COMPARATIVE STUDY OF HEPATITIS B AND HEPATITIS C
VIRUSES AMONG HIV-1 INFECTED INTRAVENOUS DRUG USERS
AND NON-USERS IN MOMBASA COUNTY

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P150/20439/2010

A research thesis submitted in partial fulfilment of the requirements for the
award of the degree of Master of Science Infectious Diseases (Medical Virology)
in the School of Medicine of Kenyatta University

September 2017
DECLARATION

This thesis is my original work and has never been presented for any award in any other University.

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DEDICATION

This thesis is dedicated to my immediate family; my husband, Cromwell Mwiti, our son, Chase Karani Mwiti, our lovely sweet daughters Sherry Karimi Mwiti and Shereen Mukami Mwiti. Thank you for your patience and company.
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ACRONYMS AND ABBREVIATIONS

ALP  Alkaline phosphatase
ALT  Alanine transaminase
Anti-HBs  Antibody to hepatitis B surface antigen
ARV  Antiretroviral therapy
AST  Aspartate aminotransferase
CCC  Comprehensive Care Centres
CD4  Cluster of Differentiation 4
CDC  Centres for Disease Control and prevention
CPGH  Coast Provincial General Hospital
CTLs  Cytotoxic T Lymphocytes
ELISA  Enzyme Linked Immunosorbent Assay
FDA  Food and Drug Administration
HAART  Highly active antiretroviral therapy
HBeAg  Hepatitis B envelope antigen
HBsAg  Hepatitis B surface antigen
HBV DNA  Hepatitis B deoxyribonucleic acid
HBV  Hepatitis B Virus
HCC  Hepatocellular carcinoma
HCV  Hepatitis C Virus
HIV  Human Immunodeficiency Virus
IDUS  Intravenous Drug Users
IFN  Interferon
IRIS  Immune reconstitution inflammatory syndrome
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
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<tr>
<td>LDL</td>
<td>Low-density lipoprotein</td>
</tr>
<tr>
<td>LFTs</td>
<td>Liver function tests</td>
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<tr>
<td>MSM</td>
<td>Men Who Have Sex with Men</td>
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<td>NASCOP</td>
<td>National AIDS and STI Control Programme</td>
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<tr>
<td>NDUs</td>
<td>None drug users</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PEP</td>
<td>Post Exposure prophylaxis</td>
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<tr>
<td>PI</td>
<td>Protease Inhibitor</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
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<tr>
<td>RT</td>
<td>Reverse Transcriptase</td>
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<tr>
<td>S.O.P</td>
<td>Standard Operating Procedures</td>
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<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
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<tr>
<td>tRNA</td>
<td>Transfer Ribonucleic Acid</td>
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<tr>
<td>UNEP</td>
<td>United Nations Environmental Programme</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>y-GT/GGT</td>
<td>Gamma-Glutamyl Transpeptidase/Transferase</td>
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ABSTRACT

HIV/AIDS is a debilitating disease associated with high mortality and morbidity globally. In Kenya, it is the major cause of mortality across all gender and age groups, in effect, putting a huge demand on the healthcare system and the economy. The HIV 1 positive population faces major challenges such as the drug resistance, severe hepatic coupled with immunological deficiencies and toxicity. The problems are aggravated by co-infection with blood borne diseases, varied responses to the infection and therapy among vulnerable groups. This study purposed to determine and compare; the CD4 counts, HIV viral loads, liver enzyme markers and the prevalence of viral hepatitis among the HIV-1 positive IDUs and NDUs. A cross-sectional study was carried out in Mombasa County. A structured questionnaire was used to collect information on HIV AIDS, Hepatitis and social demographics. Blood samples were collected, screened and analyzed for HIV, HBV and HCV, CD4+ cell, HIV viral load and liver enzyme markers. Pearson’s Chi square, Student T test and one way Anova were used to analyze data. A P value ≤ 0.05 was considered statistically significant. A total of 200 participants consisting of 78 males and 122 females were recruited. Sero-prevalence of Hepatitis B and Hepatitis C was at 16% and 20% respectively among the IDUs and 11% and 8% respectively among the NDUs. The sero-prevalence of HIV+HBV+/HCV infection among IDUs and NDUs was 6% and 4% respectively. The liver enzyme markers (Alkaline phosphate, Aspartate aminotransferase and Alanine transferase) were markedly elevated among the co-infected participants than the mono-infected in both the NDU and IDU groups. IDUs showed elevated mean liver enzymes than the NDUs. The IDUs had a lower mean CD4+ cell count of 350.2 (±225.27) cells/µl than the NDUs 485.9 (±243.38) cells/µl (P˂0.0001). Participants who were co-infected showed remarkably low mean CD4+ cell counts of 192.91(±84.08) cells/µl than the mono-infected with mean count of 536.79 (±218.76) cells/µl (P=<<0.0004). A statistically significant high mean viral load of 153392.97(±395699.65) copies/ml was reported among co-infected participants than the mono-infected at 2435.05(±5596.88) copies/ml (P<0.0001) across the study population. The study established that the co-infection rates with HBV and HCV was higher among the IDUs than the NDUs. The liver profile indices indicated elevated liver enzymes among IDUs than NDUs. Co-infected participants had statistically significant higher liver enzyme markers than the mono-infected. Immunologically, CD4+ cell counts were lower among IDUs than the NDUs. Co-infected individuals had a lower mean CD4+ cell count than the mono-infected. IDUs had a statistically significant higher HIV viral load than the NDUs. The co-infected also indicated a statistically significant higher mean viral load than the mono-infected. This study proposes routine baseline screening of HBV and HCV for IDUs and NDUs owing to the high frequency of co-infections. A people driven campaign is also necessary to create awareness on the effects of the use of substances of abuse in relation to viral infections and treatment. Also the campaign is necessary to create awareness on the HBV vaccination.
CHAPTER ONE: INTRODUCTION

1.1 Background information

Hepatitis B virus, hepatitis C virus and Human Immunodeficiency Virus are blood borne viruses with similar epidemiological characteristics; these include risk populations and transmission route. Human immunodeficiency virus 1 infection is one of the leading causes of morbidity and mortality in the world. In Kenya, approximately 1.6 million people are infected with HIV and an estimated 77,647 new cases were reported in 2016 (Kenya National AIDS Control Council, 2016). The risks and incidents of HIV infection remain high among certain demographics globally, including injection drug users, commercial sex workers and men who have sex with other men, truck drivers and cross-border mobile populations (Papworth et al., 2013; Butler et al., 2014). HIV infection rates coupled with the emerging twin epidemic of HIV and IDU are worsening the already significant burden of high infection rates in Africa (Desalegn, 2013; Papworth et al., 2013).

An estimated 1.7 million of people living with AIDS are injection drug users (WHO, 2012). East and Southern Africa accounts for up to 15.5% HIV positive intravenous drug users (WHO, 2012). The increase in injection drug use in Kenya especially in the larger towns and cities like Mombasa and Nairobi is notable (Beckerleg et al., 2005; Strathdee et al., 2010). The reports of 2016 on the prevalence of HIV-1 among IDUs in Kenya stands at 18.3% (KNACC, 2016). The high burden of HIV-1 infections among IDUs is attributable largely to an increased exposure through sexual and parenteral routes, non-adherence to treatment, increased antiretroviral resistance, drug dependence and the presence of co-morbidities such as Tuberculosis and Hepatitis among others (Mizukoshi et al., 2008).
The transmission routes for HIV and HBV include sexual intercourse, percutaneous, and vertical, from mother to child. The transmission of HBV is more efficient than that of HIV (Nyirenda et al., 2008). HIV-1 and HBV pose a serious health threat worldwide (Athena et al., 2012). Both have no cure currently thus may translate into chronic disease, preclude cancer and are a source of increased morbidity and mortality. Resistance to antiviral drugs will usually manifests during or after a period of therapy and continued treatment becomes clinically ineffective (Hoffmann et al., 2009). Co-infection with the two viruses complicates the negative effects (Athena et al., 2012). In sub-Saharan Africa, the major route of HBV transmission is vertical, from mother to child or in early childhood. A positive Hepatitis B surface antigen test signifies the presence of HBV and it is estimated that 5 – 15% of HIV patients globally have chronic HBV infection (Sulkowski, 2008).

In sub-Saharan Africa, HBV-HIV-1 co-infection is estimated at 8-20% (Day et al., 2013). In Kenya, statistics indicate that the prevalence of HBV has risen and surpassed that of HIV three-fold in a span of two years (Odiwuor, 2014). A nationwide research conducted in 2013 revealed that out of about 150,000 people who donated blood, 1,200 were HIV-1 positive while 3,000 were diagnosed with HBV (Odiwuor, 2014). The prevalence of HBV in Kenya stands at 8.8% with a range of 8% - 30% experienced in urban areas (Okoth et al., 2006; Harania et al., 2008; Mutuma et al., 2011). More recent co-infection rates reports indicate a prevalence range of 3.6% to 10% among the general population (Muriuki et al., 2013; Kerubo et al., 2015; Kilongosi et al., 2015). Prevalence in co-morbidities of these viruses has continued to increase with an increase in intravenous drug users. Among the intravenous drug users the prevalence is between 8% and 15% (Kibaya et al., 2015; Kilongosi et al., 2015).
During the course of infection a greater risk of developing chronic HBV is associated with the failure to sero-convert in HIV-HBV co-infection and especially in intravenous drug users compared with HIV sero-negative individuals. This accelerates the progression rate and complications of viral hepatitis in the co-infected (Puoti et al., 2006; Thio, 2009). It follows therefore that in the presence of HIV-HBV co-infection, chronic hepatitis is much more likely than in HIV sero-negative persons (Gatanaga et al., 1998). When anti-retroviral therapy (ART) is instituted, immune reconstitution inflammatory syndrome (IRIS) may occur, this may worsen liver disease and result in hepatic decompensation among other symptoms (Drake et al., 2004). Hepatitis B may also be reactivated when an ART dose with anti-HBV agents is stopped (Bellini et al., 2009). Reports have indicated that HIV co-infection may speed up the advancement of HBV associated hepatitis. As a result the effects of co-infections is of great importance in areas of high ART use (Hoffmann et al., 2009). The increased use of ARTs in HBV endemic regions makes liver disease in chronic HBV-HIV co-infected a likely emergent public health risk. This is due to the increased life expectancy associated with their use among the HIV infected individuals (Hoffmann et al., 2009). Clifford et al. (2008) report that HIV infected individuals with low CD4 counts are at a higher risk of developing Hepato-cellular Carcinoma.

It is estimated that there are 71 million individuals suffering from chronic Hepatitis C virus infection (Hanafiah et al., 2012). In this infection, predisposing factors include transfusion of blood products, tattooing and intravenous drug use (Muasya et al., 2008). There is evidence that the parenteral route of transmission is the most common. Majorly, intravenous drug abusers, hemophiliacs and administration of unscreened blood are of major concern (Merat et al., 2000).
Hepatitis C virus transmission via sexual intercourse and vertically via the uterus and perinatal has been shown to occur but the risks are considered to be very low and incidences are rare (Muasya et al., 2008). Currently, injection drug use is the most prominent risk factor in the transmission of HCV virus. The practice of sharing the injection drugs is a common phenomenon where drugs are prepared and common needles and syringes are used in the administration of the drugs making it a common means of blood borne virus transmission (Hahn et al., 2002). In addition, flash-blood practices and engagement in sexual orgies magnifies the HCV, HBV and HIV transmission risk of sharing needles.

In Kenya, the recent prevalence of HIV/HCV among the population of drug injectors ranges between 15% to 25% (Muasya et al., 2008; Mwatelah et al., 2015), while in the general public the sero-prevalence is between 0.31% and 11% (Harania et al., 2008; Muriuki et al., 2013). While the interaction between HCV and HIV-1 is complex and poorly depicted, some studies have shown that HCV promotes a rapid decrease in the CD4 count and speeds up the disease progression during HIV-1 co-infection (Lichtfuss et al., 2011). The HCV-HIV-1 co-infection increases the rate of HCV hepatitis progression and may lead to ART associated hepato-toxicity (Tedaldi et al., 2003).

In HIV infection, it is notable that co-infection with viral hepatitis leads to a more pronounced rate of liver damage as opposed to hepatitis infections in the non-HIV infected individuals. In therapy, the adverse effects occasioned by the interaction between highly active anti-retroviral therapy (HAART) and HIV/HCV/HBV co-infections are becoming apparent (Sulkowski et al., 2007). There is however no consensus on the reverse effect on the progression of HIV/AIDS disease when there is co-infection with HCV or HBV (Konopnicki et al., 2005). The use of ART is credited
with a prolonged life expectancy among people living with HIV/AIDS (PLWA).
However, co-infection with HCV and HBV leading to an increase in liver disease has been a major blow to this achievement. These have caused adverse and life threatening complications among this group (Lodenyo et al., 2000).

The objective of this study was to determine the differences in prevalence of viral hepatitis, immunological markers (CD4+ count), virological markers (HIV viral load) and liver profile among the HIV-1 co-infected intravenous drug users and non-drug users.

1.2 Statement of the problem

Co-infection with HIV, HBV and HCV is a major health problem among PLWA and mostly among the IDUs. Currently, the urgency of HIV prevention in the general population and the risk groups such as IDUs overshadows the so called silent epidemic of viral hepatitis. The effects associated with hepato-tropic viruses are myriad and lethal. More-so, a co-infection with HIV complicates the health status, treatment and management of the diseases. The evidence of increasing prevalence of co-infections of HIV/HBV and HIV/HCV is seemingly treated as unimportant and very little has been published to outline the risk and frequency of intra and extra-exposure among the IDUs. In addition, little information exists on immunological, virological characterization and liver enzyme profiling features that could help guide on diagnosis, treatment and care for IDUs co-infected with HIV and HCV or HIV and HBV viruses or the three viruses.

1.3 Justification

The HBV, HCV and HIV viruses have debilitating effects on body mechanisms. They share similar patterns of transmission therefore the probability of intra and extra
exposure from the risk population to the general population is high. HIV co-infections have over the years posed a challenge during the formulation of treatment options and especially in the control of development of liver cirrhosis and subsequently hepatocellular carcinoma. HIV infection has been known to exacerbate the course of disease in HBV and HCV associated chronic hepatic disease. The use of HAARTs has greatly reduced the incidences of HIV/AIDS associated opportunistic infections. Liver disease is today emerging as a significant cause of morbidity and mortality in Africa. In Kenya and indeed worldwide, several studies have been conducted on HIV/HBV/HCV co-infections, these are however not enough and therefore there is still limited information on the impact and intervention strategies among the IDUs in Kenya.

1.4 Research questions

i. Is there any difference in the prevalence of HBV and HCV among the HIV-1 positive IDU and non-DU subjects in relation to gender and age?

ii. How do the CD4+ cell count and viral load compare among study population in relation to injection drug use and hepatitis virus infection status?

iii. What is the degree of elevation of liver enzyme markers and how do they compare among study population in relation to injection drug use and hepatitis virus infection status?
1.5 Study objectives

1.5.1 Broad objective

To determine and compare the hepatitis B and hepatitis C virus infection rates, CD4+ cell count, HIV viral load and the degree of elevation of liver enzyme markers among HIV-1 co-infected IDUs and NDUs in Mombasa County

1.5.2 Specific objectives

1. To determine and compare the prevalence of HBV and HCV infections among HIV-1 positive IDU and non-DU subjects

2. To determine and compare CD4+ cell count and HIV load among HBV and HCV co-infected HIV-1 positive IDU and non-DU subjects.

3. To determine and compare the degree of elevation of liver enzyme markers among HBV and HCV co-infected HIV-1 positive IDU and non-DU subjects

1.6 Significance of the study

This study is geared at establishing any variation in prevalence, immunological, Virological characterization and liver enzyme profiling features among IDUs and NDUs co-infected with HIV and HCV, or HIV and HBV, or the three viruses. The results obtained from this study will clearly give guidelines on the interventions that can be put in place in order to curb the intra and extra exposure of blood borne viral diseases. The outcomes will also give a clear picture on whether the clinical management among IDUs needs some extra consideration in comparison to the NDUs. The information will help the policy makers on prioritizing and strategizing on
interventions which assist in prevention, treatment and management of HIV/HBV/HCV co-infections.

1.7 Limitation and Delimitation of the study

The study was a cross sectional study hence could not zero down to the specific causal reasons of certain study outcomes such as the impact of the ART use on CD4+ cells, HIV viral load and liver enzyme markers over time. Also the study did not perform toxicological analysis which could truly ascertain the use of drugs and other substances of abuse instead it relied on self-report and scarification among the IDUs.
CHAPTER TWO: LITERATURE REVIEW

2.1 Hepatitis

Hepatitis refers to the inflammation of the liver resulting from factors that include, an immunological attack of the liver also referred to as autoimmune hepatitis, viral infections especially hepatitis A, B and C viruses, parasitic infections, alcohol and other hepatotoxic chemicals, poisonous mushrooms and other poisons. Inflammation of the liver can also result from medications such as an overdose of acetaminophen (Giannini et al., 2005).

Hepatitis viruses are classified into five main groups, which are types A, B, C, D and E. These are of great public health concern and constitute a major source of hepatic disease burden arising from their epidemic nature and high mortality rates (WHO, 2016). Valdiseri and Koh (2014), have reported that viral hepatitis are among the top ten infectious killer diseases, and account for the highest incidences of liver cirrhosis. In the era of ART and their increased availability in resource sufficient communities, liver disease has been shown to be the leading non-HIV/AIDS cause of morbidity and mortality in HIV-infected persons (Lodenyo et al., 2000).

2.1.1 Hepatitis B Virus

Structure of HBV virus

Hepatitis B virus is also called Dane particle after the investigator who first described its characteristic appearance under the electron microscope (Seeger & Mason, 2015). HBV is a hepadnavirus with a partially double stranded DNA. The virion has a 42nm outer lipid envelope containing the surface antigen (HBsAg) and a 27nm protein icosahedral nucleo-capsid core (HBcAg) (Seeger & Mason, 2000, Figure 2.1). Enclosed
in the nucleo-capsid are the viral DNA and a reverse transcriptase-DNA polymerase (Locarnini, 2004). Viral binding and cell entry are facilitated by proteins embedded on the outer envelope (Locarnini, 2004).

The HBV is one of the smallest enveloped animal virus with the ability to infect human hepatocytes (Harrison, 2009). Howard et al. (1990), also notes that other than Danes particles, serum from infected individuals’ express filamentous and spherical bodies that do not contain a core. These are non-infectious lipid and surface antigen particles excessively produced during the viral life cycle. The genomes full-length strand is 3020–3320 nucleotides long and 1700–2800 nucleotides long for the short length-strand (Zhang et al., 2016). Soon after infection viral DNA maybe observed in the nucleus of the infected cell.

