Addis Ababa University

School of Graduate Studies College of Natural Sciences

Prevalence and phylogenetic variant of GBV-C on disease progression of HCV, HBV and HIV under HAART

By

Mohamed Farouk Mirghani Saeed (MSC.)

A thesis Submitted to The Department of Microbial, Cellular and Molecular Biology for the Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy of Science

(Applied Microbiology)

April, 2019

Addis Ababa, Ethiopia

DECLARATION

I, the undersigned, declare that this thesis is my own original work, has not been presented for a degree in any other university, and that all sources of materials used for the thesis have been duly acknowledged.

Name: Mohamed Farouk Mirghani Saeed

Date: _____

Signature: _____

Supervisor:

Name: Dr. Mekuria Lakew

Date: _____

Signature: _____

Dedication

To my father Eng. Farouk Mirghani Saeed, my mother, the entire family and relatives

ACKNOWLEDGEMENT

Firstly, I would like to express my sincere gratitude to my advisor Dr. Mekuria Lakew for tirelessly advising me throughout the research process. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my Ph.D. study.

Very special thanks after god to my father Farouk Mirghani for his moral and financial support without which, this research would not have been successful.

I would like to thank Dr. Gurja Belay, Dr. Dereje, Dr. Desta Kassa, Dr. Abraham Tesfaye, the staff of Adera Higher Clinic and the staff of Addis Ababa City Administration Health Research Bureaus Laboratory for their help during this research.

I immensely thank Prof. Muntaser El Tayeb Ibrahim and the staff of the Institute of Endemic Diseases, University of Khartoum for their technical assistances during the sequencing of the viral genome.

ABSTRACT

Prevalence and phylogenetic variant of GBV-C on disease progression of HCV, HBV and HIV under HAART

Mohamed Farouk
Addis Ababa University, 2019

Despite the advancements in health system management and technology, Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) remain a serious global public health challenge. GB virus (GBV-C) is a virus in the *Flaviviridae* family with HCV that was isolated from patients with liver disease. It has the same mode of transmission with HIV, HBV and HCV. Several studies from different countries have reported the positive role of GBV-C in improving the clinical infection and treatment outcomes of HIV patients, while its impact among HBV and HCV patients is controversy. Although the virus has been isolated from patients with fulminant hepatitis, but other studies failed to prove any association. Co-infection of GBV-C with one or more hepatotropic viruses like HBV or HCV may have negative or positive effect on the disease evolution. In Ethiopia, there has not been any study made on the prevalence or the clinical impact of GBV-C in circulation. Therefore, the aim of this study was to determine the prevalence, predominant genotype and the association between GBV-C and the clinical outcome among viral hepatitis and HIV patients under HAART.

Serum samples were collected from different population living in Addis Ababa from March 2014 to February 2016. The subjects of this study comprised a total of 252 participants of all ages, and were divided into distinct groups according to their health status. The first group comprised a total of 81 HIV samples that were sent to the health research laboratory in Addis Ababa for follow-up studies, the second group comprised a total of 101 serum frozen samples collected from Adera Internal Medical Specialty Center for patients with viral hepatitis, and 70 frozen serum samples of healthy individuals collected from the Ethiopian Public Health and Research Institutes and adult volunteers. To determine the prevalence of GBV-C, RNA was extracted, reversed transcribed, and amplified by Real Time polymerase chain reaction (PCR), using primers for 5- untranslated region (5-UTR) of the HGV/GBV-C. Among the HIV patients the CD4+ cells count and plasma viral load were performed. Liver function test and abdominal ultra sound were investigated in all viral hepatitis patients. The prevalence of GBV-C RNA were detected in 20 (11.27%) of the patients with viral hepatitis and HIV, while all the healthy subjects were negatives.

Among HIV patient's comparison of the mean CD4+ count was found to be significantly different between HGV/GBV-C positive and negative patients at ($P < 0.05$). GBV-C and HIV coinfected patients were categorized in the first and second WHO clinical staging system. Furthermore, positive and negative GBV-C patients were sub divided into small groups based on age, sex and date of starting HAART. The number of CD4+ cells over time increased more rapidly in GBV-C positive patients compared to GBV-C negative patients. Among viral hepatitis patients the prevalence of GBV-C were slightly higher among HBV patients, however, there was no significant difference ($P > 0.05$) in the liver enzymes level among GBV-C negative and positive individuals. Our study found that GBV-C infection had no influence on the severity of chronic liver disease among HBV and HCV coinfection. While among HIV patients the

coinfection reduces the viral load, increases the number of CD4+ cells and improves response to treatment.

To determine the predominant genotype, serum samples were recollected on 2017, from the same patients who were GBV-C positive in 2014. After performing various molecular techniques on the samples, the virus was detected but at a low level in the patient's serum, which indicates the clearance of the virus. Therefore it was not possible to sequence the virus.

To the best of our knowledge, this is the first report of GBV-C in Ethiopia. Understanding the mechanisms between GBV-C and HIV could lead to develop novel treatment/vaccine.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
HIV	Human Immunodeficiency Virus
HBV	Hepatitis B Virus

HCV	Hepatitis C Virus
HCC	Hepatocellular Carcinoma
TB	Tuberculosis
WHO	World Health Organization
UNAIDS	United Nations Program on HIV/AIDS
CD	Cluster of Differentiation
HAART	Highly Active Antiretroviral Therapy
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NtRTIs	Nucleotide Reverse Transcriptase Inhibitors
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
PI	Protease Inhibitor
RNA	Ribo Nucleic Acid
cDNA	Complementary DNA
FIs	Fusion Inhibitor
CRIs	Co-receptor Inhibitors
INIs	Integrase Inhibitor
DNA	Deoxyribo Nucleic Acid
CDC	Centers for Disease Control and Prevention
GBV-C	GB Virus C
HGV	Hepatitis G Virus
STDs	Sexually Transmitted Diseases
MOH	Ministry of Health
UNGASS	United Nations General Assembly Special Session on HIV/AIDS
IFN	Interferon
SIV	Simian Immunodeficiency Virus
kb	kilo base
p	Protein
gp	Glycoprotein
mRNA	messenger RNA
PBMC	Peripheral Blood Mononucleated Cells
HLA-DR	Human Leukocyte Antigen-D Related
VL	Viral Load
T-tropic	T-cell-tropic
M-tropic	macrophage-tropic
Tat	Trans-activator of transcription
Rev	Regulator of virion
Nef	Negative regulatory factor
Vif	Viral infectivity factor
Env	Envelop
Vpu	Viral protein u

Vpr	Viral protein r
CCR5	C-C chemokine receptor type 5
CXCR4	CXC chemokine receptor type 4
MIP-1α	Macrophage Inflammatory Protein-1 alpha
MIP-1β	Macrophage Inflammatory Protein-2 beta
RANTES	Regulated on Activation, Normal T-cell Expressed and Secreted
ADCC	Antibody Dependent Cellular Cytotoxicity
bNAbs	Broadly Neutralizing Antibodies
NK	Natural Killers Cells
GALT	Gut Associated Lymphoid Tissue
TGF−β	Transforming Growth Factor- Beta
LPS	Lipopolysaccharide
pDCs	plasmacytoid DCs
DC	Dendritic Cell
Th	T helper Cells
IL	Interleukin
TNF	Tumor Necrosis Factor
CTLs	cytotoxic T lymphocytes
Ig	Immune globulin
CAF	Cell Antiviral Factor
MHC	Major Histocompatibility Complex
PrEP	Pre-Exposure Prophylaxis
PEP	Post-Exposure Prophylaxis
ART	Antiretroviral Therapy
Ad5	Adenovirus 5 vector
HVTN	HIV Vaccine Trails Network
PEG	Poly Ethylene Glycol
PD-1	Programmed Death receptor
siRNA	Single RNA
Tregs	regulatory T cells
NHL	Non-Hodgkin’s lymphomas
FDA	Food and Drug Administration
PMTCT	Prevention of Mother To Child Transmission
OI	Opportunistic Infections
VCT	Voluntary Counseling and Testing
GB	G Barker
HAV	Hepatitis A Virus
HDV	Hepatitis D Virus
HEV	Hepatitis E Virus
GBV-A	GB virus A

GBV-B	GB virus B
GBV-D	GB virus D
nm	nano meter
HBsAg	Hepatitis B surface Antigen
HBcAg	Hepatitis B core Antigen
HBeAg	Hepatitis e Antigen
anti-Hbc	Hepatitis B core antibody
pegIFN-α	pegylated-interferon alpha-2a
SVR	Sustained Virological Response
ORF	Open Reading Frame
UTR	Un-Translated Regions
IRES	Internal Ribosomal Entry Site
NS	Non Structural protein
NTPase	Nucleotidase
RT-PCR	Reverse-Transcription Polymerase Chain Reaction
anti-E2	anti-E2 antibody
ALT	Alanine Transaminase
AST	Aspartate Transaminase
AHF	AIDS Health Care Foundation
HCT	HIV Counseling and Testing
EPHI	Ethiopian Public Health Institute
EHNRI	Ethiopian Health and Nutrition Research Institute
SOD	Super Oxide Dismutase
ALP	Alkaline phosphatase
SGPT	Serum Glutamate Pyruvate Transaminase
SGOT	Serum Glutamic Oxaloacetic Transaminase
BIL-T	Total bilirubin
ALB	Albumin
LDH	lactate dehydrogenase

CHAPTER 1: INTRODUCTION

1.1. Background of the study

Despite the advancements in health system management and technology, Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) remain serious global public health challenge. HIV, as the name shows is an immunological and systematic disease. It causes Acquired Immunodeficiency Syndrome (AIDS), by impairing the natural defense system of the host against infections (Lucas *et al.,* 1993). While chronic infection by HBV and HCV leads to slow progressive liver disease that over a period of up to 30 years may result in cirrhosis, chronic liver failure and hepatocellular carcinoma (HCC) (Igor *et al.*, 2011).

HIV is a slow acting virus that takes years to produce illness in a person. It impairs infected person's defense system and, over time, other viral, bacterial, fungal, and parasitic infections that take advantage of the opportunity and manifest themselves in different types of illnesses, such as tuberculosis (TB), pneumonia, candidiasis, diarrhea and cancer. This is why the infectious conditions found in HIV infected individuals are called opportunistic (De Cock *et al*., 2011; Maartens *et al*., 2014).

According to the World Health Organization (WHO) approximately 37 million people are currently living with HIV of which 24 million suffer from accumulated AIDS-related deaths and 2.6 million are new infections (WHO, 2015). Sub-Sahara Africa is by far the worst affected in the world by the epidemic. The region harbor about 12% of the world's population, but is home to over 68% of people living with HIV, and accounts for 70% of new HIV infections in 2010 (UNAIDS, 2015; Piot *et al.*, 2015).

The pathogenesis of HIV infection is marked by a latent period of 7 to 10 years from initial infection to clinical AIDS in adults, though AIDS may be manifested in less than two years or be delayed in onset beyond 10 years. The phases and events along with their associated markers are depicted in figure 1.

Acute HIV infection occurs at two to-six weeks, post infection, and is characterized by the onset of flu-like symptoms, and HIV viremia. During this time CD8+ T cells are activated to kill HIV-1 infected cells, CD4+ T cell count decreases due to large quantities of cell death within the gut-associated lymphoid tissue. Following the acute phase of HIV-1 infection, the level of virus which is maintained in the blood plasma, known as the viral set point, has been shown to be indicative of future HIV disease progression. The asymptomatic latent phase of HIV infection can last between six months and twenty years, and on average it lasts about ten years (Murphy, 2012). Although infection is asymptomatic during this time, HIV-1 infection persists and numbers of CD4+ T cells decline. The persistence of HIV-1 infection during this time has been associated with the up regulation of markers of CD4+ and CD8+ T cell activation (Boasso and Shearer, 2008). Moreover, chronic immune activation is associated with the persistence of HIV infection and helps drive CD4+ T cell depletion (Souasa *et al.*, 2002; Paiardini and Muller, 2013).

Once CD4+ T cells decline, typically from normal levels of 800-1200 to levels of ≤500 cells per milliliter of blood, the symptomatic phase begins and opportunistic infections or cancer begins to occur. Once CD4+ T cells reach levels of 200 cells per milliliter in an individual he is clinically diagnosed with AIDS (Murphy, 2012; Hazenberg *et al*., 2000).

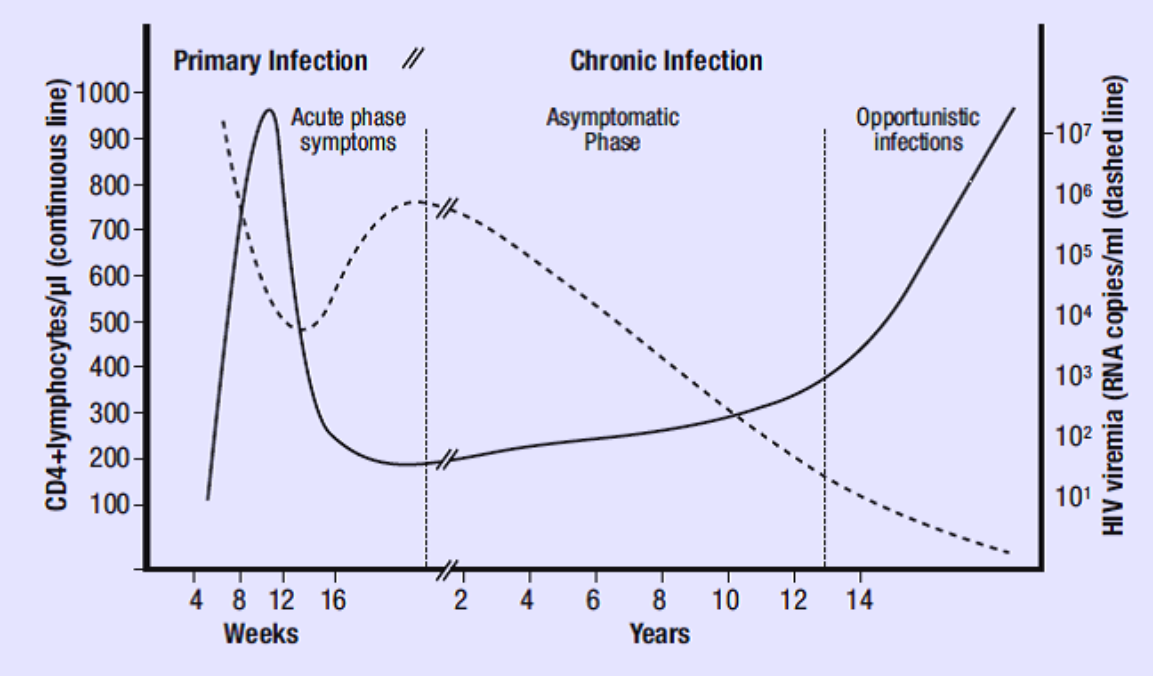

Figure 1: Clinical course of HIV infection.

At present, there are combinations of drugs which increase the survival of infected individuals by suppression of HIV replication and restoration of immune function. These drugs collectively called Highly Active Antiretroviral Therapy (HAART), and are distributed into distinct classes based on their molecular mechanisms and resistance profiles. Two of the classes, Nucleoside/nucleotide Reverse Transcriptase Inhibitors (NRTIs/NtRTIs) and Non-Nucleotide Reverse Transcriptase Inhibitors (NNRTIs), target the process of reverse transcription of genomic RNA into cDNA by the enzyme reverse transcriptase (Sarafianos *et al*., 2009). The other four classes target the proteolytic cleavage of viral poly proteins by the enzyme protease into their respective functional proteins (Protease Inhibitors [PIs]), viral entry through virus–cell fusion (Fusion Inhibitor [FIs]) and interaction of the virus with its (co-)receptors (Co-receptor Inhibitors [CRIs]), and integration of the proviral DNA into the host cell genome with the help of the enzyme integrase (Integrase Inhibitor [INIs]) (Sarafianos *et al.*, 2009).

These drugs have made a profound decrease in HIV associated morbidity and mortality and enhanced the quality of life among HIV patients (Detels *et al.,* 1998; Gulick *et al*., 2003).

It is given to all HIV cases with severe or advanced HIV clinical disease (WHO clinical stage 3 or 4), individuals with CD4+ counts of ≤350 cells/mm^3, pregnant and breast feeding women, children less than 5 years old, and chronic HBV, HCV or TB coinfection. In addition, all symptomatic patients regardless of CD4+ count, who are at risk of disease progression, are treated with HAART (WHO, 2016; Thompson *et al.,* 2010). However, HAART is not able to eradicate the virus from the body completely because the HIV can recover and replicate from resting CD4+ T-lymphocytes (Hantano *et al*., 2010).

The emergences of drug resistant strains to HAART are becoming common occurrence. This can have a wide range of adverse effects on patients. The undesirable side effects from the long term use, ends in damages of the kidney, liver, and pancreas whereas the changes in fat metabolism lead to increased risk of strokes and heart attacks. Discontinuing the treatment will end up in an increase of the viral load, beside the undesirable side effects, which may lead to hepatotoxicity especially in the case of co-infection with hepatitis viruses (Barbara *et al*., 2014; Geeraert *et al*., 2008; UK Collaborative Group on HIV Drug Resistance, 2005). Furthermore, the drug is too expensive for the countries that are in need. From 2015 onward it is estimated to be requiring 22- 24 billion dollars annually (WHO, 2013).

Coinfection with HBV and HCV among HIV patients is common because they more or less share the same mode of infection, sexual, parenteral, and vertical routes of transmissions (Mallet *et al*., 2011; Lacombe and Rockstroh, 2012). Approximately 5–25% of HIV patients in Sub- Saharan African are either coinfected with HBV and/or HCV. Studies show that co-infections of hepatitis viruses complicate the management of HIV by reducing the survival

rates and increased the risk of liver disease and hepatotoxicity as a result of antiretroviral therapy (Barth *et al.,* 2010; WHO, 2013; CDC, 2014).

However, not all the co-infections among HIV patients have deleterious effects. In the mid-90s, novel viruses that were identified from patients with liver disease by two independent groups and labeled as GB virus C (GBV-C) and hepatitis G virus (HGV). Genome analysis showed that the two viruses shared 96% of the amino acid sequence and 86% of the nucleotide sequences and this suggest that they are different isolates of the same virus, and the term GBV-C is the commonly used since they are not associated with any disease (Simons *et al*., 1995a; Simons *et al.,* 1995b; Linnen *et al.,* 1996; Leary *et al.,* 1996b). Although, GBV-C is the most closet virus to HCV and they are sharing approximately 30% amino acid homology, but unlike HCV that replicates in the hepatocytes, GBV-C is a lymphotropic virus and not associated with any disease by itself (Cheung *et al.,* 1997; Stapleton, 2003).

The interest in this virus started when Toyoda and his group observed that GBV-C co infected HIV patients were found with lower viral load than mono-infected individuals (Toyoda *et al*., 1998). Later, two large independent studies with long follow ups showed that GBV-C is also associated with longer survival rate and improved clinical conditions (Tillmann *et al.,* 2001; Xiang *et al.,* 2001). Other studies have also shown that the protections include high CD4+ counts, low viral load, higher survival rate, good response to HAART and late progression to AIDS (Heringlake *et al.,* 1998; Souza *et al.,* 2006; Gilles *et al.,* 1999; Rodriguez *et al*., 2003; Williams *et al.,* 2004; Bjorkman *et al.,* 2007).

GBV-C does this, by competing for the receptor and co-receptor molecules used by HIV. Likewise, it also enhances the outcomes of HAART by reducing the activation of CD4+ and

CD8+ T-cells that would otherwise serve HIV replication and increase the viral load in circulation (Maidana-Giret *et al*., 2009; Stapleton *et al.,* 2009). With increase in GBV-C, the levels of HIV decreases in inverse proportion whereas, it is directly proportional to the increase of GBV-C RNA fragments. The optimal treatment effects of HAART are obtained in GBV-C/HIV coinfected patients under treatment (Bjorkman *et al*., 2007).

However, the above-mentioned effects are not seen in all GBV-C viruses found everywhere. There are multiple GBV-C genotypes and subtypes in individuals that may have different impacts on HIV disease (Ruiz *et al*., 2010; Schwarze-Zander *et al.,* 2012). In Ethiopia the situation is not known, there are no reports on the prevalence or the genotype of GBV-C among HIV patients under HAART or on the degree of protection of GBV-C.

Thus, this study focuses on studying: 1) the prevalence, 2) the impact of GBV-C on viruses and immunity of the host, 3) the association between GBV-C and the clinical outcome among viral hepatitis and HIV patients under HAART, 4) characterize the genotypes and subtypes of GBV-C in the circulation.

1.2. Rational and significance of the study

The countries of the sub-Sahara Africa including Ethiopia are by far the worst affected in the world by the HIV pandemic, poverty, malnutrition, drought, limited healthcare, and other infectious diseases could be the major factors in enhancing progression of AIDS.

HIV was first detected in Ethiopia in stored sera collected in 1984 (Tsegae *et al*., 1988) and the first two HIV cases were documented in 1986 (Lester *et al.*, 1988; Seyoum *et al*., 1988). The unprotected heterosexual transmissions accounts for approximately 88% of all HIV infections while the maternal transmission account for 8-10%. There are important factors that promote the spread of the disease. These factors include multiple sexual partners, prostitutions, alcohol and sexually transmitted diseases (STDs) (MOH/HAPCO, 2006). According to data collected from the Ministry of Health (MOH), the overall prevalence of HIV in the population is 1.1% which accounts for about 790,000 HIV positive persons in all age groups. The highest prevalence rate occurs in the age group 15-24 and those between 15 to 49 years, which are the economically productive segments of the population (Gemechu *et al*., 2015).

In 2003, based on the recommendations of the United Nations General Assembly Special Session on HIV/AIDS (UNGASS), the government of Ethiopia lunched ART program to reduce HIV associated morbidity and mortality and to limit the transmission of the virus (FDRE, 2006). Despite all efforts to limit its distribution, the virus is still in circulation in the country as in the rest of the world. The reasons are due to drug resistant strains and also the fact that many infected patients do not use the drug because of its undesirable side effects due the long term use. HAART is given, to all HIV cases with CD4 count below 200 cells/mm3, HIV viral load > 100,000 copies/ml, pregnant and breast feeding women, children less than 5 years old, chronic HBV, HCV or TB coinfection (WHO, 2013; WHO, 2014). However,

discontinuing the treatment even for few weeks will end up in high increase of the viral load (Geeraert *et al.,* 2008).

On the other hand, HBV and HCV are hepatotropic viruses that spread mainly through contaminated blood and blood products, sexual contact and contaminated needles. Chronic infection by these viruses leads to slow progressive liver disease that over a period of up to 30 years may result in cirrhosis, chronic liver failure and hepatocellular carcinoma (HCC). According to the WHO there are 350 million people with chronic HBV infection and 170 million people with chronic HCV infection worldwide. Furthermore, HBV is estimated to result in 563,000 deaths and HCV result in 366,000 deaths annually (Abel, 2013).

HIV, HBV, and HCV infections share similar transmission routes and therefore co-infection is common. These viruses are prevalent in different parts of the world including Ethiopia (Dessie *et al*., 2007; Shimelis *et al*., 2017). There for this study shows the burden of these viruses on Ethiopia.

The presence of the above problems and the lack of preventive vaccine against HIV made the development of new drugs a compulsory step but remains a major area of challenge. To this end many approaches are being tested. Of these, the use of new pharmacologic agents and microorganisms like Newcastle Disease Virus (Sunil *et al*., 2011), Adenovirus (Robert *et al*., 1992) and GBV-C.

To this effect this thesis target to study the prevalence and to identify and characterize the genotype of GBV-C virus in the circulation in Ethiopia and find its influence on the HBV, HCV and HIV viruses and treatment outcome.

In the past few years, studies had shifted from looking at GBV-C as a pathogenic suspect to a beneficial modulator of HIV infection, disease progression and treatment. They focused on the identification and establishment of the molecular mechanisms of interaction between the two viruses and the immune system to find different targets of intervention to HIV infection.

Molecular epidemiological studies to detect the incidence, impact and characteristic of GBV-C among HIV, viral hepatitis patients and general population in Ethiopia is essential for multiple reasons. First, in Ethiopia there has not been any endeavor to study the prevalence or genotype of GBV-C among high risk groups or general population. Therefore, this study will give information to governmental and non-governmental organizations which work in the area of HIV/AIDS and viral hepatitis. Second, several studies has indicated the positive influence between GBV-C infection among HIV patients and the good response to HAART(Tillmann *et al*., 2001b; Nunnari *et al.,* 2003; Williams *et al*., 2004; Tillmann *et al.,* 2004). Understanding the role of GBV-C among HIV patients and the response to HAART may help to improve or to modify the current treatment.

Third, it is known that highly divert members of GBV-C are found in different geographical locations and only limited members among them are capable of playing a protective or curative role. Studies, which focused on the importance of the genotypes influence in HIV disease progression, have found two genotypes with minor differences labeled as 2a and 2b, which could produce opposite effects on CD4+ count and disease progression (Muerhoff *et al*., 2006; Schwarze-Zander C *et al*., 2006). Other studies observed that various GBV-C genotypes reflect differences as their sensitivity to interferon (IFN), cell tropism and their ability to persist in culture (Shimizu *et al*., 1999; George *et al*., 2003).

So far there are seven different genotypes and subtypes located in different geographical regions. Genotype 1 predominant in West Africa; genotype 2 in Europe and USA; genotype 3 in Asia; genotype 4 in the Southeast Asian countries; genotype 5 in South Africa; genotype 6 in Indonesia and genotype 7 isolated in China recently (Naito *et al*., 1999; Sather *et al*., 1999a; Tucker *et al*., 1999; Smith *et al.*, 2000; Tucker and Smuts, 2000; Sather *et al.*, 2001; Feng *et al*., 2011). Due to this, it is possible that GBV-C genotype could at least partially account for the variable influence of GBV-C infection on HIV disease progression.

Therefore, in consideration of the above mentioned factors, epidemiological and molecular characterization of GB Virus C in Ethiopia is essential. In this study, we conducted a molecular epidemiological study on GBV-C from HIV, viral hepatitis patients and healthy individuals.

The information generated from this work will be disseminated to the scientific/non-scientific community through publications in national and international journals, conferences and seminars and can be utilized by various governmental and non-governmental bodies to improve healthcare delivery effort in the country.

1.3. Objectives of the study

1.3.1. General objectives

The general objective of this study is to determine the prevalence, genotype and clinical characteristic of GBV-C in the circulation, and its role in clinical outcome among viral hepatitis and HIV/AIDS patients under HAART in Addis Ababa, Ethiopia.

1.3.2. Specific objectives

1. To identify prevalence of GBV-C in Addis Ababa, Ethiopia.
2. To determine its effect on disease progression and treatment.
3. To determine and characterize the predominant genotype in Addis Ababa, Ethiopia.

CHAPTER 2: LITERATURE REVIEW

2.1. Human Immunodeficiency Virus

The first cases of AIDS were described in the early 1980s, among Californian homosexual men suffering from rare opportunistic infections and cancers (CDC, 1981; CDC, 1982). Few years later it was found that the disease is caused by two viruses, HIV-1 which is the primary cause of AIDS worldwide, and HIV-2 which is restricted in Western and Central Africa (Clavel *et al*., 1986; McCutchan, 2006). Although the two types cause AIDS, but HIV-2 is less virulent than HIV-1 and require long period to progress to AIDS (Luciw, 1996; Lucas *et al*., 1993).

HIV is an RNA virus within the genus *Lentivirus* in the family of *Retroviridea*. The viruses in this family are characterized by chronic course of diseases, longer incubation period, persistent viral replication and they use their RNA and host DNA to make viral DNA. Epidemiological data suggest that HIV-1 was derived from a similar virus within the same genus called Simian Immunodeficiency Virus (SIV) that cause AIDS in primate. There are four subtypes of HIV-1 which are found in specific geographic areas called groups M (major), N (non-M/non-O), O (outlier), and recently group P. The pandemic subtype of HIV that infects millions of peoples worldwide is group M, which is subdivided into eleven subtypes or clades (A to K) as well as several recombinant isolates (Sharp and Hahn, 2011; McCutchan, 2000).

Based on records, it's been suggested that HIV-1 emerge into human at the beginning of the twentieth century (Worobey *et al.*, 2008). The first cases were identified in samples from the Democratic Republic of Congo in 1959 (Zhu *et al.*, 1998). However, the epidemic of the viruses accelerate at the end of that century due to several factors like increased travel,

prostitution and the use of unsterilized medical equipment's (Hahn *et al.*, 2000; Gisselquist, 2003).

2.1.1. Structure and Genome Organization

The basic structure of HIV consists of an outer lipoglycoprotein bilayer which is derived from the host cell with several cellular membrane proteins such like major histocompatibility antigens, actin and ubiquitin (Arthur *et al.*, 1992). The inner cylindrical core composed of about 2000 copies of the viral capsid protein p24 enclosing a diploid copy of a single RNA stranded which is coated with the nucleocapsid protein p7. The viral genomes are about 9.7kb and present as a dimer. The conical core contains the viral enzymes required for early replication events (reverse transcriptase, p51 and p66; integrase, p32). In addition to the conical capsid, the inner portion of the viral membrane contains the viral protease p10 which is surrounded by the matrix protein p17. The outer envelope proteins as shown in figure 2 are derived from a gp160 precursor in which is cleaved into a gp120 surface protein and the transmembrane gp41. These proteins have an important role in infectivity and disease progression by mediating the recognition of specific receptor on CD4+T- lymphocytes, macrophages, and monocytes, and chemokine co- receptors, such as CXCR4 or CCR5, which facilitate the process of cell binding and viral entry (Earl *et al*., 1990; Gelderblom *et al*., 1987; Gelderblom *et al*., 1989).

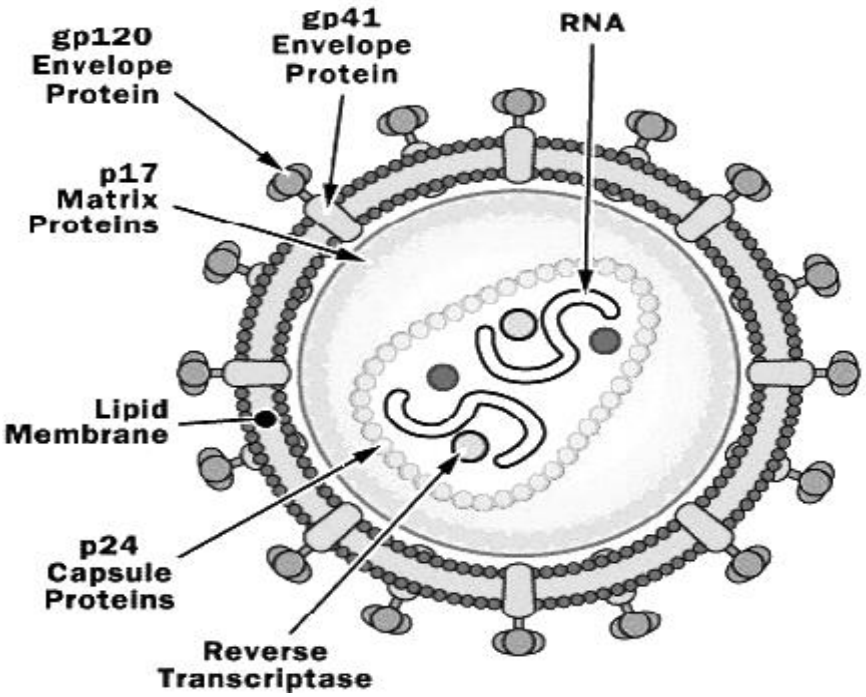

Figure 2: Structure of an HIV particle
(Adopted from http://health.howstuffworks.com with modifications).

The virus co-receptors are important binding sites for chemokines that recruit immune cells to the location of their secretion. In high concentration, these chemokines (e.g., MIP-1α; MIP-1β, RANTES) can compete for virus attachment to the chemokine receptor. Typically, T-cell-tropic (T-tropic) HIV viruses use the CXCR4 receptor, whereas macrophage-tropic (M-tropic) viruses use the CCR5 receptor (Chapham and Weiss, 1997; Moore, 1997; Doranz *et al.*, 1996).

In addition to the structural proteins, the virus contains also regulatory (Tat, Rev) and accessory proteins (Nef, Vif, Vpu, Vpr), which play key roles in modulating virus replication. The RNA binding proteins Tat (trans-activator of transcription) and Rev (regulator of virion) are expressed early in the life cycle and are essential for viral replication. Tat promotes the transcription of HIV genes and enhances the amount of proteins production, while the Rev ensures the transportation process of the messenger RNA from the nucleus to the cytoplasm.

Nef (negative regulatory factor) has multiple functions including down regulation of cell protein production which is important in cell defense and plays a major role in disease progression. Vif (viral infectivity factor) enhances the infectiveness of progeny virus particles. Vpu (viral protein u) is necessary for virus assembly and virus budding. Vpr (viral protein r) is involved in the arrest of the cell cycle and enables reverse transcribed DNA to gain access to the nucleus in non-dividing cells. Nef and Vif are closely associated with the core, while Vpu is found most likely outside the core. Vpr could be detected in virions and cells as well as in sera and cerebrospinal fluids of AIDS patients indicating that it exerts its biological functions via different mechanisms (Camaur and Trono, 1996; Greene and Peterlin, 2002; Le and Benichou, 2005).

2.1.2. HIV co-receptor interactions

The HIV co-receptors that facilitate the viral entry are either C-C chemokine receptor type 5 (CCR5) or CXC chemokine receptor type 4 (CXCR4), which belongs to the seven transmembrane G-protein coupled chemokine receptor family. The HIV isolates which are transmitted *in vivo* by using CCR5 are termed R5, while CXCR4-tropic variant that termed X4 usually appear in the late stages of HIV infection, and are associated with rapid decrease in CD4+ count. The principle co-receptor for HIV variants which are sexually transmitted and persist within the majority of infected individuals is CCR5 (Reynes *et al.*, 2001).

2.1.3. Replication

As shown in figure 3, the first event in HIV replication and infection is binding and attachments of the envelope gp 120 into the CD4+ receptor present on the surface of the cells. The viral cell entry requires the formation of a tri-molecular complex among the gp120, the primary receptor CD4, and the chemokine receptor CCR5 or CXCR4. During the entry, the virus loses its outer envelope and releases its contents into the host cell cytoplasm (Berger *et al.,* 1999; Kwong *et al.,* 1998; Wyatt and Sodroski, 1998).

The viral reverse transcriptase use the RNA genome as a template to produce an intermediate DNA then a double strand complementary cDNA, which is transported to the nucleus and integrated to the host cell genome by viral integration. The integration product called provirus which may remain inactive for years, producing few or no new copies of HIV. During the reverse transcription process a number of highly mutagenic viruses are produced due to the lack of proof-reading. These strains of viruses are able to evade neutralizing antibodies and to develop resistance against drugs antiretroviral agent (Coffin 1995; Roberts *et al.,* 1988).

Following host cell activation, the virus uses the host RNA polymerase enzyme to produce copies of a messenger RNA or mRNA which is produced complementary to the viral DNA in the nucleus. The viral enzyme protease will splice the mRNA to produce the viral proteins and finally the new virus will be produced by assembly and budding (Levy, 2007).

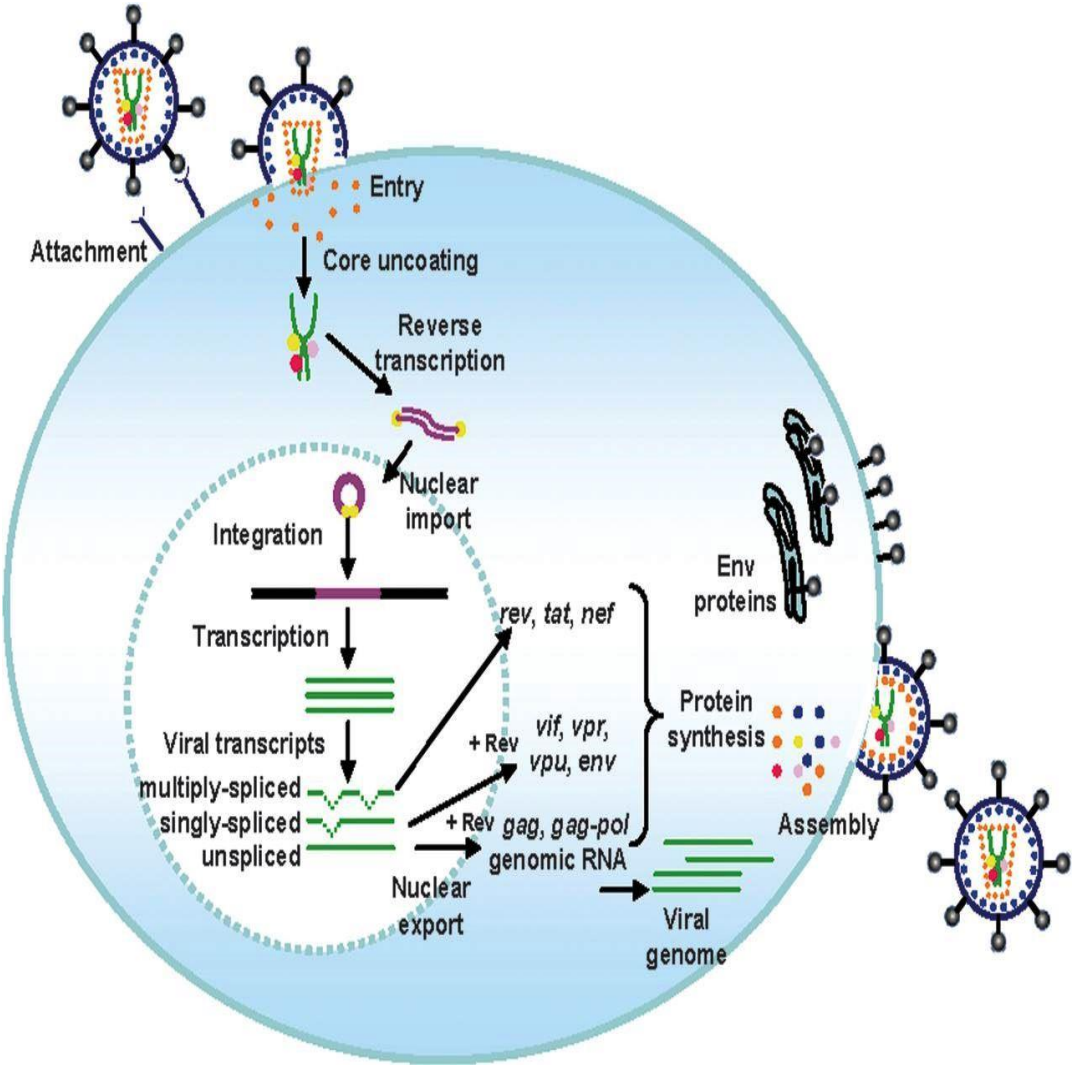

Figure 3: Events in HIV-1 life cycle (Adopted from www.HIVwebstudy.org)

2.1.4. Immune activation

Chronic HIV-related immune activation is characterized by the inappropriate production of pro-inflammatory cytokines and over expression of cellular activation and exhaustion markers. Most of these inflammatory responses induced by HIV are not directed toward HIV (Maartens *et al.*, 2014). They rather enhance susceptibility of target cells to HIV infection and enhance virus replication in already infected cells, which accelerates disease progression. This chronic, non-specific T cell activation leads to T cell exhaustion and apoptosis of CD4+ and CD8+ T cells. Increased expressions of HLA-DR and CD38 molecules on CD8+ T cells

correlate with a higher level of immune activation and constitute markers for bad prognosis, which are partly independent from actual CD4+ T count and VL. It remains unclear whether there is a single key mechanism behind this HIV-associated immune activation or not. A so-called "leaky gut syndrome" hypothesis proposes that massive loss of CD4+ T cells in the GALT may affect the protective barrier of the intestinal mucosa, allowing bacterial toxins such as lipopolysaccharide (LPS) to enter the bloodstream (Blankson, 2010). This "microbial translocation" could in consequence induce a pathological over-activation of both the innate and adaptive immune system (Maartens *et al*., 2014; Hessell *et al*., 2007).

2.1.5. HIV-specific humoral immune response

The humoral immune response is mediated by antibody producing B cells figure 4. In general, by preventing infections of the host cells, virus-specific antibodies play an important role in the control of many viral infections. This arm of the adaptive immune system is activated after uptake of viral proteins by antigen presenting cells (DCs, macrophages and B cells) that digest the proteins into small peptides and present them on MHC II molecules to CD4+ T helper (Th) cells. Specifically activated Th2 cells that produce B cell stimulating cytokines (including IL-4, IL-5, IL-6, IL-10, and TGF−β) will activate naive B cells. The latter are recognized by specific epitopes or intact virus through their surface IgM and promote B cell differentiation into plasma cells producing large amounts of IgG, IgA, IgE antibodies and memory B cells. During HIV-1 infection antibodies against gp120, gp41, the nucleocapsid (p24) and the matrix (p17) arise few weeks to several months after infection. This process is commonly referred to as Seroconversion (Winni *et al*., 2012).

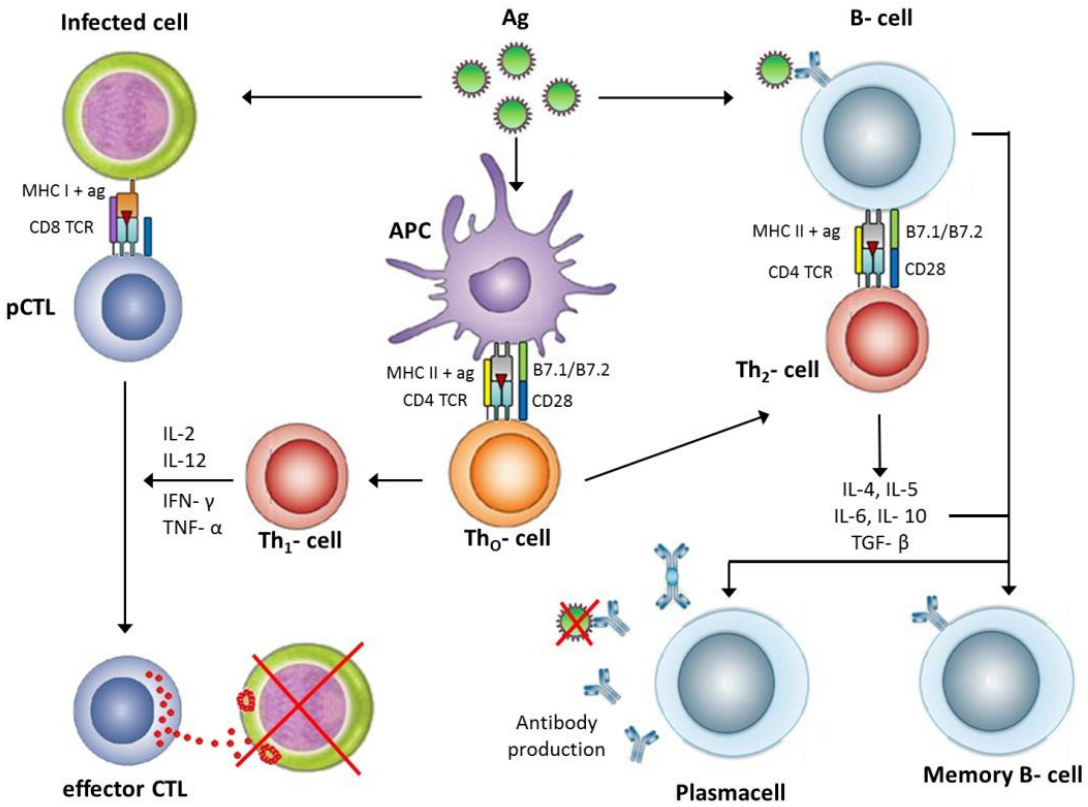

Figure 4: The humoral immune response

Virus antigens are presented by dendritic cells and B cells to T cells. Infected cells present peptides together with MHC I molecules on the plasma membrane. The peptide-MHC I complex is recognized by precursor cytotoxic CD8+ T lymphocytes (CTLs). Th1 cells, induced by antigen presenting cells, produce IL-2, IFN-γ, and TNF-α. These results in activation and differentiation of the precursor CTLs into memory or effector CTLs. Effector CTLs can directly kill infected cells by the production of perforins and granzymes. Activated Th2 cells, also induced by antigen presenting cells, produce B cell stimulating cytokines (including IL-4, IL-5, IL-6, IL-10, and TGF−β) that activate naive B cells. This facilitates/induces B cell differentiation into memory B cells and plasma cells which produce large amounts of IgG, IgA, IgE antibodies which prevent further virus infection.

