# PREVALENCE AND GENETIC DIVERSITY OF HEPATITIS B AND C VIRUSES AMONG HUMAN IMMUNODEFICIENCY VIRUS INFECTED INDIVIDUALS IN SIAYA COUNTY, KENYA

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**SEPTEMBER, 2020**

## DECLARATION

I do hereby declare that this thesis is my original work and has not been submitted for a degree or any other form of award to any other University or Institution of Higher Learning.

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## DEDICATION

I dedicate this work to my dear wife Fenny Juma, my lovely daughter Trappiere Darells Kevin and My sisters Sally and Queenter for the tremendous moral support, unceasing love and prayers. Further dedication to all HIV/HBV and HIV/HCV co-infected patients of Siaya County who made this study a reality. May God bless you.

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## ABBREVIATION AND ACRONYMS

| | |
|---|---|
| AIDS | Acquired Immunodeficiency Syndrome |
| ALT | Alanine aminotransferase |
| Anti-HCV | Antibodies to hepatitis C virus |
| ART | Antiretroviral Therapy |
| CccDNA | Covalently closed circular DNA |
| CSW | Commercial sex workers |
| DNA | Deoxyribonucleic acid |
| HAART | Highly Active Antiretroviral Therapy |
| HBcAg | Hepatitis B core antigen |
| HBeAg | Hepatitis B e antigen |
| HBsAb | Hepatitis B surface antibody |
| HBsAg | Hepatitis B surface antigen |
| HBV | Hepatitis B virus |
| HBxAg | Hepatitis B x protein |
| HCC | Hepatocellular carcinoma |
| HCV | Hepatitis C virus |

| | |
|---|---|
| IgM | Immunoglobulin M |
| IDUs | Injecting Drug Users |
| MSM | Men who have sex with men |
| ORF | Open Reading Frame |
| PCR | Polymerase Chain Reaction |
| PLHIV | People Living with HIV |
| PgRNA | Pregenomic RNA |
| RNA | Ribonucleic acid |
| RPM | Revolution per minute |
| SCRH | Siaya County Referral Hospital |
| SD | Standard Deviation |
| SPSS | Statistical Package for Social Sciences |
| UNAID | Joint United Nations Program on HIV/AIDS |
| WHO | World Health Organization |

## ABSTRACT

Viral hepatitis B and C co-infections among Human immunodeficiency virus infected patients is significantly becoming a worrying public health problem worldwide. The three infectious viruses' shares common routes of transmission including, blood transfusion, sexual intercourse, and injecting drug users among others, which could be the reason for the co-infections observed in the previous studies in sub-Sahara Africa. In Kenya, the documented data from the studies conducted indicate that Nyanza recorded the highest HIV prevalence at 15.1% with Siaya County at 17.8% of the total national population. The invention of Highly Active Antiretroviral Therapy was thought to reduce morbidity and mortality rate of HIV patients, however, HIV patients continue to suffer from liver related illness due to the co-infections with hepatitis B and C viruses, in addition to the emergence of drug resistant strains. Despite this there is scarcity of information on hepatitis B and C co-infections, circulating virus genotypes and drug resistant strains in Kenya. This study evaluated the co-infections of viral hepatitis B/C and genetic diversity and drug resistance of HBV among HIV infected individuals in Siaya County. Approval to conduct this study was sought from Kenyatta University Research and Ethical Review Committee and SCRH Institutional review Committee. This was a hospital based cross-sectional study in which a total of 225 blood samples were aseptically collected from consenting participants. The blood samples were separated and plasma used for serological assays. Serological detection of HBsAg and anti-HCV IgM was performed using On Site Rapid Test Kits as prescribed by the manufacture (CTK Biotech, Inc, San Diego, USA).Viral DNA was extracted from positive HBsAg plasma samples using Qiamp$^{TM}$DNA Mini kit as per the manufactures' instructions. HBV-*pol* gene was amplified by nested PCR using specific primers and the amplicons directly sequenced by automated ABI 377 DNA sequencer (Applied Biosystem, Foster City, USA) using BigDye Terminator Kit (Applied Biosystem®). Generated sequences were phylogenetically analyzed together with references sequences using Molecular Evolutionary Genetics Analysis (MEGA X version 10.0.4) software. Of (225) individuals who participated in this study, 157(69.8%) were female and 68(30.2%) were males. Their ages were ranged between 3 and 76 years with mean of 38.26 years. Majority of the participants were married (146/225) with most of them having secondary education level (116/225). Gender, age and level of education were not significantly associated with HBV infection. However, place of residence was associated with HBV infection. In addition, only gender and marital status were significantly associated with HCV infections. Overall prevalence for HBV/HIV was 6.2% (14/225); HCV/HIV was 4.0% (9/225) while that of HIV mono-infection was 89.8% (202/225). Nevertheless, none of the study participants was infected with all the three viruses. HBV drug resistance mutation rt169F was detected in one participant. However, the rest of the 10 Individuals were infected with HBV drug susceptible strains. Of the 11 samples that were successfully sequenced, the phylogenetic analysis revealed the sequences belonged to HBV genotype A1. The study findings reveal that the levels of HBV/HIV and HIV/HCV co-infections could be higher than reported here with circulating strains remaining susceptible to treatment. There is therefore a need for continuous surveillance of HBV, HCV infections and monitoring circulating trends of these viral genotypes and drug resistance in this region in order to guide vaccine design and optimize treatment.

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# CHAPTER ONE

# INTRODUCTION

## 1.1 Background Information

One of the greatest health challenges globally remains to be Human Immunodeficiency Virus (HIV) since its advent with some countries including Kenya having declared it a national disaster by 1999 (Ndambuki, 2006). Globally it is estimated that about 36.9 million people are living with HIV as of 2017 with approximately 1.8 million people reported to be newly infected yearly (UNAIDS, 2018). In sub-Sahara Africa HIV infection estimation stands at 25.6 million people and about 16% new infection in 2017.Worldwide 35.4 million people are estimated to have died from AIDS-related illness with 1.3 million deaths in the year 2017 (UNAIDS, 2018).

In Kenya, HIV/AIDS still remains a serious health problem and the prevalence of the infection is estimated on the regional basis with Nyanza region recording the highest prevalence of 15.1% (National AIDS Control Council, 2014). HIV/AIDS predispose the infected individuals to a variety of opportunistic infections due to the immune system suppression by the HIV virus (Douek *et al*., 2009). Acute and chronic liver disease are majorly caused by viral hepatitis B and C infections, moreover, HBV/ HCV co-infected individuals develop severe liver disease (Liu and Hou, 2006).

Viral hepatitis is critically important to the global public health with an infection rate of about 2 billion people estimated to be HBV infected, 130 million people infected with HCV and about 350 - 400 million people have chronic hepatitis (Amiri *et al*., 2016). In Kenya, previous studies indicate that anti-HCV and HBsAg prevalence rate among blood donors and patients attending clinics is at <1% to 4.4% and 5-8% respectively (Ly *et al.*, 2016; Ochwoto *et al.,* 2016). The survival of individuals co-infected with HIV/HBV and/or HCV is significantly threatened

worldwide due to hastened liver disease progression, an effect attributed to higher viral load in the infected patients and drug hepatotoxicity (Wambani *et al.,* 2015). The overlapping transmission routes such as sexual intercourse, blood-blood contact and intravenous drug use, explains why viral hepatitis B/C and HIV co-infections are becoming more prevalent (Muriuki *et al*., 2013).

World health organization (2017) reported a 7.4% and 6.2% prevalence of chronic viral hepatitis B and C in patients infected with HIV respectively, majority being in Africa (WHO, 2017). Hepatitis B and C co-infections in HIV infected individuals has been found to significantly lead to high morbidity and mortality rates most especially among intravenous drug users (IDUs) (Flores *et al*., 2016).

Genetic diversity of HBV in HIV infection is of great clinical significance with previous studies having successfully identified ten HBV genotypes so far (A-J) (Webale *et al.,* 2015). In Kenya, genotype A is the predominant HBV genotype. However, genotypes D and E have been detected in coastal region (Webale *et al.,* 2015). Response to treatment, progression of hepatocellular carcinoma and liver cirrhosis (LC) significantly vary across the HBV genotypes (Sunbul, 2014).

In Kenya, the scarcely available data on HBV and HCV prevalence and hepatitis B virus genetic diversity among HIV infected individuals shows variable observations. However, Siaya County is among counties in which this data is not available. This study was therefore conducted to provide evidence based information on the co-infection prevalence of HIV, HCV and/or HBV and HBV serotypes in Siaya County. Due to financial constrain, the study focused only on HBV genotypes. The information shall be critical in developing guidelines on viral hepatitis B and C surveillance, treatment and HIV management in relation to hepatitis B and C co-infections.

**1.2 Statement of the problem**

Despite of the existing control and preventive measures, Human immunodeficiency virus (HIV) remains a scourge in Siaya County with high prevalence rate of 17.8% (National AIDS Control Council, 2014). Hepatitis B and C co-infection in HIV is increasingly becoming more common owing to the fact that they share routes of transmission. HCV and HBV infection rate among HIV-infected persons varies significantly among different HIV-infected populations with the IDUs being the highest risk population (Muriuki *et al.,* 2013). Researchers have done a number of studies with the aim of evaluating epidemiology of HIV, HCV, and HBV in various countries in Sub-Sahara Africa which bears the greatest burden of the epidemics. However, little data exist on the HCV/ HBV co-infection prevalence among individuals infected by HIV in Kenya and particularly in Siaya County.

The mortality and morbidity rate among people living with HIV (PLHIV) in Kenya has reduced greatly following the invention and introduction of antiretroviral drugs allowing the appearance of liver associated complications due to HBV and HCV infections. Liver infections related to HCV and HBV are accelerated to chronic liver diseases in presence of HIV infection (Ranjbar *et al.,* 2011). In addition, the emergence of HBV drug resistant strains now threatens the survival of HIV patients co-infected with HBV as a result of treatment failure (Mabeya *et al.,* 2017). Moreover, HBV and HCV co-infections among HIV-infected individuals is becoming rampant (Ranjbar *et al.*, 2011).

The detected Hepatitis B virus strains in Kenya include strains A, D and E with strain A being the most predominant strain (Mwangi *et al.,* 2008). However, the information on the circulating hepatitis B virus genotypes and subtypes in Siaya County remains elusive. The data collected from the previous studies suggest that Nyanza region has the highest HIV prevalence rate in

Kenya (National AIDS Control Council, 2014). Therefore, it is important to conduct the study in order to examine the extent of HCV and HBV co-infection as well as HBV genetic diversity and drug resistance in HIV-infected individuals taking antiretroviral drugs in Siaya County.

### 1.3 Justification of the study

Hepatitis B and C have similar routes of transmission and risk factors with HIV yet routine screening of HBV and HCV in HIV patients is still a challenge in resource limited nations. HIV/HBV and/or HCV co-infection have been found to increase morbidity and mortality rate as compared to mono-infection with any of the three viruses (Muriuki *et al.*, 2013). The increased morbidity and mortality in co-infections is associated with hepatotoxicity due to drugs, HBV reactivation, higher viral load and rapid progression to advanced liver disease (Wambani *et al.*, 2015).

So far, there are no documented studies on extent and strains of hepatitis B and C co-infections among people living with HIV in Siaya County despite the fact that HIV prevalence rate is high (17.8%) in the county (National AIDS Control Council, 2014). With the advent of antiretroviral drugs, many human immunodeficiency virus-infected individuals now survive longer, thus chronic HCV and HBV infections are now becoming the greatest cause of death following AIDS-related complications, among HIV-infected patients (Ubajaka *et al.*, 2015). In Kenya, many people are unaware of the epidemics and more significantly in HIV-infected individuals. Therefore, this study shall provide evidence-based data to allow for estimation of the extent of the co-infection of HCV/HBV; genetic diversity of HBV as well as HBV drug resistance among HIV infected individuals in Siaya County in order to optimize treatment.

## 1.4 Research question

i. What is the extent of viral hepatitis B/C co-infections among HIV patients attending HIV comprehensive clinic of Siaya County Referral hospital?

ii. What is the genetic diversity of hepatitis B virus in HIV patients attending HIV comprehensive clinic of Siaya County Referral hospital?

iii. What are the HBV drug resistance mutations among HBV/HIV co-infected individuals attending comprehensive HIV clinic of Siaya County Referral hospital?

## 1.5 Objectives

### 1.5.1 General Objective

To determine the co-infections prevalence rates of HCV, HBV, genetic diversity and drug resistance of HBV among HIV-infected individuals attending HIV comprehensive clinic of Siaya County Referral hospital.

### 1.5.2 Specific Objectives

i. To determine the prevalence of hepatitis B and C viruses in HIV-infected individuals attending comprehensive HIV clinic of Siaya County Referral hospital.

ii. To determine the genetic diversity of Hepatitis B virus (HBV) among HIV infected individuals attending comprehensive HIV clinic of Siaya County Referral hospital.

iii. To determine HBV drug resistance among HBV/HIV co-infected individuals attending comprehensive HIV clinic of Siaya County Referral hospital.

### 1.6 Significance of the study

The study shall provide more information regarding the co-infection rate of HCV/HIV, HBV/HIV, HIV/HBV/HCV, and HBV strains and drug resistance among individuals infected with HIV in the County. The findings of the study shall provide concrete data for formulation of guidelines for management, prevention as well as strategies for treatment of HIV in relation to viral hepatitis B and/or C co-infections which shall go along with planning health programmes in the county. The findings of the study shall also help in the documentation of the epidemic to aid the development of public health intervention such as viral hepatitis surveillance in Siaya County.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Epidemiology of Hepatitis B, C and HIV co-infections

Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) has been described as one of the devastating disease to human (UNAIDS, 2000). Currently there is no cure for HIV; however, with better access to antiretroviral therapy (ART) now readily available the survival rate for HIV patients have been increased. Studies reveal that with 100% drug adherence the viral load can significantly be reduced and hence increased life expectancy (Bhatti *et al*., 2016). In spite of antiretroviral therapy HIV infection remains a scourge with ever expanding prevalence worldwide prompting accelerated morbidity and mortality among HIV infected patients especially in developing countries (Bhatti *et al.,* 2016).