Four genes are encoded by the genome and include the C, X, P, and S genes (Figure 2.1). The core protein is encoded by the gene C whose start codon is led by an upstream in-frame AUG start codon that produces the pre-core protein. The HBeAg is produced by a proteolytic processing of the pre-core proteins. The DNA polymerase is encoded by the P gene while the S gene is responsible for the HBsAg (Figure 2.1). The S gene consists of three in-frame ATG start codons and provides multi-start mechanisms. According to (Ganem & Varmus, 1987), these result in large middle or small polypeptides resulting from combinations of the three sections i.e. pre-S1, pre-S2 and S and giving rise to either Pre S1+ pre S2 or Pre S2 + S or S proteins (Figure 2.1). The gene X encodes a protein that has been linked to the occurrence of hepatic carcinoma seemingly stimulating growth of genes that promote cell growth and while inactivating growth-regulating molecules (Li et al., 2010).
Figure 2.1: Hepatitis B structure and genome

HBV is a partially double stranded, enveloped DNA virus. It measures 42-47 nm in diameter and is spherical in shape (Nahigan et al., 2011).

2.1.1.1 Hepatitis B virus Life cycle

Hepatitis B virus is a para-retrovirus that replicates through reverse transcription process. The host cells targeted by HBV are hepatocytes and the binding receptor is the sodium taurocholate co-transporting polypeptide on the cell surface (Yan et al., 2012). The HBV virions utilize the pre-S domain occurring on the viral surface antigen for host binding and its internalization is via clathrin-dependent endocytosis (Yan et al., 2012). The pre S-specific receptors are mainly expressed on hepatocytes but some viral DNA and proteins have been demonstrated in extra-hepatic sites, supporting the view that extra-hepatic cellular receptors for HBV do exist (Coffin et al., 2011).
In the HBV replication, host protein chaperones are involved in the transfer of the viral DNA into the nucleus of the cell. The viral DNA is made from host-facilitated RNA (Hollinger & Liang, 2001). Hollinger and Liang (2001) recorded that, the partly double stranded DNA is covalently transformed into a fully double stranded DNA and used as a template for the four viral RNAs that are 3.5, 2.4, 2.1 and 0.7kb long. These transcripts are then transported to the cell cytoplasm after polyadenylation where they are decoded to form the nucleo-capsid, the pre-C, viral polymerase, the envelope small, medium and large and the trans-activating proteins (Liang, 2009). The pre-genomic 3.5kb mRNA, polymerase RNA and a protein kinase are packaged together and serve as the reverse transcriptional material for a negative-strand DNA. The HBV RNA is converted to DNA within the virions (WHO, 2016). The new mature nucleo-capsids then either amplify viral genome inside the cell or form and secrete new virions (Patient et al., 2009; Seeger & Mason, 2000).

In the assembly pathway, the nucleo-capsids associate with the envelope proteins within the endoplasmic reticulum (ER), bud within the ER lumen and are secreted via the cellular golgi apparatus (Hollinger & Liang, 2001). Genome amplification is as a result of the nucleo-capsid delivery of the covalently closed circular DNA into the intranuclear pool (Liang, 2009). The pre-core HBeAg is formed when the pre-core polypeptides’ amino acid and carboxyl termini are cleaved and a resultant protein antigen secreted. Seeger and Mason (2000) also note that the HBV replication, interaction with transcription factors and capability to stimulate cell proliferation and death is influenced greatly by the viral X-Protein.
2.1.1.2 Hepatitis B virus genotypes

There are four HBV serotypes that have been characterized. These include \textit{adr}, \textit{adw}, \textit{ayr} and \textit{ayw}. The nucleotide sequence of the genome has also been used to classify the virus into ten genotypes A, B, C, D, E, F, G H, I, and J (Kramvis \textit{et al.}, 2005) that have been isolated in distinct geographical regions. Genotypic differences affect the severity of disease, disease progression, complications that may arise, vaccine and treatment regimens and are also useful in tracking disease development, evolution and spread (Magnius \& Norder, 1995). Norder \textit{et al.} (1994), recorded approximately 8% sequence differences between genotypes.

Most of the HBV genotypes have been characterized according to some discreet properties and common geographic locations (Schaefer, 2007). The sub-genotype distribution may overlap in certain areas. The genotype A is common in America, Africa, the Indian sub-continent and West Europe. The genotype B and C are the most prevalent in parts of Asia and the United States of America. In South Europe, India and the United States, genotype D is common while in South and West Africa genotype E is prevalent (Arauz-Ruiz \textit{et al.}, 2002; Kurbanov \textit{et al.}, 2005). In Latin America and the Caribbean genotype F is commonly found while in France the genotype G which is also prevalent in the USA is common. This genotype has been found to have a 36 nucleotide insert in its core gene. (Cotelesage \textit{et al.}, 2011) have identified unique properties due to this insertion. The genotype H is common in California (USA) and in south-central America (Deinstag \& Isselbacker, 2001). Five of the predominant genotypes A-E are found in Africa among which genotypes A-D are common in South Africa, D in Egypt and Tunisia, A, B and E in Kenya, Egypt and Nigeria respectively (Arauz-Ruiz \textit{et al.}, 2002; Kurbanov \textit{et al.}, 2005; Kramvis \& Kew, 2007; Ng’ang’a \textit{et al.}, 2013)
2.1.1.3 Pathogenesis of Hepatitis B Virus

In HBV infections, hepatic disease is mainly as a result of viral clearance and liver damage occasioned by host immune responses. This may be attributed to the tendency of the virus to replicate within the hepatocytes (Chisari et al., 2010). Although Fisicaro et al. (2009) suggest that innate response is not majorly involved in hepatocellular damage in these viral infections, (Iannacone et al., 2007; Han et al., 2013) show otherwise. Virus-specific cytotoxic T-Cells are the predominant actors in the pathogenesis of HBV and contribute a great deal towards liver injury (Rehermann & Nascimbeni, 2005; Malhi & Gores, 2008). CTLs produce antiviral cytokines that have been linked to purging of HBV from hepatocytes and viral particle killing (Iannacone & Guidotti, 2015). Iannacone et al. (2007), also suggest that non-antigen specific inflammatory cells may worsen CTL-induced viral clearance, and activate platelets that help increase levels of CTL in the liver.

2.1.1.4 Infection stages of HBV

Hepatitis B is also referred to as the “silent infection”, because clinical symptoms of the infection are somewhat hidden when first infected, or sometimes never (HBF, 2009). Approximately, 69% of people infected with HBV never manifest any signs or symptoms of the disease (HBF, 2009). Acute stages of HBV infection is characterized by among others, nausea, vomiting and general illness, body aches and mild fever, progressively darkening of the urine and jaundice may ultimately follow (Goldsmith, 2010). Viral hepatitis may also be characterized by itchy skin, an illness that may last a few weeks but from which most people gradually recover (Hoofnagle et al., 2007). Some individuals however will develop fulminant hepatitis which in most cases is life threatening (Bernaau et al., 1986; Diop et al., 2008).
On the other hand some individuals may have latent or chronic disease that last for a long period of time. Patients maybe asymptomatic or develop chronic hepatitis which may progress to cirrhosis of the liver and an increased probability of developing hepato-cellular carcinoma (Thorgeirsson & Grisham, 2002; Koziel & Peters, 2007). The probability of a chronic hepatitis B patient developing cirrhosis is 10-80 times and 30-150 times chances of developing liver cancer (Koziel & Peters, 2007). Due to the trauma on the liver, liver cancer usually develops after 25-30 years of living with HBV infection (Koziel & Peters, 2007). Approximately 10–20% of HBV-infected people may exhibit extra hepatic symptoms such as a serum-sickness like syndrome, Gianotti-Crosti syndrome in children and acute necrotizing vasculitis among others (Han, 2004). Other disorders associated with HBV are immune-mediated hematological disorders and include aplastic anemia and essential mixed-cryoglobulinemia (Schattner, 2005).

**2.1.1.5 Influence of HIV on the progression of HBV disease**

Co-infection with HIV may modify the course of acute HBV infection such that there are lower rates of spontaneous clearance and high incidence of icteric disease (Gatanaga et al., 1998). In HIV/HBV co-infection there are lower rates of HBeAg and increased levels of HBV DNA (Chien et al., 1999; Tran et al., 2015). Persons co-infected with HIV and HBV have higher levels of HBV DNA and lower rates of clearance of the HBeAg (Piroth et al., 2002; McMahon, 2009). They may also have lower serum transaminase levels than HBV-mono-infected individuals (Soriano et al., 2005). Thio et al. (2002) cautions that normal transaminase levels do not negate the presence of underlying hepatic fibrosis and that HIV infection increases the risk of cirrhosis and end-stage liver disease hence should never be used solely to conclude on the diagnosis. According to (Chauvel et al., 2007), HIV co-infection is majorly linked to frequent
episodes of increased transaminase levels which occur as a result of ART interruption and development of HIV/HBV resistance to treatment and are characterized by IRIS. End-stage liver disease has emerged as the leading cause of non-HIV related death in areas of widespread effective ART use. Chauvel *et al.* (2007), describe a high mortality rate of 14% in HIV/HBV co-infection compared to that found in HIV mono-infection which was at 6% (Bonacini *et al.*, 2004; D:A:D Study Group, 2006).

2.1.1.6 The effect of HBV on HIV to AIDS progression

Early workers of the ART era were divided on the role of HBV co-infection in HIV to AIDS disease progression. While Eskild *et al.* (1992) provided data for increased rate, Gilson *et al.* (1997) did not find any association. Recent study is still divided on the role of HBV on HIV to AIDS disease progression. Nikolopoulo *et al.* (2009), suggests that indeed the co-infection with HBV leads to an increased progression to AIDS in HIV infected patients while Núnez, (2006), determined that the increased use of ART targeting HBV may be associated with a decline in the progression of HIV to AIDS in HBV/HIV co-infected individuals. Some studies have also shown that HBV co-infection may define ART response and the occurrence of HIV/AIDS related illnesses and deaths (Konopnicki *et al.*, 2005; Rivera *et al.*, 2015).

2.1.1.7 Diagnosis of HBV in HIV Infection

The first consideration for a patient with HIV and suspicious of HBV should include history and physical examination. Specific concern should be focused on family history, previous infection with HBV, suspected HCC and previous or concurrent use of alcohol (Robinson, 1995). Diagnosis of hepatitis is made first by an evaluation of biochemical activity of the liver which includes; determination of direct and indirect bilirubin levels,
liver transaminases, alkaline phosphatase, total protein, globulin and albumin levels, full blood count and selected coagulation assays (Hollinger & Liang, 2001).

In the detection of HBV, whole blood, serum or plasma maybe used to determine the presence of HBV antigens or antibodies (Krajden et al., 2005). To correctly interpret these results, a multifaceted approach is needed (Bonino et al., 1988) (Table 2.1). Normally, the first antigen to appear in response to the infection is the hepatitis B surface antigen and it is therefore used for most detection assays (Thomson et al., 2009) (Table 2.1). However, in both early and late infection stages, the antigen may not be detectable or may be completely absent in some infected individuals (Jaroszewicz et al., 2010, Table 2.2). During this period of latency, the host may be able to clear the virus from its system successfully and the expression of Immunoglobulin M, anti-HBV core antigen is the only notable marker for the disease (Table 2.2). In this regard, diagnostic panels for hepatitis B may include HBsAg and total anti-HBc (IgM+IgG) (Thomson et al., 2009, Table 2.2). The e antigen normally occurs just after the expression of the antigen S and its expression is accompanied by increased viral replication rates and heightened infectivity. Some variants however do not express the antigen making it of limited use in diagnosis (Liaw et al., 2010).

The use of assays specifically targeting the whole virion, viral DNA or virions containing DNA polymerase in liver and serum are satisfactory but of limited use (Hollinger & Liang, 2001). For qualitative analysis of HBV DNA, two polymerase chain reaction based assays are Food and Drug Administration approved (FDA) (Table 2.1). These include the Amplicor™ Hepatitis B Virus Test, version 2.0, and the Cobas AmpliPrep™ Hepatitis B Virus Test, version 2.0 (Allice et al., 2007). The detection limits employed by these assays are low and range at about 50IU/ml. Serum alpha-
fetoprotein and liver ultrasonography are recommended for HCC screening (Bruix & Sherman, 2011). Patients with HBV replication, abnormal liver enzymes and HCV co-infection, a liver biopsy is recommended (Roche & Samuel, 2011). Puoti et al. (2004) recommend HCC surveillance for all HIV/HBV co-infected patients who may have chronic hepatitis B disease with persistence presence of HBsAg and Anti-HBc (Table 2:2). This is because it has been shown that liver disease progresses more rapidly and HCC is more aggressive in co-infection (Puoti et al., 2004).
Table 2.1 Serological markers of HBV infection

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Antibodies</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbsAg</td>
<td>anti-HBs</td>
<td>Hepatitis B surface antigen is the earliest indicator of acute infection and chronic infection if it persists for more than 6 months. It is useful for the diagnosis of HBV infection and for blood screening.</td>
</tr>
<tr>
<td>HbcAg</td>
<td>anti-HBc</td>
<td>Hepatitis B core antigen is derived from the protein envelope and is not detectable in the bloodstream. The HBCAg is a marker of the infectious viral material and it is the most accurate index of viral replication.</td>
</tr>
<tr>
<td>HbeAg</td>
<td>anti-HBe</td>
<td>Hepatitis B e antigen appearing during weeks 3 to 6 indicates an acute active infection which means that the patient is infectious. Persistence of this virological marker beyond 10 weeks shows progression to chronic infection and infectiousness.</td>
</tr>
<tr>
<td>HbxAg</td>
<td>anti-HBx</td>
<td>Hepatitis B x antigen is detected in HBeAg positive blood in patients with both acute and chronic hepatitis.</td>
</tr>
<tr>
<td>HBV DNA</td>
<td></td>
<td>HBV DNA is detectable as soon as one week after initial infection. The tests are for monitoring of antiviral treatment or detection of mutants that escape detection by current methods.</td>
</tr>
<tr>
<td>HBV DNA polymerase</td>
<td></td>
<td>Tests for the presence of HBV DNA polymerase, detectable within 1 week of initial infection.</td>
</tr>
</tbody>
</table>

The immunological response towards structural components of HBV (Thomson et al., 2009).
Table 2.2 Serological interpretation of HBV markers

<table>
<thead>
<tr>
<th>HBsAg</th>
<th>Anti-HBs</th>
<th>Anti- Hbc</th>
<th>Serology interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Susceptible to infection</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Immune due to past resolved infection</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Immune due to vaccination</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Chronic infection</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>Inconclusive</td>
</tr>
</tbody>
</table>

HBsAg- Hepatitis B surface antigen, Anti-HBs -Antibody to hepatitis B surface antigen, Anti- Hbc- hepatitis B core (Thomson et al., 2009).

2.1.1.8 HBV Therapy in the Era of ART

The anti-retroviral drugs used in HIV/AIDS therapy also contain some HBV-active components; these include lamivudine and tenofovir disoproxil fumarate that are nucleoside/nucleotide analogue. All co-infected patients irrespective of the CD4 cell count should be started on a fully active ART treatment that ensures HIV is not partly treated (Holmberg et al., 2012). The combination of tenofovir disoproxil fumarate with emtricitabine or lamivudine is suggested as a highly effective first-line treatment for HIV+HBV (Holmberg et al., 2012). Entecavir treatment is considered for individuals who cannot tolerate tenofovir disoproxil fumarate because of renal insufficiency or other intolerance. Treatment with lamivudine or emtricitabine as the only HBV-active agent in ART is not recommended due to a high risk of developing HBV drug resistance over time (Benhamou et al., 1999).
2.1.1.9 Treatment of HBV in the absence of ART

Holmberg et al. (2012) recommend that, even when HIV treatment is not indicated, HIV/HBV co-infected patients should receive ART that is fully active for both HIV and HBV. It is of necessity to avoid treatment regimens that do not suppress or have anti-HIV activity or those that may promote HIV drug resistance. Examples include telbivudine and entecavir both of which have anti-HIV-activity and contribute to HIV-drug resistance (Low et al., 2009). Lamivudine, emtricitabine, tenofovir disoproxil fumarate should also be avoided as they may have anti-HIV-activity when used for HBV therapy. In the treatment of HBV alone, adefovir may be considered though it is noteworthy since even in small doses it may have anti-HIV-activity (Low et al., 2009).

Although Pegylated interferon (IFN) may be used in HBV treatment, it is of limited efficacy and prolonged use in chronic HBV may not be feasible due to its toxicity and low tolerability. Moreover, there is limited safety data on its use and the efficacy may be limited in co-infection (McMahon et al., 2007).

2.1.1.10 Vaccination for HBV in HIV infected Individuals

It is recommended that HIV infected individuals who are negative for Hepatitis B virus should be immunized. It is postulated that patients with the response rate for the development of protective surface antibody after vaccination is as low as 18% and may not go beyond 71% as opposed to a 90-100% rate in the uninfected (Shire et al., 2006; Kim et al., 2009). Low CD4 cell counts, detectable HIV-RNA and co-infection with HCV have all been linked to diminished sero-conversion (Rey et al., 2000; Fonseca et al., 2005). Tayal and Sankar (1994) have associated the decrease in mean HBV surface antibody titre and its decline over time to HIV infection. Double dose HBV vaccine at 40mcg is associated with a 47% increase in vaccine effectiveness and especially in
HIV patients with CD4 counts greater than 350 cells/microliters (Fonseca et al., 2005). Immunization should not be deferred even in advanced HIV/AIDS patients because some individuals have been found to develop immunity even with low CD4 counts. Patients who do not achieve the 10µL should be considered for re-immunization using higher doses. Post-immunization checks are recommended after a 30 – 60 day period (Mast et al., 2006; Holmberg et al., 2012). Regular checks for persons who develop immunity are recommended and it is suggested that yearly levels of HBV surface antibodies are checked (Holmberg et al., 2012). The use of adjuvants are a promising source of HBV antibody response boosters (Law et al., 2004; Kim et al., 2009).

2.1.1.11 Prevention of HBV Transmission

Information and counselling on the risks of transmission of HBV to close contacts should be availed to all HBV-positive patients. The higher infectivity of HBV in HIV/HBV co-infection should be pointed out to sex partners and other household members. Such members of the household with close physical contact, sharing drug equipment and other personal items should be checked and vaccinated if not already infected. Use of protective condoms, single use of non-shared equipment for drug injection greatly reduces the risks of infection (Mast et al., 2006; Daniels et al., 2009; Holmberg et al., 2012). The transmission of HBV can to a greater degree be contained with surveillance. This can be achieved when disease incidences are monitored, infection source and transmission modes carefully evaluated and most importantly investigating epidemiology, outbreak recognition and spread containment and the use of post exposure prophylaxis (PEP) in identified contacts (Mast et al., 2006).

