+</sup> count is the main test routinely done to follow HIV disease progression. The study clearly showed the pattern of CD4<sup>+</sup> changes considering different factors during the time of follow up. The information obtained in this study may promote our understanding of the impact

of HCV infection on immunohaematological parameters and liver enzyme levels among HIV/HCV co-infected individuals before and after initiation of HAART. In addition, with the limitations mentioned, we believe that this cohort may provide an accurate reflection of current clinical trends regarding this dual infection.

## **4.2 Recommendations**

The present study has indicated the impact of HCV in co-infected patients especially its impact on liver enzyme levels which are the best indicators of liver injury. Based on these findings the following recommendations are made:-

1. The centers caring for HIV infected patients should develop clear strategies for ensuring all HIV infected patients undergoing testing for HCV regardless of their anti-retroviral treatment (HAART) status. Usually hepatitis A, and B screening is also recommended as a general principle for better management of HIV infection.
2. Further study should be conducted on various HIV/HCV co-infected population groups to clarify the impact of HCV on HIV and to determine its interaction with HIV.
3. HCV and HBV transmission occur by mainly the same paths, thus, HIV infected individuals be vaccinated against those hepatoviruses for which they demonstrate no immunity to minimize HCV/HBV co-infection burden.
4. Based on phylogenetic analysis of nucleotide sequences, multiple genotypes and subtypes of HCV have been identified. The genetic heterogeneity varies in geographical distribution, disease outcome, and response to therapy. Therefore, knowledge on genotypes of this virus in certain locality could potentially be used to select effective treatment, and decide length of

therapy. Thus, further study on this area is waiting to answer the issue of HCV genotypes in Ethiopian context for the proper treatment and management of HCV infection.

5. Further study should be conducted on the impact of HIV nucleoside analog drugs on the multiplication of HCV.
6. Extensive cohort study should be conducted on HIV/HCV co-infection in order to clearly indicate the impact of HCV on CD4<sup>+</sup> and CD8<sup>+</sup> T-cells and liver enzymes.

## References

- Alatrakchi, N., Graham, C., Van der Vliet, H., Sherman, K., Exley, M. and Koziel, M. (2007). Hepatitis C Virus (HCV)-Specific CD8+ T Cells Produce Transforming Growth Factor That Can Suppress HCV-Specific T-Cell Responses. *J. Virol.* **81(11)**:5882–5892.
- Alessandro, P., Costanza, S., Luigi, A., Stella, G., Carla, A., Ciro, E., Antimo, D. and Oreste, P. (2006). Lower CD4+ T-Cell Number Expressing CD7+ Characterizes HIV/Hepatitis C Virus Coinfection Compared With HIV and Hepatitis C Virus Monoinfection. *J. AIDS* **43(4)**: 504-506.
- Al-Sherbiny, M., Osman A., Mohamed, N., Tarek-shata, M., Abdel-Aziz, F., Abdel-Hamid, M., Abdel-Wahab, S., Mikhail N., Stoszek, S., Ruggeri, L., Folgori, A., Nicosia, A., Prince, A., and Strickland, T. (2005). Exposure to hepatitis C virus induces cellular immune responses without detectable viremia or seroconversion. *Am. J. Trop. Med. Hyg.* **73(1)**:44–49.
- Anderson, K., Guest, J. and Rimland, D. (2004). Hepatitis C Virus Coinfection Increases Mortality in HIV-Infected Patients in the Highly Active Antiretroviral Therapy Era: Data from the HIV Atlanta VA Cohort Study. *Clin. Infect. Dis.* **39**:1507-1513.
- Antonucci, G., Girardi, E., Cozzi-Lepri, A., Capobianchi, M., De Luca, A., Puoti, M., Petrelli, E., Carnevale, G., Rizzardini, G., Grossi, P., Viganò, P., Moioli M., Carletti, F., Solmone, M., Ippolito, G. and Monforte, A. (2005). Role of hepatitis C virus (HCV) viremia and HCV genotype in the immune recovery from highly active antiretroviral therapy in a cohort of antiretroviral-naïve HIV-infected individuals. *Clin. Infect. Dis.* **40(12)**:101-109.
- Arizcorreta, A., Márquez, M., Gutiérrez, C., Guzmán, E., Brun, F., Iglesias, M. and González, J. (2006). T cell receptor excision circles (TRECs), CD4+, CD8+ and their CD45RO+ and CD45RA+ subpopulations in hepatitis C virus (HCV)-HIV-co-infected patients during treatment with interferon alpha plus ribavirin: analysis in a population on effective antiretroviral therapy. *Clin. Exper. Immunol.* **146**:270–277.