The virus neutralization is characterized by the interaction of specific antibodies with the viral envelope spikes. This interferes with virus attachment or viral entry in target cells and

results in the inhibition of infection. Only a minority of anti-HIV Env antibodies, at any time, exerts immune pressure by autologous neutralization. However, the virus easily mutates and readily escapes from these potentially protective immune responses. During the chronic course of infection only 20% of the infected individuals will generate broadly neutralizing antibodies (bNAbs) having the ability to neutralize heterologous viruses. In addition to classical neutralization, antibodies can attach to HIV infected cells and kill them via antibody dependent cellular cytotoxicity (ADCC) mediated through their Fc moiety and natural killers cells (NK) (Stamatatos *et al*., 2009; Winni *et al*., 2012).

2.1.6. HIV-specific cellular immune response

The cellular immune response is the other arm of the adaptive immune system as shown in figure 5 and it is crucial to combat viral infections. CD8+ cytotoxic T lymphocytes (CTLs), which eliminate infected cells, play a key role in this process. The initial step involves processing of intracellular antigens by the proteasome. The resulting peptides are then presented together with MHC I molecules on the membrane of infected somatic cells. The peptide-MHC I complex is recognized by precursor cytotoxic CD8+ T lymphocytes (CTLs). Also in this case a CD4+ T cell help, induced by antigen presenting cells, is crucial. In this case the so-called Th1 cells, producing IL-2, IFN-γ, and TNF-α, activate and differentiate the CTLs into memory or effector CTLs. Effector CTLs can directly kill infected cells by the production of perforins and granzymes (Winni *et al*., 2012). Alternatively, CTLs can induce apoptosis of the infected cells after interaction of Fas ligand on CTLs with Fas receptor on infected T cells. CD8+ T cells also display a non-cytotoxic antiviral activity involving several cytokines, chemokines and a yet unidentified soluble CD8+ cell antiviral factor (CAF).

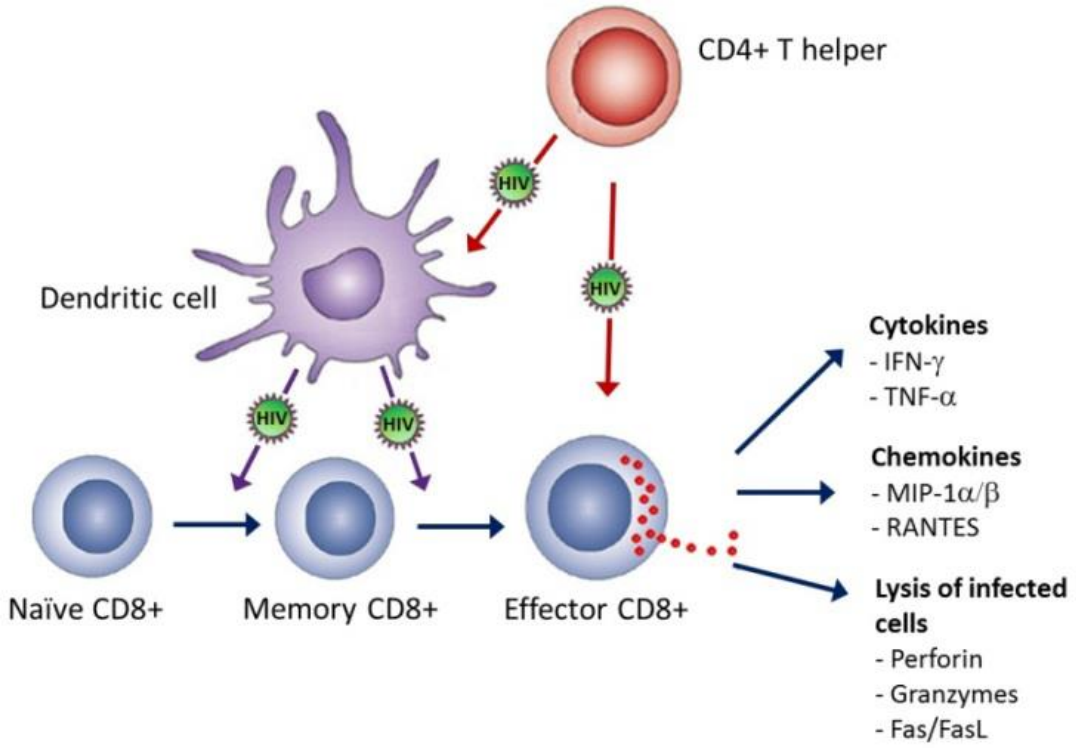

Figure 5: The cellular immune response

HIV specific CD8+ effector cells produce chemokines and cytokines in order to eliminate infected cells. CD4+ T helper cells help to stimulate both dendritic cells and CD8+ T-cells to maintain a CD8+ T-cell memory response. HIV interferes with this supportive function of CD4+ T-cells. The first T cell responses during HIV infection arise when the viremia peak is approached and reach maximum 1-2 weeks later. In non-controllers, the virus evades the CD8+ mediated T cell response by introducing mutations in CTL epitopes, by Nef-mediated down-regulation of MHC I and by influencing cytokine production and T-cell signaling. Since an optimal CD8+ T cell response, similar to the B cell response, depends on help of CD4+ T lymphocytes, the deterioration of CD8+ mediated viral control is also related to the weakening of CD4+ T cell function (Winni *et al*., 2012).

There are many indications that HIV-specific CD8+ T cell responses are responsible for at least partial VL control. In the macaque model, depletion of CD8+ T cells during SIV infection resulted in an increased viral load. In HIV-infected human subjects, who initially control the virus, escape mutations in specific CD8+ T cell epitopes were responsible for the loss of control and increase in VL (Winni *et al*., 2012).

2.1.7. Transmission and Epidemiology

HIV is mainly transmitted via contaminated blood or blood product, sexual intercourse, and from infected mother to child. The virus can be found in many body fluids with different concentration, thereby, the risk of infection is based upon the viral load in the body fluid which the individual is exposed to. The main fluids of transmission with high virus concentrations are peripheral blood mononucleated cells (PBMC), semen, cervical and vaginal secretions, breast milk and blood or blood products. The rates of transmission from infected patient to uninfected individual depend on the mode of transmission and other specific conditions. The HIV virus has a short half-life outside the host. This is due to the large size of the virus (Busch *et al.*, 1996; Quinn, 1996; Shepard *et al.*, 2000).

The WHO estimated that approximately 37 million people are living with HIV worldwide (WHO, 2015), and about 39 million people have died from AIDS since the beginning of the epidemic (UNAIDS, 2014). In 2014 there were 1.2 million AIDS-related deaths worldwide (UNAIDS, 2015).

The prevalence of HIV varies from region to another as shown below in figure 6.This is due to poverty, malnutrition, and drought. The Sub-Saharan in Africa harbor 70% of HIV (Piot *et al.*, 2015).

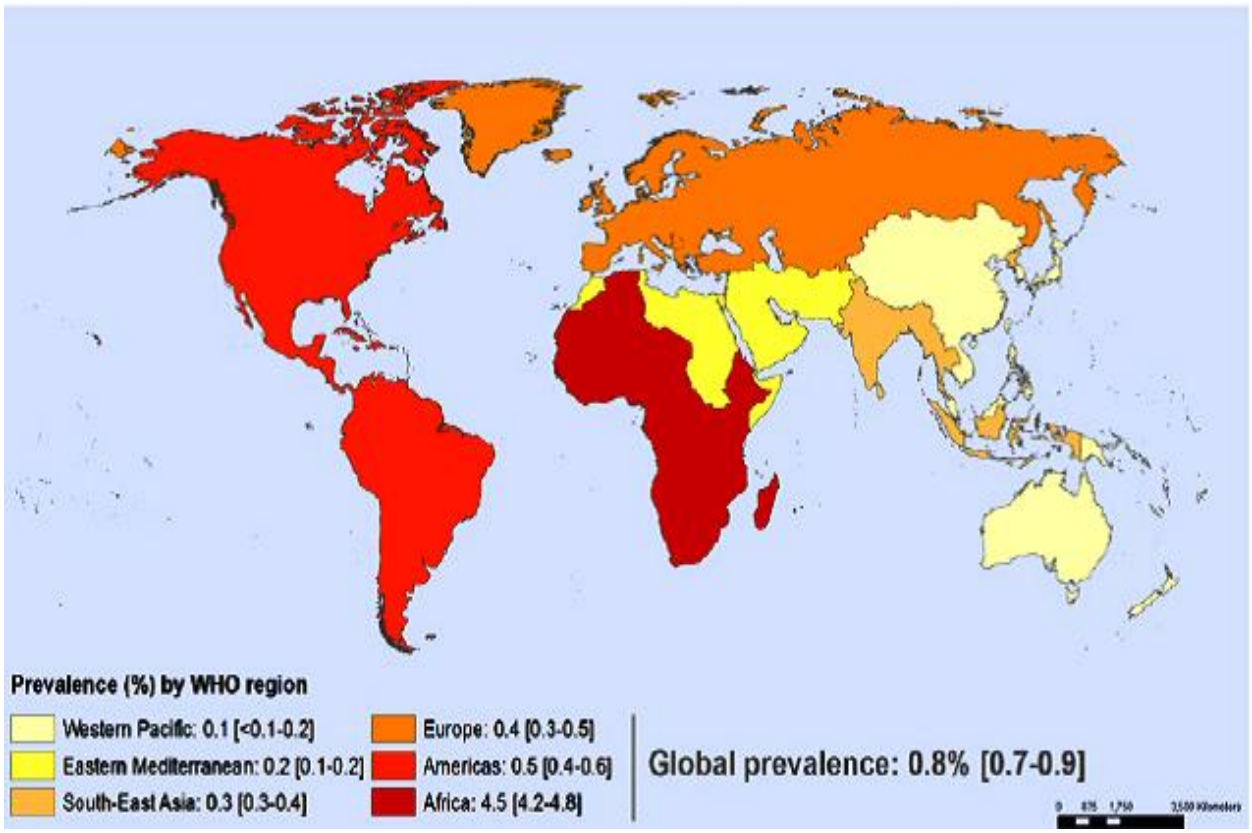

Figure 6: Global HIV Prevalence (15-49 years) By Region (Adopted from WHO, http://www.who.int/gho/hiv/hiv_013.jpg?ua=1)

2.1.8. Pathogenesis of HIV

On average, there is a latent period of 7 to 10 years from initial infection to clinical AIDS in adults, though AIDS may be manifested in less than two years or be delayed in onset beyond 10 years. The phases and events along with their associated markers are illustrated in figure 7.

Acute HIV infection occurs at two to-six weeks post infection, and is characterized by the onset of flu-like symptoms, and HIV viremia. During this time CD8 T cells are activated to kill HIV-1 infected cells, CD4 T cell count decreases due to large quantities of cell death within the gut-associated lymphoid tissue, and antibody production marks seroconversion. HIV infection then disseminates and establishes infection in lymphoid tissues throughout the

body. Following the acute phase of HIV-1 infection, the level of virus which is maintained in the blood plasma, known as the viral set point, has been shown to be indicative of future HIV disease progression (Murphy, 2012).

The asymptomatic latent phase of HIV infection can last between six months and twenty years, and on average it lasts about ten years (Murphy, 2012). Although infection is asymptomatic during this time, HIV-1 infection persists and numbers of CD4+ T cells decline. The persistence of HIV-1 infection during this time has been associated with the up regulation of markers of CD4+ and CD8+ T cell activation (Boasso and Shearer, 2008). Moreover, chronic immune activation is associated with the persistence of HIV infection and helps drive CD4+ T cell depletion (Souasa *et al.*, 2002; Paiardini and Muller, 2013).

Once CD4+ T cells decline, typically from normal levels of 800-1200 to levels of $\leq$ 500 cells per milliliter of blood, the symptomatic phase begins and opportunistic infections or cancer begins to occur. Once CD4+ T cells reach levels of 200 cells per milliliter in an individual, he is clinically diagnosed with AIDS (Murphy, 2012).

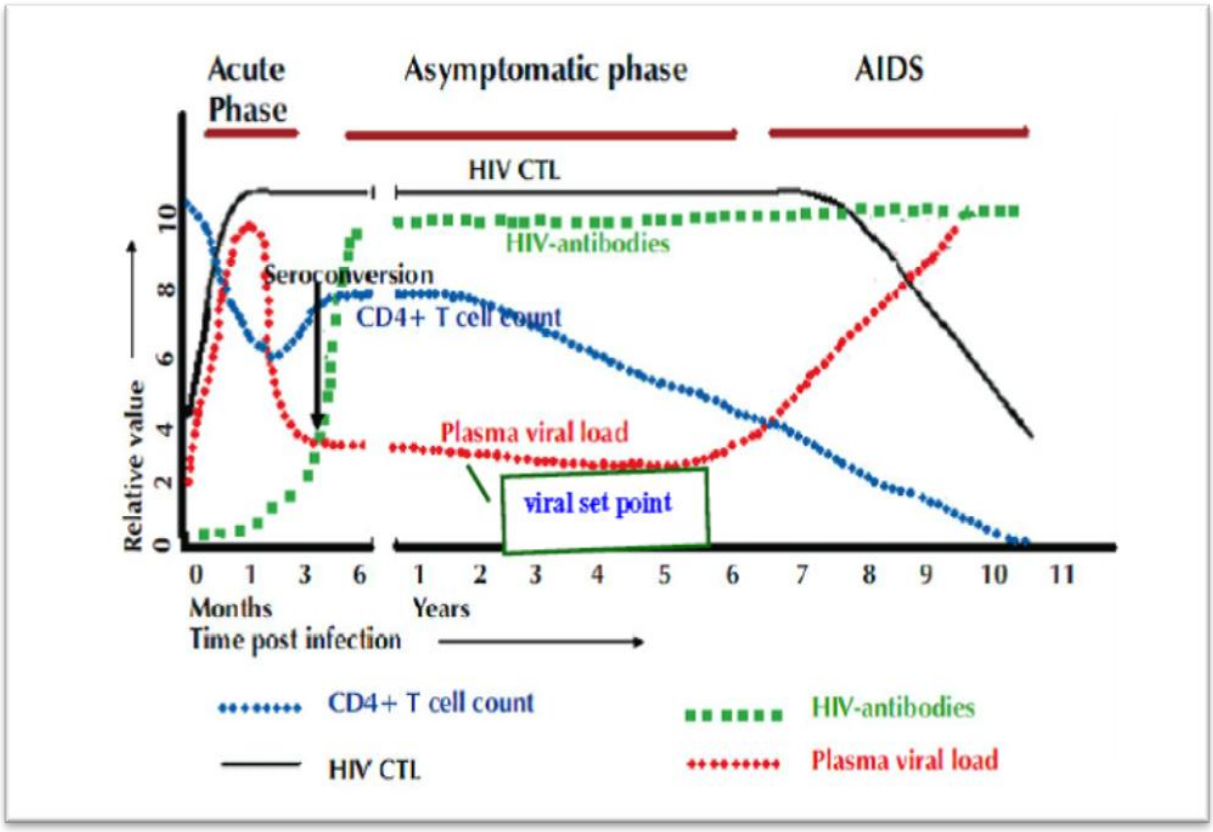

Figure 7: Phases of HIV Infection (Adopted from WHO, 2009).

2.1.9. Prevention and control of HIV

The effective methods for preventing and controlling HIV infection are either Pre-exposure prophylaxis (PrEP) that reduces the chance of infection, or PEP (post-exposure prophylaxis) which controls the infection after the exposure to the virus.

2.1.9.1. Pre-exposure prophylaxis

Several approaches have been development to prevent or reduce the risk of HIV infection among high risk groups. Medical male circumcisions have been shown to prevent up to 60% of HIV transmissions (Cohen *et al.,* 2008; Klausner *et al.,* 2008). Recent microbicide which are substances that could be applied rectally or vaginally proves to reduce the rate of sexual transmission by approximately 30%, while the use of the condoms as a physical barrier can

reduce the rate of transmission by up to 95% when it is available and used consistently (Cutler and Justman, 2008; Padian *et al.,* 2008).

2.1.9.2. HIV vaccine

Vaccine is one of the most potent and cost effective strategy to end the epidemic of HIV. For example, widespread vaccination against smallpox, measles and polio has eradicated the disease and save millions of lives. There are two main types of vaccines based on their use, either prophylactic or therapeutic. Vaccine that protects the person from getting the disease is prophylactic and is the major type of vaccine. On the other hand, therapeutic vaccines are used to increase the host immunity to fight disease during treatment. An HIV vaccine would theoretically have two goals. First, it could be given as prophylactic vaccine to healthy people to protect them from HIV infection. While the therapeutic vaccine could reduce the viral load and increase the CD4+ cells. The clinical trials database from the International AIDS Vaccine Initiative (IAVI) indicates that a total of 218 clinical trials have been conducted since 1988, but only 5 vaccines (Table 1) have advanced to Phase IIb and IIIb clinical trials, including the VAX003, VAX004, Step/Phambili, RV144 and HVTN505 trials (McElrath and Haynes, 2010; McMichael *et al.,* 2013; Esparza, 2013).

This is due to the high mutation rate of the virus such like the envelope protein, which is the key target for neutralizing antibodies. The protein has mutated in such a way that the circulating antibodies cannot neutralize it. New antibodies are induced, but new mutations repeatedly enable the virus to evade the immune system (Burton *et al.,* 2004). Furthermore, unlike other viruses, HIV rapidly integrates itself into the DNA of the host cell, where, in some cells, it remains latent and essentially invisible to the immune system. Latency makes the eradication of the virus impossible, even in patients receiving HAART for extended periods (Walker and Burton, 2008).

Currently, there are close to 30 clinical trials of HIV vaccines, using different targets and strategy against the virus, underway in over 20 different countries around the world, but they are still in Phase I and II safety studies.

These trials included antibody responses against Env or more recently, cellular immune responses to Tat and Nef (Paolo *et al.,* 2014; Andrew *et al.,* 2013). The findings of the RV144 trial, in which a combined regimen of T and B cell vaccines were modestly effective in preventing HIV infection, suggest that targeting both antibody and cellular immunity against HIV might constitute a better approach to induce protection, particularly if, this immunity is targeted to conserved regions within the HIV proteome (Winni *et al*., 2012; Deeks *et al*., 2013).

Table 1: HIV Vaccines Trails

Trial	Product	Candidate	NO Volunteers	Phase	Immune Response	Result
VAX003	AIDSVAX	Recombinant gp120 Protein	5,417	III	T cell, Abs	No efficacy
VAX004	AIDSVAX	Recombinant gp120 Protein	2,546	III	T cell, Abs	No efficacy
STEP	MRK-Ad5	Ad5 gag/pol/nef	3,000	IIb	T cell	No efficacy
PHAMBILI	MRK-Ad5	Ad5 gag/pol/nef	801	IIb	T cell	No efficacy
RV144	ALVAC-HIV and AIDSVAX	Recombinant gp120 Protein + Canary pox vector	16,402	III	T cell, Abs	31.20%
HVTN505	DNA and Ad5	DNA Ad5 gag/pol/ Nef	2,500	IIb	T cell, Abs	No efficacy

2.1.9.3. Vaccination strategies against HIV-1

2.1.9.3.1. HIV immunotherapy

Immunotherapy, aimed at reducing inflammation, preventing immune activation by HIV-1, or promoting effective immune responses, is currently being investigated (Table 2). Diverse approaches are being studied, and a number of trials are completed or under way (Vikram *et al*., 2014; Bhawna, 2013).

Table 2: Recent immunotherapy for HIV-1 infection

Immunotherapy	Treatment	Responses	Reference
Promoting immune responses			
Cytokines	IL-2 during ART treatment interruptions	No evidence of improved HIV-1-specific immunity	(Abrams *et al.,* 2009)
	IL-7 dose escalation trial in ART-treated patients	Increases in CD4+ T-cell numbers	(Pallikkuth *et al.,* 2013)
	IL-21 administered to SIV-infected Rhesus macaques	Increases in natural killer-cell and CD8+ T-cell function and Th17 cells and a reduction in bacterial translocation and immune activation	(Pallikkuth *et al.,* 2013; Lévy Y *et al., 2012*)
Drugs	Immuno modulatory drugs	Enhanced T-cell responses to dendritic cells electroporated with GAG or NEF mRNA	(De Keersmaecker *et al.,* 2012)
Correcting immune activation and dysfunction			
Drugs	Aspirin	Reductions in CD38, HLA-DR, and sCD14	(O'Brien M *et al.,* 2013)
	COX-2 inhibitor celecoxib	Celecoxib reduced the expression CD38 and inflammatory markers	(Pettersen FO *et al.,* 2011)
Antibodies	Anti-PD-1	Blocking PD-1 results in increased cytokine expression in HIV-1 patients	(Porichis *et al* .,2013)
	Anti-CCR5 Maraviroc	Reduction in CD38 HLA-DR-expressing CD4+ T cells and normalized CD8+ T-cell skewing	(Westrop *et al.,* 2012)
Probiotics	Symbiotic plus dietary fiber	No alteration in bacterial translocation	(Schunter M *et al.,* 2012)
	Prebiotic and probiotic	Reductions in bacterial DNA, CD4+ T-cell counts, and IL-6	(González-Hernández *et al* .,2012)

2.1.9.3.2. Cytokine-based therapies

IL-2

Although initial studies showed that intermittent infusions of IL-2 led to increased CD4+ T cell count, recent trials using daily IL-2 administration in combination with either single or dual nucleoside therapy or therapeutic vaccination showed no benefit. Peter *et al*. reported that despite significant increases in CD4+ T cell count in the IL-2 plus antiretroviral therapy (ART) arm, no clinical benefit was observed in two IL-2 clinical trials (Peter *et al*., 2014).

IFN-α

Clinical trials show that polyethylene glycol (PEG)-ylated IFN-α has anti-HIV activity. However, plasma viremia was not significantly different from baseline 6 weeks after stopping therapy, and no change was observed in CD4+ T cell numbers at the end of IFN-α treatment. Moreover, post-treatment lymphocyte proliferation in response to different antigens was not different from baseline (Bernard and Charles, 2010).

2.1.9.3.3. Blockade of inhibitory receptors

The discovery of inhibitory receptors, such as the programmed death receptor (PD)-1, and their role in T-cell dysfunction in the setting of chronic viral infections has led to *in vitro* studies blocking their regulatory ability. The *in vivo* blocking of PD-1 in an SIV macaque model showed an enhancement of virus-specific poly functional CD8+ T-cell responses that correlated with a decrease in plasma viremia. Although PD-1 blockade showed a novel way of boosting immune response to control the virus, the increase in the response was transient. By day 43 post-treatment, plasma viremia had returned to baseline levels. Breton et al. after observing an up regulation of the PD-1 ligands (PD-L1 and PD-L2) in monocyte-derived DCs following maturation attempted a specific knockdown of the ligands by electroporation with a single siRNA sequence during the monocyte and immature stages of DC development. These DC were when cultured with peripheral blood mononuclear cells and pools of Gag

peptides, the investigators noted a modest enhancing effect (Macatangay and Rinaldo, 2009; Kassu *et al.,* 2010).

2.1.9.3.4. Therapeutic vaccination

Unlike cytokine-based strategies and blockade of inhibitory receptors, therapeutic vaccination is geared towards a more HIV-specific boosting of the immune response. Several clinical trials have utilized the potent antigen-presenting capability of DCs that allows them to activate pathogen-specific T-cell responses. Studies were aimed at enhancing anti-HIV responses in infected subjects by loading autologous DCs with HIV-specific peptides, RNA, or autologous, inactivated virus (Bernard and Charles, 2010). Connolly et al. used autologous DCs loaded with HIV peptides in ART-treated subjects and showed a transient increase in the frequency of IFN-γ-producing T cells following vaccination (Connolly *et al.,* 2008). Lu et al. loaded DCs with autologous inactivated virus and showed decreased plasma HIV viremia (median of 80%) during the first 112 days following administration of the vaccine (Lu W *et al.,* 2004). The antiviral effect was shown to correlate with HIV-specific IL-2 and IFN-γ-producing CD4+ T cells.

Significant research has been conducted on the role of regulatory T cells (Tregs) in HIV infection. To date, however, it is not clear whether Tregs plays a detrimental role or a beneficial role in the pathogenesis of HIV infection. In fact, a number of immunotherapeutic strategies to control HIV infection have revealed a possible antagonistic role for Tregs. This necessitates investigating ways to counteract the suppressive functions, such as through Treg depletion or blockade of specific Treg immunosuppressive mechanisms, without further increasing the cellular immune activation associated with chronic HIV infection. Simply applying Treg immunotherapeutic strategies used in diseases other than HIV may pose problems due to the complexity of HIV immunopathogenesis. Studies are therefore necessary to elucidate the different immunoregulatory networks in HIV infection in order to determine

the specific cellular or molecular pathways that can be altered to boost the body's immune control of HIV (Bhawna, 2013; Yves, 2010).

2.1.9.4. Post-exposure prophylaxis

Currently the post-exposure prophylaxis against HIV infection is a combinations of drugs, collectively called highly active antiretroviral therapy (HAART) (May and Ingle, 2011).

This medication suppresses the replication of HIV, improving immunity and delaying mortality. HAART is delivered as part of a comprehensive care, which includes voluntary counseling and testing (VCT), the diagnosis and treatment of sexually transmitted infections (STIs), Tuberculosis (TB), other opportunistic infections (OI), and the prevention of mother to child transmission (PMTCT) as well as the treatment of pregnant women. Over the last 20 years, antiretroviral therapy has moved from almost ineffective mono-therapy to a combination of multidrug regimens that can virtually suppress viral replication in most HIV infected patients. As a consequence, the nature of HIV infection has been changed from a fatal disease into chronic illness requiring lifelong therapy (Hima and Naga, 2011; Lorenza *et al.*, 2009).

To date, an arsenal of 25 Food and Drug Administration (FDA) approved drugs are available for the treatment of HIV infection. These drugs are distributed into distinct six classes based on their molecular mechanisms and resistance profiles (Table 3).

Two of the classes, Nucleoside/nucleotide Reverse Transcriptase Inhibitors (NRTIs/NtRTIs) and Non-Nucleotide Reverse Transcriptase Inhibitors (NNRTIs), target the process of reverse transcription of genomic RNA into cDNA by the enzyme reverse transcriptase (Sarafianos *et al.*, 2009). The other four classes target the proteolytic cleavage of viral polyproteins by the enzyme protease into their respective functional proteins (Protease Inhibitors [PIs]), viral entry through virus–cell fusion (Fusion Inhibitor [FIs]) and interaction of the virus with its

(co-)receptors (Co-receptor Inhibitors [CRIs]), and integration of the proviral DNA into the host cell genome with the help of the enzyme integrase (Integrase Inhibitor [INIs]) (Sarafianos *et al*., 2009).

According to the International AIDS Society-USA Panel guideline which has been issued in 2010, the treatment should be initiated in certain conditions. It is given to all HIV cases with CD4 count below 200 cells/mm3, HIV viral load > 100,000 copies/mL, pregnant and breast feeding women, children less than 5 years old, chronic HBV, HCV or TB coinfection. In addition, all symptomatic patients regardless of CD4 count which are at risk of disease progression can be treated with HAART (Thompson *et al*., 2010). However, HAART is not able to eradicate the virus from the body completely because the HIV can be recovered and replicate from resting CD4+ T-lymphocytes (Hantano *et al*., 2010).

Beside the emergence of drug resistant strain, HAART can have a wide range of adverse effects on patients. The undesirable side effects from the long term use ends up in damages of the kidney, liver, and pancreas whereas the changes in fat metabolism lead to increased risk of strokes and heart attacks (Barbara *et al.,* 2014; Geeraert *et al.,* 2008; UK Collaborative Group on HIV Drug Resistance, 2005).

Table 3: Classes of antiretroviral drugs approved for therapeutic use

NRTIs	NNRTIs	PIs	Fusion inhibitors	Co-receptor Inhibitors	Integrase inhibitors
Dideoxycytidine (ddC)	Delaverdine (DLV)	Ritonavir (RTV)	Enfuvitride (ENF)	Maraviroc (MRV)	Raltegravir (RAL)
Zidovudine (AZT)	Nevirapine (NVP)	Nelfinavir (NFV)			
		Lopinavir (LPV/r)			
Lamivudine (3TC)	Etravirine (ETV)	Atazanavir (ATV)			
Tenofovir (TDF)	Efavirenz (EFV)	Saquinavir (SQV)			
Didanosine (ddI)		Darunavir (DRV)			
		Tipranavir (TPV)			
Stavudine (d4T)		Indinavir (IDV)			
Abacavir (ABC)		Amprenavir (APV)			
		Fosamprenavir (FPV)			

2.2. HIV and viral co-infection

Infection with two or more viruses is common worldwide and considered a major public health problem and recognized worldwide (Koziel and Peters, 2007). Among HIV patient's co-infection may have positive or negative impact.

Human herpes viruses are associated with chronic immune activation and inflammation in HIV patients. Some studies found association between herpes virus and the development of non-Hodgkin's lymphomas (NHL) and Kaposi Sarcoma (Carr and Tomanek, 2006; Strong *et al.,* 2013).

Co-infection with HIV and HBV or HCV is common due to shared routes of transmission, including sexual contact, mother to fetus, injection drug use, and blood (Mallet *et al.,* 2011; Lacombe and Rockstroh, 2012). Approximately 5–25% of HIV patients are either coinfected with HBV and/or HCV especially in Sub- Saharan African countries. Studies showed that the rates of HBV and HCV among people living with HIV were reported as 15% and 7%, respectively (Barth, *et al*., 2010; WHO, 2013). Recent study has found that about 2.3 million of HIV individuals are coinfected with HCV globally (Plattl *et al.,* 2016). These three viruses are the most common chronic viral infections and among the top ten causes of mortality in the world (WHO, 2005). Unfortunately, the majority of HIV patients have not been tested for hepatitis B or C, which will have a negative influence in the treatment. In Africa, including Ethiopia, the status of viral hepatitis among HIV patients is not well known (Soriano *et al*., 2010).

These hepatotropic viruses complicate the management of HIV patients by reducing the survival rates and increased risk of liver disease and fibrosis following the initiation of HAART (CDC, 2014). HCV infection is considered an important cause of non-AIDS deaths

among HIV patients especially in developed countries which accounts up to 45% (Rosenthal *et al*., 2003; Salmon-Ceron *et al*., 2005; Rockstroh and Spengler, 2004).

On the other hand another member of the hepatitis virus called GB Virus C (GBV-C) has appositive impact on HIV patients by slowing the disease progression, and better health outcomes in HIV-1 positive individuals. Its positive impact was first observed in 1998 when the GBV-C co infected HIV patients were found with lower viral load than mono-infected individuals (Toyoda *et al*., 1998). Later, two large independent studies with long follow up showed that GBV-C is also associated with longer survival rate and improved clinical conditions (Tillmann *et al*., 2001a; Xiang *et al*., 2001).

2.3. Hepatitis Viruses

For decades, hepatitis was a major significant public health problem afflicts millions of people. The first records were written by Hippocrates (400 BC). Although Greeks and Romans reported several cases of epidemic jaundice, but the description of the disease appears more likely to be malaria or leptospirosis (Zuckerman AJ, 1983; Oon GCJ, 2012).

Jaundice Outbreak was common and often occurred in crowded, unsanitary conditions and in armies especially during wars. However, first observation in the nature of the disease was made by the end of the 19th century as a result of the epidemics of jaundice that occurs in peoples after been vaccinated against smallpox prepared from human lymph (Lurman, 1885).

Absence of studies on animal models of infection delayed understanding the nature of the infection, and this led to study of the disease in human volunteers. Significant advances on hepatitis research were achieved by the studies of the epidemics which occurred during and after the Second World War. Following a series of observation studies in volunteers, scientists were able to classify viral hepatitis based on the clinical and epidemiological criteria into hepatitis A and hepatitis B (MacCallum, 1947; Anonymous, 1947).

In the late sixties, Deinhardt and his group reported the first successful serial transmission of a virus from a 34-year old surgeon G. Barker (GB) into tamarins, a type of New World monkey (*Saguinus labiatus*) that developed self-limiting hepatitis. The patient was suspected to be infected with hepatitis A, but further studies showed that neither hepatitis A nor hepatitis B were present and the transmissible agent was named (GB) based on the initial of the surgeon and was suggested to be latent virus of the marmosets with no any impact on humans, and it was ignored at that time (Deinhardt *et al*, 1967; Parks and Melnick, 1969; Deinhardt *et al*, 1975).

The discovery of Hepatitis B Surface antigen (the Australian antigen) and hepatitis A virus (HAV) and the development of sensitive serological test for their detection led to increase the evidence of the existence of other types of hepatitis (non- A, non-B hepatitis) responsible for post transfusion hepatitis (Blumberg *et al*, 1965; Bayer *et al*, 1968; Blumberg *et al*, 1969; Feinstone *et al*, 1973; Feinstone *et al*, 1975; Prince *et al*, 1974).

In 1977 Mario Rizzetto's lab in Italy reported the detection of a novel antigen like virus (Delta antigen) in liver biopsy of HBV patient and the studies showed that it is a defective RNA virus requiring the helper function of HBV for its replication. It was named hepatitis D virus (HDV) (Rizzetto *et al*, 1977). In the late 1980s and 1990s, two other viruses were discovered. Hepatitis C virus (HCV) the major cause of chronic and post transfusion hepatitis (Choo *et al*, 1989; Kuo *et al*, 1989) and hepatitis E virus (HEV) which was quickly established as the major cause of acute epidemic water-born hepatitis, especially in developed countries (Reyes *et al*, 1990; Dawson *et al*, 1992).

Despite the great advance in the molecular biology and serology assays, there were still cases of hepatitis with possible viral etiology which were undefined. About 10-20 % of acquired and transfusion acute non-A non-B hepatitis were not due to HCV or HEV. More than 30%

of chronic hepatitis and hepatitis associated aplastic anemia and cirrhosis was not related to (A-E) viral hepatitis (Alter & Bradley, 1995; Brown *et al,* 1997a).

If the above cases were caused by a single agent, it will probably be designated as hepatitis F. Although there were reports from Japan of cases caused by hepatitis F virus, but it was a mutant form of hepatitis B virus (Unchida *et al,* 1994). The non A-E were diagnosed only after excluding the known viruses, drugs, alcohol abuse and any condition that damage the liver.

In 1995, after evaluation in molecular technique, a group of researchers from Abbott Laboratories identified two GB agents in plasma of tamarins which had been inoculated with the GB agent. The two new viruses were classified within the flaviviruses due to similar genome organization with HCV and named GBV-A and GBV-B (Muerhoff *et al,* 1995; Simons *et al,* 1995a). A third GB like virus was isolated in sera of West African patient with acute hepatitis, and based on nucleotide sequence similarities with the two primate viruses, it was named GB virus C (Simons *et al.,* 1995b).

GBV-A and GBV-B were not identified in any human sera, although GBV-B was serially transmitted in tamarins and caused hepatitis, but GBV-A could not be readily transmitted to tamarins (Schaluder *et al,* 1995). Few months later researchers at Genelabs Technologies discovered a virus in a patient with HCV infection which they named hepatitis G virus (HGV) (Linnen *et al*., 1996).

Genome analysis showed that the two viruses shared 96% of the amino acid sequence and 86% of the nucleotide sequences and this suggests that they are different isolates of the same virus, and the term GBV-C is the commonly used name since they are not associated with any disease (Simons *et al.,* 1995a; Linnen *et al.,* 1996; Leary *et al.,* 1996).

Recently another GB agent was discovered in a serum of health bats in Bangladesh, tentatively named GB virus D (GBV-D). This virus is more related to GBV-A and GBV-C and represents a new member within the family Flaviviridae (Jonathan *et al.,* 2010).

2.3.1. Hepatitis B Virus

Hepatitis B virus is considered to be second to tobacco among the carcinogens (WHO, 2002; Vandamine and Herck, 2007). Humans are the only host, and the virus may remain infectious in the environment for several days (Gavilanes *et al.*, 1982).

The virus is small double-shelled virus in the family Hepadnaviridae with unusual features similar to retroviruses. HBV has a small circular DNA genome of about 3.2 kilo base (kb) pairs which is partially double-stranded (Vandamine and Herck, 2007; Glebe and Urban, 2007).

Based on sequence analysis, it is classified into eight genotypes, A to H with distinct geographic distribution. Three types of viral particles are visualized in infectious serum by electron microscopy. Two of the viral particles are smaller spherical structures with a diameter of 20 nm and filaments of variable lengths with a width of 22 nm as shown in figure 8. The spheres and filaments are composed of hepatitis B surface antigen (HBsAg) which is used in the diagnosis and lipids which is derived from the host cells (Gavilanes *et al.*, 1982). The infectious HBV virion which is also called Dane particle has a spherical, double-shelled structure 42 nm in diameter, consisting of a lipid envelope containing HBsAg which surrounds an inner nucleocapsid which contains hepatitis B core antigen (HBcAg), a DNA polymerase reverse transcriptase, the viral genome as well as cellular proteins. Hepatitis e antigen (HBeAg) is secreted from infected cells into blood and indicates transmissibility of the disease (Thomas *et al.,* 2007).

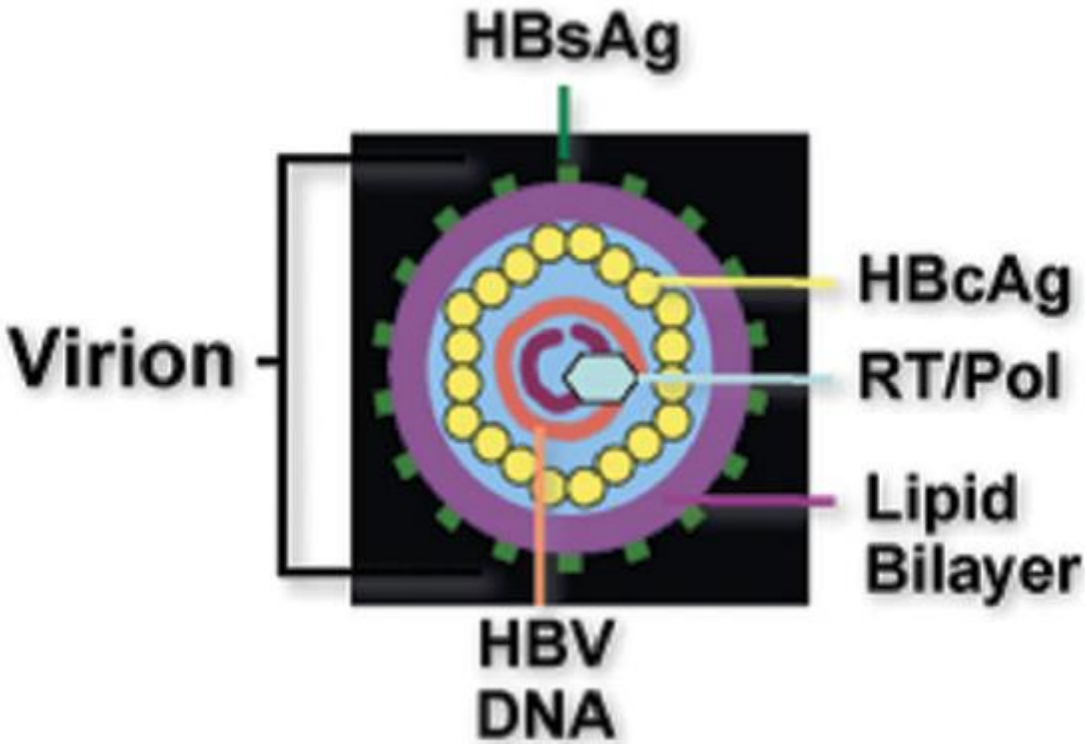

Figure 8: Structure of HBV (Adopted from T. Jake Liang, 2009).

HBV is transmitted through exposure to blood or body fluids. The virus is present in blood, semen, saliva, vaginal secretions, menstrual blood and as small amount in tears and breast milk of infected persons. The mode of transmission includes sexual intercourse, from mother to child, blood transfusions and the use of contaminated needles (Mahoney *et al.,* 2004).

At the initial evaluation, patients should be screened for HBsAg, hepatitis B core antibody (anti-Hbc), and anti-HBs. The first viral antigen detectable in the blood of infected patient is HBsAg, before the elevation of the transaminase levels, especially during the incubation period and during acute clinically apparent disease. If HBsAg is positive, then HBeAg, anti-HBe, and HBV DNA should be measured. The active chronic infection requires active HBV viral replication with presence of HBV DNA. Presence of HBsAg without detectable HBV DNA or HBeAg defines chronic carrier state (Table 4). These patients usually have anti-

HBeAg and normal liver parameters. Small amounts of HBV DNA might be detected as long as HBsAg antigens are present (Brunetto *et al.,* 2010).

Although the prophylaxis vaccine against HBV is available, however the disease is still considered a major public health problem. The World Health Organization estimates that 240 million people are chronically infected and approximately about 600,000 of them die every year due to the acute or chronic complications of the virus. Based on the prevalence of HBV markers the incidence of infection can be divided into areas of low, intermediate, and high as shown below in figure 9 (WHO, 2012). The prevalence of liver disease in Ethiopia and other Sub-Saharan Africa is high and accounts for 12% of the hospital admissions and 31% of the mortality in hospitals (Tsega, 2000).

A sero-epidemiological study of the prevalence of hepatitis B markers prevalence conducted in Ethiopian males from all regions of the country shows that the rates of HBsAg were 10.8% (Abebe, 2003).