2.1.1.12 Epidemiology
Hepatitis B exposure and infection amongst the world population stands at over two billion. These are people who at one point in their life time have been infected with HBV. Chronic HBV infections stand at over 257 million while it is estimated that up to three thousand six hundred people succumb to HBV related disorders daily (Schweitzer et al., 2015). Of individuals co-infected with HIV about 10% suffer chronic HBV which is ten times higher than in the uninfected population (Mast et al., 2006). In sub-Saharan Africa, HBV occurs in about 80% of patients presenting with sporadic hepatitis. Ayuk et al. (2013) showed that about 28% of HBV patients were co-infected with HIV; the median co-infection rate in expectant mothers was about 4% while that of children below 17 years was approximately 7%. Ladep et al. (2013) indicated co-infection rates of 12% in West Africa, (5%) in Southern Africa and (4%) in Eastern Africa. In Kenya, co-infection rates have been placed at a median range of 9% with up to 30% co-infection rates in some urban areas (Okoth et al., 2006; Mutuma et al., 2011). Okoth et al. (2006) recorded a 34% HBsAg positive rate among children aged 5 – 10 years in his study. The study also showed that HCC was the commonest solid tumor affecting the population with an estimated 80% of all liver-associated tumors being HCC and 70% of these were positive for HBsAg. Other causes of liver tumors were cholangiocarcinoma (8%) and hepato-blastomas (2%) common in children (Okoth et al., 2006).
2.1.2 Hepatitis C Virus

2.1.2.1 Virus Structure

Choo and Kuo first characterized *Hepatitis C virus* in 1989 (Houghton, 2009). The virus was initially described as a post transfusion hepatitis causative virus and referred to as the non-A, non-B type (Mohamoud et al., 2013). *Hepatitis C virus* is a Hepacivirus of the family Flaviviridae. It is an enveloped RNA virus. The genome contains a 9.6kb single open-reading strand with positive polarity with about 9033 to 9099 nucleotides. The nucleotides have a 5’ and 3’ non-coding regions flanking at each end (Deinstag & Isselbacker, 2001). The main product of the genome is a long polypeptide estimated to contain about 3000 amino acid chains (Lindenbach & Rice, 2005). The virus employs co-translational and post-translational mechanisms to process this long polyprotein into its core, envelope E1 and E2 structural proteins (Figure 2.2). The HCV virus also processes non-structural (NS) proteins from this polyprotein. Some of its recognized NS proteins include NS1/p7, NS2, NS3, NS4A&B and NS5A&B (Simmonds et al., 2005; Wen, 2011, Figure 2.2). The outer protein surface of the virus is composed of the two envelope proteins which are responsible for host cell entry, the variable NS5B region codes for an RNA-dependent RNA polymerase (Figure 2.2). Because of its lack of proof reading capability, the HCV RNA-Polymerase may modify sensitivity to IFN, anti-viral resistance, viral pathogenicity and the detection mechanisms (Lohmann et al., 1997; Yamane et al., 2014)
HCV is an enveloped single stranded RNA virus. It measures 55-65nm in diameter. It has viral envelope glycoproteins E1 and E2. It also has an open reading from that is translated during replication (Losikoff et al., 2012).

2.1.2.2 Hepatitis C Virus Life cycle

Viral replication mainly occurs in the intracellular lipid membranes of the hepatocytes (Dubuisson et al., 2002). Other cells that have been implicated in HCV replication include mononuclear cells in peripheral circulation (Egger et al., 2002). The viral entry into host cells is a complex mechanism involving many receptors and molecules. This include the LDL receptor, SR-BI receptor CD81 surface marker and the Occludin and Claudin-1 molecules (Zeisel et al., 2009; Kohaar et al., 2010).
Grakoui et al. (1993) has documented that the virion takes control of intracellular mechanisms to facilitate its replication after its entry into the cell. First, the approximately 3000 amino acid long single protein strand is produced. Proteases, both cellular and viral are then involved in processing of this long chain into structural and non-structural viral proteins. (Lindenbach & Rice, 2005; Scheel & Rice, 2013). An alternative reading frame protein may also be produced when a frame shift mutation occurs in the core protein (Xu et al., 2001; Branch et al., 2005).

Hepatitis C encodes two major proteases, a serine protease and a cysteine auto-protease associated with NS3-4A and NS2 regions respectively (Lindenbach & Rice, 2005, Figure 2.1). The host cytoplasmic membranes are rearranged into an RNA replication complex, the viral genome is then recruited into the complex via the NS- viral proteins (Boulant et al., 2008). NS5B and the viral polymerase then helps produce the negative strand RNA intermediate as a first major step towards HCV RNA replication (Lohmann et al., 1997). This negative strand RNA is the basis for the formation of new positive stranded HCV genomes (Scheel & Rice, 2013). New genomes are then translated, undergo more replication or are enclosed into new virions which bud off the cell through a cellular secretory pathway (Boulant et al., 2008).

2.1.2.3 Hepatitis C virus genotypes

Nakano et al. (2012) have identified seven genotypes of hepatitis C virus named numerically 1–7. The classification is based on genetic differences between HCV strains, with several subtypes within each genotype. Further sub-divisions yield quasispecies based on HCV gene variations. Genotypic differences are in the region of 30 to 35% nucleotide regions from the main genome while the subtype differences range from 20-25% (Ohno et al., 1997). Globally, about 60% of all HCV infections are
associated with the 1a and 2a subtypes. In Kenya genotypes 1 and 4 are common with the genotype 4 being the most common (Deinstag & Isselbacker, 2001; Muasya et al., 2008; Scheel & Rice, 2013)

**2.1.2.4 Hepatitis C virus Pathogenesis**

The disease turns chronic in about 85% of all infections. The invasion of mononuclear peripheral blood cells makes the infection show a wide spectrum of clinical manifestation (Yano et al., 1996; Egger et al., 2002). About 15% of all cases may resolve, with the chronic infection manifesting with near normal liver function (Craxì et al., 2008). Fulminant disease is characterized by severe chronic hepatitis, cirrhosis and HCC (Farci et al., 1996). The non-availability of reliable animal models is a great hindrance to HCV chronicity study. The mechanisms for infection persistence and HCC development are therefore still poorly understood (Craxì et al., 2008; Mizukoshi et al., 2008; Lingala & Ghany, 2015).

**2.1.2.5 Hepatitis C Virus Transmission**

The main transmission mode for HCV is blood contact. Common sources include blood contaminated equipment, blood products and shared injection equipments. Rarely does mother to child infection occur although transmission risks increase with HIV co-infection (Mast et al., 2005; Dao et al., 2007; Urbanus et al., 2009). The risk of infection in heterosexual contact has low incidences of transmission but this risk is increased by HIV co-infection (Urbanus et al., 2009). In men who have sex with men, co-infection with HIV and other sexually transmitted infections appear to increase the risk of transmission via sexual contact (Urbanus et al., 2009). Other factors that may increase the risk include; syphilis, use of drugs, sexual practices that injure the epithelia including fisting (Luetkemeyer et al., 2006). Fomite transmission though rare has been
documented with tattoos, body piercing and shared drug consumption equipment (Liang & Ghany, 2002).

2.1.2.6 Signs and Symptoms

Acute Hepatitis C virus infection is asymptomatic in most individuals. This short illness occurs during six months of exposure and the symptoms if produced are generally mild or vague (Wilkins et al., 2010). Up to 30% of adults contracting the disease will show clinical symptoms 3 to 12 weeks post exposure (Mast et al., 2005). These individuals present with among others jaundice, diarrhoeal, abdominal pain, myalgia and fatigue. Elevated liver enzymes and elevated bilirubins characterize their laboratory diagnoses. (Bashawari et al., 2004).

Thomson et al. (2009) estimate that 85% of HIV co-infected individuals are likely to develop chronic HCV infection. This underscores the need to treat these individuals promptly as they are less likely to clear the HCV without therapy. Vogel et al. (2010), have however reported a 40% spontaneous clearance in a study involving HIV infected populations of MSM. The clinical presentation of acute hepatitis C maybe quite severe, however development of fulminant hepatic failure is rare (Farci et al., 1996; Bailey, 2010). Other symptoms of Chronic HCV include fatigue and mild cognitive problems (Forton et al., 2005; Maheshwari et al., 2008). In late disease, cirrhosis and HCC may develop (Rosen, 2011). Cirrhosis may lead to portal hypertension, ascetic disease, concomitant bleeding with easy bruising, jaundice and cognitive impairment syndrome (Ozaras & Tahan, 2009).

2.1.2.7 Hepatitis C Virus Diagnosis
Routine assays for evidence of HCV infection in HIV-infected individuals are recommended. Screening tests for the presence of HCV antibodies include enzyme immunoassay (EIA) and chemiluminescence immunoassay (CIA) (Wilkins et al., 2010). These antibodies are detectable only 4 – 12 weeks after exposure. This large period of latency may hinder HCV antibody detection even with active viral presence. Interestingly, some patients with chronic HCV may yet have undetectable levels of HCV (Wilkins et al., 2010). Chamie et al. (2007) have reported that HIV immunosuppression with CD4 counts less than 200 cells/microliter may be linked to HCV sero-negativity thus in highly suspected HCV cases, RNA test is a useful diagnostic method. To check for spontaneous clearance and confirm persistent viremia, running a confirmatory HCV-RNA is important on all antibody positive individuals (Chamie et al., 2007). The FDA have currently approved ampilicor™ HCV assay version 2.0 and the Cobas-ampilicor™ HCV PCR based tests for the qualitative testing of HCV-RNA (Alter et al., 2003; Allice et al., 2007). Treatment of HCV antigen positive patients also require baseline results for among others thyroid stimulating hormones (TSH), complete haemogram, serum aminotransferases, creatinine levels, prothrombin time test, bilirubin and alkaline phosphatase (Chamie et al., 2007). Before treatment, other causes of hepatitis such as autoimmune hepatitis and hemochromatosis should be ruled out (Yano et al., 1996).

2.1.2.8 Effect of HIV co-infection on HCV disease progression

Graham et al. (2001) indicate that fibrosis progresses up to three times faster in HCV/HIV co-infection than when patients only have HCV. The presence of HIV has been found to boost the rate at which HCV replicate with a resultant increase in liver and serum HCV RNA levels. As a result there is a much shorter time from infection to development of cirrhosis (Graham et al., 2001; Thomson et al., 2009). Sulkowski et al.
(2007) report indicates that up to 24% of co-infected individuals had two fibrosis stages in a span of 3 years when serial biopsies were performed. HIV/HCV co-infection may contribute to an increased risk of death in comparison to mono-infection with hepatitis C virus (Anderson et al., 2004).

2.1.2.9 Effect of Hepatitis C Virus on HIV to AIDS progression

There is no substantial conclusion up to date on the role of HCV infection on HIV progression to AIDS (Luetkemeyer et al., 2006). Most study outcomes have remained controversial with results from both sides of the divide sufficing. Although HCV infection may be found to suppress CD4 cell rejuvenation, liver cirrhosis has also been cited as a source of CD4 cell suppression irrespective of HCV or HIV infection (Luetkemeyer et al., 2006; McGovern et al., 2007). No study has conclusively established a direct link between HCV infection as a mechanism that alters HIV course or its progression to AIDS (Karki et al., 2009). The increased mortality in HIV/HCV co-infection seemingly result from the accelerated liver disease (Kim & Chung, 2009) and may also be due to complications of injecting cultures among the IDUs rather than as a result of the impact of HCV on HIV to AIDS progression (Vlahov et al., 1998).

2.1.2.10 Prevention of HCV Transmission

It is important that individuals co-infected with HIV/HCV be tested for other viral agents of hepatitis such as HBV, thereafter immunization strategies be effected on those previously non-immunized for HBV (Luetkemeyer et al., 2006). HCV-infected patients should be informed and cautioned on the use of hepato-toxins, such as alcohol, excessive acetaminophen and marijuana, which contributes to steatosis and fibrosis (Hézode et al., 2008). Awareness should be mounted and pointed out clearly among patients and the public on ways of minimizing risks of HCV transmission, potential
risks emanating from IDU injecting paraphernalia sharing and engagement in unprotected sex (Urbanus et al., 2009). It is desirable that HCC patients and those with advanced cirrhosis be tested for disease progression using ultrasonography and levels of α-fetoprotein (Luetkemeyer et al., 2006).

### 2.1.2.12 Hepatitis C Virus Therapy

The treatment of Hepatitis C has evolved over the years. Initially interferon mono-therapy was developed and subsequently a combination of ribavirin and interferon was formulated, later polyethylene glycol molecule was added to give the Pegylated interferon therapy (Agarwal et al., 2016). Sofosbuvir an oral NS5B polymerase inhibitor that suppresses the replication of HCV genotypes 1, 2, 3, and 4 was approved by the FDA as an important component of the currently used regimen (Koff, 2014). The combination of ledipasvir/sofosbuvir is the first oral regimen without INF and ribavirin approved by the FDA for HCV (Asselah et al., 2016). In 2014, FDA approved Daclatasvir (Daklinza), a NS5A inhibitor, for use with sofosbuvir for chronic HCV genotype 3 infections in treatment-naïve or treatment-experienced patients (Sulkowski et al., 2014). Later in 2014 the FDA approved the combination of one tablet fixed dose of Ombitasvir/paritaprevir/ritonavir and a separate tablet of dasabuvir a combination called Viekira Pak. The combination was indicated for the treatment of genotype 1 chronic hepatitis C infection in adults including those with compensated cirrhosis. It can also be used in HIV/HCV co-infection. The oral regimen can be used with or without ribavirin (FDA, 2016).

In 2015, FDA approved a combination of Viekira without the dasabuvir which was called Technivie. This was formulated for the treatment of genotype 4 chronic HCV infection without cirrhosis in patients who were either treatment naïve or did not
achieve a virological response with prior treatment with Pegylated interferon/ribavirin. The formulation is recommended to be used in combination with ribavirin, although it can be used for treating the treatment-naïve patients who cannot tolerate ribavirin (FDA, 2016).

2.1.2.13 Anti-Retroviral Therapy in HIV/HCV Co-infection

Antiretroviral therapy in these co-infections may result into hepato-toxicity with the highest risk to patients with hepatic disease (Kaplan et al., 2009). The risk of drug toxicity to the liver is in particular associated with tipranavir, didanosine, stavudine and nevirapine but may also occur in other classes of ARTs. It is important to strictly monitor and periodically assay liver function during therapy (Chauvel et al., 2007). In HCV/HIV co-infection on ART, a five-fold increase of transaminase levels should be flagged for other causes of liver injury such as toxicity associated with concomitant use of alcohol and other drugs (Ozaras & Tahan, 2009). ART-related hepato-toxicity risks are higher in patients having abnormal transferases before therapy and in HBV co-infection and may be reduced with successful HCV treatment (Raison et al., 2005). HCV infection associated immune reconstitution inflammatory syndrome may also occur after ART initiation but this finding should normally be used as an exclusion diagnostic method (Mauss et al., 2004). Ghany et al. (2009) recommend that the use of ART should be sustained without breaks unless hypersensitivity occurs.

2.1.2.14 Commencement of ART in respect of HCV therapy

The timing for initiation of HIV therapy in HIV/HCV co-infection is still controversial. It is however logical to initiate ART in patients with higher CD4 cell counts
(<500 cells/μL). This emanates from studies showing increased response of HCV patients to treatment and avoidance of complications that may arise from untreated HIV (Ho et al., 1995; Bräu, 2003; Akinbami et al., 2012). HIV therapy has been found to reduce progression of HCV-associated fibrosis and may also improve the immune-modulatory effects of interferon based hepatitis C treatment. The reason for starting ART before starting HCV treatment is that treatment of HIV may slow progression of HCV-induced fibrosis or may also improve the immune modulatory effects of IFN-based HCV treatment (Mauss et al., 2004). Conversely, Interferon use in untreated HIV infections lowers leucocyte counts, downgrades CD4 cell production so that counts are difficult to interpret (Rodriguez-Torres et al., 2009).

Laufer et al. (2008) suggest that increased hepato-toxicity due to ART in untreated HCV is the main reason for deferring ART treatment in co-infected patients with high CD4 cell counts. Successful treatment of HCV on the other hand is associated with reduced ART related hepato-toxicity (Raison et al., 2005). Consideration of one therapy prior to the other reduces overlaps in drug toxicity and resistance. There have not been randomized control trials that evaluate these overlaps or toxicities in relation to co-infection treatment. In practice ART is initiated before HCV treatment irrespective of CD4 count (Rodriguez-Torres et al., 2009).

### 2.2 Human Immunodeficiency Virus

#### 2.2.1. Human Immunodeficiency Virus structure

HIV-1 is a characteristically spherical, 120nm wide lentivirus. Genetically, the virion is enclosed in a conically shaped capsid consisting of the p24 viral protein that encloses
a pair of non-covalently bound, non-spliced, positive-sense single strand RNA (Chermann, 1990). Ratner et al. (1985) estimate the length of the RNA to contain approximately 9749 nucleotides. The RNA has a 5’ cap (GPPP) and a 3’ poly (A) tail and has been shown to have many open reading frames (Mahy & Van Regenmortel, 2010). Encoding of structural proteins is facilitated by long open reading frames while that of other proteins including attachment, membrane fusion, replication and assembly regulators are processed by small open reading frames (Mahy & Van Regenmortel, 2010). The nucleo-capsid proteins p7 and assembly protein p6 are bound tightly with the RNA. Essential enzymes for virion development such as reverse transcriptase and integrase are also included in this tight binding (Chermann, 1990; Paranjape, 2005).

