PREVALENCE, GENETIC DIVERSITY AND DRUG RESISTANCE OF HEPATITIS B VIRUS AMONG HIV-INFECTED PATIENTS ATTENDING KISII TEACHING AND REFERRAL HOSPITAL, KISII COUNTY, KENYA

PHINEHAS GUTHUA MUGO NJERU (B.Sc. MICROBIOLOGY)

I56/25943/2014

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (MEDICAL MICROBIOLOGY) IN THE SCHOOL OF PURE ANDAPPLIED SCIENCES OF KENYATTA UNIVERSITY

JUNE, 2025

DECLARATION

I 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.

Signature…… Date…….

Phinehas Guthua Mugo Njeru (BSc Microbiology)
I56/25943/2014
Department of Biochemistry, Microbiology and Biotechnology

Supervisors

We confirm that the work reported in this thesis was carried out by the student under our supervision as the university supervisors.

Signature……... Date…….

Prof. Anthony Kebira
Department of Biochemistry, Microbiology & Biotechnology
Kenyatta University, Nairobi, Kenya

Signature……... Date…….

Dr. John Maingi
Department of Biochemistry, Microbiology& Biotechnology
Kenyatta University Nairobi, Kenya

DEDICATION

This work is dedicated to my best friend and wife Teresia, our sons Bob Osteen and Benny Moen, and daughters Victoria, Melissa and Victoria Jane for their insurmountable love and support.

ACKNOWLEDGEMENTS

I am incredibly grateful to God for giving me this chance to pursue this course. He has provided the financial resources to realize this dream and sustained me in good health. I'm deeply thankful to my dedicated supervisors, Prof. Anthony Kebira and Dr. John Maingi, for their time to guide and review this work. To my wife Teresia Guthua, I appreciate the push and constant urge to complete this course.

The Kisii Teaching and Referral Hospital team is hereby appreciated for granting me a chance to conduct this study. The product of this study wouldn't have been realised without the input of the patients who volunteered to respond to my questions. Thank you for your cooperation.

The generosity of Kenya Medical Research Institute (KEMRI) who allowed me to use their laboratories and instruments to conduct this research is hereby acknowledged. I am particularly deeply indebted to your guidance, correction, criticism and moral support while I conducted laboratory work within your institution.

ABBREVIATIONS AND ACRONYMS

ADF	Adefovir
ALT	Alanine Aminotransferase Test
ART	Antiretroviral Therapy
ARV	Antiretroviral
cccDNA	Covalently Closed Circular DNA
ccDNA	Closed Circular DNA
CTL	Cytotoxic T-Lymphocytes
DNA	Deoxyribonucleic Acid
DTG	Dolutegravir
EFV	Efavirenz
ELISA	Enzyme-Linked Immunosorbent Assay
FH	Fulminant Hepatic
HBcAg	Hepatitis B Core Antigen
HBeAg	Hepatitis B e Antigen
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HIV	Human Immuno Deficiency Virus
IFN	Interferon
IG	Immunoglobulin
IgM	Immunoglobulin M
KTRH	Kisii Teaching and Referral Hospital
L-dT	Telbevudine
LFT	Liver Function Test
LMV	Lamivudine
mRNA	Messenger RNA
NRTIs	Nucleoside Reverse Transcriptase Inhibitors
NTCP	Sodium-Taurocholate Cotransporting Polypeptide
NVP	Nevirapine
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
POL	Polymerase
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
TAE	Tris-Acetate EDTA
TFV	Tenofovir
TDF	Tenofovir disoproxil fumarate
UV	Ultra Violet
VCT	Voluntary Counseling and Testing
WHO	World Health Organization
WMHBV	Wooly Monkey Hepatitis B Virus

ABSTRACT

The Hepatitis B Virus (HBV) infections continue to pose a global public health concern. The emergence of liver cirrhosis and liver cancer incidences have been increasing. HBV co-infection with HIV-1 has been on an upward trajectory, exacerbating the accompanying severe complications that include liver cirrhosis and, eventually, cancer that could lead to mortalities. Based on the shared transmission routes of HBV and HIV infections and the upsurge of incidences of infections associated with these viral infections, the need to confirm the status of disease burden in the country, particularly Kisii County, is inevitable. This study was conducted to assess the prevalence, genetic diversity, and drug resistance of HBV among HIV-infected patients at Kisii Teaching and Referral Hospital in Kisii County, Kenya. A cross-sectional study was conducted, and participants were randomly recruited. Administration of a structured questionnaire was conducted, and demographic data was acquired from 400 consenting eligible HIV-infected patients. Blood samples were drawn, and HBV infection HBsAg serostatus was confirmed. The samples confirmed to be HBsAg-positive were isolated, and viral DNA was extracted using the Qiagen Viral DNA Mini Kit, following the manufacturer's guidelines. The HBV–*pol* gene was then amplified and directly sequenced using the automated ABI 377 DNA sequencer (Applied Biosystem, Foster City, USA). The produced sequences were phylogenetically analysed using the Molecular Evolutionary Genetics Analysis (MEGA X version 10.4) software. The risks associated with HBV infections were statistically analyzed using two-way ANOVA. Of the 400 individuals who participated in this study, 221(55.2%) were female and 179 (44.8%) were male, all aged between 18 and 69 with an average age of 40.09 years. Majority of the study particpants (301/400) had tertiary levels of education. Age, gender and marital status were identified as significant risk factors associated with HBV infections. The overall prevalence of 11.75% (47/400) HBV-HBsAg was detected in this study. The analysis of the phylogenetic relationships of the 47 samples revealed that all sequences were of HBV genotype A. HBV nucleos(t)ide drug resistance mutations; rt1814V (1), rt180FSQ rt202L/E, I169K/L, rtV173K/G (2), rt202H/F/K (3), 180Q/S/F (3), and rt181G (4) were detected in 4 (8.5%) patients. This study, therefore, confirms that HBV genotype A is the most predominant in the country, with a low proportion of HBV-HIV co-infected patients being infected with drug-resistant strains. In addition, this study shows that in order to entirely suppress infections in co-infected individuals with HIV and HBV, dual antiviral therapy is required.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER ONE

INTRODUCTION

1.1 Background

Hepatitis B Virus (HBV) attacks the liver leading to acute and chronic ailments (WHO, 2018). HBV poses global healthcare menace. There are about 350,000 long-term carriers worldwide. Up to 25% of the infected die due to HBV-associated complications, such as liver cirrhosis and liver cancer. According to WHO, the occurrence of HBV has been found to be higher in the Western Pacific regions at 6.2%, while the African region spread rate stands at 6.1% among adults. The Eastern Mediterranean prevalence has 3.3%, the South East Asia Section has 2.0%, while the European area has 1.6% of the general population infected. Recent research studies set the HBV menace in Kenya prevalence at 5–8 % (Mabeya *et al.,* 2017). The co-infections of HIV and HBV has sparked a lot of interest in the recent past. This has been attributed to the opportunistic nature of HBV due to the compromised immunity manifested by HIV-infected patients. In the year 2012, the HIV infection rate within the constituency of Kisii recorded 8.6% which was high compared to the nationwide scale of 5.6% (Mokua *et al*., 2019). Deaths caused by HIV-related complications such as HBV Co-infection in Kisii County were recorded in 594 adults and 118 children in the year 2015 (Mokua *et al*., 2019).

Studies across various regions in the country have shown a varied proportions of HBV infections. In the eastern Kenya, for instance, HBsAg prevalence has been documented ranging between 8.8% and 11.2%, in an expansive County of Turkana. In Nairobi, a study conducted at Kenyatta National Hospital liver clinic, among chronic hepatitis and

liver ailment patients showed 77% of these victims infected (Nyairo, 2018). The HBV is a highly infectious agent that can endure 6-7 days outside the body cells, hence contributing to its increased capacity to be transmitted. In the endemic areas, perinatal transmission is common. Blood transfusion, accidental needle injuries, sharing of needles is common amongst intravenous drug users, multiple infected sexual partners, prisoners and other institutionalized people (Kleinman *et al*., 2009).

Preventive measures against HBV infections are necessary due to the rising incidence of HBV infections. It is demonstrated that immunization of all infants at regular intervals could effectively stop perinatal transmission (Schillie, 2018). Based on the efficacy of HBV vaccination, all high-risk and active young grownups should therefore be incorporated into HBV vaccination programs. For those who are already infected, an aggressive program to monitor the flare-up of HBV infection should be conducted. It is expected that the patient should be put on treatment immediately upon diagnosis and continuously monitored for treatment response and any risk for cancer development. For the HBV HIV infection, viral loads must also be monitored to understand the recovery progress (Hyams *et al*., 1989). Though in Kenya, HBV genotype A remains to be predominant, HBV genetic diversity requires to be studied further. The Northern and Coastal regions have also been detected with circulating HBV genotypes D and E (Mokua *et al*., 2019). There is a likelihood that other genotypes of HBV could be existing.

Various conditions may be attributed to HBV's fast advancement, including ignorance, whereby the general populace has no idea about transmission and behavior that may be linked to alcoholism. The HBV opportunistic infection amongst HIV patients due to loss of body immunity is a significant factor contributing to co-infection. This study explores more of these factors and documents the HBV-HIV relationship amongst HIV subjects within Kisii County.

1.2 Statement of the problem

The HIV and HBV are two common viruses that share transmission mechanisms that lead to severe chronic infections. These viruses are most often transmitted through , sharing of sharps, needles and syringes amongst intravenous drug users (IDUs), sexual or blood contact routes. The HBV/HIV Co-infection aggravates morbidity as well as mortality. Studies have proven that the natural course of HIV disease progression can be adversely affected by HIV a co-infections (Mabeya *et al.*, 2017). While this is the case, HBV infection has been documented to have accelerated to chronic liver disease amongst HIV patients. The recent emergence of drug-resistant HBV has scaled down the survival chances of people with HIV coinfected with HBV (Mabeya *et al.*, 2017). Hepatotoxicity experienced during treatment is also known to have contributed to the reactivation of hepatitis disease (Mabeya *et al*., 2017).

This study shows that the end stages of acute liver disease and cirrhosis may be accelerated by HBV and HIV co-infection (Thio *et al*., 2002). Hepatic-related complications and disease have been documented as one of the principal factors leading

to death among people not infected with HIV globally. Studies have also proven that in HBV/HIV-co-infected cases, the death percentage due to liver complications is higher by 3-6% globally. Acute HBV normal course may also be interfered with by the presence of HIV, whereby a co-infection results in greater intensities of HBV DNA and poor rate of elimination of Hepatitis B e Antigen (HBeAg) (Lukhwareni, 2008).

HBV screening should be incorporated into the testing for all HIV-positive cases. This would ease the management of the HBV-related complications that usually arise amongst HIV-infected patients. This has not been the practice in many parts of Kenya. Although there are effective therapeutic options available for treating mono-infections., the emergence of HBV/HIV co-infections adversely interrupts the normal course of infection. This complexity calls for individualized treatments (Cheng *et al*., 2021).

These patients are often initiated on Tenofovir disoproxil fumarate (TDF) + 3TC + Dolutegravir (DTG) (or TDF + 3TC + Efavirenz (EFV)) (CAP_CQUIN_Kenya-ARV-Guidelines- 2018-Final_20th Aug2018, n.d.; NASCOP, 2018). The development of drug resistance often limits this utilized option. It requires the inclusion of dual drugs combinations for optimal response to treatment. Therefore, including both treatment administration forms is recommended for managing HBV drug resistance (Chang *et al*., 2021). There is still elusive data on the current status of HBV disease burden and distribution of HBV genotypes, including drug resistance.

Therefore, this study determined the prevalence of HBV/HIV infections, specifically amongst HIV infected individuals attending Kisii Referral Hospital, highlighting the

need for intervention strategies that could be applied in inhibiting and controlling HIV-HBV co-infection in this county.

1.3 Justification of the study

The association between HIV and HBV is a global concern. This co-infection is aggravated by the shared means of transmission and spreading of these viruses. There are several risk factors, including risky sexual behaviors, sharing of infected syringes and needles, and blood-borne transmission that have supported the disease endemicity (Khalid *et al*., 2013). Severe chronic liver disease due to Heptatis B virus infection causes high morbidity and mortality amongst individuals on antiretroviral therapy (ART). This may be due to hepatotoxicity attributed to ARTs (Mohammadi *et al*., 2009). This accelerated liver disease suppresses the success of ART amongst co-infected patients. While this fact persists, ample testing and scrutiny are not carried out due to scarcity of resources, thus giving a blind eye to the possibilities of a co-infection (Mathews *et al*., 2014). Pronounced liver disease progression varies with the virus strain and genotype involved. Breaking down the genetic variations of the HBV would be critical in informing the management protocols to be applied.

The harmful effects of the co-infection, if not well studied, will contribute to loss of prevention and awareness opportunities, misdiagnosis, and lost treatment opportunities, escalating the disease burden and unyielding treatment or therapy programs. Prevention is cheaper than cure. With little information and low research activities on HBV infection in Kisii, this study sought to explore for this data with aim of optimizing the

control of its co-infection with HIV and reduction in drug resistance amongst individuals seeking treatment in the Kisii Hospital.

1.4 Research Questions

i) What risk factors are associated with HIV HBV co-infection within Kisii County?

ii) What is the HIV and HBV disease burden in Kisii County?

iii) What could be the HBV genetic variation amongst the HIV infected?

iv) Could there be HBV drug resistance amongst the HIV HBV co-infected?

1.5 Hypotheses

i) The HIV HBV co-infection poses no risk to patients in Kisii County

ii) There is no existence of any HBV HIV co-infections in Kisii County.

iii) There is no any circulating HBV strains in Kisii county.

iv) There is no HBV drug resistance in Kisii county.

1.6 Objectives

1.6.1 General Objective

To determine the prevalence, genetic diversity and drug resistance of HBV among HIV-infected patients attending Kisii Teaching and Referral Hospital.

1.6.2 Specific Objectives

i. To determine the risk elements associated with HBV HIV co-infections.

ii. To determine the prevalence of HBV amongst HIV-infected individuals visiting Kisii Teaching and Referral Hospital.

iii. To determine the HBV genetic diversity amongst HIV-infected individuals visiting Kisii Teaching and Referral Hospital.

iv. To determine HBV drug resistance amongst the HBV HIV co-infected patients.

1.7 Significance of the study

Understanding the impact of genetic variation of the HBV on the course and liver disease progression shall be a fundamental achievement in the formulation of management protocols. This key information shall also be employed in optimizing control. This study shall divulge the importance of ample testing and scrutiny of all HIV patients presenting with symptoms of liver disease in Kisii County.

CHAPTER TWO

LITERATURE REVIEW

2.1 Epidemiology of HBV

Human chronic liver infections have long been a global concern. The largest percentage of these infections are caused by HBV infection. Therefore, about 800,000 fatalities worldwide have been associated with liver cirrhosis and hepatocellular carcinima. (Lavanch *et al*., 2016). By end of 2014, HBV was ranked 15th cause of global mortality and was categorized as a global health menace in 2014 according to WHO (World Health Organization). Of all infected patients, 15-40% progress to hepatic cirrhosis, cancer and culminating in liver failure. 15-25% of the infected percentage will succumb to the illness. Globally, it is believed that HBV affects almost 2 billion individuals. Of these infected persons, studies show that 240 million are chronic carriers. Advancing interest in studying and analyzing specific data on HBV infection has promoted awareness and availed research data and improved testing. The development of vaccines and immunization programs has also progressively improved, even though there is still a lot to be done to curb new infections (Schillie, 2018).

Studies have shown that 70-90% of HIV-infected persons within the endemic areas have a co-infection with HBV (Rodríguez *et al*., 2000). Approximately 5-10% cases of individuals with HIV also have a co-infection with HBV, a proportion that is extra advanced than the overall populace (Alter *et al.*, 2006). Persons who have had a past infection and exhibit core antibody presence have a very high threat of HBV activation (Lacombe *et al*., 2006). Immunocompromised individuals, those undergoing

chemotherapy, and those subjected to prolonged steroid drug usage have all been connected with the risk of reactivation, too (Lacombe *et al*., 2006). HBV is categorized within a cluster of viruses called Hepadnaviridae. It is mainly hepatotropic sparking persistent infection which culminates to chronic liver disease (Tiollais *et al*., 1985).

2.2 The Hepatitis B virus structure

The HBV structure exhibits stable circular covalently closed (CCC) DNA that can endure within the liver for a prolonged period. Three viral particle types can be visualized using an electron microscope. Two of these particles consist of minute sphere-like structures, 20nm in diameter and filaments of varying lengths, 22nm in width (Bodworth *et al*., 1989). The highly virulent particle of the HBV (Dane Particle) is composed of a dual-shelled sphere-shaped configuration that is 42 nm in span, consisting HBsAg, HBcAg, HBcAg, and the viral DNA genome (Bodworth *et al*., 1989). The nucleocapsid offers protection and acts as an enclosure for the viral DNA as well as the DNA polymerase. It has an envelope comprising of complex proteins used while binding to the host and hence assist in gaining entry into the hosts cells (Bodworth *et al*., 1989).