Hepatitis B is a non cytopathic virus, but the inflammations and injury of the liver are the result of the host immune response against the HBV-infected hepatocytes. The infection can be either acute or chronic, and may range from asymptomatic infection or mild to severe disease and rarely fulminant hepatitis. Among immunocompetent adults the infection is acute and usually self-limited with transient liver disease and subsequent viral clearance which is achieved in more than 95% of adults with a case fatality rate of 0.5–1%, whereas more than 90% of neonates exposed to HBV at birth become persistently infected, because the age plays a major role in the chronic state of the infection.

Table 4: Hepatitis B serological markers (Adapted from Morbidity and Mortality Weekly Report, 2005).

Tests	Results	Interpretation
HBsAg	Negative	Susceptible
anti-HBc	Negative	
anti-HBs	Negative	
HBsAg	Negative	Immune due to natural infection
anti-HBc	Positive	
anti-HBs	Positive	
HBsAg	Negative	Immune due to hepatitis B vaccination
anti-HBc	Negative	
anti-HBs	Positive	
HBsAg	Positive	Acutely infected
anti-HBc	Positive	
IgM anti-HBc	Positive	
anti-HBs	Negative	
HBsAg	Positive	Chronically infected
anti-HBc	Positive	
IgM anti-HBc	Negative	
anti-HBs	Negative	
HBsAg	Negative	Interpretation unclear; four possibilities:
anti-HBc	Positive	1.Resolved infection (most common)
anti-HBs	Negative	2. False-positive anti-HBc, thus susceptible
		3. “Low level” chronic infection
		4. Resolving acute infection

Chronic hepatitis B infection encompasses a spectrum of disease, and is defined as persistent HBV infection with the presence of HBsAg in the blood or serum for longer than six months, with or without associated active viral replication and evidence of hepatocellular injury and inflammation (Chisari and Ferrari, 1995; Ganem and Prince, 2004; Guidotti and Chisari, 2006).

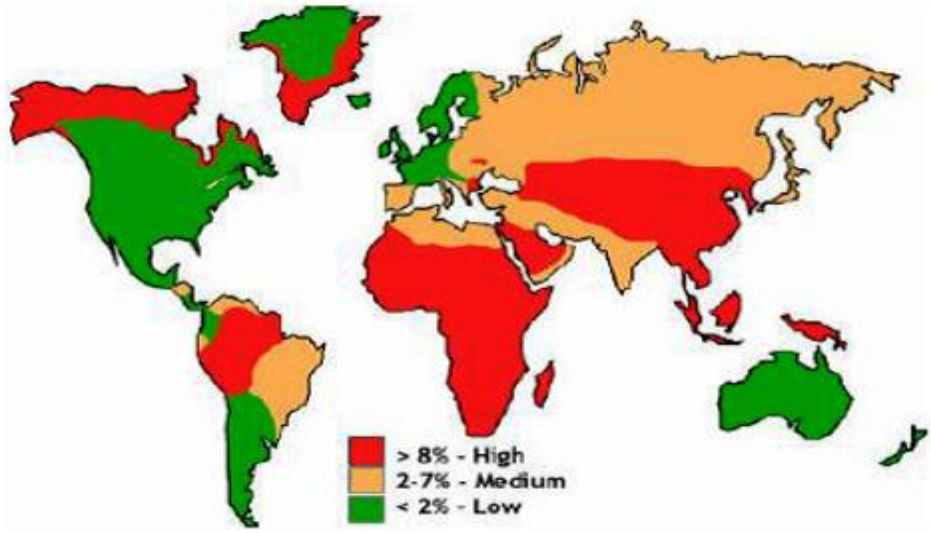

Figure 9: Geographical distribution of hepatitis B infection (Adopted from Ott *et al.*, 2012)

The natural history and spectrum of the disease are diverse. In some people, the virus is inactive and does not lead to significant liver disease. While in others, it may cause progressive liver fibrosis, leading to cirrhosis with end-stage liver disease, and a markedly increased risk of hepatocellular carcinoma (HCC), independent of the presence of cirrhosis generally years after initial infection (Lok and McMahon, 2007).

Currently, seven drugs have been approved for the treatment of chronic hepatitis B infection, which include two formulations of alpha-interferon (standard and pegylated) that enhance the antiviral immune response of the host and five nucleoside or nucleotide analogs (lamivudine, adefovir, entecavir, telbivudine, and tenofovir) that inhibit HBV DNA polymerase (Zulim *et al.,* 2009). At present, the preferred first-line treatment choices are pegylated-interferon alpha-2a (pegIFN-α), tenofovir and entecavir because of their great antiviral efficacy (Gish *et al.,* 2012).

2.3.2. Hepatitis C Virus

Hepatitis C or the silent killer is a blood-borne virus which was previously referred to as non-A/non-B hepatitis. It is a small enveloped virus with a ~ 9.6 kb positive sense single-stranded RNA, a member of the Flaviviridae family within the genus *Hepacivirus* (Choo *et al*., 1989).

HCV genome encodes a single poly protein which is processed and translated into three structural and seven non-structural proteins. The viral core protein forms the capsid, which is surrounded by a lipid bilayer containing the envelope glycoproteins, E1 and E2 on the external surface which initiate the infection in the host cells. While the nonstructural proteins direct the intracellular processes of the virus life cycle (Tanji *et al*., 1994; Grakoui *et al*., 1993).

HCV has been classified into six distinct genotype and several subtypes which differ from each other by 31–33% at the nucleotide level. Genotype 1, 2, and 3 appears to have a worldwide distribution and their relative prevalence varies from one geographic area to another as shown in figure 10 (Pawlotsky, 2004). HCV genotype 4 appears to be prevalent in North Africa and the Middle East, and genotype 5 and 6 seem to be confined to South Africa and Hong Kong respectively (Alavian, 2009; Simmonds, 2001).

HCV is transmitted by multiple routes: direct blood-to-blood contact, injected drugs use and unsafe transfusions and therapeutic injections. It can be transmitted sexually and at a less degree from mother to her child which accounts for 6%. Healthcare workers are at risk for HCV infection because of the needle stick accidents and unavoidable situations that may result in direct contact with blood from an infected individual (Hughes and Mahy, 1998).

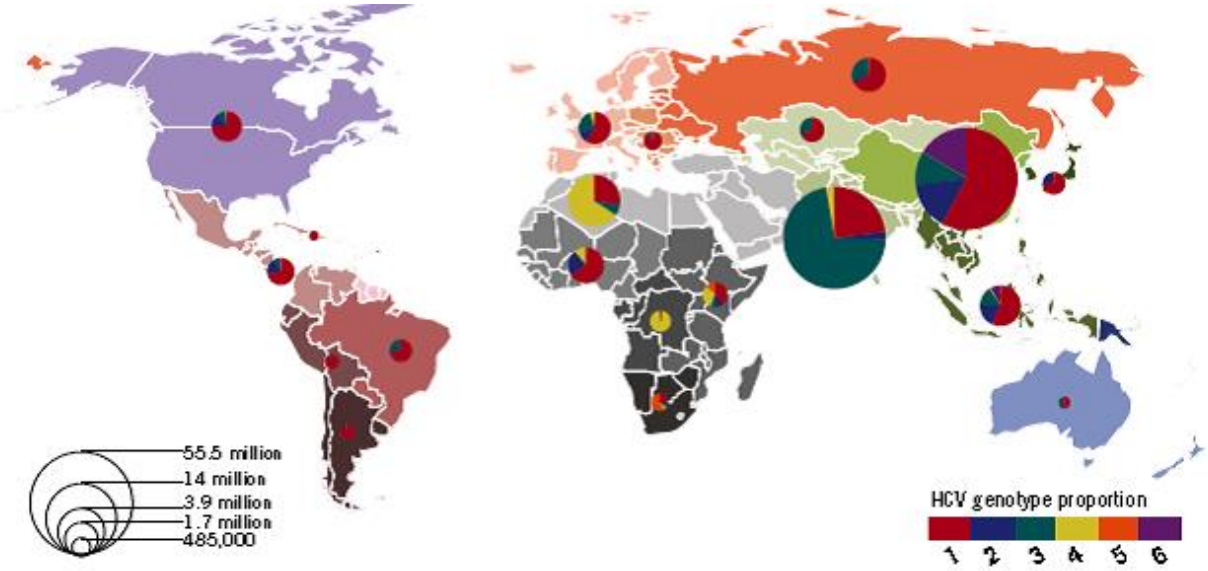

Figure 10: Distribution of HCV genotypes (Adopted from Messina *et al.*, 2015)

Epidemiological studies show that death due to HCV is increasing. In 1990 there were 333,000 deaths compared to 704,000 in 2013 deaths due to HCV-related diseases. HCV is four to five times more prevalent than HIV globally (Lozano *et al.*, 2012). In 2013 it was estimated that there were 185 million having anti-HCV antibody which indicates history of infection. Among those about 130–150 million are HCVRNA positive which indicate chronic infection (Mohd *et al.*, 2012). A more recent systematic review which excluded previous studies, estimated that 110 million persons are HCV-antibody positive and 80 million have chronic infection (Gower *et al.*, 2014). The highest prevalence of HCV is in Sub-Saharan Africa which accounts for (5.3%). Only Limited data is available in Ethiopia. The overall seroprevalence of HCV in 1,580 Ethiopian individuals representing urban and rural populations was reported to be 2.0% (Madhava *et al.*, 2002; Frommel *et al.*, 1993).

HCV infects only chimpanzees and humans and may cause acute or chronic infections. Patients infected with HCV have an 80 to 85% chance that the HCV infection will persist and that they will probably develop a chronic HCV infection. The chronic infection leads to progressive liver disease associated with inflammation and fibrosis and in some patients may

progress to cirrhosis. End liver stage or cirrhosis is associated with multiple complications including bleeding, ascites, encephalopathy, and hepatocellular carcinoma, which is the common cause of mortality and morbidity in HCV infected patients. The majority of HCV infections are asymptomatic and probably, undiagnosed. In acute infections, the immune system clears the virus without any treatment within six months of the infection and is rarely life-threatening (WHO, 2016; Sultan *et al.*, 2009).

Currently there is no vaccine against HCV because of the high mutability of the virus genome. The therapy of HCV is generally supportive, consisting of bed rest and balanced diet with small frequent nitrous meals. The goal of treatment is to achieve a sustained virological response (SVR), as defined by the absence of HCV RNA 12 weeks (SVR12) or 24 weeks (SVR24) after treatment (Arase *et al.*, 2013; Van der *et al.*, 2012).

2.3.3. GB Virus C

Based on sequence and genome organization, the GBV-C is classified together with HCV as a member in the Flaviviridae family, which has three known genera: *Flavivirus*, *Pestivirus* and *Hepacivirus*. Since the discovery of GBV-C it was not assigned to any of the known genera as shown in figure 11, but recently it was proposed to be classified as GBV-C together with GBV-A and GBV-D in a new fourth genera called *pegivivirus* based on phylogenetic relationships and pathogenic features (Stapleton JT *et al.*, 2011; Reshetnyak *et al.*, 2008).

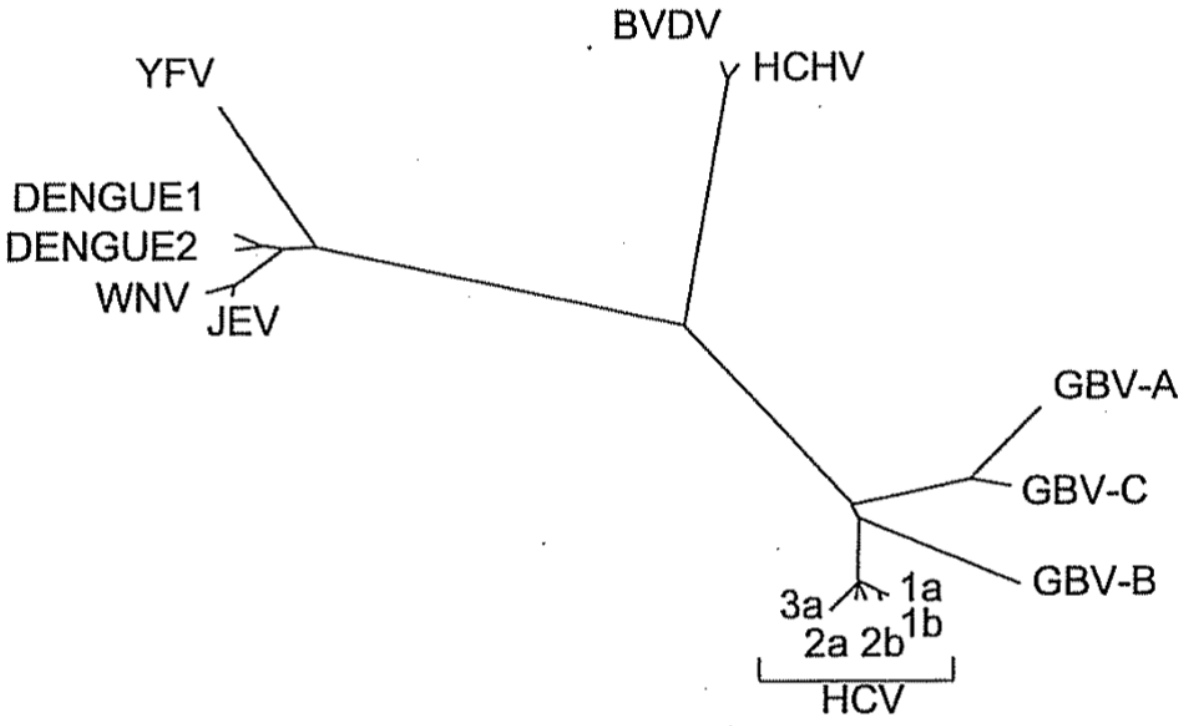

Figure 11: Phylogenetic relationship of the family Flaviviridae: HCV genotypes 1a, 1b, 2a, and 3a2,West Nile virus (WNV), yellow fever virus (YFV), Japanese encephalitis virus (JEV), dengue virus types 1 and 2, hog cholera virus (HCHV) and bovine viral diarrhea virus (BVDV), (Adopted from Simons *et al.*, 2000).

This virus is an enveloped single stranded RNA with positive polarity. The genome of the virus is represented by 9.4 Kb and approximately 9500 nt, organized similar to HCV. The different isolated viral genome of GBV-C have between 9103 and 9395 nucleotides and contains one open reading frame (ORF) that encodes a large polyprotein precursor of approximately 3`000 amino acids (Table 5). The open reading frame is located between untranslated regions (UTR) at the 5' and 3' ends of the viral genome (Mohr and Stapleton, 2009).

The 5' UTR of GBV-C is conserved even higher than HCV, it contains the internal ribosomal entry site (IRES), which is responsible for the RNA translation.

Table 5: Comparison between GBV-C and other members of the Flaviviridae family (adapted from Linnen *et al.*, 1996 and Simmons *et al.*, 1996.)

	GBV-C	HCV	GBV-A	GBV-B
Genome	9103-9395	9401	9653	9143
3' NCR (nt)	313-315	72	198	83
5' NCR (nt)	281-551	341	594	445
Host	Humans	Humans	Tamarins	Tamarins
Core	Absent	Present	Absent	Present

The activity of GBV-C IRES is lower than HCV, and it is essential for translation of the viral genome and allows the direct connection of the 40s subunit of the ribosome (Muerhoff *et al.*, 2006).

The polyprotein is cleaved by a combination of cellular and viral proteases to form two structural and five nonstructural proteins. The structural proteins of GBV-C are encoded in the N-terminal region of the ORF. The structural proteins is a capsid derived whose coding regions has not yet been completely identified, two envelope glycoproteins, E1 and E2 forming a heterodimer and are located on the surface of the viral particle, as shown in figure 12 (Mohr and Stapleton, 2009).

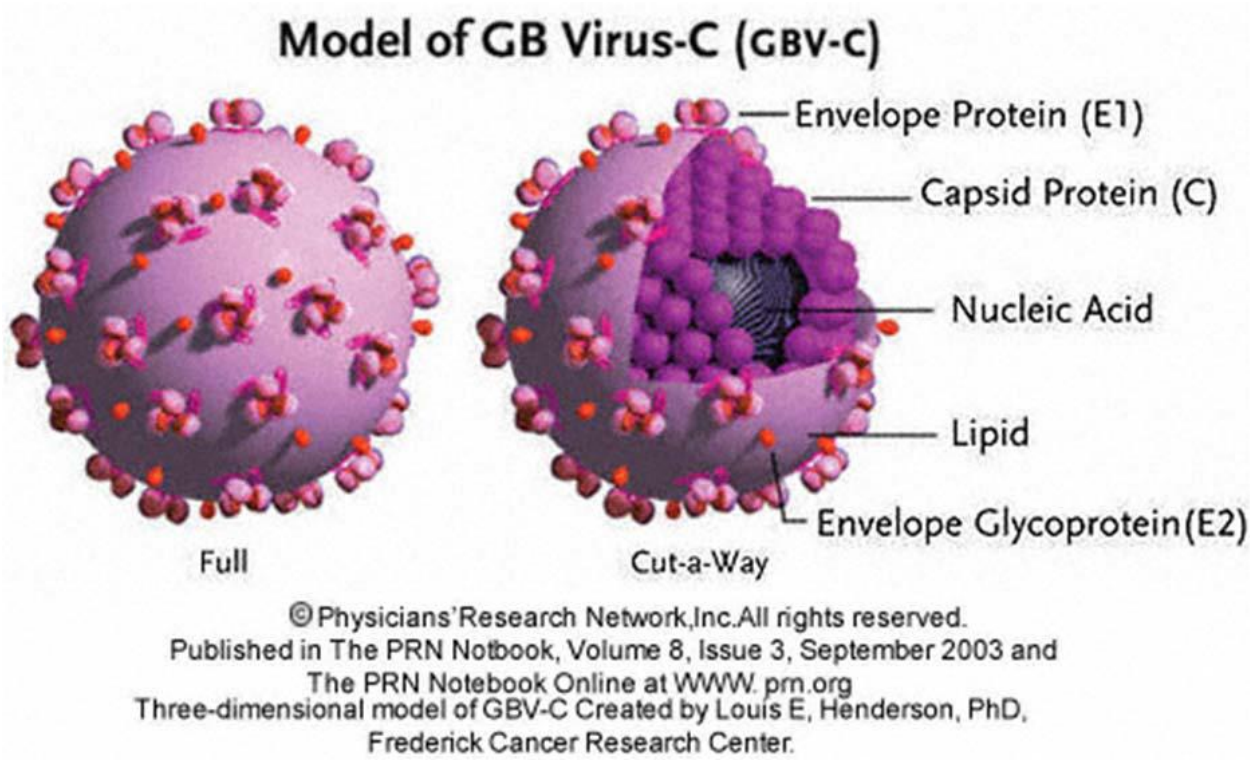

Figure 12: Model of GB Virus-C (GBV-C)

The nonstructural proteins of GBV-C are encoded in the C-terminal portion of the ORF as shown in figure 13, the nonstructural protein are NS2 , NS3 , NS4a, NS4B, NS5a and NS5b, with molecular weights of 20, 70, 28, 55, and 57 kDa, respectively. These proteins perform various functions and activity such as protease, helicase, and RNA-dependent RNA-polymerase (Kim & Fry, 1997).

After cleavage by the cellular peptidases, NS2 is first protease activated. Its C-terminal portion contains a protease domain, which together with the N-terminal domain of NS3 catalyzes the cleavage between NS2 and NS3 **(**Kim and Fry, 1997).

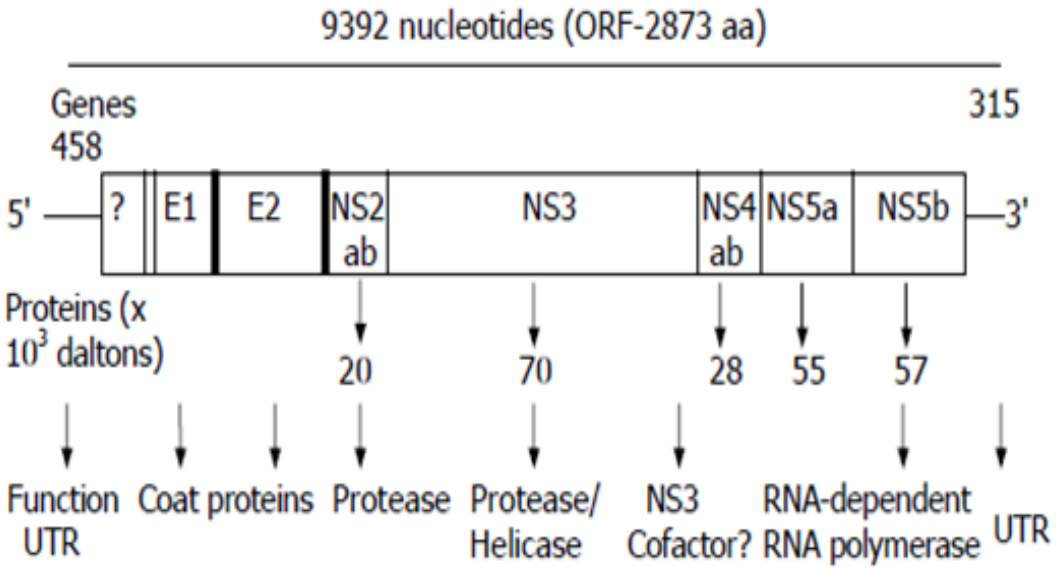

Figure 13: The Genome of GBV-C (Adopted from Kim and Fry, 1997).

It is believed that in the GBV-C the NS2 functions, such as: inhibition of apoptosis, modulation of gene expression and phosphorylation of NS5A. NS3 is versatile and has serine protease activity, RNA helicase and nucleotidase (NTPase). Its helicase domain related to the initiation of RNA synthesis by dissociation of the embryonic RNA. NS4A is a cofactor of the serine protease and is responsible for NS3/NS4a cleavage of all subsequent proteins. The NS5a and NS4B are poorly characterized, but it is known that NS4B protein recruits other components of the replicase to the site of viral replication and that NS5a interacts with the innate immune system. The NS5b is the RNA -dependent RNA polymerase, which is essential for RNA replication machinery (Leary *et al*., 1996).

2.3.3.1. Genotype and cell tropism

Using samples from around the world, the phylogenetic analysis of the genomic sequences of GBV-C revealed the existence of seven genotypes and several subtypes based on the differences in (5′UTR). These genotypes are directly related to the geographic region of origin as shown in figure 14. Genotype 1 predominates mainly in West Africa, genotype 2 in

Europe and USA, genotype 3 in Asia, genotype 4 in the Southeast Asian, genotype 5 in South Africa, genotype 6 in Indonesia and recently genotype 7 was isolated in China (Feng *et al.*, 2011; Sathar *et al*., 2001; Muerhoff *et al.,* 2006).

Figure 14: Genotypic distribution of GBV-C viremia (adapted from Mohr and Stapleton, 2009).

Within a given genotype, additional diversity exists. For example, intra genotype genetic distances may range from 13 to 19%, and multiple subtypes within a genotype have been reported. Moreover, mixed infections and recombinant viruses have been detected. Within an individual, distinct variants of GBV-C have also been identified implying that viral adaptation may occur within individuals as well (Ruiz V *et al*., 2010).

Interestingly, interferon sensitivity and cell tropism may differ among such variants (Schwarze-Zander *et al.*, 2012). Similarly, clinical isolates of GBV-C also vary in their ability to replicate in culture, suggesting that genotypic diversity may impact the virus phenotype. While the biological consequences of recombination and intra patient diversity to GBV-C pathogenesis have not been examined. Studies of HIV suggest that viral diversity

may result in altered cell tropism, virulence and/or drug susceptibility (Schwarze-Zander *et al*., 2012).

GBV-C is produced by T and B-lymphocytes removed from infected patients, however, the cellular receptor for GBV-C is unknown. Nattermann et al. suggested that the envelope protein E2 of GBV-C interacts with the cells by CD81 (Nattermann *et al.,* 2003), however, following studies failed to prove this theory (George *et al.,* 2006; Kaufman *et al.,* 2007).

The primary site of GBV-C replication is not completely understood. Some studies have found the virus RNA in liver samples, while other studies found the viral load of GBV-C in blood compared to liver was higher. Furthermore, the level of hepatotropic viruses is usually higher in the liver than blood circulation, and this suggests that the liver is not the replication site of GBV-C (Mohr and Stapleton, 2009).

Moreover the negative strand of GBV-C was found in the spleen and the bone marrow, and it has never been found in the peripheral blood lymphocytes in some studies, which indicates an active cellular viral transcription, and this indicates that the virus replicate in the hematopoietic site (Mohr and Stapleton, 2009; Rong and Perelson, 2009).

2.3.3.2. Prevalence and transmission of GBV-C

GBV-C is mainly transmitted through parental exposure to blood or blood products, sexual contact, and less frequently through mother-to-child transmission. The parental route is the most important. The first verification of this fact was the experiments dealing with inoculation of primates with the blood of the surgeon who fell ill in 1966 (Linnen J *et al.,* 1996).

GBV-C infection occurs worldwide and it is common, with a rate of approximately 1-3 in US blood donors, and much higher in HIV, chronic HCV and HBV 20-40%, 10-25%, 10%

respectively and this is because they share the same mode of transmission (Mohr and Stapleton, 2009; Bhattarai and Stapleton, 2012).

Prevalence studies suggest that 1–4% of healthy blood donors in most developed countries are viremic at the time of blood donation, and another 5– 13% have anti-E2 antibodies, indicating prior infection (Monica *et al.*, 2011; Hossein *et al*., 2012). In developing countries, blood donors have higher viremia prevalence, approaching 20% in some regions of the world (reviewed by Mohr and Stapleton, 2009; Polgreen *et al*., 2003). GBV-C is more prevalent among people with blood borne or sexually transmitted infections (Yue Feng *et al*., 2014; Davoe *et al*., 2013). In one study of HIV infected homosexual men in USA, 39.6% viremia and 46% E2 protein (anti-E2) antibody against GBV-C were detected (Hossein *et al*., 2012; Nunnari *et al.*, 2003).

2.3.3.3. Diagnosis and detection of GBV-C

Currently, unlike HCV there is no serological markers indicative of ongoing or degree of GBV-C infection. The detection of the viral RNA indicates active infection; the immunecompetent individuals usually clear the virus within the first years after infection. The clearance of the viral RNA is usually associated with the development of the antibodies which are produced against the viral envelope glycoproteins (anti-E2) protein, so it's uncommon to detect both of them at the same sample (Tanaka *et al.*, 1998a).

Antibodies against GBV-C E2 glycoprotein could be detected by using ELISA and Western Blot techniques. Unfortunately, these methods are not useful because only 25% of individuals infected with GBV-C develop antibody which makes the interpretation of the result difficult (Bourlet *et al.*, 1999; Pilot-Matias *et al.*, 1996).

The only diagnostic tool available to detect GBV-C RNA in human serum, plasma, or tissues is by reverse-transcription polymerase chain reaction (RT-PCR). The first step is the

synthesis of cDNA by using random hexameres with reverse transcriptase, to generate relatively large number of DNA sequences to which GBV-C specific primers would anneal. Then by using thermo stable DNA polymerase the cDNA will be amplified. Because some of the genomic regions were not well conserved among various GBV-C isolates, degenerate primers containing bases with more than one nucleotide at a certain position were used. A thermal cycling strategy named "touchdown" PCR was used to increase the specificity of the annealing between mismatched nucleotide sequences. The annealing temperature is incrementally decreased with each PCR cycle, preferentially allowing the best matched primer template to form (Leary *et al.,* 1996).

There are also assays that use primers targeting the NS5B region of the GBV-C genome, and the PCR product is then detected by semi-quantitative enzyme-linked test (Enzyrnun-Test) or dot blot hybridization (Genelabs Technologies INC, USA and Boehringer Mannheim, Germany). Another strategy amplifies two regions such as NS5A and 5' NCR from the same sample to avoid false negative results (Schlueter *et al.,* 1996).

The first RT-PCR generation assays used primers from the NS5 and NS3 regions, which results in a number of false negative results due to high mutation rate in these regions (Simons *et al,* 1995b; Linnen *et al,* 1996a). Later on two different target sites were used, NS5A because it's highly conserved regions (Linnen *et al.,* 1996a) or 5' UTR since it appears to be more sensitive than the previous ones (Schlueter *et al.,* 1996; Kao *et al.,* 1997a).

2.3.3.4. Pathogenicity & Replication

GBV-C is lymphotropic virus and the initial replication occurs in mononuclear cell of the peripheral blood as CD4+, CD8+, bone marrow and spleen (Li C *et al.,* 2006). The replication site of the virus protein has not been clarified, but probably it occurs in the cytoplasm like the other positive sense single strand RNA viruses, which synthesis an RNA

with negative polarity, which serve as a template for the production of the positive strands (Luisa *et al*., 2013).

In healthy immunocompetent individuals the RNA level of GBV-C can be up to 10^7 copies/ml, while among immunosuppressed or HIV patients the virus load increases the transmissibility of the virus (Ohto *et al.,* 2000). The outcome of GBV-C infection can lead to persistent viremia for years, and this occurs in about 25-50% of healthy individuals (Alter, 1997b), or clearance of the infection and develop anti-E2 antibody (anti-E2), and this occurs in 33-58% of individuals which usually gain the virus through blood transfusion (Alter, 1997b; Xiang *et al.,* 1998). Clearance of the virus requires cellular and humoral immune response (George and Varmaz, 2005), usually immunocompetent individuals clear the infection within two years (Tanaka *et al.,* 1998a; Alter, 1997a).

2.3.3.5. Biology of GBV-C

There are contradictory results regarding the impact of interferon (IFN) therapy on GBV-C. Several studies have found the effect of INF is similar to HCV (Jarvis *et al.,* 1999; Tanka *et al.,* 1996; Orito *et al.,* 1997; Berg *et al.,* 1996b), while others found no association between GBV-C and IFN (Umlauft *et al*., 1997; McHutchison *et al.,* 1997; Nagayama *et al.,* 1997).

The GBV-C RNA decrease to undetectable level in most patients under Alfa-IFN therapy (Martinot *et al.,* 1997; Jarvis *et al.,* 1999; Nagayama *et al*, 1997; Saiz *et al,* 1997), but the viral RNA returned after discontinuing the therapy. Several factors play a role on the efficacy of the treatment among HCV patients such like the viral load, genotype, IFN dose and the minor genetic differences in the viral proteins (Shiratori *et al.,* 1997).

Among other RNA viruses the genetic differences between genotypes reflective biological consequences regardless of the similarity in the genome structures and replication strategies. For instance, it is postulated that the mechanism of the disease vary among HCV genotypes,

(Adinolfi *et al.*, 2001), furthermore, genotypic variation in HIV can influence clinical variables such as drug resistance profiles, diagnosis, perinatal transmission rates, viral promoter activity, disease prognosis, and level of the viral load, as well as partially predict chemokine co-receptor use, and disease free survival times (Essex, 1999; Kanki *et al.*, 1999).

Unfortunately, only few studies had focused on the impact of the genotypes and subtypes differences of GBV-C pathogenic potential and its influence on disease progression among HIV patients (Xiang *et al.*, 2000).

George et al. noted the ability to persist in peripheral blood mononuclear cell, (PBMC) cultures is differing among variants GBV-C isolates and this due to variations in regulatory regions of the virus genome (George *et al.*, 2003).

Therefore, the genetic diversity between GBV-C genotype and subtypes has a critical role on the GBV-C behavior, and this suggests that these differences in the virus within populations may produce different impact in the progression of HIV and response to the treatment (Berzsenyi *et al.*, 2005; Muerhoff *et al.*, 2003; Kaye *et al.*, 2005).

Muerhoff et al. studied the influence of GBV-C genotypes in HIV coinfection and has found that the number of CD4+ tended to be lower in individuals infected with genotype 2a compared with those with genotype 2b (Muerhoff *et al.*, 2003). However, there are no other genotypes in the study for further comparison. Based on two other large scale studies it was found that GBV-C genotype 2 was associated with higher CD4+ cell counts compared with GBV-C genotype 1 (Schwarze-Zander *et al.*, 2006; Alcalde *et al*., 2010).

For that reason, it is likely that GBV-C genotype could at least partially account for the variable influence of GBV-C infection on HIV disease progression. Earlier analyses of GBV-C isolates of different regions have shown a different evolutionary pattern for these viruses

(Erker *et al.,* 1996; Muerhoff *et al.,* 1996; Pickering *et al.,* 1997; Gimenez-Barcons *et al.,* 1998).

Xiang et al. found that different genotypes have different effects on HIV development. The non-structural proteins NS5A phosphoprotein of GBV-C genotypes 1, 2, 3, and 5 only were able to inhibit HIV replication through inhibiting the co-receptor CXCR4 by increasing its ligand SDF-1 (Xiang *et al.,* 2007).

2.3.3.6. Interactions between GBV-C and hepatitis viruses

The impact of GBV-C in liver disease is controversy. Primarily, GBV-C was thought to be a possible cause of a transmissible hepatitis because it was isolated from patients with liver diseases (Table 6). However, several studies among different groups at high risk for liver disease, when controlled for transmission exposure risks, show that there was no association between GBV-C and hepatitis or any other disease found (Stapleton, 2003; Arie *et al.,* 2009l Halasz *et al*., 2001). Co-infection of GBV-C with one or more hepatotropic viruses like HBV or HCV may have negative or positive effect on the disease evolution (Chams *et al.,* 2003). Berzsenyi et al. have found that GBV-C exerted a beneficial effect on patients with HCV-related liver cirrhosis with lower compensated and decompensated cirrhosis, and longer cirrhosis-free survival in subjects with HIV/HCV coinfection (Berzsenyi *et al.,* 2007). Furthermore, recent studies have found that GBV-C viremia is associated with reduction in the level of ALT and AST in patients with chronic HCV and HIV coinfection (Yue Feng *et al.,* 2014).

Table 6: Detection rate of GBV-C RNA in liver disease

Clinical Group	Country	RNA^+ No. (%)	Author
Acute/Chronic HBV	Europe	72(9.7)	(Linnen *et al*. 1996)
	Japan	83(4)	(Sugai *et al*. 1997)
	US	100(32)	(Alter *et al.* 1997c)
	South Africa	106(26.4)	(Mphahlele *et al.* 1998)
Acute/Chronic HCV	China	24 (33.33%)	(Yue *et al.* 2014)
	Japan	88(8)	(Sugai *et al*. 1997)
	US	116(20)	(Alter *et al.* 1997a)
	South Africa	82(30.5)	(Mphahlele *et al*. 1998)
Chronic Liver Disease	South Africa	92(12)	(Sathar *et al.* 1999b)
	US	326(12.2)	(Linnen *et al.* 1996a)
Hepatocellular Carcinoma	Europe	57(7)	(Brechot *et al*. 1998)
	South Africa	135(14)	(Lightfoot *et al.* 1997)
Fulminant hepatitis	Japan	6(50)	(Yoshiba *et al*. 1995)
	US	36(38.8)	(Munoz *et al.* 1999)

2.3.3.7. GBV-C and HIV co-infection

The initial attention in GBV-C declined, because there was no association between the virus and any human disease. In 1998 a group of scientists reported that GBV-C decrease the mortality and the viral load among HIV co-infected patients (Toyoda *et al.*, 1998, Heringlake *et al*., 1998). Later on, several studies confirmed the association between GBV-C infection and the favorable prognosis for HIV-infected patients and slowing the disease progression (Yeo *et al.,* 2000; Xiang *et al.,* 2001; Tillmann *et al.,* 2001a; Nunnari *et al.,* 2003; Williams *et al.,* 2004; Zhang *et al.,* 2006). Lefrere et al. observed that only 39% of patients with active infection of GBV-C required starting HAART, whereas 68% of HIV mono infection needs antiretroviral therapy (Lefrere *et al.,* 1999).

Although few studies failed to confirm the positive impact of GBV-C on HIV co-infected patients, because it were either performed during the early stages of the HIV infection or after wide spread use of the therapy (Birk *et al.,* 2002; Bjorkman *et al.,* 2004; Kaye *et al.,* 2005; Ryt-Hansen *et al.,* 2006).

However, clearance of GBV-C RNA accelerated the course of HIV, worsen the disease prognosis and increase mortality than those with no evidence of past or present infection with GBV-C. Williams et al. evaluate data from multicenter AIDS cohort study and found that the rate of mortality is 2.8 times more in patients who lacked persistent GBV-C viremia compared to GBV-C negative individuals (Williams *et al*., 2005). Other longitudinal study performed on HIV homosexual individuals for eight years, showed that the survival rate is better among patients remained consistently GBV-C negative in comparison to patients that lose the GBV-C viremia during the study period. The authors hypothesized that the loss of GBV-C RNA is a result of insufficient number of CD4+ (Van der *et al.,* 2005).

However, the abundance of studies examine the relationship between GBV-C, HIV and HAART, have found that the coinfection is associated with increase in CD4+ cell counts, better survival, and less treatment failure than GBV-C negative individuals (Rodriguez *et al.*, 2003; Antonucci *et al.*, 2005; Souza *et al.*, 2006; Farnaz *et al.*, 2012; Ernst *et al.*, 2014). Furthermore, GBV-C viremia was found to reduce HIV transmission from mothers to children (Supapol *et al.*, 2008; Palomba *et al.*, 1999; Sather *et al.*, 2004; Bhanich *et al.*, 2009).

2.4. Interactions between GBV-C and HIV

There is strong evidence and several hypotheses which try to explain the mechanisms by which GBV-C enhances the course of HIV infections, although these mechanisms are not completely understood [reviewed by (Luisa *et al*., 2013)] as summarized in figure 15.

2.4.1. Direct inhibition of HIV replication by GBV-C proteins

Xiang J, and his colleges in 2001, hypothesized that GBV-C inhibit HIV replication by direct action since the two virus replicate in CD4+, CD8+ and B lymphocytes. This inhibition could be by affecting the initial stages of HIV cycles due to competition between the two viruses (Xiang J *et al*, 2001). It has been proposed also that the GBV-C E2 protein modifies HIV disease progression by inhibition of the entry of HIV into CD4+ cells by targeting the gp41of HIV and blocks its entry in vitro. However, this may suggest the possibility of structure similarity between HIV particles and GBV-C E2 proteins. Broadly neutralizing Abs to HIV is well described, but still identification of antigen able to elicit this Abs has proven difficult. Antibodies induced against GBV-C E2 protein were able to neutralize HIV particles and enveloped pseudo virus particles (Jung *et al.*, 2005; McLinden *et al.*, 2006; Emma *et al.*, 2010; Eissmann *et al.*, 2013). Furthermore vitro studies have found that the NS5A phosphoprotein of GBV-C also inhibit HIV replication during the entry step. This phosphoprotein is able to down regulates the expression of CXCR4 and release the CXCR4

ligand (SDF-1) in CD4+T cells, and reduce the CD4+ expression by decreasing the steady state CD4 mRNA levels (Xiang *et al.,* 2008; Xiang *et al.,* 2006). These data indicates that a heterologous viral protein can induce HIV neutralizing Abs.

2.4.2. Induction of a cytokine profile favoring HIV infection

Other hypotheses trying to explain the way in which GBV-C minimizes the progression of HIV is by stimulating the production of IL-2, IL-12 and IFN gamma by mononuclear cells. This will lead to increasing the response Th1, demonstrating that progression of HIV disease is associated with a shift from Th1 to Th2 cytokine profile, which can be reversed after initiating HAART, compared with HIV patients without GBV-C infection in whom Th1 cytokine levels decreased and shifted towards Th2 cytokines (IL-4 and -10) over time. GBV-C infection results in increased cell number and activation levels of circulating CD80+ pDCs in HIV patients, which are a major source of Th1 cytokines. It has been shown that pDCs are important in controlling HIV replication and high levels of HIV viral load are associated with pDC cell death via apoptosis and necrosis. By preserving and boosting the innate antiviral response to infection with HIV, GBV-C may stabilize the antiviral response to HIV (Nunnari G *et al.,* 2003; Capobianchi MR *et al.,* 2006; Lalle *et al.,* 2008).

Progression of HIV disease is linked to the depletion of T-helper1 cytokines, which usually restored after initiating antiretroviral therapy. The two subset of T-helper play a vital role in response to HIV infection (Siegal, 2003).

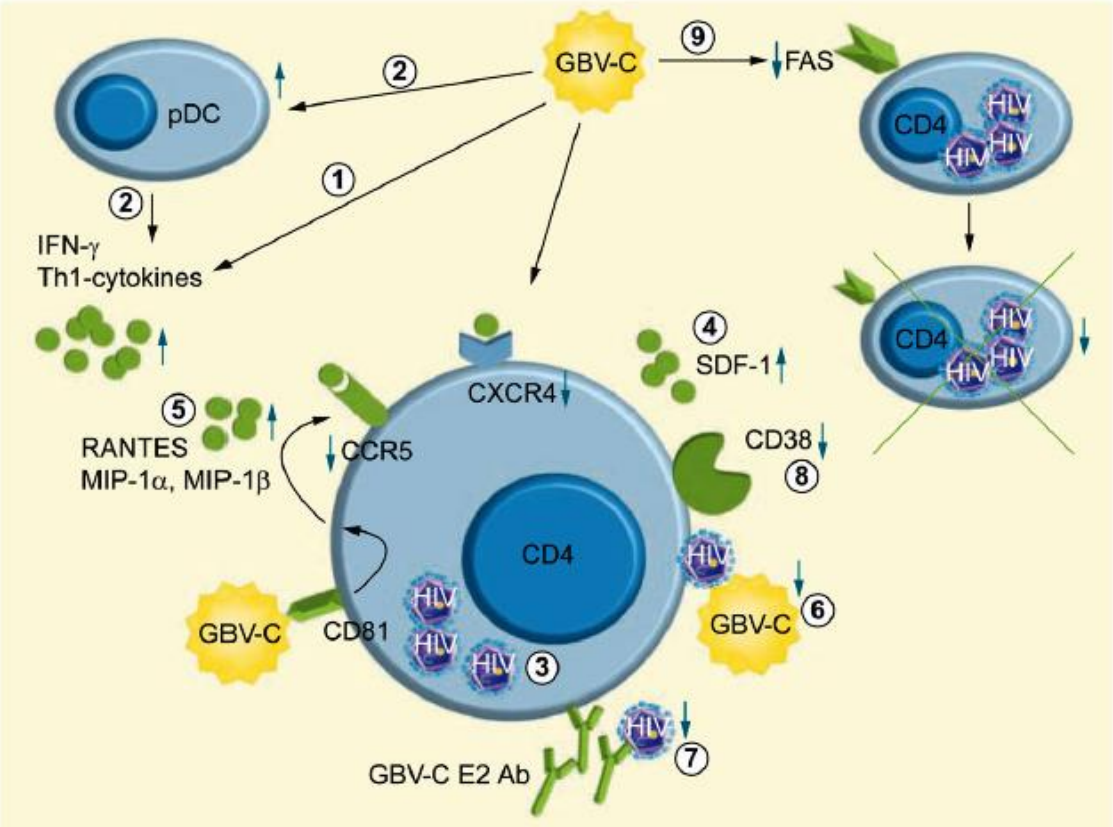

Figure 15: Proposed molecular interactions of GB virus C with HIV (Adopted from Schwarze-Zander *et al.*, 2012).