The reverse transcriptase utilizes a magnesium dependent pathway primed by lysine tRNA (Mahy & Van Regenmortel, 2010). The genomic RNA is protected from nuclease digestion by its association with the nucleo-capsid. The virion also encloses viral protease, Vif, Vpr, and Nef. The viral p17 protein forms a matrix around the capsid and helps maintain virion integrity. An envelope of host cell membrane origin surrounds the matrix and consists of the glycoprotein 41 and glycoprotein 120. This viral envelope is responsible for host cell binding and penetration. Its spikes consist of a glycoprotein 120 trimer and glycoprotein 41 heterodimer (Zhu et al., 1996).
Figure 2.3: The life cycle of HIV

HIV attacks major cells of the human immune system which have the CD4 cell-surface receptor molecules such as CD4+ T cells, macrophages, and dendritic cells and the life cycle is started and continued as depicted above (National Institute of Allergy and Infectious Diseases, 2016).
**2.2.1.1 Pathogenesis of HIV**

HIV viral infection and disease is largely dependent on host and viral factor interactions (Paranjape, 2005). During its life cycle, the virus target’s CD4 cells in the body and destroys these by complex mechanisms including apoptosis (Fisher, 1998). This loss of CD4 cells renders the infected person unable to recognize and deal with opportunistic infections and other subsequent infections to which resistance relies on CD4 cell populations (Ho et al., 1995; Gougeon et al., 1997). The HIV virus is able to recruit host mechanisms to aid its progression. Among these include, HLA polymorphisms that are known to affect both the progression of disease and susceptibility (Naif, 2013) and the innate immunity that has been shown to aid progression of disease (Paranjape, 2005). The host adaptive immunity plays a critical role in the control and prevention of disease progression. Specific HIV-host responses that may influence disease progression include HIV-specific CD4 and CTL responses (Levy, 2011). Although neutralizing antibodies have been characterized, their role and action in relation to HIV disease progression is yet to be fully understood (Richman et al., 2003). Mucosal immune response is another grey area though it has been demonstrated that HIV infection impairs the activity of the mucosal epithelial barrier (Nazli et al., 2010). Belkaid and Rouse (2005), described the outcome of HIV infection as an orchestrated act of the different immune system mechanisms.

**2.2.1.2 Clinical Stages of HIV**

**2.2.1.2.1 Acute infection**

A characteristic flu-like illness is experienced within 14 to 28 days post exposure. This sickness may last for weeks depending on the host’s natural immune response to HIV. This is the most contagious period with increased amounts of virus in the body.
period of acute infection may be unnoticed in most individuals as they do not experience these mild symptoms. Laboratory diagnostic tests useful in confirmation of disease at this stage are the fourth generation Ab-Ag tests or Nucleic acid testing (NAT) (Buttò et al., 2010).

2.2.1.2.2 Clinical latency

This period of dormancy or HIV inactivity is also considered to be the asymptomatic or chronic HIV infection stage. During latency, HIV reproduction is limited to very low levels and victims of the infection are quite asymptomatic. In patients not on ART, the duration of this stage is varied from a few months to decades (Caldwell et al., 1994). For patients who are immediately put on ART, this phase may last for decades. The individuals are however still able to transmit the virus but the risk decreases greatly with the use of ART. The end stage for this asymptomatic phase is characterized by lower CD4 cell counts and an increased viral load (Chermann, 1990; Aberg et al., 2004).

2.2.1.2.3 Acquired immunodeficiency syndrome (AIDS)

This is the most severe stage of the HIV virus infection. During this period, the victims immune modulatory and response mechanisms are severely damaged, opportunistic infections become unchecked and patient survival averages about 3 years without therapy (Aberg et al., 2004). Characteristic laboratory diagnosis of AIDS involves a positive HIV test and a high viral load with CD4 levels below 200cells/mm. Clinical symptoms include weight loss, swollen lymph glands, general body weakness, night sweats, fever and chills. Due to the elevated viral load, these individuals remain highly infective (Vlahov et al., 1998; Aberg et al., 2004; Clifford et al., 2008).
2.2.1.3 Diagnosis of HIV

The most common detection methods for HIV in early infection are antibody based diagnostic assays. These HIV antibodies are detectable in a majority of individuals at around 3 to 6 weeks post exposure. Sero-conversion is almost universally within 12 weeks of infection (CDC, 2006). There is however a small percentage of individuals who do not develop antibody within this period and even for months. In order to capture the most number of infections in the exposed, serological tests have been developed that employ highly sensitive assays such as enzyme-linked immunosorbent assay (ELISA), solid-phase enzyme immunoassay (EIA) and chemiluminescence immunoassay (CIA). The rapid techniques are used for preliminary screening and detection and confirmation is then followed by highly specific tests such as Western blot and Line Immunoassays (LIA) (Oelemann et al., 2002; Basavaraju et al., 2010; Buttò et al., 2010). The use of oral fluids and urine among other body fluids may be employed in HIV detection provided such methods are validated (Oelemann et al., 2002).

Test results maybe scored as either reactive, non-reactive or indeterminate while in the final analysis, test result interpretation is recorded as either positive, negative or inconclusive (CDC, 2001). Many workers have elucidated the different reasons for indeterminate or inconclusive results ranging from operator errors, manufacturing faults and host-virus related factors (Constantine & Zink, 2005; Olowookere & Adewole, 2011). Viral antigenic properties may also be employed in the detection of HIV infection (CDC, 2001). During the latency period, viral antigens appear in plasma only intermittently and may not be a good diagnostic tool (Buttò et al., 2010).
Assays that may detect viral nucleic acid such as pro-viral cDNA in leucocytes and viral RNA in the cell free area are currently also achievable. Most of these tests are commercially available and employ Real-Time PCR, PCR, Branched DNA, Nucleic Acid Sequence-based Amplification, and Ligase Chain Reaction (CDC, 2001; Buttò et al., 2010). The down-side of these tests is however the unavailability due to cost constraints, specific laboratory equipment, specialized laboratory skills and stringent controls required for the assays. They are thus a challenge to perform in the current clinical laboratory set-ups (Buttò et al., 2010).

2.2.1.4 Markers associated with progression of HIV-1 infection

The natural progression of disease in HIV infection is mainly characterized by an initial acute viremia phase that translates into a latent stage of varying duration when there is little or no viral activity. The final stage of the infection entails a stable or slowly declining number of CD4+ T-helper cells, qualitative T-cell function defects and increasing viral load. HIV-1 pathogenesis consists of a close interaction between host immune mechanism and the virus. Throughout the infection therefore, host immune processes are activated (Malone et al., 1990). Molecules that correlate with HIV-1 disease progression are normally available in serum and can be used to monitor levels of immune system activation. These include among others serum and cellular markers and molecules such as β2-macroglobulin and neopterin (Fahey et al., 1990).

HIV/AIDS disease has a non-conformational asymptomatic phase for different individuals. This coupled with a varied sero-conversion period and AIDS disease development has made non-clinical patient management the most important parameter for therapy and patient management (Dar & Singh, 1999). The use of viral load and other direct viral characteristics have therefore been adopted widely for clinical use and
in anti-retroviral drug trials (Saag et al., 1996). The relationship between stages of disease progression and viral turnover, activation of host immune system and viral replication and time taken for ART-resistance development have been delineated by the use of these virus-activity measurements (Matee, 1999).

Viral load determination has been quite instrumental in the management of HIV infections. In early infection at the set point, viral load measurements helps in assessing the likely course of the infection and therefore appropriate treatment decisions are made (Carpenter et al., 1997). The short-term efficacy of a specific anti-retroviral treatment can then be assessed by measuring the reduction in virus concentrations achieved within the first 2 to 4 weeks after treatment initiation (Carpenter et al., 1997). Long-term efficacy is achieved when virus levels continuously decrease below the levels of detection and remain undetectable using the PCR or NAT testing (Saag et al., 1996).

The CD4 count is the most commonly used cellular marker for HIV infection progression. It is however a vague indicator for disease progression, its wide use by clinicians notwithstanding (Fahey et al., 1990). As noted by Malone et al. (1990) one abnormal result is insufficient reason to alter treatment. This is because factors such as psychological state, time of sample collection, co-infection and exercise have been known to affect the CD4 count. A well-established correlation exists between high levels of plasma HIV-1 RNA and lower baseline CD4+ T-cell count. A rapid decrease in CD4+ T-cell counts has also been correlated with rapid infection progression. It has been shown that patients with a viral load of 100,000 copies/ml in 6months after seroconversion are ten times more likely to progress to AIDS within 5-years than those having less copies per ml of plasma (Deeks et al., 2007). When plasma RNA viral load is maintained at below 10,000 copies/ml, the risk of progression to AIDS is
substantially reduced. This is however not true in patients with advanced disease with reports showing that 30% of such individuals progress to AIDS even with viral load below 10,000 copies/ml (O’Brien et al., 1996; Saag et al., 1996).

### 2.2.1.5 Treatment of HIV

Therapy against the HIV virus is currently abundant. HIV regimen choice therefore depends on factors such as possible side effects including allergies and potential drug interactions. There are six HIV-drug categories depending on the phase of the HIV life cycle targeted by the drug. These include integrase strand transfer inhibitors, fusion and entry inhibitors, protease inhibitors, pharmacokinetic enhancers, non-nucleoside reverse transcriptase inhibitors and reverse transcriptase inhibitors (Arts & Hazuda, 2012; De Clercq & Li, 2016). Because each drug takes care of only one point in the HIV life cycle which the virus is sometimes able to circumnavigate, it takes more than one drug to offer protection against the HIV virus (Hoffmann et al., 2009). During HIV replication sometimes imperfect copies are made. These may be non-responsive to drugs taken for HIV control. Three drug regimens are the most commonly used in the prevention of HIV replication and keeping the infection at bay. Nucleotide reverse transcriptase inhibitors (NRTIs) block HIV replication by acting as faulty building blocks for viral DNA. This then inhibits the ability to use the reverse transcriptase enzyme and prevents the virus from replicating its own DNA materials (Dao et al., 2007). On the other hand, the non-nucleoside reverse transcriptase inhibitors (NNRTIs) block reverse transcriptase enzyme activity; these offer a direct blockade to enzyme activity thus making it difficult for the virus to replicate. Protease inhibitors (PIs) are able to stop genome splicing by the protease enzyme, indirectly preventing the formation of structural and no-structural proteins (Roberts et al., 1990).
### Table 2.3: Drugs commonly used in treatment of HIV

<table>
<thead>
<tr>
<th>Nucleoside reverse transcriptase inhibitors (NRTIs)</th>
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</thead>
<tbody>
<tr>
<td>Stavudine (d4T)</td>
<td>Lamivudine (3TC)</td>
</tr>
<tr>
<td>Zidovudine (AZT)</td>
<td>Didanosine (ddl)</td>
</tr>
<tr>
<td>Abacavir (ABC)</td>
<td>Emtricitabine (FTC)</td>
</tr>
<tr>
<td>Zalcitabine (ddc)*</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Nucleotide reverse transcriptase inhibitors (NNRTIs)</th>
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</thead>
<tbody>
<tr>
<td>Tenofovir disoproxil fumarate (TDF)</td>
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</table>

<table>
<thead>
<tr>
<th>Non-nucleoside reverse transcriptase inhibitors (NNRTIs)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nevirapine (NVP)</td>
<td>Efavirenz (EFV)</td>
</tr>
<tr>
<td>Delavirdine (DLV)*</td>
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</table>

<table>
<thead>
<tr>
<th>Protease inhibitors</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lopinavir (LPV) with Ritonavir (RTV)</td>
<td>Nelfinavir (NFV)</td>
</tr>
<tr>
<td>Saquinavir (SQV)</td>
<td>Atazanavir (ATV)</td>
</tr>
<tr>
<td>Indinavir (IDV)</td>
<td>Amprenavir (APV)*</td>
</tr>
<tr>
<td>Fosamprenavir (f-APV)*</td>
<td>Tipranavir (TPV)*</td>
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</table>

<table>
<thead>
<tr>
<th>Fusion/ Entry inhibitors</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Enfuvirtide (T20)*</td>
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</table>

The starred drugs are not available in Kenya. In the use of the antiretroviral drugs, the drugs are administered in regimen whereby there is the first line regimen for adults and adolescents made up of Stavudine or Zidovudine + Lamivudine + Efavirenz or Nevirapine. There is also a standardized national second line regimen for adults and adolescents made up of Didanosine + Abacavir + Lopinavir/Ritonavir (Kaletra)² or Tenofivir + Abacavir + Lopinavir/Ritonavir (Kaletra)² (Ministry of Health, 2016).
Table 2.4: Alternative first line and second line antiretroviral regimen

<table>
<thead>
<tr>
<th>Alternative First line regimen</th>
<th>Subsequent Second line regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZT/3TC/EFV or NVP</td>
<td>ddI/ABC³/Kaletra¹ OR TDF/ABC³/Kaletra¹</td>
</tr>
<tr>
<td>ddI/D4t/EFV or NVP</td>
<td>TDF/3TC/Kaletra¹ OR ABC/3TC/Kaletra¹</td>
</tr>
<tr>
<td>TDF²/3TC/EFV or NVP</td>
<td>AZT/TDF/Kaletra¹ OR AZT/Kaletra</td>
</tr>
<tr>
<td>TDF²/FTC/EFV or NVP</td>
<td>NNRTI/Kaletra¹/TDF OR NNRTI/Kaletra/ddI</td>
</tr>
<tr>
<td>ABC/3TC/AZT</td>
<td>TDF/3TC/Kaletra¹ OR ABC/3TC/Kaletra</td>
</tr>
<tr>
<td>D4t/ddI/IDV</td>
<td>TDF/ABC/Kaletra OR ddI/ABC/Kaletra</td>
</tr>
</tbody>
</table>

In case of a failure in the primary first line and second line of ART administration, an alternative drug combination as shown above is recommended in Kenya (Ministry of Health, 2016).

2.2.1.5.1 Side effects

Adverse effects in the use of antiretroviral drugs may vary in various individuals and depending on the regimen. Owing to these, people on the same regimen are not likely to experience the same adverse effects. These effects include the following among others, headache and occasional dizziness which can be termed mild reactions to severe life threatening effects such as hepatic damage, glossitis and swelling of the mouth (Brinkman et al., 1998; Catz et al., 2000).
2.2.1.5.2 Drug interactions

The effects of drug interactions maybe both positive and negative. In any one of the prescribed regimen, drug interactions may reduce host response to medicine and disease or increase it. Drug interactions have also been known to cause adverse reactions (Mofenson et al., 2009).

2.2.1.5.3 Drug resistance

The HIV virus is highly mutagenic. This propensity to mutate is responsible for the development of ART resistance in persons undergoing therapy (Loeb et al., 1999). One of the most important factors affecting drug resistance and causing treatment failure is poor adherence to HIV ART therapy. After a mutation, the new HIV virus are not responsive to the previous ARTs and the patient’s regimen may have to be changed (Gamell et al., 2016).

2.2.1.6 Epidemiology of HIV

HIV/AIDS disease is a recurrent devastating health concern in many parts of the globe today. Since the year 2000, up to 25.3 million people are reported to have died of AIDS related illnesses. Global estimates of new infections are in excess of 2 million new cases and rising annually. In the year 2014, about 36.9 million were living with AIDS and 15.8 million were on HIV ART. Approximately 1.2 million died of AIDS related disease (Fettig et al., 2014; Liu et al., 2015).

Africa south of the Sahara is the most affected region in the world. By the year 2010, an estimated 68% of all HIV/AIDS cases occurred here, and up to 66% of all AIDS related deaths were reported (Fettig et al., 2014). This translates to 5% of the total adult population being affected by the disease (Johnson et al., 2006; Larney et al., 2015).
South Africa is considered to be the country with the highest population of people living with AIDS in the world (Ayuk et al., 2013). It is quite contrasting that women are the most affected by the disease in sub-Saharan Africa while in other parts of the world the greatest majority are men (Larney et al., 2015).

In Kenya the prevalence rate of HIV is approximately 6%, with about 1.6 million people living with HIV infection (KNACC, 2016). It is one of the six countries in Africa that has recorded a high HIV burden. Western Kenya, through the counties of Homabay, Siaya and Kisumu are the most affected with HIV rates of 26%, 24.8% and 19.9% respectively (KNACC, 2016). The counties with the least infection rates are Wajir, Tana River and Marsabit with rates of 0.4%, 1.9% and 1.4% (KNACC, 2016).

2.2.1.7 The liver during HIV and viral hepatitis co-infection

The liver is a large, complex organ that is well designed for its central role in carbohydrate, protein and fat metabolism. It is the organ responsible for the detoxification of waste products of metabolism through the process of deamination, hence urea production (Giannini et al., 2005). The destruction of old red blood cells is a shared function between the liver and the spleen. The catabolism involves the recirculation of essential red cell components. The liver is also responsible for synthesis and secretion of among others, bile, plasma proteins and lipoproteins and clotting factors (Green & Flamm, 2002). Gluconeogenesis, glycogenesis and glycogenolysis are all processes controlled by the liver which helps regulate and maintain blood glucose levels (Giannini et al., 2005).

The various activities that are central to the body function and are controlled by the liver makes its functional processes a key component of disease detection. Liver function tests are therefore significant non-specific markers of disease (Gopal & Rosen,
2000; Limdi & Hyde, 2003). The interpretation of abnormal LFTs and its links to underlying disease are common practice in primary health care (Krier & Ahmed, 2009). It is not easy to diagnose or locate disease by a single measurement of LFTs. However, test result patterns and abnormality characteristics may help define and determine the origin. The clinical picture arrived at in LFT as used in clinical diagnosis can be grouped into non-hepatic, hepatocellular or cholestatic abnormality states (Rochling, 2001). A differential diagnosis is usually possible when a combination of symptoms (current and previous), clinical history and drug history are considered (Limdi & Hyde, 2003; Lenzini et al., 2010). Abnormality in liver function tests is not always indicative of underlying liver function abnormality. Normal values with a standard deviation of ±2 are deceptive in the investigation of hepatic disease. This is so owing to the fact that liver abnormality is mostly asymptomatic and if not detected early can lead to chronicity and carcinoma. The traditional normal outliers in LFT measurements should not be left uninvestigated in order to stem increased liver disease and failure (Sherwood et al., 2001; Limdi & Hyde, 2003).

2.2.1.8 Interpreting the liver enzyme markers from blood test results

In the interpretation of liver enzyme markers, Gamma-Glutamyl Transferase (GGT) level is used as a marker of healthy liver. γ-GT levels do not normally exceed 45mmol/l. When the levels increase beyond 100iu/l, other liver enzymes are checked out and used to work out possible causes of liver damage (Giannini et al., 2005). Abnormally high liver enzyme combinations and their possible interpretations include the following

\[ \gamma\text{-GT} > 100, \ALT < 80 \text{ and ALP < 200}, \]

These levels may be indicative of excessive alcohol consumption, recreational drug use such as heroin may also be implicated. Other conditions include diabetes and a fatty
liver. When coupled with high levels of triglycerides, it is indicative of liver stimulation by barbiturates or other prescription drugs such as warfarin, immunosuppressant’s, pain-killers benzodiazepines, anticonvulsants or anti-depressants (Delladetsima et al., 2001).