Averill, L., Lee, W. and Karandikar, N. (2007). Differential Dysfunction in Dendritic Cell Subsets During Chronic HCV Infection. *Clin. Immunol.* **123(1)**:40–49.

Balasubramanian, A., Groopman, J. and Ganju, R. (2008). Underlying Pathophysiology of HCV Infection in HIV Positive Drug Users. *J. Addict. Dis.* **27(2)**:75–82.

Barnes, E., Salio, M., Cerundolo, V., Francesco, L., Pardoll, D., Klenerman, P. and Cox, A. (2008). Monocyte derived dendritic cells retain their functional capacity in patients following infection with hepatitis C virus. *J. Viral Hepat.* **15**:219–228.

Barnes, E., Lauer, G., Walker, B. and Klenerman, P. (2002). T Cell Failure in Hepatitis C Virus Infection. *J. Viral Immunol.* **15(2)**:285–293.

Barth, H., Schnober, E., Neumann-Haefelin, C., Thumann, C., Zeisel, M., Diepolder, H., Hu, Z., Liang, T., Blum, H., Thimme, R., Lambotin, M. and Baumert, T. (2008). Scavenger Receptor Class B Is Required for Hepatitis C Virus Uptake and Cross-Presentation by Human Dendritic Cells. *J. Virol.* **82(7)**:3466–3479.

Baye, G. and Yohannes, M. (2008). The prevalence of HBV, HCV and malaria parasites among blood donors in Amhara and Tigray regional states. *Ethiop. J. Heal. Dev.* **22(1)**: 1-5.

Bensch, B., Spangenberg, H., Kersting, N., Neumann-Haefelin, C., Panther, E., Von Weizsäcker, F., Blum, H., Pircher, H. and Thimme, R. (2007). Analysis of CD127 and KLRG1 Expression on Hepatitis C Virus-Specific CD8 T Cells Reveals the Existence of Different Memory T-Cell Subsets in the Peripheral Blood and Liver. *J. Virol.* **81(2)**:945–953.

Bertoletti, A. and Ferrari, C. (2003). Kinetics of the Immune Response during HBV and HCV Infection. *J. Hepat.* **38(1)**: 4-13.

Blackard, J., Shata, M., Shire, N. and Sherman, K. (2008). Acute Hepatitis C Virus Infection: A Chronic Problem. *J. Viral Hepat.* **47(1)**:321–331.

Blackard, J. and Sherman, K. (2008). HCV/ HIV Co-infection: Time to Re-evaluate the Role of HIV in the Liver? *J. Viral Hepat.* **15(5)**: 323-330.

Brass, V., Moradpour, D. and Blum, H. (2006). Molecular Virology of Hepatitis C Virus (HCV). *Int. J. Med. Sci.* **3**:29-34.

Caetano, J., Martinho, A., Paiva, A., Pais, B., Valente, C. and Luxo, C. (2008). Differences in Hepatitis C Virus (HCV)-Specific CD8 T-Cell Phenotype during Pegylated Alpha Interferon and Ribavirin Treatment Are Related to Response to Antiviral Therapy in Patients Chronically Infected with HCV. *J. Virol.* **82(15)**:7567–7577.

Capa, L., Soriano, V., Garcí'a, J., Nuñ ez, M., Romero, M., Cascajero, A., Muñ oz, F., Gonza'lez, J. and Benito, J. (2007). Influence of HCV Genotype and Co-Infection With Human Immunodeficiency Virus on CD4<sup>+</sup> and CD8<sup>+</sup> T-Cell Responses to Hepatitis C Virus. *J. Med. Virol.* **79**: 503–510.