Globally an estimated population of people infected with HIV stood at 36.9 million in 2017 and approximately 1.8 million people reported to be newly infected with HIV worldwide in the same year (UNAIDS, 2018). The global prevalence ranges between <1% in about 91 countries and >10% in 8 countries (Kates, 2018). The infection is so severe in Africa such that in every 25 adults 1(4%) is most likely to be HIV infected and this account for about two-thirds of the global HIV infection (Mayer, 2005). HIV/AIDS still remains a major health challenge globally, with sub-Sahara Africa bearing the largest HIV epidemic at an estimated number of 25.6 million people living with HIV and about 16% new infection in 2017 (UNAIDS, 2018).

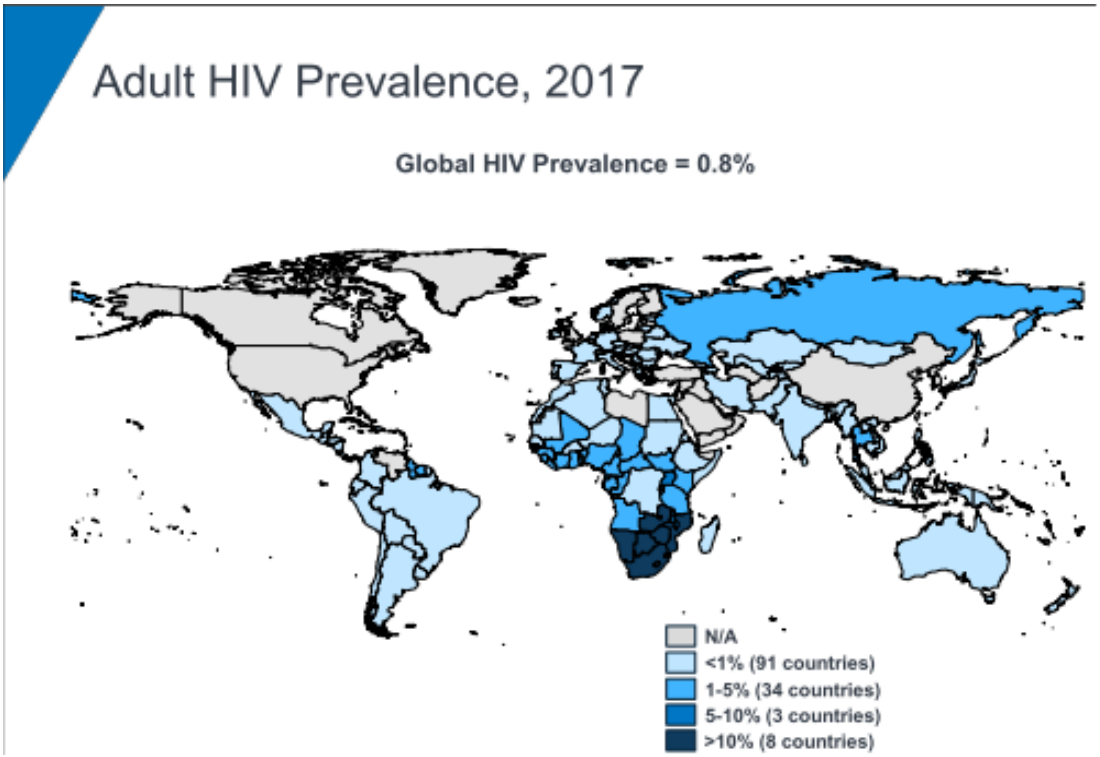


**Figure 2.1:Global prevalence of HIV/AIDS among Adults (15-49) (Kates, 2018)**

In Kenya HIV epidemic was first diagnosed in 1984 and since then it has remained the major cause of mortality. The prevalence reached a peak of 10.5% between 1995 and 1996. Currently, the epidemic has a prevalence range between 5.6% to 6.7% (MOH, 2015). However, in Nyanza and precisely Siaya County the prevalence of the epidemic still remains relatively high. HIV prevalence data is documented on county basis in the country with Homabay (27.1%), Kisumu (18.7%) and Siaya (17.8%) counties leading whereas Tana River (2%), Mandera (1.3%) and Lamu (1.3%) counties showing relatively low prevalence rates (National AIDS Control Council, 2014). Despite several measures that the Kenyan government has put in place to curb the epidemic Nyanza region and more particularly Siaya County continues to experience high HIV prevalence. Therefore, there is need to determine the prevalence of the associated viruses (HBV and HCV) in the County for better clinical management of HIV patients and to develop appropriate preventive measures in the County.

Hepatitis B virus is known to be one of the infectious viruses responsible for acute and chronic hepatitis with about 35% to 40% perinatal transmission of all new cases of infection globally (Zamani *et al*., 2001). Despite the successful introduction of HBV vaccine, new cases of infection is still on the rise more so in HIV infected persons (Thimm *et al*., 2005). The virus (HBV) still remains of major health concern worldwide and it is estimated to have infected about 2 billion people globally and a further 350 million chronic cases translating to about 600,000 deaths each year (Ranjbar *et al.,* 2011). Hepatitis B virus infection is of significant risk factor for deaths associated with liver cirrhosis and liver cancer (Ott *et al*., 2012). The HBV prevalence rate ranges from 0.5-2%, 2-7% and ≥8% in low, intermediate and high endemicity world regions respectively (Jinlin *et al*., 2005; Maclachlan and Cowie, 2015). North America, Europe, Asia and Japan are considered low endemic regions and they make up to 12% of the world population whereas intermediate endemic world regions include Latin America, East and South Europe, Middle East, South Asia and North Africa that constitute about 40% of the global population (Maclachlan and Cowie, 2015). Sub-Saharan Africa, Kenya inclusive is designated as a high endemic area with 70% to 95% of the total population showing present or past HBV infection serological evidence (Franco *et al*., 2012).

The previous studies conducted in Kenya have consistently reported inconsistent HBV prevalence across the country and mainly urban settings. The reported prevalence ranges between 5% and 8% and in some cases as high as 50.6%. A number of these studies are conducted among high risk population such as injecting drug users, commercial sex workers and men who have sex with men as well as jaundice and HIV patients (Muriuki *et al.,* 2013; Webale *et al.,* 2015; Mabeya *et al.,* 2016; Ochwoto *et al.,* 2016).


**Figure 2.2: Worldwide prevalence of hepatitis B virus infection (Maclachlan and Cowie, 2015)**

Hepatitis C virus is a global leading cause of liver cirrhosis and hepatocellular carcinoma as well as liver transplant, unlike hepatitis B virus and hepatitis A virus, hepatitis C virus vaccine is unavailable (Halliday *et al.,* 2017). Hepatitis C virus is known to be a prevalent pathogen worldwide causing morbidity and mortality. Persistent HCV infection is connected to rapid progression to cirrhosis, hepatocellular carcinoma, liver failure and death, furthermore, HCV is now the major cause of death among HIV patients on highly active antiretroviral therapy (HAART) (Messina *et al.,* 2015). HCV shares similar routes of transmission with HIV and HBV, however, HCV is less transmitted by a single unit of exposure to potential risk factor (Bruguera and Tapias, 2000). Little data exist on HCV prevalence and as such the global prevalence is based on regional estimates considering only a number of countries (Petruzziello *et al.,*2016). The global prevalence of HCV is estimated at 2% - 3% (130 - 170 million) people infected with HCV, however, the epidemiology varies throughout the world which ranges from < 1% to > 10% prevalence rates (Averhoff *et al*., 2012). In Kenya the research done so far reveals disparity in

the prevalence of HBsAg and anti-HCV infections with Kerubo, *(*2015) reporting the prevalence of 13.3% and 0.7% respectively, whereas Muruiki *et al*., (2013) reporting the prevalence of HBsAg and anti-HCV as 6% and 10.3% respectively. Ochwoto *et al.* (2016) in their study reported the HCV prevalence ranging from <1% to 4.4%.

**Table 2.1: Regional prevalence of HCV and Viraemic rate (Petruzziello *et al.*, 2016)**

| Regions | Anti-HCV prevalence (%) | Viraemic rate (%) |
|---|---|---|
| Central Sub-Saharan Africa | 6.0 | 68.5 |
| EastSub-Saharan Africa | 2.4 | 65.0 |
| Southern Sub-Saharan Africa | 0.9 | 69.0 |
| WestSub-Saharan Africa | 2.4 | 79.6 |
| North Africa and Middle East | 2.7 | 68.8 |
| North America, High Income | 1.2 | 75.7 |
| Caribbean | 1.5 | 70.0 |
| Andean Latin America | 1.2 | 70.0 |
| Central Latin America | 1.4 | 75.8 |
| Southern Latin America | 1.5 | 79.5 |
| Tropical Latin America | 1.6 | 80.2 |
| Central Asia | 5.8 | 48.7 |
| East Asia | 2.8 | 63.6 |
| Pacific Asia, High-income | 1.1 | 70.5 |
| South Asia | 2.5 | 78.5 |
| Southeast Asia | 1.6 | 60.5 |
| Australasia | 1.8 | 74.8 |
| Europe, Central | 1.3 | 76.6 |
| Europe, Eastern | 3.1 | 69.6 |
| Europe, Western | 0.9 | 71.0 |

Dual or tri-infection of HIV/HBV and/or HCV is significantly common due to the shared routes of transmissions including sexual, perinatal (carrier mother to the baby), parenteral (tattooing, injecting drug use and blood transfusion) (Jinlin. *et al.*, 2005). The co-infection prevalence of HBV and HCV in persons infected with HIV varies significantly among different HIV-infected populations with men who have sex with men, intravenous drug users (IDUs) and commercial sex workers (CSW) as the highly affected risk populations (Flores *et al*., 2016). Globally, an

approximated population of two-four million HIV infected patients shows chronic hepatitis B co-infection and about 4-5 million are HCV infected, although the prevalence rate varies subject to the region with time. Viral hepatitis B,C and HIV endemicity are high in Africa with variable prevalence rate among different African countries (Kerubo *et al*., 2015). The previous studies conducted in some Sub-Sahara African countries detected HBV/HIV co-infection prevalence range between < 2% and 25% whereas that of anti-HCV ranges between 0.1% and 13.6% (Zampino *et al.,* 2015; Obadahn and Kamal, 2017). However, triple co-infection prevalence of HBV/HCV/HIV in Sub-Sahara Africa as well as Kenya remain largely below 1% (Muriuki *et al.*, 2013; Kerubo *et al.,* 2015).

**2.2 Risk factors associated with Hepatitis B, C and HIV co-infections**

These three blood borne viruses share similar routes of transmission and hence may also be sharing common risk factors for infection (Muriuki *et al.,* 2013; Kerubo *et al.,* 2015). Some of the risk factors associated with hepatitis B, C and HIV infections that have been reported in previous studies include age, sex, surgical history, multiple sexual partners, intravenous drug use, blood transfusion, tattooing, marital status and history of hepatitis among others (Ashraf *et al.,* 2010; Kerubo *et al.,* 2015; Jacqueline *et al.*,2016; Dabsu, 2018). Risk factors for Hepatitis B, C and HIV infections may also vary based on socioeconomic status, education and sociocultural variables of the study populations (Joukar *et al.,* 2018). However, it is important to note that previous studies conducted in different parts of the world have reported inconsistent association of the above named risk factors with HBV, HCV and HIV co-infections. The observed inconsistency could be associated with level of awareness of the three infectious viruses among the general population, health workers and government health policies such as HBV vaccination in various study populations (Ashraf *et al.,* 2010; Joukar *et al.,* 2018).

### 2.3 Impact of HIV on Viral Hepatitis B and C disease progression

The previous studies reveal that the concurrent infection with viral hepatitis B, C and HIV seems to influence the natural history of the other and that co-infections are common owing to the fact that the three viruses share similar routes of transmission (Franco *et al.,* 2012). HIV patients shows rapid liver disease progression in presence of HBV or HCV co-infection, an effect attributed to the HIV suppression of the immune system of the patient (Ranjbar *et al.,* 2011). The HBV clearance rate is at 90% for an immune competent individual, however, in presence of HIV, the risk of HBV reactivation is high which may cause chronic infections (Ubajaka *et al.,* 2015). HBV/HIV co-infection increases HBV DNA concentration; reduces liver enzyme level and low clearance of HBeAg. Coupled with the increase in HBV replication as well as poor inflammatory response to chronic HBV, the end result is that the individuals co-infected with HBV/HIV have increased development of liver disease (Ranjbar *et al.,* 2011). Furthermore studies have revealed that men co-infected with HIV-HBV are at risk of death 17 times more than men mono-infected with HBV due to liver related diseases (Chakravarty, 2015). In addition HIV infection significantly modifies the natural history of HBV resulting into high rate of occult HBV as compared to HBV mono-infected patients (Klein *et al.,* 2011).

As stated earlier, simultaneous infection with HCV and HIV is common as a result of the common transmission routes. Hepatitis C virus replication increases considerably in HCV-HIV co-infection thus resulting in increased level of serum and liver HCV RNA. This decreases the response to HCV antiviral therapy and hence accelerating development of liver infection in the affected individuals (Lin and Scott, 2012). Studies have also shown that comparatively HIV-HCV dual infected patients are three times at risk of developing cirrhosis or advanced liver disease than HCV mono-infected patients (Thimme *et al.,* 2005).

**2.4 Impact of Viral hepatitis B and C on HIV disease progression**

Some studies suggested that Hepatitis B virus and HIV co-infection leads to faster progression to AIDS, although the current studies have found no significant evidence to correlate HBV-infection with HIV progression (Rosemary, 2008). Thus, the effect of HBV on natural history of HIV remains elusive. Nevertheless, the study of HBV/HIV co-infected cohort group revealed a slower response to HAART during the first few weeks as compared to HIV mono-infected patients (Idoko *et al.,* 2010; Ubajaka *et al.,* 2015). On the same note, the impact of HCV co-infection on HIV disease progression still remains oblique with conflicting information from the studies conducted so far. The Swiss HIV cohort study reported a rapid progression to AIDS with reduced survival rate among HCV co-infected patients, while the United States HIV cohort study found an insignificant correlation between HCV- infection, survival and progression to AIDS in patients infected with HCV (Cheng *et al*., 2007).