A circular DNA molecule that is 3.2 kilobases long makes up the HBV genome and is relatively incomplete double-stranded (rcDNA) (Alter *et al*., 2006). The cccDNA has a 5' cap structure. It is polyadenylated and unspliced. Pregenomic RNA (pgRNA) has a different 5' end from the pre-core RNA, both of 3.5kb. The template responsible for reverse transcription is the pregenomic RNA (pgRNA). Pre-core RNA controls how the

pre-core gene is translated. Depending on the genotype, the genomes' length varies from 3182bp to 3248bp. The genome comprises 4 Overlapping Reading Frames (ORFs) (Mabeya *et al*., 2016). The four are polymerase (pol ORF), core (c ORF), XORF and envelope (SORF) (Figure 2.1) (Kao *et al*., 2011).

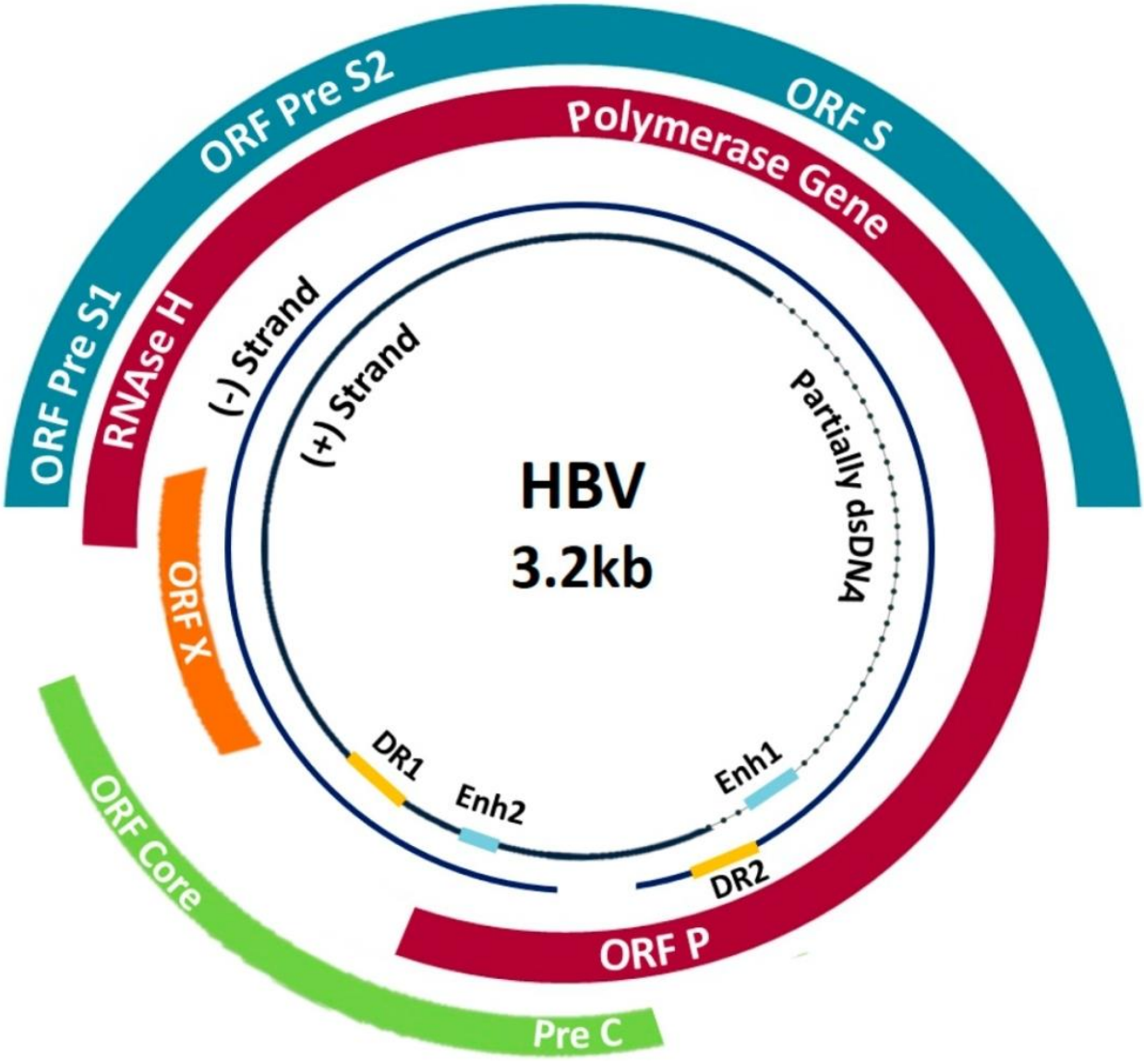

Figure 2.1 The Hepatitis B virus (HBV) genome, its structure and organisation (Kao, 2011)

2.3 Life Cycle

The HBV belongs to the Orthohepadnavirus genus and is of the family Hepadnaviridae and double stranded. Its life cycle can be summarized in five essential stages. In the attachment stage, it binds to the receptors of the target cell, then gains entry via clathrin-mediated endocytosis (Coffin *et al.,* 2018). Initially, the virus binds to heparin sulphate proteoglycan. It then binds tightly to sodium taurocholate co-transporting polypeptide (NTCP), which is commonly present within the sinusoidal casing of the hepatic cells (Figure 2.2) (Ganem *et al*., 2001).

HBV then joins with the hosts' cell membrane after being endocytosed by the hepatic cells through the penetration stage. The nucleocapsid of the HBV is then discharged into the cytoplasm (Ganem *et al*., 2001). During the uncoating phase, HBV replicates through an RNA acquired from host enzymes. Core proteins are then dissociated out of the viral DNA that is moderately dual-stranded (Figure 2.2). Then it is completely converted to double-stranded DNA (cccDNA). This is a prototype which enhances copying of four mRNAs (Ganem *et al.*, 2001).

Replication follows whereby new genome copies, capsid primary proteins as well as RNA-dependent DNA polymerase are made from the mRNA (Ganem *et al*., 2001). At the assembly stage, the resultant four transcripts undergo further processing, forming progeny virions freed from the cell (Bruss *et al*., 1991). The process of release follows where the mRNA is, at that moment, conveyed back to the cytoplasm, where reverse transcriptase activity synthesizes DNA through the Virion P protein (Bruss *et al*., 1991).

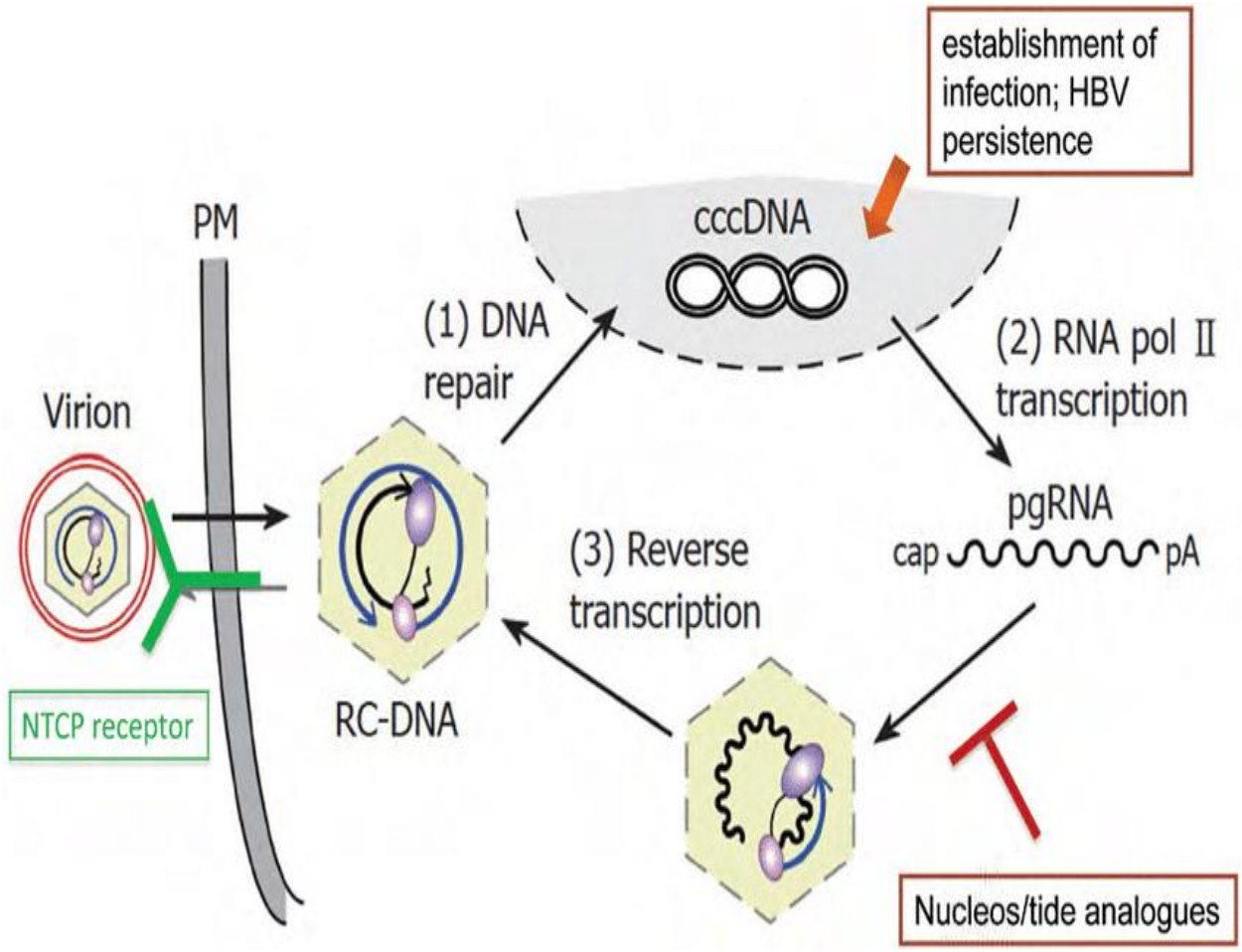

Figure 2.1 Summary of the HBV Lifecycle (Coffin et al., 2018).

2.4 Hepatitis B Virus Pathogenesis

The HBV thrives within the liver cells, thus affecting the normal operation of this vital organ. The viral infection leads to persistent infection, chronic infection, liver cirrhosis, hepatocellular carcinoma (HCC) and immune complex syndrome (Razavi *et al*., 2018). This virus doesn't in itself cause lysis of the hepatocytes, it is non-cytolytic. During infection, the body mounts an immune response, specifically by recruiting Cytotoxic T lymphocytes (CTL), with aim of getting rid of the infected cell. The mounted strong response by the CTL often results into clearance of the infection, albeit with an intense

episode of jaundice. However, incase of a weaker response, the infection would advance hence increasing the chances of development of hepatocellular carcinoma (Cameron *et al*., 2005).

2.4.1 Acute Hepatitis B Virus

The HBV infection could present with mild, subclinical and usually asymptomatic illness in approximately two-thirds of the infected individuals (Liang, 2009). This stage often passes without detection. The other third of infected individuals could exhibit hepatitis signs including jaundice, nausea and fever. About 1% of victims with acute HBV progress to acute liver failure and jaundice (Bastug *et al*., 2019). Sudden fever, jaundice, vomiting, abdominal pain followed by confusion, incomprehension, and coma marks the onset of fulminant hepatitis (Ichai *et al*., 2008).

2.4.2 Chronic Hepatitis B Virus

During the early stages, HBsAg, HBeAg and HBV DNA are often elevated in concentration, while serum aminotransferase may be slight or modest. The immune tolerance phase may kick in with elevated levels of HBV DNA and HBeAg, or an inactive carrier state may result in diminished undetectable titres of HBeAg which also causes a drop of the HBV DNA (Balaji, 2015). Intensity of this disease provides the general prognosis of persistent hepatitis. The endurance rate for victims suffering from severe chronic hepatitis and cirrhosis is about 50% (Wlodzimirow *et al*., 2013). Those with severe cirrhosis will show signs such as jaundice, splenomegaly, and gastrointestinal bleeding (Su *et al*., 2022). About one-third of chronic HBV patients will

eventually suffer long-term complications like HCC. The individual's immune status, age, genetic constitution and gender are host-specific conditions that could affect the advancement of chronic hepatitis (Kadiri *et al*., 2022).

2.5 HBV genetic diversity

The HBV has a sequence heterogeneity characteristic which is a a prototype of the Hepadnaviridae family (Kramvis, 2014). The virus is categorized into 10 genotypes, designated A through J, grounded on its phylogenetic history, with up to 8% variation in its nucleotide sequence. (Kramvis, 2014). Further, as many as thirty-five additional subgenotypes have been identified for A, D, F, H, and "I.". (Kramvis, 2014). Genotypes and subgenotypes are therefore critical determinants of HBV responses to NRTIs and essentially could be used in distinguishing HBV geographical prevalence (Kramvis, 2014). The various genotypes and subgenotypes are also instrumental in human migration tracking, predicting potential risk and preparedness, and in designing strategies to handle the emergence of infection (Kramvis, 2014).

According to a phylogenetic model based on the whole genome sequence of subgenotype A strains, only three categories, A1, A2, and A6, have been classified as variants or subgenotypes. The analysis identified four independent clusters confirmed by high bootstrap values (Pourkarim *et al*., 2010). The A3, "tentative A4", and A5 subgenotypes, which were previously considered separate groups, have now been merged into a single stem and are referred to as "quasi-subgenotypes." These three groups could not be distinguished as distinct subgenotypes based on genetic distances.

These findings support the revision of the HBV genotype A classification to A1, A2, "quasi-subgenotype A3," and A4 subgenotypes (Pourkarim *et al*., 2010). A2 is predominantly found in North America and Northern Central Europe, while subgenotypes A1, A4, and the quasi-subgenotype A3 are predominant in Africa (Kramvis, 2014).

Six B subgenotypes have been reclassified as B1, B2, B4-B6, and quasi-subgenotype B3 based on phylogenetic analysis and sequence divergence of >4%. The B1 is a subgenotype that was likely carried by the native populations who migrated from Alaska and Siberia to Greenland and North America, which later evolved into B5 (Osiowy *et al*., 2011).

The HBV genotype C is oldest among all geneotypes with a long history of endemicity in humans occurring in multiple subgenotypes ranging from C1 to C16 (Pancawardani *et al*., 2012). It has been shown that Indonesia happens to have a higher significant number of HBV subtype C subgenotypes. Nevertheless, the indigenous people of northern Australia, descendants of a pioneer group that left Africa have been shown as the only regions with carriers of subgenotype C4 (Hudjashov *et al*., 2007).

Genotype D has been confirmed to have six subgenotypes in previous studies. These subgenotuypes are distinguished from based on their signature amino acids and unique clustering with strong bootstrap support (Yousif *et al*., 2013). The study also found that "D8" was a genotype D/E recombinant and not a subgenotype. Additionally,

subgenotypes D3 and "D6" were reclassified as a single subgenotype D3. In Sudan, a subgenotype D4 recombinant has been discovered, and D4 is though to have first emerged in Africa (Yousif *et al*., 2013).

Genotype E is distinct from genotypes AD, F, H, and I by a 3-nucleotide deletion in the preS1 region. Genotype E is characterized by the distinct serological subtype ayw4 (Andernach *et al*., 2013). With little genetic diversity, this genotype is limited to western and central Africa. Except among those who descended from Africans, genotype E is only encountered in Africa (Kramvis *et al*., 2007). The four subgenotypes, F1-F4 that comprise the genotype F isolates are all members of the serological subtype adw4. This subtype is predominant among the Amerindian from Alaska, the Central region of America, and Southern parts of America (Kramvis, 2014).

The 36-nucleotide insert that lies 3′ of position 1905 and the two translation stop codons that halt HBeAg production at positions 2 and 28 in the pre-core/core region define genotype G (Kramvis *et al*., 2005). Chronic infection only occurs when genotype A is present to supplement HBeAg in trans. Another significant risk determinant factor is gayism. The only similarities between genotypes G and E are the three nucleotide deletions in the core region and the unique sequence in preS. Even though genotype G has not yet been found in Africa, it is believed to have originated there (Kramvis *et al*., 2005). Genotype H is prevalent amongst mestizos and Aztecs in Mexico, with genotype F being closely associated with it (Panduro *et al*., 2013).

In 2008, a ninth Vietnamese-related genotype, I, was proposed (Arankalle *et al*., 2010). Recombinant genotypes A/C/G and an unknown genotype make up genotype I. In the polymerase, genotype A is clustered with respect to genotype C on examination of the primary genome (Arankalle *et al*., 2010).

Genotype J was first discovered in Hepatocellular carcinoma (HCC) in a man of Japanese descent (Tatematsu *et al*., 2009). Full genomic clusters of nonhuman HBV, including gorillas and chimpanzees, are found. Upon further investigation, the 'J' genotype has actually been discovered to be a genotype C recombinant (Locarnini *et al*., 2013). This genotype is also understood to manifest and maintain itself by transmitting across different primates (Locarnini *et al*., 2013).

2.6 Diagnosis

Laboratory diagnosis and testing to confirm HBV infection often focuses on detecting the HBsAg, HBeAg, HBcAg, anti-HBcAg, anti-HBsAg and IgM markers using enzyme immunoassays, immunodiffusion techniques, dot blot hybridization and PCR. Positive results for HBsAg and IgM indicate that there is an acute HBV infection. Throughout the early stage of infection with HBV, Individuals usually tests positive for HBeAg, symbolizing a high viral replication rate (WHO, 2018). Upon testing positive for HBeAg's in bodily fluids and blood, the patient is considered to be potentially infectious (WHO, 2018).