Carbonero, M., Barreiro, P., Jim, G., Berriguete, R., Lahoz, J. and Soriano, V. (2007). Clearance of Hepatitis C Virus in HIV-Infected Patients with Multiple Chronic Viral Hepatitis. *J. Viral Hepat.* **14(6)**:392-395.

Carbonero, M., Soriano, V. and Valencia, E. (2001) Increasing impact of chronic viral hepatitis on hospital admissions and mortality among HIV infected patients. *AIDS Research and Human Retroviruses.* **17(16)**:1467-1471.

Carcelain, G., Li, T. and Autran, B. (1999). Immune Reconstitution under Highly Active Antiretroviral Therapy (HAART). *AIDS Rev.* **1**:51-56.

Chung, R. (2004). Management of HIV/HCV conifection. *The PRN notebook.* **9(1)**:14-19.  
<http://www.prn.org>

Cox, A., Mosbrugger, T., Lauer, G., Pardoll, D., Thomas, D. and Ray, S. (2005). Comprehensive Analyses of CD8<sup>+</sup> T Cell Responses during Longitudinal Study of Acute Human Hepatitis C. *J. Hepat.* **42(1)**:104–112.

DeSimone, J., Pomerantz, R. and Babinchak, T. (2000). Inflammatory Reactions in HIV-1 Infected Persons after Initiation of Highly Active Antiretroviral Therapy. *Ann. Intern. Med.* **133**:447-454.

Dhoot, J. (2003). Hepatitis C management in HIV infected patients. *Ann. Intern. Med. J.* **138**: 197-207.

Dolganiuc, A., Norkina, O., Kodys, K., Catalano, D., Bakis, G., Marshall, C., Mandrekar, P. and Szabo, G. (2007). Viral and host factors induce macrophage activation and loss of Toll like Receptor tolerance in chronic HCV infection. *J. Gastroenterol.* **133(5)**:1627–1636.

Drexler, J., Kupfer, B., Petersen, N., Grotto, R., Rodrigues, S., Grywna, K., Panning, M., Annan, A., Silva, G., Douglas, J., Koay, E., Smuts, H., Netto, E., Simmonds, P., Pardini, M., Roth, W. and Drosten, C. (2009). A Novel Diagnostic Target in the Hepatitis C Virus Genome. *PloS Med.* **6(2)**:210-220.

Dziora, P., Figlerowicz, M., Formanowicz, P., Alejska, M., Jackowiak, P., Malinowska, N., Fraczak, A. and Blazewicz, J. (2005). Computational methods in diagnostics of chronic hepatitis C. *Bull. Pol. Ac.: Tech.* **53(3)**:273-281.

Elmowalid, G., Qiao, M., Jeong, S., Borg, B., Baumert, T., Sapp, R., Hu, Z., Murthy, K. and Liang, T. (2007). Immunization with hepatitis C virus-like particles results in control of hepatitis C virus infection in chimpanzees. *PNAS* **104(20)**:8427–8432.

Fan, Z., Huang, X., Kalinski, P., Young, S. and Rinaldo, C. (2007). Dendritic Cell Function during Chronic Hepatitis C Virus and Human Immunodeficiency Virus Type 1 Infection. *Clin. Vacc. Immunol.* **14(9)**:1127–1137.

Forbi, J., Gabadi, S., Alabi, R., Iperepolu, H., Pam, C., Entonu, P. and Agwale, S. (2007). The role of triple infection with hepatitis B virus, hepatitis C virus, and human immunodeficiency

virus (HIV) type-1 on CD4+ lymphocyte levels in the highly HIV infected population of North-Central Nigeria. *Mem. Inst. Oswaldo Cruz* **102(4)**:1-3.