2.4.3. Decreased expression of chemokine receptors

The entry of HIV into target cells depends on T cell receptor CD4+ and its secondary receptors, the C-C chemokine receptor type CCR5 or CXCR4 which are very important for the progression of HIV transmission. Some strains of HIV use the CCR5 receptor for entry into target cells, and the others use CXCR4 co-receptor. Previous study has found logarithmically correlation between the loss of CD4+ in the advance stages of HIV infection and increasing in CCR5 density (Reynes *et al*., 2001).

Xiang et al. in 2004, has found that the coinfection with GBV-C leads to 23% reduction in HIV replication. They performed an in vitro study where GBV-C and HIV where inoculated in mononuclear cells. GBV-C leads to the secretion of cytokines such as RAN TES, MIP-1α,

MIP-1β, natural ligands of CCR5 and SDF-1, thus reducing the expression of CD4, CCR5 and CXCR4 used by HIV for cell entry (Xiang *et al.,* 2004). GBV-C coinfection is associated with up-regulation of CCR5 and CXCR4 expression in HIV patients with advanced disease (Schwarze-Zander *et al.,* 2010)

2.4.4. Modulating T-cell activation

Chronic T-cell activation enhances HIV replication, pathogenesis and contributes to the depletion of T cells by induced cell death (Stevenson *et al.,* 1990; Douek, 2003; Badley *et al.,* 1999; Douek, 2007; Hunt *et al.,* 2003). The degree of activation is measured by the co-expression of CD38 and HLA-DR on CD4+ and CD8+ T cells, correlates with the disease progression (Giorgi *et al.,* 1999; Hazenberg *et al*., 2000). Although HAART reduces T- cell activation but the level never returned as in healthy subjects (French *et al.,* 2011; Lederman *et al.,* 2011).

GBV-C viremia inhibits IL-2 signaling pathways and T cells activation which is thought to contribute to the immune dysfunction observed in HIV patients (Robert *et al.,* 2012). Stapleton *et* al. has also found that GBV-C viremia is associated with reduced activation, and proliferation of T-cells, and suggested that GBV-C may provide benefit and better out come together with antiretroviral therapy (Stapleton *et al.,* 2012; Maidana-Giret *et al*., 2009). Recent data showed that GBV-C reduced global T cell activation through the structural protein E2 that interfere and compete with lymphocytes specific protein tyrosine kinase (Lck) after the engagement with T-cell receptor (Stapleton *et al.,* 2013).

2.4.5. Influencing Fas-mediated Apoptosis

Expression of Fas-mediated apoptosis contributes to the eradication and depletion of CD4+ cells in the course of HIV disease (reviewed by [Dianzani *et al.,* 2003]). In HIV infected individuals the frequency of Fas-expressing T cells is high compared to healthy individuals

(Moenkemeyer *et al.,* 2008). The progression of HIV disease leads to increase in the expression of Fas in CD4+ and CD8+ which leads to Fas mediated cell death (Silvestris *et al.,* 1996), but once the patients start the HAART the Fas mediated apoptosis is reduced (Gougeon *et al.,* 1999).

Study on GBV-C/HIV coinfection in patients not receiving HAART has showed that the percentage of Fas expressing cells was low compared with HIV mono infection. Lower expression of Fas on T cells and reduced Fas-mediated apoptosis may contribute to the beneficial effect of GBV-C co-infection on HIV disease progression (Moenkemeyer M *et al*., 2008).

CHAPTER 3: MATERIALS AND METHODS

3.1. Site Description

Ethiopia

Ethiopia lies in the Horn of Africa, which is the Eastern part of the African continent. Ethiopia, with a population of over 105 million, is the second most populous country in sub-Saharan African. It covers 1, 104, 300 square kilometers (1 million km^2 land area and 104,300 km^2 water) (CSA, 2014; WWP, 2017). Its borders are Eritrea on the north, on the east by Djibouti and Somalia, on the south by Kenya, and on the west by Sudan and South Sudan (CSA, 2014). Administratively, the country is divided into 9 regions and two administrative cities. The country has a great geographical diversity and varied topography from high peaks of 4,550 m above sea level to a low depression of 110 m below sea level. The predominant climate type is tropical monsoon, with temperature climate on the plateau and hot in the lowlands.

According to data from the Ministry of Health (MOH), the overall prevalence of HIV in the population today is 1.1% (790,000) HIV positive persons. The highest prevalence is in the age group 15 to 49 years (Gemechu, *et al*., 2015).The data also show that, 12% of medical admission and 31% of mortality in Hospitals, medical wards, is due to liver disease (Tsega, 2000).

Addis Ababa

Addis Ababa situated at an altitude of around 2,400 m (7, 874 ft) and an approximate area of 526.99 $km^{2,}$ is the capital and largest city of Ethiopia. According to the 2007 census, Addis Ababa has a population of 3, 384, 569, of whom 1, 305, 387 are men, and an annual growth rate of 3.8% (CSA, 2007). The city has a subtropical highland climate with fairly uniform year round temperature, daily maximum temperatures averaging 20-25 ^{o}C (68-77 ^{o}F) which

are constant from month to month. The seasons in Addis Ababa are largely defined by rainfall, with a dry season from October to February, a light rainy season from March to May, and a heavy rainy season from June to September.

The city is divided into 10 sub-cities and 99 wards *(kebeles)*, with 12 hospitals and 26 health centers. The 10 sub cites are: Addis Ketema, Akaky-Kaliti, Abrade, Bole, Gullele, Kirkos, Kolfe-Keranio, Ledeta, Nifas Silk-Lafto and Yeka.

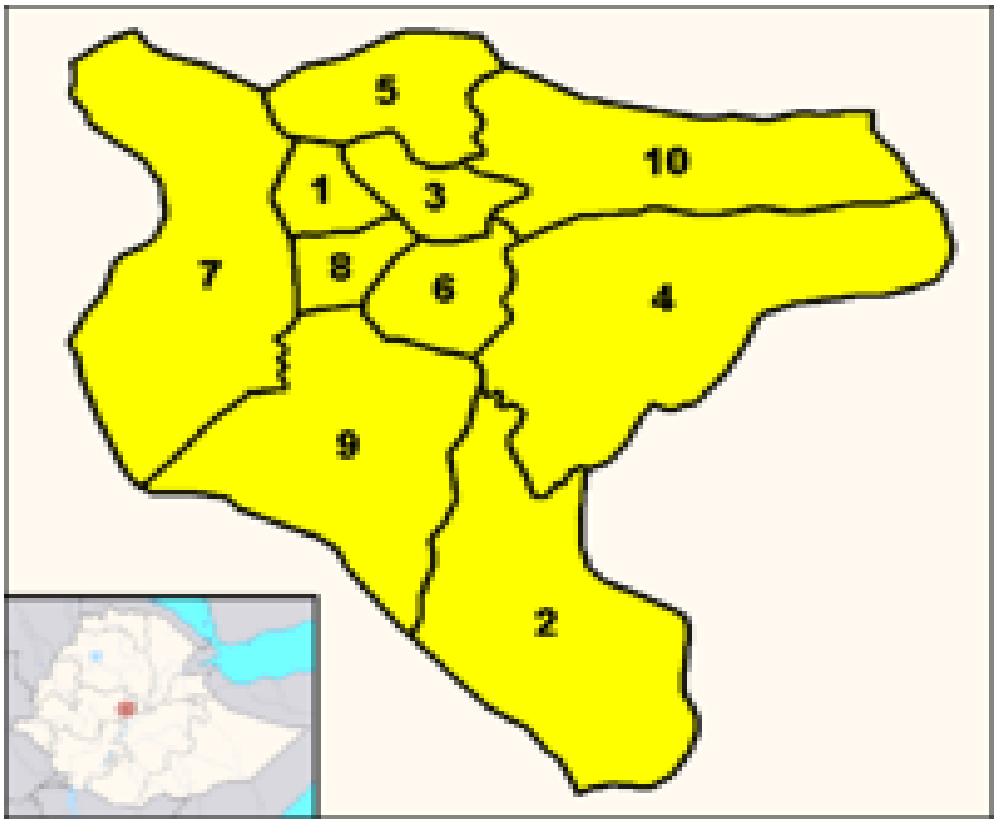

Figure 16: District map of Addis Ababa (Adopted from Addis Ababa city website; site map).

The following are the Health centers where this study was conducted.

A. Zewditu memorial hospital

Zewditu memorial hospital is a government hospital administered under health bureau of the city government of Addis Ababa. It is located in Kirkos sub city (no. 6 of Figure 16). Currently, the hospital has a total of 174 beds and provides referral medical services for patients coming from all sub cities and remote areas out of Addis Ababa. Zewditu memorial hospital, established in 1925, is affiliated with the oldest Nursing school. On average, the hospital serves nearly 378 patients per day and it is Ethiopia's leading hospital in the treatment of ART patients. Currently more than 18,000 people had been enrolled for HIV care, support and treatment, of these 6500 were adults put on ART regimen. CDC Ethiopia helped the launch Ethiopia's first ART program at Zewditu in July 2003, and in March 2005 it received technical assistance from Johns Hopkins University's (JHU) TSEHAI Program. Zewditu became the largest HIV clinic in Ethiopia, with 14,000 patients in its care. Since, ART programs have been initiated in other hospitals around the country, the pressure on the hospital is relieved (CSA, 2007; Asemahagn *et al*., 2018).

B. AIDS Health Care Foundation (AHF)

AIDS Health Care Foundation (AHF)-Ethiopia, with operations in Addis Ababa currently runs two HIV care centers facilities namely AHF-Yeka and AHF-Kolfe Clinics. These care centers are situated in two different sub-cities, Yeka Sub-city (no. 10 of Figure 16) and Addis Ketema Sub-city (no. 2 of Figure 16) respectively, where a large number of people reside and there is high day-to-day population movement. It is also believed that these areas have relatively higher HIV service needs. Generally, AHF-Ethiopia provides free comprehensive HIV care services including ART, treatment of opportunistic infections, HIV Counseling and Testing (HCT), condom

promotion and distribution, tuberculosis diagnosis and management, care for HIV exposed infants and family focused care, integrated family planning services, provision of preventive care package, and nutritional and psychosocial support program. To date, AHF-Ethiopia has provided HIV care and treatment to 3,949 individuals in the two sites of which 1,124 are children and adolescents. Of those patients enrolled to care, 2,816 have received ART and related treatment support. AHF-Ethiopia also provided HIV testing to 85,946 individuals in 2015, and 120,133 condoms were distributed alongside other HIV prevention activities the same year. AHF-Ethiopia persistently strives to improve its collaboration with partners and is planning to expand its service to other government and non-government sites this year. Moreover, they are partnering with local civil society groups to work on HIV service advocacy with the objective of improving access to HIV prevention, treatment, and care to the needy beneficiaries (AHF-Ethiopia, 2016).

C. Kirkos health center

Kirkos health center is one of the five governmental health facilities found in Kirkos sub city (no. 6 of Figure 16). The sub city has a total area of 14.62 km2 and population of 235,441 of which 109,607 are males. Under this sub city there are different health facilities including 4 private hospitals and 82 clinics (CSA, 2007).

D. Ethiopian Public Health Institute (EPHI)

In 2014, the Ethiopian Public Health Institute (EPHI) was established in Gullele sub city (no. 5 of Figure 16). EPHI's work builds upon the strong foundation of the Ethiopian Health and Nutrition Research Institute (EHNRI) which opened in 1996 with the goal of using health research to update national health policy. Valid health information is crucial as the basis for effective decisions to improve health and general well-being of the population, and to improve and develop health-related

research activities in the country. EPHI's expanded operations include its enhanced role in health research based on the priority health and nutrition problems identified in the national public health research agenda. Based on the prevalence of health and nutrition problems and by taking public needs into considerations, the Institute had been setting priority strategies to address the public demands. Currently the Institute is focusing on diseases research and strengthening the national public health laboratory services in the country.

E. Adera Internal Medical Specialty Center

Adera Internal Medical Specialty Center is a private internal medicine clinic that provides healthcare to a great number of patients. The clinic is in Kirkos sub city (no. 6 of Figure 16).

3.2. Sample and data Collection

The study was done on a total of 252 blood samples collected from participants living in Addis Ababa between March 2014 to February 2016. The first group comprised a total of 81patients under investigation for drug resistant HIV strains Zewditu Memorial Hospital and two health center: AIDS Health Care Foundation, and Kirkos Health Center.

The second group comprising of 101 serum samples collected from Adera Internal Medical Specialty Center for patients with viral hepatitis. The patients were there seeking medical care or treatment. The center is an internal medicine clinic that provides healthcare to a large number of patients with liver diseases.

The last, 70 frozen serum samples from healthy individuals collected from adult Ethiopians volunteer at the Public Health and Research Institutes and Addis Ababa University.

Following collections, the serum samples were divided into 2 micro tubes and transported in an icebox to the Immunology and Molecular laboratories at the department of Microbial

Cellular & Molecular biology, Addis Ababa University. Demographic data, including age, gender, medical history and health status were collected from the patient's cards and archives from the hospital or clinic. All HIV patients were classified into stages according to the WHO staging (Table 7) (WHO, 2006). Repeated freezing and thawing was avoided, and all the regulation was strictly followed during packaging and transportation.

Table 7: Classification of HIV infection stages

WHO Stage	Clinical Monitoring	CD4 Monitoring
1: Asymptomatic	No HIV-related symptoms	CD4 cell count ≥500cells/mm3
2: Mild	➢ Unexplained weight loss ➢ Respiratory infections ➢ Herpes zoster ➢ Oral ulceration ➢ Seborrhoeic dermatitis ➢ Fungal nail infection	➢ HIV-related symptoms ➢ CD4 count ≥500cells/mm3
3: Advanced	➢ Severe weight loss ➢ Chronic diarrhea ➢ Persistent fever ➢ Oral candidiasis ➢ Pulmonary TB, ➢ Severe bacterial infection	➢ HIV-related symptoms ➢ CD4 count 200-499 cells/mm3
4: Severe (AIDS)	➢ HIV wasting syndrome ➢ Pneumonia, ➢ Chronic herpes simplex ➢ Extra-pulmonary TB ➢ Kaposi sarcoma ➢ HIV encephalopathy	➢ AIDS-defining conditions ➢ CD4 count <200 cells/mm3

3.3. Viral load assays and CD4 + T cells count

The HIV viral load and CD4 + T cells count of HIV serum samples were measured at the Addis Ababa City Administration Health Research laboratory.

3.3.1 CD4+ T Cell Count

The CD4+ T cell count was done using FACScan Flow Cytometery (Becton Dickinson Immunocytometry System, San Jose, CA., USA) and FACS Count CD4/3 SW Version 1.0 08/05 flow cytometer system (Becton Dickinson Immunocytometry Systems [BD], San Jose, Calif.) at Addis Ababa City Administration Health Research laboratory.

3.3.2. Viral load assay

Viral load assay was done by using Real-time HIV-1 assay, which has an internal Quality Control system, with automated Abbott *m*2000*rt*™ System (Abbott Molecular Inc., Des Plaines, IL 600018, USA) at Addis Ababa City Administration Health Research laboratory according to the manufacturer's instruction. However, sample preparation for the automated quantification assay was done manually following the manufacturer's instruction in the same laboratory by extracting and concentrating the target RNA molecules from 200 µl plasma to make the target accessible for amplification and to remove potential inhibitors of amplification from the extract. The upper detection limit of the assay is10 million copies/ml, while its lowest limit for the amount of input sample used in this study was 150 copies per ml, although this could be further lowered to 40 copies per ml if the amount of input samples were at least 600 µl.

3.4. Serological assay

Serum of liver disease patients was tested for anti-HCV IgG antibodies and Hepatitis B Surface antigen.

3.4.1. HBV Surface Antigen ELISA

The Cobas® HBsAg II and Roche Modular Analytics E170 were used for the qualitative determination of hepatitis B Surface Antigen in serum (Roche Diagnostics GmbH, Mannheim). Biotinylated anti-HBsAg antibodies and anti-HBsAg antibodies labeled with a ruthenium complex are mixed with the sample to form a sandwich complex. This complex binds to a streptavidin-coated micro-particle via the interaction of streptavidin with biotin. This complex is magnetically captured onto the surface of an electrode. Unbound substances are removed. A voltage applied across the electrode induces a chemiluminescent signal which is measured by a photomultiplier. The software automatically determines the result by comparing this signal to calibrated signal-cutoff values.

The kit contains controls and a calibrator for quality control purposes. The specificity and sensitivity of the assay are stated to be 100% (Package insert: Cobas® HBsAg II, Roche Diagnostics GmbH, Mannheim).

3.4.2. HCV Antibody ELISA

A commercially available 3rd generation ELISA (ABBOTT AxSYM® HCV v 3.0, Abbott Laboratories, Abbott Park, IL, USA) was utilized according to the manufacturer's protocol. This is a micro-particle enzyme immunoassay for the qualitative detection of anti-HCV antibodies in human serum or plasma. The assay uses proteins coated on a micro-particle solid phase to capture antibodies against structural and nonstructural proteins of HCV. The bound is detected by an antibody coupled to an enzyme. This enzyme acts on a substrate to produce a fluorescent signal. The fluorescent signal is proportional to the amount of analyte present in the initial sample.

Several recombinant proteins are included in the assay. Collectively they contain sequences of putative core structural proteins and nonstructural proteins NS3, NS4 and NS5 bound to superoxide dismutase (SOD).

The kit contains positive and negative controls and a calibrator for quality control purposes. The specificity and sensitivity of the assay are stated to be 99.84% and 100%, respectively (Package insert - ABBOTT AxSYM® HCV v 3.0, Abbott Laboratories, Abbott Park, IL, USA).

3.5. Molecular based assays

3.5.1. Extraction of GBV-C RNA

Viral RNA was extracted from all plasma samples. It was performed manually using Ribo Virus kit (Sacace Biotechnologies, Italy) according to the manufacturer's instruction. Briefly, a 150-µL thawed plasma sample was added onto a 600-µl prepared Buffer RAV1 containing carrier RNA and internal control in a 1.5-ml micro-centrifuge tube. All the tubes were mixed by pulse-vortexing for 15 seconds and the mixture was incubated at 70°C for 5min. RAV1 is a lysis buffer containing guanidine thiocyanate, and the internal control serves as amplification control to identify possible reaction inhibition. Six hundred microliter of ethanol (96–100%) was added and tubes were mixed and centrifuged at 6000xg (8000 rpm) for 1 minute. These processes allow lysis of the samples under highly denaturing conditions to inactivate RNase and to ensure isolation of intact viral RNA. Carrier RNA improves binding and recovery of the low-concentrated viral RNA. The mixture was transferred into Ribo Virus columns containing silica membrane. This was followed by repeated washing and centrifugation by ethanolic buffers RAW and finally RAV3 to remove contaminations and potential PCR inhibitors like salts, metabolites and soluble macromolecular cellular components. Finally, 50 µl of Elution Buffer was added to the dried silica pellet, resuspended by vortexing and incubated at 56°C for 10 minutes. It was then centrifuged at 10,000g for 2

minutes to get the nucleic acid eluted. Purified RNA was either used immediately or stored at -20°C.

3.5.2. Reverse Transcription (RT-PCR), Amplification and Sequencing

One-step reverse transcription of the extracted RNA and PCR amplification of the cDNA was performed by using (V2-FRT, Sacace, Italy), which includes all the components to generate RT-PCR amplified products from GBV-C RNA. Briefly, all the components of the reaction mix were first thawed and vortexed thoroughly. The total reaction volume was 25 ul, containing 10 ul of extracted RNA and 15 ul of the master mix which was prepared based on the manufacturer's instructions. The master mix contains reagent for the RT and the amplification reaction (Table 8), DNA polymerase and primers that amplify the 5'UTR region (Table 9) (Sheng *et al.,* 2007; Valinciute *et al.,* 2013).

The synthesis of the complementary DNA (cDNA) was performed at 50°C for 15 min, followed by inactivation of the reverse transcriptase enzyme at 95°C for 5 min. The two PCR rounds are the same. The amplification was done in 5 cycles for the first round (95°C for 5 s, 60°C for 20 s, 72°C for 15 s) and 40 cycles for the second rounds of the PCR (95°C for 5 s, 60°C for 30 s, 72°C for 40 s with a final extension at 72°C for 15 s). The fluorescence detection and analysis of the PCR product was performed during the final extension by using the FAM, JOE/HEX channels of the Mx3000P instrument. Negative control of amplification and positive control of amplification and extraction were maintained as provided by the kit. Internal control, which serves as amplification control, was used to identify possible reaction inhibition.

The kit allows detecting GBV-C in 100% of the tests with a sensitivity not less than 100 copies/ml. The analytical specificity of the primers and probes was validated with negative samples. They did not generate any signal with the specific GBV-C primers and probes. The

specificity of the kit was 100%. The potential cross-reactivity of the kit was tested against the group control (HAV, HCV, HBV, HDV, HIV, HSV ½, EBV, CMV and other ones). It was not observed any cross-reactivity with other pathogens.

Nested PCR was performed using Taq PCR Master Mix Kits with the same primers and thermal profile but with various annealing temperatures of 50, 52, 57 and 60 degrees Celsius.

3.5.3. Agarose Gel Electrophoresis

A 2% agarose gel was poured by melting 2.0g of agarose in I00 ml of Ix TBE buffer. Five microliters (5ul) of Ethidium bromide (50ug/ml) was added to the gel before it was casted. Five microliters (5ul) of each PCR product was added to 5ul of bromophenol blue loading gel buffer (50% glycerol; 1x TAE buffer; 1% bromophenol blue: 1% Xylene cyanole ff). Two microliters (2ul) of molecular weight marker (1Kb DNA Ladder, (Gibco-RL, Life Technologies, Paisley, UK), was diluted in 3 ul of sterile DEPC-H20 and added to 5ul of loading buffer. Ten microliters (10ul) of each reaction along with the molecular weight marker was loaded into the appropriate wells in the gel. Electrophoresis was performed at a constant 100V for approximately 1 hr. The gel was visualized on a UV transilluminator and photographed.

Table 8: Reagents of the RT and amplification reaction

Component name
Moloney Murine Leukemia virus reverse transcriptase
RNase inhibitor
RT buffer [mM Tris-HCl, mM $MgCl_2$, 3M KCl, mM dithioerythritol
random hexameres
DNTPs

Table 9: Sequence and location of primers used for amplification of the 5' UTR of the GBV-C

Polarity*	Sequence 5' to 3'
+	GGC CAA AAG GTG GTG GAT GG
-	ATT GAA GGG CGA CGT GGA CC
+	GTG ATG ACA GGG TTG GTA GG
-	GTA CGT GGG CGT CGT TTG CC
• Direction of primer sequence (+ sense, - anti-sense)	

3.6. Liver Function Test

The following parameters were measured: Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Total bilirubin (BIL-T), and Albumin (ALB).

The device used for the sample analysis was Olympus 640 (Olympus Diagnostica GmbH, Hamburg, Germany) auto-analyzer. All reagents were prepared to fit the required volumes and concentration. Reagents used in Olympus auto-analyzer machine were in specific containers referred to as "reagent cartridges". The reagent cartridges were bar-coded for the identification by the machine.

3.6.1. Alkaline phosphatase (ALP)

Alkaline phosphatase reagent was used to measure the activity of ALP enzyme by a kinetic UV method using a 2-amino-2-methyl-1-propanol (AMP) buffer. In the reaction, ALP catalyzed the hydrolysis of the colorless organic phosphate ester substrate, p-Nitro phenyl phosphate to the yellow colored product, p-nitro phenol and phosphate. The reaction occurred at an alkaline pH of 10.3. A volume of 5µL from the serum was reacted with 250 µl of the

reagent. The change in absorbance was monitored at 409 nm and this change was directly proportional to the activity of ALP. The activity was calculated and expressed in U/L. The reaction took place at 37^{o}C for three minutes.

3.6.2. Alanine aminotransferase (ALT)

The ALT reagent was used to measure Alanine aminotransferase in the sample by an enzymatic kinetic UV rate method. In the assay reaction, the ALT catalyzed the reversible transamination of L-Alanine and α-ketoglutarate to pyruvate and L-glutamine. The pyruvate then reduces to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of β-Nicotinamide Adenine Dinucleotide (reduced form) (NADH) to β-Nicotinamide Adenine Dinucleotide (NAD). Pyridoxal-5-phosphate was required in this reaction as a cofactor that was required for transaminase activity by binding to the enzyme using Schiff-base linkage. A sample of 10µL was reacted with 110µL of the reagent. The change in absorbance was monitored at 340 nm and this change was directly proportional to the activity of ALT. The activity was calculated and expressed in U/L. The reaction took place at 37^{o}C for three minutes.

3.6.3. Aspartate aminotransferase (AST)

Aspartate aminotransferase (AST) reagent was used to measure aspartate aminotransferase activity by an enzymatic kinetic UV rate method. In the reaction aspartate aminotransferase catalyzed the reversible transamination of L-aspartate and alpha -keto-glutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase (MDH) with the concurrent oxidation of reduced beta-nicotinamide adenine dinucleotide (NAD). A sample of 10µl was reacted with 110µl of the reagent. The change in absorbance was monitored at 340 nm and this change was directly proportional to the activity of ALT. The activity was calculated and expressed in U/L. The reaction took place at 37oC for three minutes.

3.6.4. Total bilirubin (BIL-T)

Bilirubin and related compound are measured in body fluid through chromatography, capillary electrophoretic and photometric methods. Stabilized diazonium salt (3, 5-dichlorophenyldiazonium tetrafluoroborate (DPD), reacted with conjugated bilirubin directly and with unconjugated bilirubin in the presence of an accelerator (caffeine) to form azobilirubin (purple).A sample of 8 µl was reacted with 280 µl of reagent and the change in absorbance was monitored at 552nm.This change was directly proportional to the concentration of T BILI in the sample and was used to calculate and express concentration in µmol/L. The reaction took place at 37oC for two minutes.

3.6.5. Albumin (ALB)

Albumin reagent was used to measure albumin concentration by a timed endpoint method and the reaction took place at 37^{o}C for one and half minutes. Albumin combined with Bromocresol green to form a colored product. A sample of 3 µl was reacted with 300 µl of reagent and the change in absorbance was monitored at 552nm.Thischange was directly proportional to the concentration of ALB in the sample and was used to calculate and express concentration in g/l.

3.7. Data interpretation and statistical analyses

The data of HIV and hepatitis patients were analyzed separately by using SPSS v. 20. Data was entered and analysis done by using SPSS v. 20. Student t- test was used to determine the differences of continuous variables. Chi-square tests were used to compare categorical variables. The prevalence for HBV and HCV was expressed in percentages for the entire study group and results obtained were presented in tables, figures and graphs. Analysis of covariance was used to compare GBV-C positive and negative patients with respect to CD+4 count and viral load, adjusting for age, sex year of acquiring HIV and date of starting HAART. P value < 0.05 was considered statistically significant.

3.8. Ethical Considerations

Ethical approval for this study was obtained from three levels. National Research Ethics Review Committee, faculty of Natural and Computational Science Review Committee and the Department Ethical Review Committee. This was a laboratory-based study utilizing sera for HIV, and hepatitis patients with their clinical data which obtained from the patients' hospital records. There was no direct patient interaction or contact. No additional blood samples were taken and the only mentioned tests and analysis techniques were performed. The results were notified to the centers and clinic where samples were collected.

CHAPTER 4: RESULT

4.1. Profiles of the study populations

4.1.1. HIV patients under HAART

A total of 81 HIV patients' blood samples on HAART treatment for at least 24 weeks were recruited in this study to detect the prevalence and impact of GBV-C in the disease progression and treatments. Gender distribution was 44 (54.3%) and 37 (45.7%) for females and males respectively. The majority of the participants 31 (38.3 %) were aged between 31 - 40 years, and 20(24.7%) were in the age group 41-50 years as shown in (Figure 17).

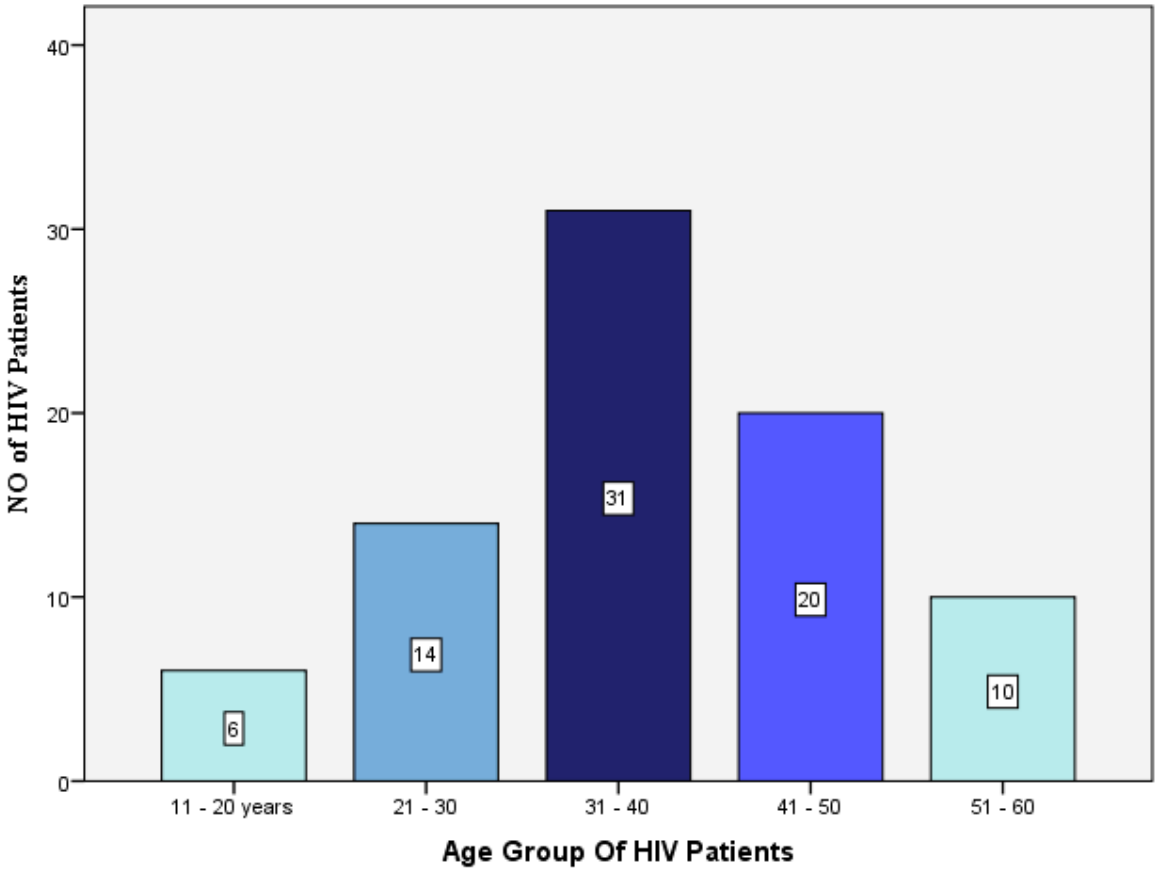

Figure 17: Age distribution of HIV patients under treatment

Regarding disease stages of the participants, recorded data showed that 22/44 (50%) of the women and 21/37 (56.75%) of the men participants were in the advanced WHO clinical stages (stages III or IV). While 22 of the women and 16 of the men belonged to WHO stage I and II (Table 10).

Table 10: Age and gender distribution of HIV patients in WHO staging system

Age	**Clinical Status**								**Total**
	Stage 1		**Stage 2**		**Stage 3**		**Stage 4**		
	Gender		**Gender**		**Gender**		**Gender**		
	Male	Female	Male	Female	Male	Female	Male	Female	
11 - 20 years	0	1	1	1	2	0	0	1	**6**
21 - 30	0	1	1	5	1	4	1	1	**14**
31 - 40	3	4	3	7	4	6	1	3	**31**
41 - 50	3	0	5	0	4	2	2	4	**20**
51 - 60	0	2	0	1	4	1	2	0	**10**
Total	**6**	**8**	**10**	**14**	**15**	**13**	**6**	**9**	**81**

Of the total 81 participant 47 (58%) were married, and the majority 46 (56.8%) were illiterate or receive the primary education only as shown in (Figure 18).

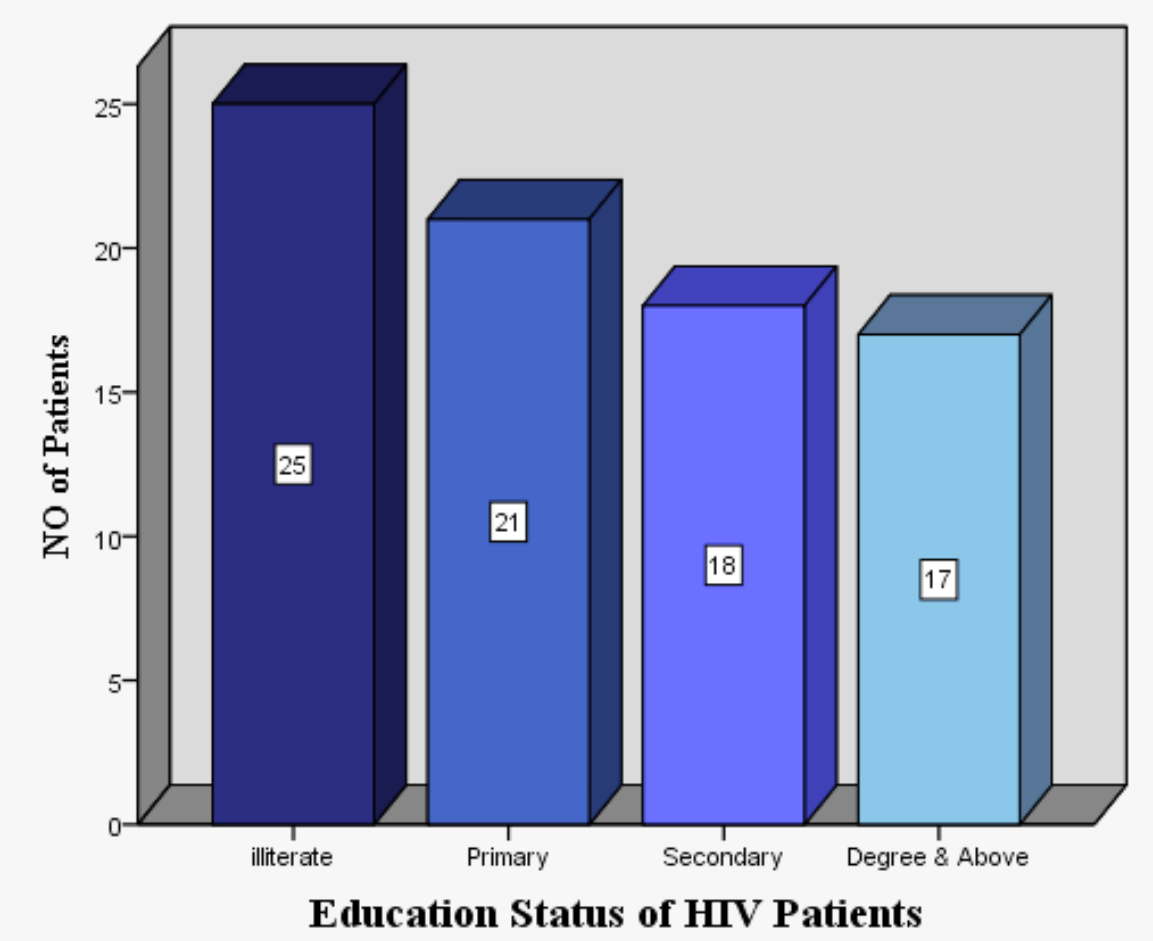

Figure 18: Education status of HIV patients under treatment

Plasma viral RNA load and CD4+ count was performed for all HIV patients under HAART. For ease of analysis, both plasma viral RNA and CD4+ cell count results were grouped into six and five categories, respectively.

The minimum and maximum values of the plasma viral load were 150 and 165,482 RNA copies/ml respectively, whereas in 18(22.2%) of the patients, the HIV RNA were not detectable.

The most frequent 28 (34.57%) RNA group was the one with the viral load over 10,000 copies/ml followed by the group with 600 to 5,000 copies/ml among 15(18.5%) of the HIV patients as shown below in (Figure 19).

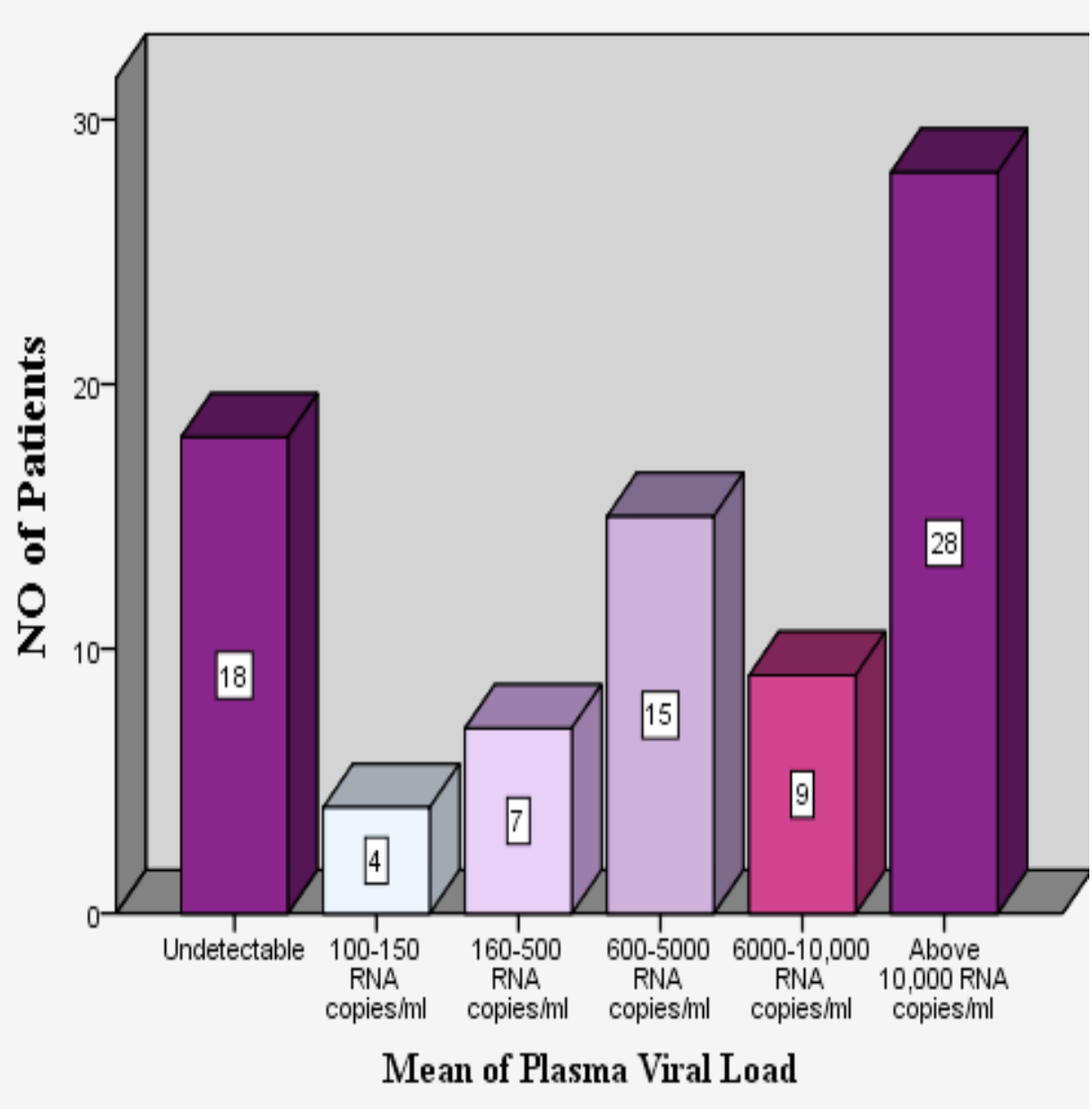

Figure 19: Mean of Plasma Viral Load

There were no significant differences in the level of the plasma viral load between males and females. However, in four groups the plasma viral load were more among females, in one group the male were more than females, and the numbers were equal in the last group as shown in (Figure 20).

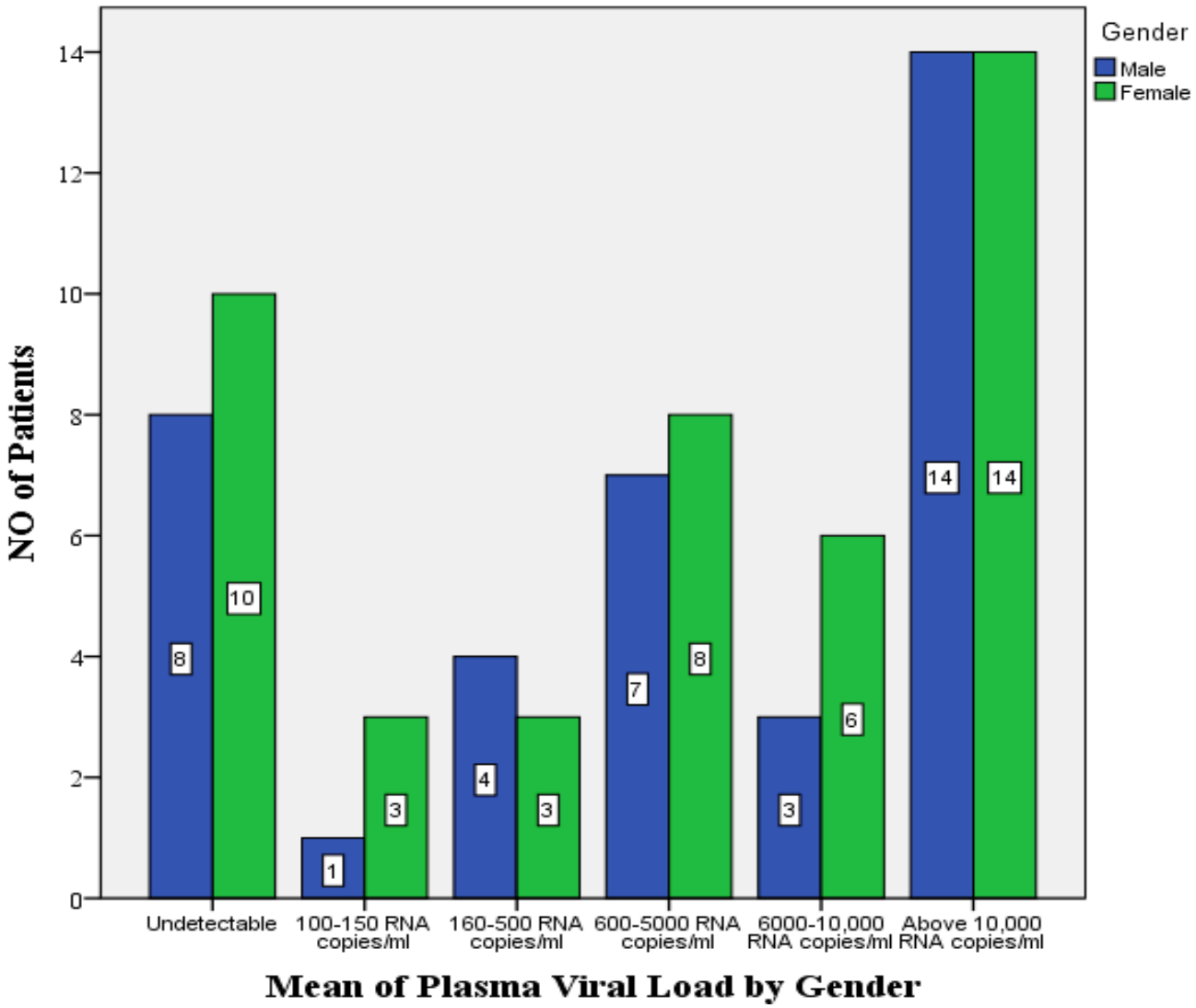

Figure 20: Average of the of Plasma viral load between males and females

The minimum and maximum CD4+ cells values were 29 and 771 cells/mm^3, while the median and mean counts were 193 and 218.49 cells/mm^3 respectively. The most frequent 29 (35.8%) of the patients fall in the CD4+ group (101-200 cells/mm^3), followed by the group with (201-300 cells/mm^3) among 19(23.5%) of the HIV patients as shown below in (Figure21).