2.7 Worldwide patterns of HBV genotypes and subtypes

The HBV genotype has a definite association with HBV endemicity, transmission methods, and clinical manifestations (Kao, 2011). It's also noted that HBV genome variations have clinical and epidemiological implications (Kao, 2011). The various genotypes have distinct geographical distributions. Ten HBV genotypes ranging from A-J have been identified. The HBV genotype A is mainly found in Africa, America, India and Europe and is considered the most common strain within these areas. Genotypes B and C are commonly found in the Asia-Pacific region. The genotype D is commonly found in various world regions such as Africa, Europe, the Mediterranean, and India. Genotype E is found exclusively in West Africa, while genotype F is typically in South and Central America. The genotype G has been detected in Germany, America, and France. Genotype H has been detected in Mexico and Central America, while Genotype I has been traced in Vietnam and Laos (Tran *et al*., 2008; Phung *et al*., 2010). The HBV has several genotypes commonly found in different regions of the world. Genotype A has a strong presence in Africa, India, America, and Europe , and is considered the predominant strain in these regions (Tran *et al*., 2008; Phung *et al*., 2010).

On the other hand, Genotypes B and C are frequently observed in the Asia-Pacific region. The D genotype is commonly found in various world regions such as Africa, Europe, the Mediterranean, and India. The E genotype is found exclusively within West Africa, while the F genotype is typically in South and Central America. The genotype G has been detected in Germany, America, and France. Genotype H has been detected in Mexico and Central America, while Genotype I has been traced in Vietnam and Laos.

Lately, Japan was where a novel HBV genotype J was initially identified (Kiem *et al*., 2007). According to a molecular epidemiological study conducted in Taiwan, two types of HBV strains are prevalent in the region. These strains are adw (70%) and adr (30%). All adr strains were found to belong to genotype C, whereas for adw strains, 81% belong to B genotype and 12% to C genotype respectively (Kim *et al*., 2007).

Similarly, a study conducted in Korea found that HBV genotype C2 had an overwhelming prevalence in patients with 92.3% of the cases being of the adr serotype and 5.7% of the adw serotype. The HBV genotype J, a newly discovered variant, was found in Japan (Tatematsu *et al*., 2009). Two types of HBV strains are prevalent in the Taiwan region. These strains are adw (70%) and adr (30%). All adr strains were found to belong to genotype C, whereas for adw strains, 81% and 12% correspond to the respective genotypes B and C (Table 2.1) (Liu *et al*., 2002). A survey conducted in Korea found that HBV genotype C2 had an overwhelming prevalence in patients with CHB; The proportion of adr and adw serotypes were, respectively, 92.3% and 5.7 percent (Kim *et al*., 2007).

Different transmission modes of the HBV are evident in various parts of the world, depending on the HBV genotypes present in those regions (Kao and Chen, 2002). For instance, HBV genotypes B and C are widespread in highly endemic areas, mainly in Asian countries, where mother-to-infant or perinatal transmission is the primary mode of spreading HBV. However, the rest of the genotypes are found in areas predominated with horizontal transmission. However, the most common routes to HBV infection are

adult sexual interactions, blood transfusions, and intimate personal contact between young children. Specific HBV genotypes show geographical distribution based on transmission modes. A study conducted in Taiwan utilized HBV genotyping to determine the HBV transmission mechanisms within families (Lin *et al*., 2005). The study revealed that prevalence of HBsAg carriage was noticeably higher in kids from households with clustering HBV carriers compared to the general population (77.8% vs. 15%). It was discovered that HBV can be transmitted within families by determining that carriers' parents and offspring share the same HBV genotype (Lin *et al*. 2005). When there is no effective national hepatitis B vaccination program, this mode of transmission may also affect the prevalence of HBV. A good number of acute HBV patients seeking treatment in Japan have exhibited HBV genotype A, which has been linked to their promiscuous sexual behaviours. (Yotsuyanagi *et al*. 2005).

The proportion of patients with HBV genotype A presenting with chronic HBV infection rose from 1.7% to 3.5% in Japan. Genotyping of the HBV genome is important since it could be used to correlate the distribution of various genotypes with the multiple means of transmission associated with communal and behavioural interactions. Therefore, genotyping of the HBV virus is an important epidemiological pointer to predict demographic distribution based on social interactions (Matsuura *et al*., 2009). However, many regions of the globe are inaccessible making studies to be based on a minimal number of patients examined. Further, the distribution of HBV genotypes may differ with span and people migration, as exemplified in a current study in the United

States. Again, subtypes have now been identified within some genotypes (Fung *et al*., 2004). Kramvis *et al*. state that there are at least 24 subtypes (Table 2.1).

Table 2.1: Variants and Genotypes of the Hepatitis B Virus (Lin et al., 2005).

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

2.8 The HBV prevalence in East Africa

Recent HBV epidemiological and pooled prevalence studies reported a typically high rate of (6.03%), with studies in Kenya yielding (8.54%). In comparison, Uganda showed a rating of (8.454%). Amongst gravid women, Ethiopia had recorded a prevalence rate

of (4.8%), Rwanda at (3,7%) and Tanzania at (5.2%). HIV seropositivity, promiscuous sexual behaviours, male gender factor and blood transfusion were identified as the significant driving factors towards HBV infections. (Kafeero *et al*., 2021). Studies conducted between 2014 and 2016 amongst the adversely risky populations of western Kenya came up with a prevalence of 10.7% (Karoney *et al*., 2020).

A recent research on HBV prevalence among HIV-coinfected individuals in Kenya, yielded a prevalence rate of 8.2% (Makokha *et al*., 2023). The study reported a high heterogeneity, which was attributable to the prevailing risk factors and the specificity of behaviours in certain study regions. In another study conducted in Nairobi, HBV seroprevalence of 7.25% was detected with HBV genotype A as the most prevalence HBV strain (Mabeya *et al.,* 2016). In previous studies conducted in a rural set-up population of Kenyans of varied age groups, HBV prevalence of 8%, have been detected (Mutuma *et al*., 2011). However, 9.3% prevalence of HBV infection among preganct women in eight regions of Kenya, namely Nairobi, Coast, Nyanza/Western, Rift Valley, Eastern, and Central, has also been detected (Okoth *et al.,* 2006). The detected levels of HBV prevalence in expectant women increases fears about the risk of transmission towards newborns, and the potential of neonates chronic carrier states. Nevertheless, in studies conducted among the HBV-HIV coinfected individuals presenting with jaundice in Nyanza, the HBV of 47% was detected which is very high (Otedo, 2004).

2.9 HBV disease progression and treatment

The HBV infection causes liver inflammation which manifests in varying severity. This can then advance to prolonged persistent infection, which slowly presents with cirrhosis, with some progressing to carcinoma (Rossner, 1992). It is expected that upon HBV infection, these individuals should start treatment right away to prevent the condition from advancing to the chronic stage.

Due to the high cost of medications, HIV patients in Kenya and other low-income countries sometimes receive regimens that only include lamivudine as the primary active agent against HBV (Kim *et al.*, 2011). Consequently, when employing single nucleoside or nucleotide medicines, the efficacy of HBV treatment deteriorates following drug resistance (Mabeya *et al.,* 2017). To manage HIV-HBV co-infection and prevention of the establishment of medication resistance, it is recommended to employ a combined therapy consisting of lamivudine (3TC), tenofovir (TDF) and efavirenz (EFV) (Mabeya *et al.,* 2017).

2.9.1 Acute and Chronic hepatitis

An acute infection may result upon first time contact with HBV. Most adults with sound health can cure without the manifestation of any symptoms. The acute phase can last six months. During this period, the infected may be with or without any signs or symptoms. It is also highly transmissible to others. It is uncommon among adults and the elderly since their immune system is more robust, unlike the young, and opportunities for infection are minimal, respectively. Risk factors among the elderly include sharing bath

towels and brushes, shavers, sexual intercourse, and syringes (Thompson *et al*., 2009; Tajiri *et al*., 2013). Clinical symptoms among the elderly are similar to those in the younger generation. A study conducted among the elderly domiciled at a nursing home for the old confirmed that most of the infected were asymptomatic and did not require specialized treatment in a hospital (Kondo *et al*., 1993). Simple, rapid tests can ascertain the presence of an infection with HBV. Frequent checks on liver health and functions should be strictly observed during the six months. A negative HBV report is expected after six months (Kondo *et al*., 1993). Patients with confirmed positive infection should be advised to protect others from contamination. Close relatives and friends should also be encouraged to get tested for HBV and partake in immunization if not infected (Kondo *et al*., 1993).

Acute infection can manifest with symptoms like jaundice, nausea, anorexia, myalgia, pyrexia, vomiting, and tummy discomfort. These signs manifest within 60 to 150 days of contamination. Hospitalization may be recommended for those with severe symptoms. A rare presentation of the disease "fulminant hepatitis", which is fatal, can occur in case of a re-infection with acute HBV. Urgent hospitalization would be paramount in such cases to avert sudden liver failure (Chang, 2007). Strict adherence to a healthy diet and avoiding the use of drugs such as cigarettes and alcohol can promote faster recovery (Chang, 2007).

Acute HBV harbours no permanent effects. A successful recovery from acute HBV infection means that detection of the the virus in blood is no longer viable but may

prevail within the liver in a dormant state. In such a state, infectivity is null, while re-activation may happen if the patient's immunity is compromised (Chang, 2007). Immunosuppressant drugs should be avoided at all costs by early disclosure to healthcare providers of a past infection (Chang, 2007).

All tests that turn positive after the sixth month of infection are considered chronic infections. HBV infection anchors in the blood circulation and the liver when the patients immune system cannot mount a robust response (Simonetti *et al*., 2010). The age at which a person is initially exposed to chronic HBV may have an impact on the illness's course. At least 90% of all infants infected progress to chronic disease, while children aged 1 to 5years have a 50% chance of advancing to chronic status. 90% of all infected adults recover, meaning about 10% progress to chronic HBV infection. Most people who test positive for chronic HBV live a healthy life (Simonetti *et al*., 2010).

Although chronic HBV infection may be asymptomatic, the affected individuals have a higher possibility of progressing to liver cirrhosis which could results into hepatocellular carcinoma (Yu *et al*., 1997). In order to avert this, regular liver function tests (LFTs) should be done by a specialist, avoid the use of substances that would aggravate the situation, such as alcohol and cigarettes and observe a healthy diet (Yu *et al*., 1997).

2.9.2 HBV Natural Course of Infection and Consequent Immune Responses

The HBV results in a severe infection that may progress to either acute or chronic stages of the disease. This infection without recovery or treatment could lead to liver cirrhosis

and in few cases causing liver cancer which risk death. It has a high prevalence in the western Pacific and African regions at around 6%. Due to the massive vaccination uptake in American regions, HBV is rarely seen. (Hennessey *et al*., 2013). The transmission of HBV from mother to her newborn can happen at the time of giving birth, horinzontal route, contaminated needles, and through body fluids vaginal secretions, blood, semen, saliva and mentral fluids. Most people do not present with symptoms, while others present with a serious illness characterized by symptoms like, extreme fatigue, jaundice, the urine turns dark, nausea, abdominal upset and vomiting. A small percentage of the infected subjects may present with acute liver failure and death. In certain individuals, HBV can result in chronic persistent liver failure, cirrhosis, and liver cancer. (Williams, 2006).

The host cell provides the HBV virus with the necessary metabolic requirements for the generation of new progeny. Hepatitis B Virus has a protective outer shell or capsid and contains DNA inside it. The HBV attaches on hepatoctytes on liver cells, which are also referred to as hepatocytes. It gets engulfed into the cell, where the casing is taken apart, and the DNA is moved to the cell nucleus, which serves as the cell's control center. It utilizes the hepatocytes' cellular machinery to replicate itself by employing its own DNA. The HBV then leaves the liver cell once it has achieved the necessary proteins and the DNA is successfully assembled. At this stage, the virus is ready to infest another hepatocyte. (Lamontagne *et al*., 2016).

As HBV invades and spreads through hepatocytes, the body's immune system is activated and begins attacking the infested cells. This attack marks the commencement of recruitment of immune cells such as Cytotoxic T-cells. These cells act as killer cells whose mode of action is by targeting only the cells infested by HBV (Stanley,2009). The process is called cell-mediated adaptive immune response (Stanley, 2009). T-cell activation occurs when the T-cell receptor and a co-stimulatory molecule identify an antigen presented by the major histocompatibility complex (MHC) (Huard *et al*., 1996).

Major Histocompability Complexes (MHCs) are on the surface of antigen-presenting cells (APCS). They isolate and mark the virus as a potential threat to their well-being, break it down converting it into a form that can be recognized by T-cells. Following this, the T-cells transform into T helper cells, which generate cytokines that elicit evolution of cytotoxic T lymphocytes (CTLs) (Schuch *et al*., 2014). Cytotoxic T lymphocytes (CTLs) generate toxic proteins which functions as toxins for target cells. Holes are poked into all the infected cells, causing their contents to leak out and ultimately resulting into the cell's death (Huard *et al*., 1996). The hepatocytes die in a fashion that imparts destruction of the liver and the symptoms, as mentioned earlier, seen in hepatitis B (Iannacone *et al*., 2022).

Specifically, the CD8 T-cells form a significant component that is effectively coined as cell-mediated adaptive immune response. They typically play a role in defending against pathogens including cancer cells. There are two principal signals needed for correct

activation of CD8 cells; first, the identification of MHC-1-Antigen complex on APCs, and second, the generation of co-stimulatory signs by the APCs (Schuch *et al*., 2014).

There are specific CD8 T cells that are significant in eradication of HBV infection. The best viral load clearance has been associated with higher titres of CD8 cells that are HBV-specific (Schuch *et al*., 2014). Minimal self cells destruction is experienced when HBV-specific CD8 T-cells are recruited (Schuch *et al*., 2014). This is mediated by the secretion of inflammatory cytokines such as IFN Gamma and TNF by the CD8 T-cells. However, virus-specific CD-8 T cell responses are rarely detectable in chronically HBV-infected individuals (Schuch *et al*., 2014). This is because of their functional impairment, which is linked to decreased expression of co-stimulatory molecules, a reduced capacity for proliferation, and compromised cytokine secretion (Schuch *et al*., 2014). This prevents the clearance of HBV infection and results in the aforementioned more chronic severe symptoms. Patients with high levels of viremia from HBV infection exhibit a more severely dysfunctional CD8 T-cell profile; therefore, the CD8 T-cell response plays a key role in eliminating HBV (Heim *et al*., 2021; Schuch *et al*., 2014).

In chronic hepatitis B, multiple mechanisms culminates to impaired role of HBV-specific T cells (Ye *et al*., 2015). The result is exhausted T cells characterized by dysfunctional proliferation, diminished cytokine production and heightened apoptosis or cell death as the viral load rises (Ye *et al*., 2015). These dysfunctional cells increasingly express inhibitory factors such as Protein Disulphide Isomerase (PD1), Cytotoxic T-Lymphocyte- associated Protein 4 (CTLA-4) and TIM3, exacerbating their lack of

responsiveness, eventually resulting in a shortage of CTLs capable of attacking the HBV (Kumar *et al*., 2021; Murakami et al., 2014).

Ultimately in severe stages of T-cell exhaustion, virus-specific T cells become entirely deleted, and thus the virus is now cleared, contributing to the chronic nature of the disease (Wherry, 2011). Regarding prevention, a vaccine that offers 98–100% protection against HBV infection is currently accessible and safe. The WHO advises that all newborns should be given the HBV vaccine within the first 24 hours of birth (CDC, 2008). Prompt vaccination can help halt perinatal transmission. However, knowing that this is just a preventative measure is essential. Currently, there is no definitive cure for acute HBV. Providing comfort and ensuring adequate nutrition balance, including replacing fluids lost from vomiting and diarrhoea, should be the primary concern of the caregivers. Chronic hepatitis B can be managed with medicine, such as oral antiviral drugs. Therapy can stall the advancement of cirrhosis, hence improving survival chances (Martin, 2014).

2.9.3 Pathogenesis of Liver Disease in HBV/HIV co-infection

Studies reveal that progression of HBV ailment is associated with various factors, including the virus, host, and environment characteristics. When an individual is coinfected with both HBV and HIV, their immunity gets compromised, triggering accelerated progression of HBV infection (McGovern, 2007). Chances of developing chronic HBV are higher among those who have HIV (Koblin *et al*., 1992). Additionally, people experience slim chances of clearing HBeAg and HBcAg, and a lower rate of

developing anti-HBe and anti-HBs antibodies, while having higher titres of HBV DNA (Di Martino *et al*., 2002; Modise, 2019).

The acceleration of liver damage leading to cirrhosis and HCC represents the most extreme outcome. Individuals who are co-infected with HBV/HIV face an increased risk of developing HCC, up to five to six times higher, especially when it is accompanied by cirrhosis (Robbins *et al*., 2014). Moreover, HIV/HBV co-infection hastens the progress of liver cirrhosis (Soriano *et al*., 2010). CD4+ T cells are critical for recognising viral antigens and regulating the activities of other immune cells. Host immunosuppression, which is characterised by exhaustion of CD4+ T cells is crucial for HIV to change the typical progression of HBV, resulting in a rise in liver-related deaths (Cheng *et al*., 2021; Joshi *et al*., 2011). Inhibiting the host's immune response can greatly enhance HBV replication, causing severe liver damage (Cheng *et al*., 2021; Soriano *et al*., 2010).