Gatti, F., Nasta, P., Matti, A., Manno, D., Mendeni, M., Puoti, M. and Carosi, G. (2007). Treating Hepatitis C Virus in HIV Patients: Are Side Effects a Real Obstacle? *AIDS Rev.* **9**:16-24.

Grimm, D. and Kay, M. (2006). Therapeutic short hairpin RNA expression in the liver: viral targets and vectors. *Gene Therapy* **13**:563–575.

Guha, C., Lee, S., Chowdhury, N. and Chowdhury, J. (2005). Cell Culture Models and Animal Models of Viral Hepatitis. Part II: Hepatitis C. *Lab Animal* **34(2)**:39-46.

Guideline for treatment of ARV FMOH and National PMTCT guideline Ethiopia, 2007/2008.

Guo, F., Wei, L., Han, Y., Qiu, Z., Zuo, L., Li, Y. and Li, T. (2010). Impact of Hepatitis C Virus Coinfection on HAART in HIV-Infected Individuals: Multicentric Observation Cohort. *J. AIDS*, **54(2)**: 137-142.

Helbig, K., Ruszkiewicz, A., Lanford, R., Berzsenyi, M., Harley, H., McColl, S. and Beard, M. (2009). Differential Expression of the CXCR3 Ligands in Chronic Hepatitis C Virus (HCV) Infection and Their Modulation by HCV In Vitro. *J. Virol.* **83(2)**:836–846.

Heller, T., Saito, S., Auerbach, J., Williams, T., Moreen, T., Jazwinski, A., Cruz, B., Jeurkar, N., Sapp, R., Luo, G. and Liang, T. (2005). An in vitro model of hepatitis C virion production. *PNAS* **102(7)**:2579–2583.

Highleyman, L. (2009). Antiviral Agents with Activity against Both HIV and Hepatitis C Virus. *HIV and Hepatitis.com*

Highleyman, L. (2008). Does HIV-HCV Coinfection Increase the Risk of Liver Disease Progression and Worsen Clinical Outcomes? *HIV and Hepatitis.com*

Highleyman, L. (2007). HIV/HCV Coinfection. [www.hivandhepatitis.com](http://www.hivandhepatitis.com)

Jarlais, D., Perlis, T., Arasteh, K., Torian, L., Hagan, H., Beatrice, S., Smith, L., Wethers, J., Milliken, J., Mildvan, D., Yancovitz, S. and Friedman, S. (2005). Reductions in hepatitis C virus and HIV infections among injecting drug users in New York City, 1990–2001. *AIDS* **19**(suppl 3):S20–S25.

Kanda, T., Steele, R., Ray, R. and Ray, B. (2007). Hepatitis C Virus Infection Induces the Beta Interferon Signaling Pathway in Immortalized Human Hepatocytes. *J. Virol.* **81**(22):12375–12381.

Katie, B., Anderson, J., Guest, L. and Rimland, D. (2004). Hepatitis C Virus Coinfection Increases Mortality in HIV-Infected Patients in the Highly Active Antiretroviral Therapy Era: Data from the HIV Atlanta VA Cohort Study. *Clin. Infect. Dis.* **39**:1507-1513.

Kindrick, A. (2002). HIV and Hepatitis C Co-infection. <http://www.ucsf.edu/hivcntr>

Koziel, M. (2002). New Insights in HIV and Hepatitis C Virus Co-infection. *Perspective - HIV and Hepatitis C Virus.* **10**(2):16-21.

Kuroki, M., Ariumi, Y., Ikeda, M., Dansako, H., Wakita, T. and Kato, N. (2009). Arsenic Trioxide Inhibits Hepatitis C Virus RNA Replication through Modulation of the Glutathione Redox System and Oxidative Stress. *J. Virol.* **83**(5):2338–2348.

Larsen, C., Pialoux, G., Salmon, D., Antona, D., Le Strat, Y., Piroth, L., Pol, Rosenthal, S., Neu, D., Semaille, C., Delarocque, E. (2008). Prevalence of hepatitis C and hepatitis B infection in the HIV-infected population of France. *Eurosurveillance*, **13**(22): 109-112.