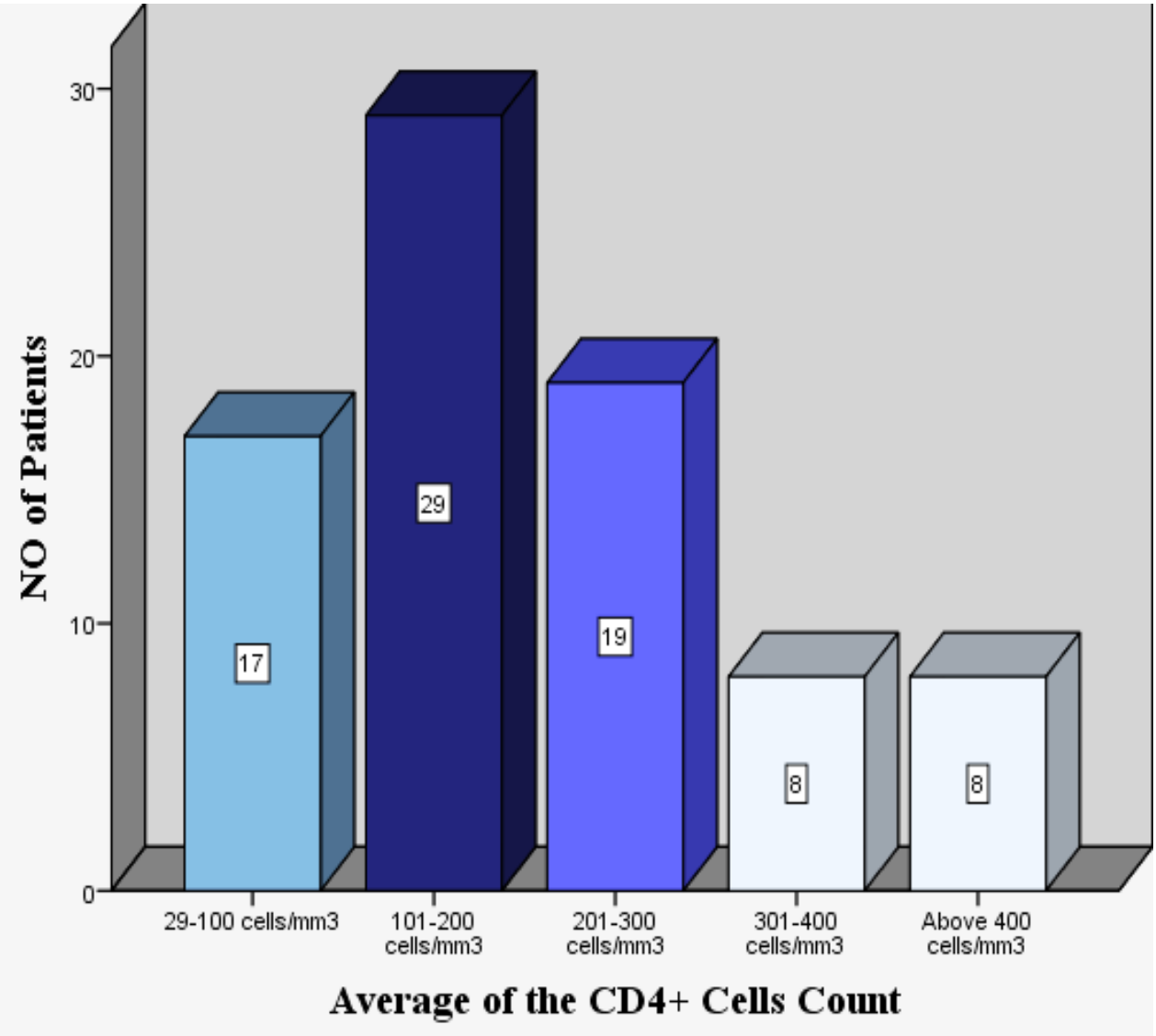

Figure 21: Average of the CD4+ cells count

4.1.2. Viral hepatitis patients

A total of 101 subject's samples with liver disease were included in this study collected from Adera internal medical specialty center, to study the role of GBV-C in liver disease progression.

Sero-prevalence analysis have shown that 83 (82.17%) were HBV positive, while the remaining 18 (17.8%) were HCV positive and two samples among them were co-infected with HBV and HIV respectively (Figure 22).

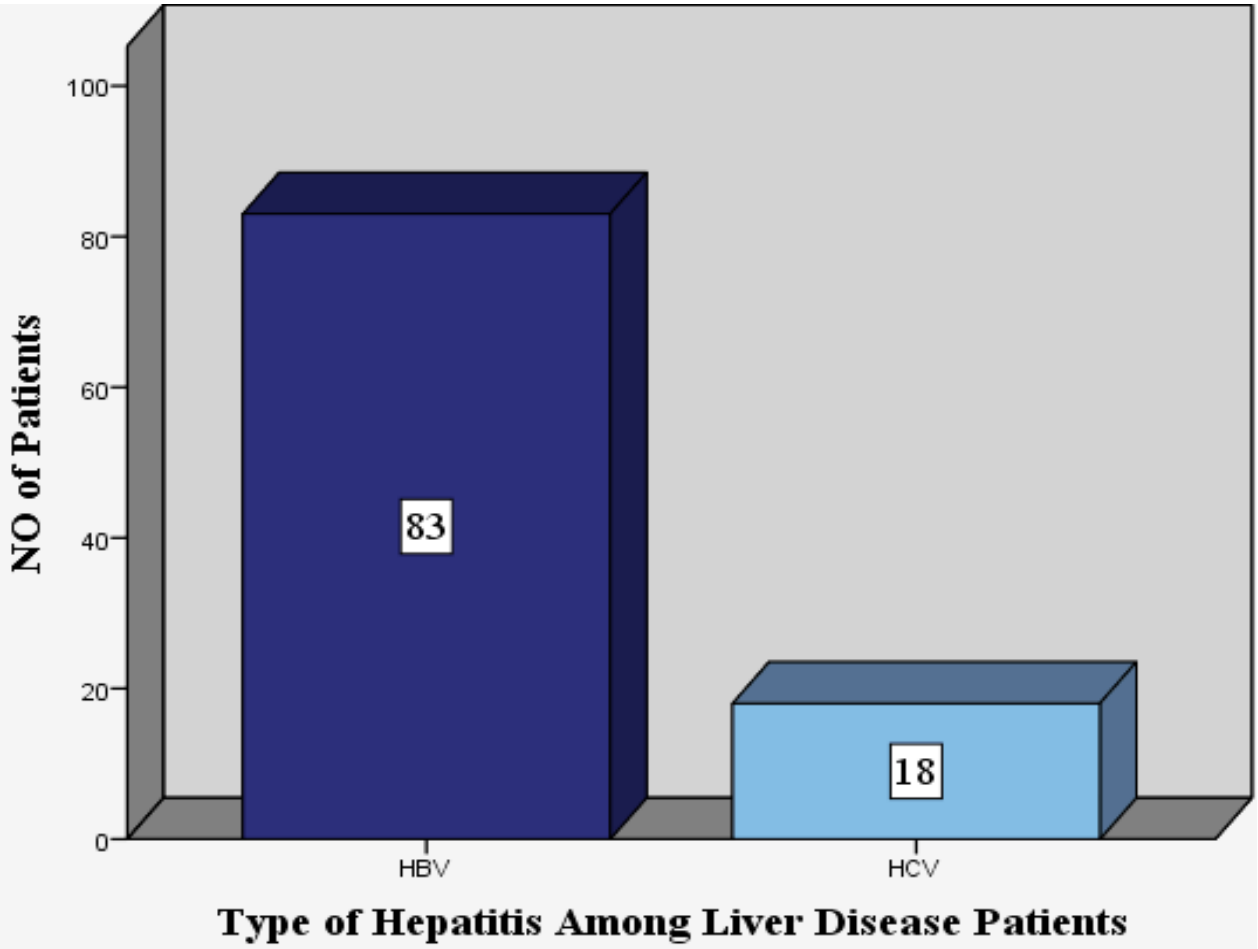

Figure 22: Type of hepatitis among liver disease patients

The socio-demographic characteristics of hepatitis patients, as shown in (Figure 23), shows that the majority 52 (62.7%) of HBV patients were male while 31 (37.3%) were female. Most of the HBV participant 58 (69.87%) were aged between (25 – 44) years. While among HCV patients there were 11 (61.1%) females and 7 (38.9%) males. Among HCV participants 12 (66.7%) were aged between (45-60) years and 6 (33.3%) were in the age group (35 – 44) years.

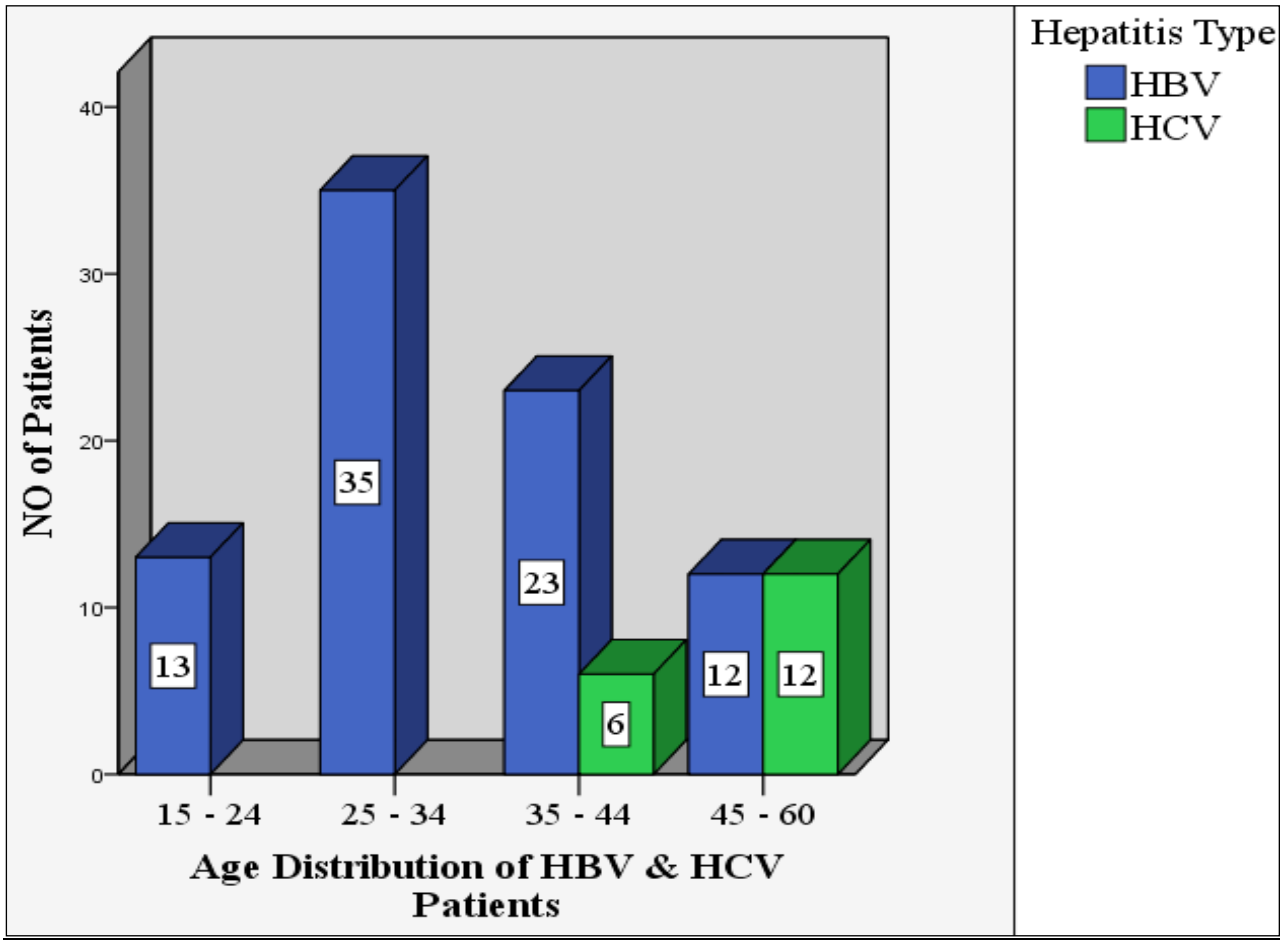

Figure 23: Age distribution of HBV and HCV patients

Based on status of the disease and the degree of the liver damage of the cases of HBV and HCV, patients were categorized respectively into four and two groups as shown in (Figure 24).

Most of HBV patients 33 (39.8%) had mild liver injury. Their level of the liver cell enzymes in the circulation were in the upper limit and with normal Liver scan, and 25 (30.1%) were asymptomatic. The cases of HCV were categorized into two groups: 11 (61.1%) patients who were suffering from cirrhosis and or hepatocellular carcinoma. The last group of HBV patients were 4 (4.8%) with moderate liver injury which included those with sever fatty liver and raised level of the liver enzymes in the circulation have been found in 21 (25.3%) of HBV in contrast 7 (38.9%) of HCV patients.

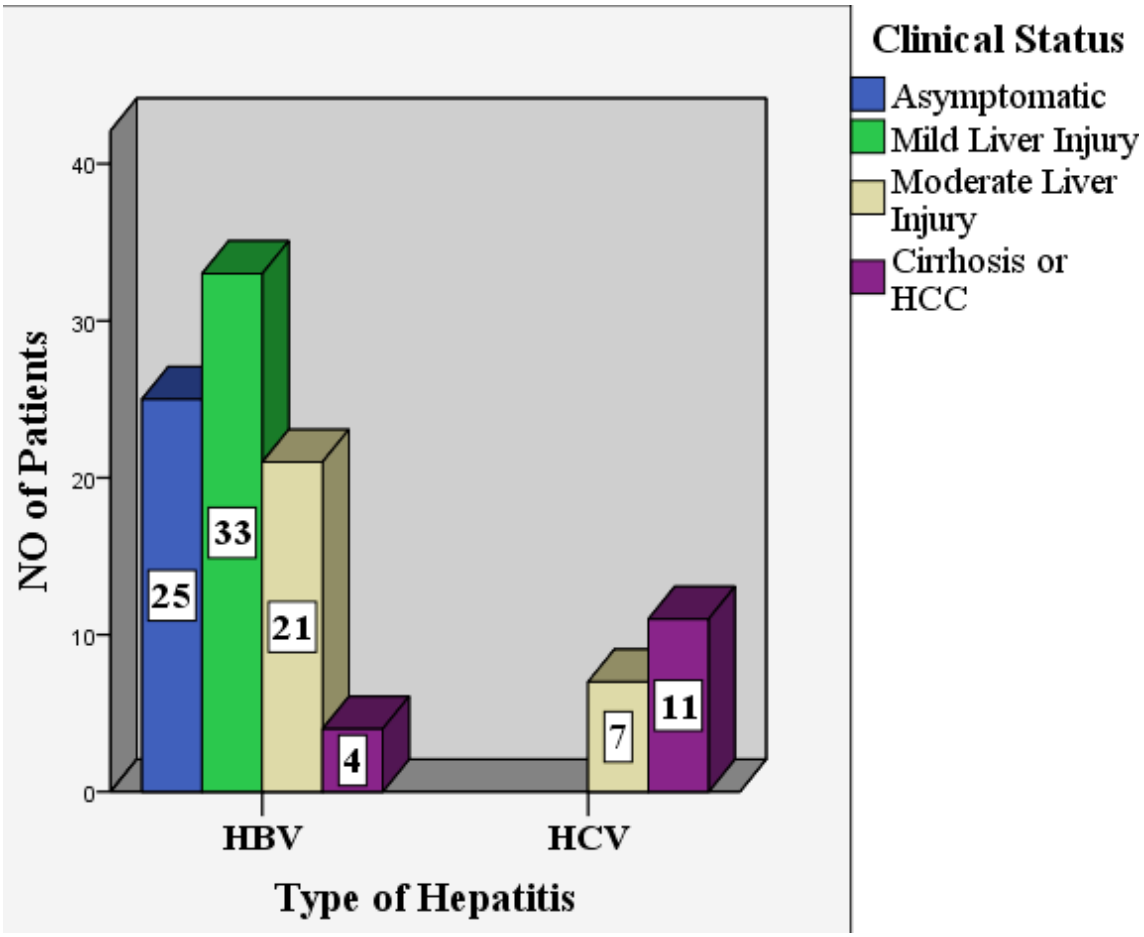

Figure 24: Clinical states of the hepatitis patients

4.1.3. Healthy control individuals

A total of 70 samples of healthy adult volunteers from Ethiopian Public Health and Research Institutes and Addis Ababa University were collected. Of the 70 samples, 50 (71.4%) were males and 26 of them were above 32 years. The remaining 20 (40%) were from females, most of them 12 (60%) were age between 27-32 years old (Figure 25).

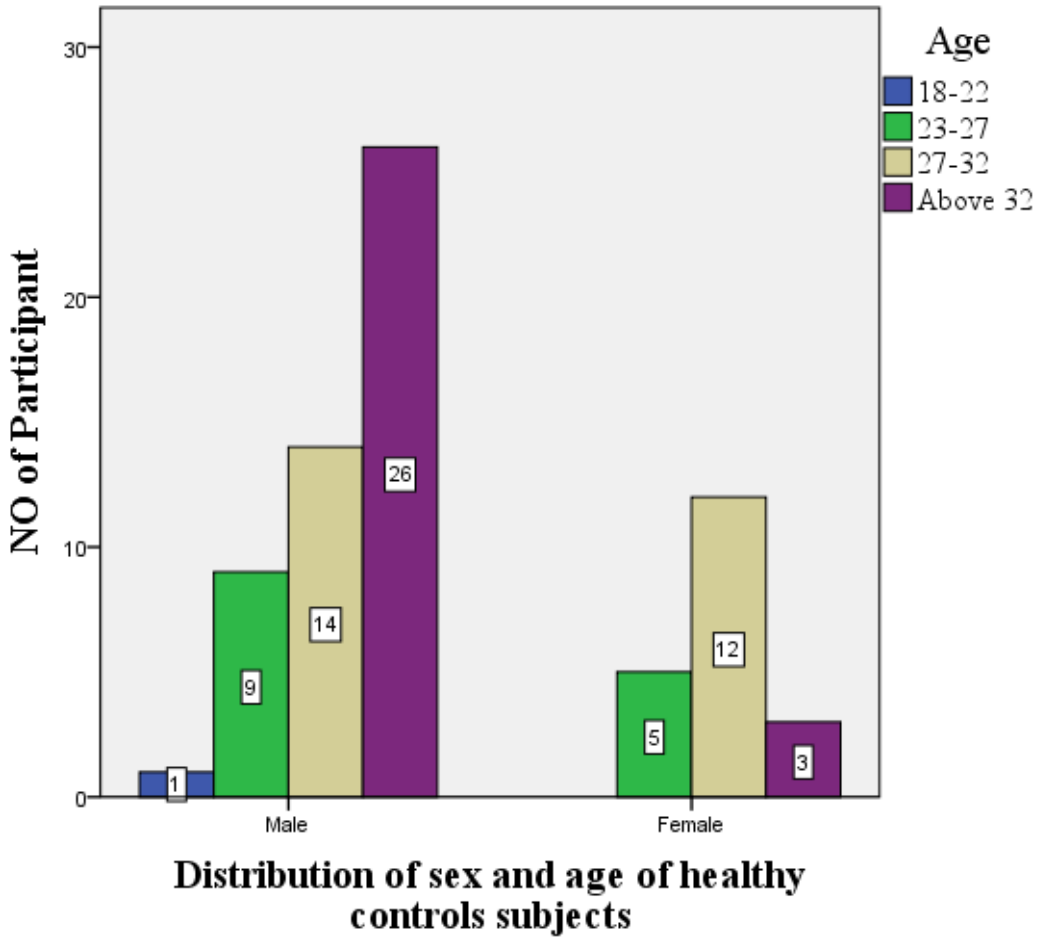

Figure 25: Distribution of sex and age of healthy controls subjects

Among the study subjects, 44 were single, 26 were married. Most 52 (74.3) of the study subjects have attained secondary school or above.

4.2. Prevalence, impact and GBV-C genotype

During the study period, 252 serum samples were screened for GBV-C by RT-PCR. GBV-C RNA were detected in 20 (11.27%) of the patients with viral hepatitis and HIV, while all the healthy subjects were negatives.

4.2.1. GBV-C among HIV patients under HAART

The overall prevalence of GBV-C RNA in this study was among 7 (8.6%) patients out of the 81 samples of HIV patients under HAART. GBV-C incidence was relatively higher in the age group of 31 – 40 years and in female, but not statistically significant *($p > 0.05$).* Similarly higher prevalence as shown in (Table 12) was seen among illiterate, primary school and married participants but not statistically significant.

Table 12: Prevalence of GBV-C by socio-demographic variables among HIV patient under HAART

Variables	**GBV-C RNA**		
Age group	**Positive no (%)**	**Negative no (%)**	**Total no (%)**
11 – 20	1 (17)	5 (83)	6 (8)
21 – 30	1 (7)	13 (93)	14(17)
31 – 40	3 (10)	28 (90)	31 (38)
41 – 50	1 (5)	19 (95)	20 (25)
51 – 60	1 (10)	9 (90)	10 (12)
Sex			
Male	3 (8.1)	34 (91.9)	37 (45.7)
Female	4 (9)	40 (91)	44 (54.3)
Marital status			
Married	5 (10.4)	43 (89.6)	48 (59)
Single	2 (6)	31 (94)	33 (41)
Education			
Illiterate	2 (8)	22 (92)	24 (30)
Primary school	2 (9)	20 (91)	22 (27)
Secondary school	1 (6)	17 (94)	18 (22)
Diploma and above	2 (12)	15 (88)	17 (21)

GBV-C positive patients displayed markedly lower HIV viral load (54 vs. 14,676 Copies/ ml). Of the seven GBV-C positive patients 5 (71.4%) were having undetectable plasma HIV RNA, and the two remaining there plasma viral load were 150 and 226 copies/ml respectively as shown in (Figure 26).

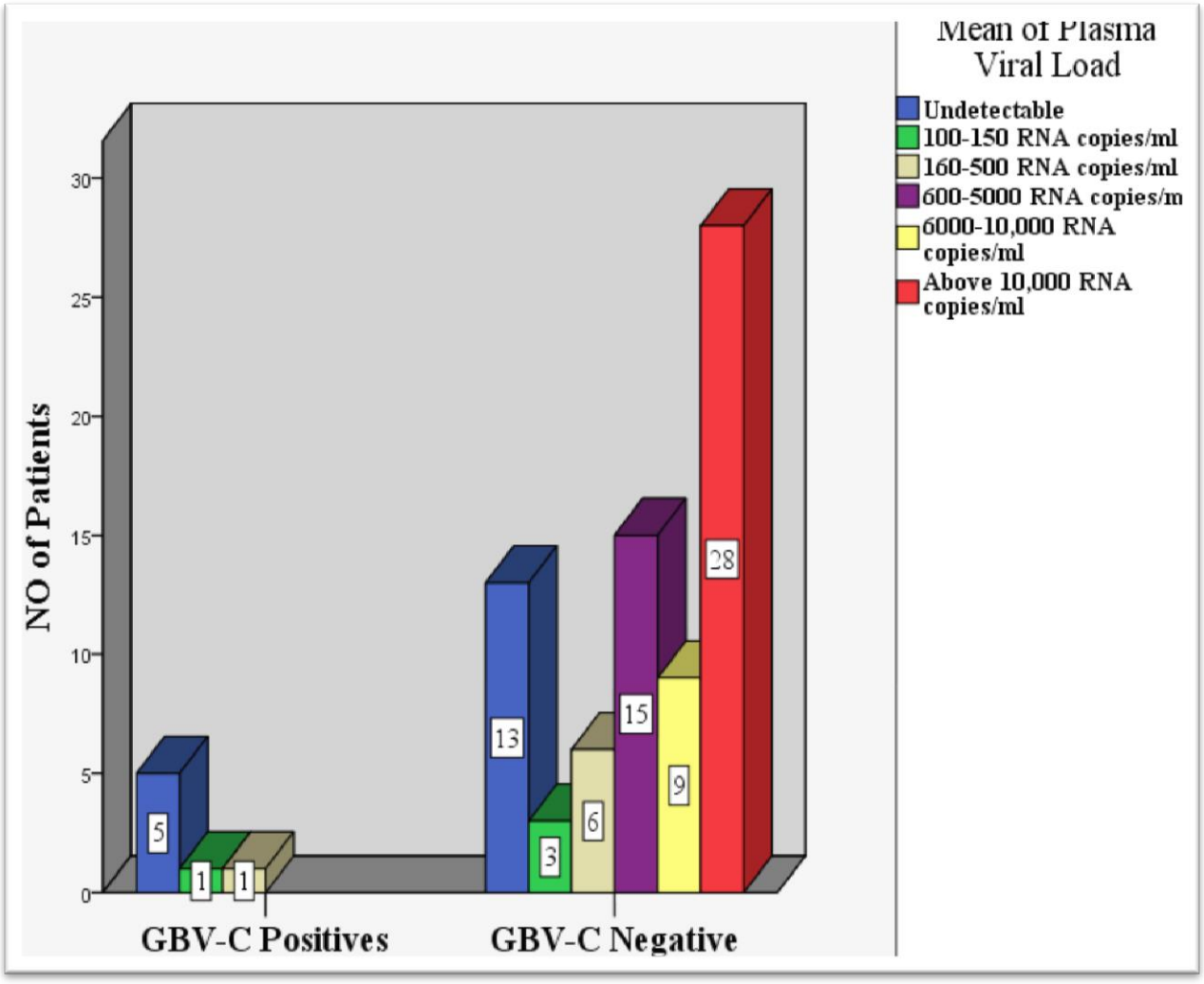

Figure 26: Average of the plasma viral load between GBV-C positive and negative

The mean number of CD4+ cells count between the GBV-C positive and GBV-C negative groups in HIV positive patients showed significant difference ($p < 0.05$). Of the seven GBV-C positive patients 3 (42.9%) of them, their CD4+ cells number were classified in the group (above 400 cells/mm^3), 2 (28.6%) in the group (301-400 cells/mm^3) and 2 (28.6%) in the group (201-300 cells/mm^3) as shown in (Figure 27).

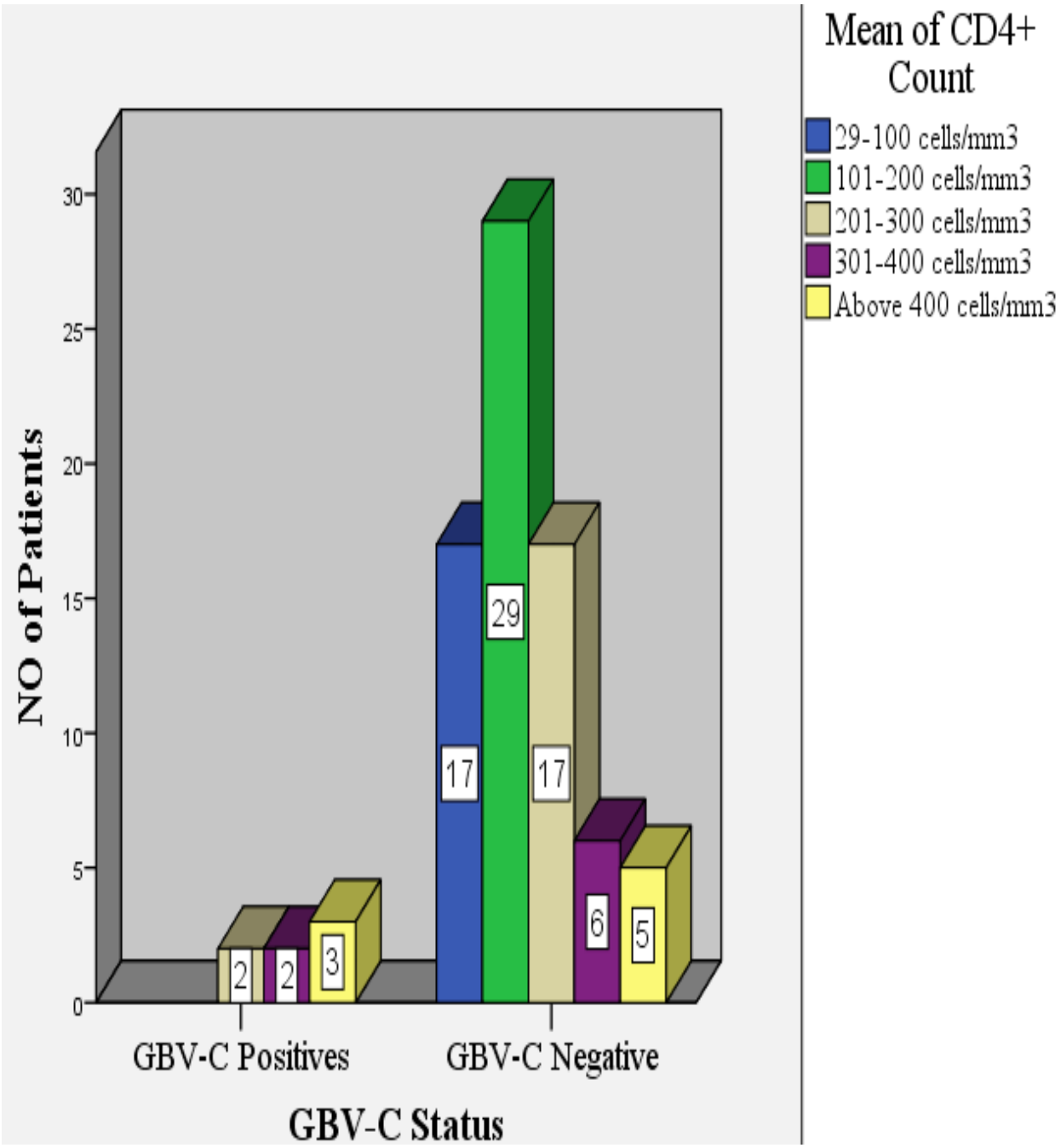

Figure 27: Average of the CD4+ count between GBV-C positive and negative

Moreover, as shown in (Table 13), five of the GBV-C positive participants are categorized in the first WHO clinical stage and two in the second stage based on the patient's symptoms which range from night sweat, acute diarrhea and cough. While GBV-C negative patients suffer from more sever diseases such like extra-pulmonary TB, severe bacterial infection, Kaposi sarcoma and others (Figure 28).

Table 13: Immunologic, virologic, and clinical outcomes

Characteristics	**GBV-C RNA**	
	Positive	**Negative**
Clinical Status		
WHO Clinical Stage 1	5	8
WHO Clinical Stage 2	2	21
WHO Clinical Stage 3	0	27
WHO Clinical Stage 4	0	18
CD4 Count (mean)	411 cells/mm3	200 cells/mm3
HIV Viral Load (mean)	54 Copies/ ml	14.676 Copies/ ml

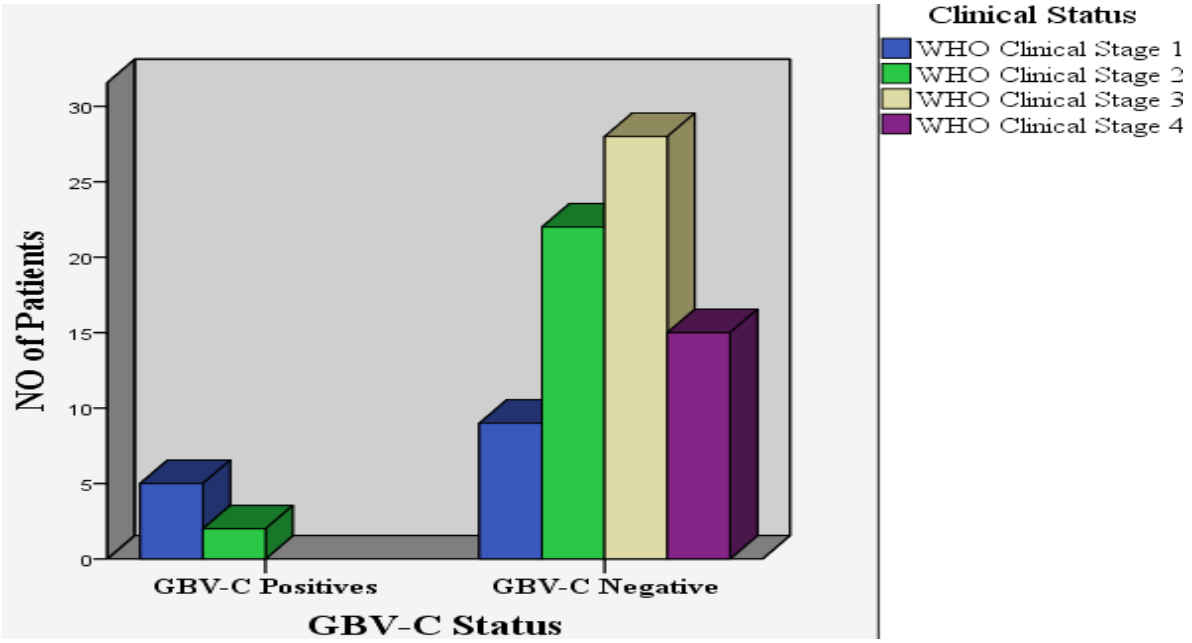

Figure 28: Classification of HIV patients according to the WHO clinical stages

Positive and negative GBV-C patients were sub divided into small groups based on age, sex and date of starting ART. The CD4 cell counts were compared in this sub groups to evaluate the impact of GBV-C on progression and treatment of HIV patients. The mean of CD4+ cells count over time increase more rapidly in GBV-C positive patients compared to GBV-C negative patients as shown in (Figure 29).

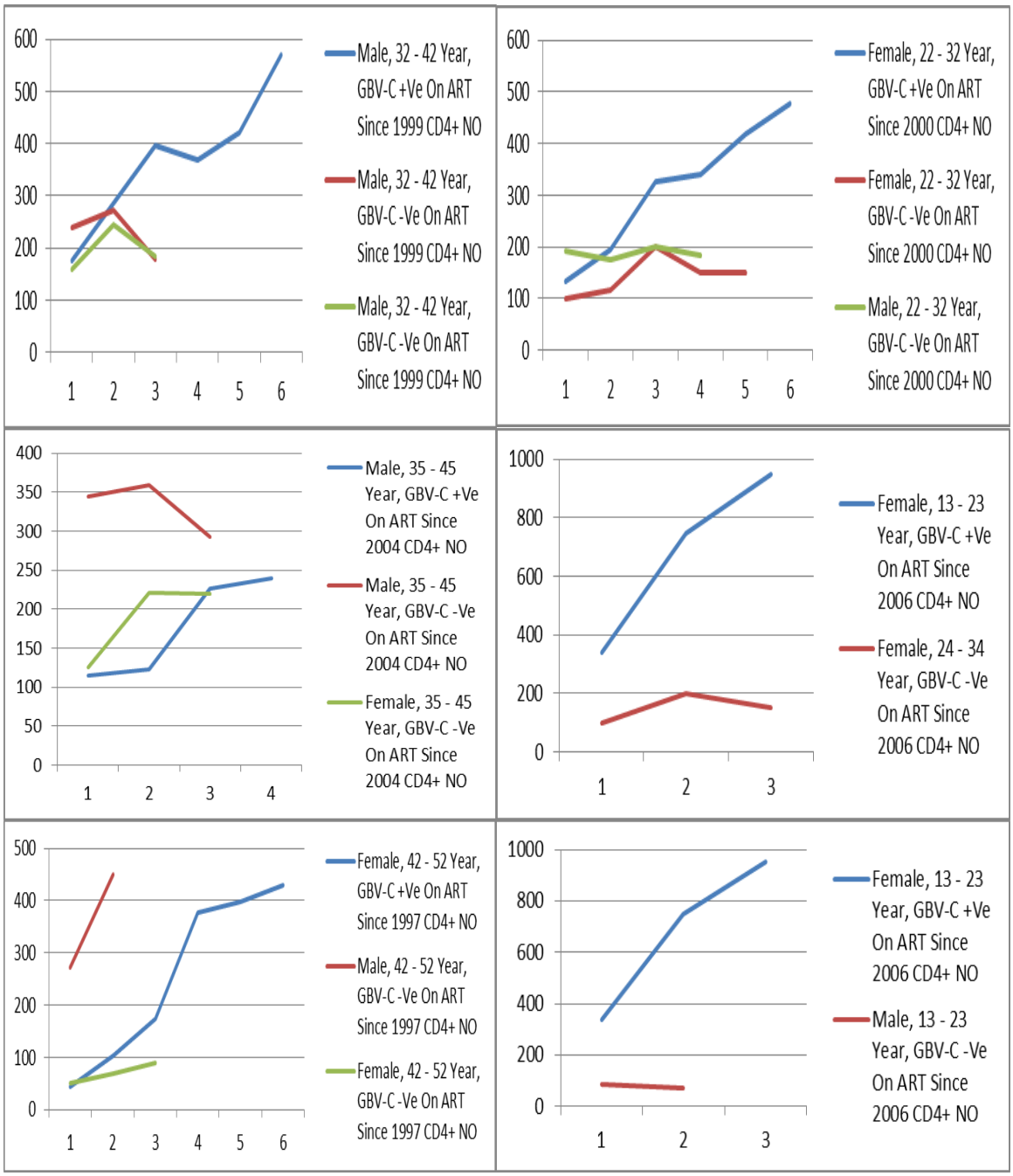

Figure 29: Comparison of CD4+ count between GBV-C positive and negatives

4.2.2. Viral hepatitis patients

The overall prevalence of GBV-C RNA were detected in 13 (12.9%) of the hepatitis patients, 2 (11.1%) of the HCV and 11 (13.25%) of the HBV patients as shown in (Figure 30). The rate of GBV-C RNA was significantly higher among HBV patients.

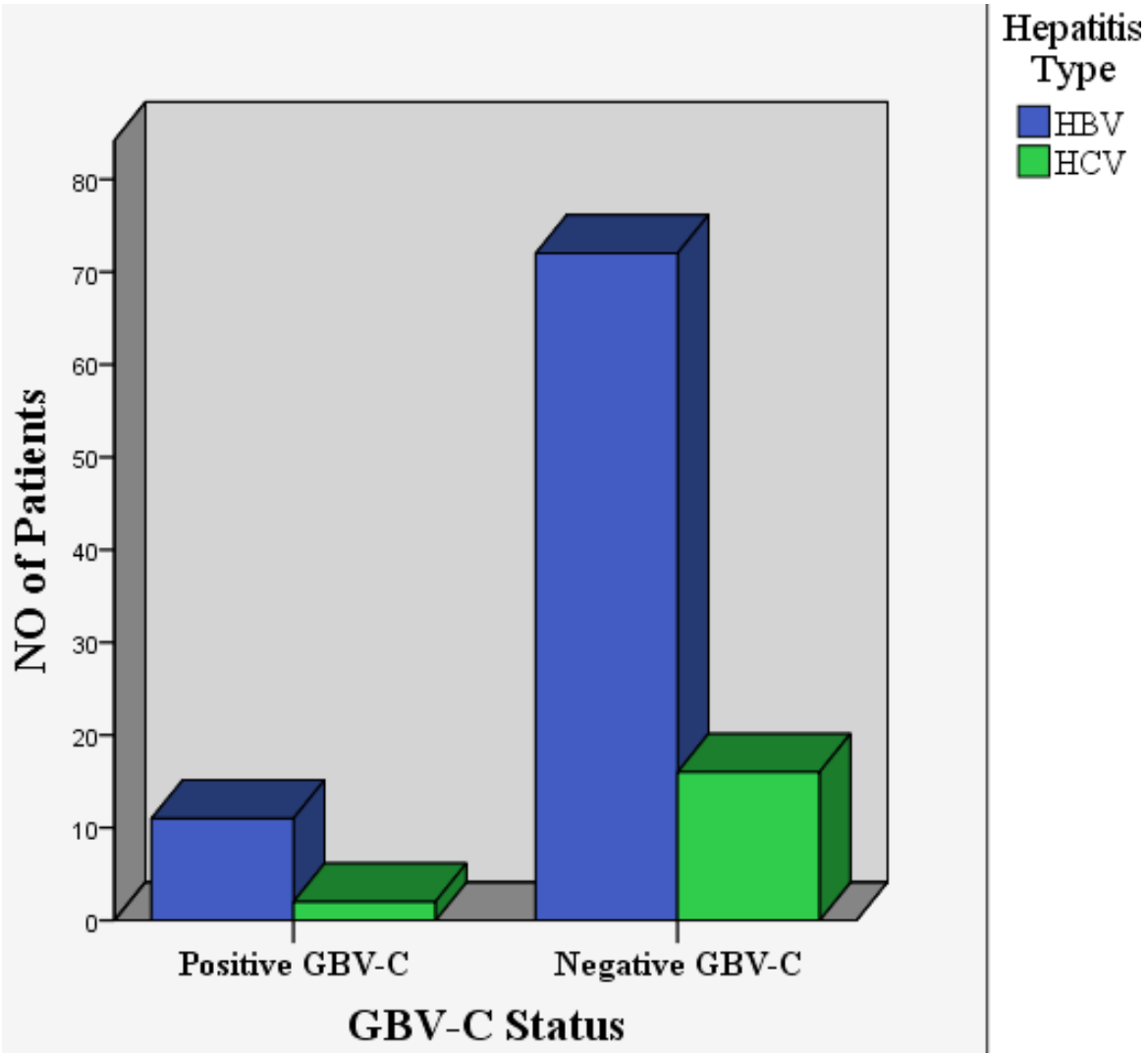

Figure 30: Prevalence of GBV-C among patients with viral hepatitis

Patients with HCV and GBV-C co-infection were in the age range 45-60 and there were no significant differences in sex and clinical status because all the HCV patients were in the end stage liver disease. Among the 83 patients with hepatitis B, there were no significant differences in sex, age and the clinical status between those with and without GBV-C infection (Table 14). GBV-C infection had no influence on the severity of chronic liver disease, because there were no significant differences in the clinical picture between GBV-C positive and negative patients.