\( \gamma\)-GT > 100, ALT < 80, ALP > 200

The enzyme levels point towards slow-down of bile flow or its obstruction. Possible causes may include presence of gallstones or severe bile-duct inflammation, extra-hepatic tumour pressing down on the ducts, excessive drug or alcohol abuse, and liver scarring. Some bone marrow diseases will also result in increased ALP levels. In bile flow obstruction, plasma bilirubin is elevated above 20 mmols/l and the patient is jaundiced (Yu et al., 2015).

\( \gamma\)-GT > 100, ALT > 80, ALP < 200

The major causes of this picture are the Hepatitis viruses (A, B or C) the Epstein Barr virus which may cause inflammation of the liver cells. The consumption of excess alcohol, a fatty liver and use of hepato-toxic drugs are also implicated (Pirro et al., 2011).

\( \gamma\)-GT > 100, ALT > 80 and ALP > 200

This picture is indicative of hepato-cellular damage. Characteristic infections that present with these liver enzyme levels include acute and chronic viral hepatitis, drug and alcohol toxicity, auto immune disorders, hepatic and non-hepatic tumors resulting in bile flow obstruction and liver scarring. The enzyme AST is used to differentiate alcoholic liver disease in this picture as its levels may rise even higher than those of ALT (Schuppan & Afdhal, 2008).
2.3 Intravenous Drug Use

Certain demographics globally remain at a high risk of contracting blood borne viruses such as HIV, HBV and HCV. Among the risk population are Injection drug users, commercial sexual workers and men who have sex with men. Kenya stands among the top countries with high prevalence of HIV AIDS in sub – Saharan Africa. East Africa leads in opiates use in Africa with Kenya being among the top heroin using county (Mwatelah et al., 2015). In Kenya the major towns are affected but in particular the coastal towns including Mombasa.

At least 18% of the IDUs were reported to have contracted HIV AIDS by the year 2014 (KNACC, 2016). The escalated prevalence was associated with the behavior patterns among IDUs which are characterized by sharing of injecting paraphernalia, blood-flashing and engaging in sexual orgies (Kerosi et al., 2015). In this account, IDUs stand a high risk of contracting HIV, HBV and HCV due to the shared transmission routes by the viruses (Kerosi et al., 2015). Co-infection of HBV and HCV in HIV infected individuals may lead to several health challenges such as liver dysfunction, immune system dysregulation, dilemma in formulating the drugs, treatment and management hurdles characterized by negative effects caused by drug interaction (Were et al., 2014; Puri et al., 2017).
CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

This study involved subjects seeking treatment, counselling, and HIV-1 comprehensive services at Bomu Medical Centre for non-DUs and the IDUs population was from dropping points of the Mewa rehabilitation Centre, outpatient clinics of Bomu and Port Reitz all in Mombasa County.

Mombasa County, with an estimated area of 229.7sq.Km and surrounding 65sq.Km water mass is the smallest of the Kenyan counties located along the Indian Ocean Coast. The county straddles Mombasa Island and parts of the mainland to the north, west and south bordering Kilifi and Kwale. To the East is the Indian Ocean (Mombasa county Government, 2013). The island is encompassed by Tudor creek and the Kilindini harbor and connected to the mainland through the Makupa causeway to the west and the Nyali Bridge to the north. The Likoni ferry service connects the island to the southern mainland across the Kilindini harbor (Mombasa county Government, 2013). The population of Mombasa is cosmopolitan with multiple religious groups evident. The main religions include Hindu, Christian and Muslim groups (Oparanya, 2009). The predominant ethnic communities are the Mijikenda, and Swahili people. Other communities include Akamba and Taita, Luo and the Luhya. Traders from other regions, including Asia, the Indian subcontinent have over the centuries settled in Mombasa. The city is the center of the coastal tourism industry. It has a total population of 939,370 and 268,700 households (Oparanya, 2009). The population density is 4,292/km2 and 37.6% of the population live below the poverty line.
3.2 Study design

This was a cross-sectional design which was conducted during the study period April 2014- September 2014.

3.3 Study variables

The independent variables were; HIV and hepatitis status, Injection drug use status and social demographics. The dependent variables were; CD4+ cell count, HIV viral load count and the liver enzyme markers. The hepatitis status was treated as a dependent variable when dealing with the prevalence.

3.4 Target population

The target population was HIV positive intravenous drug users and HIV positive non-drug users (subjects who have never used drugs before) in Mombasa County.

3.5 Sampling method and Sample size determination

3.5.1 Study Criteria

3.5.1.1 Inclusion criteria

- HIV 1 positive IDUs and NDUs in clinical stage two or three of treatment were eligible.
- Patients who were on first line of antiretroviral regimen were recruited for the study.
- Patients aged 16 years and above who gave their informed consent were recruited
3.5.1.2 Exclusion criteria

- Subjects on first line regimen with nevirapine were excluded

3.5.2 Sampling design

The study participants were recruited from Bomu Medical Centre, dropping points of Mewa rehabilitation Centre, outpatient clinics of Bomu and Port Reitz all in Mombasa County using simple random sampling. Direct recruitments were done whereby the physicians talked with the eligible patients seeking treatment, counselling, and HIV-1 comprehensive services. Also referral method was used where by health social workers were used to recruit the participants from non-medical based areas. Only subjects who met the study criteria standards were considered.

A structured questionnaire was administered to each participant who consented and met the study criteria before the blood sample was taken.

3.5.3 Sample size calculation

The sample size was calculated by the formulae; (Fisher, 1998).

\[ n = \frac{Z^2pq}{d^2} \]

Where:-:

- \( n \) - The desired sample size (assuming the population is greater than 10,000)
- \( z \) - The standard normal deviation, set at 1.96, which corresponds to 95% confidence level
- \( p \) - The proportion in the target population estimated to have a particular characteristic.
- If there is no reasonable estimate, then use 50 percent (the study used 7.4% based on the HIV prevalence in Mombasa county 2013 (NASCOP, 2014).
q = 1.0 – p

d = the degree of accuracy desired, here set at 0.05 corresponding to the 1.96.

**Table 3.1: Calculation of the sample size**

<table>
<thead>
<tr>
<th>$Z^2$</th>
<th>P</th>
<th>Q</th>
<th>$d^2$</th>
<th>$n = \frac{Z^2pq}{d^2}$</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1.96)$^2$</td>
<td>0.074</td>
<td>1-0.074</td>
<td>(0.05)$^2$</td>
<td>$3.84 \times 0.074(1-0.074)$</td>
<td>$0.0025$</td>
</tr>
</tbody>
</table>

Thus, 100 participants from each study group were used for the study.

### 3.6 Research instrument

A structured questionnaire was used as a data collection tool. The tool was pretested prior to rolling out the study. Based on the outcomes of the pilot study, the questionnaire was revised accordingly to enhance reliability of the final study findings.

### 3.7 Sample collection and transportation

Twelve millilitres of whole blood was collected through venepuncture, 4mls of blood was distributed into lavender tops EDTA vacutainers, and two red tops plain vacutainer tubes. The blood in anti-coagulated tubes was mixed up and down gently for the anticoagulants to mix with blood. These samples were then transported from the site of collection to the laboratory in an icebox for analysis. On arrival to the laboratory the blood in plain tubes was stood for 30-60 minutes for the cells to coagulate and settle, this blood was then spun at 2,500rpm for 10 minutes to separate the serum. For the blood in EDTA tubes, the analysis of CD+ cells was done, and then the remaining blood was centrifuged at 1048g to separate plasma. The Serum and plasma were aliquoted in appropriately labelled storage tube. All the remaining serum was stored at temperatures between 2-8$^0$C and plasma at -70$^0$ C.
3.8 Laboratory analysis

3.8.1 HIV confirmatory test

HIV status was confirmed using Determine™ (Abbott Labs, Abbott Park, IL, USA) and Unigold™ rapid diagnostic techniques (Trinity Biotech Plc, Bray, Ireland). The whole blood was placed at the tip of the device and a drop of the buffer was added and allowed to diffuse along a nitrocellulose strip with a capture site containing HIV specific antigens that are conjugated to the selenium colloid. This was allowed to flow through the strip according to the manufacturer’s guide. Samples containing anti-HIV antibodies reacted with the antigen-selenium colloid. As the antigen-antibody-selenium complexes flowed past the capture site, a visible red line was formed, which confirmed assay validity. The results were evaluated after 10-15 minutes. Study participants with positive results for both Determine and Unigold were considered HIV infected (NASCOP, 2010).

3.8.2 CD4 Count

The whole blood collected in the purple top EDTA tubes was used for the analysis. The CD4 positive cell count was done within 48 hours after blood collection. Enumeration of the CD4 positive cells was done using the BD™ FACS Caliber™ (Becton Dickson New Jersey USA) according to the manufacturer’s protocols (Lyons & Parish, 1994). In the test procedure, 20 μL of Mutitest Reagent was pipetted into TruCOUNT™ tubes, labeled according to the samples. Fifty microlitres (50 μL) of the well-mixed blood sample was added into corresponding tubes and gently vortexed. The mixtures were then incubated in the dark at room temperature for 15 minutes. Four hundred and fifty micro-litres (450μL) of lysing solution (FACS Lysing Solution™) was then added to the tubes and the mixture re-vortexed (BD Biosciences, San Jose, CA, USA). A 1:10
part dilution of the Lysing fluid and de-ionized water was mixed at room temperature to form a 1part concentrate of FACS Lysing Solution for use during the assays. After addition of lysing fluid, the tubes were further incubated in the dark for 15 minutes. Thorough low speed vortexing to reduce aggregation was then done before tubes were placed onto the automatic sample loader on the FACS Caliber™ system. Samples were then analyzed using the FACS system and lymphocyte subpopulations assayed (BD Biosciences, San Jose, CA, USA). CD4 cell counts for each sample were obtained and result entries done (CPGH laboratory manual, 2014).

3.8.3 Hepatitis B and Hepatitis C Analysis

Architect i2000sr was used in the assay of the Hepatitis B surface antigen marker from a cell free serum sample (Abbott Architect HBsAg Confirmatory V.1, USA). The HBsAg was assayed using chemiluminescent micro-particle assay which uses specific antibody neutralization technique. The daily Quality control procedures and calibration were run and proceeded to analyze specimen only when QC had passed. The micro-particle bottles were re-suspended by inverting thirty times and the cap discarded. The architect HBsAg reagents were loaded in the system as per the manufacturer’s instructions. One hundred micro-litres (100µl) of the serum specimen were loaded on the architect system in accordance with the manufacturer’s instructions (Abbott Architect HBsAg Confirmatory V.1, USA). Details of the specimen such as the identification numbers were keyed in as the loading of the specimen was going on. Using the appropriate system software module the samples were run and the result output was recorded (CPGH laboratory manual, 2014).

Architect i2000sr was also used in the detection of antibodies to Hepatitis C virus (anti-HCV) (Abbott Architect Laboratories, USA). The assay is a chemiluminescent micro-
particle assay which quantitively detects antibodies to structural and non-structural proteins to HCV genome in human fibrin and cell free serum specimen. The daily Quality control procedures and calibration were run and proceeded to analyze specimen only when QC had passed. The micro-particle bottles were re-suspended by inverting thirty times and the cap discarded. The architect Anti- HCV reagents were loaded in the system as per the manufacturer’s instructions (Abbott Architect Laboratories, USA). One hundred micro-litres (100µl) of the serum specimen were loaded into the sample carrier and placed in the sample load queue. Details of the specimen such as the ID numbers were keyed in as the loading of the specimen was going on. Using the appropriate system software module the samples were run and the result output was recorded (CPGH laboratory manual, 2014).

3.8.4 Liver Profile Analysis

Cobas Integra 400 plus (Roche Diagnostics Limited, USA) was used for the liver profile analysis which uses the fluorescence polametry and absorbance photometry. Serum was used for the liver profile tests. The daily quality control procedures and calibration were run and proceeded to analyse specimen only when QC was passed. The liver profiling reagent packs were loaded into the system as per the manufacturer’s instructions. Using the sample cups one hundred micro-litres (100 µl) of serum specimen was loaded onto racks according to the manufacturer’s instructions. The details of the specimen such as the ID numbers were keyed in as the loading of the specimen was going on. The loaded racks were inserted through the rack windows, using Cobas system station computer, the appropriate software module was used to run the samples and the result output was recorded (CPGH laboratory manual, 2014).
3.8.5 Viral Load Count

Cobas® AmpliPrep/COBASTM® TaqMan® (Roche Molecular Systems, Branchburg, USA) was used to quantitatively determine viral load in the cell-free plasma. Briefly, the quantification of HIV-1 viral RNA was done using Quantitation Standard RNA (Puren, et al., 2010). The HIV-1 Quantitation Standard with known copy number was included in the individual specimen. These were then carried through to the specimen preparation, reverse transcription, amplification hybridization and detection step. The Cobas® AmpliPrep Analyzer was then used to calculate the levels of HIV-1 RNA in the samples by a comparison of the HIV-1 signal to the Quantitation Standard signal for each sample. At the completion of analysis, the result reports were printed, error messages and flags checked and the interpretation counterchecked for all the results (CPGH laboratory manual, 2014).

3.9 Ethical considerations

The study was approved by the Kenyatta University Ethics Review Committee (Reference No: KU/R/COMM/51/282) (Appendix 11) and clearance permit obtained from the National Council of Science and Technology (NACOSTI) (Permit No: NACOSTI/P/14/9730/871) (Appendix 111) before it was executed. All participants were required to give a written informed consent before being enrolled into the study.

3.10 Data analysis

All data obtained from the study was entered into a specially prepared database, cleaned and statistical analysis done using SPSS software (21.0 version). Frequencies and Pearson’s chi square were used in calculating and comparing the prevalence of HBV and HCV infections and the questionnaire responses among the study groups. Means were used for summing up the continuous variables such as CD4 positive cells, viral
load counts and the liver profile results. Inter-group comparison of continuous data was
done using Student T tests for the two means comparison and one way analysis of
variance (ANOVA) for more than two means comparison. All tests were two-tailed and
a \( P \) value \( \leq 0.05 \) was considered statistically significant.
CHAPTER FOUR: RESULTS

4.1 Demographics

The study comprised of 200 participants with 78(39%) males and 122(61%) females. The age ranged from 16-70 years with mean age of 36.53 (±9.79) years. The study participants were divided into two cohorts, 100 IDUs and 100 Non-DUs. The IDU group had 57 (57%) females with a mean age of 34.54(±8.89) and 43(43%) males with a mean age of 36.74(±9.28). The Non IDU group had 65 (65%) females with a mean age of 35.17(±9.49) and 35 (35%) males with a mean age of 43.1(±10.39). The two cohorts were further sub divided into four categories based on the viral status, HIV only, HIV+HBV, HIV+HCV, HIV+HBV+HCV depending on the co-infection.

4.2 The social demographics of the study subjects

Though statistically insignificant, a high prevalence of co-infection with HIV+HBV was seen in females than in the males at 55.6% and 44.4% respectively (P=0.382). A similar trend was observed with the HIV+HCV and HIV+HBV+HCV co-infection. The co-infection rate of HIV+HCV was 53.6% among females and 46.4% among males and that of HIV+HBV+HCV both gender had a co-infection rate of 50% (P=0.279 and P=0.360) respectively.

A statistically insignificant high co-infection rate of HIV+HBV was seen among the age’s 31-40 years at 37% (P=0.912), the Muslim believers at 55.6% (P=0.174) and those who were at the primary and secondary level of education at 40.7% each (P=462). A statistically insignificant higher prevalence of HIV+HBV was observed among the married at 62.9% (P=0.777) and the casuals at 51.9% (P=0.504).
In the HIV+HCV co-infection, a statistically insignificant high prevalence rate was observed among individuals who were Muslim believers at 46.4% (P=0.867) and secondary education level at 39.3% (P=0.851). A non-significant high prevalence rate with HIV+HCV was seen among participants of ages 41-50 at 35.7% (P=0.613), the singles and married at 19.6% (P=0.219) and those who were classified as casuals by occupation at 51.9% (P=0.077).