Lawson, A. (2010). Hepatitis C virus-infected patients with a persistently normal alanine aminotransferase: Do they exist and is this really a group with mild disease? *J. Viral Hepat.* **17**(1): 51-58.

Levin, J. (2005). Hepatitis C Virus (HCV) and HCV/ HIV Co-infection. <http://www.natap.org>.

Lindenbach, B. and Rice, C. (2005). Unraveling hepatitis C virus replication from genome to function. *Nature* **436(18)**:933-938.

Lusk, H. (2006). Converging Epidemics: HIV and HCV Coinfection. *J. Focus* **21(5)**:1-8

Machida, K., Kondo, Y., Huang, J., Chen, Y., Cheng, K., Keck, Z., Fong, S., Dubuisson, J., Sung, V. and Lai, M. (2008). Hepatitis C Virus (HCV)-Induced Immunoglobulin Hypermutation Reduces the Affinity and Neutralizing Activities of Antibodies against HCV Envelope Protein. *J. Virol.* **82(1)**:6711–6720.

Machida, K., Cheng, K., Sung, V., Levine, A., Fong, S. and Lai, M. (2006). Hepatitis C Virus Induces Toll-Like Receptor 4 Expression, Leading to Enhanced Production of Beta Interferon and Interleukin-6. *J. Virol.* **80(2)**:866–874.

Maier, I. and George, Y. (2002). Hepatitis C and HIV co-infection: a review. *World J. Gastroenterol.* **8(4)**:577-579.

Manns, M., Foster, M., Rockstroh, J., Zeuzem, S., Zoulim, F. and Houghton, M. (2007). The way forward in HCV treatment-finding the right path. *Nature Rev./Drug Discovery* **6**:991-1000.

Marina, N. and Vincent, S. (2003). How effective is HAART in HCV and HIV coinfection? *Clin. Infect. Dis.* **37**:1678-1685.

Mizukoshi, E., Eisenbach, C., Edlin, B., Newton, K., Raghuraman, S., Weiler-Normann, C., Tobler, L., Busch, M., Carrington, M., McKeating, J., O'Brien, T. and Rehermann, B. (2008). Hepatitis C Virus (HCV)-Specific Immune Responses of Long-term Injection Drug Users Frequently Exposed to HCV. *J. Infect. Dis.* **198(2)**:203–212.

Mohsen, A. and Easterbrook, P. (2003). Hepatitis C testing in HIV infected patients. *J. Sex. Trans. Inf.* **79(76)**:11-36.

Murillo, K., Rodríguez, M., Rodríguez, H., Saldaña, H., Padilla, F., Jiménez, J. and Estilla, A. (2004). Experimental models for hepatitis C virus (HCV): New opportunities for combating hepatitis C. *Ann. Hepat.* **3(2)**:54-62.

Nascimbeni, M., Mizukoshi, E., Bosmann, M., Major, M., Mihalik, K., Rice, C., Feinstone, S. and Rehermann, B. (2003). Kinetics of CD4<sup>+</sup> and CD8<sup>+</sup> Memory T-Cell Responses during Hepatitis C Virus Rechallenge of Previously Recovered Chimpanzees. *J. Virol.* **77(8)**:4781-4793.

Novick, D. (2000). The Impact of Hepatitis C Virus Infection on Methadone Maintenance Treatment. *Mount Sinai J. Med.* **67(5)**:437-443.

Pawlotsky, J. and Gish, R. (2006). Future therapies for hepatitis C. *Antiviral Therapy* **11(4)**:1-12.

Quiroga, J., Llorente, S., Castillo, I., Iñigo, E., Pardo, M. and Carreño, V. (2006). Cellular Immune Responses Associated with Occult Hepatitis C Virus Infection of the Liver. *J. virol.* **80(22)**:1972-1979.