Table 14: Socio-demographic and clinical characteristics of HBV & HCV patients with and without GBV-C infection

Characteristics	**GBV-C RNA (+)**		**GBV-C (-)**	
	HBV +	**HCV +**	**HBV +**	**HCV +**
No. (%)	11 (13.25%)	2 (11.1%)	72 (86.7%)	16 (88.9%)
Sex M/F	8/3	1/1	44/28	6/10
Age group				
15-24	2	0	11	0
25-34	3	0	32	0
35-44	5	0	18	6
45-60	1	2	11	10
Liver Status				
Asymptomatic	4	0	21	0
Mild Liver Injury(1)	5	0	28	0
Moderate Liver Injury(2)	2	0	19	7
Cirrhosis / HCC	0	2	4	9

(1): Normal liver scan and the level of the liver enzyme in the upper limit
(2): Fatty liver and high liver enzymes

4.2.3. GBV-C Genotype

To determine the predominant genotype, blood samples were recollected on 2017, from the same patients who were GBV-C positive in 2014. The virus was detected at a low level in some patient's serum. The samples show faint ~210bp band in agarose gel (Figure 31, 32).

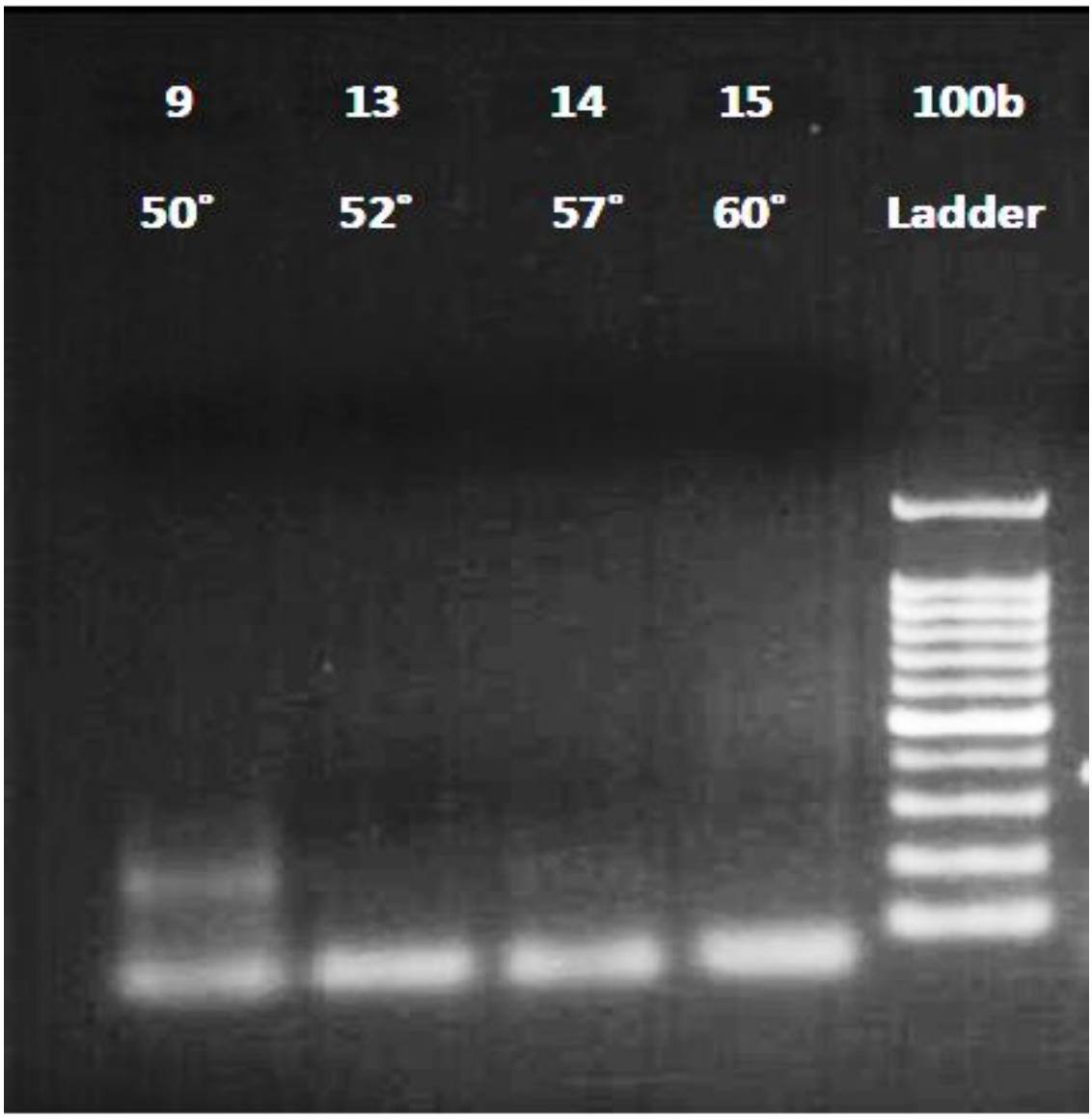

Figure 31: Gradient PCR (1st PCR)

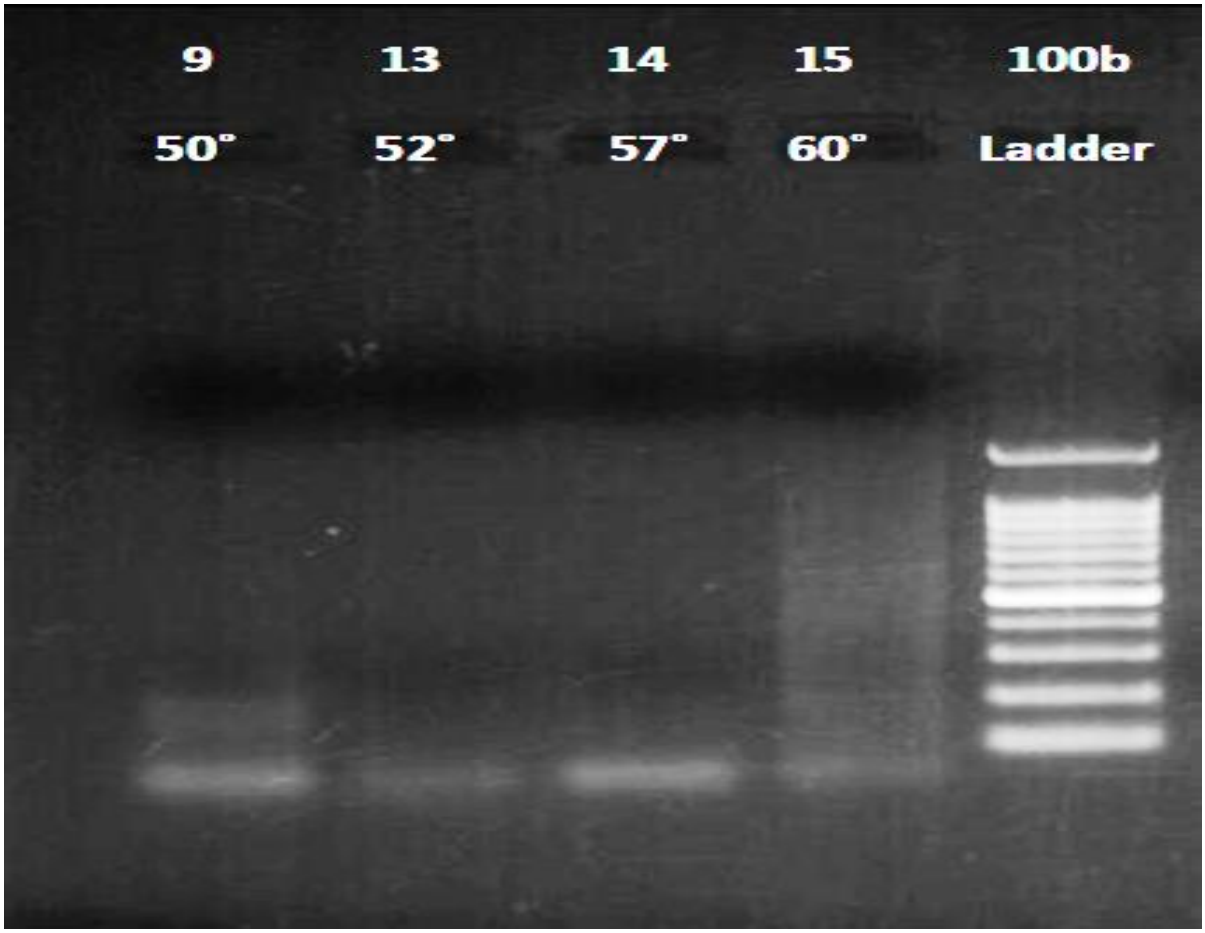

Figure 32: Gradient PCR (2nd PCR)

CHAPTER 5: DISCUSSION

5.1. General discussion

The human immunodeficiency virus (HIV) since its discovery in early 1980's has killed more than 40 million of persons in a world and despite efforts to control its spread is still stretched /projected worldwide. Despite 30 years of intense researches, it has not been possible to produce a vaccine, capable of providing protection against HIV its infection as yet. It is a retrovirus belonging to the genus Lentivirus and the Family of Retroviridea. These group of viruses use their RNA as template to produce complementary DNA that incorporate itself into host DNA and replicate themselves using the machineries' of the eukaryotic host cells. They are known for their long incubation period. They after one month of fast replication undergo to long clinical latency of 7-10years and could not be traced from circulation. During this period, they cause severe damage the immune and non-immune cells, including those in the mucosal surfaces; mainly depleting the CD4+ T-cells that choose and support the appropriate types of humoral and cellular responses against the specific infectious agents. At this stage of the infection the presence of the viruses is noticed only from the antibodies produced against them in plasma. Over time, the host response declines and the viral infection gains momentum both in its replication and suppression of the immunity of the host. At the final stage signs and symptoms of the disease AIDS where the individual becomes susceptible to all kinds of infections cancer (manifest, Maartens *et al*., 2014).

The AIDS pandemic evolved over time, in four main phases of evolution. It started patchily distributed in the population and spread to include large portion of the urban populations. Because of the risk involving behavior of individuals and groups accelerated to include those in sexual promiscuity and injection drug use, and finally escalated at an accelerated rate in definable risk groups in Asia and Africa. The last phase of stabilization occurred in some regions of the world: Western Europe, North America, and Australia, where success stories

in the control measures were documented. However, in other continents, Africa and Asia, the pandemic continued to escalation through the 1990's into the 21st century (Cohen *et al*., 2008).

With the advent of HAART, it was possible to change the alarming and acute disease to a manageable chronic phase all over the world. That has made a major impact on survival of HIV patient's dramatic morbidity and mortality in low and middle income countries as well. Other viral infections like HBV and HCV that share similar routes of transmissions with HIV: sexual, parenteral and perinatal, though they infect and replicate in hepatocytes remained a major clinical and public health challenges in HIV patients (Kourtis*et al.,* 2012; Ocama and Seremba, 2011; Leerantanpetch*et al.,* 2008; Modi and Feld, 2007).

Different from the above viruses is GBV-C, the major subject matter of this discussion. It is frequently found with the pathogenic hepatotropic viruses (HCV and HBV), and HIV but have never been established to have a role in disease causation (Yoshiba *et al.,* 1995; Alter, 1997a). Instead epidemiological, clinical and experimental observations have shown that they have no effect on the hepatotropic, HCV and HBV but HIV(Rey *et al.,* 1999; Wachtler*et al.,* 2000; Rendina*et al.,* 2001; Chams*et al.,* 2003; Alvarado *et al*., 2011; Rey *et al.,* 1999; Bhattarai and Stapleton, 2012). The very presence of the GBV-C improves the clinical and treatment outcomes of HIV infection and improves the life expectancy of infected individuals. In fact some strains are shown to have more pronounced impact on patient's survival (Heringlake *et al*., 1998; Lefrere *et al*., 1999; Yeo *et al.,* 2000; Xiang *et al.,* 2001; Tillmann *et al.,* 2001; Rodriguez *et al.,* 2003; Tillmann *et al.,* 2004; Antonucci*et al.,* 2005; Mosam*et al*., 2007; Bjorkman *et al.,* 2007; Maidana*et al.,* 2009).

It, more or less, shares the mode of transmission with all the three viruses mentioned above i.e. the blood-borne route is the commonest mode, although vertical and sexual transmission

is well documented and is cosmopolitan in distribution. As a result co infection with the hepatotropic viruses ranges between 12-15%. Its prevalence in the general population is 10 fold higher in African countries than in non-African countries. High prevalence of GBV-C is commonly found in subjects with frequent parenteral exposure and in groups at high risk of exposure to blood and blood products. Phylogenetically it is more related to HCV. Variant strains are found in different geographic regions. However it does not infect hepatocytes nor does it influence the severity of hepatitis due to other viruses. It instead infects and replicates like HIV in immune cells (Monocytes and Lymphocytes) and establishes an acute monotropic and chronic lymphotropic phases of infections and is sensitive to interferon's and therefore mobilize Innate and Acquired Immune Responses. The acquired immunity include: humoral-antibodies against envelop proteins labeled as Ab E1 and Ab E2 and cellular immune responses TH1 and TH2 CD_{4+}cells, and CD_{8+} and NK cells. Humans exhibit immunological diversity as suggested by the fact that different diseases follow a different course of illness and resolution in different individuals. This depends upon host factors such as genetic makeup and immunological response to an attack along with the capacity of pathogen viral genome to attack. What remains under examined is the role of potentially beneficial organisms which have the capacity to alter the course of illness by interacting with the body and its other normal residents or the new invaders (Sheikh e*t al*., 2014).

A couple of immunomodulatory or antiviral mechanisms can be induced by GBV-C and may play an interacting role with HIV coinfection. GBV-C-infected peripheral blood cells decreased expression of chemokine receptors (CCR5 and CXCR4) that found on the surface of CD4+ and CD8+ T-cells. A potential pathomechanism for this down-regulation of chemokine receptors is the E2-protein-induced release of RANTES from T lymphocytes by its binding to the CD81 receptor. Chemokine receptors are targets for HIV. Therefore, the result of decreased chemokine receptor expression is a decrease in HIV replication.

Surprisingly, anti-E2 antibodies were also able to inhibit HIV replication in vitro (Xiang 2006b), which is in contrast to the observation that anti- E2 seroconversion accelerates the clinical HIV progression. Another study showed that a peptide consisting of a 69-amino acid subunit from NS5A (which is a viral protein from GBV-C) was able to induce RANTES in vitro and therefore down regulates HIV replication. Complex disturbances of the cytokine profile have been described in HIV-infected individuals in vivo, but are less prevalent in individuals with GBV-C/HIV coinfection. Focusing on the innate immunity, normalized levels of CD69 (Fas-ligand) could be demonstrated on NK cells and were less pronounced on lymphocytes in GBV-C viremic HIV infected individuals, resulting in down-regulation of apoptosis (Bhattarai and Stapleton, 2012).

Moreover, the genetic diversity between GBV-C genotype and subtypes has a critical role on the GBV-C behavior, and this suggests that these differences in the virus within populations may produce different impact in the progression of HIV and response to the treatment (Berzsenyi *et al.,* 2005; Muerhoff *et al.,* 2003; Kaye *et al.,* 2005).

For that reason, it is likely that GBV-C genotype could at least partially account for the variable influence of GBV-C infection on HIV disease progression. Earlier analyses of GBV-C isolates of different regions have shown a different evolutionary pattern for these viruses (Erker*et al.,* 1996; Muerhoff *et al.,* 1996; Pickering *et al.,* 1997; Gimenez-Barcons*et al.,* 1998).

In Ethiopia, the land features are ranging from high mountains stand at 4550 m above the sea level (Ras Dashen) to Danakil 125 m below the sea level. Depression is also interesting to capture unique strains of GBV-C.

Therefore, the aims of this project are to study the impact, prevalence and genetic variant of GBV-C among various clinical statuses of HIV patients under HAART that includes: (HIV co-infected with TB, HIV mono infection), HBV, HCV and healthy control individuals.

5.2. Prevalence of GBV-C

This is the first study determining the frequency, genetic variant of GBV-C on disease progression of HCV, HBV, HIV patients under HAART and healthy controls in Ethiopia.

The patients were there seeking medical care or treatment. The study was conducted on HIV patients under HAART, collected from different referral hospitals and health centers in Addis Ababa. In this retrospective cross-sectional study GBV-C RNA was detected in 7 (8.6 %) patients out of the 81 HIV patients.

Our data was slightly less from other reports that show GBV-C prevalence of 14–45% among HIV patients (Kozal*et al.,* 2002). This could be due to the number of patients and the laboratory systems in the detection of the viral RNA and the antibody which was one of our limitations because the study was self-sponsored, and isolation of the virus was our goal in 150 serum sample of patients with HIV and TB, HIV mono-infection and HIV negative samples. Unfortunately, none of the sera were found to be positive for GBV-C. The reason could be due to mistake in identification of the sample because poor specimen handling and ambiguity in the labels of many samples, repeat thawing them as they were used for other studies as well before us. Moreover, we have not used the Ab detection because of budget limitation. The detection of the viral RNA with reverse transcription polymerase chain reaction (Ruiz *et al.,* 2006), indicated active infection, however the clearance of the virus is associated with the development of antibodies against envelope glycoprotein (E2) which were supposed to be detected by immunoassays (Thomas *et al.,* 1998). This method has the power to identify 30 - 65% of GBV-C antibodies against the virus E2 enveloped protein from

HIV infected patients; it would have indicated prior GBV-C infection (Thomas *et al.,* 1998; Tillmann *et al.,* 2001b). Furthermore, in a study on GBV-C persistent among HIV-Infected Men, E2 antibody were not developed in most of patients (Williams *et al*., 2004).

Transmission is another important factor for the high incidence of GBV-C. The virus is more prevalent among homosexuals rather than heterosexuals individuals (Scallan*et al.,* 1998). The low prevalence in not a surprise since there is no homosexual or drug abuse participant among our samples. GBV-C incidence was relatively higher in the age group of 31 – 40 years and in female, but not statistically significant *($p > 0.05$).* Our results were similar to those reported by (Rey *et al.,* 1999; Keyvanil*et al.,* 2010) which showed no difference in GBV-C RNA prevalence between male and female and their mean ages were similar in GBV-C positive and GBV-C negative groups. However, the prevalence of GBV-C RNA was lower in patients over 50 years compared to other age groups. In our study, there were ten patients in the age group (51-60 years) and only one was GBV-C RNA positive. Similarly higher prevalence was seen among illiterate, primary school and married participants but also not statistically significant.

To study the prevalence of GBV-C among HBV and HCV patients, a total of 101 subject's samples with liver disease were included in this study collected from Adera internal medical specialty center.

Analysis of the viral hepatitis patients sera showed 83(82.17%) to be positive for HBsAg antigen and 18 (17.8%) for HCV antibodies. Most of the HBV patients were male 52 (62.7%) and 58 (69.87%) of the participant were aged between (25 – 44) years. HCV patients 11 (61.1%) were females and 7 (38.9%) males, 12 (66.7%) were aged between (45-60) years and the remaining were in the age group (35 – 44) years.

Previous reports from Ethiopia (Abel and Solomon, 2013) have documented seroprevalence of HBsAg and HCV Ab is 35.8% and 22.5% respectively among hepatitis patients. In this study seroprevalence of HCV in the area over the last 3 year period was much lower than the previous studies. Interestingly, in this study we observed that most of HCV patients were females and older than HBV patients as in the other studies by (Abel and Solomon, 2013). In relation to HBsAg the reasons for the relatively higher rate of seroprevalence in our study compared with other studies cannot be completely discerned.

From the 83 HBsAg positive samples, 11 (13.25%) were also positive for GBV-C RNA while from the 18 anti-HCV positive samples, 2 (11.1%) were positive for GBV-C. Our study showed that the co-infection rate of GBV-C among hepatitis patients was significantly higher ($P < 0.05$) in HBV than in HCV patients.

Our findings were consistent with other reports; Birgit et al. reported the incidence of co-infection with GBV-C in chronic HCV to be 11% (Birgit *et al.,* 1998). A study on the northeastern Thai blood donors, the prevalence of GBV-C among HBsAg and anti-HCV positive patients were 10% and 11%, respectively (Barusruk*et al*., 2006), which in the case of HCV samples is close to our findings but lower than the co-infection rate of GBV-C and HCV in our study.

Prevalence studies of GBV-C co-infection with HCV and HBV varies from place to other. Colombian blood donor had only 5.06% of HBsAg-positive and 3.2% of HCV Ab positive patients had GBV-C co-infection (Alvarado-Mora *et al.,* 2011).

Hofer et al. reported the prevalence of GBV-C among chronic hepatitis C patients undergoing antiviral therapy as 12.2% by (Hofer *et al.,* 2011) which is close to our findings. In a study on hemodialysis patients in Iran, 5% of GBV-C infected patients were also coinfected by HCV (Ziaee*et al.,* 2007), much lower than our results. Whereas in Turkey it was 7% for HCV and

29% for HBV (Alcali*et al.*, 2006), which is dissimilar from the results of the present study. While in United Arab Emirates the co-infection of this GBV-C with HBV and HCV was 5.7% and 14.3%, respectively (Abu Odeh*et al.,* 2005), close to our findings.

There is a large difference in the prevalence of GBV-C infection in various geographical regions. This variation could be due to the size of the sample population involved in the study, methodology used to detect GBV-C infection, demographic and clinical features of patients, and different patterns of transmission of virus around the world.

The prevalence of GBV-C in our study is higher among HBV than HCV; this might be duo to the impact of HCV treatment on GBV-C RNA clearance (Masaru *et al* 1998; Berg *et al.,* 1996b). The presence of GBV-C infection among hepatitis patients was not associated with sex and age, which correlated with other reports (Alvarado-Mora *et al.,* 2011; Tanaka *et al.,* 1998b). GBV-C incidence was relatively higher in the age group of 35 – 44 years and in male, but the difference was not statistically significant ($p > 0.05$) similar to other report (Jeng-Fu *et al.,* 2006).

Among Healthy controls, the data about the prevalence of GBV-C among healthy system is very limited. There available reports were done among blood donors from different countries around the worlds. The mean prevalence of GBV-C in blood donors is 4.8% and is different in diverse world regions (Ramezani*et al.,* 2008). The immunecompetent individuals usually clear the virus within the first years after infection, and only 25% of individuals infected with GBV-C develop antibody which makes the interpretation of the result difficult (Bourlet *et al.,* 1999; Pilot-Matias *et al.,* 1996). However, the high mean prevalence of GBV-C among healthy individuals was found by using anti-E2 antibody (Kleinman*et al.,* 2001).

In this study all the 70 healthy participants were GBV-C RNA negative by RT-PCR. A recent case-control study to determine the association between GBV-C and unknown chronic

hepatitis in Iran, the GBV-C were positive in one participant out of the 35 hepatitis patients. However, GBV-C was negative among the 50 control group, which is similar to our finding. None of the healthy subject in this study had a history of blood transfusion, were intravenous drug users, or patients on hemodialysis. Behavior is the main source of variability in the assessment of GBV-C prevalence.

Moreover, the samples those were collected from Public Health and Research Institutes, were stored and been used for research purpose for several years. High prevalence of GBV-C were reported in subjects such as haemodialysed patients (6.9%-16%), hemophiliacs (14.3%-35.2%), thalassemics (35%), intravenous drug users (25%-28.8%) and patients with aplastic anemia (26.3%) (Brown *et al.,* 1997b; Hayashi *et al.,* 1998; Lamproy*et al.,* 1999; Kondili*et al.,* 2001; Wu *et al.,* 1997).

However, in our study the control groups were healthy and with no history of blood transfusion and this could be the fact that all of them were GBV-C RNA negative beside the small sample size.

5.3. Impact of GBV-C among HIV patients under HAART

The mean CD4+ count and viral load was significantly different among GBV-C positive patients in comparison to the negative patients. The CD4+ were higher among GBV-C positive patients and the HIV viral load was low or undetectable, which is in agreement with previous reports findings of GBV-C on CD4+ and HIV viral load (Heringlake *et al.,* 1998; Yeo *et al.,* 2000). Previous studies suggested that HIV patients with GBV-C coinfection who received HAART had a more favorable outcome, with delayed progression to AIDS, compared to those with HIV mono infection (Souza *et al.,* 2006; Tillmann *et al.,* 2001a; Lefrere *et al.,* 1999; Yeo *et al.,* 2004; Williams *et al.,* 2004; Xiang *et al.,* 2001).

Long-term persistence of GBV-C viremia seems to be the key component for the beneficial outcome of GBV-C to HIV coinfected patients (Williams *et al.,* 2004; Xiang *et al.,* 2001). The mechanisms of GBV-C interference with HIV replication within immune cells (Tillmann *et al.,* 2005) and immunologically delay progression of HIV infection through induction of various cytokines and other soluble factors (Xiang *et al.,* 2004) or by maintaining an intact T-helper-1 cytokine profile (Nunnari *et al.,* 2003).

The interference of GBV-C viremia can produce the beneficial effects on HIV progression and responses to HAART has now been controverts by many studies (Brumme*et al.,* 2002; Van der *et al.,* 2005; Rodriguez *et al.,* 2003; Antonucci*et al.,* 2005; Toyoda *et al.,* 2005). The differences in degree of response in the findings of the studies may originate from differences in patients recruited, at the design level, assays methods of virologic and immunologic responses to HAART employed, and persistence strains of GBV-C coinfection. All our patients are under HAART. To assess the effect of GBV-C, the HIV patients under HAART were followed by their fluctuation on the CD4+ and HIV viral load over two years' time.

Attempt was also made to compare the GBV-C positive and negative on the basis of HAART starting date, age group and gender. Our data showed a high increase in the CD+ number among all the GBV-C positive patients compared to GBV-C negative patents that show decline in the CD4+ number or slow improvement. Moreover, five of the GBV-C positive participants were categorized in accordance with WHO clinical staging system of the HIV patients in the first stage, while the two remaining in the second stages. However, this classification is based on the patient's symptom which ranges from night sweating, acute diarrhea and cough while the GBV-C negative patients suffer from more severe diseases.

To study the impact of GBV-C clearance among the seven HIV patients that were GBV-C positive before four years, the CD4+ count, viral load and general clinical status of the

patients required continues evaluation. The clinical statuses of five of the patients were generally good and stable. The CD4+ count of 3 patients were above 530 cell/mm^3, and the other two patients were 300 and 320 cell/mm^3 respectively.

During the 4 years observation time the CD4+ count of five of the seven patients were as from the beginning of the study, and the HIV viral load were below the detection level. The clearance of GBV-C among those patients was not associated with a short-term loss of HIV control. The remaining two patients were having a drop in the CD4+ count from 480 and 302 to 360 and 186 respectively, while their viral load that were at the beginning of the study 150 and 226 copies/ml, had raised to 418719 and 15083 copies/ml respectively. Moreover, their frequency of visit to the hospital was almost double the visit time of the above five patients who come on their six month regular checkup.

Several studies have suggested that the effect of GBV-C co-infection on HIV disease is not uniform. It changes during the course of HAART treatment. Persistence of GBV-C RNA over five to six years after seroconversion was associated with a significant survival benefit, whereas the clearance of GBV-C RNA were associated with poor HIV prognosis (Williams *et al*., 2004), like the two patients in our case. On the other hand, among the other five HIV patients GBV-C most likely was present, but the result cannot be attributed to be completely due to the presence of GBV-C all alone, for the patients were under HAART during the four years follow up period. Similar results have been reported elsewhere (Pirothet *al*., 2008).

Since HAART does not reduce the levels of immune activation to that seen in healthy individuals, GBV-C may provide clinical benefit to HIV patients under treatment. It is, therefore, of great interest to understand the mechanisms involved in this interaction, because it could result in progress in our understanding of viral pathogenesis and in a contribution towards the development of novel HIV-1 treatment strategies. Understanding the

mechanism(s) by which GBV-C might reduce CD4+ activation may provide novel approaches to address the persistent T cell activation observed in HIV-infected people.

5.4. Impact of GBV-C on HBV and HCV

Although, GBV-C has been isolated from patients with liver disease during the search of non-A, non-B, non-C, non-E hepatitis, but its impact on liver disease is still controversial (Simons *et al.,* 1995a; Linnen *et al.,* 1996).

Regarding the liver status of the hepatitis patients, we found that 33 (39.8%) of the HBV patients were with mild liver injury, because their level of the liver enzymes was in the upper limit, while 25 (30.1%) were asymptomatic and their liver enzyme level were within the normal range. In contrast there were 11 (61.1%) of HCV patients were suffering from cirrhosis and or hepatocellular carcinoma while among HBV patients there are only 4 (4.8%) had complication and there enzyme levels were out of the normal range. Moderate liver injury which include sever fatty liver and raised level of the liver enzymes have been found in 21 (25.3%) of HBV and 7 (38.9%) of HCV patients.

A higher prevalence of GBV-C infection was found in patients with hepatocellular carcinoma (Muller *et al.,* 1997). Another studies supported the hypothesis of an association between GBV-C infection and HCC, but the causality of the association was unclear (Tagger *et al.,* 1997). Jeng-Fu et al. found that GBV-C coinfection increase the risk of HCC development in HBV patients, but not in HCV carriers (Jeng-Fu *et al.,* 2006) in contrast to our study.

In the present study, there were no significant differences among GBV-C positive and negative concerning the health status. Five of HBV and GBV-C positive patients were suffering from mild liver injury and the remaining where asymptomatic. On the other hand, the two GBV-C patients coinfected with HCV were suffering from HCC and they were the ones described by the physician as that they are at the end stage of the liver disease. In

agreement with our study, Kao et al. and Lightfoot et al. showed that GBV-C infection dose not play part in HCC development (Kao *et al.,* 1997b; Lightfoot *et al.,* 1997).

Some studies have shown that GBV-C infection is associated with significantly less compensated and decompensated cirrhosis, and with improvement in cirrhosis-free survival among patients with HBV or HCV (Chams*et al.,* 2003; Berzsenyi *et al.*, 2007). In agreement with previous reports GBV-C infection has no influence on the severity of chronic liver disease among HBV and HCV co-infection (Alter, 1997a; Tanaka *et al.,* 1998a; Grassi*et al.*, 2000; Ramezani*et al.,* 2008).

Some research stated that GBV-C infection led to reduced liver disease in HIV/HCV coinfected patients but not in HCV mono-infection (Berzsenyi *et al.,* 2007; Schwarze-Zander *et al.,* 2006; Berzsenyi *et al.,* 2011).

Similar to other reports, our study demonstrated that GBV-C did not appear to increase liver injury in patients with chronic HCV infection, as measured by the levels of ALT and AST (Tan *et al.,* 1999; Pereira *et al.*, 2002).

In China, a study of 41 acute and 67 chronic hepatitis patients with unknown etiology, GBV-C RNA was detected in 6 (14.6%) and 12 (17.9%) patients, respectively (Wang *et al.*, 1997). More recent studies among patients with acute unknown hepatitis, the prevalence of GBV-C were (29.1%) and they were suffering from very severe clinical syndrome (Tassopoulos *et al.,* 2008).

However, similar to our findings, numerous studies have expressed their doubt on the fact that GBV-C was responsible for chronic hepatitis (Hollingsworth *et al.,* 1998; Guilera*et al.,* 1998).

5.5. GBV-C genotype in Addis Ababa

This component of the thesis work was done in Korea and Egypt. The plasma from GBV-C positive patients were stored at -20, and transported to Institute of Endemic Diseases at Khartoum University. There, RNA was extracted and cDNA was synthesized for gene expression analysis. Since there is no sequencing machine at the Institute, the final step, which includes the amplification and sequencing of the viral genome, was sent to Macrogen laboratories in South Korea for gene expression analysis. However, the sequencing result was poor, possibly due to low copy number of the virus. Therefore it was thought to use fresh samples from the same patients whom the previous samples were taken to alleviate any problem.

The second blood samples for the purpose of this was collected from the same GBV-C positive patients and relatively better volume of plasma was harvested. The rest of the work was done at the Color Medical Lab in Egypt. Different extraction methods of RNA, were employed to make sure enough viral products are availed for amplification and sequencing. Unfortunately the result was the same. The result showed only two of the seven HIV positive patients, which indicates the clearance of the virus. Therefore it was not possible to sequence the virus and its limitation of the thesis work.

CHAPTER 6: SUMMARY AND RECOMMENDATIONS

Humans are infected continuously by a vast of viruses that not associated with any obvious pathologic outcome and/or even provide some unrecognized benefits. Infection with two or more viruses is phenomenon common worldwide. It is considered a major public health problem and plays a major role in disease progression (Koziel and Peters, 2007). HIV, HBV and HCV are among the most common chronic viral infections and among the top ten causes of mortality in the world (WHO, 2005). HBV and HCV infections increase mortality and morbidity in HIV patients. The coinfection between these viruses increases the progression of liver fibrosis to cirrhosis and hepatocellular carcinoma which is more common among HIV-HBV or -HCV coinfection (Maller*et al.,* 2011; Lacombe and Rockstroh, 2012). Furthermore, human herpesvirus infection is associated with chronic immune activation and inflammation among HIV patients (Carr and Tomanek, 2006).

On the other hand infection with GBV-C has appositive impact on HIV patients by slowing the disease progression, and better health outcomes in HIV-1 positive individuals (Tillmann *et al*., 2001a; Xiang *et al*., 2001). The aim of this study was to determine the prevalence, predominant genotype and the association of HGV/GBV-C in the clinical outcome among viral hepatitis patients and HIV patients under HAART.

This study showed that the prevalence of GBV-C among viral hepatitis and HIV patients under HAART were 12.9% (n=13) and 8.6% (n=7) respectively, while all the healthy control individuals were negative.

Among HIV patients with GBV-C, patients have shown good response to HAART as measured by significantly higher CD4+ count and low viral load. Furthermore, among the seven GBV-C positive patients, five of them were categorized in the first WHO clinical stage and the two remaining in the second stage, none were in stage 3 and 4 where as those with

HIV virus only. GBV-C prevalence was relatively higher in the age group of 31 – 40 years and in female, thought the difference was not statistically significant. Likewise, higher prevalence was seen among illiterate, primary school and married participants.

Among viral hepatitis patients, from 83 HBsAg positive samples, 11 (13.25%) were positive for GBV-C and while from 18 anti-HCV positive samples 2 (11.1%) were positive for GBV-C.

Our study showed that the co-infection rate of GBV-C RNA among viral hepatitis patients was slightly higher among HBV patients 13.25% (n=11) than HCV patients 11.1 (n=2). Mostly in the age group of 35 – 44 years and in males, but the difference was not statistically significant. GBV-C infection among HBV positive patients had no influence on the severity of the disease in comparison with the negative patients. While among HCV positive patients, the fact that the liver function test and abdominal ultrasound were similar in all chronic HCV patients regardless of GBV-C infection suggests that GBV-C didn't cause observable additive hepatic injury in those patients. Based on these observations one can say that GBV-C infection had no influence on the severity of chronic liver disease among HBV and HCV patients. While among HIV patients the coinfection reduces the viral load, increase the number of CD4+ cells and better response to treatment.

Therefore, based on this study the following recommendations are made:

1. In order to learn the pathology, diversity, biology and the prevalence of GBV-C as desired a cohort study on a large population over a long period among various viral diseases is necessary.
2. A long follow up study is required to characterize the acute and long-term impact of GBV-C acquisition on virological, immunological, and clinical consequences of HIV infection.

3. Determine whether GBV- C could be used as an alternative vector to express HIV antigens such as adenovirus or canary pox vectors that were used in RV144, the vaccine trial that showed 31% effective in preventing HIV infection.
4. In order to have a comprehensive picture of GBV-C strain distributed in the country, it is necessary to characterize the predominant genotype in all regions of Ethiopia.
5. To determine the role of GBV-C in HIV progression, humanized model animal would allow conducting controlled experiment.
6. Identification of the mechanisms GBV-C uses to inhibit HIV progression to be used as a novel therapeutic agent in combination with HAART.
7. Vaccination against HBV is instrumental in the prevention of viral chronic liver disease, and is recommended for all newborns and individuals who are at increased risk for infection.
8. GBV-C remain an attractive agent for intervention in viral infections not only HIV but also Ebola or any emerging viruses, because of its unique features: sharing the mode of many pathogenic viruses and able to induce both the innate and acquired immunity (harmless vector).
9. The land features of Ethiopia ranging from Ras Dashen mountain to Danakil depression below sea level is also interesting to capture unique strains of GBV-C.

REFERENCES

Abebe A (2003). Sero-epidemiology of Hepatitis B Virus In Addis Ababa, Ethiopia: Transmission Patterns And Vaccine Control. *Epidemiol Infect.* **131**:757 –770.

Abel Girma Ayele and Solomon Gebre-Selassie (2013). Sero-prevalence of HBV and HCV among chronic liver disease patients visiting OPD in public hospitals in Addis Ababa. *ISRN*. (563821):1-7.

Abrams D, Lévy Y, Losso MH, Babiker A, Collins G, Cooper DA, Darbyshire J, Emery S, Fox L, Gordin F, Lane HC, Lundgren JD, Mitsuyasu R, Neaton JD, Phillips A, Routy JP, Tambussi G and Wentworth D (2009). Interleukin-2 therapy in patients with HIV infection. *N Engl J Med.* **361**:1548-59.

Abu Odeh RO, Al-Moslih MI, Al-Jokhdar MW and Ezzeddine SA (2005). Detection and genotyping of GBV-C virus in the United Arab Emirates. *J Med Virol.* 76: 534- 40.

Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R and Ruggiero G (2001). Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology*. **33**:1358–64.

AIDS Health care Foundation (2016). *Facts at a Glance*, (Africa/ Ethiopia). www.aidshealth.org/#/countries

Akcali S, Sanlidag T and Ozbakkaloglu B (2006). Prevalence of GBV-C/ hepatitis G virus viremia among chronic hepatitis B, chronic hepatitis C and hemodialysis patients in Turkey. *Ann Saudi Med.* **26**: 68-9.

Alavian S (2009).Hepatitis C in hemodialysis patients. *Gastroenterol*. **15**(6):641-6.

Alcalde R, Nishiya A, Casseb J, Inocencio L, Fonseca LA, and Duarte AJ (2010). Prevalence and distribution of the GBV-C/HGV among HIV-1-infected patients under anti-retroviral therapy. *Virus Res.***151**:148–52.

Alter HJ, and Bradley, D.W (1995). Non-A, non-B hepatitis unrelated to the hepatitis C virus (non - ABC). *Sem. Liv. Dis.* **15**:110-120.

Alter HJ (1996). The cloning and clinical implications of HGV and HGBVC. *N Engl J Med.* **334**: 1536-7.

Alter HJ (1997a). G-pers creepers, where'd you get those papers? A reassessment of the literature on the hepatitis G virus. *Transfusion.* **37**(6):569-572.

Alter HJ, Nakatsuji, Y, Melpolder, J, Wages, J, Wesley, R, Shih, and Kim JP. (1997b). The incidence of transfusion-associated hepatitis G virus infection and its relation to liver disease. *N EngI. J. Med*. **336**: 747-754.

Alter MJ, Gallagher M, Morris, TY Moyer, LA Meeks EL, Krawczynski K, Kim IP, Margolis H and for the Sentinel Counties Viral Hepatitis Study Team (1997c). Acute non-A-E Hepatitis in the United States and the role of Hepatitis G Virus infection. *N Eng. J. Med*. **336**: 741-746.

Alvarado-Mora MV, Botelho L, Nishiya A, Neto RA, Gomes-Gouvea MS, Gutierrez MF, Carrilho FJ and Pinho JR (2011). Frequency and genotypic distribution of GB virus C (GBV-C) among Colombian population with Hepatitis B (HBV) or Hepatitis C (HCV) infection. *Virol J*. **8**: 345.

Andrew M. Tager, Michael Pensiero, and Todd M. Allen (2013). Recent Advances in Humanized Mice: Accelerating the Development of an HIV Vaccine. *JID.* (2):208.

Anonymous (1947). Homologous serum hepatitis. *Lancet*. **2**: 691e692.

Antonucci G, Girardi E, Cozzi-Lepri M. R. Capobianchi, G. Morsica, P. Pizzaferri, N. Ladisa, L. Sighinolfi, A. Chiodera, M. Solmone, E. Lalle, G. Ippolito and A. Monforte (2005). Response to HAART and GB virus type C coinfection in a cohort of antiretroviral-naive HIV-infected individuals. *Antivir Ther (Lond).* **10**(1):109-117.

Arase Y, Kobayashi M, Suzuki F, Suzuki Y, Kawamura Y, Akuta N, Kobayashi M, Sezaki H, Saito S, Hosaka T, Ikeda K, Kumada H, and Kobayashi T (2013). Effect of type 2 diabetes on risk for malignancies includes hepatocellular carcinoma in chronic hepatitis C. *Hepatology*. **57**:964–973.

Arie J, Jangu E, Paul G, Barry S, Philip M (2009). Principles and practice of clinical virology, 6th edition. *John Wiley & Sons Ltd, Singapore.* 321-324.

Arthur LO, Bess Jr JW, Sowder 2nd RC, Benveniste RE, Mann DL, Chermann JC, Henderson LE (1992). Cellular proteins bound to immunodeficiency viruses: implications for pathogenesis and vaccines. *Science.* **258**:1935–8

Asemahagn MA, Aragaw A, Agumas Y, Molla M, Mulugeta Y, Mekonnin A and Degu G (2018). Perceived Risk Factors of HIV Infection and ART Adherence at Zewditu Memorial Hospital, Addis Ababa, Ethiopia: A Survey of People Living with HIV/AIDS Experiences. *Ann Med Health Sci Res*. 8: 105-110.

Badley AD, Parato K, Cameron DW, Kravcik S, Phenix BN, Ashby, A. Kumar, DH Lynch, J Tschopp and JB. Angel (1999). Dynamic correlation of apoptosis and immune activation during treatment of HIV infection. *Cell Death & Diff*. **6**: 420– 32.

Barbara Ensoli, Aurelio Cafaro, Paolo Monini, Simone Marcotullio and Fabrizio Ensoli (2014). Challenges in HIV vaccine research for treatment and prevention. *FIMMU.* (5)417.