However, HIV mono-infected patients experiences up to over 5700 copies/ml decrease in HIV viral load averagely with an average increase in CD4 cell count of 111cells/$mm^3$ contrary to patients with double infection of HIV-HCV who experiences low decrease in HIV viral load of 606 copies/ml and about 53cells/$mm^3$ increase in CD4 cell count (Ubajaka *et al.*, 2015).

**2.5 Viral Hepatitis B and C Serological Markers**

Understanding the sero-markers for both hepatitis B and C viruses is of great importance. This will go along with assessing the clinical significance of the infection. Hepatitis B virus is characterized by five Serological markers. In screening of HBV infection, it is recommended that all the five serological markers are screened so as to assess the phase of infection (Webale *et al.*, 2015). The presence of hepatitis B surface antigen (HBsAg) in the serum during the first four to ten weeks defines an acute infection; however, HBsAg persistence in the serum for over six

months defines chronic infection (Odimayo *et al.,* 2016). The hepatitis B surface antibody (HBsAb) detection in the serum presents a successful immune response or successful vaccination against hepatitis B virus, whereas Hepatitis B core antibody (HBcAb) is not a protective antibody and remains in the serum for life following exposure to HBV hence a good measure of previous or ongoing HBV infection (Odimayo *et al.*, 2016). The presence of hepatitis B envelop antigen (HBeAg) in the blood sample is an indicator of active viral replication and it defines high infectivity. Just like HBcAb, hepatitis B pre-core antibody (HBeAb) is not a protective antibody but its presence shows low viral replication defining end-point of treatment (Webale *et al.*, 2015). The HCV serological marker is the antibody to HCV (anti-HCV) where the detection in the serum may indicate the previous exposure or current infection and as such do not confirm the current infection.

**2.6 Hepatitis B Virus Structure and Genomic organization**

Hepatitis B virus (HBV) is one of the smallest known animal viruses with a Dane diameter of 42nm (Cento *et al.,* 2013). The virus is an enveloped DNA virus of genus *Orthohepadna* virus belonging to the *Hepadnaviridae* family (Stuyver *et al.,* 2000). The Dane particle (virion) is made of lipid (outer envelope) and a protein made icosahedral nucleocapsid core. The nucleocapsid functions to enclose the viral DNA and a DNA polymerase responsible for reverse transcriptase. The outer envelope of the virus is surrounded with vital proteins for binding and entry into the susceptible host cells (Stuyver *et al.,* 2000). The three structurally related envelope proteins (Figure 2.3), small (S), middle (M) and large (L) respectively holds the main viral antigenic domains.

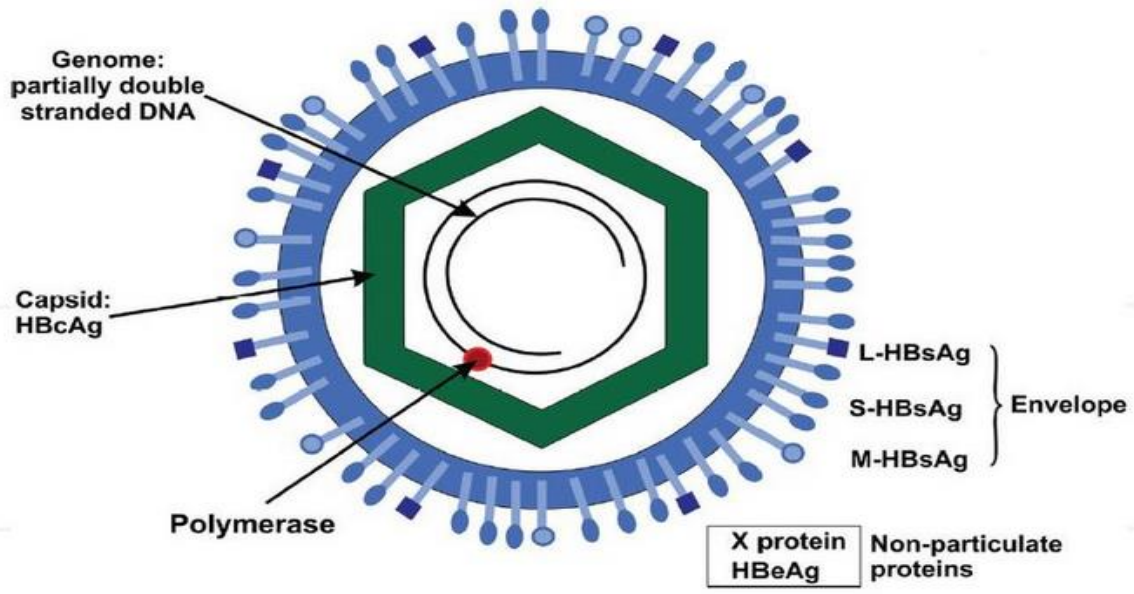


**Figure 2.3: Morphological structure of hepatitis B Virus (Datta *et al.*, 2012)**

The genome holds a relaxed, circular; incompletely-double stranded DNA (rcDNA) that replicates by reverse transcription of RNA intermediate, the genomic RNA (pgRNA). Genomic length ranges between 3182bp to 3248bp depending on the genotype (Cento *et al.*, 2013). The genome is constituted into four overlapping reading frames (ORFs) encoding the core/precore (C ORF), polymerase (*pol* ORF), envelope (S ORF) and X ORF that are expressed into viral core protein, reverse transcriptase (RT) or polymerase, surface protein and HBx protein respectively (Caligiuri *et al.*, 2016; Mabeya *et al.*, 2016). The PreS/S ORF overlaps fully with the Pol ORF while Pre-core/Core and XORF overlap partially with the Pol ORF (Pollicino *et al.*, 2014).

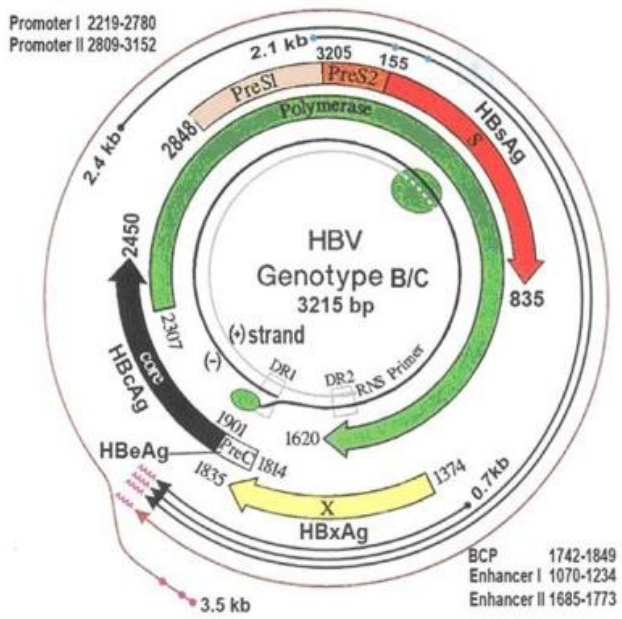


**Figure 2.4: Organization of Hepatitis B virus genome ( Lin and Kao, 2015)**

## 2.7 Genetic Diversity of Hepatitis B Virus

Hepatitis B virus is globally distributed and is endemic in many parts of the world with sub-Sahara Africa designated as hyper-endemic region of the virus. Acute and chronic hepatitis is majorly caused by hepatitis B virus more significantly in Asia and Africa which are considered hyper endemic regions for HBV (Sunbul, 2014; Wambani *et al.,* 2015; Onyango *et al.,* 2018). Hepatitis B virus genotypes are classified based on the genomic sequences and antigenic properties of surface antigen (HBsAg) are used to further classify HBV into subtypes (Xue *et al.,* 2017). The previous studies have successfully identified ten HBV genotypes (A-J) based on sequence variation greater than 8% in the whole HBV genome and over 30 sub-genotypes with 4% to 8% nucleotide variation distributed along geographical lines worldwide (Sunbul, 2014). Some of the identified sub-genotypes include sub-genotype A1 to A7, B1 to B9, C1 to C16, D1

to D9, F1 to F4 and I1 to I2, however, sub-genotypes of G, H and J are not yet discovered to date (Kramvis, 2014; Sunbul, 2014).

Genotypes A, G, F and H are found to be predominant in North America, whereas genotype F and H are predominantly found in South America (Tanaka *et al.*, 2007; Sunbul, 2014; Velkov *et al.*, 2018). The most prevalent HBV genotypes in Europe has been found to be genotype A, B, C, D and G with seven HBV-D sub-genotypes (D1-D7) in circulation (Gabanelli *et al.*, 2012; Velkov *et al.*, 2018). In Asia the prevalent Hepatitis B Virus genotypes are genotype B and C with HBV-B expressing two sub-genotypes B1 and B2, however, HBV sub-genotypes A, D, C, I and J are also common in some parts of Asia (Mohebbi *et al.*, 2008; Kramvis, 2014; Velkov *et al.*, 2018). In Africa HBV genotypes are distributed such that strain A, D and E are the most prevalent genotypes in Africa (Kramvis *et al.*, 2007; Kramvis, 2014; Sunbul, 2014; Webale *et al.*, 2015; Velkov *et al.*, 2018). In East Africa, HBV genotype A is the predominant genotype with circulating sub-genotype A1 (Forbi *et al.*, 2015; Velkov *et al.*, 2018). According to Webale and colleagues (2015) in Kenya, genotype A is the most prevalent genotype with sub-genotypes A1 and A2 being present, however, genotypes D and E have also been detected, though are less prevalent (Mwangi *et al.*, 2008; Webale *et al.*, 2015). **(Table 2.2)**

**Table 2.2: Worldwide distribution of Hepatitis B virus Genotypes and Subtypes ( Lin and Kao, 2015)**

| Genotypes | Serotypes | Subtypes | Geographic location |
|---|---|---|---|
| A | adw | A1 | Sub-Saharan Africa and India |
| | | A2 | Northern Europe and India |
| | | A3 | Western Africa |
| B | adw, ayw | B1 | Japan |
| | | B2–5 | East Asia, Taiwan, China, Indonesia, Vietnam, and the Philippines |
| | | B6 | Alaska, Northern Canada, and Greenland |
| C | adw, ayr, adr | C1–3 | Taiwan, China, Korea, and Southeast Asia |
| | | C4 | Australia |
| | | C5 | The Philippines and Vietnam |
| | | C6–11 | Indonesia |
| D | ayw | D1–6 | Africa, Europe, Mediterranean countries, India, and Indonesia |
| E | ayw | | Restricted to West Africa |
| F | adw | F1–4 | Central and South America |
| G | adw | | France, Germany, and the United States |
| H | adw | | Central America |
| I | adw | | Vietnam and Laos |
| J | | | Japan |

Nevertheless, the information on genetic diversity of HBV in Kenya remain little despite the fact that Kenya is considered as one of the countries with high HBV infection prevalence in Africa (Mwangi *et al*., 2008). In Siaya County the data on HBV strains among HIV infected patients is lacking. Therefore this study aimed at determining the HBV serotypes among HIV infected persons in Siaya County.

### 2.8 Hepatitis B Virus Pathogenesis

Viral hepatitis is a liver inflammatory disease of varied severity characterized by prolonged HBV infection and can gradually progress to cirrhosis and hepatocellular carcinoma (HCC) due to poor adaptive immune response (Chirasi, 2011). The body fluids such as blood, semen, vaginal secretion and saliva are some of the carriers of HBV infection; however, blood is the major

carrier of HBV infection (Mabeya *et al.,* 2016). However, Vertical and horizontal transmission are the major routes of infection in high endemic zones whereas unprotected sexual intercourse and sharing of unsterilized syringes among injecting drug users (IDUs) remains the major routes of transmission in regions of low endemicity ( Muriuki *et al.,* 2013; Mabeya *et al.,* 2016). Blood transfusion of infected or unscreened blood, tattooing and usage of unsterilized instruments are also other known risk factors for HBV infection (Mabeya *et al.,* 2016). Hepatitis B virus then enters the susceptible host cell with the aid of viral binding proteins producing a variety of clinical outcome in different HBV infected patients ranging from asymptomatic to chronic hepatitis diseases (Stuyver *et al.,* 2000; Mabeya *et al.,* 2016). Hepatitis B virus infection is more severe in patients co-infected with HIV as opposed to immune competent HBV mono-infected patients whose HBV clearance is estimated at 90% (Ubajaka *et al.,* 2015). It is also observed that HBV persistent infection in infected infants at birth is at 90% and reduces to 20 to 50% of infection occurring between the ages of 1-5 years and is much reduced to less than 5% of infection occurring during adult ages ( Mabeya *et al.,* 2016).

### 2.8.1 Natural history of HBV Infection

Hepatitis B virus infection is known to cause acute and chronic hepatitis leading to a wide range of liver diseases including cirrhosis, hepatocellular carcinoma and liver cancer (Liang, 2009). Acute HBV infection is usually asymptomatic in most patients and as such remains undetected; however, few cases develop clinical symptoms ranging from mild fatigue and nausea to jaundice and occasionally liver failure and even death, during which the levels of alanine aminotransferase (ALT), HBeAg and HBV DNA considerably rises in the serum and this may persist for some weeks (Zhang *et al.,* 2016).

Hepatitis B virus infection in infants and children <5 years have high degree of developing chronic hepatitis compared to adults. About 90% to 95% of acute hepatitis B virus infection in adults is cleared with only about 5% developing to chronic hepatitis (Thimme *et al.,* 2005; Zhang *et al.,* 2016). Chronic HBV infection is classified into immune tolerance, immune clearance, inactive and reactivation phases. Immune tolerant is characterized by HBeAg serum positivity, normal levels of ALT and increased HBV DNA level in serum (> 20,000,000IU/Ml) common with infected infants through perinatal transmission from mothers positive for HBeAg and this can last for years or even decades (Kim *et al.,* 2011). However, infected child with genotype A1, E, D, A2, or D1 from Africa or Western Europe where these genotypes are prevalent, this phase is much shortened during which the risk of developing cirrhosis and hepatocellular carcinoma is greatly reduced although presence of high level HBV DNA for several years may accelerate chances of HCC with time (Kim *et al.,* 2011).