Hepatocytes infected with HBV generally do not display any discrete cellular damage or viral cytopathic effects (Lokhande *et al*., 2011). However, individuals with co-infection of HBV and HIV may experience fibrosing cholestatic hepatitis. Changes in the hepatic cytokine environment characterise this condition (Revill *et al*., 2007). Earlier studies have demonstrated that HIV glycoproteins stimulate the production of tumour necrosis factor-related apoptosis inducing ligand (TRAIL) in hepatocytes, culminates to programmed cell death (Babu *et al*., 2009).

In addition, HIV can trigger programmed cell death utilizing mechanisms that do not require caspases or enzymes that are crucial in apoptosis. Liver cells death can also be induced by HIV through macrophages and hepatic stellate cells. This leads to inflammation and fibrosis of the liver (Cheng *et al*., 2021; Jeyarajan *et al*., 2020).

Previous study in this area confirms that HBV/HIV-coinfected individuals experience higher manifestations of hepatocyte apoptosis as opposed to individuals with HBV mono-infection. HBV can infect hepatocytes without necessarily causing immediate or obvious harm to the cells themselves, a key characteristic of HBV's ability to establish chronic infections in some individuals (Cheng *et al*., 2021). However, individuals co-infected with HBV and HIV may exhibit fibrosing cholestatic hepatitis (Revill *et al*., 2010). When the liver is co-infected with HIV and HBV, the interaction between these viruses can result in an intricate immune response, potentially causing alterations in the generation and control of cytokines (Svegliati-Baroni *et al*., 2010).

Reports indicate that HIV glycoproteins prompt hepatocytes to produce the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which leads to termination by death of the affected liver cells (Cheng *et al*., 2021; Babu *et al*., 2009). Hu7 cells death can also be triggerd by the HIV envelope protein without involvement of the caspase activation (Cheng *et al*., 2021; Vlahakis *et al*., 2003). HIV infection causes the death of liver cells by promoting their engulfment by macrophages leading to inflammation and liver scarification (Cheng *et al*., 2021). Hepatocyte apoptosis levels

are higher in patients with both HBV and HIV infections compared to those with only HBV infection (Cheng *et al*., 2021; Iser *et al*., 2011).

The HIV gp120 is a protein that enables the virus to penetrate host cells and induce infection. Investigations have revealed that it can provoke inflammation in the liver by boosting the production of a molecule called IL-8. Similarly, the X protein of HBV (HBx) can also augment IL-8 levels through characteristic signalling pathways (Cheng *et al*., 2021; Crane *et al*., 2012). IL-8 is a critical molecule that attracts white blood cells to the liver and helps sustain the inflammatory environment that plays a role in emergence of liver cancer. (Yu *et al*., 2011). In addition to IL-8, HBx can also increase the production of another molecule called COX-2, which is typically highly expressed in cirrhosis cases (Mohammed *et al*., 2004).

Interleukin 8 can also trigger COX-2 activity, leading to an even more inflammatory environment. The liver's COX-2 and IL-8 are activated by HBV proteins, leading to ongoing inflammation and liver damage. (Zhang *et al*., 2014). One consequence of this inflammation is the secretion of a molecule called CXCL10, which is connected to the harshness of liver deterioration induced by viral hepatitis. CXCL10 attracts immune cells to the liver, contributing to the ongoing inflammation. Levels of CXCL10 are elevated amongst HBV HIV coinfected individuals, suggesting this molecules involvement in accelerating liver disease (Singh *et al*., 2020; Cheng *et al*., 2021).

2.9.4 Hepatitis B Virus Treatment

Individuals presenting with chronic HBV manifesting conditions such as decompensated cirrhosis, co-infections (HIV, HCV, HDV), individuals undergoing hemodialysis, experiencing renal failure, or in immunosuppressed conditions (such as transplant recipients, children, and pregnant women) should begin antiviral treatment as promptly as possible (Zhou *et al*., 2017). It does not matter if the symptoms are at the clinical stage or if the CD4+ cell count is still normal (Cheng *et al*., 2021; Zhou *et al*., 2017). This is paramount because advanced immunodeficiency may lead to inefficiency of HBV treatment. (Nunez *et al*., 2003). Mendes-Corrêa *et al*. (2010) note that exclusive use of antiviral drugs against HBV can lead to HIV drug resistance. Dual anti-HIV and anti-HBV are recommended (Nunez *et al*., 2010).

The available antiviral choices are categorized into host-based action and virus-based action. These comprise monoclonal antibodies and immunomodulators and nucleoside/ nucleotide reverse transcriptase inhibitors (NRTIs), respectively (Cheng *et al*., 2021). HBV and HIV comparative lifecycles can be utilized in the production of dual antiviral agents. The structures and functions of polymerase found in HBV and reverse transcriptase in HIV are analogous, suggesting that drugs targeting these proteins can disrupt the life cycle of both viruses (Nassal *et al*., 2008; Manendez-Arias *et al*., 2014).

The NRTIs principal mechanism of action is to disturb the function of enzyme polymerase as well as reverse transcriptase in HBV and HIV respectively, by establishing competition with innate nucleotide substrates that fuses with DNA

sequences (Cheng *et al*., 2021). They function as chain terminators due to lack of the 3'-OH and thus stop DNA synthesis (Cameron *et al*., 2009; Bazzoli *et al*., 2010). Nucleoside reverse transcriptase inhibitors (NRTIs) block HIV's replication ability by inhibiting reverse transcriptase enzyme, which converts the virus's RNA into DNA (Menéndez-Arias *et al*., 2014) (Figure 2.3). Cyclophilin A (Cyp A) is an enzyme that helps fold and assemble proteins (Gürtler *et al*., 2013). It has been reported to enhance the replication of various viruses, which include HIV and HBV, and is also associated with the pathogenesis of these viruses. Therefore, blocking the action of Cyp A could be a potential strategy for combating infections caused by HIV and HBV (Tian *et al*., 2010).

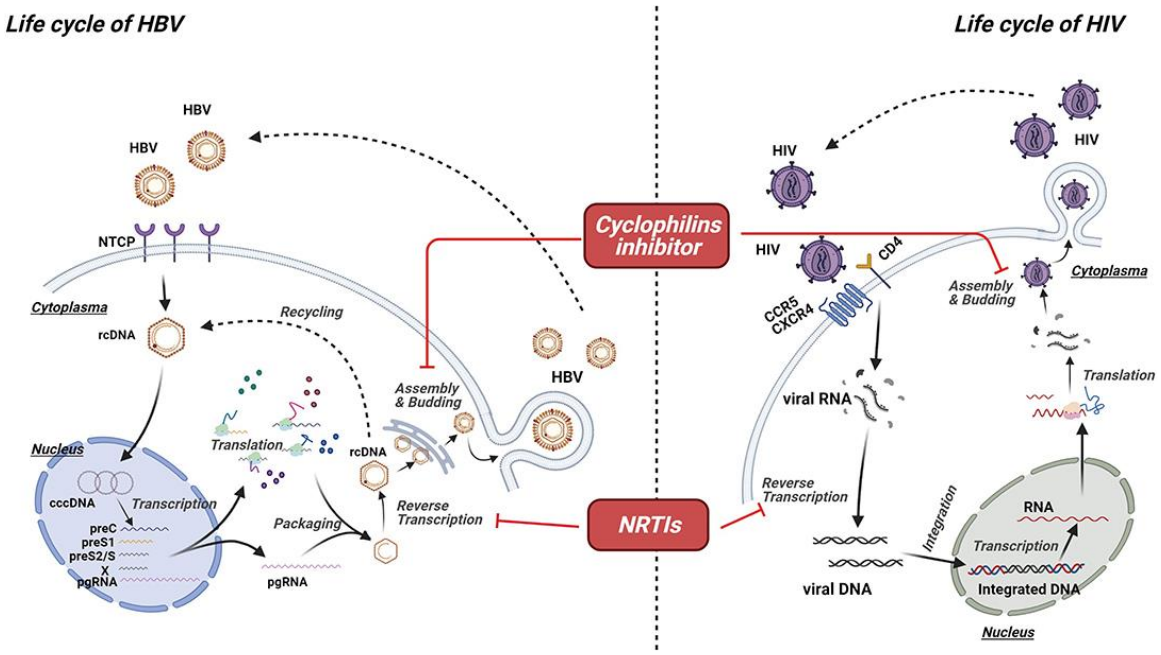

Figure 2.2 Mechanism of virus-targeted (Cheng *et al*., 2021).

When the body detects invasion by viruses, an immune response is instigated, activating transcription elements and, discharging antiviral agents such as interferons (IFN) and cytokines (Sato *et al*., 2015). IFN triggers receptor associated enzymes such as JAK1 and Tyk 2 leading to formation of a homo/heterodimer complex. The heterodimer complex initiates production of viral restriction enzymes with high potential of viral replication inhibition (Yuen *et al*., 2018).

The IFN's capacity to enhance the hosts' immune response provides a platform for the management of viral infections like HBV and HIV. Adaptive immunity which is highly dependent on T cells which triggers programmed cell death (PD-1) can also be employed in viral infection control by reversing their function of detecting compromised immune response to enhance a corresponding high iantiviral effects (Feray *et al*., 2019). HBV infection can cause severe liver damage and is a and is a major contributor to mortality among coinfected people. Therefore, HBV treatment's main goal is to reduce liver inflammation by suppressing its replication. There are various antiviral medications available for both mono and dual infection cases. However, the best option for coinfected patients is combination antiviral therapy (cART) (Cheng *et al*., 2021). Lamivudine (3TC), whose mode of action involves chain termination, virus regeneration suppression, and viral load replication, has antiviral factors on both HIV and HBV. This is a type of cytosine analog called dideoxynucleoside (Scot, 2020).

Lamivudine agents have a half inhibitory concentration of 0.002 to 1.14uM, giving them potent antiviral ability against diverse HIV strains (Shinazi *et al*., 1992). Lamivudine

Single-tablet regimen (STR) proves to be successful in HIV patients Cahn., 2017). Despite Lamivudine's potent antiviral abilities, they exhibit drug resistance, a typical characteristic of nucleoside analog agents (Ryon *et al*., 2020). It is, therefore, advisable to apply combination therapy with TDF and TAF for all coinfected cases (Ryon *et al*., 2020).

Emtricitabine is an NRTI which, in combination with other ART, is used to treat HIV-1, HIV-2 and HBV (Muller *et al*., 2023). It is used in combination with other medications in controlling HIV spread as well as for pre-exposure prophylaxis (PrEP) (Muller *et al*., 2023; Saravolatz *et al*., 2006). NRTI emtricitabine is an influential ART for HIV-infected gravid women administered along with Lamivudine, Tenofovir and Abacavir (Muller *et al*., 2023; Saag *et al*., 2018). It is paramount to administer these ARTs early enough to curb neonatal infection. Emtricitabine effectively treats patients who have both HIV and hepatitis C infection (HCV), as it has minimal interactions with HCV medications (Muller *et al*., 2023; Saag *et al*., 2018). It doesn't cause teratogenesis and other affiliated abnormalities.

Tenofovir Disoproxil Fumarate (TDF), along with other agents of medication, is used in treating HIV infections. It is also used to manage chronic HBV infections (Avihingsanon *et al*., 2023). Tenofovir Alafenamide (TAF) is also effective for managing chronic HBV infections. Tenofovir is an NRTI whose mode of action involves decreasing HBV and HIV DNA in the blood (Avihingsanon *et al*., 2023). It may not cure HIV, but it

decreases the chances of aggravating to immunodeficiency syndrome and other related symptoms (Avihingsanon *et al*., 2023).

Several other NRTIs, such as Entecavir, telbivudine and adefovir, exhibit strong antiviral responses against HBV, though with minimal effect on HIV (Bethesda, 2012). However, their remarkable suppressive results on HBV replication qualify them for combating HBV/HIV co-infection in combination with other agents (Bethesda, 2012). Adefovir has potent antiviral activity against HBV with minimal resistance reported (Tillmann, 2007). However, it causes nephrotoxicity in HIV cases (Soriano *et al*., 2007). Additionally, Adefovir improves the conversion of HBeAg to anti-HBe, stabilizes ALT levels, and delays liver damage and cirrhosis (Benhamou *et al*., 2001).

Entecavir suppresses HBV DNA in serum more effectively than Emtricitabine and Adefovir. It has been proven to be active in the elimination of HBV strains that may be resistant to emtricitabine and also adefovir (Soriano *et al*., 2011).

The CRV431 is a non-immunosuppressive cyclophilin inhibitor which triggers an interruption of the interaction of the cyclophilin A, thus resulting in HBV DNA seroconversion (Bobardt *et al*., 2020). Various trials have also achieved ameliorated liver tumor burden and fibrosis, marking CRV431 as a potential novel therapy for liver ailments (Sun *et al*., 2021). When a host cell detects the presence of a pathogen, it produces defensive proteins like IFN to fight off the invaders. In chronic cases of HBV infection, induction treatment comprises IFN-α and PEG-α, known for inhibiting viral

replication and enhancing the host's defence mechanisms (Wilkins *et al*., 2013; Lee *et al*., 2017).

2.9.5 Hepatitis B Virus Drug Resistance

Studies on HBV therapy and consequent development of polymerase inhibition based antiviral agents has enhanced treatment of this infection (Zoulim, 2011). Zoulim notes that the virus's ingenious survival tactics against the antiviral agents continue to challenge effective treatment. Worsening liver disease has been attributed to resistance to antiviral nucleoside agents, which gets worse with the continuous administration of drugs, leading to multi-drug resistance (Lok *et al*., 2009). Resistance to NRTIs and cross-resistance are specific to HBV structure. Five NAs that have been certified can be classified into three major groups (Warner *et al*., 2014).

The first group comprises L-nucleoside analogues, including telbivudine (L-dT) and lamivudine (LMV), along with their 5-fluoro derivatives, emtricitabine and adefovir (ADV). The second group encompasses acyclic nucleoside phosphonates, defined by the dAMP correspondent tenofovir (TFV) and adefovir (ADV) (Warner *et al*., 2014). The third group involves deoxyguanosine analogues like entecavir (ETV), in which a cyclopentane sugar derivative takes the place of the deoxyribose molecule (Warner *et al*., 2014).

The development of unresponsiveness to certain analogues is linked to distinct mutations present around reverse transcriptase regions of the HBV polymerase (Warner *et al*.,

2014). Heightened replication then follows as a link to compensatory mutations. Incorporation of NRTIs as a distortion to HBV DNA replication is thus hampered, causing a malfunction of the intended result. Viral replication progresses undeterred, enhancing high-yield viral DNA. Multidrug resistance may occur owing to multiple hybrids of mutations. Many viruses may also alter the surface proteins through the overlapping surface gene, making them unresponsive to NRTIs (Warner *et al*., 2014).

Tenofovir disoproxil fumarate (TDF) is now known to be highly effective against HBV. However, before TDF was confirmed and endorsed, Lamivudine was widely used without combination in the treatment of HIV patients (Benhamou *et al*., 1999). According to Benhamou *et al*., patients who received Lamivudine monotherapy before the TDF regimen faced a higher risk of developing lamivudine resistance. It was observed that single mutations at rtM204V/I, double mutations at rtL180M + rtM204V, and triple mutations at rtV173L + rtM204V + rtL180M occurred among patients who received Lamivudine monotherapy (Delaney *et al*., 2003). Triple mutants that occasion surface mutations sE164D and sI195M act as vaccine escape modes, triggering the debate of possible infections among those already vaccinated (Delaney *et al*., 2003). These mutations in HBV occur because of the overlapping reading frames (Torresi *et al*., 2002).

ADV resistance is much slower to develop than LMV. It is commonly associated with HBeAg-negative CHB patients and with patients who are attempting to manage LMV failure. ADV resistance can be provoked by any substitution at rtA181V/T and/or

rtN236T through nucleotide transformations (Warner *et al*., 2014). TFV resistance can result from substitutions at rtA194T, but only if LMV resistance is also triggered. This is often seen in patients who have a co-infection with HIV-1 (Warner *et al*., 2014). Patients with a prior history of LMV failure are frequently associated with ETV failure. This condition arises when mutations occur, leading to alterations in nucleotides in at least one of rtM250V, rtT184G, rtS202I, or rtI169T (Warner *et al*., 2014).