Barth RE, Huijgen Q, Taljaard J and Hoepelman AI (2010). Hepatitis B/C and HIV in sub-Saharan Africa: an association between highly prevalent infectious diseases. A systematic review and metaanalysis. *Int J Infect Dis.* **14**: e1024–e1031.

Barusruk S and Urwijitaroon Y (2006). High prevalence of HGV coinfection with HBV or HCV among northeastern Thai blood donors. *Southeast Asian J Trop Med Public Health.* **37**:289-293.

Bayer M.E, Blumberg B.S, and Werner B (1968). Particles associated with Australia antigen in the sera of patients with leukaemia, Down's syndrome and hepatitis. *Nature*. **218** (5146), 1057e1059.

Berg T, Naumann U, Fukumoto T, Lobeck W, Bechstein P, Neuhaus P, Lobeck H, Hohne M, Schreier E and Hopf U (1996a). GB virus C infection in patients with chronic hepatitis B and C before and after liver transplantation. *Transplantation*. **62**: 711-714.

Berg T, Dirla U, Naumann U, Heuft HG, Kuther S, Lobeck H, Schreier E and Hopf U (1996b). Responsiveness to interferon alpha treatment in patients with chronic hepatitis C coinfected with hepatitis G virus. *J. Hepatol*. **25**: 763-768.

Berger EA, Murphy PM and Farber JM (1999). Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. *Annu Rev Immunol.* **17**:657-700.

Bernard JC Macatangay1 and Charles R Rinaldo (2010). Regulatory T cells in HIV immunotherapy. *HIV Ther.* **4**(6): 639–647.

Berzsenyi MD, Bowden DS and Roberts SK (2005). GB virus C: insights into co- infection. *J Clin Virol*. **33**:257–66.

Berzsenyi MD, Bowden DS, Kelly HA, Watson KM, Mijch AM, Hammond RA, Crowe SM and Roberts SK (2007). Reduction in hepatitis C-related liver disease associated with GB virus C in human immunodeficiency virus coinfection. *Gastroenterology.* **133**(6):1821–30.

Berzsenyi MD, Woollard DJ, McLean CA, Preiss S, Perreau VM, Beard MR, D Scott Bowden, BC Cowie, S Li, AM Mijch and SK Roberts (2011). Down- regulation of intra-hepatic T-cell signaling associated with GB virus C in a HCV/HIV co-infected group with reduced liver disease. *J Hepatol*. **55**(3):536–44.

Bhanich WS, RS Remis, J Raboud, M Millson, J Tappero, R Kaul, P Kulkarni, MS McConnell, PA Mock, JM McNicholl, N Vanprapar, S Asavapiriyanont, N Shaffer and S Butera (2009). Mother-to-Child Transmission of GB Virus C in a Cohort of Women Coinfected with GB Virus C and HIV in Bangkok, Thailand. *Journal of Infectious Diseases*. **200**:227–35.

Bhattarai N and Stapleton JT (2012). GB virus C: the good boy virus? *Trends Microbiol*. **20**(3):124–30.

Bhawna Poonia (2013). Immunotherapy in HIV Infection. *J Infect Dis Ther*. **1**:1.

Birk M, Lindback S and Lidman C (2002). No influence of GB virus C replication on the prognosis in a cohort of HIV-1-infected patients. *AIDS*. **16**(18):2482-2485.

Birgit Kallinowski, Christine Buhrmann, Stefanie Seipp, Tobias Goeser, Wolfgang Stremmel, Gerd Otto and Lorenz Theilmann (1998). Incidence, Prevalence, and Clinical Outcome of Hepatitis GB-C Virus Infection in Liver Transplant Patients. *Liver Transplantation and Surgery*. **1** (4): 28-33.

Bjorkman P, Flamholc L, Molnegren V, Marshall A, Guner N and Widell A (2007). Enhanced and resumed GB virus C replication in HIV-1-infected individuals receiving HAART. *AIDS*. **21**(12): 1641–1643.

Bjorkman P, Flamholc L, Naucler A, Molnegren V, Wallmark E and Widell A (2004). GB virus C during the natural course of HIV-1 infection: Viremia at diagnosis does not predict mortality. *AIDS.* **18**(6):877-886.

Blankson JN (2010). Effector mechanisms in HIV-1 infected elite controllers: highly active immune responses. *Antiviral Res.* **85**:295-302.

Blumberg BS, Alter HJ and Visnich S (1965). A "new" antigen in leukemia sera. *JAMA*.**191**: 541e546.

Blumberg BS, Sutnick A and London WT (1969). Australia antigen and hepatitis. *JAMA*. **207**(10): 1895e1896.

Boasso A and Shearer G (2008). Chronic innate immune activation as a cause of HIV-1 immunopathogenesis. *Clin Immunol*. **126**(3):235-42.

Bourlet T, Guglielminotti C, Evrard M, Bertelot P, Grattard F, Fresard A, Lucht FR and Pozzetto B (1999). Prevalence of GBV-C/hepatitis G virus RNA and E2 antibody among subjects infected with human immunodeficiency virus type 1 after parenteral or sexual exposure. *J Med Virol*. **58**(4):373-377.

Brechot C, Jaffedo F, Logorce D, Meyer zum Buschenfelde K, Papakonstontinou A, Hadziyannis S, Romeo R, Colombo M, Rodes J, Bruix J, Williams R and Naoumov N (1998). Impact of HBV, HCV and GBV-C/HGV on hepatocellular carcinomas in Europe: Results of a European concerted study. *J. Hepatol*. **29**: 173-183.

Brown K E, Tisdale J, Barrett J, Dunbar CE and Young NS (1997a). Hepatitis associated aplastic anaemia. *N Eng. J. Med.* **336**: 1059-1064.

Brown KE, Wong S and Young NS (1997b). Prevalence of GBVC/ HGV, a novel "hepatitis" virus, in patients with aplastic anaemia. *Br J Haematol.* **97**:492-6.

Brumme ZL, Chan KJ, Dong WWY, Theresa Mo, Brian W, Robert SH, Julio SG, Michael VO and P Richard Harrigana (2002). No association between GB virus- C viremia and virological or immunological failure after starting initial anti- retroviral therapy. *AIDS.* **16**:1929–33.

Brunetto MR, Oliveri F, Colombatto P, Moriconi F, Ciccorossi P, Coco B, Romagnoli V, Cherubini B, Moscato G, Maina AM, Cavallone D and Bonino F (2010). Hepatitis B surface antigen serum levels help to distinguish active from inactive hepatitis B virus genotype D carriers. *Gastroenterology*. **139**(2):483-90.

Burton DR, Desrosiers RC, Doms RW, Wayne C Koff, Peter D Kwong, Moore JP, Nabel GJ, Sodroski J, Wilson IA and Wyatt RT (2004). HIV vaccine design and the neutralizing antibody problem. *Nat Immunol.* **5**: 233-6.

Busch MP, Operskalski EA, Mosley JW, TH Lee, D Henrard, S Herman, Sachs DH, M Harris, W Huang, Stram DO and Transfusion Safety Study Group (1996). Factors influencing human immunodeficiency virus type 1 transmission by blood transfusion. Transfusion Safety Study Group. *J Infect Dis.* **174**: 26-33.

Camaur D and Trono D (1996). Characterization of HIV-1 Vif particle incorporation. *J Virol.* **70**: 6106-6111.

Capobianchi MR, Lalle E, Martini F, D'Offizi G, Antonucci G, Abbate I and Dianzani F (2006). Influence of GBV-C infection on the endogenous activation of the IFN system in HIV-1 co-infected patients. *Cell Mol Biol (Noisy-le-grand).* **52**(1):3–8.

Carr D and Tomanek L (2006). Herpes simplex virus and the chemokines that mediate the inflammation. *Curr Top Microbiol Immunol.* (303):47-65.

Centers for Disease Control and Prevention (1981). Pneumocystis pneumonia - Los Angeles. *MMWR Morb Mortal Wkly Rep.* **30**: 1-3.

Centers for Disease Control and Prevention (1982). Epidemiologic Notes and Reports Pneumocystis carinii Pneumonia among Persons with Hemophilia A. *MMWR Morb Mortal Wkly Rep.* **31**: 365-367.

Centers for Disease Control and Prevention (2014). HIV/ AIDS and viral Hepatitis. CDC, Atlanta, USA

Chams V, Fournier-Wirth C, Chabanel A, Hervé P and Trépo C (2003). Is GB virus-C alias "hepatitis G" involved in human pathology. *Trans Clin Bioi.* **10**: 292 306.

Chapham PR and Weiss RA. (1997). Spoilt choice of co-receptors. *Nature*. **388**: 230-231.

Cheung RC, Keeffe EB and Greenberg HB (1997). Hepatitis G virus: Is it a hepatitis virus? *West J Med*. **167**:23–33.

Chisari FV and Ferrari C (1995). Hepatitis B virus immunopathogenesis. *Annu Rev Immunol*. **13**: 29–60.

Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW and Houghton M (1989). Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science*. **244**: 359–362.

Chow R, Krajden M, Yu A, Braybrook H, Lai A, Mak A, Cook D, Tellier R, Petric M, Gascoyne RD, Connors JM, Brooks-Wilson A, Richard PG and John J Spinelli (2010). GBV-C/hepatitis G virus infection and non-Hodgkin lymphoma: a case control study. *Int J Cancer.* **126**(12):2885-92.

Clavel F, Guetard D, Brun-Vezinet F, Chamaret S, Rey MA, M. O. Santos-Ferreira, Laurent AG, Dauguet C, Katlama C, Rouzioux C, Klatzmann D, JL Champalimaud and Luc Montagnier (1987). Isolation of a new human retrovirus from West African patients with AIDS. *Science*. **233**:343-6.

Coffin JM (1995). HIV population dynamics in vivo: implications for genetic variation, pathogenesis, and therapy. *Science*. **267**:483-9.

Cohen MS, Hellmann N, Levy JA, DeCock K and Lange J (2008). The spread, treatment, and prevention of HIV-1: evolution of a global pandemic. *J. Clin. Invest.* **118**: 1244–1254.

Connolly NC, Whiteside TL, Wilson C, Kondragunta V, Rinaldo CR and Riddler SA (2008). Therapeuticimmunization with human immunodeficiency virus type 1 (HIV-1) peptide-loaded dendritic cells is safe and induces immunogenicity in HIV-1-infected individuals. *Clin Vaccine Immunol.* **15**(2):284–292.

CSA (2014). Federal Demographic Republic of Ethiopia Central Statistical Agency-Population Projection of Ethiopia for All Regions at Wereda Level from 2014-2017. *Poulation and Housing Census of Ethiopia*.

CSA (2007). "Country Level". *Poulation and Housing Census of Ethiopia.*

Cutler B and Justman J (2008). Vaginal microbicides and the prevention of HIV transmission. *Lancet Infect. Dis.* **8**: 685–697.

Davod Javanmar, Makvandi, Hajiani, Khalafkhany and Samarbaf Zadeh (2013). Hepatitis G virus and its prevalence and genotypes in patients with hepatitis B and C in Ahvaz, southwestern Iran. *Turk J Med Sci*. **43**: 474-478.

Dawson GJ, Chan KH, Cabal CM, Yarbough PD, Reyes GR and Mushahwar IK (1992). Solid-phase enzyme-linked immunosorbent assay for Hepatitis E virus IgG & IgM antibodies utilizing recombinant antigens and synthetic peptides . *J Virol Meth.* **38**: 175-186.

Deeks SG, Lewin SR and Havlir DV (2013). The end of AIDS: HIV infection as a chronic disease. *Lancet.* **382**(9903): 1525-33.

De Keersmaecker B, Allard SD, Lacor P, Schots R, Thielemans K and Aerts JL (2012). Expansion of polyfunctional HIV-specific T cells upon stimulation with mRNA electrophoresed dendritic cells in the presence of immunomodulatory drugs. *Virol.* **86**:9351-60.

De Cock KM, Jaffe HW and Curran JW (2011). Reflections on 30 years of AIDS. *Emerg Infect Dis*. **17**(6):1044-1048.

Deinhardt F, Peterson D, Cross G, Wolfe L and Holmes A W (1975). Hepatitis in marmosets. *Am J Med Sci.* **270**: 73-80.

Deinhardt F, Holmes AW, Capps RB and Popper H (1967). Studies on the transmission of disease of human viral hepatitis in marmoset monkeys. Transmission of disease, serial passage and description of liver lesions. *J Exp Med.* **125**:673-678.

Dessie A, Abera B and Wale F (2007). Seroprevalence of major blood-borne infections among blood donors at Felege Hiwot referral hospital, northwest Ethiopia. *Ethiop J Health Dev*. 21:68–69.

Detels R, Munoz A, McFarlane G, Kingsley LA, Margolick JB, Giorgi J, Schrager LK and Phair JP (1998). Effectiveness of potent antiretroviral therapy on time to AIDS and death in men with known HIV infection duration. Multicenter AIDS Cohort Study Investigators. *JAMA.* **280**: 1497–1503.

Dianzani U, T Bensi, A Savarino, S Sametti, M Indelicato, R Mesturini and A Chiocchetti (2003). Role of FAS in HIV infection. *Curr. HIV Res*. **1**:405–417.

Doranz BJ, Rucker J, Yi Y, Smyth RJ, Samson M, Peiper SC, Parmentier M, Collman RG and Doms RW (1996). A dual-tropic primary HIV-1 isolate that uses fusin and the b-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell*. **85**: 1149-1158.

Douek DC (2003). Disrupting T-cell homeostasis: how HIV-1 infection causes disease. *AIDS Rev.* **5**: 172–177.

Douek DC (2007). HIV disease progression: immune activation, microbes, and a leaky gut. *Top HIV Med.* **15**: 114–7.

Earl PL, Doms RW and Moss B (1990). Oligomeric structure of the HIV-1 envelope glycoprotein. *Proc Natl Acad Sci USA.* **87**: 648-652.

Eissmann K, Mueller S, Sticht H, Jung S, Zou P, S Jiang, A Gross, J Eichler, B Fleckenstein and H Reil (2013). HIV-1 Fusion Is Blocked through Binding of GB Virus C E2D Peptides to the HIV-1 gp41 Disulfide Loop. *PLoS ONE*. **8**(1): e54452. doi:10.1371/journal.pone.0054452.

El-Zayadi AR, Abe K, Selim O, Naito H, Hess G and Ahdy A (1999). Prevalence of GBVC/ hepatitis G virus viraemia among blood donors, health care personnel, chronic non-B non-C hepatitis, chronic hepatitis C and hemodialysis patients in Egypt. *J Virol Methods.* **80**: 53-58.

Emma L Mohr, Jinhua Xiang, James H McLinden, Thomas M Kaufman, Qing Chang, David C Montefiori, Donna Klinzman and Jack T Stapleton (2010). GB Virus Type C Envelope Protein E2 Elicits Antibodies That React with a Cellular Antigen on HIV-1 Particles and Neutralize Diverse HIV-1 Isolates. *J Immunol*. **185**(7): 4496–4505.

Erker le, Simons IN, Muerhoff AS, Leary TP, Chalmers ML, Desai SM and Mushahwar IK (1996). Molecular cloning and characterization of a GB virus C isolate from a patient with non-A-E hepatitis. *J. Gen Virol*. **77**:2713-2720.

Ernst D, M Greer, R Akmatova, S Pischke, H Wedemeyer, H Heiken, HL Tillmann, RE Schmidt and M Stoll (2014). Impact of GB virus C viraemia on clinical outcome in HIV-1-infected patients: a 20-year follow-up study. *HIV Medicine*. **15**: 245–250.

Esparza J (2013). A brief history of the global effort to develop a preventive HIV vaccine. *Vaccine*. **31**(35):3502–18.

Essex M (1999). Human immunodeficiency viruses in the developing world. *Adv Virus Res*. **53**:71–88.

Ezechi OC, Kalejaiye OO, Gab- Okafor CV, Oladele DA, Oke BO and Musa ZO (2014). Seroprevalence and factors associated with Hepatitis B and C co- infection in pregnant Nigerian women living with HIV infection. *Pan African Medical Journal*. **17**: 197.

Fallahian F, Alavian SM and Rasoulinejad M (2010). Epidemiology and transmission of hepatitis G virus infection in dialysis patients. *Saudi J Kidney Dis Transpl*. **21**(5):831-4.

Farnaz Vahidnia, Maya Petersen, Jack T. Stapleton, George W. Rutherford, Michael Busch and Brian Custer (2012). Acquisition of GB Virus Type C and Lower Mortality in Patients with Advanced HIV Disease. *Clinical Infectious Diseases*. DOI: 10.1093/cid/cis589.

Federal Democratic Republic of Ethiopia (2006). Report on Progress Towards Implementation of the Declaration of Commitments on HIV/AIDS. Addis Ababa.

Feinstone SM, Kapikian AZ and Purcell RH (1973). Hepatitis A: detection by immune electron microscopy of virus like antigen associated with acute illness. *Science*. **200**: 365–73.

Feinstone S, Kapikian A, Purcell R, Alter H and Holland P (1975). Transfusion associated hepatitis not due to viral hepatitis type A or B. *N Engl J Med.* **292:** 767–70.

Feng Y, Zhao W, Dai J, Li Z, Zhang X, Liu L, Bai J, Zhang H, Lu L and Xia X (2011). A novel genotype of GB virus C: its identification and predominance among injecting drug users in Yunnan, China. *PLoS One*. **6**: e21151.

French MA, King MS, Tschampa JM, da Silva BA and Landay AL (2011). Serum immune activation markers are persistently increased in patients with HIV infection after 6 years of antiretroviral therapy despite suppression of viral replication and reconstitution of CD4+ T cells. *J Infect Dis.* **200**: 1212–5.

Frommel D, Tekle-Haimanot R, Berhe N, Aussel L, Verdier M, Preux PM and Denis F (1993). A survey of antibodies to hepatitis C virus in Ethiopia. *Am J Trop Med Hyg.* **49**:435-39.

Ganem D and Prince AM (2004). Hepatitis B virus infection-natural history and clinical consequences. *N Engl J Med*. **350**: 1118–1129.

Gavilanes F, Gonzales-Ros A and Peterson D (1982). Structure of hepatitis B surface antigen: characterization of the lipid components and their association with the viral proteins. *J Biol Chem.* **257**:7770– 7777.

Geeraert L, Kraus G and Pomerantz RJ (2008). Hide-and-seek: the challenge of viral persistence in HIV-1 infection. *Annu Rev Med*. **59**:487–501.

Gelderblom HR, Hausmann EH, Ozel M, Pauli G and Koch MA (1987). Fine structure of HIV and immunolocalization of structural proteins. *Virology*. **156**: 171-176.

Gelderblom HR, Ozel M and Pauli G (1989). Morphogenesis and morphology of HIV. Structure-function relations. *Arch Virol.* **106**: 1- 13.

Gemechu Kejela, Lemessa Oljira, Yadeta Dessie and Direslgne Misker (2015). Comprehensive HIV/AIDS knowledge level among out-of-school youths in Wayu Tuka district, Western Ethiopia. *European Journal of Preventive Medicine*. **3**(1): 11-16.

George SL, Xiang J and Stapleton JT (2003). Clinical isolates of GB virus type C vary in their ability to persist and replicate in peripheral blood mononuclear cell cultures. *Virology*. **316**:191–201.

George SL and Varmaz D (2005). What you need to know about GB virus C. *Curr Gastroenterol Rep*. **7**(1):54-62.

George SL, Varmaz D and JT Stapleton (2006). GB virus C replicates in primary T and B lymphocytes. *J Infect Dis.* **193**:451-454.

Giannoulis E, Economopoulos T, Mandraveli K, Giannoulis K, Nikolaides C, Zervou E, Papageorgiou E, Zoulas D, Tourkantonis A, Giannopoulos G and Fountzilas G (2004).The prevalence of hepatitis C and hepatitis G virus infection in patients with B cell non-Hodgkin lymphomas in Greece: a Hellenic Cooperative Oncology Group Study. *Acta Haematol*. **112**(4):189-93.

Gilles N, Christian R and Pascal V (1999). Clearance of hepatitis G viremia in a Human immunodeficiency virus -positive patient by high-activity antiretroviral therapy. *Clin Infect Dis*. **29**:1332–1333.

Gimenez-Barcons M, Ibanez A, Tajahuerce A, Sanchez-Tapias IM, Rodes J, Martinez MA and Saiz JC (1998). Genetic evolution of hepatitis G virus in chronically infected individual patients. *J Gen Viral.* **79**: 2623-2629.

Giorgi JV, Hultin LE, McKeating JA, Johnson TD, Owens B, LP Jacobson, R Shih, J Lewis, DJ Wiley, JP Phair, SM Wolinsky and R Detels (1999). Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. *J Infect Dis.* **179**: 859–70.

Gish R, Jia JD, Locarnini S and Zoulim F (2012). Selection of chronic hepatitis B therapy with high barrier to resistance. *Lancet Infect Dis.* **12**:341–53.

Gisselquist D (2003). Emergence of the HIV type 1 epidemic in the twentieth century: comparing hypotheses to evidence. *AIDS Res Hum Retroviruses.* **19**:1071-8

Glebe D and Urban T (2007). Viral and cellular determinants involved in hepadna viral entry. *World Journal of gastroenterology*. **13**(1): 22-38.

Gonzalez-Hernández LA, Jave-Suarez LF, Fafutis-Morris M, Montes- Salcedo KE, Valle-Gutierrez LG, Campos-Loza AE, Enciso-Gómez LF and Andrade- Villanueva JF (2012). Synbiotic therapy decreases microbial translocation and inflammation and improves immunological status in HIV-infected patients: a double-blind randomized controlled pilot trial. *Nutr J.* **11**:90.

Gougeon ML, Lecoeur H and Sasaki Y (1999). Apoptosis and the CD95 system in HIV disease: impact of highly active anti-retroviral therapy (HAART). *Immunol Lett*. **66**(13):97–103.

Gower E, Estes C, Blach S, Razavi-Shearer K and Razavi H (2014). Global epidemiology and genotype distribution of the hepatitis C virus infection. *J Hepatol*. **61**(1):S45– 57.

Grakoui A, Wychowski C, Lin C, Feinstone SM and Rice CM (1993). Expression and identification of hepatitis C polyprotein cleavage products. *J Virol.* **67**:1385-95.

Grassi M, Raffa S, Traditi F, Ferrazzi M, Cioschi S, Fontana M, Pezzella M (2000). Detection and clinical evaluation of GBV-C/HGV in plasma from patients with chronic hepatitis of unknown etiology. *Clin Ter*. **151**(4): 241-5.

Greene WC and Peterlin BM (2002). Charting HIV's remarkable voyage through the cell: Basic science as a passport to future therapy. *Nat Med.* **8**: 673-680.

Guidotti LG and Chisari FV (2006). Immunobiology and pathogenesis of viral hepatitis. *Annu Rev Pathol Mech Dis*. **1**: 23–61.

Guilera M, Saiz JC, Lopez-Labrador FX, Olmedo E, Ampurdanes S, Forns X, Bruix L, Pares A, Sanchz-Tapias IM, Jimenez de Anta MT and Rodes I (1998). Hepatitis G virus infection in chronic liver disease. *Gut.* **42**(1):107-11.

Gulick RM, Meibohm A, Havlir D, Eron JJ, Mosley A, Chodakewitz JA, Isaacs R, Gonzalez C, McMahon D, Richman DD, Robertson M and Mellors JW (2003). Six year follow-up of HIV-1-infected adults in a clinical trial of antiretroviral therapy with indinavir, zidovudine, and lamivudine. *AIDS*. **17**: 2345–2349.

Hahn BH, Shaw GM, De Cock KM and Sharp PM (2000). AIDS as a zoonosis:scientific and public health implications. *Science.* **287**:607-14

Halasz R, Weilanda O and Sällberg M (2001). GBV virus C/hepatitis G virus. *Scandinavian Journal of Infectious Diseases.* **33**:572-80.

Hantano H, Delwart EL, Norris PJ, Lee TH, Neilands TB, Kelley CF, Hunt PW, Hoh R, Linnen JM, Martin JN, Busch MP and Deeks SG (2010). Evidence of persistent low-level viremia in long term HAART-suppressed, HIV-infected individuals. *AIDS*. **24**: DOI:10.1097/QAD.0B013E32833DBA03.

Hazenberg MD, Hamann D, Schuitemaker H and Miedema F (2000) T cell depletion in HIV-1 infection: how CD4+ T cells go out of stock. *Nat Immunol* **1**: 285–9.

Hayashi J, Furusyo N, Sawayama Y, Kishihara Y, Kawakami Y, Ariyama I, Etoh Y and Kashiwagi S (1998). Hepatitis G virus in the general population and in patients on hemodialysis. *Dig Dis Sci.* **43**:2143-8.

Hessell AJ, Hangartner L, Hunter M, Havenith CEG, Beurskens FJ, Bakker JM, Caroline MS Lanigan, Gary Landucci, DN Forthal, Paul WParren, Preston A and Dennis R Burton (2007). Fc receptor but not complement binding is important in antibody protection against HIV. *Nature.* **449**(7158):101-4.

Heringlake S, Ockenga J, Tillmann HL, Trautwein C, Meissner D, Stoll M, Hunt J, Jou C, Solomon N, Schmidt R and Manns MP (1998). GB virus C/hepatitis G virus infection: a favorable prognostic factor in human immunodeficiency virus-infected patients? *Journal of Infectious Diseases*. **177**:1723-6.

Hima Bindu and Naga Anusha (2011). Adverse Effects of Highly Active Anti-Retroviral Therapy (HAART). *J Antivir.* **3**(4):060-064.

Hnatyszyn HJ (2005). Chronic hepatitis C and genotyping: the clinical significance of

determining HCV genotypes. *Antivir Ther.* 10:1–11.

Hofer H, Aydin I, Neumueller-Guber S, Mueller C, Scherzer TM, Staufer K, Steindl-Munda P, Wrba F and Ferenci P (2011). Prevalence and clinical significance of GB virus type C/hepatitis G virus coinfection in patients with chronic hepatitis C undergoing antiviral therapy. *J Viral Hepat.* **18**: 513–7.

Hollingsworth RC, Minton EJ, Fraser-Moodie C, Metivier E, Rizzi PM, Irving WL, Jenkins D and Ryder SD (1998). Hepatitis G infection: role in cryptogenic chronic liver disease and primary liver cell cancer in the UK. Trent Hepatitis C virus Study Group. *Journal J Viral Hepat*. **5**(3):165-9.

Hossein Keyvani, Avid Mohammadi, Masoud Sabouri Ghannad, and Mahboobeh Hajabdolbaghi (2012). The Effect of GBV-C Infection on CD4 Count and Viral Loads in Patients Infected With HIV. *Hepat Mon.* **12**(1): 39–42.

Hughes J and Mahy B (1998). Recommendations for prevention and control of hepatitis C virus infection and HCV-related chronic disease. *CDC MMWR Recomm Rep*. **47**:1-39.

Hunt P, Martin J, Sinclair E, Bredt B, Hagos E and S Deeks (2003). T-cell activation is associated with lower CD4+ T cell gains in human immunodeficiency virus infected patients with sustained viral suppression during antiretroviral therapy. *J Infect Dis.* **187**: 1534–43.

Igor R, Evropi T, Harish N, Ana M and Harry C (2011). Reducing the burden of maternal and neonatal infections in low-income settings. *J Global.* **1**(2): 1211-1218.

Jake Liang T (2009). Hepatitis B: The Virus and Disease. *Hepatology*. **49**(5): S13–S21.

Jarvis LM, Bell H , Simmonds,P, Hawkins A, Hellum,K, Harthug S, Maeland A, Ritland S, Myrvang B, von der Lippe B, Raknerud N and Skaug K (1999). The effect of treatment with alpha-interferon on hepatitis G/GBV-C viraemia. *J Gastroenterol*. **33**:195-200.

Jeng-Fu Yang, Chia-Yen Dai, Wan-Long Chuang, Wen-Yi Lin, Zu-Yau Lin, Shinn-Cherng Chen, Ming-Yuh Hsieh, Liang-Yen Wang, Jung-Fa Tsai, Wen-Yu Chang and

Ming-Lung Yu (2006). Prevalence and Clinical Significance of HGV/GBV-C Infection in Patients with Chronic Hepatitis B or C. *Jpn. J. Infect. Dis*. **59**: 25-30.

Jonathan H Epstein, Phenix-Lan Quan, Thomas Briese, Craig Street, Omar Jabado, Sean Conlan, Shahneaz Ali Khan, Dawn Verdugo, M. Jahangir Hossain, Stephen K Hutchison, Michael Egholm, Stephen P Luby, Peter Daszak and W Ian Lipkin (2010). Identification of GBV-D, a Novel GB-like Flavivirus from Old World Frugivorous Bats (Pteropus giganteus) in Bangladesh. *PLoS Pathog* **6**(7): e1000972. doi:10.1371/journal.ppat.1000972.

Jung S, Knauer O, Donhauser N, Eichenmuller M, Helm M, Fleckenstein B and Reil H (2005). Inhibition of HIV strains by GB virus C in cell culture can be mediated by CD4 and CD8 T-lymphocyte derived soluble factors. *AIDS*. **19**(12):1267-1272.

Kanki PJ, Hamel DJ, Sankale JL, Hsieh C, Thior I, Barin F, Woodcock SA, Guèye-Ndiaye A, Zhang E, Montano M, Siby T, Marlink R, Doye IN, Essex ME and MBoup S (1999). Human immunodeficiency virus type 1 subtypes differ in disease progression. *J Infect Dis*. **179**:68–73.

Kao J, Chen P, Chen W, Hsiang C, Lai M and Chen D (1997a). Amplification of GB virus-C /hepatitis G virus RNA with primers from different regions of the viral genome. *J. Med. Viral*. **51**:284-289.

Kao J, Chen P, Lai M , Chen W, Liu D, Wang J, Shen M and Chen D (1997b). GB virus-C/hepatitis G virus infection in an area endemic for viral hepatitis, chronic liver disease, and liver cancer. *Gastroenterology*. **112**:1265-1270.

Kassu A, Marcus RA, D'Souza MB, Kelly-McKnight EA, Akkina R, Fontenot AP, Wilson CC and Palmer BE (2010). Regulation of virus-specific CD4+ T cell function by multiple costimulatory receptors during chronic HIV infection. *J Immunol*. **185**(5):3007–3018.

Kaufman TM, JH McLinden, J Xiang, AM Engel and JT Stapleton (2007). The GBV- C envelope glycoprotein E2 does not interact specifically with CD81. *AIDS*. **21**:1045-1048.

Kaye S, Howard M, Alabi A, Hansmann A, Whittle H and Schim Loeff M (2005). No observed effect of GB virus C coinfection on disease progression in a cohort of African woman infected with HIV-1 or HIV-2. *Clin Infect Dis*. **40**:876–8.

Kim JP and Fry KE (1997). Molecular characterization of the hepatitis G virus. *J Viral Hepat.* **4**: 77-79.

Klausner JD, Wamai RG, Bowa K, Agot K, Kagimba J and Halperin DT (2008). Is male circumcision as good as the HIV vaccine we've been waiting for? *Future HIV Ther.* **2**(1): 1–7.

Kleinman S (2001). Hepatitis G virus biology, epidemiology, and clinical manifestations: Implications for blood safety. *Transfus Med Rev*. **15**(3):201-12.

Kondili LA, Chionne P, Dettori S, Badolato MC, Grosso A, Vania A and Rapicetta M (2001). GB virus/hepatitis G virus exposure in Italian pediatric and young adult thalassemic patients. *Infection*. **29**:219-21.

Kourtis AP, Bulterys M, Hu DJ and Jamieson DJ (2012). HIV-HBV coinfection–a global challenge. *N Engl J Med.* **366**: 1749–1752.

Kozal MJ, Friedland G, Chiarella J, Kyriakides T, Concato J, Klinzman D and Stapleton JT (2002). Prevalence of HIV and GBV-C coinfection and the likelihood of genotypic resistance development. *9th Conference on Retroviruses & Opportunistic Infections*. Seattle, WA, USA.

Koziel MJ and Peters MG (2007). Viral hepatitis in HIV infection. *N Engl J Med.* **356**: 1445-1454.

Kuo G, Choo QL, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, Miyamura T, Dienstag JL, Alter M, Stevens CE, Tegtmeier GE, Bonino F, Colombo M, Lee WS, Kuo C , Berger K, Shuster IR, Overby LR, Bradley DW and Houghton M (1989). An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science*. **244**: 362-364.

Kwong PD, Wyatt R, Robinson J, Sweet RW, Sodroski J and Hendrickson WA (1998). Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature*. **393**:648-59.

Lacombe K and Rockstroh J (2012). HIV and viral hepatitis co-infections: Advances and challenges. *Gut.* **61**(1): i47–i58.

Lalle E, Sacchi A, Abbate A, Vitale, F Martini, G D'Offizi, G Antonucci, C Castilletti, F Poccia and MR Capobianchi (2008). Activation of interferon response genes and of plasmacytoid dendritic cells in HIV-1 positive subjects with GB virus C co-infection. *Int J Immunopathol Pharmacol*. **21**:161–71.

Lamproye A, Delwaide J, Vaira D, Maggipinto G, Gèrard C, Sondag-Thull D, Rentier B, Rorive G and Belaiche J (1999). Prevalence of hepatitis G virus in a haemodialysis unit. *Acta Gastroenterologica Belgica*. **62**:13-5.

Laskus T, Radkowski M, Wang LF, Vargas H and Rakela J (1997). Lack of hepatitis G virus replication in the livers of patients coinfected with hepatitis C and G viruses. *J. Virol.* **71**: 7804-7806.

Leary TP, AS Muerhoff, JN Simons, TJ Pilot-Matias, JC Erker ML Chalmers, GG Schlauder, GJ Dawson, SM Desai and IK Mushahwar (1996). Sequence and

genomic organization of GBV-C: a novel member of the flaviviridae associated with human non-A-E hepatitis. *J Med Virol.* **48**:60-67.

Lederman MM, Calabrese L, Funderburg NT, Clagett B, Medvik K, Hector Bonilla, Barbara Gripshover, Robert A. Salata, Alan Taege, Michelle Lisgaris, Grace A. McComsey, Elizabeth Kirchner, Jane Baum, Carey Shive, Robert Asaad, Robert C Kalayjian, Scott F Sieg and Benigno Rodriguez (2011). Immunologic failure despite suppressive antiretroviral therapy is related to activation and turnover of memory CD4 cells. *J Infect Dis.* **204:** 1217–26

Leerantanpetch N and Suseangrut W (2008). Hepatitis B virus and Hepatitis C virus co-infection with HIV patients at Khon Kaen Hospital. *Khon Kaen Hospital Medical Journal*. 229-238.

Lefrere JJ, Roudot-Thoraval F, Morand-Joubert L, Petit JC, Lerable J, Thauvin M and Mariotti M (1999). Carriage of GB virus C/hepatitis G virus RNA is associated with a slower immunologic, virologic, and clinical progression of human immunodeficiency virus disease in coinfected persons. *J Infect Dis*. **179**(4):783-789.

Le Rouzic E and Benichou S (2005). The Vpr protein from HIV-1: distinct roles along the viral life cycle. *Retrovirology*. **2**:11.

Lester FT, Ayehunie S and Zewdie D (1988). Acquired immunodeficiency syndrome: seven cases in an Addis Ababa hospital. *Ethiop Med J.* **26**(3):139-45.

Levy JA (2007). HIV and the pathogenesis of AIDS. 3rd ed. Washington DC

Levy Y, Sereti I, Tambussi G, Routy JP, Lelièvre JD, Delfraissy JF, Molina JM, Fischl M, Goujard C, Rodriguez B, Rouzioux C, Avettand- Fenoël V, Croughs T, Beq S, Morre M, Poulin JF, Sekaly RP, Thiebaut R and Lederman MM (2012). Effects of recombinant human interleukin 7 on T-cell recovery and thymic output in HIV infected patients receiving antiretroviral therapy: results of a phase I/IIa randomized, placebo-controlled, multicenter study. *Clin Infect Dis.* **55**:291-300.

Li C, Collini P, Danso K, Owusu-Ofori S, Dompreh A, Candotti D, Opare-Sem O and Allain JP (2006). GB virus C and HIV-1 RNA load in single virus and co-infected West African individuals. *AIDS.* **20**:379-86.

Lightfoot K, Skelton M, Kew MC, Yu MC, Kedda MA, Coppin A and Hodkinson J (1997). Does hepatitis GB virus C infection cause hepatocellular carcinoma in black Africans? *Hepatol*. **26**: 740-742.

Linnen J, J Wages, ZY Zhang-Keck, KE Fry, KZ Krawczynski, H Alter, E Koonin, M Gallagher, M Alter, S Hadziyannis, P Karayiannis, K Fung, Y Nakatsuji, JW Shih, L Young, M Piatak, C Hoover, J Fernandez, S Chen, JC Zou, T Morris, KC

Hyams, S Ismay, JD Lifson, G Hess, SK Foung, H Thomas, D Bradley, H Margolis and JP Kim (1996). Molecular cloning & disease association of hepatitis G virus: a transfusion-transmissible agent. *Science*. **271**:505-508.

Liu H, Wu X, Newman M, Shaw G, Hahan AH and Kappes JC (1995). The Vif protein of human and simian immunodeficiency viruses is packaged into virions and associates with viral core structures. *J V irol*. **69**: 7630-7638.

Lok AS and McMahon BJ (2007). Chronic hepatitis B. *Hepatology*. **45**(2):507–39.

Lorenza Nogueira Campos, Cibele Comini César, Mark Drew and Crosland Guimarães (2009). Quality of life among hiv-infected patients in Brazil after initiation of treatment. *CLINICS*. **64**(9):867-875.

Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartelsn DH, Bell M, Benjamin EJ, et al (2012). Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. **380**(9859):2095–128.

Lu W, Arraes LC, Ferreira WT and Andrieu JM (2004). Therapeutic dendritic-cell vaccine for chronic HIV-1 infection. *Nat Med*. **10**(12):1359–1365.

Lucas, SB, Hounnou A, Peacock C, Beaumel A, Djomand G, N'Gbichi JM, Yeboue K, Honde M, Diomande M, Giordano C, Doorly R, Brattegaard K, Kestens L, Smithwick R, Kadio A, Ezani N, Yapi A, De Cock KM (1993). The mortality and pathology of HIV infection in a West African city. *AIDS*. **7**:1569-79.

Luisa Dias da Mota, Fabiana Nunes Germano, Maria Fernanda Martinez Barral, Nayle Maria Oliveira da Silva, and Ana Maria Barral Martinez (2013). Hepatitis G Virus and Coinfection in Seropositive for Human Immunodeficiency Virus – Review. Rev Bras Cien Med Saude. **2**(2):23-32

Lurman A (1885). Eine Icterus epidemic. *Berl Klin Wochenscr*. **22**: 20-30.

Maartens G, Celum C and Lewin SR (2014). HIV infection: epidemiology, pathogenesis, treatment, and prevention. *Lancet*. **384**(9939):258-271.

Macatangay BJ and Rinaldo CR (2009). PD-1 blockade: a promising immunotherapy for HIV. *Cellscience*.**5**(4):61–65.

MacCallum F (1947). Homologous serum hepatitis. *Lancet*. **2:** 691–2.

Madhava V, Burgess C and Drucker E (2002). Epidemiology of chronic hepatitis C virus infection in sub-Saharan Africa. *The Lancet Infec Dis*. **2**:293-302.

Mahoney K, Stewart H and Coleman M (2004). Progress toward the elimination of hepatitis B virus transmission among health care workers in the United States. *Internal Medicine.* **157**(22) 2601–2605.

Maidana-Giret MT, TM Silva, MM Sauer, H Tomiyama, JE Levi, KC Bassichetto, A Nishiya, RS Diaz, EC Sabino, R Palacios and EG Kallas (2009). GB virus type C infection modulates T-cell activation independently of HIV-1 viral load. *AIDS.* **23**:2277-2287.

Mallet V, Vallet-Pichard and AS Pol (2011). The impact of human immunodeficiency virus on viral hepatitis. *Liver Int.* **31**(1): 135–139.

Martinot M, Marcellin P, Boyer N, Detmer J, Pouteau M, Castelnau C, Degott C, Auperin A, Collins M, Kolberg J, Wilber J, Benhamou P and Erlinger S (1997). Influence of hepatitis G virus infection on the severity of liver disease and response to interferon-alpha in patients with chronic hepatitis C. *Ann Intern Med*. **126**:874-881.

Masaru E, Shuhei S, Katsuhiko F, Tetsuo K, Motoharu T, Shuzo O, Masayuki O and Takeyuki M (1998). Characteristics of Patients with Hepatitis C Virus with and Without GB Virus C/Hepatitis G Virus Co-infection and Efficacy of Interferon Alfa. *HEPATOLOGY*. (27)5.

May MT and Ingle SM (2011). Life expectancy of HIV-positive adults: a review. *Sex Health*. **8**: 526–533.

McCutchan FE (2000). Understanding the genetic diversity of HIV-1. *AIDS.* **14**(l3): S31-S44.

McCutchan FE (2006). Global epidemiology of HIV. *J Med Virol.* **78** (1):S7-S12.

McElrath MJ and Haynes BF (2010). Induction of immunity to human immunodeficiency virus type-1 by vaccination. *Immunity*. **33**(4):542–54.

McHutchison J, Nainan OV, Alter MJ, Sedghi-Vaziri A, Detmer J, Collins M and Kolberg I (1997). Hepatitis C and G co-infection: Response to interferon therapy and quantitative changes in serum HGV-RNA. *Hepatol*. **26**:132-1327.

McLinden JH, Thomas MK, Jinhua X, Qing C, Donna K, Alfred ME, Georg H, Urban S, Michael H and Stapleton JT (2006). Characterization of an Immunodominant Antigenic Site on GB Virus C Glycoprotein E2 That Is Involved in Cell Binding. *J of Virology*. **80**(24): 12131–12140.

McMichael A, Picker LJ, Moore JP and Burton DR (2013). Another HIV vaccine failure: where to next? *Nat Med*. **19**(12):1576–77.

Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG and Barnes E (2015). Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology*. **61**(1):77–87.

Moenkemeyer M, Schmidt RE, Wedemeyer H, Tillmann HL and Heiken H (2008). GBV-C co-infection is negatively correlated to Fas expression and Fas-mediated apoptosis in HIV-1 infected patients. *J Med Virol*. **80**(11):1933–1940.

Modi AA and Feld JJ (2007). Viral hepatitis and HIV in Africa. *AIDS Rev*. **9**: 25-39.

MOH/HAPCO (2006). *AIDS in Ethiopia. 6th Report*. September 2006. Addis Ababa, Ethiopia. Available at: http://www.etharc.org./aidsinEth6th_en.pdf.

Mohd Hanafiah K, Groeger J, Flaxman AD and Wiersma ST (2012). Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology*. **57**(4):1333–42.

Mohr EL and Stapleton JT (2009). GB virus type C interactions with HIV: the role of envelope glycoproteins. *J Viral Hepat*. **16**: 757-768.