Infection occurring during childhood or adulthood may progress to immune clearance phase soon after exposure and this associated with increased ALT and HBV DNA levels in serum and liver inflammation. This is preceded by seroconversion of HBeAg to anti-HBe, normalized ALT level and reduced HBV DNA load to undetectable level in serum. Interestingly studies shows that activities of immune clearance phase are HBV genotype dependent with patients infected with HBV genotype B in Asia experiencing higher HBeAg seroconversion as opposed to genotype C and D infected patients (Mohebbi *et al.,* 2008; Kim *et al.,* 2011; Zhang *et al.,* 2016).

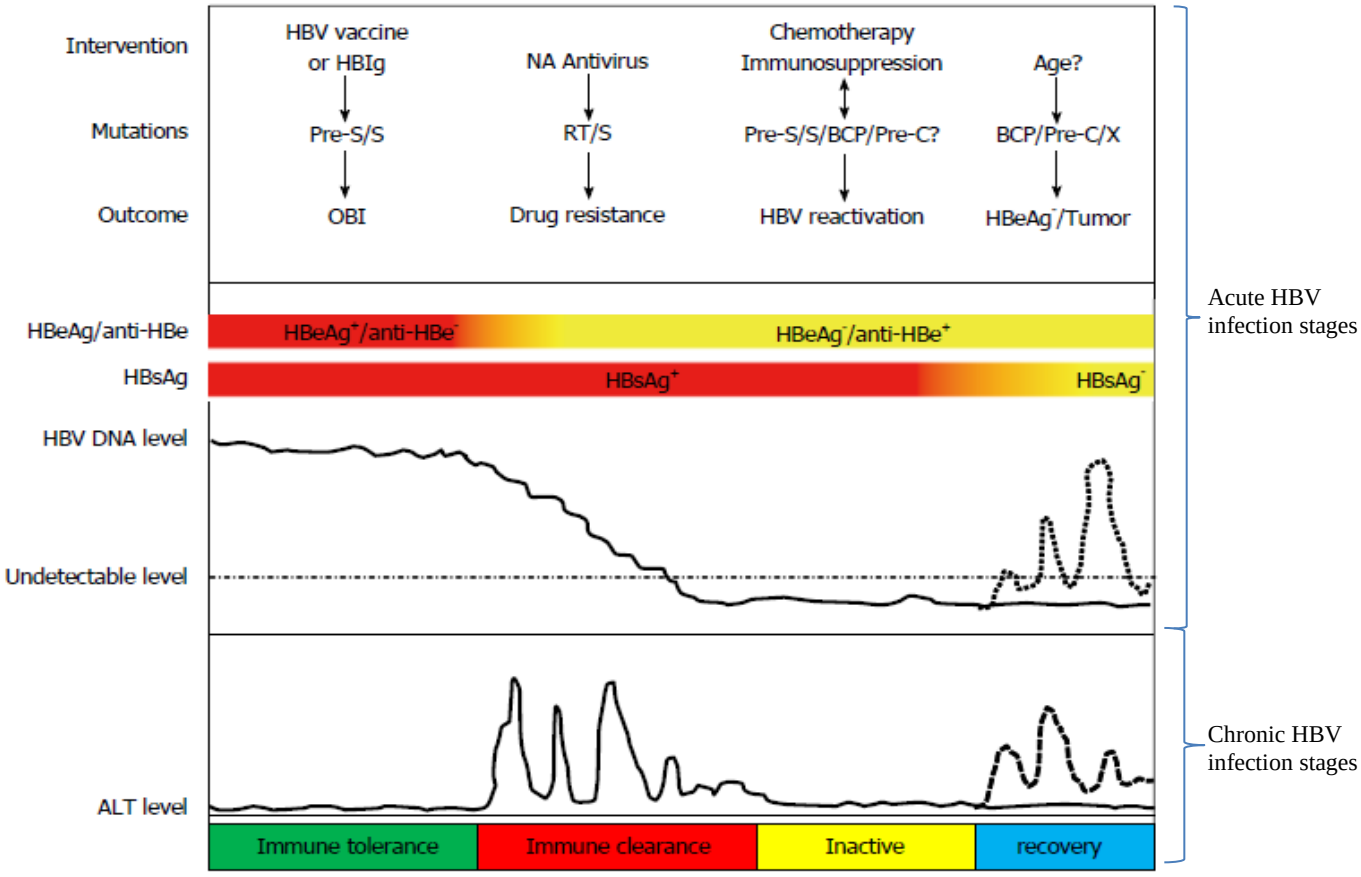


**Figure 2.5: Natural history of Hepatitis B infection (Zhang *et al.*, 2016)**

The successful HBeAg seroconversion stimulates the beginning of inactive phase distinguished by anti-HBs and HBeAg positivity and negativity respectively; low HBV DNA levels, constant ALT levels and minimal fibrosis. Most HBV infected patients remain in this phase almost their entire life with few exemptions that may develop reactivation occasioning reactivation phase (Kim *et al.*, 2011; Lin *et al.*, 2015; Zhang *et al.*, 2016). Reactivation phase is equally depicted by high levels of serum HBV DNA, anti-HBe positivity, HBeAg negativity and increased ALT levels. Reactivation phase has been partly attributed to HBV genotypes and reactivation cases reported in Europe, Mediterranean and parts of Africa are largely attributed to HBV-D the prevalent genotype in the said regions and is known for precore mutation (Kim *et al.*, 2011).

**2.9 Hepatitis C Virus structure and Genomic organization**

Hepatitis C virus (HCV) is an enveloped RNA virus of *Hepacivirus* genus belonging to the *Flaviviridae* family whose members present a common basic and virological features and a diameter of about 55 to 65nm (Cisneros, 2014; Kalinina and Dmitriev, 2015; Shi and Suzuki, 2018). The envelope is made of a lipid bilayer anchoring two or even more envelope proteins. The nucleocapsid encloses a number of copies of small basic proteins (Core or C) containing the viral RNA genome (Ashfaq *et al.*, 2011; Kalinina and Dmitriev, 2015). In addition core protein is an activator of transcription and is involved in both transportation of viral particle during entry to host cell and virion assembly whereas the envelope glycoprotein E1 and E2 facilitate binding process to the receptor sites during entry into the hepatocytes (Kalinina and Dmitriev, 2015).

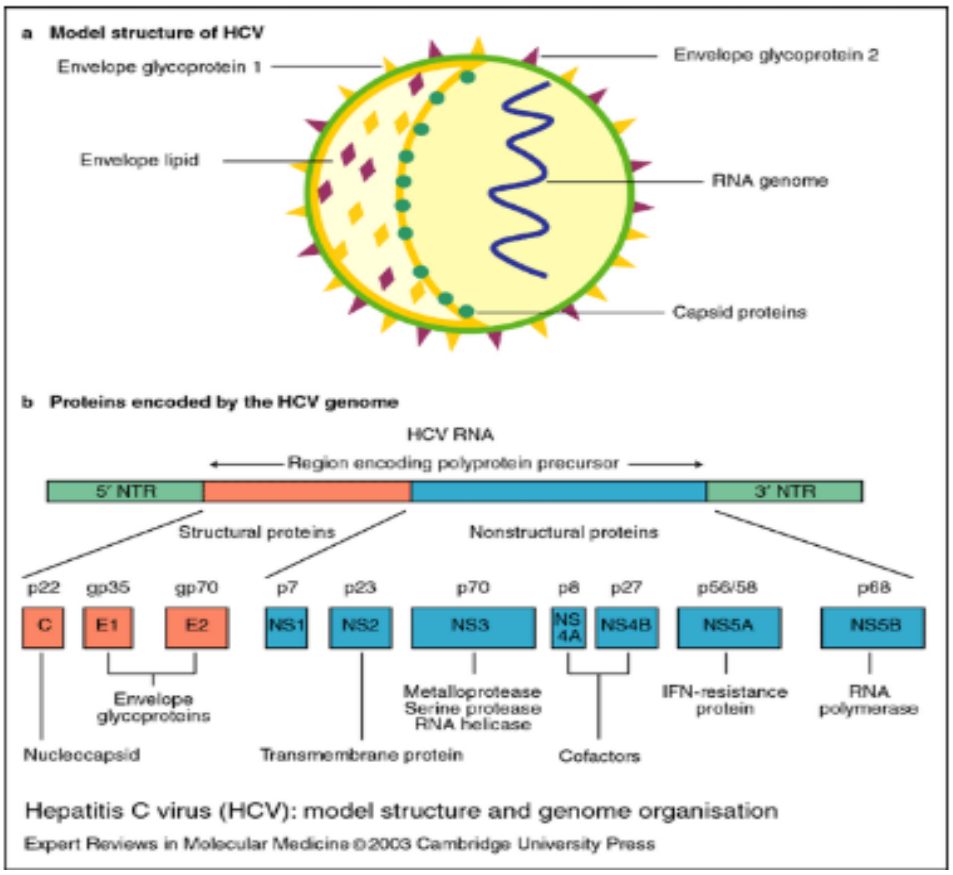


**Figure 2.6: Hepatitis C virus morphology and genome organization (Cisneros, 2014)**

The genome of hepatitis C virus consist of a plus single stranded RNA with genomic length of about 9600 nucleotides (Kalinina and Dmitriev, 2015). The genome is made of a single long open reading frame (ORF) encoding a polypeptide protein that is cleaved into ten other proteins with the first three structural proteins being core, E1 and E2 and the replication complex is formed by the other remaining five including NS3 (helicase/protease), NS4A, NS4B, NS5A and NS5B (RNA-dependent RNA-polymerase) (Cisneros, 2014; Pirakitikulr *et al.*, 2016).

### 2.9.1 Genetic diversity of HCV

Hepatitis C virus is prevalent across the world and is one of the leading causes of death and morbidity worldwide as a result of liver related diseases. The previous studies have revealed

seven different HCV genotypes (1-7) based on phylogenetic and sequence analysis of whole genome with more than 70 sub-genotype distributed globally (Messina *et al.,* 2015). The genotypes differ at 30% to 35% nucleotide sites while sub-genotypes differ at 15% nucleotide sites (Petruzziello *et al.,* 2016). The global genetic diversity of HCV remains complex, however, in North America genotype 1 (G1) is the predominant genotype although genotypes 2 (G2), 3 (G3), 4 (G4) and 7 (G7) have also been reported (Messina *et al.,* 2015; Petruzziello *et al.,* 2016). Hepatitis C virus genotypes 1 (G1), 2 (G2) and 3 (G3) are prevalent in South America (Donald *et al.,* 2016; Petruzziello *et al.,* 2016). The predominant HCV genotypes in Europe include G1, G2, G3 and G4 whereas in Asia genotypes G1-G6 are prevalent (Muasya and Kulundu, 2008; Petruzziello *et al.,* 2016). The prevalent genotypes in Africa include G1- G5 with G1 and G4 being prevalent in East Africa and in Kenya the data on common genotypes remains scanty, however, genotype 1(G1a) and 4 (G4) have been detected in the previous studies conducted in Kenya (Muasya and Kulundu, 2008; Petruzziello *et al.,* 2016).

### 2.9.2 Hepatitis C virus Pathogenesis

Despite recent advances in modern antiviral treatment HCV infection is still the major cause of cirrhosis, hepatocellular carcinoma and liver failure globally (Kwon *et al.,* 2014). Hepatitis C virus is majorly transmitted through percutaneous exposure via injecting drug use as the principal mode of transmission with blood as the main carrier of HCV infection and can also be transmitted through transfusion of infected blood, however, sexual transmission still remains minimal (Liang *et al.,* 2000). Hepatitis C virus attaches to the host cell receptors through E1 and E2 binding proteins, enters the host through endocytosis and remains suspended in the cytoplasm although this remains a complex mechanism that is not yet fully understood (Zeisel *et al, 2011).* Hepatitis C virus has a cytoplasmic centered life cycle and as such do not integrate with host

genome and hence involves various indirect mechanisms for pathological outcomes (Kwon *et al.*, 2014). Hepatitis C virus is one of the main causes of chronic liver disease and infected patients show a wide range of clinical outcomes from asymptomatic to liver cancer and are largely influenced by host factors, environmental factors and genetic factors (Westbrook and Dusheiko, 2014; Kwon *et al.*, 2014; Lingala *et al.*, 2018). Nevertheless, most HCV infected patients (80%) are likely to develop chronic HCV infection and have high probability of progression to cirrhosis and hepatocellular carcinoma (Kwon *et al.*, 2014).

### 2.9.3 Natural history of HCV infection

Hepatitis C virus infection just like HBV infection is known to be one of the major end stage liver illnesses such as cirrhosis, hepatocellular carcinoma and liver cancer globally. Acute HCV is usually mild and largely remains undetected in most patients; nonetheless, in some few cases symptoms may develop in a period of 2-14 weeks after exposure which may include fever, fatigue, dark urine, abdominal pain, loss of appetite, vomiting, joint pain, nausea and jaundice during which the levels of alanine aminotransferase (ALT) rises significantly (Hajarizadeh *et al.*, 2013). The viral dynamics in acute infection is very important in diagnosis and prediction of spontaneous clearance of acute HCV infection. The spontaneous clearance of acute HCV infection is a highly synchronized interaction between the virus and the host cell which is associated with various genetic factors and can as well be reactivated after clearance (Westbrook and Dusheiko, 2014).

Natural history of chronic HCV remains elusive, however, it usually caused by persistent hepatic inflammation leading to liver cirrhosis in about 20-30% of infected patients and hepatocellular carcinoma (Lingala *et al.*, 2018). The studies reveal that about 4-24% of chronic HCV infection develops to cirrhosis after 20 years of infection while about 1% to 3% of chronic HCV infection

progress to hepatocellular carcinoma (HCC) after a period of 30 years of infection (Hajarizadeh *et al.,* 2013). The outcome of chronic hepatitis C virus is found to be influenced by a number of host, environmental, and viral factors such as age at infection, gender, race, HCV-RNA level, HCV-genotype, co-infection with HBV, alcohol and smoking among others (Westbrook and Dusheiko, 2014; Lingala *et al.,* 2018). The spontaneous resolution of chronic HCV is very uncommon although it is a possibility in few cases (Westbrook and Dusheiko, 2014).