Participants of the ages 31-40 and 41-50 years, Christian believers, those who had the secondary and tertiary level of education and those who were classified as casuals by occupation showed a statistically high prevalence with HIV+HBV+HCV co-infection at 30% (P=0.611), 60% (P=0.482), 70% (P=0.672) and 60.7% (P=0.267) respectively. A statistically insignificant high co-infection rate with HIV+HBV+HCV was observed among the participants who were single at 50% (P=0.566) (Table 4.1).
Table 4.1: The social demographics of the study participants

<table>
<thead>
<tr>
<th>Demographic</th>
<th>HIV Mono</th>
<th>HIV+HBV</th>
<th>P value</th>
<th>HIV Mono</th>
<th>HIV+HCV</th>
<th>P value</th>
<th>HIV Mono</th>
<th>HIV+HBV+HCV</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>48(35.6)</td>
<td>12(44.4)</td>
<td>0.382</td>
<td>48(35.6)</td>
<td>13(46.4)</td>
<td>0.279</td>
<td>48(35.6)</td>
<td>5(50)</td>
<td>0.360</td>
</tr>
<tr>
<td>Females</td>
<td>87(64.4)</td>
<td>15(55.6)</td>
<td></td>
<td>87(64.4)</td>
<td>15(53.6)</td>
<td></td>
<td>87(64.4)</td>
<td>5(50)</td>
<td></td>
</tr>
<tr>
<td>Age groups</td>
<td></td>
<td></td>
<td>0.912</td>
<td></td>
<td></td>
<td>0.613</td>
<td></td>
<td></td>
<td>0.611</td>
</tr>
<tr>
<td>≤20</td>
<td>6(4.4)</td>
<td>2(7.4)</td>
<td></td>
<td>6(4.4)</td>
<td>1(3.6)</td>
<td></td>
<td>6(4.4)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>21-30</td>
<td>33(24.4)</td>
<td>6(22.2)</td>
<td></td>
<td>33(24.4)</td>
<td>6(21.4)</td>
<td></td>
<td>33(24.4)</td>
<td>3(30)</td>
<td></td>
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<td>31-40</td>
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<td>10(37.0)</td>
<td></td>
<td>57(42.2)</td>
<td>9(32.1)</td>
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<td>57(42.2)</td>
<td>3(30)</td>
<td></td>
</tr>
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<td>41-50</td>
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<td>6(22.2)</td>
<td></td>
<td>29(21.5)</td>
<td>10(35.7)</td>
<td></td>
<td>29(21.5)</td>
<td>2(20)</td>
<td></td>
</tr>
<tr>
<td>&gt;50</td>
<td>10(7.4)</td>
<td>3(11.1)</td>
<td></td>
<td>10(7.4)</td>
<td>2(7.1)</td>
<td></td>
<td>10(7.4)</td>
<td>2(20)</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td>0.777</td>
<td></td>
<td></td>
<td>0.219</td>
<td></td>
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<td>Single</td>
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<td>6(22.2)</td>
<td></td>
<td>41(30.4)</td>
<td>10(19.6)</td>
<td></td>
<td>41(30.4)</td>
<td>5(50)</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>74(54.8)</td>
<td>17(62.9)</td>
<td></td>
<td>74(54.8)</td>
<td>10(19.6)</td>
<td></td>
<td>74(54.8)</td>
<td>4(40)</td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>13(9.6)</td>
<td>2(7.4)</td>
<td></td>
<td>13(9.6)</td>
<td>5(17.8)</td>
<td></td>
<td>13(9.6)</td>
<td>1(10)</td>
<td></td>
</tr>
<tr>
<td>Widowed</td>
<td>7(5.2)</td>
<td>2(7.4)</td>
<td></td>
<td>7(5.2)</td>
<td>3(30)</td>
<td></td>
<td>7(5.2)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>Religion</td>
<td></td>
<td></td>
<td>0.174</td>
<td></td>
<td></td>
<td>0.867</td>
<td></td>
<td></td>
<td>0.482</td>
</tr>
<tr>
<td>Christian</td>
<td>62(45.9)</td>
<td>12(44.4)</td>
<td></td>
<td>62(45.9)</td>
<td>13(46.4)</td>
<td></td>
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<td></td>
</tr>
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<td>Muslims</td>
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<td></td>
<td>59(43.7)</td>
<td>13(46.4)</td>
<td></td>
<td>59(43.7)</td>
<td>4(40)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>14(10.4)</td>
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<td></td>
<td>14(10.4)</td>
<td>2(7.1)</td>
<td></td>
<td>14(10.4)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>Education levels</td>
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<td></td>
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<td></td>
<td>0.851</td>
<td></td>
<td></td>
<td>0.672</td>
</tr>
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<td>Primary</td>
<td>41(30.4)</td>
<td>11(40.7)</td>
<td></td>
<td>41(30.4)</td>
<td>10(35.7)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>56(41.5)</td>
<td>11(40.7)</td>
<td></td>
<td>56(41.5)</td>
<td>11(39.3)</td>
<td></td>
<td>56(41.5)</td>
<td>4(40)</td>
<td></td>
</tr>
<tr>
<td>Tertiary</td>
<td>38(28.1)</td>
<td>5(18.5)</td>
<td></td>
<td>38(28.1)</td>
<td>7(25)</td>
<td></td>
<td>38(28.1)</td>
<td>4(40)</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td>0.504</td>
<td></td>
<td></td>
<td>0.077</td>
<td></td>
<td></td>
<td>0.267</td>
</tr>
<tr>
<td>Civil</td>
<td>14(10.4)</td>
<td>2(7.4)</td>
<td></td>
<td>14(10.4)</td>
<td>3(10.7)</td>
<td></td>
<td>14(10.4)</td>
<td>0(0)</td>
<td></td>
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<tr>
<td>Business</td>
<td>5(3.7)</td>
<td>0(0)</td>
<td></td>
<td>5(3.7)</td>
<td>3(10.7)</td>
<td></td>
<td>5(3.7)</td>
<td>1(10)</td>
<td></td>
</tr>
<tr>
<td>Social worker</td>
<td>14(10.4)</td>
<td>2(7.4)</td>
<td></td>
<td>14(10.4)</td>
<td>0(0)</td>
<td></td>
<td>14(10.4)</td>
<td>1(10)</td>
<td></td>
</tr>
<tr>
<td>Secretary</td>
<td>10(7.4)</td>
<td>0(0)</td>
<td></td>
<td>10(7.4)</td>
<td>0(0)</td>
<td></td>
<td>10(7.4)</td>
<td>1(10)</td>
<td></td>
</tr>
<tr>
<td>Housewives</td>
<td>36(26.7)</td>
<td>9(33.3)</td>
<td></td>
<td>36(26.7)</td>
<td>5(17.9)</td>
<td></td>
<td>36(26.7)</td>
<td>0(0)</td>
<td></td>
</tr>
<tr>
<td>casuals</td>
<td>56(41.5)</td>
<td>14(51.9)</td>
<td></td>
<td>56(41.5)</td>
<td>17(60.7)</td>
<td></td>
<td>56(41.5)</td>
<td>7(70)</td>
<td></td>
</tr>
</tbody>
</table>
On the awareness of any information pertaining the Hepatitis in general and specificity, 10% of the mono-infected participants and 13.5% of co-infected participants had an idea of viral hepatitis while 76.5% of the study population did not know anything pertaining the viruses. Participants with a past diagnosis with Hepatitis disease was 2% while 98% had never been diagnosed with the disease or were not aware of any past diagnosis. On vaccination against HBV, 9% of the participants admitted to have been vaccinated while 91% admitted not to have been vaccinated or were not sure on the vaccination status (Table 4.1.2).

**Table 4.2: Hepatitis awareness**

<table>
<thead>
<tr>
<th></th>
<th>HIV only N=200</th>
<th>Co-infected N=200</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge on Hepatitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knowledgeable</td>
<td>20(10%)</td>
<td>27(13.5%)</td>
<td>47</td>
</tr>
<tr>
<td>No knowledge</td>
<td>115(57.5%)</td>
<td>38(19%)</td>
<td>153</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>135</td>
<td>63</td>
<td>200</td>
</tr>
<tr>
<td>Past diagnosis with Hepatitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosed</td>
<td>0(0%)</td>
<td>2(1%)</td>
<td>2</td>
</tr>
<tr>
<td>Not diagnosed</td>
<td>135(67.5%)</td>
<td>63(31.5%)</td>
<td>198</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>135</td>
<td>63</td>
<td>200</td>
</tr>
<tr>
<td>No vaccinated against HBV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinated</td>
<td>10(5%)</td>
<td>8(4%)</td>
<td>18</td>
</tr>
<tr>
<td>Not vaccinated</td>
<td>125(62.5%)</td>
<td>57(28.5%)</td>
<td>182</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>135</td>
<td>65</td>
<td>200</td>
</tr>
</tbody>
</table>

HIV only – Mono-infection, HIV+HBV- Dual infection, HIV+HCV- Dual infection and HIV+HBV+HCV- Triple infection.

On the injecting pattern among the IDUs, the substance of abuse the participants were using was majorly heroine whereby 58% of the HIV mono-infected and 48% of co-infected were using the heroine. The activeness in the use of substance of abuse varied, whereby 82% were actively injecting the substance of abuse while 18% had stopped injecting at least six months before the study was carried out. It was also reported that
among the IDU participants, 50% had never shared the injecting paraphernalia while 50% had shared, with 40% reporting to have shared in the past while 10% of the same study population was still sharing different injecting paraphernalia as at the time of study. IDUs reported administering the drugs different number of times in a day. At least 38% administered the substance once in a day, 32% administered twice a day and 30% injected three or more times in a day.

Table 4.3: Injecting behaviour among IDUs

<table>
<thead>
<tr>
<th></th>
<th>HIV only</th>
<th>Co-infected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=100</td>
<td>N=100</td>
<td></td>
</tr>
<tr>
<td><strong>Substance in use</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heroine</td>
<td>(58)58%</td>
<td>42(42%)</td>
<td>100</td>
</tr>
<tr>
<td>Cocaine</td>
<td>(0)0%</td>
<td>(0)0%</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>(0)0%</td>
<td>(0)0%</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td><strong>Duration of injection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current Injectors</td>
<td>56(56%)</td>
<td>26(26%)</td>
<td>82</td>
</tr>
<tr>
<td>Ex - injectors</td>
<td>2(2%)</td>
<td>16(16%)</td>
<td>18</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td><strong>Sharing of injecting tools</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past</td>
<td>10(10%)</td>
<td>30(30%)</td>
<td>40</td>
</tr>
<tr>
<td>Current</td>
<td>0(0%)</td>
<td>10(10%)</td>
<td>10</td>
</tr>
<tr>
<td>Never</td>
<td>46(46%)</td>
<td>4(4%)</td>
<td>50</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td><strong>No. of times injected/day</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>30(30%)</td>
<td>8(8%)</td>
<td>38</td>
</tr>
<tr>
<td>Two</td>
<td>18(18%)</td>
<td>14(14%)</td>
<td>32</td>
</tr>
<tr>
<td>≥ three</td>
<td>10(10%)</td>
<td>20(20%)</td>
<td>30</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

Legend of the table;

Past; have shared the injecting materials but had stopped at least six months before the study was carried out. Current; were still sharing. Never; have never shared.
All the study participants were aware of their HIV status. On the regularity in attending the comprehensive care clinics the IDUs had a 65% regular attendance and 35% attended irregularly. Among the NDUs 90% attended the CCC regularly while 10% had irregular attendance. On the ART adherence, 45% of the IDUs observed strict adherence while 55% flouted on the intake patterns. Among the NDUs, 85% of the participants had strict adherence on the ART intake while 15% were flouters. (Table 4.1.4).

Table 4.4: Assessment on HIV

<table>
<thead>
<tr>
<th></th>
<th>IDUs</th>
<th>NDUs</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Knowledge on HIV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Know the status</td>
<td>100(100%)</td>
<td>100(100%)</td>
<td>200</td>
</tr>
<tr>
<td>Not Know the status</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Attendance of CCC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regularly</td>
<td>65(65%)</td>
<td>90(90%)</td>
<td>155</td>
</tr>
<tr>
<td>Irregularly</td>
<td>35(35%)</td>
<td>10(10%)</td>
<td>45</td>
</tr>
<tr>
<td>Never attends</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Adherence to ARTs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strict</td>
<td>45(45%)</td>
<td>85(85%)</td>
<td>130</td>
</tr>
<tr>
<td>Not strict</td>
<td>55(55%)</td>
<td>15(15%)</td>
<td>70</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>200</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Strict adherence- were using drugs according to the schedule throughout. Not strict- had times when they did not follow the strict timing of drug intake.
4.3 Prevalence of HIV/Hepatitis co-infection among IDUs and NDUs

The overall prevalence of co-infection among the study population was 32.5%. The prevalence of viral hepatitis (HBV and HCV) among the IDUs was 21% and 11.5% among the NDUs. The sero-prevalence of HBV and HCV was 16% and 20% respectively among the IDUs. Among the NDU group, the prevalence of HBV and HCV was 11% and 8% respectively. The sero-prevalence of triple infection, (HIV/HBV/HCV) among the IDUs and the NDUs was 6% and 4% respectively. (Table 4.3)
<table>
<thead>
<tr>
<th>DRUG USE STATUS</th>
<th>HIV only</th>
<th>HIV+HBV</th>
<th>HIV+HCV</th>
<th>HIV+HBV+HCV</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>COUNT</td>
<td>77</td>
<td>11</td>
<td>8</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>NDU % CO-INFECTION</td>
<td>77%</td>
<td>11%</td>
<td>8%</td>
<td>4%</td>
<td>100%</td>
</tr>
<tr>
<td>IDU COUNT</td>
<td>58</td>
<td>16</td>
<td>20</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>% CO-INFECTION</td>
<td>58%</td>
<td>16%</td>
<td>20%</td>
<td>6%</td>
<td>100%</td>
</tr>
</tbody>
</table>

HIV only – Mono-infection, HIV+HBV- Dual infection, HIV+HCV- Dual infection and HIV+HBV+HCV- Triple infection.

IDUs- Intravenous Drug Users, NDUs- Non- Drug Users.
4.4 Prevalence of HIV/Hepatitis co-infection by age groups

In relation to the age groups, ages 31-40 years indicated the highest co-infection rate of 15.4% while ages >60 had the lowest co-infection rate of 1.5% among the NDUs. In the IDU study group, ages 41-50 had the highest co-infection at 21.5%, while ages ≤20 and >60 indicated the lowest co-infection rate of 1.5%. The prevalence of co-infection in relation to age groups among the NDUs and IDUs was statistically insignificant (P=0.282) (Figure 4.1).

Figure 4.1: The co-infection rate in relation to age among the study groups
IDUs- Intravenous Drug Users, NDUs- Non-Drug Users. P ≤0.05 was considered statistically significant.
4.5 Prevalence of HIV/Hepatitis co-infection by Gender

In relation to gender, the co-infection rate was high among the female NDUs than the male NDUs at 21.5% and 13.8% respectively (P=0.145). Among the IDUs both the females and males had equal prevalence of 32.3%. Comparing the prevalence among NDUs and IDUs, the male and female IDUs had a higher co-infection rate of 32.3% than their counterpart NDUs who had a co-infection rate of 13.8% among males and 21.5% among females. The difference was statistically insignificant among females (P=0.167), while among the males the difference was statistically significant (P=0.013). Cumulatively, males reported a lower prevalence than the females though the difference was statistically insignificant (P=0.382) (Figure 4.2).

![Figure 4.2: The co-infection rate in relation to gender among the study groups](image)

IDUs- Intravenous Drug Users, NDUs- Non-Drug Users. P ≤ 0.05 was considered statistically significant. (±) is the standard deviation.
4.6 The CD4 positive cell count of the study participants

The mean CD4+ cell count for all the IDU participants in the study was 350.12 (±225.3) cells/µl while that of NDUs was 485.94 (±243.38). The difference in the CD4+ cell count among IDUs and NDUs was statistically significant (P=0.0004). IDU males who were co-infected had a lower mean CD4+ cell count of 162.86(±63.22) cells/µl compared to the male NDUs with a mean count of 227.00(±94.47.14) cells/µl. The difference was statistically significant (P= 0.022). A similar pattern was observed with the female participants (P= 0.035). However, across the gender, males had a statistically significant lower mean CD4 positive cell count of 366(±210) cells/µl than the females who had a mean count of 450(±259) cells/µl (P=0.021) (Figure 4.3).

**Figure 4.3: Mean CD4+ cell count in relation to co-infection and gender**

Co-infected- having HIV+HBV/HIV+HCV/HIV+HBV+HCV. Mono-infected- having HIV only. P≤0.05 was considered statistically significant. Normal range; CD4 count 410-1590cells/µl. (±) is the standard deviation.
Mono-infected subjects had a statistically significant higher mean CD4+ cell count of 536.79 cells/µl (±218.76) than the co-infected subjects whose mean count was 192.91 cells/µl (±84.08) (P<0.0001). Participants who were co-infected with the three viruses had a statistically significant lower mean CD4+ cell count of 92.17 cells/µl (±45.556) than the dual co-infected among IDUs (P=0.001). Among the NDUs, the same pattern was reported whereby triple co-infected indicated the lowest mean CD+ cell count of 104.50 cells/µl (±74.661) (P=0.001). The HIV+HBV co-infected had a statistically insignificant higher mean CD4+ cell count of 184.25 cells/µl (±50.195) among IDUs and 267.00 cells/µl (±89.379) among the NDUs than the HIV+HCV co-infected (P=0.800 and P=0.879 respectively). The difference in mean CD4+ cell count among IDUs and NDUs in relation to viral co-infection, was statistically significant (P=0.0001) (Figure 4.4).

Figure 4.4: Mean CD4 Count in relation to co-infection status among the IDUs and NDUs
P≤0.05 was considered statistically significant. Normal range; CD4+ cell count 410-1590 cells/µl. (±) is the standard deviation.
Participants aged 61 years and above recorded a statistically insignificant lowest mean CD4+ cell count of 247 cells/µl (±7.071) among the IDUs in comparison to the other age groups (P= 0.788) and 375 cells/µl (±243.382) among the NDUs (P= 0.303). IDUs aged ≤20 years had a statistically insignificant higher mean CD4+ cell count of 426.75 cells/µl (±228.812) than the other age groups (P= 0.788). Among the NDUs, the highest mean CD+ cell count was among ages 21-30 years at mean count of 591.39 cells/µl (±319.432). (Figure 4.5).

![Figure 4.5: Mean CD4 Count in relation to age groups among the IDUs and NDUs](image)
P≤0.05 was considered statistically significant. Normal range: CD4+ cell count; 410-1590 cells/µl. (±) is the standard deviation.

It was observed that participants who were using the regimen containing Stavudine (D4T) had the lowest mean CD4+ cell count of 141.8 (±69.03) cells/µl among the IDUs (P<0.0001) and 179.64 (±102.86) cells/µl among the NDUs (P<0.0001). The IDUs on Tenofovir disoproxil fumarate (TDF) regimen also indicated a low mean CD4 positive
cell count of 292.7(±168.43) cells/µl while in NDUs the CD4 +cell count was 484.27(±161.56) cells/µl (P<0.0001). Both the IDUs and NDUs using regimens with Zidovudine (AZT) showed a statistically significant higher mean CD4+ cell count of 451.4(±231.92) cells/µl and 529.4(±234.29) cells/µl. respectively in comparison to those on other NRTIs (P<0.0001). (Figure 4.6)

![Figure 4.6: Mean CD4 Count in relation to antiretroviral drugs among the IDUs and NDUs](image)

**KEY;** AZT- Zidovudine, D4T- Stavudine, TDF- Tenofovir disoproxil fumarate. Normal range: CD4+ cell count; 410-1590 cells/µl. P≤0.05 was considered statistically significant. (±) is the standard deviation.

**4.7 The HIV Viral Load of the study participants**

Participants who were using the intravenous drugs had a statistically significant higher HIV viral load of 43,781.08 (±100466.3) copies/ml compared to the non-drug users who had a HIV viral load of 26347.18 (±82646.8) copies/ml (P=0.05). Female IDUs had a statistically insignificant higher viral load of 121,328 (±311812.4) copies/ml than
the female NDUs whose mean viral load was 55,490.79 (±57157.39) (P=0.442). Male IDUs had a statistically insignificant higher viral load of 275025.6 (±303983.10) copies/ml than the male NDUs whose mean viral load was 96694.22 (±195818.46) copies/ml (P=0.400) (Figure 4.7).

Figure 4.7: Mean HIV viral load in relation to IDUs and NDUs

Co-infected- having HIV+HBV/HIV+HCV/HIV+HBV+HCV. Mono-infected- having HIV only. P≤0.05 was considered statistically significant. Normal range; Undetectable (≤20copies/ml). (±) is the standard deviation.