On multidrug resistance, Warner *et al* asserts that, only two mutations, rtA181 T and rtL180M + rtM204V, are associated with multidrug resistance out of the eight codons (rtI169T, rtT184G, rtL180M, rtA181T/V, rtS202I, rtM204V/I, rtN236LT, and rtM250V) related to primary drug resistance (Warner *et al*., 2014). Several other factors enhance the barrier to resistance, such as viral fitness, virulence mechanisms, the ability to establish cross-resistance, inconsistency in patient adherence to therapy, the potency of the antiviral agent, and virus genetic barrier mechanisms. Continuous research is paramount to establish new viral and immune targets that can be employed in the production of even more effective novel antiviral drugs (Zoulim, 2011). Using potent antiviral analogues from the onset is critical to eliminating the infection. Unfortunately, many regions have not achieved this due to cost-related factors.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The Kisii Teaching and Referral Hospital, located in the county of Kisii Kenya and coordinates 0°40′14″S 34°46′17″E / 0.670658781297077 3°S 34.77137216690011°E, is a public hospital that holds an accreditation of Level 6 by Kenya's Ministry of Health, making it the most comprehensive hospital in the County (Arasa, 2019). It functioned as a district hospital for several years before being elevated to a Level 5 hospital in 2007 and then to a Level 6 hospital in 2014 (Herbert *et al*., 2017). It is presently associated with the Kisii University School of Medicine (Herbert *et al*., 2017; Waithera *et al*., 2017). It is 320 km from Nairobi City. It borders Nyamira to the northeast, Narok to the south, and Homabay and Migori to the west (Figure 3.1). This county has nine constituencies (Mokua *et al*., 2019). The area is comprised of 9 sub-counties which are divided into 24 divisions, 75 locations, and 190 sub-locations, spanning an expanse of 1,317.5 square kilometres (Opano, 2013). The County's estimated population in 2012 was 1,236,966, with 597,934 males and 639,032 females (Opano, 2013). According to estimates, In 2012, the county's population totalled 1,236,966, with 597,934 males and 639,032 females. (Opano, 2013).

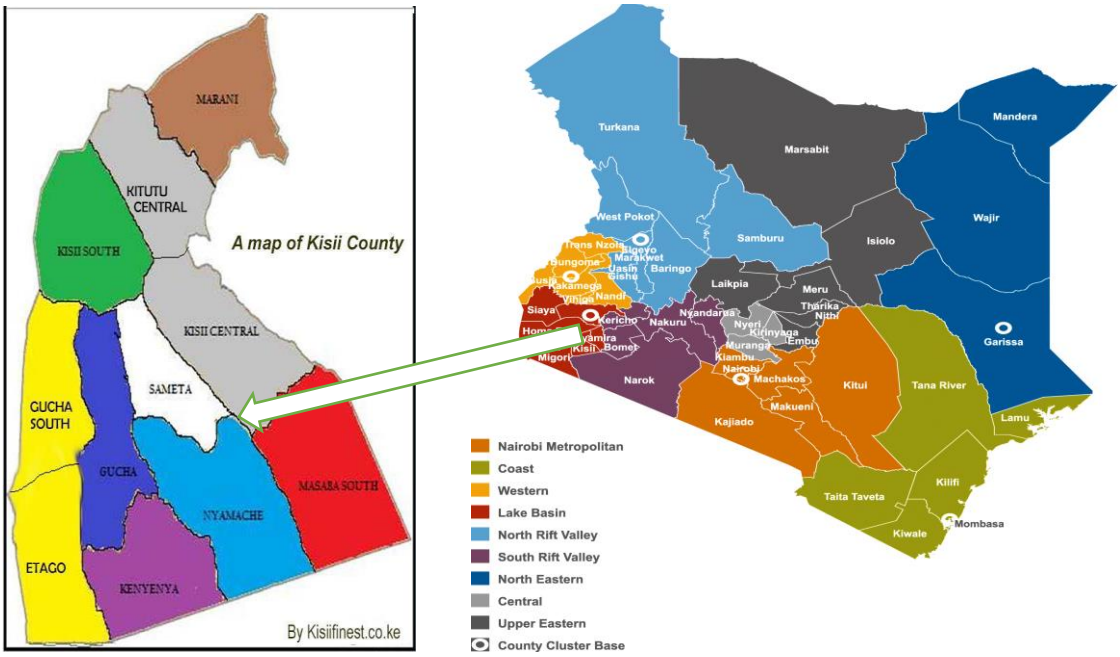

Figure 3.1 A map of Kenya showing Kisii County (Google Maps, 2018).

3.2 Study Population

The target study population involved patients who had already tested positive for HIV, attending Kisii Teaching and Referral Hospital.

3.3 Study design

This study was conducted in a hospital environment. The cross-sectional inquiry was executed among patients with HIV who sought treatment at the comprehensive care clinic of Kisii Level 5 Hospital during July and August 2019.

3.4 Sampling design

Random sampling was used in recruiting HIV-infected patients visiting the HIV-1 care program of Kisii Teaching and Referral Hospital. Those newly diagnosed from the VCT

counselling centre were also included. All HIV-positive patients attending Kisii Teaching and Referral Hospital were given an equal chance to be sampled.

3.5 Sample size

Desired sample size was obtained using a formula developed by (Naing *et al*., 2006). There is no known proportion of HBV infections among HIV-positive infected individuals in this region. Therefore, 50% was assumed and used for calculation of sample size. Thus,

$n=Z^2P(1-P)/d^2$

Where n= size of the sample,

Z=Z statistic for a level of confidence (Naing *et al*., 2006) (Z= 1.96 standard error)

P= expected prevalence (for 50%, *p*= 0.50)

d= Precision

Thus, $n= Z^2P(1-P)/d^2$

$=1.96^2 \times 0.50\ (1-0.50)/0.05^2$

$= 384$

3.6 Selection Criteria

3.6.1 Inclusion criteria

Any patient attending Kisii Teaching and Referral Hospital, regardless of gender, has tested positive for HIV infection and did not know their hepatitis B infection history were included.

Any HIV patient who gave consent within the facility.

3.6.2 Exclusion criteria

Any patient attending Kisii Teaching and Referral Hospital who knew their HBV infection history or was vaccinated against it. Those who didn't give consent were excluded from this study. Those who consented, were positive with HIV but didn't seek attention from Kisii Teaching and Referral Hospital. Patients who confirmed having been vaccinated against Hepatitis B and those who had HBV infection but not infected with HIV.

3.7 Patients recruitment

After explaining the study's objectives, the facility management and research personnel aided in enlisting volunteers. Four hundred individuals (400) aged 16-68 years, including pregnant women, were randomly sampled after providing consent. A questionnaire was distributed to volunteers, along with instructions to provide a signed consent form. The demographic data included information on education level, age, whether married or not, history of female genital mutilation, history of dental surgery,

piercing of body parts especially, tattooing, blood transfusion and history of surgical procedures while giving birth.

3.7.1 Collection of blood samples

About 5ml samples of venous blood was aseptically collected via venipuncture from consenting participants in an EDTA BD vacutainer ™ anticoagulant tube (BD, Franklin Lakes, USA). Centrifugation at 2000rpm for 5 Min was done to obtain plasma which was later used in HBV serology. The collected samples were refrigerated at -20˚C until the time for analysis (Fujii, 2013). The collected blood samples were utilized to conduct serological profiling (Mabeya *et al.*, 2016).

3.7.2 HBsAg detection

A rapid analysis kit for HBV, was used to analyze all samples based on screening for antibodies for HBsAg based on five sero-markers. They include HBsAg, HBsAb, HBeAg, HBeAb and HBcAb-IgM. Serum laboratory reaction of samples was measured by use of one-step Lumiquick HBV-5 panel kit. Manufacturers stipulations were adhered to (Castella *et al*., 2006). Briefly, approximately 2-3 drops (80-120) µl of plasma was added to each of the five sample wells designated for specific markers, and then buffer was added (Castella *et al*., 2006). Following incubation for a period of 20 Min at room temperature HBsAg reactions were checked and filed. Two to three drops of buffer were added to each well, and HBV seromarker reactivities were recorded after 20 Min of incubation at room temperature.. Samples positive for HBsAg were subjected to DNA extraction and hence sequencing (Kaminska *et al*., 2015).

3.7.3 DNA extraction

Blood was mixed to homogenize it, and 200µl of samples were used for DNA extraction using QIAamp DNA Mini Kit, pursuing the producer's guidelines and instructions. Lysing of the red and white blood cells is achieved by proteinase K in the kit (Carpi *et al*., 2011). According to Chacon-Cortes *et al*. (2014), "the lysis'' step discharges nuclei and mitochondria out of the white blood cells. Centrifugation was then done to collect them. Protein contaminants were then removed. A denaturation buffer which contains salt and a protease, is used to treat the white cell nuclei and mitochondria (Carpi *et al*., 2011). The buffer instantaneously denatures the proteins leaving the genomic DNA and digested proteins in the solution (Carpi *et al*., 2011).

The DNA purification procedure comprised four steps using QIAamp Mini spin columns in a standard microcentrifuge (Carpi *et al.,* 2011). The DNA was extracted using alcohol precipitation and eluted inTris EDTA buffer solution based QiaAmp ™ DNA Mini kit from Qiagen Inc. Valencia, USA. (Santos *et al*., 2010) according to manufacturers instructions. Briefly, in eppendorf tube, 200µl of plasma was mixed with 25µl of protease K and 200µl of Carrier RNA buffer and the contesnts incubated at . 56°C for 15 Min. After a quick spin, 250µl of 96-100% was later ethanol and vorted for 15 Sec, and let to stand for 5 Min at room temperature. Resultant contents were then transferred into wash tubes. These were then spun again at 10,000 rpm for a period of 2 Min followed by filtration. Spin columns were placed in wash tubes. The contents were washed with AW1 wash buffer (500µl) by soinning it at 10,000RPM for 2 Min, with filtrate poured off. The washing was repeated again using 500µl (AW2) wash buffer by centrifuging at

10,000rpm for 2 Min. A further 500µl of ethanol (96-100%) was put in wash tubes and span at 10,000 rpm for 2 Min and filtrate poured off. After replacing the wash tubes, the contents were centrifuged at full speed for 3 Min. The sample was then incubated for 3 Min at 56°C to for the membrane to dry completely. Wash tubes were then superseded with recovery tubes, and 50µl of elution buffer (AVE) added to the centre of the membrane. This was incubated at room temperature for 5 Min followed by 2 Min cetrifugation. The resultant extracted DNA was stored for future use at a temperature of -20°C (Santos *et al*., 2010).

3.7.4 Gel electrophoresis

The integrity of the DNA collected was tested using gel electrophoresis. This procedure separates DNA by size, determined by the length of base pairs. An electric field was used to migrate the negatively charged DNA through 1% agarose gel towards the positively charged electrode. For ease of visualization under ultraviolet (UV) Light, the DNA was impregnated with Ethidium Bromide. A molecular ladder was loaded on the first lane of the gel for comparison to infer DNA sizes (Salazar *et al.,* 1998). The sample was loaded onto the visualization equipment using an ultra violet transilluminator. The electrophoretic tank was set at 100 volts and left to run for 45 Min to allow fragment separation (Viljoen *et al*., 2005). The reverse transcriptase gene had a molecular weight of 692 base pairs.

3.7.5 Nested PCR

The DNA was taken through PCR process for amplification of HBsAg whereby 5 µl of DNA was mixed with 4 µl of master mix containing FIREPol® DNA polymerase, 5x Reaction Buffer B 0.4 M Tris-HCl, 0.1 M (NH4)2SO4, 0.1% w/v Tween-20, 7.5 mM MgCl2 1x PCR solution – 1.5 mM MgCl2, 2 mM dNTPs of each 1x PCR solution – 200 µM dATP, 200 µM dCTP, 200 µM dGTP and 200 µM dTTP. Reverse primer HBPr135 (803-822, 5'-CAAAGACAAAAGAAAATTGG-3 ') and forward primer HBPrl (position: 2850-2868, 5 '-GGGTCACCATATTCTTGGG- 3') was used. For nested PCR, Reverse primerHBPr3 (Position: 3226-3246, 5'CCACTGCATGGCCTGAGGATG-3) and forward primer, HBPr2(position2867-2888, 5'GAACAAGAGCTACAGCATGGG-3 was used for second PCR'.

Once the master mix were prepared, amplification was conducted in both both rounds of the PCR conditions based on: one initial cycle at 94°C for ten Min, then 40 cycles consisting of 30 Sec at 94°C, 30 Sec at 50°C, and 1 minute at 72°C. The last extension step was then performed at 72°C for 10 Min. The PCR product acquired was then visualized using 2% agarose gel.

3.8 Sequencing and analysis

The chain termination method, also known as Sanger Sequencing, was used to determine DNA nucleotide sequences (Vlab amrita, 2011). Applied Biosystems genetic analysis system was used (Macrogen, Europe). (Vlab amrita, 2011). The sequences were then

analyzed phylogenetically using MEGA X and the HBV drug resistance-associated mutations were determined through the Stanford HBV drug resistance database.

3.9 Data Analysis

Descriptive and inferential statistical data analysis was done using the one-way SPSS version 19 frequency tables were generated. The prevalence markers for HBV were presented as a percentage and organized in a table format. Two-tailed chi-square tests with significance set at *a p-value* of < 0.05 were used to compare how the association between socio-demographic elements, for instance gender, age and marital status, could impact HBV infection, sero-markers and HBV infection stages across the study participants. Generated sequences were phylogenetically analyzed using MEGAX. Sequences were aligned using Clustal W 1.6.6 version and joined by neighbor software, and the Tree was viewed using Tree View Software.

3.10 Ethical Approval

After acquiring ethical approval from the Kenyatta University Ethical Review Committee, the study was executed in conformity with the Helsinki Declaration. (Parsa-Parsi *et al.,* 2014). Informed consent was obtained before collecting samples and demographic data from each participant. During the entire study period, confidentiality was maintained and respected. (Parsa-Parsi *et al.,* 2014).

CHAPTER FOUR

RESULTS

4.1 Study participants' demographics

The total number of patients who participated in this study was 400, whereby 179 (44.75%) were male, and 221 (55.25%) were female respondents. The ages of the study participants were ranged between 18 and 69 years with an average of 40 years and a standard deviation of 12.17 years. The average age of male participants was 42.41 with a standard deviation of 12.21 years while females was 38.31 years with a standard deviation of 11.83 years. Majority of the participants were either married, 183 (45.8%) or single, 179 (44.8%). All participants were on antiretroviral drugs administered in combination with Lamivudine (3TC) and Nevirapine (NVP) (Table 4.1)

Table 4.1: Study participants' demographics of HIV infected individuals in Kisii.

Demography	**Category**	**(N = 400)**	**Frequency**
Gender	Male	179	44.75
	Female	221	55.25
Marital status	Single	179	44.8
	Married	183	45.8
	Divorced	2	0.5
	Separated	15	3.8
	Widow	11	2.8
	Widower	10	2.5
	Ages ranges		**Average (SD)**
Male	(18-67) yrs		42.41 ± 12.21
Female	(21-69) yrs		38.21 ± 11.83

4.2 The HBV prevalence among HIV-infected individuals in Kisii

In an estimated 400 individuals who took part of this, overall, HBV-HBsAg seroprevalence of 11.75% (47/400) was detected among HIV-infected Kisii populations. Among the sampled population, the status of HBV revealed that 47 (11.75%) hence had HIV/ HBV co-infection while 88.2% HIV mono-infections. Among the males, 24 (13.4%) were HBV infected while 10.4% were females. This demonstrated that both males and females could be co-infected, but there was no notable correlation between HBV infection and the gender of the respondents ($\chi^2 = 0.859$, $p = 0.435$ (Table 4.2)

Table 4.2: The HBV prevalence among HIV-infected individuals in Kisii

Demography	**Category**	**Frequency (N = 400)**	**χ^2 value**	***P* value**
Gender	Male	24 (6%)	0.859	0.435
	Female	23 (5.75%)		
Marital	Single	17(4.25%)		
	Married	15(3.75%)		
	Divorced	1(0.25%)		
	Separated	6(1.5%)		
	Widow	4(1%)		
	Widower	5(1%)		

4.3 Co-infection across the gender of various marital status

Among the single participants, 64.7% were female whereas among the married group, 53.3% were females. These formed the majority of those who had co-infections. However, gender had no significant association in co-infection ($\chi^2 = 7.186$, $p = 0.207$).

Nevertheless, as described in Table 4.3, the burden of co-infections, following the WHO age groups categorizations, young adults, had 9.1% HIV/ HBV co-infection while adults were, 13.5%.

Table 4.3: Cross-tabulation of the gender and the marital status of the co-infected participants

	Category	**Male**	**Female**	**χ^2 value**	**P value**
Marital	Single	6(35.3%)	11(64.7%)	7.186	0.207
	Married	7(46.7%)	8(53.3%)		
	Divorced	1(100%)	0(0.0%)		
	Separated	4(66.7%)	2(33.3%)		
	Widow	2(50.0%)	2(50.0%)		
	Widower	4 (100%)	0(0.0%)		
	Category	**HBV positive**	**HBV Negative**	**χ^2 value**	***P* value**
Age	Young adults	4 (9.1%)	40 (90.9%)	0.337	0.804
	Adults	49 (13.5%)	313 (86.5%)		

4.4 The risk factors associated with HBV infection among HIV-infected individuals

The demographic characteristics of the study participants were analysed to determine if they could influence HBV infections. Demographics, gender, marital status and age of the patients were analysed. However there was a significantly correlation between marital status (χ^2 = 31.594, P = 0.0001) and HBV infections. A significantly high number of patients who were co-infected were single (36.2%) followed by married

31.9% group. Only 2.1% of the co-infection cases were divorced. More males (51.1%) were co-infected. This could be associated with their sexual behaviours depicted by multiple sexual partners and drug and substance abuse, especially alcoholism. Poor feeding leads to malnutrition, which lowers immunity and may also be attributable to the ease of co-infection (Alter *et al*., 2006). However, gender did not significantly influence HBV infections $\chi^2 = 0.859$, P = 0.435 (Figure 4.1).