### 2.9.4 Hepatitis B virus treatment and drug resistance

The prime aim for chronic hepatitis B therapy is to minimize the probability of rapid liver disease progression by suppressing viral replication. HIV patients shows rapid liver disease progression in presence of HBV infection, an effect attributed to the HIV suppression of the immune system of the patients (Ranjbar *et al.,* 2011). The HBV clearance rate is estimated to be 90% for an immune competent individual, however, in presence of HIV, the risk of HBV reactivation is high which may cause chronic infections (Ubajaka *et al.,* 2015). Thus chronic hepatitis B infection is more common among HBV/HIV co-infected patients a justification of high mortality observed in HBV/HIV co-infected patients as a result of HIV clinical consequences prior to the introduction of highly active antiretroviral therapy (Patassi *et al.*, 2016). Nevertheless, with the introduction of HAART, HBV/HIV co-infected patients now die out of HBV related illness due to accelerated progression of HBV chronic liver disease (Thio, 2011).

The primary treatment for chronic HBV infection is normally achieved by administering nucleotide/nucleoside analogues (NA) such as lamivudine (3TC), tenofovir (TDF), telbuvidine (LdT), entecavir (ETV) and adenofovir dipivoxil (ADV) to suppress viral replication by inhibiting HBV reverse transcriptase (RT) as they incorporate into DNA strand and initiate chain

termination (Tacke and Kroy, 2016). However, in Kenya and most developing world, due to cost and availability, the commonly administered regimen for antiretroviral therapy of human immunodeficiency virus (HIV) include stavudine, nevirapine and lamivudine, with lamivudine being the only active agent against hepatitis B virus (HBV) (Kim *et al.,* 2011). Lamivudine have been reported to have the least genetic barrier for HBV resistance and hence associated with rapid emergence of resistance when administered alone (Kim *et al.,* 2011).

Quite often, chronic HBV treatment with any single nucleotide/nucleoside analogues significantly suppresses rapid viral replication in the short-term, however, long-term treatment with these nucleoside analogues have resulted in the emergence of HBV drug resistant strains explaining the incidence of treatment failures observed in the past with lamivudine monotherapy (Mabeya *et al.,* 2017). This result in an increased viral load and liver enzyme level which may be fatal in some patients, therefore, lamivudine monotherapy for HBV treatment is not advisable (Mabeya *et al.,* 2017). The previous studies conducted in the country have reported inconsistency in their findings with regard to HBV drug resistance (Kim *et al.,* 2011; Day *et al.,* 2013; Mabeya *et al.,* 2017).

In Kenya, the ministry of health (MOH) recommends a combination of lamivudine (3TC), efavirenz (EFV), and tenofovir (TDF) for treatment of patients co-infected with HBV/HIV-1 in order to prevent the emergence of drug resistant HBV variants (MOH, 2016). The ministry further proposes immediate commencement of treatment of HBV/HIV-1 co-infected patients irrespective of their CD4 cell count or stage of liver disease to suppress rapid progression to liver cirrhosis and associated deaths (MOH, 2016). HBV drug resistance presents a significant clinical challenge as failure of nucleotide/nucleoside analogues treatment leaves limited treatment options (Shaw *et al.,* 2006). In western countries, all HIV infected patients are currently tested

for drug resistance prior to antiretroviral therapy following a reported 10% prevalence of transmission of HIV drug resistant strains (Trevin *et al.,* 2009). However, such report on transmission of HBV drug resistant strains among HIV patients remains elusive in Kenya where HBV prevalence is relatively high (Mabeya *et al.*, 2017). Therefore, it would be prudent to conduct baseline drug resistance in newly diagnosed HIV/HBV co-infected patients.

Drug resistance is associated with mutations that usually emerge during antiviral therapy with nucleotide/nucleoside analogues prompting treatment failure. Several mutations in the RT gene of HBV are associated with classical antiviral resistance whereas mutations of rt180, rtm204 and rtN236 are associated with primary resistance (Lei *et al.*, 2013). Previous studies have categorized genotypic resistance patterns in HBV polymerase into five predictable major pathways which include L-nucleoside pathway (rtM204I/V), the acyclic phosphate pathway (rtN236T), shared pathway (rt180T/V) of both L-nucleoside and acyclic phosphate pathway, ETV resistance pathway (rtL180M + rtM204 with one of rtT184, S202, or M250 residue change), multidrug resistance pathways (rtA181T + rtI123V + rtM250L) (Lei *et al.,* 2013). However, primary mutation is inseparable with secondary/ compensatory mutation thus secondary mutation could occur to restore RT activity inhibited by primary drug resistance mutation (Tacke and Kroy, 2016). The position of secondary/ compensatory mutation and drug resistance development partially differ with respect to hepatitis B virus genotypes (Tacke and Kroy, 2016).

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study Site

The study was conducted at Siaya County Referral Hospital (SCRH) in Siaya town. Siaya town is located in the western region of Kenya and 89Km/55.3 miles from Kisumu City. The facility provides the referral medical services to the neighboring Sub-counties of Rarieda, Bondo, Gem, Ugunja, and Ugenya. Siaya County has an estimated population of 751,677 as per the 2009 census (KNBS, 2010). The socioeconomic activities of the population include fishing, subsistence farming, small scale trading, livestock keeping and rice farming (Siaya County Health Management Team, 2017). Infection with HIV is a major health challenge in Siaya County with 17.8% as the estimated prevalence rate (National AIDS Control Council, 2014).

## 3.2 Study Design

This was a hospital based cross-sectional study design and it was carried out between the months of August and December, 2018.

## 3.3 Study Population

The study population consisted of HIV-infected patients attending comprehensive HIV clinic of Siaya County Referral hospital.

### 3.3.1 Inclusion Criteria

i. HIV-infected patients regardless of age or gender attending comprehensive HIV clinic of Siaya County Referral hospital.

ii. Those who gave consent.

### 3.3.2 Exclusion Criteria

i. Patients who did not consent to be part of the study.

ii. Infected individuals but not attending comprehensive HIV clinic of Siaya County Referral hospital.

iii. Any other patient who was HBV or HCV infected but not HIV infected.

iv. Any other patient who was HBV vaccinated.

## 3.4 Determination of Sample Size

Sample size determination was based on the Cochran formula (Cochran, 1977) with assumed prevalence of 17.8% (National AIDS Control Council, 2014).

$$\boldsymbol{N = \frac{Z^2\, P(1-P)}{C^2}}$$

Where: **N**= is the sample size **Z**= 1.96 at 95% interval (Standard error)

**P**= is the estimated prevalence (17.8%).

**C**= is the Confidence interval expressed in decimal (0.05).

$$\boldsymbol{N = \frac{1.96^2 0.178(1-0.178)}{0.05^2}}\ \mathbf{N= 225}$$

## 3.5 Sampling Design

The study utilized the systematic random sampling technique during which the patients were grouped into a cluster of five and in each cluster the first, third and fifth patient were recruited to take part in the study. This continued until the target sample was achieved.

### 3.6 Recruitment of the patients

With the help of the trained counselors appointed by the lead researcher through the facility management, the volunteers were recruited to take part in this study. The volunteers were taken through the objectives of the study after which the consenting volunteers were interviewed using a structured questionnaire and were required to hand in the duly signed consent form.

### 3.7 Blood Sample Collection

About 5ml of venous puncture blood was collected into 10ml vacutainer tubes (Becton Dickson, San Jose, California) to be used for HBV and HCV serological assays. The venipuncture was done aseptically and blood samples drawn from each consenting participant and the plasma separated by centrifugation at 2500 rpm for 10minutes. Thereafter, blood plasma was used for the HBV and HCV serology. In expected delay, the samples were stored at -20$^{o}$C for later analysis (Muriuki *et al.*, 2013).

#### 3.7.1 Serological Assays

The Serological marker detection was done using HBV and HCV Onsite Rapid Test Kit manufactured by CTK Biotech, Inc USA and distributed locally by High-Ridge Pharmaceutical Kenya. This was done with strict adherence to the manufacturers' prescribed instructions.

#### 3.7.2 Onsite HBsAg Assay

The presence of HBsAg in the blood plasma was detected using Onsite HBsAg Rapid Test Cassette (CTK Biotech, Inc, San Diego, USA), sourced from High-Ridge Pharmaceuticals. The test is based on a lateral flow chromatographic immunoassay for the detection of hepatitis B virus surface antigen in human serum or plasma samples (Chameera *et al*., 2013). In brief, the test cassette and the sample specimens were brought to room temperature and the samples

thoroughly mixed after thawing for the refrigerated samples. The Cassette was then removed from the pouch and placed on a clean flat surface and labeled with samples' identity number. Using a pipette dropper, 80µl of the plasma was added to the sample wells and the timer set immediately. The test results were read after 15 minutes of the test.

Two distinct red bands in the Test Zone (T) and Control Zone (C) indicated HBsAg reactivity (positive). However, if no red band observed in the Test Zone (T) within 10 minutes of the test, then there is no reactivity and that no HBsAg detected (negative). Or a single red band in the Control Zone (C). But if there is no red band at all, then the test is invalid and is to be repeated with new kit and new plasma sample (Chameera *et al*., 2013).

### 3.7.3 Anti-HCV Assay

Onsite Rapid Test Kit for qualitative detection of anti-HCV in human serum or plasma (CTK Biotech, Inc, San Diego, USA) was used to detect the anti-HCV antibody with strict adherence to the manufacturers' instruction .The test is based on immune chromatographic principles. Briefly, the pouch and the samples were brought to room temperature and cassette removed from the pouch just before testing. The device was placed on a clean level surface and 25µl of plasma added into the sample window. 80µl of diluent was then added to the samples and the timer set. The test results were read after 15minutes of the test.

Two distinct coloured bands observed, one in the control zone (c) and the other in the test zone (T) indicated reactivity (positive). The intensity of the colour in the test zone (T) differed with the concentration of HCV antibodies in the sample and thus any shade of red or pink band in the test zone (T) was considered positive. One band in the control region (c) or no red or pink band appearing in the test region (T) indicated negative results and thus no antibodies to hepatitis C

virus detected in the sample. The result was considered invalid if no band was observed in the control zone (T) and this was repeated using a new test cassette (Waheed *et al*., 2017).

### 3.7.4 HBV DNA Extraction

The viral DNA was extracted from the plasma samples positive for HBV surface antigen (HBsAg) using QiaAmp$^{TM}$DNAMini kit (QiagenInc. Valencia, USA) following manufacture's guidelines (Dokanehiifard and Bidmeshkipour 2010). The extracted viral DNA was stored at -20$^{o}$C for later analysis. Briefly, 200µl of each plasma sample was added into an eppendorflysis tube containing 25µl of protease K followed by 200µl of buffer containing carrier RNA. The mixture was incubated at 56$^{o}$C for 15 minutes in a heating block. Brief spin was done; 250µl of ethanol (96-100%) added vortexed for 15 seconds and incubated for 5 minutes at room temperature. This was transferred in wash tubes and centrifuged at 10,000rpm for 2 minutes filtered and spin columns placed in clean wash tubes. 500µl of wash buffer (AW1) was added and centrifuged for 2 minutes at 10,000RPM filtrate discarded and wash tubes replaced. A volume of 500µl of wash buffer (AW2) was added and centrifuged at 10,000 rpm for 2 minutes followed by replacement of wash tubes and addition of 500µl of ethanol (96-100%). Further centrifugation was done at 10,000RPM for 2 minutes and the filtrate discarded. Wash tubes were replaced and content centrifuged at full speed for 3 minutes and incubated for 3 minutes at 56$^{o}$C to dry the membrane completely. Wash tubes were then replaced with recovery tubes and 50µl of elution buffer (AVE) added to the centre of the membrane. The mixture was incubated for 5 minutes at room temperature followed with full speed centrifugation for 2 minutes. The DNA extract were stored at -20$^{o}$C for later analysis (Dokanehiifard and Bidmeshkipour 2010).

### 3.7.5 Amplification of HBV-*pol*

Nested polymerase chain reaction was used to amplify HBV-*pol* gene. The amplification was carried out using specific sense and antisense primers for both rounds of the PCR. The specific primers HBPr1 (position: 2850-2868, 5'-GGGTCACCATATTCTTGGG-3') and HBPr135 (position: 803-822, 5'-CAAAGACAAAAGAAAATTGG-3') (Stuyver *et al.,* 2000) was used for the first round while primers HBPr2 (position: 2867-2888, 5'-GAACAAGAGCTACAGCATGGG-3') and HBPr3 (position: 3226-3246, 5'-CCACTGCATGGCCTGAGGATG-3') (Stuyver *et al.,* 2000) was used in the second round of the PCR with the products of outer reaction serving as the DNA template for nested PCR (Webale *et al.,* 2015). Briefly, to each PCR reaction mix containing 12.5µL of 2 X phusion high-fidelity master mixes, 5.0µL of DNA was added followed by 0.625µL of each sense and antisense primers. To make a total final volume of 25µL, 6.25µL of distilled water was added to the mixture. The mixture was then placed in a thermo cycler with the following set conditions for both rounds of the PCR: one cycle at 94$^{o}$C for 10 minutes, 40 cycles at 94$^{o}$C for 30 seconds, 50$^{o}$C for 30 seconds, and 72$^{o}$C for 1 minute. The final extension was performed at 72$^{o}$C for 10 minutes (Mwangi *et al.*, 2008; Webale *et al*., 2015).

### 3.7.6 Confirmation of PCR products by Gel electrophoresis

The agarose gel electrophoresis method was used to visualize and confirm nested PCR products during which the PCR amplicons were stained with ethidium bromide (0.05%) and loaded for visualization using transilluminator (UVP, San Gabriel, A, USA). The gel electrophoresis was allowed to run for 45 minutes in electric field at 100 volts for the fragments to separate ( Lee *et al*., 2012). An average molecular weight of 692 bp for the reverse transcriptase gene was expected.

### 3.7.7 PCR Products Sequencing and Genotyping

The products of the second round nested PCR were subjected to direct sequencing at Macrogen Europe Laboratory, Netherlands by automated DNA sequencer ABI 377 (Applied Biosystems, Foster City, USA) using Big Dye Terminator kit (Applied Biosystems). The specific primers HBPr2 and HBPr3 for sense and antisense sequencing reactions were used respectively.