Radziewicz, H., Ibegbu, C., Hon, H., Osborn, M., Obideen, K., Wehbi, M., Freeman, G., Lennox, J., Workowski, K., Hanson, H. and Grakoui, A. (2008). Impaired Hepatitis C Virus (HCV)-Specific Effector CD8<sup>+</sup> T Cells Undergo Massive Apoptosis in the Peripheral Blood during Acute HCV Infection and in the Liver during the Chronic Phase of Infection. *J. Virol.* **82(20)**:9808–9822.

Rehermann, B. and Nascimben, M. (2005). Immunology of hepatitis B Virus and hepatitis C Virus Infection. *Nature Rev. | Immunol.* **5**:215-229.

Randall, G., Panis, M., Cooper, J., Tellinghuisen, T., Sukhodolets, K., Pfeffer, S., Landthaler, M., Landgraf, P., Kan, S., Lindenbach, B., Chien, M., Weirh, D., Russo, J., Jug, J., Brownstein, M., Sheridan, R., Sander, C., Zavolan, M., Tusch, T. and Rice, C. (2007). Cellular cofactors affecting hepatitis C virus infection and replication. [www.pnas.org](http://www.pnas.org)

Rodríguez, R., García, J. and Soriano, V. (1999). Hepatitis C, an Emerging Problem in HIV Infected Patients. *AIDS Rev.* **1**:22-28.

Sabry, A., El-Dahshan, K., Mahmoud, K. and El-Husseini, A. (2009). Effect of HCV infection on hematocrit and hemoglobin level in Egyptian hemodialysis patients. *Int. Urol. Nephrol.* **41**: 189–193.

Schaller, T., Appel, N., Koutsoudakis, G., Kallis, S., Lohmann, V., Pietschmann, T. and Bartenschlager, R. (2007). Analysis of Hepatitis C Virus Superinfection Exclusion by Using Novel Fluorochrome Gene-Tagged Viral Genomes. *J. Virol.* **81**:4591–4603.

Semmo, N., Lucas, M., Krashias, G., Lauer, G, Chapel, H. and Klenerman, P. (2006). Maintenance of HCV-specific T-cell responses in antibody-deficient patients a decade after early therapy. *Am. J. Haemat.* **107**(11):4570-4571.

Sillanpää, M., Melén, K., Porkka, P., Fagerlund, R., Nevalainen, K., Lappalainen, M. and Julkunen, I. (2009). Hepatitis C virus core, NS3, NS4B and NS5A are the major immunogenic proteins in humoral immunity in chronic HCV infection. *J. Virol.* **6(84)**:1-12.

Sookoian, S. (2003). New therapies on the horizon for hepatitis C. *Annal. Hepat.* **2(4)**:164-170.

Su, A., Pezacki, J., Wodicka, L., Brideau, A., Supekova, L., Thimme, R., Wieland, S., Bukh, J., Purcell, R., Schultz, P. and Chisari, F. (2002). Genomic analysis of the host response to hepatitis C virus infection. *PNAS* **99(24)**:15669–15674.

Sugimoto, K., Stadanlick, J., Ikeda, F., Brensinger, C., Furth, E., Alter, H. and Chang, K. (2003). Influence of Ethnicity in the Outcome of Hepatitis C Virus Infection and Cellular Immune Response. *J. Hepat.* **37(93)**:590-599.

Sulkowski, M. (2002). Hepatitis C Virus Infection in HIV-infected Patients. *Current Hepatitis Reports* **1**:16–22.

Sulkowski, M., Thomas, D., Chaisson, R. and Moore R. (2000). Drug-associated Liver Disease during HAART: Impact of HCV Coinfection. *JAMA*, **284**: 74-80.

Sun, J., Tumurbaatar, B., Jia, J., Diao, H., Bodola, F., Lemon, S., Tang, W., Bowen, D., McCaughan, G., Bertolino, P. and Chan T. (2005). Parenchymal Expression of CD86/B7.2 Contributes to Hepatitis C Virus-Related Liver Injury. *J. Virol.* **79(16)**:1730-1739.

Tedaldi, E., Chen, L., Markowitz, N., Kelly, L. and Abrams, D. (2005). Effect of IL-2 on hepatitis C virus RNA levels in patients co-infected with human immunodeficiency virus receiving HAART. *J. Viral Hepatitis* **12**:414–420.