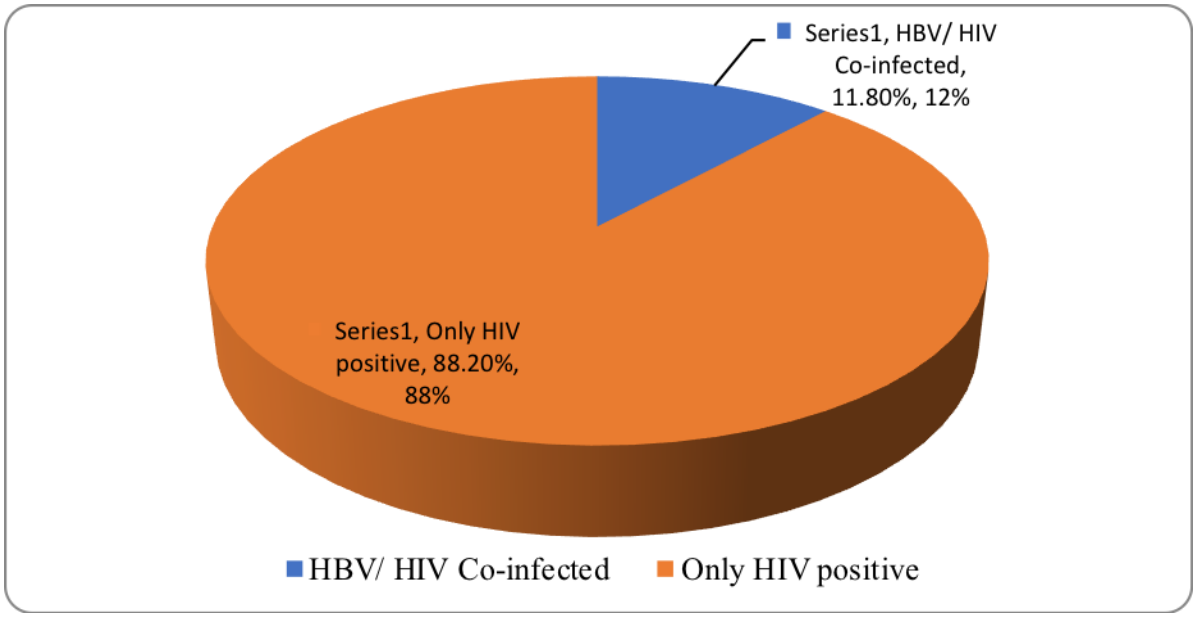

Figure 4.1 Number of participants Co-infected by HBV and HIV in Kisii County

In this study, gender and marital status were analysed to determine if they could influence any infection of HBV. In this study, Considering the age groupings according to WHO, most of the respondents, adults, 91.5% were most infected compared to young adults, 8.5%. However, even in these categorized ages, there was no significant correlation with HBV infection. Nevertheless, according to this study, marital status significantly influences HBV infection p= 0.0001.

Table 4.4: HBV/ HIV co-infection status of the infected individuals

	Category	**HBV Positive**	**HBV Negative/HIV mono-infected**	**χ^2 value**	**P value**
Gender	Male	24 (51.1%)	155 (43.9%)		
	Female	23 (48.9%)	198 (56.1%)	0.859	0.435
	Category	HBV positive	HBV negative	**χ^2 value**	***P* value**
Age	Young adults (15-24)	4 (8.5%)	40 (11.3%)	0.337	0.804
	Adults (>25yrs)	43 (91.5%)	313 (88.7%)		
Marital	Single	17 (36.2%)	162 (45.9%)		
	Married	15 (31.9%)	168 (47.6%)		
	Divorced	1 (2.1%)	1 (0.3%)		
	Separated	6 (12.8%)	9 (2.5%)	31.594	0.0001*
	Widow	4 (8.5%)	7 (2.0%)		
	Widower	4 (8.5%)	6 (1.7%)		

KEY: *significant association

4.4 The HBV Genetic diversity

All 47 samples that were positive for HBV infection, HBV -pol gene was was effectively amplified and directly sequenced. From the phylogenetic analysis, all the sequences disclosed that all the sequences belonged to HBV genotype A. The alignment and phylogenetic clusters further revealed four main clusters that showed close

relatedness with references sequences from Kenya and other neighboring countries of Sudan and Ethiopia.

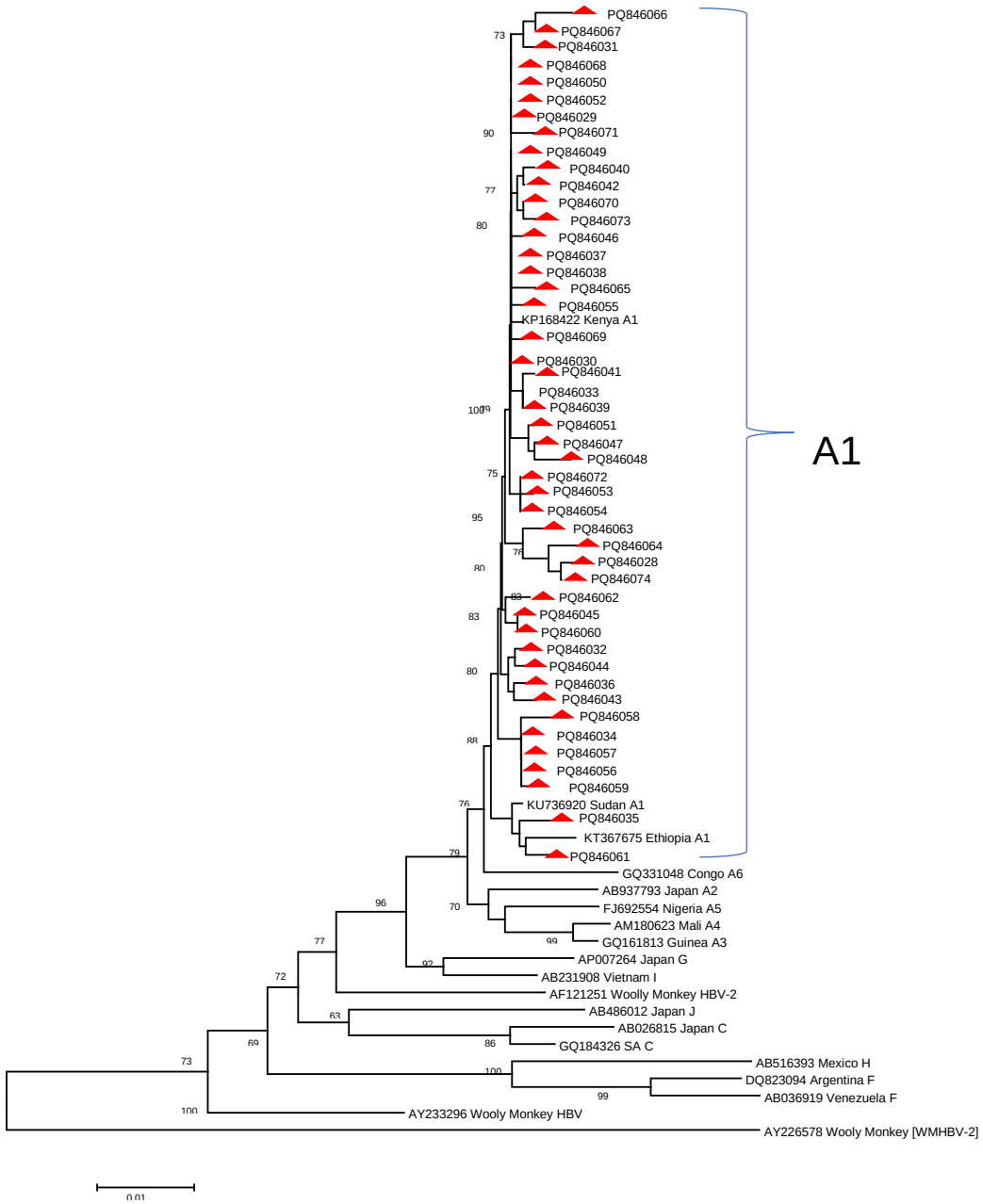

Figure 4.2 Phylogenetic analysis of HBV-pol sequences generated from Kisii site, Kenya, marked in red. A neighbor-joining method based on 1,000 bootstrap replicates was used. Wooly monkey HBV (AY226578-WMHBV) was used as the outgroup, and bootstrap values >70% are indicated.

4.5 HBV drug resistance

Four (4) of the 47 sequences that were analyzed for HBV drug resistance showed drug resistance mutations associated with Lamivudine and Telbivudine in HBV/HIV-coinfected patients. The triple nucleoside analogue associated mutations, which mainly occur at position 204 (rtM204) with or compensatory mutations at position 173 (rt V173K) and or at position 180 (rt180M) mutations, were detected in three patients. These patients were infected with viral strains resisting Lamivudine, Telbivudine, Elbivudine and Entecavir. One patient has 81T mutations that are associated with Adefovir, Telbivudine Lamivudine and Tenofovir resistance.

Table 4.5: The Hepatitis B virus drug resistance mutation patterns in Kisii HBV/HIV coinfected patients visiting Kisii teaching & Referral hospital, Kisii, Kenya

Sequence ID	HBV genotypes	combined mutations pattern	Associated drug
KHB001	A	AI81T	LMV, LdT, ADF/TDF
KHB010	A	V173K, L180M, A181S, 184V, S202K, M204V	LMV, LdT
KHB021	A	A169L, V173G, L180M, A181C, S202F, M204V	LMV, LdT ETV, ADF/TDF
KHB038	A	A169K, L180F, A181G, S202H	LMV, , LdT, ADF/TDF

Key
ETV: Entecavir, LMV: Lamivudine, LDT: Telbivudine, ADF: Adefovir, TDF: Tenofovir

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

5.1.1 HBV Prevalence among participants in the study

From a total of 400 samples that were screened, the overall seroprevalence of HBV infection 11.75% detected in Kisii region. The detected prevalence indicated that co-infection among the HIV-infected population was highly endemic as compared to those obtained the sampled in Garissa at 8.6% (HBsAg. This observation was founded on WHO categorization, where low (<2%), intermediate (2-7%) and high (>8%) than expected (Meldal *et al*., 2009; Mabeya *et al.,* 2016; Hou *et al*., 2020).

This burden of HBV infection was slightly higher than expected proportion of intermediate endemicity levels among the general population. (Downs *et al.,* 2023). This finding was compatible with previous studies that has been conducted elsewhere in diverse regions of Kenya. The results concurred with those that have been conducted in Nyanza 11% (Greer *et al.,* 2016). This colloborative findings were also comparable to those obtained from other countries, like Nigeria (11.9%) and Botswana (10.6%) (Weter *et al.,* 2006; Otegbayo *et al.,* 2008).

However, these findings, could be higher than those from other regions in Country. Rift Valley was 9.7% (Okoth *et al.,* 2017), Nyanza was 6.2% (Onyango *et al.,* 2021), Central and Nairobi was 5.5% (Mabeya *et al.,* 2016), Maina *et al.* (2015) was 5.8%, Chakraborty *et al.* (2003) was 6.1%, and Kim *et al.* (2011) was 6.9%. A study in South Africa recorded similar high rates (Lodenyo *et al.,* 2000).

However, the observed prevalence was also marginally lower than that of coastal regions (12.5%; Kibaya *et al.,* 2015; Kerosi *et al.,* 2015). The high-risk population of IDUs evaluated along the coast may be linked to the high rates. The realized prevalence was low (17.1%) compared to other national study statistics in Kenya. This was obtained from a multi-region study (17.1%) from a national survey (29%), according to Too *et al.* (2018). Reports from various parts of Africa and beyond likewise portrayed high HBV levels. Tanzania 17.3% (Nagu *et al.,* 2008), South Africa 19.7% and Ethiopia 14.4% (Rahlenbeck *et al.,* 1997). The observed variance depends on the intended target population and sample size.

The result aligns with earlier studies carried out in various hospitals across the country, with prevalences of 9.3 % (Okoth *et al.,* 2006) and 9.4% (Gatheru *et al.,* 2018) being recorded. Most of these studies have been conducted among other populations other than just among gravid women. HBV infections have also been confirmed to fall within intermediate infection levels, with prevalence ranging between 2% and 5% (Downs *et al.,* 2023).

In Ethiopia, HBV endemicity has been reported to be 8% among women (Umer *et al*., 2023), 9.51% in Chinese women (Zhang *et al*., 2013), 9% in Côte d'Ivoire (Rouet *et al*., 2004), and 9.2% in Gambia (Bitayye *et al*., 2019). These levels of HBV infections were relatively higher than studies conducted at Mbagathi, Nairobi, 3.8% (Ngaira *et al.,* 2016) also elsewhere in Haiti (Tohme *et al.,* 2016), 4.3% South Ethiopia (Yang *et al*., 2006), 5.7%, 6.5% DR Congo and Zambia respectively (Mohammed & Solomon, 2005) and

7.5% in Limbe and Muyuka Health Districts of South West of Cameron (Eyong *et al*., 2019). Amongs gravid ladies from Ethiopia, much lower rates of 1.35% were recorded (Mohammed & Solomon, 2005), Mexico at 3.7% (Jose *et al.,* 2003), Rwanda at 2.4% (Pirillo *et al*., 2007) and Israel 0.88% (Bogomolski *et al.,* 1991).

Previous reports indicate higher rates of HBV infection, in contrast to this study. Increased HBV infection rates have been reported in other parts of Nigeria 11.6% Hong Kong China 10%, Papa New Guinea 11%, Yemen (10.2%) (Murad *et al.,* 2013) and others on intensely high like Cameroun 25.3% (Mohammed & Solomon, 2005). These variations in HBV Sero-prevalence could be associated with discrepancies attributed to the socioeconomic ranks of diverse populations, geographical and cultural contrasts, sexual conduct across the study zones, their adopted HBV preventive efforts or the sample sizes of the population (Bahati *et al*., 2021).

According to Mabeya *et al.* (2016), the HBV 5 panel (HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb) has been utilized to guide the correct diagnosis of HBV, including the infectious illness stage. Using HBsAg markers instead of proper testing due to the high cost is a risky practice that can result in missing cases of occult hepatitis. (Kilongosi *et al.,* 2015). With single screening methods of HBsAg as a marker for HBV infection, oversight is highly probable, leading to failure in the detection of occult hepatitis, thus potentially increasing the chances of gravid women transmitting the virus. Findings of 2.7% of occult hepatitis cases concurs with earlier studies (Mabeya *et al.,* 2016). In this population, low proportions of women had been vaccinated, leading to the vulnerability

of this population to HBV infection. With such low HBV vaccination coverage, this study shows a notable requirement to increase HBV vaccination. It is worth noting that the current vaccination schedule for HBV, which starts at six weeks in children, should be revised to include a birth dose. "This measure will effectively eliminate the persistent threat of exposure to HBV." (Mabeya *et al.,* 2016).

5.1.2 HBV drug resistance

All participants were on ART containing 3TC (208) TDF (13) monotherapy with 3TC/TDF drug combinations. Though 3TC and TDF monotherapy were predominantly used, while a few cases were on 3TC/TDF drug combinations as recommended in cases of suspected risk of emerging HBV drug resistance (Benhamou, 2000). The non-adherence could be associated with a lack of frequent screening for HBV in HIV-infected patients as one way to improve treatment response (Lieveld *et al*.,2013). These reults reveal that, of the 11.75% (47/400) participants that were HBV/HIV co-infected, 29 participants were treated with antiretroviral drugs containing 3TC, one on TDF while 12 patients were on 3TC/TDF drug combinations. Five (5) patients were not on anti-HBV, while four patients presented with drug-resistant strains of 3TC (Lieveld *et al*.,2013).

These results confirms the conclusions from previous studies (Suzuki *et al.,* 2007; Mabeya *et al.,* 2017). All the patients were infected with viral strains carrying mutations that confer cross-resistance. The triple nucleoside analogue associated mutation lrtV2004M, which is a mutation located at position 204 of YMDD motify, often leads to

Lamivudine resistance. Since this mutation is often accompanied by compensatory mutations, like (rtL80V/I, rtI169T, rtV173L, rtL180M, rtT184S/G, rtS202I and rtQ215S), cross-resistance to Lamivudine, Telbivudine and Entecavir often occurs. (Bartholomeus & Locarnini, 2006). Further, in previous studies, the occurrence of drug resistance to Lamivudine to other drugs, such as Entecavir, was detected due to cross-resistance (Kim *et al.*, 2011; Mabeya *et al.*, 2017). Despite Entecavir having high genetic barrier (Chang *et al.,* 2010), the co-occurrence of rtL180M + rtM204V mutations confirmed this resistance (Zoulim & Lorcaninii, 2009).

In this study, three patients had a viral infection with rtA181T/V mutations that confer Lamivudine (LMV), Telbivudine(LdT), and Adefovir (ADF)/ Tenofovir disoproxil fumarate (TDF) resistance. The occurrence of drug resistance concurred with previous studies conducted locally and around the regions (Mabeya *et al.*, 2017), though in lower proportions in China. (Kim *et al.*, 2011; Lei *et al.*, 2013; Mabeya *et al.*, 2017).