## 3.8 Sequence and Data Analysis

### 3.8.1 Phylogenetic Analysis

Pair wise contiguous sequences were generated using DNA Baser sequence assembler version 4.20.0 (Heracle Software, Germany). The generated sequences were aligned with viral hepatitis B strains A-J reference sequences from Gene bank to construct phylogenetic tree based on partial HBV *pol* gene by CLUSTAL W (version 1.81) and neighbour-Joining method, following Kimura's two- parameter distances based on 1000 bootstrap replicates using Molecular Evolutionary Genetic Analysis (MEGA X version 10.0.4) (Kumar *et al.,* 2018).

### 3.8.2 HBV drug resistance Analysis

Hepatitis B virus-*pol* sequences were analyzed for drug resistance using *insilico* tool platforms; https://hbvdb.ibcp.fr/HBVdb/HBVdbResistance (Hayer *et al.,* 2012), and (http://hivdb.stanford.edu/HBV/HBVseq/development/HBVseq.html) (Rhee *et al.,* 2010). The HBV isolate sequences (query sequences) were aligned with reference reverse transcriptase (RT) sequences and computed. The algorithm searches for mutations in the HBV isolate sequences that define known resistance to entecavir, lamivudine, telbivudine, tenofovir and adefovir drugs. The detected mutations are reported with the associated drug, resistance status and mutation positions in the query sequence (Hayer *et al.,* 2012).

### 3.8.3 Statistical data Analysis

Data analysis was done using scientific programme for social sciences (SPSS) version 24.0 and co-infections prevalence rate of HBV/HIV, HCV/HIV and HBV/HIV/HCV expressed in percentages. The correlation between the presence of HBV/HIV, HCV/HIV and HBV/HIV/HCV co-infections with socio-demographic variables was determined using Chi-square test with the significant p-value of $p \leq 0.05$.

## 3.9 Ethical Consideration

The study was executed upon approval by Kenyatta University Research and Ethical Review Committee (KU-ERC), cleared by Siaya County Department of Health and Sanitation and permitted by Siaya County Referral Hospital Institutional Review Committee (IRC).

# CHAPTER FOUR

# RESULTS

## 4.1 Demographic information of the enrolled participants

Among the 225 patients who participated in this study, male patients were 68 accounting for 30.2% while the female patients visiting the clinic formed the majority, 69.8%. The youngest patient was 3 years old while the oldest was 76 years of age. The average age of the patients who attended this clinic at this time was 38.26 ± 15.46 years (mean ± SD) whereas the mean age for females and males were 36.29 years (SD± 15.21) and 42.82 years (SD±15.18) respectively. Using WHO age grouping (<5 young children, 5 – 15 children, 15 – 24 young adults, above 25 adults), most of these patients were adults (80.9%). The young children were 0.9% whereas the children were 7.1% as indicated in table 4.1. All patients were on average treatment duration of 4.76 years (SD± 3.30). However, on the treatment line, only 11(4.9%) of the participants were on second-line treatment of HIV infection whereas majority 214(95.1%) were on first-line treatment of HIV infection.

**Table 4.1: Socio-demographic characteristics of the study participants**

| Categories | All n= 225 | Male | Female |
|---|---|---|---|
| Age(yrs) {Mean (S.D)} | 38.26 (±15.46) | 42.82 (±15.18) | 36.26 (±15.21) |
| Range (Years) | 3yrs - 76 yrs | | |
| **Gender** | | 68 (30.2) | 157 (69.8) |
| Treatment Duration(yrs) {mean (S.D)} | 4.76 (± 3.30) | | |
| **Age group (years)** | | | |
| < 5 | 2 (0.9) | 0 (0.0) | 2 (1.3) |
| 5 – 15 | 16 (7.1) | 6 (8.8) | 10 (6.4) |
| 15 - 24 | 25 (11.1) | 1 (1.5) | 24 (15.3) |
| >25 | 182 (80.9) | 61 (89.7) | 121 (77.1) |
| **N(%)** | 225 | 68 (100) | 157 (100) |
| **Marital Status** | | | |
| Married | 146(65) | 44 (30.1) | 102 (69.9) |
| Single | 38(17) | 8 (21.1) | 30 (78.9) |
| Divorced | 19(8) | 10 (52.6) | 9 (52.6) |
| Widowed | 22(10) | 6 (27.3) | 16 (72.7) |
| **Academic Level** | | | |
| Primary | 56(25) | 13 (23.2) | 43 (76.8) |
| Secondary | 116(52) | 44 (37.9) | 72 (62.1) |
| Post-secondary | 48(21) | 10 (20.8) | 38 (79.2) |
| None | 5(2) | 1 (20) | 4 (80) |
| **Place of Residence** | | | |
| Township | 99(44) | 21 (21.2) | 78 (78.8) |
| North Alego | 24(11) | 7 (29.2) | 17 (70.8) |
| Central Alego | 23(10) | 10 (43.5) | 13 (56.5) |
| West Alego | 24(11) | 9 (37.5) | 15 (62.5) |
| S.E Alego | 28(12) | 12 (42.9) | 16 (57.1) |
| Usonga | 17(8) | 8 (47.1) | 9 (52.9) |
| Others | 10(4) | 1 (10) | 9 (90) |
| **ART Reginem** | | | |
| TDF+3TC+EFV | 141(62.6) | 34(24.1) | 107(75.9) |
| AZT+3TC+EFV or NVP | 73(32.4) | 52(71.2) | 21(28.8) |
| TDF+3TC+ATV/r | 7(3.1) | 5(71.4) | 2(28.6) |
| AZT+3TC+LPV/r | 4(1.8) | 3(75) | 1(25) |

**KEY:**

TDF: tenofovir; 3TC: lamuvidine; EFV: efavizenz; AZT: zidovudine; NVP: nevirapine; ATV/r: atazanavir/ritonavir; LPV/r: lopinavir/ritonavir.

The mean treatment duration of the patients was 4.76 (±3.3) years with most patients (62.6%) being on TDF/3TC/EFV ART regimen **(Table 4.1).** Among the patients who were on first line of HIV treatment 10.3% had co-infections with HBV or HCV whereas those on second line of HIV treatment 9.1% were HBV or HCV co-infected **(Table 4.2).**

### 4.2 HBsAg and anti-HCV sero-prevalence among the study participants

From the two hundred and twenty five patients recruited in the study, 202 (89.8%) were HIV mono-infected whereas 23 (10.2%) had co-infection with 14 (6.2%) and 9 (4.0%) being HIV-HBV and HIV-HCV co-infected respectively. The study found no co-infection with the three viruses (HIV, HBV and HCV) among the patients attending Comprehensive HIV Clinic of Siaya County Referral Hospital. HIV co-infection was found to be higher ($P$ = 0.059) among the male patients (16.2%) as compared to the females (7.6%), though this was not scientifically significant. Among the different age groups, HIV co-infection was more among the children between the ages of 5 and 15 years old (18.8%), followed by young adults (12.0%) and adults above 25 years of age (9.3%) respectively. In spite of the differences, this was not statistically significant. In addition the study found no significant difference in HIV/HBV and HIV/HCV co-infections by the patients' line of HIV treatment **(Table 4.2).**

#### 4.2.1 Prevalence of Hepatitis B virus and HIV co-infections

Of the 225 plasma samples from the study participants who were screened for HBsAg, 14(6.2%) were HBsAg reactive. The prevalence of HBV/HIV co-infection among both male and female patients showed that, 7.4% of the males and 5.7% of the females were co-infected respectively. However, there was no significant difference in HBV/HIV co-infection by gender ($P$ = 0.764). Both males and females were infected. Within the various age groups, the most HBV/HIV co-infected age group was young adults in the ages of 15–24 years (8.0% prevalence). This was

followed by the children in the ages of 5–15 years (6.3% prevalence) and adults in the ages above 25 years which had prevalence of 6.0%. There was however, no significant differences in the HBV/HIV co-infection prevalence in the ages ($P$ = 0.964). Other demographic factors such as marital status and education were also found to be insignificantly associated with the risk of being HIV/HBV co-infected. On the other hand residential place was found to be a risk factor with most co-infected patients from North Alego. This was statistically significant at $P$ = 0.023.

### 4.2.2 Prevalence of Hepatitis C virus and HIV co-infections

The prevalence of anti-HCV among Human Immunodeficiency Virus infected individuals attending comprehensive HIV clinic of SCRH was found to be 4.0% (9/225) anti-HCV (IgM) positive. Most of the detected HIV/HCV co-infection occurred among adults. The prevalence of HCV-HIV co-infection among male and female patients was found to be 8.8% and 1.9% respectively. Furthermore there was a significant difference in HCV-HIV co- infection by gender ($P$ = 0.024). Within the various ages, the highest co-infection by HCV-HIV was recorded among children in the ages of 5 – 15 years (12.5% prevalence). This was followed by the young adults in the ages 15 - 24 years (4.0% prevalence) and adults in the ages above 25 years which had 3.3% prevalence. The differences in HCV-HIV co-infection prevalence among the ages observed was however, not scientifically significant ($P$= 0.344). The prevalence of HCV/HIV co-infection among the single study participants was significantly high ($P$= 0.013) **(Table 4.2**).

**Table 4.2: Hepatitis B and C virus co-infections prevalence among study participants**

| Categories | N = 225 | HIV-Mono | HIV-Co | *P-Value* | HIV/HBV | *P-Value* | HIV/HCV | *p-value* |
|---|---|---|---|---|---|---|---|---|
| **Gender** | | | | | | | | |
| Males | 68 (30.2) | 57 (83.8) | 11(16.2) | | 5 (7.4) | | 6 (8.8) | |
| Females | 157 (69.8) | 145 (92.4) | 12(7.6) | 0.059 | 9 (5.7) | 0.764 | 3 (1.9) | **<0.024*** |
| **Age group (yrs)** | | | | | | | | |
| < 5 | 2 (0.9) | 2 (100) | 0 (0.0) | | 0 (0.0) | | 0 (0.0) | |
| 5 – 15 | 16 (7.1) | 13 (81.2) | 3 (18.8) | 0.629 | 1 (6.3) | 0.964 | 2 (12.5) | 0.344 |
| 15 - 24 | 25 (11.1) | 22 (88.0) | 3 (12.0) | | 2( 8.0) | | 1 (4.0) | |
| >25 | 182 (80.9) | 165 (90.7) | 17 (9.3) | | 11 (6.0) | | 6 (3.3) | |
| **Marital Status** | | | | | | | | |
| Married | 146(65) | 133 (91.1) | 13 (8.9) | | 10 (6.8) | | 3 (2.1) | |
| Single | 38(17) | 34 (89.5) | 4 (10.5) | 0.312 | 1 (5.3) | 0.961 | 3 (15.8) | **<0.013*** |
| Divorced | 19(8) | 15 (78.9) | 4 (21.1) | | 2 (5.3) | | 3 (7.9) | |
| Widowed | 22(10) | 20 (90.9) | 2 (9.1) | | 1 (4.5) | | 0 (0.0) | |
| **Academic Level** | | | | | | | | |
| Primary | 56(25) | 51 (91.1) | 5 (8.9) | | 3 (5.4) | | 3 (5.4) | |
| Secondary | 116(52) | 105 (90.5) | 11 (9.5) | | 5 (4.3) | | 6 (5.2) | |
| Post-secondary | 48(21) | 42 (87.5) | 6 (12.5) | 0.801 | 6 (12.5) | 0.224 | 0 (0.0) | 0.409 |
| None | 5(2) | 4 (80) | 1 (20) | | 0 (0.0) | | 0 (0.0) | |
| **Residence** | | | | | | | | |
| Township | 99(44) | 93 (93.9) | 6 (6.1) | | 3 (3) | | 4 (4) | |
| North Alego | 24(11) | 18 (75) | 6 (25) | | 5 (20.8) | | 1 (4.2) | |
| Central Alego | 23(10) | 20 (87) | 3 (13) | | 3 (13) | | 0 (0.0) | |
| West Alego | 24(11) | 23(95.8) | 1 (4.2) | 0.053 | 0 (0.0) | **<0.023*** | 1 (4.2) | 0.497 |
| S.E Alego | 28(12) | 23 (82.1) | 5 (17.9) | | 2 (7.1) | | 3 (10.7) | |
| Usonga | 17(8) | 16 (94.1) | 1 (5.9) | | 1 (5.9) | | 0 (0.0) | |
| Others | 10(4) | 9 (90) | 1 (10) | | 0 (0.0) | | 0 (0.0) | |
| **Regimen** | | | | | | | | |
| Line-One | 214(95.1) | 192 (89.7) | 22 (10.3) | 1 | | | | |
| Line-Two | 11(4.9) | 10 (90.9) | 1 (9.1) | | | | | |

### 4.3: Genetic Diversity of Hepatitis B virus

In this study, of the 14 samples that were serologically positive for HBsAg, only a total of eleven samples were successfully amplified and directly sequenced. The phylogenetic analysis revealed almost all (11) sequences were hepatitis B virus genotype A with a single sequences being a HBV sub-genotype A1. From the analysis all the eleven DNA isolates sequences clustered closely with sub-genotype A1 from Kenya, Sudan and Ethiopia **(Figure 4.1).**