Mono-infected participants had a statistically significant lower mean viral load of 2435.05 (±5596.88) copies/ml than the co-infected participants whose mean viral load was 153392.97 (±395699.65) copies/ml (P < 0.0001). The triple co-infected subjects indicated the highest mean HIV viral burden of 755,433 (±1199794) copies/ml as compared to the HIV+HBV with 75034 (±169675.7) copies/ml and HIV+HCV co-infected with 101231.4 (±132062.2) copies/ml respectively among IDUs (P<0.0001).
A similar pattern was evident among NDUs with 381589.7(±481869) copies/ml among triple co-infected, 65693.27(±107465.9) copies/ml among HIV+HBV and 84,259.35(±132724.9) copies/ml among and HIV+HCV co-infected respectively. The participants with dual co-infection of HIV +HBV, had the lower mean HIV viral load than those with dual co-infection of HIV+HCV in both study groups. The difference in the mean viral load between the co-infected groups of both study groups was statistically significant (P=0.0001) (Figure 4.10)

![Figure 4.8: Mean viral load in relation to hepatitis co-infection among IDUs and NDUs](image)

P≤0.05 was considered statistically significant. Normal range; Undetectable (≤20copies/ml). (±) is the standard deviation.

Participants who were using the regimen containing Stavudine (D4T) had the highest mean HIV viral load of 82788.1(±127227.16) copies/ml among the IDUs and 45518.3(±36257.45) copies/ml among the NDUs (P < 0.0001). Those who were using
regimens with Zidovudine (AZT) had the lowest load of 20769.1\(\pm\)66895.27 copies/ml among the IDUs and 20135.3\(\pm\)85875.87 copies/ml among the NDUs (P < 0.0001). The study participants who were on Tenofovir disoproxil fumarate (TDF) regimen showed a higher mean viral load than those on AZT (P < 0.0001). (Figure 4.11)

Figure 4.9: Mean viral load in relation to antiretroviral drugs among IDUs and NDUs

P\leq0.05 was considered statistically significant. Normal range; Undetectable (\leq20copies/ml). (\pm) is the standard deviation.

In the correlation of CD4 positive cell count and viral load among the study participants, higher HIV viral load correlated with lower CD4 positive cell counts among IDUs (r= -0.48) and among the NDUs (r= -0.68) (Figure 4.8 and 4.9).
Figure 4.10: Correlation between the CD4 cell count and viral load among NDUs
Normal range; Undetectable (≤20copies/ml).

Figure 4.11: Correlation between the CD4 cell count and viral load among IDUs
Normal range; Undetectable (≤20copies/ml).
4.8 Liver enzyme markers of the study participants

The mean liver enzymes markers (Alkaline phosphate, Aspartate aminotransferase and Alanine transferase) were mildly more elevated among the co-infected participants than the mono-infected participants. Participants who had triple co-infection indicated the highest mean values of the three liver enzyme markers ALP, AST and ALT. Participants with a dual co-infection of HIV+HBV had a more elevated liver enzyme markers than those co-infected with HIV+HCV. The difference in the mean liver enzyme markers elevation was statistically insignificant at \( P = 0.245 \) for ALP, \( P=0.945 \) for AST and \( P=0.805 \) for ALT (Figure 4.12).

**Figure 4.12: Mean liver enzyme markers level in relation hepatitis co-infection status**

Normal ranges: ALP - 40-150U/L. AST - 5-34U/L. ALT- 0-55U/L. (±) is the standard deviation.
IDUs had a statistically non-significant elevated mean ALP levels of 170.95(±64.78) U/L than the NDUs who had a mean level of 161.8(±78.03) U/L (P=0.368). Higher mean levels of AST and ALT were observed among the IDUs at 42.63(±16.73) U/L and 63.69(±42.55) U/L than the NDUs 36.05(±20.17) U/L and 49.86(±28.89) U/L. The difference was statistically significant (P=0.013 and P=0.001) respectively (Figure 4.13).

Figure 4.13: Mean liver enzymes in relation to IDUs and NDUs
Normal ranges: ALP - 40-150U/L. AST - 5-34U/L. ALT- 0-55U/L. P ≤0.05 was considered statistically significant. (±) is the standard deviation.

The study participants who were on D4T regimen indicated elevated mean liver enzymes whereby the mean ALP was 175.66 (±60.79)U/L, AST was 86.0 (±36.53) U/L and ALT was 74.35 (±42.54) U/L among the IDUs and ALP was 166.47 (±51.08) U/L, AST was 37.2(±17.74) U/L and ALT was 55.63 (±31.45) U/L. The NDU participants using TDF indicated a slight elevation of ALP and AST while those on AZT had a mild
elevation of ALP and AST. The mean liver enzyme markers were elevated among the IDUs regardless of the regimen they were on (Figure 4.14).

Figure 4.14: Mean liver enzymes in relation to antiretroviral drugs among IDUs and NDUs

KEY; AZT- Zidovudine, D4T- Stavudine, TDF- Tenofovir disoproxil fumarate. Normal range: CD4+ cell count; 410-1590 cells/µl. P≤0.05 was considered statistically significant. Normal ranges: ALP - 40-150U/L. AST - 5-34U/L. ALT - 0-55U/L.

4.9 Differential profiling using the liver enzymes

The clinical picture was grouped into non-hepatic, hepatocellular and Cholestatic patterns of abnormality using the GGT as the reference. This was combined with a clinical history, medication and drug history of the participants. IDUs showed more cases of liver abnormality than the Non-DUs. 5% of IDUs had none-hepatic abnormality which is characterized by GGT>100 ALT<80 ALP<200 compared to 2% of Non-DUs. Cholestatic abnormality characterized by GGT>100 ALT<80 ALP>200 was seen in 1.5% of IDUs and 1% Non-DUs and 1.5% of IDUs had hepatocellular
abnormality characterized by GGT>100 ALT>80 ALP>200 compared to the 0.5% of Non-DUs (Figure 4.15).

Figure 4.15: Subjects with different type of liver abnormalities

KEY; GGT- Gamma-glutamyl transferase ALP- Alkaline phosphatase, ALT- Alanine aminotransferase

On the liver enzyme profiling in relation to the hepatitis status, triple infected individuals indicated a higher rate of non-hepatic abnormality of 2% among IDUs and 1% NDUs as compared to the dual infected and the mono-infected participants. The Cholestatic abnormality was reported among IDUs with dual and triple infection at 0.5% and 0.5% among NDUs with HIV+HCV and HIV+HBV+HCV.
Table 4.6: Profiling among study participants in relation to hepatitis status

<table>
<thead>
<tr>
<th>Non-hepatic abnormality</th>
<th>(GGT&gt;100, ALT&lt;80)</th>
<th>ALP&lt;200</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDUs</td>
<td>NDUs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mono-infected HIV only</td>
<td>1(0.5%)</td>
<td>0(0%)</td>
<td>1</td>
</tr>
<tr>
<td>HIV+HBV</td>
<td>2(1%)</td>
<td>1(0.5%)</td>
<td>3</td>
</tr>
<tr>
<td>Co-infected HIV+HCV</td>
<td>3(1.5%)</td>
<td>1(0.5%)</td>
<td>4</td>
</tr>
<tr>
<td>HIV+HBV+HCV</td>
<td>4(2%)</td>
<td>2(1%)</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>4</td>
<td>14</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cholestatic abnormality</th>
<th>(GGT&gt;100, ALT&lt;80)</th>
<th>ALP&gt;200</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDUs</td>
<td>NDUs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mono-infected HIV only</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0</td>
</tr>
<tr>
<td>HIV+HBV</td>
<td>1(0.5%)</td>
<td>0(0%)</td>
<td>1</td>
</tr>
<tr>
<td>Co-infected HIV+HCV</td>
<td>1(0.5%)</td>
<td>1(0.5%)</td>
<td>2</td>
</tr>
<tr>
<td>HIV+HBV+HCV</td>
<td>1(0.5%)</td>
<td>1(0.5%)</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hepatic abnormality</th>
<th>(GGT&gt;100, ALT&lt;80)</th>
<th>ALP&gt;200</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDUs</td>
<td>NDUs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mono-infected HIV only</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0</td>
</tr>
<tr>
<td>HIV+HBV</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0</td>
</tr>
<tr>
<td>Co-infected HIV+HCV</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0</td>
</tr>
<tr>
<td>HIV+HBV+HCV</td>
<td>3(1.5%)</td>
<td>1(0.5%)</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

Non-hepatic abnormality was characterized by GGT>100, ALP<200, and ALT <80

Cholestatic abnormality was characterized by GGT>100, ALP>200, and ALT <80

Hepatic abnormality was characterized by GGT>100, ALP>200, and ALT >80
CHAPTER FIVE: DISCUSSION

The sero-prevalence of HBV among the IDUs was at 16%. The findings were higher than in previous studies done in Kenya on similar populations by (Kerosi et al., 2015; Kibaya et al., 2015; Webale et al., 2015) where the findings were 9.6%, 13.89% and 14.3% respectively. The difference in the prevalence outcome could have been due to variation in the population sampling, sensitivity and specificity of the diagnostic techniques whereby this study did not undertake the molecular diagnostics which are considered more specific and sensitive. Also in migration of cases could contribute to the variation as Mombasa is one of tourist attraction centre both locally and internationally. Among the NDUs, the HIV/HBV sero-prevalence was found to be 10%. This was higher than previously found in Kenyan general populations by Muriuki et al. (2013) at 6%, Ochwoto et al. (2013) at 6% and Kerubo et al. (2015) at 8.9% but lower than the findings by Otedo (2004) which were 53%. The study recruited participants from various health facilities and the communities thus differences in the prevalence rates as compared to those of previous studies could be attributed to demographic and population dynamics over time, sample size and population sampling differences and an immigration of cases and susceptible individuals. Also the variation could be as a result of assay specificity and sensitivity whereby this study did not undertake the molecular diagnostics which are considered more specific and sensitive.

Among the IDUs the sero-prevalence of HCV was 20% higher than the findings by (Mwatelah et al., 2015) which was 16.4%, but lower than the findings of Muasya et al. (2008) which was at 22.2%. The co-infection rate among the NDUs with HIV/HCV was 8%, a finding higher than that of previous studies done by (Harania et al., 2008) at 1.1% and Kerubo et al. (2015) at 0.7% but lower than the findings of Muriuki et al.
(2013) which was at 10.3%. The variation in the prevalence rates in comparison to the other previous studies could be due to demographic dynamics across the study sites, variation in population sampling and sample size. Also the difference could be an indicator of a cross transmission between the IDUs and the NDUs.

The sero-prevalence of triple infections, (HIV+HBV+HCV) among the IDUs and the NDUs was 6% and 4% respectively. The findings were higher than the previous studies done in Kenya among the IDUs and NDUs and the variation could have been due to the differences in the population dynamics and population sampling methods.

This study revealed that there was sufficient knowledge on HIV among the study population as all, knew about their HIV status, had information on ARTs, the CCC attendance and the transmission modes. On the hand, the study population had little or handily no information on blood borne viral hepatitis. Majority did not know whether they have ever been diagnosed with hepatitis B or hepatitis C neither did they know whether they were vaccinated against hepatitis B. This was an indication of a possible gap in the knowledge about possible organisms which can be contracted along-side HIV. These findings are in-line with reports from previous studies by (Kerosi et al., 2015; Umutesi et al., 2017).

All the IDUs participants in this study were consuming heroin as the injecting drug. Despite the introduction and the ongoing programmes of needle/syringe provision, the sharing of various injecting paraphernalia was still evident. Current injectors had a higher number of co-infected participants than the ex-injectors. This could be an indication of an active transmission through either sharing of various injecting paraphernalia or other shared routes of transmission. The study also reported a higher
number of co-infection among participants who injected the drugs more times in a day than those who had lesser injections. The heroin use and the dosages have been linked to immune compromisation as it enhances viral infection via immune suppression (Ilić et al., 2005; Were et al., 2014).

The findings of assessment of the demographic characteristic; the occupation, showed a significant high prevalence of co-infection among the casuals. This strengthens the assertion that poverty levels contribute significantly to the transmission of diseases. This was consistent with the propositions from a previous report by (Kerosi et al., 2015). The fact that co-infection was low among those with tertiary education as compared to the other levels of education indicates that there is a gap in the knowledge about the viral diseases and the modes of transmission. The outcome is also supported by the report from this study, on the hepatitis awareness whereby there was poor knowledge pertaining the hepatitis infection. The finding is in consistent with reports by (Umutesi et al., 2017). The study findings based on marital status indicated that the married and the singles were more prone to the infections than the divorced and the windowed. The findings are in line with reports on “Poverty drives some Kenyans to rent out their wives,” (Onyulo, 2018) and the report on “The child sex trade is booming in this Kenyan port city” (Onyulo, 2016). The Muslim believers indicated a higher co-infection rate of HIV+HBV than the Christians and other believers. This outcome could be due to close familial interaction practiced by the community. The outcome is supported by the reports of Hatami et al. (2013).

Notably, the co-infection rate was not clustered among the age group 21-30 who are known to have more idle time, easily influenced by peer pressure and have the urge and excitement of experimenting on new experiences and lifestyles such as drug and alcohol
abuse or cigarette smoking. These tendencies have been attributed to the frustrations of unemployment which is high among the age group. The observations were in contrast with a previous study done by Muasya et al. (2008). High co-infection rates were however indicated in the 31-40 years age group among NDUs and 41-50 years among the IDUs. From this study, it was reported that more individuals who had a past history of sharing of injecting paraphernalia indicated a higher rate of co-infection, thus the outcome could suggest that the older people may have been infected much earlier and could have stayed with the disease for much longer thus posing a greater risk of disease transmission to the younger population. This is so because of the age sexual disparate relationship evident in most countries (Schaefer et al., 2017).

Co-infection rate was higher among the females in comparison to the males among the NDUs. This was contrary to studies by Hamza et al. (2013) and Muriuki et al. (2013), who reported a higher prevalence among males than females. The findings probably may be due to the polygamous nature of a portion of the population in coastal region. Also transactional sex and age disparate sexual relationships could have led to the outcome (Schaefer et al., 2017). The study outcome is in part, consistent with the reports of the outreach rehabilitation centre 2016 and CSIS 2011 that reported a higher HIV prevalence among women than men (Mombasa County Strategic Plan, 2014; UNAIDS, 2018). Among IDUs the co-infection rate was at par in males and females. The outcome could possibly be due to the patterns of recruitments in drug use and the sharing of the injecting paraphernalia among closely associated individuals. Blood borne viruses share similar transmission routes hence this could majorly account for the outcome (Benyawa, 2016).
The mean CD4+ cell count between the two cohorts was significantly different whereby IDUs had a significantly lower CD4+ cell count than the NDUs. The differences are attributable to among others, the individual immunity which is markedly different across individuals. The heroin use could also result to immune compromisation as it enhances viral infection via immune suppression (Ilić et al., 2005). Viral hepatitis and antiretroviral drug regimen adherence which the IDU populations are known to flout could to lead to immune suppression. This was quite evident from the questionnaire responses. Participants who had co-infections expressed lower mean CD4+ cell counts compared to those who were HIV mono-infected. The findings were similar to previous studies done by Otedo, (2004), Adewole et al. (2009); Wondimeneh et al. (2013). This finding could be due to the high viral burden experienced during co-infections. Documentations by Nikolopoulos et al. (2009) and Day et al. (2013), argue that co-infection is a major contributory factor in immunological derangement in the affected subjects which can be expressed by reduction in CD4+ cells. Co-infection is further known to exacerbate the development of HIV to AIDS and has been shown to inversely affect CD4 values in relation to HIV AIDS disease progression (Day et al., 2013).

The study also found that males had a lower CD4+ cell count than the females in both cohorts. Similar results were reported by Tugume et al. (1995) in Uganda, Akinbami et al. (2012) in Nigeria and Wondimeneh et al. (2013) in Ethiopia. These findings may be accounted to the daily activities, nutrition and hard labour the males put up with (Malone et al., 1990; Wondimeneh et al., 2013; Onyulo, 2016). It is also notable that males are less likely to accept their disease status and as a result, psychological stress may develop causing a further strain in the immunological processes. This in turn increases the risk of disease progression and reduced CD4 cell counts (Leserman et al.,
1999; Wondimeneh et al., 2013; Onyulo, 2016). The study analyzed Mean CD4 positive cell counts for various population age subsets. The mean CD4 positive cell count was found to be higher in the younger age brackets as compared to the older persons in both males and females. Similar finding were reported by (Wondimeneh et al., 2013). The outcome could imply that there was diminishing immunity as a result of increased dysregulation of the adaptive immunity as the age progressed (Wondimeneh et al., 2013). In the treatment of the HIV AIDS, it was observed that participants who were using the antiretroviral drug regimen with the Stavudine had lower mean CD4+ cell count compared to those on the Zidovudine and Tenofovir disoproxil fumarate across the study population. This observation was also reported elsewhere by (Mwatelah et al., 2015). The finding suggests that the Stavudine exacts negative effects on the immunological responses which in turn can lead to the disease progression. The negative effect could be due to cross resistance between compounds of the same class especially in circumstances where Stavudine is considered as an alternative to Zidovudine and Tenofovir disoproxil fumarate (Bygrave et al., 2011; Velen et al., 2013).

The viral load count was higher among the IDUs in comparison to the NDUs. Similar report was made by (Pal et al., 2011; Kumar et al., 2015), who argued that drugs of abuse could lead to reduction in the efficacy of the antiretroviral drugs leading to toxicity, treatment failure and an increase in the viral loads. Also factors such as differences in the immunity levels across individuals, multiple viral infections, and adherence to antiretroviral drugs could contribute to the outcome. Males had a higher viral load burden than the females among the study participants and this could have been due to daily activities, nutrition and hard labor which contribute to immune-
compromisation leading to an increase in the viral load. Males are also less likely to accept their disease status and as a result, psychological stress may develop causing a further strain in the immunological processes leading to increase in the HIV viral load. Comparison of groups within the study cohorts, indicated a significant difference with the triple infected group having the highest mean viral load, followed by the HIV+HCV, HIV+HBV and HIV mono-infected had the lowest mean viral load count. The mean viral load values are representative of the increased risk of disease, marked increase in disease progression after sero-conversion and increased derangement of the immune system occasioned by multiple co-infections. These factors render the body susceptible to rapid disease advancement.