5.1.3 HBV diversity

Revill *et al*., (2020) found that HBV diversity may affect treatment, immune response, and population spread. Upon phylogenetically analyzing the 47 sequences, it was confirmed that the entire group matched with HBV genotype A. Although these isolates clustered with those from Kenya and neighbouring countries, the analysis revealed minimal variation among them. This confirms that genotype A of HBV persists as the most dominant genotype in the country (Revill *et al*., (2020).

These findings are in agreement with those obtained from other parts of the country, such as Nairobi (Mwangi *et al*., 2009; Kim *et al*., 2011; Mabeya *et al*., 2017), Siaya (Onyango *et al*., 2021), Mombasa (Webale *et al*., 2015), and the general Kenyan population as a whole (Langat *et al*., 2023). Though the clustering of these sequences was aligned with those from local and neighbourhood countries, the analysis confirmed minimal diversity across the isolates. However, more HBV genotypes such as; genotypes D and E have been detected throughout the country, albeit in small proportions (Mabeya *et al.*, 2017), and across various groups such as IV drug users, blood donors and commercial sex workers (Mwangi *et al.*, 2009; Kim *et al.*, 2011; Kibaya *et al.*, 2015; Webale *et al.*, 2015). This phenomenon is still likely associated with the homogeneous population sampled and the nature of the studied population, which incorporates intravenous drug users and sexual workers who are regarded as high-risk populations for HIV. There is possible existence of these risk population in the Kisii community; these genotypes were not found in this study.

5.2 Conclusions

i. Age, gender and marriage status were identified as potential risk factors for HBV infections.
ii. HBV prevalence of 11.75% was detected among HIV infected individuals visiting Kisii Level Five hospital.
iii. The most predominant HBV sub-genotype detected among HIV patients sampled was sub-genotype A1.

iv. Low proportion 4 (8.5%) of HBV-HIV co-infected patients were infected with HBV drug resistance strains to lamuvidine

5.3 Recommendations

i. In order to effectively manage HBV emergence and prevalence, it is prudent to diagnose all HIV patients for HBV infections regularly.

ii. This study shows that in order to entirely suppress infections in co-infected individuals with HIV and HBV, dual antiviral therapy is required.

iii. Regular surveillance of HBV genotypes, mutations, and drug resistance would help formulate effective management measures.

iv. This study further recommends advanced research in various cohorts in the region to derive a more dependable prevalence rate of HBV.

REFERENCES

Alter, M. J. (2006). Epidemiology of viral hepatitis and HIV co-infection. *Journal of hepatology*, *44*, S6-S9.

Andernach, I. E., Hunewald, O. E., & Muller, C. P. (2013). Bayesian inference of the evolution of HBV/E. *PloS one*, *8*(11), e81690.

Arankalle, V. A., Gandhe, S. S., Borkakoty, B. J., Walimbe, A. M., Biswas, D., & Mahanta, J. (2010). A novel HBV recombinant (genotype I) similar to Vietnam/Laos in a primitive tribe in eastern India. *Journal of viral hepatitis*, *17*(7), 501-510.

Avihingsanon, A., Lu, H., Leong, C. L., Hung, C. C., Koenig, E., Kiertiburanakul, S& Li, T. (2023). Bictegravir, emtricitabine, and tenofovir alafenamide versus dolutegravir, emtricitabine, and tenofovir disoproxil fumarate for initial treatment of HIV-1 and hepatitis B co-infection (ALLIANCE): a double-blind, multicentre, randomised controlled, phase 3 non-inferiority trial. *The Lancet HIV*, *10*(10), e640-e652.

Babu, C. K., Suwansrinon, K., Bren, G. D., Badley, A. D., & Rizza, S. A. (2009). HIV induces TRAIL sensitivity in hepatocytes. *PLoS One*, *4*(2), e4623.

Balaji, G. (2015). A Study on Role of HBsAg Quantification in patients with Chronic Hepatitis B Infection and Predicting its Response to Treatment (Doctoral dissertation, Kilpauk Medical College, Chennai).

Bastug, A., & Bodur, H. (2019). Acute Hepatitis B. Viral Hepatitis: Acute Hepatitis, 25-44.

Bazzoli, C., Jullien, V., Tiec, C. L., Rey, E., Mentré, F., & Taburet, A. M. (2010). Intracellular pharmacokinetics of antiretroviral drugs in HIV-infected patients, and their correlation with drug action. *Clinical pharmacokinetics*, *49*, 17-45.

Benhamou, Y., Bochet, M., Thibault, V., Calvez, V., Fievet, M. H., Vig, P., ... & Poynard, T. (2001). Safety and efficacy of adefovir dipivoxil in patients co-infected with HIV-1 and lamivudine-resistant hepatitis B virus: an open-label pilot study. *The Lancet*, *358*(9283), 718-723.

Benhamou, Y., Bochet, M., Thibault, V., Di Martino, V., Caumes, E., Bricaire, F., ... & Poynard, T. (1999). Long-term incidence of hepatitis B virus resistance to lamivudine in human immunodeficiency virus–infected patients. *Hepatology*, *30*(5), 1302-1306.

Bethesda, L. (2012). Clinical and Research Information on Drug-Induced liver injury [Internet]. *National Institute of Diabetes and Digestive and Kidney Diseases*.

Bobardt, M., Hansson, M. J., Mayo, P., Ure, D., Foster, R., & Gallay, P. (2020). Structurally distinct cyclosporin and sanglifehrin analogs CRV431 and NV556 suppress established HCV infection in humanized-liver mice. *PLoS One*, *15*(8), e0237236.

Bodsworth, N., Donovan, B., & Nightingale, B. N. (1989). The effect of concurrent human immunodeficiency virus infection on chronic hepatitis B: a study of 150 homosexual men. *Journal of Infectious Diseases*, *160*(4), 577-582.

Bruss, V., & Ganem, D. (1991). The role of envelope proteins in hepatitis B virus assembly. *Proceedings of the National Academy of Sciences*, *88*(3), 1059-1063.

Cahn, P., Rolón, M. J., Figueroa, M. I., Gun, A., Patterson, P., & Sued, O. (2017). Dolutegravir–lamivudine as initial therapy in HIV-1 infected, ARV-naive patients, 48-week results of the PADDLE (Pilot Antiretroviral Design with Dolutegravir LamivudinE) study. *Journal of the International AIDS Society*, *20*(1), 21678.

Cameron, A. M., Truty, J., Truell, J., Lassman, C., Zimmerman, M. A., Kelly Jr, B. S., ... & Busuttil, R. W. (2005). Fulminant hepatic failure from primary hepatic lymphoma: successful treatment with orthotopic liver transplantation and chemotherapy. *Transplantation*, *80*(7), 993-996.

Cameron, C. E. (2009). Viral genome replication. M. Götte, & K. D. Raney (Eds.). Boston, MA, USA:: Springer.

Castella, V., Dimo-Simonin, N., Brandt-Casadevall, C., & Mangin, P. (2006). Forensic evaluation of the QIAshredder/QIAamp DNA extraction procedure. Forensic science international, 156(1), 70-73.

Chang, M. H. (2007, June). Hepatitis B virus infection. In *Seminars in fetal and neonatal medicine* 12, (3) 160-167). WB Saunders.

Cheng, Z., Lin, P., & Cheng, N. (2021). HBV/HIV co-infection: impact on the development and clinical treatment of liver diseases. *Frontiers in Medicine*, *8*, 713981.

Coffin, C. S., Fung, S. K., Alvarez, F., Cooper, C. L., Doucette, K. E., Fournier, C., ... & Villeneuve, J. P. (2018). Management of hepatitis B virus infection: 2018 guidelines from the Canadian Association for the Study of Liver Disease and Association of Medical Microbiology and Infectious Disease Canada. Canadian Liver Journal, 1(4), 156-217.

Crane, M., Iser, D., & Lewin, S. R. (2012). Human immunodeficiency virus infection and the liver. *World journal of hepatology*, *4*(3), 91.

Delaney IV, W. E., Yang, H., Westland, C. E., Das, K., Arnold, E., Gibbs, C. S., ... & Xiong, S. (2003). The hepatitis B virus polymerase mutation rtV173L is selected during lamivudine therapy and enhances viral replication in vitro. *Journal of virology*, *77*(21), 11833-11841.

Di Martino, V., Thevenot, T., Colin, J. F., Boyer, N., Martinot, M., Degos, F., ... & Marcellin, P. (2002). Influence of HIV infection on the response to interferon therapy and the long-term outcome of chronic hepatitis B. *Gastroenterology*, *123*(6), 1812-1822.

Féray, C., & López-Labrador, F. X. (2019). Is PD-1 blockade a potential therapy for HBV?. *JHEP Reports*, *1*(3), 142-144.

Fung, S. K., & Lok, A. S. (2004). Hepatitis B virus genotypes: do they play a role in the outcome of HBV infection? Hepatology, 40(4), 790-792.

Ganem, D., & Schneider, R. J. (2001). The molecular biology of the hepatitis B viruses, p. 2923-2970. *Fields virology*, *2*.

Gürtler, C., & Bowie, A. G. (2013). Innate immune detection of microbial nucleic acids. *Trends in microbiology*, *21*(8), 413-420.

Herbert, A., Wijlaars, L., Zylbersztejn, A., Cromwell, D., & Hardelid, P. (2017). Data resource profile: hospital episode statistics admitted patient care (HES APC). International journal of epidemiology, 46(4), 1093-1093i.

Hudjashov, G., Kivisild, T., Underhill, P. A., Endicott, P., Sanchez, J. J., Lin, A. A., ... & Forster, P. (2007). Revealing the prehistoric settlement of Australia by Y chromosome and mtDNA analysis. *Proceedings of the National Academy of Sciences*, *104*(21), 8726-8730.

Hyams, K. C., Morrill, J. C., Woody, J. N., Okoth, F. A., Tukei, P. M., Mugambi, M., ... & Gray, G. C. (1989). Epidemiology of hepatitis B in Eastern Kenya. *Journal of medical virology*, *28*(2), 106-109.

Ichai, P., & Samuel, D. (2008). Etiology and prognosis of fulminant hepatitis in adults. Liver Transplantation, 14(S2), S67-S79.

Iser, D. M., Avihingsanon, A., Wisedopas, N., Thompson, A. J., Boyd, A., Matthews, G. V., ... & Lewin, S. R. (2011). Increased intrahepatic apoptosis but reduced immune activation in HIV-HBV co-infected patients with advanced immunosuppression. *Aids*, *25*(2), 197-205.

Joshi, D., O'Grady, J., Dieterich, D., Gazzard, B., & Agarwal, K. (2011). Increasing burden of liver disease in patients with HIV infection. *The Lancet*, *377*(9772), 1198-1209.

Kadiri, D. D., Peela, S., & Ganguli, D. (2022). Effect of cirrhosis and hepatitis on the prognosis of liver cancer. In Theranostics and Precision Medicine for the Management of Hepatocellular Carcinoma (pp. 51-72). Academic Press.

Kafeero, H. M., Ndagire, D., Ocama, P., Kudamba, A., Walusansa, A., & Sendagire, H. (2021). Prevalence and predictors of hepatitis B virus (HBV) infection in east Africa: evidence from a systematic review and meta-analysis of epidemiological studies published from 2005 to 2020. *Archives of Public Health*, *79*(1), 1-19.

Kao, J. H. (2011). Molecular epidemiology of hepatitis B virus. *The Korean journal of internal medicine*, *26*(3), 255-

Karoney, M. J., Some, F. F., Dip, H. I. L., Dip, E. W., & Murgor, L. K. (2020). Burden of hepatitis B infection among high risk populations in western Kenya. *J Infect Dis Epidemiol*, *6*, 132.

Khalid, F. A., Eltayeb, A. A., & Elbadawi, N. E. (2013). Awareness and knowledge of hepatitis B and HIV/AIDS, among the University of Kassala students, Sudan.

Kleinman, S. H., Lelie, N., & Busch, M. P. (2009). Infectivity of human immunodeficiency virus-1, hepatitis C virus, and hepatitis B virus and risk of transmission by transfusion. Transfusion, 49(11), 2454-2489.

Koblin, B. A., Taylor, P. E., Rubinstein, P., & Stevens, C. E. (1992). Effect of duration of hepatitis B virus infection on the association between human immunodeficiency virus type-1 and hepatitis B viral replication. *Hepatology*, *15*(4), 590-592.

Kondo, Y., Tsukada, K., Takeuchi, T., Mitsui, T., Iwano, K., Masuko, K., ... & Mayumi, M. (1993). High carrier rate after hepatitis B virus infection in the elderly. *Hepatology*, *18*(4), 768-774.

Kramvis, A. (2014). Genotypes and genetic variability of hepatitis B virus. *Intervirology*, *57*(3-4), 141-150.

Kramvis, A., & Kew, M. C. (2007). Epidemiology of hepatitis B virus in Africa, its genotypes and clinical associations of genotypes. *Hepatology Research*, *37*, S9-S19.

Kramvis, A., Kew, M., & François, G. (2005). Hepatitis B virus genotypes. *Vaccine*, *23*(19), 2409-2423.

Kumar, A. R., Devan, A. R., Nair, B., Vinod, B. S., & Nath, L. R. (2021). Harnessing the immune system against cancer: current immunotherapy approaches and therapeutic targets. Molecular biology reports, 1-21.

Lacombe, K., Massari, V., Girard, P. M., Serfaty, L., Gozlan, J., Pialoux, G., ... & Carrat, F. (2006). Major role of hepatitis B genotypes in liver fibrosis during co-infection with HIV. *Aids*, *20*(3), 419-427.

Lavanchy, D., & Kane, M. (2016). Global epidemiology of hepatitis B virus infection. Hepatitis B virus in human diseases, 187-20

Lavanchy, D., & Kane, M. (2016). Global epidemiology of hepatitis B virus infection. *Hepatitis B virus in human diseases*, 187-203.

Lee, S., & Baldridge, M. T. (2017). Interferon-lambda: a potent regulator of intestinal viral infections. *Frontiers in immunology*, *8*, 749.

Liang, T. J. (2009). Hepatitis B: the virus and disease. Hepatology, 49(S5), S13-S21.

Liaw, Y. F., Tai, D. I., Chu, C. M., & Chen, T. J. (1988). The development of cirrhosis in patients with chronic type B hepatitis: a prospective study. *Hepatology*, *8*(3), 493-496.

Lieveld, F. I., van Vlerken, L. G., Siersema, P. D., & van Erpecum, K. J. (2013). Patient adherence to antiviral treatment for chronic hepatitis B and C: a systematic review. Annals of hepatology, 12(3), 380-391.

Locarnini, S., Littlejohn, M., Aziz, M. N., & Yuen, L. (2013, December). Possible origins and evolution of the hepatitis B virus (HBV). In *Seminars in cancer biology* 23 (6);561-575.

Lok, A. S., & McMahon, B. J. (2009). Chronic hepatitis B: update 2009. *Hepatology*, *50*(3), 661-662.

Lokhande, M. U., Miquel, J., Benito, S., & Larrubia, J. R. (2011). HBV & HCV Immunopathogenesis. Viral Hepatitis–Celected Issue of Pathogenesis and Diagnostics, 1-40.

Lukhwareni, A. (2008). Exploring the Impact of Human Immunodeficiency Virus on Hepatitis B Virus Diagnosis, Prevention and Control in Co-infected Adult South African Patients on Highly Active Antiretroviral Therapy (Doctoral dissertation, University of Limpopo (Medunsa)).

Mabeya, S. N., Ngugi, C., Lihana, R. W., Khamadi, S. A., & Nyamache, A. K. (2017). Predominance of hepatitis B virus genotype A among treated HBV HIV-infected patients experiencing high hepatitis B virus drug resistance in Nairobi, Kenya. AIDS research and human retroviruses, 33(9), 966-969.

Makokha, G. N., Zhang, P., Hayes, C. N., Songok, E., & Chayama, K. (2023). The burden of Hepatitis B virus infection in Kenya: A systematic review and meta-analysis. *Frontiers in Public Health*, *11*, 986020.

McGovern, B. H. (2007). The epidemiology, natural history and prevention of hepatitis B: implications of HIV co-infection. *Antiviral therapy*, *12*(3_suppl), 3-13.

Menéndez-Arias, L., Álvarez, M., & Pacheco, B. (2014). Nucleoside/nucleotide analog inhibitors of hepatitis B virus polymerase: mechanism of action and resistance. Current opinion in virology, 8, 1-9.

Mohammadi, M., Talei, G., Sheikhian, A., Ebrahimzade, F., Pournia, Y., Ghasemi, E., & Boroun, H. (2009). Survey of hepatitis B virus (HBsAg) and hepatitis C virus (HCV-Ab) co-infection among HIV-positive patients. Virology journal, 6(1), 1-5.

Mohammed, N. A., El-Aleem, S. A., El-Hafiz, H. A., & McMahon, R. F. T. (2004). Distribution of constitutive (COX-1) and inducible (COX-2) cyclooxygenase in postviral human liver cirrhosis: a possible role for COX-2 in the pathogenesis of liver cirrhosis. Journal of clinical pathology, 57(4), 350-354.

Mokua, G. N., Maroko, G. M., Nyakundi, A. O., & Onyambu, M. O. (2019). HIV/AIDS in Kisii County: Current Status and Challenges.

Muller, J. T., & Al Khalili, Y. (2023). Emtricitabine. In StatPearls [Internet]. StatPearls Publishing.