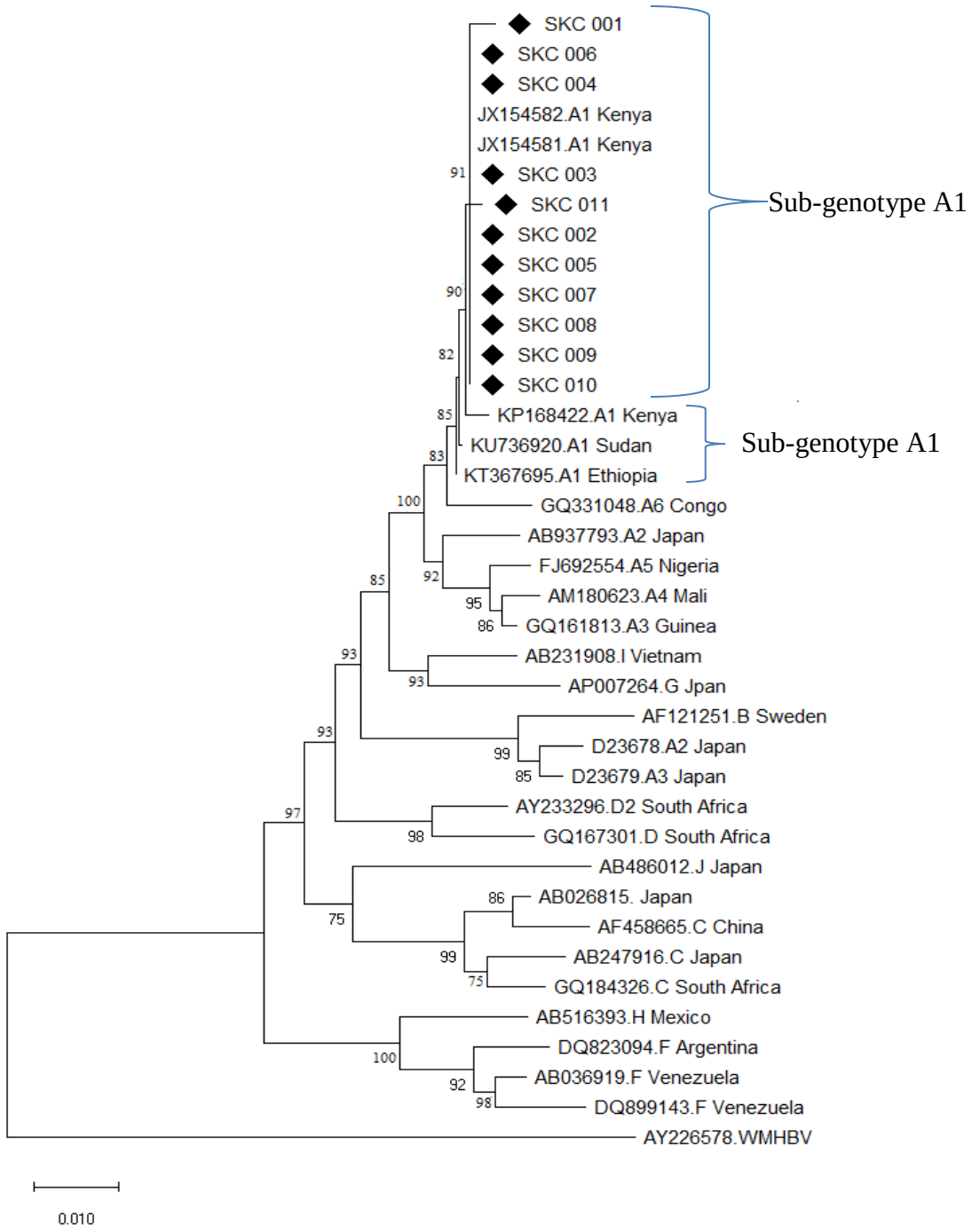


**Figure 4.1: Phylogenetic tree of HBV-pol gene sequences**. Neighbour-joining method was used to generate the phylogenetic tree at 1000 times bootstrap resampling. Wooly Monkey HBV (AY226578-WMHBV) was used as an out group. HBV isolates from study participants are indicated with diamond sign.

### 4.4 Hepatitis B virus Drug resistance

From the two hundred and twenty five (225) patients that were recruited to participate in this study, 214 (95.1%) of the patients were on first-line of HIV antiretroviral therapy, a combination that contains lamivudine (TDF+3TC+EFV). Mutation in the reverse transcriptase (RT) region of hepatitis B virus polymerase, mutation rt169F was detected in one participant. However, the rest of the 10 Individuals were infected with HBV drug susceptible strains. The mutation rt169F is a mutation that is not yet confirmed to confer drug resistance.

**Table 4.3: Patterns of hepatitis B virus mutations among study participants**

| Mutation patterns | n(11) | Frequency (%) | Drug associated |
|---|---|---|---|
| rt169F | 1 | 9 | No drug associated |
| No major mutations | 10 | 91 | Susceptible |

# CHAPTER FIVE

# DISCUSSION, CONCLUTION AND RECOMMENDATION

## 5.1 DISCUSSION

### 5.1.1 Prevalence of HBV virus among the study participants

Detected prevalence of HBV infections in the sampled population in Siaya was 6.2% which was much higher compared to the prevalence (0.83-2.88%) previously reported in Nyanza region (Ly *et al.,* 2016). The high prevalence observed in this study may be associated largely with socio-cultural practices in Siaya County such as widow inheritance, community rites involving sex and polygamy (Siaya County Health Management Team, 2017). The findings are however, comparably consistent with similar studies previously conducted in Kenya; Nairobi 7.2% (Mabeya *et al.,* 2016), 6.0% (Muriuki *et al.,* 2013), and 5.7% Eldoret (Wambani *et al.,* 2015) . This was also found to be consistent with similar study findings from sub-Saharan countries; Rwanda (5.7%), Nigeria (5.7%) Tanzania (7.3), Zambia (7.6%) and Mozambique (9.1%) (Opaleye *et al.,* 2014; Ramírez *et al.,* 2016; Wandeler *et al.,* 2016; Mutagoma *et al.,* 2017; Chambal *et al.,* 2017). General observation from these findings, the levels of infections of HBV could be also associated with target study population of HIV infected patients seeking medical care in the sampled regions following improved access to antiretroviral therapy that has led to their prolonged life span hence increased risk to HBV infection.

The observed prevalence in this study was however, in contrast with those studies previously conducted in the country. For instance, a study conducted in informal settlement in Nairobi, Kerubo *et al., (*2015) reported a significantly lower prevalence rate of 0.7% and 1.1% in Korogocho and Viwandani slums in Nairobi respectively. This findings corresponds with similar study conducted in Senegal (1.13%), Mali (1.13%) Ethiopia (2.0%) and Tanzania (2.3%)

(Tounkara *et al.,* 2009; Abera *et al.,* 2014; Kamenya *et al.,* 2017). The low HBV prevalence in these regions could be associated with the differences in risk of exposure to HBV infection, area of study as well as difference in the selected study population. Nevertheless, the detected levels of HBV infection in this study was significantly lower compared to those previously obtained in some regions or areas in Kenya. About 50.6% of HBV infections levels have been detected in Nairobi, Mombasa, Kisumu and Eldoret (Ochwoto *et al.,* 2016). The high levels of HBV infection could be type of the patients screened. This study involved patients already presenting with jaundice inpatients hence explaining probably the Likely hood of HBV leading to the clinical presentation of jaundice. In comparison with previous studies elsewhere from Kenya, higher prevalence has also been detected in Malawi (20.4%), Gambia (12.2%), Guinea-Bissau (16.3%), Cameroon (23.7%) and Nigeria (26.3%) (Mustapha, 2004; Nyirenda *et al.,* 2008; Jobarteh *et al.,* 2010; Langhoff *et al.,* 2014; Noubiap *et al.,* 2015; Ochwoto *et al.,* 2016). The detected high prevalence of HBV in these countries being more associated with probable high risk population or low levels of immunization against HBV( Mabeya *et al.,* 2016).

In this study, there was a difference in the levels of HBV/HIV co-infection across gender with males being most affected. However, there was no significant difference. This observation was similar to that observed in previous study conducted in Nairobi (Mabeya *et al.,* 2016). Nevertheless, the observed levels of infection among men could be associated with male sexual behavior, drug and substance abuse (Mabeya *et al.,* 2016). Contrary observation was observed in study conducted in Eldoret in Kenya and Uganda where females were most affected with vulnerability to unprotected sex across the ages being one of the associated factor (Wambani *et al.,* 2015; Baseke *et al.,*2015) .

Nevertheless, participants' residential area was significantly associated with HBV/HIV co-infections (Table 4.2) in Siaya. Most of the HBV/HIV co-infected patients were from North Alego, a region that is largely rural. The high HBV/HIV co-infection prevalence detected in this region could be associated with cultural practices such as widow inheritance, polygamy, multiple sexual partners and permissiveness in boy sexuality (Siaya County Health Management Team, 2017). Other demographic characteristics tested in this study such as age, marital status and level of education did not show any significant association with HBV/HIV co-infection although level of education is usually associated with infections. Patients of low levels of education are expected to be more affected probably because of poor access to health services such as health education and vaccination.

### 5.1.2 Prevalence of HCV virus among the study participants

The present study observed an overall HCV-HIV co-infection prevalence of 4.0%. This was considerably higher compared to the National prevalence of 2% (Ly *et al.*, 2018). The recorded levels were similarly higher than current levels of HCV infection in most of East sub-Saharan African countries especially in general population (Petruzziello *et al.*, 2016). The detected high HCV-HIV prevalence suggests the possibility of risk behaviors of the studied population of Siaya. Studies have shown that, population of this are involved in injecting drug use (IDUs), men who have sex with men (MSM) or prostitution or sex for fish that could be predisposing them to these infections (Siaya County Health Management Team, 2017). Based on gender, males were most affected, a finding that concurs with previous study conducted in the country especially in high risk population of IDUs (Ochwoto *et al.*, 2016). It is postulated that possible similar risk groups and means of exposure could be significantly leading to this level of HCV infection. Similar findings have also been detected elsewhere in Malawi (5%), Ethiopia (5.5%),

Cameroon (7.2%) and Italy (7.8%) (Nyirenda *et al.,* 2008; Abera *et al.,* 2014; Noubiap *et al.,* 2015; Puglia *et al.,* 2016). Similarly, the high prevalence detected in these study settings were also linked to indulgent in risk behaviors like injecting drug use and prostitution. On the other hand, HCV-HIV prevalence reported in this study was in contrast with the previous findings in some parts of Kenya (0.4%) Korogocho, Viwandani slums (Kerubo *et al.,* 2015) and 1.6% urban centres of Eldoret (Wambani *et al.,* 2015). In comparison with other African countries the findings were contrastingly equally higher to those obtained in Gambia (0.6%), Botswana (0.8%), Libya (0.9%), and Nigeria (1.7%) (Mboto *et al.,* 2009; Geretti *et al.,* 2010; Daw *et al.,* 2014; Durowaye *et al.,* 2016). The population behavious and possible underlying cases of IDUs could explain the observed trend of HCV infection in this area of Siaya town which is also known for sex-based socio-cultural practices such as widow inheritance and community rites involving sex (Siaya County Health Management Team, 2017).

On the other hand HCV-HIV co-infection prevalence reported in this study was found to be lower compared to HCV-HIV co-infection prevalence reported by Muriuki *et al.,* (10.3%) (Muriuki *et al.,* 2013). The large difference in co-infection prevalence observed between the present study and that of Muriuki *et al.,* (2013) could be that the former study that was conducted in urban center, Nairobi and sample size used (Muriuki *et al*., 2013). In addition the findings were also found to be lower compared to findings from other African countries, Gabon (9.2%), Burundi (11.3%), Cameroon (13.8%), Nigeria (16.9%) and Egypt (17.5%) and a replica in Vietnam (35.4%). High HCV/HIV co-infection prevalence observed in all these cases was associated with high risk populations (Karoney, 2013; Huy *et al*., 2014; Obadahn and Kamal, 2017). The detected HIV/HCV co-infection prevalence in this study just like the previous studies may have been contributed by a number of factors but not limited to vulnerability to unprotected

sex among women, multiple sexual partners among men, polygamous behaviour, widow inheritance drug and substance abuse such as Waragi from Uganda, HCV prevalence background and injecting drug use. However, there is scarcity of information about injecting drug use in Siaya County.

In addition, no triple co-infection with HIV/HBV/HCV was detected in this study. This finding is similar to one previously conducted in some part of the country like Eldoret (Wambani *et al.,* 2015). The same finding has also been reported in Iran (Amiri *et al.,* 2016). However, triple infection with HIV/HBV/HCV have been detected in other parts of the country like Nairobi 0.15%- 1% (Yerly *et al.,* 2004; Muriuki *et al.,* 2013; Kerubo *et al.,* 2015) and 1.1% in Ethiopia, (Wondimeneh *et al.,* 2013). Nevertheless, high levels of triple infection have been detected in China (7.1%) although among IDUs (Province *et al.,* 2012).

This study revealed a significant difference across gender in HCV infection (Table 4.2). Males most infected with HIV/HCV co-infection. However, this was not significantly different across the ages. Despite this, the high level of infection among males could be associated with risky sexual behaviours, possible cases of IDUs and prostitution. This finding was consistent with those previously observed in other urban centres of the country like Nairobi and Eldoret (Muriuki *et al.,* 2013; Wambani *et al.,* 2015). In comparison to other East African countries and elsewhere, similar findings have been obtained in Uganda, Vietnam and Iran (Zahedi *et al.,* 2014; Huy *et al.,* 2014; Baseke *et al.,*2015). Marital status was found to be a risk factor for HCV infection among study participants. Similarly those single were also found to be significantly co-infected with HCV/HIV (Table 4.2). This trend could be associated with sexual behavior of these categories of population which shows high level of infections among married and those teenagers or youths.

However, age of the patients was not associated with HIV/HCV co-infection in this study, although HCV/HIV co-infection was insignificantly common among adults and increases with increase in age. This was comparably similar to a study among pregnant women in Rwanda (Mutagoma *et al.,* 2017). Nonetheless, the findings contradict those previously reported in Kenya, Cameroon, Zambia and Poland (Kerubo *et al.,* 2015; Noubiap *et al.,* 2015; Peebles *et al.,* 2015; Grzeszczuk *et al.,* 2015). The observed variations in co-infection with HCV/HIV detected in various studies listed here could be primarily associated to sample size, exposure to risk to factors, sensitivity of the test kits used as well as HCV prevalence backgrounds (Kamenya *et al.,* 2017).

Hepatitis B and C virus infection poses significant clinical implication in choosing first-line antiretroviral therapy for HIV patients which must be done with much consideration to drug-drug interaction and likewise overlapping toxicities (Dybul *et al.,* 2002; Patel *et al.,* 2011).