The mean CD4 and mean viral load count were correlated and the outcome showed that the increase in the viral load was counteracted with a decrease in the mean CD4 count. The difference was statistically significant. Consistent with these findings, previous studies have reported a well-established correlation between high levels of plasma HIV-1 RNA and lower baseline CD4+ cell count (Malone et al., 1990). Dorrucci et al. (2007) and Deeks et al. (2013) have also documented that there is a direct relationship between decreasing CD4+ cell counts and increased viral load in respect to progression of HIV to AIDS. The study also observed that participants who were using Stavudine in the regimen had low mean CD4+ cell count than the participants on the regimen containing Zidovudine and Tenofovir disoproxil fumarate, an effect which was observed with a counter increase in HIV viral load among the same participants. This study outcome suggests that Stavudine does not contribute to positive treatment hence more investigative studies on the drug should be carried out to check for its efficacy.
The mean liver enzyme markers, alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase showed significant difference between the co-infected and the mono-infected among both the study participants. The findings were consistent with previous studies done by (Otegbayo et al., 2008; Anbazhagan et al., 2010; Olawumi et al., 2014). The outcome of this study can be attributed to the hepatitis viral infection among the co-infected and to a lesser extent the use of antiretroviral drugs as all the subjects were on the first line regimen of antiretroviral therapy whose combination is associated with minimal hepato-toxicity. Additionally it is documented that HIV viruses may invade hepatic cells and Kupffer cells in the liver (Cao et al., 1992), hence this attribute further complicates the disease and may increase the risk of hepato-fibrosis and in turn elevated liver enzyme levels. The mean liver enzyme markers also increased in a pattern whereby participants with triple co-infection showed the highest mean liver enzyme markers followed by the HIV+HBV then the HIV+HCV co-infected. This finding conforms to the findings by Lodenyo et al. (2000) in South Africa where HIV/HBV and HIV/HCV patients had both ALT and AST levels increased by 70% and ALP by 56%. The findings were also similar to those reported by Otedo, (2004). The findings contradicted those of Tripathi et al. (2007) in India where ALT was 14% higher in HIV/HBV and 20% higher in HIV/HCV co-infections. The IDUs had elevated mean liver enzyme markers than the NDUs. The finding can be linked to the fact that other than the hepatitis status and the antiretroviral drugs, the IDUs could have had a high chance of drug induced hepato-toxicity from the heroin use. It is documented that a highly common complication of intravenous heroin abuse is the chronic viral and active hepatitis which induces a significant morphological and functional liver damage (Ilić et al., 2005) a phenomenon seen in this study by the elevated liver enzymes levels. Other possible causes are differences in the duration and stages of viral disease and
other study participant conditions such as hepato-toxicity induced by use of alcohol and other substances of abuse.

Antiretroviral drugs are associated with the hepato-toxicity. This study established that participants using the regimen containing Stavudine, had higher mean liver enzyme markers than those using Zidovudine and Tenofovir disoproxil fumarate. The negative effect could be due to cross resistance between compounds of the same class especially in circumstances where Stavudine is considered as an alternative to Zidovudine and Tenofovir disoproxil fumarate (Bygrave et al., 2011; Velen et al., 2013). It was also observed that there was marked elevation of the mean liver enzyme markers among IDUs despite the regimen the participants were using. This outcome could be attributed to the heroin use and the HBV and HCV co-infection an outcome supported by (Farooqi et al., 2016). The study was a cross sectional study, hence could not conclusively argue on the specific cause of hepato-toxicity among the study population as the duration and the drug formulation changes could not be established. This study acknowledges the WHO guidelines on the use of ARTs and the use of Tenofovir disoproxil fumarate in treatment of HBV infections. Despite a population of this study being on TDF therapy, results on elevated liver enzyme markers was evident. This finding could have been attributed to the timing of ART initiation whereby the drug has a higher efficacy when given as a pre- exposure prophylaxis than as a post exposure prophylaxis (WHO, 2013). The population of IDUs were more affected as compared to the NDUs. This may have been due to negative interaction between the ARTs and the substance of abuse (heroin) the IDUs were using (Were et al., 2014).

In this study, the liver enzymes, alkaline phosphatase, and alanine aminotransferase were associated with the gamma glutamyl transpeptidase to find out the number of
participants who had different liver conditions (Giannini et al., 2005). These parameters were considered because; ALT has been shown to be a more specific marker of hepatocellular damage as opposed to the assay of ALP which is largely a non-specific marker for liver disease. Gamma glutamyl transpeptidase was also used although there may be increased levels in alcoholism and other hepatotoxic substances. The exclusion of AST was due to its increased levels in cardiovascular and muscular disease and non-specificity to hepatic diseases (Giannini et al., 2005). The qualifiers were GGT>100, ALT<80 and ALP<200 characterizing non hepatic abnormality and 5% of the IDUs and 2% of the NDUs were in the category. This outcome signified the use of recreational drugs or alcohol a common practice in IDUs as compared to NDUs. GGT>100, ALT<80 and ALP>200 characterizing cholestatic abnormality had 1.5% of IDUs and 1% of NDU. The abnormality is an indication of an obstruction of the biliary ducts, hepatic disease and diseases of the bones, this is due to ALP’s association with bone disease ((Yu et al., 2015)) More IDUs were in this category as compared to the NDUs. GGT>100, ALT>80 and ALP>200, is an indication of damage of the liver cells and a sign of slow bile flow or obstruction. From the study, 1.5% of IDUs had the indication than 0.5% of NDUs. The outcome could be largely attributed to cirrhosis of the liver, toxicity arising from alcoholism or drug use, acute and chronic viral hepatitis and hepatic or extra-hepatic tumors.

On the liver enzyme profiling in relation to the hepatitis status, triple infected individuals indicated a higher number of cases with non-hepatic abnormality among IDUs and NDUs than the dual and the mono-infected participants. HIV AIDS and the treatment are associated with liver toxicity. Hepato-tropic viruses are also involved in alteration of the liver enzymes. The findings in this study could be attributed to the viral
burden resulting from the triple infection (Abulude, 2017; Puri et al., 2017). Among the IDUs, the dual and triple infected groups had higher cases of Cholestatic abnormality than the mono-infected group. The study outcome may be as a result of uncontrolled disease resulting from co-infection (Price & Thio, 2011). Non-drug users group with HIV+HCV and HIV+HBV+HCV, reported higher cases of Cholestatic abnormality than the HIV+HBV co-infected and the mono-infected group. The reported lower cases among dual co-infection with HIV+HBV in this study is similar to the reports by (Abulude, 2017). Triple infected groups within IDUs and the NDUs had a higher number of cases with hepatic abnormality than the dual infected and triple infected groups. The findings could be due to a high viral burden a resulting to immune mediated mechanisms from hepato-tropic viruses or direct cyto-toxicity due to HCV which triggers hepatocyte apoptosis via E2 protein (Price & Thio, 2011; Kaspar & Sterling, 2017; Ringehan et al., 2017).
CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The co-infection rate of hepatitis B and hepatitis C virus was higher among IDUs than NDUs. Participants aged between 31-40 years indicated the highest prevalence among NDUs while among IDUs ages 41-50 years reported the highest prevalence. Females and males who were using injecting drugs had an equal prevalence of co-infection while the females who were not using the injecting drugs indicated a higher prevalence than the male counterparts.

2. The immunological and Virological count analysis; the CD4+ cell count showed that IDUs had significantly lower mean CD4+ cell count than the NDUs. Mono-infected subjects had a higher mean CD4+ cell count than the co-infected. The triple infected participants indicated a lower mean CD4+ cell count than the dual infected. The mean HIV viral load count was higher among the IDUs than the NDUs. Mono-infected subjects had a lower mean HIV viral load than the co-infected. The triple infected participants indicated the higher mean HIV viral load than the dual infected. The viral load count correlated with the CD4+ cell count.

3. The analysis of liver enzyme markers indicated that IDUs had markedly elevated mean liver enzymes markers than the NDUs. The mean liver enzyme markers was less elevated among the mono-infected than the co-infected. The triple infected subjects had a higher mean liver enzyme markers elevation as compared to the dual infected.
6.2 Recommendations

The IDUs was a high risk population to HBV/HCV and HIV co-infections due to the behavior associated with the drug injecting. Introduction of harm reduction which includes needle/syringe programmes and opioid substitution therapy as evidence based approach is plausible but more emphasis on the approach in terms of knowledge delivery to the community is paramount. The community health workers needs to be acquitted with the necessary information synthesizable by the community.

The WHO, recommendations on handling of various outcomes in HIV infections is well stipulated but a more keen action is vital for the IDUs. There is a need for routine baseline screening for the HBsAg and anti-HCV markers among the population. The serum liver enzymes should be monitored more closely in HBV/HCV/HIV/AIDS co-infections and more so among the IDUs whose body systems may be overwhelmed by the ARTs, the co-infections and substances they abuse. There is also the need to mount treatment follow up and enforce strict adherence to the use of ARTs especially among the vulnerable groups.

The government and development partners should heighten the vaccination campaign against HBV among the high risk groups as this would reduce the high cost of treatment and the challenges associated with it. Also, the government should consider offering free vaccination to the PLWHA when applicable.

6.3 Recommendations for further research

Prospective studies are encouraged in order to understand better the variations and the impact of the interaction of HIV, Hepatitis B, Hepatitis C, substance of abuse and the ARVs on the liver enzyme markers, immunological and Virological responses.
REFERENCES


Harbor Perspectives in Medicine, 2(4).


Diop-Ndiaye, H., Touré-Kane, C., Etard, J.-F., Lo, G., Diaw, P. A., Ngom-Guye,


Fonseca, M. O., Pang, L. W., de Paula Cavalheiro, N., Barone, A. A., & Lopes, M.


Graham, C. S., Baden, L. R., Yu, E., Mrus, J. M., Carnie, J., Heeren, T., & Koziel,


response to antiretroviral therapy and HIV disease progression in the HIV-NAT cohort. *Aids*, 18(8), 1169–1177.


Lodenyo, H., Schoub, B., Ally, R., Kairu, S., & Segal, I. (2000). Hepatitis B and C virus infections and liver function in aids patients at Chris Hani Baragwanath Hospital,


Ohno, O., Mizokami, M., Wu, R.-R., Saleh, M. G., Ohba, K., Orito, E., ... Lau, J. Y. (1997). New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. *Journal of Clinical Microbiology, 35*(1), 201–207.


European Molecular Biology Organization, 20(14), 3840–3848.


APPENDICES

Appendix I: Map of Mombasa County
Appendix II: Ethical approval

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Fax: 8711242/8711575  P. O. Box 43844
Email: kuerc.chairman@ku.ac.ke  Nairobi, 00100
kuerc.secretary@ku.ac.ke  Tel: 8710901/12
Website: www.ku.ac.ke  Tel: 8710901/12

Our Ref: KU/R/COMM/51/282  Date: 31st January, 2014

Caroline Mercy Kinya,
Kenya University,
P.O Box 43844

APPLICATION NUMBER PKU/157/1 120 – “COMPARATIVE STUDY OF HEPATITIS B AND HEPATITIS C VIRUS AMONG HIV – 1 – CO – INFECTED INTRAVENOUS DRUG USERS AND NON USERS IN MOMBASA COUNTY” - Version2

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic: “Comparative study of hepatitis B and hepatitis C virus among hiv – 1 – co – infected intravenous drug users and non users in Mombasa County” dated 31st January, 2014.

2. DECISION

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines AND APPROVED that the research may proceed for a period of ONE year from 31st January, 2014.

3. ADVICE/CONDITIONS

i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.

ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.

iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.

iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.

PROF. NICHOLAS E. GIKONYO
CHAIRMAN ETHICS REVIEW COMMITTEE

1. KINZIA. .................................... accept the advice given and will fulfill the conditions therein.

Signature: ........................................ Dated this day of ........................................ 2014.

cc. Vice-Chancellor
    Director: Institute for Research Science and Technology
Appendix III: Ethical clearance

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Date:
18th March, 2014

NACOSTI/P/14/9730/871

Caroline Mercy Kinya
Kenyatta University
P.O.Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on “Comparative study of Hepatitis B and Hepatitis C Virus among HIV-1 Co-Infected Intravenous Drug Users and Non-Users in Mombasa County,” I am pleased to inform you that you have been authorized to undertake research in Mombasa County for a period ending 31st December, 2014.

You are advised to report to the County Commissioner, the County Director of Education and the County Coordinator of Health, Mombasa County before embarking on the research project.

On completion of the research, you are expected to submit two hard copies and one soft copy in PDF of the research report/thesis to our office.

DR. M. K. RUGUTU, PhD, HSC
FOR: SECRETARY/CEO

Copy to:
The County Commissioners
The County Directors of Education
The County Coordinator of Health
Mombasa County.
Appendix IV: Research permit

MOMBASA COUNTY GOVERNMENT
OFFICE OF THE COUNTY DIRECTOR OF HEALTH

Telephone Mombasa: 2319819/36
Email: msachd2013@gmail.com

When replying please quote
Ref: MSA/CH/ADM.37. VOL.1/15

Sub County Medical Officers of Health
MOMBASA & KILINDINI

RE: RESEARCH AUTHORIZATION

The bearer of this letter, Caroline Mercy Kinya is a student at Kenyatta University Nairobi.

She intends to carry out research on comparative study of Hepatitis B and Hepatitis C Virus among HIV-1 Co-Infected Intravenous Drug Users and Non-Users in Mombasa County for a period ending 31st December, 2014.

Any assistance accorded to her will be highly appreciated.

COUNTY DIRECTOR OF HEALTH

P. O. BOX 9164 - SC131.
MOMBASA COUNTY.

DR. KHADIJA S. SHIKELY, HSC
DEPUTY DIRECTOR OF MEDICAL SERVICES,
COUNTY DIRECTOR OF HEALTH
MOMBASA COUNTY.
Appendix V: Informed Consent and Assent Form

My name is CAROLINE MERCY KINYA. I am a Master’s student from Kenyatta University. I am conducting a study on “Comparative study of Hepatitis B and Hepatitis C virus among HIV-1 co-infected intravenous drug users and non-users in Mombasa County”. The information will be used by various ministries to contribute towards enhancing prioritization in order to avert the emerging public problem by the policy and decision makers.

Participant selection

We are inviting all adults aged 16 years and above seeking treatment, counselling, HIV-1 comprehensive services and non-IDUs at the medical centre. Also invited are patients in the rehabilitation using the intravenous drugs and HIV-1 positive?

Purpose of the research

The purpose of this research is to determine the infection rates and evaluate the magnitude of this problem. This study is geared at giving insight on the impact of interaction between HIV, HBV and HCV which will contribute towards enhancing prioritization in order to avert the emerging public problem by the policy and decision makers.

Procedures to be followed

Participation in this study will require that I ask you some questions and examine you in order to screen you for HIV. Some specimen will be taken from you for further tests. I will record the information from you in a questionnaire.

Voluntary participation

Your participation in this research is entirely voluntary. Whether you choose to participate or not, all the services you receive at this clinic/centre will continue and nothing will change. If you choose not to participate in this research project, you will be offered the treatment that is routinely offered in this clinic/centre, and we will tell you more about it later. You may change your mind later and stop participating even if you agreed earlier.

We have discussed this research with your parent(s)/guardian and they know that we are also asking you for your agreement. If you are going to participate in the research, your parent(s)/guardian also have to agree. But if you do not wish to take part in the research, you do not have to, even if your parents have agreed.
You may discuss anything in this form with your parents or friends or anyone else you feel comfortable talking to. You can decide whether to participate or not after you have talked it over. You do not have to decide immediately. There may be some words you don't understand or things that you want me to explain more about because you are interested or concerned. Please ask me to stop at any time and I will take time to explain).

Discomforts

The injection might hurt for just a second when it goes into your arm. It might get a little bit red and hard around the place where the injection/needle goes in. That should go away in a day. If it hurts longer than you expected, kindly inform me and your parents.

Benefits

You will not receive direct benefit from participating from in the study but test results will be released to you. However, information gained from the study will be used to develop more effective management services.

Reward

If you agree to participate in this study transport expenses will be reimbursed.

Confidentiality

We will not tell other people that you are in this research and we won't share information about you to anyone who does not work in the research study. Information about you that will be collected from the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is and we will lock that information up with a lock and key. It will not be shared with or given to anyone.

Contact information

If you have any questions, you may contact my supervisors Dr. Antony Kebira on 0735757560 or Prof. Joseph Ngeranwa on 0734572595 or the Kenyatta University Ethical Review Committee Secretariat on kuerc@ku.ac.ke.
Certificate of Consent

I have read this information/ had the information read to me I have had my questions answered and know that I can ask questions later if I have them. I agree to take part in the research.

Name of the participant …………………………………………………

______________________________ ______________
Signature or Thumbprint Date

Certificate of Assent

Name of the participating child …………………………………………………

______________________________ ______________
Signature or Thumbprint Date

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by him/her have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of Researcher/person taking the consent………………………………………………

______________________________ ______________
Signature or Thumbprint Date

Parent/ Guardian

I have witnessed the accurate reading of the assent form to the child, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.
Name of Parent/ Guardian …………………………………………………

______________________________             ________________
Signature or Thumbprint                     Date

I confirm that the child was given an opportunity to ask questions about the study, and all the questions asked by him/her have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of Researcher/person taking the assent………………………………………………

______________________________             ________________
Signature or Thumbprint                     Date
Appendix VI: Structured Questionnaire

I am Caroline Mercy Kinya a student of Kenyatta University Nairobi taking Master of Science infectious diseases (Virology). I am doing a research on COMPARATIVE STUDY OF HEPATITIS B AND HEPATITIS C VIRUS AMONG HIV-1 CO-INFECTED INTRAVENOUS DRUG USERS AND NON-USERS IN MOMBASA COUNTY. Your response will be of a great help to this research and any answers given shall be treated confidential.

Please put a tick or fill in this space provided.

SECTION A; Demographic characteristics.

1) Sex: male ☐ female ☐
2) Age.................
3) Marital status: married ☐ single ☐
   Others (specify)..................
4) Education level: primary ☐ secondary ☐ tertiary ☐
   Others (specify)........................................
5) Religious affiliation: Christian ☐ Muslim ☐
   Others (specify) ........
6) Occupation............

SECTION B: HIV status and Hepatitis awareness

7) Do you know your HIV status?
   a) Yes ☐
   b) No ☐

   If yes specify and indicate when you tested

8) Do you know or heard about Hepatitis B or C?
   a) Yes ☐
126

b) No

If yes state the Hepatitis type…………………………

9) Are you vaccinated against the Hepatitis B?
   a) Yes
   b) No

10) Have you ever been diagnosed with Hepatitis B or C?
    a) Yes
    b) No

If yes state the type of Hepatitis…………………………

SECTION C; Drug use assessment.

11) Have you ever used any substance of abuse?
    a) Yes
    b) No

    If yes specify the substance and the method of administration
    ……………………………

12) Have you injected the drug within the past six months?
    a) Yes
    b) No

    If yes when was the last time of injection?

13) How many times do you inject drugs in a day?
14) Have you ever shared any of the injecting materials

   a) Yes  
   b) No  

If yes specify the material..........................................

15) Whom have you shared the injecting material with? .....................

SECTION D; Attendance of the CCC and rehabilitation services.

16) Do you attend the Comprehensive care clinics?

   a) Yes  
   b) No  

If yes specify the service centre……………………………………

17) Are you using the ARVs? Show card with the drug combination

   a) Yes  
   b) No  

Thanks for your participation