Tellinghuisen, T., Evans, M., Von Hahn, T., You, S. and Rice, M. (2007). Studying Hepatitis C Virus: Making the Best of a Bad Virus. *J. Virol.* **8(17)**:8853–8867.

Uebelhoer, L., Han, J., Callendret, B., Mateu, G., Shoukry, N., Hanson, H., Rice, C., Walker, C., Grakoui, A. (2008). Stable Cytotoxic T Cell Escape Mutation in Hepatitis C Virus Is Linked to Maintenance of Viral Fitness. *PloS pathogen* **4(9)**:1-15.

UNAIDS report (2009). Annual report on HIV and AIDS.

Vali, B., Yue, F., Jones, R., Sheth, P., Kaul, R., Betts, M., Wong, D., Kovacs, C., Loutfy, M., Common, A., Halpenny, R. and Ostrowski, M. (2008). HIV-Specific T-Cells Accumulate in the Liver in HCV/HIV Co-Infection. *PloS ONE* **3(10)**:1-10.

Verma, S. (2006). HAART attenuates liver fibrosis in patients with HIV/HCV co-infection: fact or fiction? *J. Antimicrob. Chemotherapy* **58(3)**:496-501.

Wang, S., Huang, C., Ye, L., Wang, X., Song, L., Wang, Y., Liang, H., Huang, X. and Ho, W. (2008). Natural killer cells suppress full cycle HCV infection of human Hepatocytes. *J. Viral Hepat.* **15(12)**:855–864.

WHO/CDCreport(2003).HepatitisC.[http://www.cdc.gov/ncidod/diseases/hepatitis/slideset/hep\\_c/slide\\_21.htm](http://www.cdc.gov/ncidod/diseases/hepatitis/slideset/hep_c/slide_21.htm)

Workenesh, A., Cutts, F., Nokes, J., Fontanet, A., Abebe, A., Messele, T., Enquesslassie, F. and Dejene, A. (2002). Higher prevalence of anti-HCV antibodies among HIV-positive compared to HIV-negative inhabitants of Addis Ababa: results from a community based survey. *J. Med. Virol.* **68**:12-17.

Zhu, Q., Oei, Y., Mendel, D., Garrett, E., Patawaran, M., Hollenbach, P., Aukerman, S., and Weiner, A. (2006). Novel Robust Hepatitis C Virus Mouse Efficacy Model. *Antimicro. Agents Chemotherapy* **50(10)**: 3260-3268.

## Consent Form

I have been informed about the study which is targeted to assess “Impact of HCV infection on Immunohaematological and clinical chemistry parameters in HIV/HCV co-infected patients before and after initiation of HAART’. The aim of the project and the need for taking part of the blood samples for the study have been informed to me in the language that I comprehend well. I have also informed about the confidentiality of the information and results. Additionally I have been told that the cooperation and participation in the study is on the voluntary bases and refusal to participate involves no penalty. Apart from this, I have been informed that the specimen is used only for research purpose and I benefit from the free laboratory investigation. Therefore, with full understanding of the importance of the study, I agreed voluntarily that my left over blood samples collected for CD4<sup>+</sup> and CD8<sup>+</sup> cell counts or other clinical investigation is to be used for the further analysis of impacts of HCV infection on Immunohaematological and clinical chemistry parameters in HIV/HCV co-infected patients.

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Participant’s signature

## DECLARATION

I, the undersigned, declare that this M. Sc. thesis is my own original work, has not been presented as thesis work for a degree in this or other University and that all sources of material used for the thesis have been duly acknowledged.

### **Investigator**

Name Solomon Taye (B. Sc)

Signature \_\_\_\_\_

Date \_\_\_\_\_

This Thesis has done under my supervision and submitted for examination with my approval as a university advisor

### **Advisor**

Name Mekuria Lakew (PhD, Associate Prof. in Immunoparasitology)

Signature \_\_\_\_\_

Date \_\_\_\_\_