### 5.1.3 Hepatitis B virus genetic diversity

Phylogenetic analysis of the 11 sequences was confirmed to belong to HBV genotype A which is the most predominant HBV genotype in the studied population. This finding concurs with previous studies in the country that have confirmed sustainable predominance of this genotype in most part of the country. Similarly this genotype happens to also dominate in most of sub-Sahara African countries (Mwangi *et al.,* 2008). Sequences from this study clustered with references sequences from Kenya, Sudan and Ethiopia. This clustering confirms its possible origins. It confirms circulation of HBV genotype A1 in this region of Kenya and neighborhood countries. The drivers of the distribution of these strains in this region could be associated with population migration within these countries (Mwangi *et al.,* 2008; Ochwoto *et al.,* 2016).

The detection of only existing HBV genotype A in this region confirms its sustainability in the region by inbreeding of this infection within the region ( Mabeya *et al*., 2017). Nevertheless, Siaya being one of the counties with high HIV prevalence, by virtual of the shared transmission mechanism, the population behavior of the residents could be a driving force in facilitating of inbreeding and circulation of this genotype in this region. In addition, virtual that Siaya County being within the Lake Victoria region that is thought to harbor high rate of prostitution especially among the fishing community and widow inheritance a fact that is evident in high HIV prevalence in the region compared to other parts of the country , all these could be among factors contributing to this infections.

These finding confirms previous studies that have been conducted in most urban centres that drives the transmission networks of this virus across the country (Mwangi *et al.,* 2008; Webale *et al.,* 2015; Ochwoto *et al.,* 2016; Mabeya *et al.,* 2017). Detection of hepatitis B genotype A and sub-genotype A1 reaffirm the predominance of HBV genotype A in Kenya. The successive detection of HBV genotype A in the country is an indicator of transmission within Kenyan border (Mabeya *et al.,* 2017). Sub-genotype A1 was first detected in southern Africa and subsequently in other African countries a suggestion of possible transmission dynamics or much emphasis on monitoring HBV genotypes surveillance across countries especially with this era of HIV infections (Ochwoto *et al.,* 2013). In spite of HBV genotype A being predominant in the country, genotype D and E have also been reported in Kenya from previous studies among the jaundice and blood donors in Kisumu, Eldoret, Mombasa and Nairobi parts of the country (Mwangi *et al.,* 2008; Ochwoto *et al.,* 2016). Their detection suggests that there could be other HBV genotypes circulating in Siaya County than reported in the current study.

### 5.1.4 Hepatitis B virus Drug resistance

In this study, most patients were on first-line of HIV antiretroviral therapy, a combination that contains lamivudine (TDF+3TC+EFV). In any case of HIV/HBV co-infections, patients are always put on treatment immediately to prevent progression to chronic stage of infection. Similarly following 90 90 90 HIV strategy, patients diagnosed and put on treatment immediately. In this study, all patients were on treatment. From the drug resistance evaluation, no drug associated mutations were detected. This finding confirms good response to treatment of HBV infections hence implying possible recovery to infection. However, HBV mutation rt169F was detected in one participant, a mutation that is not yet associated with any drug resistance. The occurrence of this mutation could be due to HAART pressure on RT gene on HBV in that such mutations in the rt region of hepatitis B virus polymerase are strongly associated with drug resistance especially during antiretroviral therapy (Xu *et al.,* 2015).

Despite the long treatment period these patients were on (4 years) the virus was still susceptible and responsive to lamivudine drugs. Contrary to expectation of low genetic barrier of lamivudine combined therapy the virus had not developed drug resistance hence conforming 100% drug adherence. This was similar to previous study conducted in the country which reported very low resistance to lamivudine in spite of long period of follow-up (6 years) and thus concluded that HBV resistance during lamivudine monotherapy could be lower in some populations and that it may be appropriate to administer lamivudine monotherapy in some populations (Day *et al.,* 2013).

In comparison with similar studies conducted in the country, this finding is contrary, since a low level of lamivudine-resistant HBV were detected among patients on a 12 month follow up (Kim *et al.,* 2011) comparable to a study in Gambia (14.3%) (Stewart *et al.,* 2011). The differences

could mean a possible suggestion that most of these HBV/HIV co-infected patients could be due to previous treatment strategy for HIV failing to capture HBV from onset hence possible upsurge of drug resistance due to drug non adherence (Thio, 2011).

Nevertheless, this finding was also contrary to the previous studies in Kenya and china that reported high levels of lamivudine-resistant HBV at 46% and 89.4% respectively (Lei *et al.,* 2013; Mabeya *et al.,* 2017). The high levels of lamivudine resistance observed were associated with long treatment period and possible non adherence to treatment. Despite low lamivudine-resistant HBV observed in this study, the sustained use of 3TC drug as the primary HBV active drug for highly active antiretroviral therapy (HAART) patients could possibly result into an increased HBV resistant strains which may impact negatively on HBV treatment in the region.

## 5.2 Conclusions

(i) The prevalence of 6.2% HBV/HIV and 4.0% HCV/HIV co-infections were detected in Siaya County.

(ii) Hepatitis B sub-genotype A1 was found to be the most predominant genotype among HIV patients seeking medical care at Siaya County Referral Hospital.

(iii) All the studied populations were infected HBV susceptible viral strains with only a person who harbored HBV strain with rt169F drug associated mutation.

## 5.3 Recommendations

(i) There is need for routine screening of HIV patients for HBV and HCV infections in this region in order to optimize treatment and management of HIV-1infections.

(ii) The study recommends continuous surveillance of circulating HBV genotypes and drug resistance among HIV/HBV co-infected patients to maximize patient management.

(iii) The study recommends for further study of a similar nature to determine the circulating HCV genotypes and drug resistant strains in the region.

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# LIST OF APPENDICES

## Appendix I: Ethical approval

**KENYATTA UNIVERSITY**
**ETHICS REVIEW COMMITTEE**
**Moi Library 1st Floor, Office No. 25**

**Fax: 8711242/8711575**
**Email: kuerc.chairman@ku.ac.ke**
**kuerc.secretary@ku.ac.ke**
**secretariat.kuerc@ku.ac.ke**
Website: www.ku.ac.ke

**P. O. Box 43844,**
**Nairobi, 00100**

Tel: 8710901/12

Our Ref: **KU/ERC/APPROVAL/VOL.1** (149) Date: 14th June, 2018

**Omondi Kevin Onyango**
**P.O Box 179-40601**
**BONDO**

**Dear Mr.Omondi,**

**APPLICATION NUMBER PKU/832/I898 "HEPATITIS B AND C CO-INFECTIONS AND GENETIC DIVERSITY AMONG HUMAN IMMUNODEFICIENCY VIRUS INFECTED INDIVIDUALS IN SIAYA COUNTY, KENYA"**

1. **IDENTIFICATION OF PROTOCOL**

The application before the Committee is with a research topic "**Hepatitis B and C Co-Infections And Genetic Diversity Among Human Immunodeficiency Virus Infected Individuals In Siaya County, Kenya "** was received on 9th March, 2018 and discussed on 12th June, 2018.

2. **APPLICANT**

**Omondi Kevin Onyango**

3. **SITE**

**Siaya County, Kenya**

4. **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 Review Committee Guidelines **AND APPROVED that the research may proceed for a period of ONE year from 12th June, 2018**

**5. 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 committee 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.**
**If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.**

P. O. BOX 43844 - 00100 NAIROBI
APPROVED
14 JUN 2018

**DR. TITUS KAHIGA**
**CHAIRMAN ETHICS REVIEW COMMITTEE**

I ...Kevin Omondi Onyango.....accept the advice given and will fulfill the conditions therein.

Signature..... Dated this day of.....05/07/..... 2018.

cc.
DVC-Research Innovation and Outreach

# COUNTY GOVERNMENT OF SIAYA

## DEPARTMENT OF HEALTH AND SANITATION

E-mail: siayachd@gmail.com
ADJACENT TO JCC CHURCH
PHONE:
SIAYA TOWN

COUNTY HEALTH HEADQUARTERS
SIAYA COUNTY
P O BOX 597
**SIAYA**

**Our Ref: SYA/CHD/RESEARCH/VOL. II (86)** **23RD JULY, 2018**

The Medical Superintendent
Siaya County Referral Hospital

**RE: CLEARANCE TO CONDUCT HEAPTITIS B AND C CO-INFECTIONS AND GENETIC DIVERSITY AMONG HUMAN IMMUODEFICIENCY VIRUS INFECTED INDIVIDUALS IN SIAYA COUNTY KENYA**

Omondi Kevin Onyango is pursuing his masters degree at Kenyatta University and has received clearance to conduct the above study.

The findings of this study shall help in the documentation of the epidermic to aid in the development of Public Health Interventions such as viral hepatitis surveillance in Siaya County.

This is to notify you that the study has been approved by the office of the undersigned.

Your assistance will be highly appreciated.

Dr. Bob Awino
DCDH (Preventive & Promotive Health Services)
SIAYA

COUNTY DIRECTOR OF HEALTH
SIAYA COUNTY
23 JUL 2018
P.O. Box 597-40600,
SIAYA.

# MINISTRY OF HEALTH

Email: siayacountyRh@gmail.com
Telephone: Siaya 0717197349
When replying please quote
Our Ref: SYA/MED/VOL1(99)

SIAYA COUNTY REFERRAL HOSPITAL,
P .O. BOX 144,
SIAYA.
25TH JULY, 2018

**To**
**Mr. Omondi Kevin Onyango**
**Reg No. 156/CE/33048/2014**
**Kenyatta University**

**RE: RESERCH AUTHORIZATION**

Following your application to carry out research for your study titled '***Hepatitis B and C co-infections and genetic diversity among human immunodeficiency virus infected individuals in Siaya County Referral Hospital''***

I am pleased to inform you that you have been authorized to undertake research project in Siaya County Referral Hospital by the Siaya Hospital Institutional Review Committee (IRC)

On completion of research you are expected to submit a soft and hard copy of the result to the hospital IRC.

MEDICAL SUPERITE
SIAYA C REFERRAL
P. O. BOX 144 - 40
SIAYA

DR. CALEB OTIENO
CHAIRPERSON, INSTITUTION REVIEW COMMITTEE
**SIAYA COUNTY REFERRAL HOSPITAL**

## Appendix II -Informed Consent Form
## CONSENT TO PARTICIPATE IN A RESEARCH PROJECT

**TITLE: HEPATITIS B AND C VIRUS CO-INFECTIONS AND GENETIC DIVERSITY AMONG HUMAN IMMUNODEFICIENCY VIRUS INFECTED INDIVIDUALS IN SIAYA COUNTY, KENYA**

Dear Participant,

**Explanation of Procedures:**

You are hereby invited to voluntarily take part in a research project aimed at determining Hepatitis B and C co-infections and Genetic diversity among Human Immunodeficiency Virus infected individuals in Siaya County, Kenya. HIV infection negatively influence the natural history of Hepatitis B and C viruses leading to rapid progression of liver disease in patients co-infected with HIV and/or HBV/HCV an effect attributed to the HIV suppression of the immune system. Timely detection of HBV and/or HCV co-infections among HIV infected patients will help in the optimizing treatment as well as preventing transmission of HBV from mother to child as result of lack of information about the co-infections. The approach of this study will be through blood specimen collection. You will be examined by a trained counselor thereafter you will be required to donate 5ml of blood sample.

**Risks:**

There will be no major risk involved by accepting to participate in this study. However, there may be slight discomfort or pain caused during blood sample collection.

**Benefits:**

There will be no monetary benefits for participating in this study, however, the participants could benefit by learning his or her HBV and HCV status. This will be very handy in helping your doctor to develop sound clinical management including hepatitis B virus vaccination. The study

will also provide data on HBV and HCV co-infections among HIV infected individuals in your community which will be critical in policy making.

**Confidentiality:**

The information collected during the study shall remain confidential as the identity of the participants shall remain undisclosed to any unauthorized persons. Any reference that could reveal the identity of the participant shall be removed before the preparation of the final research report and publication. Unused specimen shall not be used for any other research of any nature and as such shall be disposed safely.

**Withdrawal from the study:**

Your participation in this study is voluntarily and therefore participating or refusing to participate will attract no form of penalty. Participant can choose to withdraw his/her consent and be discontinued from participating in this study without victimization from the research team.

**Contact Persons:**

The participant can contact Mr. Omondi Kevin Onyango (0721 241 536) for any inquiry as far as the research progress is concerned.

The questions concerning any rights issues as a participant in this study shall be directed to Dr. Kahiga Titus Muhu, the chairman Kenyatta University Research and Ethics Review Committee. Department of Pharmacy and Complementary/ Alternative Medicine.

tkahiga@gmail.com or chairman.kuerec@ku.ac.ke

Consent to participate in the study

I……..... do hereby confirm that I have read ( I have been read to ) the consent information for the study Hepatitis B and C co-infections and Genetic diversity among HIV infected individuals in Siaya County and that I have understood the explained procedures during the consent process for the study. I further confirm that the questions raised during the consent process are satisfactorily answered and that I shall provide information to authorized persons described in this consent form. I have had time and opportunity to consider my participation in this study.

I voluntarily consent to participate in the above study.

Age: ……. Sex: …….

Sub county: …….

Location: ……..

Sub location: ……

Signature: …….. Date: …… / ……. /……

Name of the witness: ……..

Signature: …… Date: …… / …… / ……..

Signature of interviewer: ……. Date: …… / …… / ……..

## Appendix III: Questionnaire

I am Omondi Kevin Onyango a Master of Science (Microbiology) student at Kenyatta University. I intend to conduct a research on Hepatitis B and C virus Co-infections and Genetic Diversity among Human Immunodeficiency Virus Infected Individuals in Siaya County. I would like to place a humble request to you to be part of this study as your views will be very important in determining the disease burden in Siaya County. You are free to answer the questions to the best of your ability and that your responses will be handled with confidentiality that they deserve. Thank you in advance for your effort and time.

Number…….....Date……..Study site……

1. Where do you reside (ward)……
2. Are you on HAART treatment?
    a) Yes …….. No……
    b) If yes, for how long have you been on treatment…….
3. Have you been vaccinated against HBV?
    a) Yes ……..
    b) No ……
4. What is your marital status?
    a) Married….. (b) Single…… (c) Divorced…….. (d) Widow……
5. What is your level of education
    (a) Primary…… (b) Secondary…… (c) Post-secondary…… (d) None……

**Thank you so much for your time.**

## Appendix IV: Isolate Sequences

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