Monica V, Alvarado M, Livia B, Anna N, Raymundo A, Neto M and Maria F (2011). Frequency and genotypic distribution of GB virus C (GBV-C) among Colombian population with Hepatitis B (HBV) or Hepatitis C (HCV) infection. *Virol J*. **8**:345.

Mosam A, Sathar MA, Dawood H, Cassol E, Esterhuizen TM and Coovadia HM (2007). Effect of GB virus C co-infection on response to generic HAART in African patients with HIV-1 clade C infection. *AIDS*. 21:1377–9.

Moore JP (1997). Coreceptors: implications for HIV pathogenesis and therapy. *Science*. **276**: 51-52.

Morbidity and Mortality Weekly Report (2005). A Comprehensive Immunization Strategy to Eliminate Transmission of Hepatitis B Virus Infection in the United States: Recommendations of the Advisory Committee on Immunization Practices. Part I: Immunization of Infants, Children, and Adolescents. **54**: RR-16.

Mphahlele M, Lauge K and Carman W (1998). HGV: the identification, biology and prevalence of an orphan virus. *Liver*. **18**: 143-155.

Muerhoff AS, Leary TP, Simons IN, Pilot-Matias T, Dawson G, Erker C, Chalmers ML, Schlauder GG, Desai SM and Mushahwar IK (1995). Genomic Organization of GB viruses A and B: two new members of the Flaviviridae associated with GB agent hepatitis. *J Virol*. **69**:(5621):5630.

Muerhoff, AS, Simons JN, Leary TP, Erker JC, Chalmers ML, Pilot-Matias TJ, Dawson GJ, Desai SM and Mushahwar IK (1996). Sequence heterogeneity within the 5'terminal region of the hepatitis GB virus C genome and evidence for genotypes. *J Hepatol*. **25**: 379-384.

Muerhoff AS, Tillmann HL, Manns MP, Dawson GJ and Desai SM (2003). GB virus C genotype determination in GB virus-C/HIV co-infected individuals. *J Med Virol*. **70**:141–9.

Muerhoff AS, Dawson GJ and Desai SM (2006). A previously unrecognized sixth genotype of GB virus C revealed by analysis of 5'-untranslated region sequences. *J Med Virol.* **78**: 105-111.

Muller C, Pfeffel F, Peck-Radosavljevic M, Petermann D, Oesterreicher C and Pidlich J (1997). Prevalence of hepatitis G virus in patients with hepatocellular carcinoma. *J Viral Hepat*. **4**: 411-414.

Munoz SJ, Alter HJ, Nakatsuji Y, Shih JW, Reddy RK, Jeffers L, Schiff ER, Reid AE, Marrone A, Rothstein K, Manzarbeitia C and Liang TJ (1999). The significance of hepatitis G virus in serum of patients with sporadic fulminant and subfulminant hepatitis of unknown etiology. *Blood*. 15;**94**(4):1460-4.

Murphy K (2012). Janeway's Immunobiology 8th Edition. New York, USA: *Garland Science*.

Nagayama R, Miyake K and Okamoto H (1997). Effect of interferon on GB virus C and hepatitis C virus in hepatitis patients with the co-infection. *J Med Virol*. **52**:156-160.

Naito H, Win KM and Abe K (1999). Identification of a novel genotype of hepatitis G virus in Southeast Asia. *J Clin Microbiol*. **37**: 1217-1220.

Nattermann J, HD Nischalke, B Kupfer, J Rockstroh, L Hess, T Sauerbruch and U Spengler (2003). Regulation of CC chemokine receptor 5 in hepatitis G virus infection. *AIDS*. **17**:1457-1462.

Nunnari G, L Nigro, F Palermo, M Attanasio, A Berger, HW Doerr, RJ Pomerantz and B Cacopardo (2003). Slower progression of HIV-1 infection in persons with GB virus C co-infection correlates with an intact T-helper 1 cytokine profile. *Ann Intern Med.* **139**:26-30.

Ocama P and Seremba E (2011). Management of HIV and hepatitis C virus infections in resource-limited settings. *Curr Opin HIV AIDS.* **6**: 539–545.

Ohto H, Ujiie N, Sato A, Okamoto H and Mayumi M (2000). Mother-to-infant transmission of GB virus type C/HGV. *Transfusion*. **40**(6):725-730.

O'Brien M, Montenont E, Hu L, Nardi MA, Valdes V, Merolla M, Gettenberg G, Cavanagh K, Aberg JA, Bhardwaj N and Berger JS (2013). Aspirin attenuates platelet activation and immune activation in HIV-1-infected subjects on antiretroviral therapy: a pilot study. *J Acquir Immune Defic Syndr*. **63**:280-8.

Oon GCJ (2012). Viral hepatitis - The silent killer. *Annals Academy of Medicine.* **41**: 279–80.

Orito E, Mizokami M, Yasuda K, Sugihara K, Nakamura M, Mukaide M, Ohba K, Nakano T, Kato T, Kondo Y, Kumada T, Yeda R and lino S (1997). Interferon-alpha therapy in patients dually infected with hepatitis C virus GB Virus C/hepatitis C virus virological response to HGV and pretreatment HGV viremia level. *J Hepatol*. **27**:603-612.

Ott JJ, Stevens GA, Groeger J and Wiersma ST (2012). Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine.* **30**:2212-2219.

Padian NS, Buve A, Balkus J and Serwadda D (2008). Cates W Jr: Biomedical interventions to prevent HIV infection: evidence, challenges, and way forward. *Lancet.* **372**: 585–599.

Pallikkuth S, Micci L, Ende ZS, Iriele RI, Cervasi B, Lawson B, McGary CS, Rogers KA, Else JG, Silvestri G, Easley K, Estes JD, Villinger F, Pahwa S and Paiardini M (2013). Maintenance of intestinal Th17 cells and reduced microbial translocation in SIV-infected rhesus macaques treated with interleukin (IL)-21. *PLoS.Pathog.* **9**:e1003471.

Palomba E, Bairo A and Tovo P-A (1999). High rate of maternal-infant transmission of hepatitis G virus in HIV-1 and hepatitis C virus-infected women. *Acta Paediatr.* **88**:1392–5.

Paiardini M and Müller-Trutwin M (2013). HIV-associated chronic immune activation. *Immunological Reviews*. **254**(1):78-101.

Paolo Palma, Lindvi Gudmundsdotter, Andrea Finocchi, Lars E. Eriksson, Nadia Mora, Veronica Santilli, Angela Aquilani, Emma C. Manno, Paola Zangari, Maria Luisa Romiti, Carla Montesano, Alba Grifoni, Andreas Brave, Karl Ljungberg, Pontus Blomberg, Stefania Bernardi, Eric Sandstrom, Bo Hejdeman, Paolo Rossi and Britta Wahren (2014). Immunotherapy with an HIV-DNA Vaccine in Children and Adults. *Vaccines.* **2**: 563-580.

Parks WP I and MeInick I L (1969). Attempted isolation of Hepatitis viruses in marmosets. *J. Infect. Dis*. **120**:539-547.

Pawlotsky J (2004). Pathophysiology of hepatitis C virus infection and related liver disease. *Trends Microbiol.* **12**: 96-102.

Pereira LM, Spinelli V, Ximenes RA, Cavalcanti MS, Melo R, Juca N, Mizokami M and McFarlane IG (2002). Chronic hepatitis C infection: influence of the viral load,

genotypes, and GBV-C/HGV coinfection on the severity of the disease in a Brazilian population. *J Med Virol*. **67**(1):27–32.

Peter Lawrence Smith, Helen Tanner and Angus Dalgleish (2014). Developments in HIV-1 immunotherapy and therapeutic Vaccination. *F1000Prime Reports.* **6**:43.

Pettersen FO, Torheim EA, Dahm AE, Aaberge IS, Lind A, Holm M, Aandahl EM, Sandset PM, Taskén K and Kvale D (2011). An exploratory trial of cyclooxygenase type 2 inhibitor in HIV-1 infection: downregulated immune activation and improved T cell-dependent vaccine responses. *J Virol.* **85**:6557-66.

Pickering IM, Thomas HC and Karayannis P (1997). Genetic diversity between hepatitis G virus isolates: Analysis ofnucleotide variation in the NS3 and putative 'core' peptide genes. *J Gen Viral*. **78**: 53-60.

Pilot-Matias TJ, Carrick RJ, Coleman PF, Leary TP, Surowy TK, Simons IN, Muerhoff AS, Bujik SL, Chalmers ML, Dawson GI, Desai SM and Mushahwar IK (1996). Expression of the GB virus C E2 glycoprotein using the semliki forest virus vector system and its utility as a serologic marker. *Virology*. **225**(2):282-292.

Piot P, Abdool Karim S, Hecht R, Legido-Quigley H, Buse K, Stover J, Resch S, Ryckman T, Møgedal S, Dybul M, Goosby E, Watts C, Kilonzo N, McManus J and Sidibe M (2015). Defeating AIDS—Advancing Global Health. *The Lancet.* **386**: (9989): 171.

Piroth L, Carrat F, Larrat S, Goderel I, Martha B, Payan C, Lunel-Fabiani F, Bani-Sadr F, Perronne C, Cacoub P, Pol S and Morand P (2008). Prevalence and impact of GBV-C, SEN-V and HBV occult infections in HIV-HCV co-infected patients on HCV therapy. *J Hepatol*. **49**(6):892– 898.

Platt L, Easterbrook P, Gower E, McDonald B, Sabin K, McGowan C, Yanny I, Razavi H and Vickerman P (2016). Prevalence and burden of HCV co-infection in people living with HIV: a global systematic review and meta-analysis. *Lancet Infect Dis*. S1473-3099(15)00485-5.

Polgreen P, Xiang J, Chang Q and Stapleton JT (2003). GB virus type C/hepatitis G virus: a nonpathogenic flavivirus associated with prolonged survival in HIV infected individuals. *Microbes Infect*. **5**:1255-1261.

Porichis F, Hart MG, Zupkosky J, Barblu L, Kwon DS, McMullen A, Brennan T, Ahmed R, Freeman GJ, Kavanagh DG and Kaufmann DE (2013). Differential impact of PD-1 and/or IL-10 blockade on HIV-1- specific CD4 T cell and antigen presenting cell functions. *J Virol.* **88**:2508-18.

Prince A, Brotman B, Grady G, Kahns WJ, Hazzi C, Levine R W and Millian SJ (1974). Long incubation post transfusion hepatitis without serological evidence of exposure to hepatitis B virus. *Lancet*. **2**: 2416.

Quinn TC (1996). Global burden of the HIV pandemic. *Lancet.* **348**: 99-106.

Ramezani A, Gachkar L, Eslamifar A, Manoochehr K, Somayeh J, Ladan A Vahid S and Rasool H (2008). Detection of hepatitis G virus envelope protein E2 antibody in blood donors. *Int J Infect Dis*. **12**: 57-61.

Rendina D, Vigorita E, Bonavolta R, D'Onofrio M, Iura A, Pietronigro MT, Laccetti R, Bonadies G, Liuzzi G, Borgia G, Formisano P, Laccetti P and Portella G (2001). HCV and GBV-c/HGV infection in HIV positive patients in southern Italy. *Eur J Epidemiol.* 801-7.

Reshetnyak VI, Karlovich TI and Ilchenko LU (2008). Hepatitis G virus. *World J Gastroenterol*. **14**: 4725-4734.

Rey D, Fraize S, Vidinic J, Meyer P, Fritsch S, Labouret N, Schmitt C, Lang JM and Stoll-Keller F (1999). High prevalence of GB virus C/hepatitis G virus RNA in patients infected with human immunodeficiency virus. *J Med Virol.* **57**: 75-9.

Reyes GR, Purdy MA, Kim P, Luk KC, Young LM, Fry KE and Bradley NW (1990). Isolation of a cDNA from the virus responsible for enterically transmitted non-A, non B hepatitis. *Science*. **247**: 1335-1339.

Reynes J, Portales P, Segondy M, Baillat V, André P, Avinens O, Picot MC, Clot J, Eliaou JF and Corbeau P (2001). CD4 T cell surface CCR5 density as a host factor in HIV-1 disease progression. *AIDS*. **15**:1627–1634.

Riaz Shah SA, Idrees M and Hussain A (2011). Hepatitis G virus associated aplastic anemia: a recent case from Pakistan. *Virol J*. **21**: 8:30.

Rizzetto M, Canese MG, Arico S, Crivelli O, Trepo C, Bonino F and Verme G (1977). Immunofluorescence detection of a new antigen/antibody system (Delta/ anti-Delta) associated with hepatitis B virus in liver and serum of HBsAg carriers. *Gut*. **18**: 997–1003.

Robert J Natuk, Pranabk C, Michaled L, Alan R, James W, Richard H, Mark W, Bheem B, Satoshi M, Sally L, Ally L, Jorg E, Robert G, Paul H and Marjorie G (1992). Adenovirus-human immunodeficiency virus (HIV) envelope recombinant vaccines elicit high titered HIV-neutralizing antibodies in the dog model. *Proc Nad Acad Sci*. **89**: 7777-7781.

Robert T Rydze, Nirjal Bhattarai and Jack T Stapleton (2012).GB virus C infection is associated with a reduced rate of reactivation of latent HIV and protection against activation-induced T-cell death. *Antiviral Therapy.* **17**:1271–1279.

Roberts JD, Bebenek K and Kunkel TA (1988). The accuracy of reverse transcriptase from HIV-1. *Science*. **242**:1171-3

Rockstroh JK and Spengler U (2004). HIV and hepatitis C virus co-infection. *The Lancet Infectious Diseases.* **4**:437-444.

Rodriguez B, Woolley I, Lederman MM, Zdunek D, Hess G and Valdez H (2003). Effect of GB virus C coinfection on response to antiretroviral treatment in human immunodeficiency virusinfected patients. *J Infect Dis.* **187**:504–507.

Rong L and AS Perelson (2009). Modeling latently infected cell activation: viral and latent reservoir persistence, and viral blips in HIV-infected patients on potent therapy. *PLoS Comput Biol.* **5**:e1000533.

Rosenthal E, Poiree M, Pradier C, Perronne C, Salmon-Ceron D, Geffray L, Myers R P, Morlat P, Pialoux G, Pol S and Cacoub P (2003). Mortality due to hepatitis C-related liver disease in HIV-infected patients in France. *AIDS.* **17**: 1803-9.

Ruiz V, Espinola L, Mathet VL, Perandones CE and Oubina JR (2006). Design, development and evaluation of a competitive RT-PCR for quantitation of GBV-C RNA. *J Virol Methods*. **136**:58-64.

Ruiz V, Giordano M, Rivero CW, Minassian ML, Cuestas ML, Trinks J, Mathet VL and Oubina JR (2010). GB virus C quasispecies detected in plasma and lymphocyte subsets in a natural human infection. *J Gen Virol*. **91**(7):1687– 1692.

Ryt-Hansen R, Katzenstein TL, Gerstoft J and Eugen-Olsen J (2006). No influence of GB virus C on disease progression in a danish cohort of HIV-infected men. *AIDS Res Hum Retroviruses*. **22**(6):496-498.

Saiz IC, Ampurdanes S, Olmedo E, Lopez-Labrador F, Foms X, Guilera M, Tassies D, Costa I, Sanchez-Tapias IM, Jimenez de Anta MT and Rodes J (1997). Hepatitis G virus infection in chronic hepatitis C: frequency, features and response to interferon therapy. *J Hepatol*. **26**:787-793.

Salmon-Ceron D, Lewden C, Morlat P, Bevilacqua S, Jougla E, Bonnet F, Heripret L, Costagliola D, May T and Chene G (2005). Liver disease as a major cause of death among HIV infected patients: role of hepatitis C and B viruses and alcohol. *J Hepatol*. **42**:799-805.

Sarafianos SG, Marchand B, Das K, Himmel DM, Michael A. Parniak MA, Hughes SH and Arnold E (2009). Structure and Function of HIV-1 Reverse Transcriptase: Molecular

Mechanisms of Polymerization and Inhibition. *Journal of Molecular Biology.* **385**:693-713.

Sathar MA, Soni PN, Pegoraro R, Simmonds P, Smith DB, Dhillon AP and Dusheiko GM (1999a). A new variant of GB virus C/hepatitis G virus (GBV-C/HGV) from South Africa. *Virus Res.* **64**: 151-160.

Sathar M, Soni P, Naicker S, Conraide J, Lockhat F and Gouws E (199b). GB Virus C/Hepatitis G Virus infection in KwaKulu Natal, South Africa. *J Med Virol*. **59**: 38-44.

Sathar MA and York DF (2001). Group 5: GBV-C/HGV isolates from South Africa. *J Med Virol*. **65**: 121-122.

Sather M, York D, Gouws E, Coutsoudis A and Coovadia H (2004). GB virus type C coinfection in HIV-infected African mothers and their infants, KwaZulu Natal, South Africa. *Clin Infect Dis.* **38**:405–9.

Scallan MF, Clutterbuck D, Jarvis LM, Scott G and Simmonds P (1998). Sexual transmission of GB virus C/hepatitis G virus. *J Med Virol.* **55**: 203-208.

Schaluder GG, Dawson GJ, Simons JN, Pilot-Matias TJ, Gutierrez RA, Heynen CA, Knigge MF, Kurpiewski GS, Buijk SL, Leary TP, Muerhoff AS, Desai S M and Mushahwar lK (1995). Molecular and serologic analysis in the transmission of the GB hepatitis agents. *J Med Virol*. **46**: 81-90.

Schlueter V, Schmolke S, Stark K, Hess G, Ofenloch-Haehnle B and Engel AM (1996). Reverse transscription-PCR detection ofhepatitis Gvirus. *J Clin Microbiol*. **34**:26602664.

Schunter M, Chu H, Hayes TL, McConnell D, Crawford SS, Luciw PA, Bengmark S, Asmuth DM, Brown J, Bevins CL, Shacklett BL and Critchfield JW (2012). Randomized pilot trial of a synbiotic dietary supplement in chronic HIV-1 infection. *BMC Complement Altern Med.* **12**:84.

Schwarze-Zander C, Blackard JT, Zheng H, Addo MM, Lin W, Robbins GK, Sherman KE, Zdunek D, Hess G, Chung RT and AIDS Clinical Trial Group A5071 Study Team (2006). GB virus C (GBV-C) infection in hepatitis C virus (HCV)/HIV-coinfected patients receiving HCV treatment: importance of the GBV-C genotype. *J Infect Dis*. **194**:410–9.

Schwarze-Zander C, Markus Neibecker, Sabrina Othman, Cristina Tural, Bonaventura Clotet, Jason T Blackard, Bernd Kupfer, Guido Luechters, Ray T Chung, Jürgen K Rockstroh and Ulrich Spengler (2010). GB virus C coinfection in advanced HIV type-1 disease is associated with low CCR5 and CXCR4 surface expression on CD4+ T-cells. *Antiviral Therapy*. 15:745–752.

Schwarze-Zander C, Blackard JT, Zheng H and Juergen K Rockstroh (2012). Role of GB virus C in modulating HIV disease. *Expert Rev Anti Infect Ther*. **10**(5): 563–572.

Seyoum A, Debrework Z, Getachew G, Fehniger T, Gezahegn A, Tebebe Y and Britton S (1988). Prevalence of anti-HIV antibodies in female population in Addis Ababa. The Ethiopian Medical Doctors' Association. Twenty fourth Annual Medical Conference.

Sharp PM and Hahn BH (2011). Origins of HIV and the AIDS pandemic. *Cold Spring Harb Perspect Med*. **1**(1):a006841.

Sheikh Asfandyar, Muhammad Farhan Khaliq and Muhammad Muslim Noorani (2014). Virus against virus (VIVI): a potential solution against HIV/AIDS. *International Archives of Medicine.* 7:19.

Sheng WH, Hung CC, Wu RJ, Wang JT, Chen PJ, Shan-Chwen Chang and Jia-Horng Kao (2007). Clinical impact of GB virus C viremia on patients with HIV type 1 infection in the era of highly active antiretroviral therapy. *Clin Infect Dis.* **44**: 584-590.

Shepard RN, Schock J, Robertson K, Shugars DC, Dyer J, Vernazza P, Hall C, Cohen MS and Fiscus SA (2000). Quantitation of HIV type 1 RNA in different biological compartments. *J Clin Microbiol*. **38**:1414-1418 (2000).

Shimelis T, Tassachew Y, Agete Tadewos, Mesfin Worku Hordofa, Anteneh Amsalu, Birkneh T and Endale T (2017). Coinfections with hepatitis B and C virus and syphilis among HIV-infected clients in Southern Ethiopia: a cross-sectional study. *HIV/AIDS –Research and Palliative Care*. **9**; 203–210.

Shimizu YK, Hijikata M, Kiyohara T, Yoshihiro Kitamura, and Hiroshi Yoshikura (1999). Replication of GB virus C (hepatitis G virus) in interferon-resistant Daudi cells. *J Virol*. **73**:8411–4.

Shiratori Y, Kato N, Yokosuka O, Irnzeki F, Hashiomoto E, Hayashi N, Nakamura A, Asada M, Kuroda H, Tanaka N, Arakawa Y, Omata M and for the Tokyo-Chiba Hepatitis Research Group (1997). Predictors for the efficacy of interferon therapy in chronic hepatitis C virus infection. *Gastroenterol.* **13**: 558-566.

Siegal F (2003). Interferon-producing plasmacytoid dendritic cells and the pathogenesis of AIDS. *Res Initiat Treat Action*. **8**:10–3.

Silvestris F, Cafforio P, Frassanito MA, Tucci M, Romito A, Nagata S and Dammacco F (1996). Over expression of Fas antigen on T cells in advanced HIV-1 infection: differential ligation constantly induces apoptosis. *AIDS.* **10**(2): 131–141.

Simmonds P (2001). Reconstructing the origins of human hepatitis viruses. *Philos Trans R Soc Lond B Biol Sci*. **356**(1411):1013–26.

Simons JN, Pilot-Matias TI, Leary TP, Dawson GI, Desai SM, Schlauder GG, Muerhoff AS, Erker IC, Buijik SL, Chalmers ML, Van Sant CL and Mushahwar IK (1995a). Identification of two flavivirus-like genomes in the GB hepatitis agent. *Proc Natl Acad Sci*. **921**: 3401-3405.

Simons JN, TP Leary, GJ Dawson, TJ Pilot-Matias, AS Muerhoff, GG. Schlauder, SM Desai and IK Mushahwar (1995b). Isolation of novel virus-like sequences associated with human hepatitis. *Nat Med*. **1**:564-569.

Simons JN, Desai SM and Mushahwar IK (1996). The GB Viruses: Isolation, characterization, diagnosis and epidemiology. *Viral Hepatitis.* **2**: 229-246.

Simons JN, Desai SM and Mushahwar IK (2000). The GB viruses. *Curr Top Microbiol Immunol*. **242**:341-375.

Smith DB, Basaras M, Frost S, Haydon D, Cuceanu N, Prescott L, Kamenka C, Millband D, and Sathar MA and Simmonds P (2000). Phylogenetic analysis of GBV- C/hepatitis G virus. *J Gen Virol.* **81**: 769-780.

Soriano V, Mocroft A, Peters L, Rockstroh J, Antunes F, Kirkby N, de Wit S, d'Arminio Monforte A, Flisiak R and Lundgren J (2010). Predictors of hepatitis B virus genotype and viraemia in HIV infected patients with chronic hepatitis B in Europe. *J Antimicrob Chemother.* **65**: 548–555

Sousa A, Carneiro J, Meier-SchellersheimM, Grossman Z and Victorino R (2002). CD4 T cell Depletion Is Linked Directly to Immune Activation in the Pathogenesis of HIV-1 and HIV-2 but Only Indirectly to the Viral Load. *J Immunol*. **169**:3400-6.

Souza IE, Zhang W, Diaz RS, Chaloner K, Klinzman D and Stapleton JT (2006). Effect of GB virus C on response to antiretroviral therapy in HIV-infected Brazilians. *HIV Med*. **7**:25–31.

Stamatatos L, Morris L, Burton DR and Mascola J R (2009). Neutralizing antibodies generated during natural HIV-1 infection: good news for an HIV-1 vaccine? *Nature Med*. **15**: 866–870.

Stapleton JT (2003). GB virus type C/Hepatitis G virus. *Semin Liver Dis.* **23**:137-148.

Stapleton JT, Chaloner K, J Zhang, D Klinzman, IE Souza, J Xiang, A Landay, J Fahey, R Pollard and R Mitsuyasu (2009). GBV-C viremia is associated with reduced CD4 expansion in HIV-infected people receiving HAART and interleukin-2 therapy. *AIDS*. **23**:605-610.

Stapleton JT, S Foung, AS Muerhoff, J Bukh and P Simmonds (2011). The GB viruses: a review and proposed classification of GBV-A, GBV-C (HGV), and GBV-D in genus Pegivirus within the family Flaviviridae. *J Gen Virol.***92**:233-246.

Stapleton JT, Chaloner K, Martenson JA, Zhang J, Klinzman D, Xiang J, Sauter W, Desai SN and Landy A (2012). GB Virus C Infection Is Associated with Altered Lymphocyte Subset Distribution and Reduced T cell Activation and Proliferation in HIV-Infected Individuals. *PLoS ONE.* **7**(11): e50563.

Stapleton JT, Nirjal Bhattarai, James H McLinden, Jinhua Xiang, Alan L Landay and Ernest T Chivero (2013). GB Virus C Particles Inhibit T Cell Activation via Envelope E2 Protein-Mediated Inhibition of TCR Signaling. *Journal of Immunology*. **190**: doi:10.4049/jimmunol.1300589.

Stevenson M, Stanwick TL, Dempsey MP and Lamonica CA (1990). HIV-1 replication is controlled at the level of T cell activation and proviral integration. *EMBO J*. **9**: 1551–60.

Strong MJ, O'Grady T, Lin Z, Xu G, Baddoo M, Parsons C, Zhang K, Taylor CM and Flemington EK (2013). Epstein-Barr virus and human herpesvirus 6 detection in a non-Hodgkin's diffuse large B-cell lymphoma cohort by using RNA sequencing. *J Virol*. **87**(23):13059-62.

Sugai Y, Nakayama H, Fukuda M, Sawada N, Tanaka T, Tsuda F, Okamoto H, Miyakawa Y and Mayumi M (1997). Infection with GB virus C in patients with chronic liver disease. *J Med Virol*. **51**: 175-181.

Sultan M, Rahman M and Begum S (2009). Epidemiology of Hepatitis C Virus Infection. *J Bangladesh Coll Phy Sur*. **27**: 160-162.

Sunil K. Khattar, Sweety Samal, Anthony L DeVico, Peter L Collins and Siba K Samal (2011). Newcastle Disease Virus Expressing Human Immunodeficiency Virus Type 1 Envelope Glycoprotein Induces Strong Mucosal and Serum Antibody Responses in Guinea Pigs. *Journal of Virology*. **85**(20): 10529–10541.

Supapol WB, RS Remis, J Raboud, M Millson, J Tappero, R Kaul, P Kulkarni, MS McConnell, PA Mock, M Culnane, J McNicholl, A Roongpisuthipong, T Chotpitayasunondh, N Shaffer and S Butera (2008). Reduced mother-to-child transmission of HIV associated with infant but not maternal GB virus C infection. *J Infect Dis.* **197**:1369-1377.

Tagger A, Donato F, Ribero ML, Chiesa R, Tomasoni V, Portera G, Gelatti U, Albertini A, Fasola M and Nardi G (1997). A case-control study on GB virus C/hepatitis G virus infection and hepatocellular carcinoma. Brescia HCC Study. *Hepatology.* **26**: 1653- 1657.

Tan De, Akihiro Matsumoto, Cathy Conry-Cantilena, Jacqueline C Melpolder, James W K Shih, Michael Leuther, George Hess, Joan W Gibble, Paul M Ness and Harvey J Alter (1999). Analysis of Hepatitis G Virus (HGV) RNA, Antibody to HGV Envelope Protein, and Risk Factors for Blood Donors Coinfected with HGV and Hepatitis C Virus. *J Infect Dis.* **179** (5): 1055-1061.

Tanaka E, Alter HJ and Nakatsuji Y (1996). Effect of hepatitis G virus infection on chronic hepatitis C. *Ann Intern Med.* **125**: 740-743.

Tanaka E, Tacke M, Kobayashi M, Nakatsuji Y, Kiyosawa K, Schmolke S, Engel AM, Hess G and Alter HJ (1998a). Past and present hepatitis G virus infections in areas where hepatitis C is highly endemic and those where it is not endemic. *J Clin Microbiol*. **36**(1):110–114.

Tanaka Y, Mizokami M, Orito E, Ohba K, Nakano T, Kato T, Kondo Y, Ding X, Ueda R, Sonoda S, Tajima K, Miura T and Hayami M (1998b). GB virus C/hepatitis G virus infection among Colombian native Indians. *Am J Trop Med Hyg.* **59**: 462-467.

Tanji Y, Hijikata M, Hirowatari Y and Shimotohno K (1994). Hepatitis C virus polyprotein processing : kinetics and mutagenic analysis of serine proteinase- dependent cleavage. *J Virol.* **68**:8418-22.

Tassopoulos NC, Papatheodoridis GV, Delladetsima I and Hatzakis A (2008). Clinicopathological features and natural history of acute sporadic non-(A-E) hepatitis. *J Gastroentrol Hepatol*. **8**(1):1208-15.

Thomas DL, Vlahov D, Alter HI, Hunt, Marshall R, Astemborski I and Nelson KE (1998). Association of antibody to GB Virus C (Hepatitis G Virus) with viral clearance and protection from reinfection. *J Infect Dis*. **177**: 539-542.

Thomas F, Robert T and Fritz V (2007). Pathogenesis of hepatitis B virus infection. *World J Gastroenterology*. **13**(1): 82-90.

Thompson MA, Aberg JA, Pedro G, Montaner JSG, Rizzadini G, Telenti A, Gatell JM, Gunthard HF, Hammer SM, Hirsch MS, Jacobsen DM, Richman DD, Volberding PA, Yeni P and Schooley RT (2010). Antiretroviral treatment of adult HIV infection, recommendation of the international AIDS society–USA PANEL. *J American Medical Association (JAMA).* **304**(3):321-333.

Tillmann HL, Heiken H, Knapik-Botor A, Heringlake S, Ockenga J, Wilber JC, Goergen B, Detmer J, McMorrow M, Stoll M, Schmidt RE and Manns MP (2001a). Infection with GB virus C and reduced mortality among HIV-infected patients. *N Engl J Med*. **345**:715-24.

Tillmann HL and Manns MP (2001b). GB virus-C infection in patients infected with the human immunodeficiency virus. *Antiviral Res.* **52**:83–90.

Tillmann HL, Manns MP, Claes C, Heiken H, Schmidt RE and Stoll M (2004). GB virus C infection and quality of life in HIV-positive patients. *AIDS Care.* **16**:736-43.

Tillmann HL, Kaiser T, Fox Z, Staszewski S, Antunes F, Monforte Ad, Vernazza P, Hill A, Dragsted UB, Lundgren JD and MaxCmin1 Trial Group (2005). Impact of coinfection with HIV-1 and GB virus C in patients receiving a ritonavir-boosted HAART regimen: a substudy to the MaxCmin1 trial. *J Acquir Immune Defic Syndr*. **40**:378–80.

Toyoda H, Fukuda Y, Hayakawa T, Takamatsu J and Saito H (1998). Effect of GB virus C/hepatitis G virus co-infection on the course of HIV infection in hemophilia patients in Japan. *Journal Acquired Immune Deficiency Syndromes*.**17:**209-13.

Toyoda H, Honda T, Katano Y, Goto H and Takamatsu J (2005). Clearance of GB virus C during highly active antiretroviral therapy and course of HIV disease progression in HIV-infected patients with hemophilia. *Eur J Clin Microbiol Infect Dis.* **24**:645–6.

Tsega E, Mengesha B, Nordenfelt E, Hansson BG and Lindberg J (1988). Serological Survey of Human Immunodeficiency Virus Infection in Ethiopia. *Ethiopian Medical Journal*. **26**:179- 184.

Tsega E (2000). Epidemiology, prevention and treatment of viral hepatitis with emphasis on new developments. *Ethiop Med J.* **38**: 131-141.

Tucker TJ, Smuts H, Eickhaus P, Robson SC and Kirsch RE (1999). Molecular characterization of the 5' non-coding region of South African GBV-C/HGV isolates: major deletion and evidence for a fourth genotype. *J Med Virol*. **59**: 52- 59.

Tucker TJ and Smuts HE (2000). GBV-C/HGV genotypes: proposed nomenclature for genotypes 1-5. *J Med Virol* . **62**: 82-83.

UK Collaborative group on HIV drug resistance (2005). HIV drug resistance in antiretroviral treated individuals in the United Kingdom. *The Journal of Infectious Diseases.* **192**:967-973.

Umlauft F, Wong DT, Underhill PA, Oefner PJ, Jin L, Urbanek M, Gruenewald K and Greenberg HB (1997). Hepatitis G virus infection in haemodialysis patients and the effects of interferon treatment. *Am J Gastroenterol.* **92**: 1986-1991.

Unchida T, Shimojima S, Gotoh K, Shikata T and Miune S (1994). Pathology of liver infected with silent hepatitis B virus mutant. *Liver.* **14**:251-6.

UNAIDS. (2014). Factsheet 2014: Global Statistics. Geneva. p. 1.

UNAIDS. (2015). Factsheet 2015: Global Statistics. Geneva. p. 1-2.

Valinciute A, Kiveryte S and Mauricas M (2013). GB Virus C Infection among Lithuanian Population with Hepatitis C (HCV) Virus Infection. *J Antivir Antiretrovir.* **5**: 132-136.

Van der Bij AK, Kloosterboer N, Prins M, Boeser-Nunnink B, Geskus RB, Lange J, Coutinho R and Schuitemaker H (2005). GB virus C coinfection and HIV-1 disease progression: The amsterdam cohort study. *J Infect Dis*. **191**(5):678-685.

Van der Meer AJ, Veldt BJ, Feld JJ, Wedemeyer H, Dufour JF, Lammert F, Duarte-Rojo A, Heathcote EJ, Manns MP, Kuske L, Zeuzem S, Hofmann WP, de Knegt RJ, Hansen BE and Janssen HL (2012). Association between sustained virological response and all-cause mortality among patients with chronic hepatitis C and advanced hepatic fibrosis. *JAMA*. **308**:2584–2593.

Vandamine P and Van Herck K. (2007). A review of the long - term protection after hepatitis A and B vaccination. *Travel Med Infect Dis*. **5**(2):79-84.

Vikram Mehraj, Mohammad-Ali Jenabian, Kishanda Vyboh and Jean-Pierre Routy (2014). Immune Suppression by Myeloid Cells in HIV Infection: New Targets for Immunotherapy. *The Open AIDS Journal*. **8**: 66-78.

Walker BD and Burton DR (2008). Toward an AIDS vaccine. *Science*. **320**:760-4.

Wachtler M, Hofmann A, Muller G, Frosner G, Nitschko H, Karwat M, Knetsch I, Emminger C and Eichenlaub D (2000). Prevalence of GB virus C/ hepatitis G virus RNA and anti-E2 glycoprotein antibodies in homosexual men with HIV coinfection. *Infection*. **28**: 297-300.

Wang HL and Jin DY (1997). Prevalence and genotype of hepatitis G virus in Chinese professional blood donors and hepatitis patients. *J Infect Dis*. **175**(5):1229-33.

Westrop SJ, Moyle G, Jackson A, Nelson M, Mandalia S and Imami N (2012). CCR5 antagonism impacts vaccination response and immune profile in HIV-1 infection. *Mol Med.* **18**:1240-8.

Williams CF, Klinzman D, Yamashita TE, Xiang, Polgreen P M, Rinaldo C,Liu C, Phair J, Margolic JB, Zdunek D, Hees G and Stapleton JT (2004). Persistent GB virus C infection and survival in HIV-infected men. *N Engl J Med*. **350:**981–990.

Williams C, Allen JB, Benning L, Gange S, Anastos K, Greenblat R, Cohen M, Levine A, Young M, Minkoff H and Stapleton J (2005). Prevalence, acquisition, and clearance of GB virus type C in the women's interagency HIV study. 12th *Conference on Retroviruses and Opportunistic Infections,Boston*, MA. Boston, MA.

Winni De Haes, Charlotte Pollard, Guido Vanham and Joanna Rejman (2012). "Wrapped Up" Vaccines in the Context of HIV-1 Immunotherapy. (http://creativecommons.org/licenses/by/3.0)

World Health Organization (2002). Hepatitis B, fact sheet. Available from: URL: http://www.who.int/entity/csr/disease/hepatitis/HepatitisB_whocdscsrlyo2002pdf.

World Health Organization (2005). The world health report. Available from: URL: www.who.int/whr/2002/annex/en/.

World Health Organization (2006). WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV related disease in adults and children. In. Geneva: World Health Organization.

World Health Organization (2012). Hepatitis B, fact sheet no.**204**. Available from: URL: http://www.who.int/mediacentre/factsheets/fs204/en/index.

World Health Organization (2013). Global Policy Report on the Prevention and Control of Viral Hepatitis in WHO Member States. Geneva:World Health Organization.

World Health Organization. (2014). Global Update on the Health Sector Response to HIV. Geneva. p. 50.

World Health Organization (2015). *HIV/AIDS Fact sheet N°360*. Geneva. Available online: http://www.who.int/gho/hiv/en/

World Health Organization (2016). Hepatitis C Fact Sheet N°164. Available from: http://www.who.int/mediacentre/factsheets/fs164/en/.

World Health Organization (2016**).** Consolidated guidelines on HIV prevention, diagnosis, treatment and care for key populations.

World Population Prospects (2017 Revision) (https://esa.un.org/unpd/wpp/) - United Nations population estimates and projections. Total population: Estimated to be consistent with the 1984, 1994 and 2007 censuses adjusted for under enumeration, and with estimates of the subsequent trends in fertility, mortality and international migration.

Worobey M, Gemmel M, Teuwen DE, Haselkorn T, Kunstman K, Bunce M, Muyembe JJ, Kabongo JM, Kalengayi RM, Marck EV, Gilbert MT P and Wolinsky SM (2008). Direct evidence of extensive diversity of HIV-1 in Kinshasa by 1960. *Nature*. **455**:661-4.

Wu RR, Mizokami M, Cao K, Nakano T, Ge XM, Wang SS, Orito E, Ohba K, Mukaide M, Hikiji K, Lau JY and Lino S (1997). GB virus C/hepatitis G virus infection in Southern China. *J Infect Dis.* **175**:168-71.

Wyatt R and Sodroski J (1998). The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. *Science*. **280**:1884-8

Xiang J, Klinzman D, McLinden J, Schmidt W.N, LaBrecque D.R, Gish R and Stapleton IT (1998). Characterization of hepatitis G virus (GB-C virus) particles: Evidence for a nucleocapsid and expression of sequences upstream of the E1 protein. *J Virol*. **72**(4):2738-2744.

Xiang J, Wunschmann S, Schmidt W, Shao J and Stapleton JT (2000). Full-length GB virus C (Hepatitis G virus) RNA transcripts are infectious in primary CD4-positive T cells. *J Virol*. **74**:9125–33.

Xiang J, Wunschmann S, Diekema DJ, Klinzman D, Patrick KD, George SL and Stapleton JT (2001). Effect of co-infection with GB virus C (hepatitis G virus) on survival among patients with HIV infection. *New England Journal of Medicine.* **345**:707-14.

Xiang J, George SL, Wunschmann S, Qing Chang, Donna Klinzman and Jack T Stapleton (2004). Inhibition of HIV-1 replication by GB virus C infection through increases in RAN TES, MIP- 1α, MIP-1β, and SDF-1. *Lancet*. **363**:2040-6.

Xiang J, McLinden JH, Chang Q, Kaufman TM and Stapleton JT (2006). An 85-aa segment of the GB virus type C NS5A phosphoprotein inhibits HIV-1 replication in CD4+ Jurkat T cells. *Proc Natl Acad Sci USA.* **103**: 15570-15575.

Xiang J, Chang Q, McLinden JH, Stapleton JT and Sather MA (2007). Expression of GB virus C NS5A protein from genotypes 1, 2, 3 and 5 and a 30 aa NS5A fragment inhibit human immunodeficiency virus type 1 replication in a CD4+ T-lymphocyte cell line. *J Gen Virol*. **88**:3341–6.

Xiang J, Mclinden JH, Chang Q, Emma L. Jordan and Jack T Stapleton (2008). Characterization of a peptide domain within the GB virus C NS5A phosphoprotein that inhibits HIV replication. *PLosONE* . **3**(7): e2580.

Yeo AE, Matsumoto A, Hisada M, Shih JW, Alter HJ and Goedert JJ (2000). Effect of hepatitis G virus infection on progression of HIV infection in patients with hemophilia. multicenter hemophilia cohort study. *Ann Intern Med*. **132**(12):959-963.

Yeni PG, Hammer S M, Carpenter C J C, Cooper DA, Fischl MA, Gatell JM, Gazzard BG, Hirsch MS, Jacobsen DM, Katzenstein DA, Montaner JS, Richman DD, Saag MS, Schechter M, Schooley RT, Thompson MA, Vella S and Volberding PA (2002). Antiretroviral treatment for adult HIV infection in 2002: updated recommendations of the international AIDS Society-USA panel. *JAMA*. **288**: 222–235.

Yoshiba M, Okamoto H and Mishiro S (1995). Detection of the GBV-C hepatitis virus genome in serum from patients with fulminant hepatitis of unknown aetiology. *Lancet*. **346**:1131-1132

Yue Feng, Li Liu, Yue-Mei Feng, Wenhua Zhao, Zheng Li, A-Mei Zhang, Yuzhu Song andXueshan Xia (2014). GB Virus C infection in Patients With HIV/Hepatitis C Virus Coinfection: Improvement of the Liver Function in Chronic Hepatitis C. *Hepat Mon*. **14**(3):e14169.

Yves Levy (2010). Immunotherapy in HIV infection; current and future challenges. *Retrovirology.* **7**(1):I13.

Zhang W, Chaloner K, Tillmann HL, Williams CF and Stapleton JT (2006). Effect of early and late GB virus C viraemia on survival of HIV-infected individuals: A meta-analysis. *HIV Med*. **7**(3):173-180.

Zhu T, Korber BT, Nahmias AJ, Hooper E, Sharp PM and Ho DD (1998). An African HIV-1 sequence from 1959 and implications for the origin of the epidemic. *Nature*. **391**:594-7-7.

Ziaee M, Zarban A, Malekinejad P and Akhbary H (2007). Evaluation of HGV viremia prevalence and its co-infection with HBV, HCV, HIV and HTLV-1 in hemophilic patients of Southern Khorassan, Iran. *Hepat Mon.* **7**: 11–4.

Zoulim F and Locarnini S (2009). Hepatitis B virus resistance to nucleos(t)ide analogues. *Gastroenterology*. **137**:1593–608.

Zuckerman AJ (1983). The history of viral hepatitis from antiquity to the present. In: Deinhardt F, Deinhardt J. eds. viral hepatitis: Laboratory and Clinical Science. New York: Marcel Dekker. 2-32.