Mutuma, G. Z., Mbuchi, M. W., Zeyhle, E., Fasana, R., Okoth, F. A., Kabanga, J. M., ... & Kaiguri, P. (2011). Prevalence of Hepatitis B Virus (HBV) surface antigen and HBVassociated hepatocellular carcinoma in Kenyans of various ages.

Naing, L., Winn, T. B. N. R., & Rusli, B. N. (2006). Practical issues in calculating the sample size for prevalence studies. Archives of orofacial Sciences, 1, 9-14.

Nassal, M. (2008). Hepatitis B viruses: reverse transcription a different way. *Virus research*, *134*(1-2), 235-249.

Núnez, M., Puoti, M., Camino, N., & Soriano, V. (2003). Treatment of chronic hepatitis B in the human immunodeficiency virus–infected patient: present and future. *Clinical infectious diseases*, *37*(12), 1678-1685.

Nyairo, E. K. (2018). Hepatitis B Virus Genetic Diversity and Surface Antigen Mutations among Voluntary Blood Donors in Kenya (Doctoral dissertation, JKUAT-COHES).

Okoth, F., Mbuthia, J., Gatheru, Z., Murila, F., Kanyingi, F., Mugo, F., ... & Wanjuki, N. (2006). Seroprevalence of hepatitis B markers in pregnant women in Kenya. East African medical journal, 83(9).

Osiowy, C., Larke, B., & Giles, E. (2011). Distinct geographical and demographic distribution of hepatitis B virus genotypes in the Canadian Arctic as revealed through an extensive molecular epidemiological survey. *Journal of viral hepatitis*, *18*(4), e11-e19.

Otedo, A. E. O. (2004). HBV, HIV co-infection at Kisumu district hospital, Kenya. *East African medical journal*, *81*(12), 626-630.

Pancawardani, P., Depamede, S. N., Wahyono, A., Jirintai, S., Nagashima, S., Takahashi, M., ... & Okamoto, H. (2012). Identification of four novel subgenotypes (C13–C16) and two inter-genotypic recombinants (C12/G and C13/B3) of hepatitis B virus in Papua province, Indonesia. *Virus research*, *163*(1), 129-140.

Panduro, A., Maldonado-Gonzalez, M., Fierro, N. A., & Roman, S. (2013). Distribution of HBV genotypes F and H in Mexico and Central America. *Antiviral therapy*, *18*(3_part_2), 475-484.

Pettigrew, N. M., Bagshawe, A. F., Cameron, H. M., Cameron, C. H., Dorman, J. M., & MacSween, R. N. M. (1976). Hepatitis B surface antigenaemia in Kenyans with chronic liver disease. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *70*(5-6), 462-465.

Piroth, L., Sène, D., Pol, S., Goderel, I., Lacombe, K., Martha, B., ... & Gervais, A. (2007). Epidemiology, diagnosis and treatment of chronic hepatitis B in HIV-infected patients (EPIB 2005 STUDY). *Aids*, *21*(10), 1323-1331.

Pourkarim, M. R., Amini-Bavil-Olyaee, S., Lemey, P., Maes, P., & Van Ranst, M. (2010). Are hepatitis B virus "subgenotypes" defined accurately?. *Journal of Clinical Virology*, *47*(4), 356-360.

Rambaldi, A., Jacobs, B. P., Iaquinto, G., & Gluud, C. (2005). Milk thistle for alcoholic and/or hepatitis B or C virus liver diseases. Cochrane Database of Systematic Reviews, (2).

Razavi-Shearer, D., Gamkrelidze, I., Nguyen, M. H., Chen, D. S., Van Damme, P., Abbas, Z., ... & Akarca, U. (2018). Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. *The Lancet Gastroenterology & hepatology*, *3*(6), 383-403.

Revill, P. A., Littlejohn, M., Ayres, A., Yuen, L., Colledge, D., Bartholomeusz, A., ... & Locarnini, S. A. (2007). Identification of a novel hepatitis B virus precore/core deletion mutant in HIV/hepatitis B virus co-infected individuals. *Aids*, *21*(13), 1701-1710.

Robbins, H. A., Shiels, M. S., Pfeiffer, R. M., & Engels, E. A. (2014). Epidemiologic contributions to recent cancer trends among HIV-infected people in the United States. Aids, 28(6), 881-890.

Rodrıguez-Méndez, M. L., Gonzalez-Quintela, A., Aguilera, A., & Barrio, E. (2000). Prevalence, patterns, and course of past hepatitis B virus infection in intravenous drug users with HIV-1 infection. *The American journal of gastroenterology*, *95*(5), 1316-1322.

Rossner, M. T. (1992). Hepatitis B virus X-gene product: A promiscuous transcriptional activator. *Journal of medical virology*, *36*(2), 101-117.

Ryom, L., Cotter, A., De Miguel, R., Béguelin, C., Podlekareva, D., Arribas, J. R., ... & Wensing, A. (2020). 2019 update of the European AIDS Clinical Society Guidelines for treatment of people living with HIV version 10.0. *HIV medicine*, *21*(10), 617-624.

Saag, M. S., Benson, C. A., Gandhi, R. T., Hoy, J. F., Landovitz, R. J., Mugavero, M. J., ... & Volberding, P. A. (2018). Antiretroviral drugs for treatment and prevention of HIV infection in adults: 2018 recommendations of the International Antiviral Society–USA Panel. *Jama*, *320*(4), 379-396.

Salazar, L. A., Hirata, M. H., Cavalli, S. A., Machado, M. O., & Hirata, R. D. (1998). Optimized procedure for DNA isolation from fresh and cryopreserved clotted human blood useful in clinical molecular testing. Clinical chemistry, 44(8), 1748-1750.

Sanger, F., Air, G. M., Barrell, B. G., Brown, N. L., Coulson, A. R., Fiddes, J. C., ... & Smith, M. (1977). Nucleotide sequence of bacteriophage φX174 DNA. nature, 265(5596), 687-695.

Saravolatz, L. D., & Saag, M. S. (2006). Emtricitabine, a new antiretroviral agent with activity against HIV and hepatitis B virus. *Clinical infectious diseases*, *42*(1), 126-131.

Sato, S., Li, K., Kameyama, T., Hayashi, T., Ishida, Y., Murakami, S., ... & Takaoka, A. (2015). The RNA sensor RIG-I dually functions as an innate sensor and direct antiviral factor for hepatitis B virus. *Immunity*, *42*(1), 123-132.

Scharschmidt, B. F., Held, M. J., Hollander, H. H., Read, A. E., Lavine, J. E., Veereman, G., ... & Thaler, M. M. (1992). Hepatitis B in patients with HIV infection: relationship to AIDS and patient survival. Annals of internal medicine, 117(10), 837-838.

Schillie, S. (2018). Prevention of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices. MMWR. Recommendations and reports, 67.

Schinazi, R. F., Chu, C. K., Peck, A., McMillan, A., Mathis, R., Cannon, D., ... & Yeola, S. U. R. E. S. H. (1992). Activities of the four optical isomers of 2', 3'-dideoxy-3'-thiacytidine (BCH-189) against human immunodeficiency virus type 1 in human lymphocytes. *Antimicrobial Agents and Chemotherapy*, *36*(3), 672-676.

Schuch, A., Hoh, A., & Thimme, R. (2014). The role of natural killer cells and CD8+ T cells in hepatitis B virus infection. Frontiers in immunology, 5, 258.

Scott, L. J. (2020). Dolutegravir/lamivudine single-tablet regimen: a review in HIV-1 infection. *Drugs*, *80*(1), 61-72.

Simonetti, J., Bulkow, L., McMahon, B. J., Homan, C., Snowball, M., Negus, S., ... & Livingston, S. E. (2010). Clearance of hepatitis B surface antigen and risk of hepatocellular carcinoma in a cohort chronically infected with hepatitis B virus. *Hepatology*, *51*(5), 1531-1537.

Singh, K. P., Zerbato, J. M., Zhao, W., Braat, S., Deleage, C., Tennakoon, G. S., ... & Audsley, J. (2020). Intrahepatic CXCL10 is strongly associated with liver fibrosis in HIV-Hepatitis B co-infection. *PLoS pathogens*, *16*(9), e1008744.

Soriano, V., Vispo, E., & Barreiro, P. (2011). New 2011 updated DHHS antiretroviral treatment guidelines and chronic hepatitis B. *AIDS*, *25*(7), 1013-1014.

Soriano, V., Vispo, E., Labarga, P., Medrano, J., & Barreiro, P. (2010). Viral hepatitis and HIV co-infection. *Antiviral research*, *85*(1), 303-315.

Su, J. R., Zhang, Q. Y., Zhao, Y., Yu, Y. H., & Lou, J. L. (2022). Characterization of the clinical features in HBV-related acute-on-chronic liver failure. Alternative Therapies in Health and Medicine, 28(2), 65-69.

Sugauchi, F., Orito, E., Ichida, T., Kato, H., Sakugawa, H., Kakumu, S., ... & Mizokami, M. (2002). Hepatitis B virus of genotype B with or without recombination with genotype C over the precore region plus the core gene. *Journal of virology*, *76*(12), 5985-5992.

Sun, S., Yang, Q., Sheng, Y., Fu, Y., Sun, C., & Deng, C. (2021). Investigational drugs with dual activity against HBV and HIV. *Experimental and Therapeutic Medicine*, *21*(1), 1-1.

Svegliati-Baroni, G., & De Minicis, S. (2010). HIV protein gp120 and chemokines receptor for. *Gut*, *59*(4), 427e428.\

Tajiri, K., & Shimizu, Y. (2013). Liver physiology and liver diseases in the elderly. *World journal of gastroenterology: WJG*, *19*(46), 8459.

Tatematsu, K., Tanaka, Y., Kurbanov, F., Sugauchi, F., Mano, S., Maeshiro, T., ... & Mizokami, M. (2009). A genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype J. *Journal of virology*, *83*(20), 10538-10547.

Thio, C. L. (2003). Hepatitis B in the human immunodeficiency virus-infected patient: epidemiology, natural history, and treatment. In *Seminars in liver disease* (Vol. 23, No.(02,) pp. 125-136). Copyright© 2002 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA.

Thompson, N. D., & Perz, J. F. (2009). Eliminating the blood: ongoing outbreaks of hepatitis B virus infection and the need for innovative glucose monitoring technologies. *Journal of diabetes science and technology*, *3*(2), 283-288.

Tian, X., Zhao, C., Zhu, H., She, W., Zhang, J., Liu, J., ... & Xie, Y. (2010). Hepatitis B virus (HBV) surface antigen interacts with and promotes cyclophilin a secretion: possible link to pathogenesis of HBV infection. *Journal of virology*, *84*(7), 3373-3381.

Tillmann, H. L. (2007). Antiviral therapy and resistance with hepatitis B virus infection. World journal of gastroenterology: WJG, 13(1), 125.

Tiollais, P., Pourcel, C., & Dejean, A. (1985). The hepatitis B virus. *Nature*, *317*(6037), 489-495.

Torresi, J., Earnest-Silveira, L., Deliyannis, G., Edgtton, K., Zhuang, H., Locarnini, S. A., ... & Jackson, D. C. (2002). Reduced antigenicity of the hepatitis B virus HBsAg protein arising as a consequence of sequence changes in the overlapping polymerase gene that are selected by lamivudine therapy. *Virology*, *293*(2), 305-313.

Toy, M., Ahishali, E., & Yurdaydın, C. (2020). Hepatitis delta virus epidemiology in the industrialized world. *AIDS Rev*, *22*(4), 203-212.

Trépo, C., Chan, H. L., & Lok, A. (2014). Hepatitis B virus infection. The Lancet, 384(9959), 2053-2063.

Uneke, C. J., Ogbu, O., Inyama, P. U., Anyanwu, G. I., Njoku, M. O., & Idoko, J. H. (2005). Prevalence of hepatitis-B surface antigen among blood donors and human immunodeficiency virus-infected patients in Jos, Nigeria. *Memórias do Instituto Oswaldo Cruz*, *100*(1), 13-16.

Viljoen, G. J., Nel, L. H., & Crowther, J. R. (Eds.). (2005). Molecular diagnostic PCR handbook. Springer science & business media.

Vlahakis, S. R., Villasis-Keever, A., Gomez, T. S., Bren, G. D., & Paya, C. V. (2003). Human immunodeficiency virus–induced apoptosis of human hepatocytes via CXCR4. *The Journal of infectious diseases*, *188*(10), 1455-1460.

Warner, N., & Locarnini, S. (2014). Mechanisms of hepatitis B virus resistance development. *Intervirology*, *57*(3-4), 218-224.

Wilkins, E., Nelson, M., Agarwal, K., Awoyemi, D., Barnes, E., Bhagani, S., ... & Papineni, P. (2013). British HIV Association guidelines for the management of hepatitis viruses in adults infected with HIV 2013. HIV medicine, 14.

Wlodzimirow, K. A., Eslami, S., Abu-Hanna, A., Nieuwoudt, M., & Chamuleau, R. A. (2013). A systematic review on prognostic indicators of acute on chronic liver failure and their predictive value for mortality. Liver International, 33(1), 40-52.

World Health Organization. (2016). *Global health sector strategy on viral hepatitis 2016-2021. Towards ending viral hepatitis* (No. WHO/HIV/2016.06). World Health Organization.

Ye, B., Liu, X., Li, X., Kong, H., Tian, L., & Chen, Y. (2015). T-cell exhaustion in chronic hepatitis B infection: current knowledge and clinical significance. Cell death & disease, 6(3), e1694-e1694.

Yousif, M., & Kramvis, A. (2013). Genotype D of hepatitis B virus and its subgenotypes: An update. *Hepatology Research*, *43*(4), 355-364.

Yu, M. W., Hsu, F. C., Sheen, I. S., Chu, C. M., Lin, D. Y., Chen, C. J., & Liaw, Y. F. (1997). Prospective study of hepatocellular carcinoma and liver cirrhosis in asymptomatic chronic hepatitis B virus carriers. *American journal of epidemiology*, *145*(11), 1039-1047.

Yu, Y., Gong, R., Mu, Y., Chen, Y., Zhu, C., Sun, Z., ... & Wu, J. (2011). Hepatitis B virus induces a novel inflammation network involving three inflammatory factors, IL-29, IL-8, and cyclooxygenase-2. *The Journal of Immunology*, *187*(9), 4844-4860.

Yuen, M. F., Chen, D. S., Dusheiko, G. M., Janssen, H. L., Lau, D. T., Locarnini, S. A., ... & Lai, C. L. (2018). Hepatitis B virus infection. *Nature reviews Disease primers*, *4*(1), 1-20.

Zhang, X., Shen, J., Man, K., Chu, E. S., Yau, T. O., Sung, J. C., ... & Yu, J. (2014). CXCL10 plays a key role as an inflammatory mediator and a non-invasive biomarker of non-alcoholic steatohepatitis. *Journal of hepatology*, *61*(6), 1365-1375.

Zhou, K., & Terrault, N. (2017). Management of hepatitis B in special populations. *Best Practice & Research Clinical Gastroenterology*, *31*(3), 311-320.

Zoulim, F. (2011). Hepatitis B virus resistance to antiviral drugs: where are we going?. Liver International, 31, 111-116.

LIST OF APPENDICES

APPENDIX I: Ethical approval

KENYATTA UNIVERSITY
CENTRE FOR RESEARCH ETHICS AND SAFETY

Fax: 8711242/8711575 **P. O. Box 43844,**
Email: chairman.kuerc@ku.ac.ke
Nairobi, 00100

Tel: 8710901/12

Website: www.ku.ac.ke
Our Ref: **KU/ERC/APPROVAL/VOL.1** Date: 5th/05 /2023

Phinehas Mugo Njeru
P.O BOX 43844-00100
Nairobi.

Dear Mr. Njeru,

APPLICATION NUMBER: PKU/2601/I11260 - PREVALENCE AND GENETIC DIVERGENCE OF HEPATITIS B VIRUSAMONG HIV INFECTED PATIENTS ATTENDING KISII LEVEL 5 HOSPITAL

This is to inform you that ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** has reviewed and approved your above research proposal. Your application approval number is **PKU/2601/I11260.** The approval period is ***5th /05 /2023 to 5th /05 /2024***.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE***
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.

vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.

vii. Submission of an executive summary report within 90 days upon completion of the study to *KENYATTA UNIVERSITY ETHICS REVIEW COMMITTEE*

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

To serve you better, researchers are kindly requested to access and complete a customer feedback form and sent it back online as you continue with research and upon completion of data collection found on the following website link; ;(https://docs.google.com/forms/d/1ytWefDwvyz5h1oz_VIn0xbxg3uGdlDzMXFWNDsMrRPQ/edit?usp=sharing

Yours sincerely

Prof. Judith Kimiywe

Director: Centre for Research Ethics and Safety

APPENDIX II: NACOSTI approval

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **138995**

Date of Issue: **14/July/2023**

RESEARCH LICENSE

This is to Certify that Mr.. PHINEHAS GUTHUA MUGO NJERU of Kenyatta University, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Kisii on the topic: PREVALENCE AND GENETIC DIVERGENCE OF HEPATITIS B VIRUS AMONG HIV INFECTED PATIENTS ATTENDING KISII LEVEL 5 HOSPITAL for the period ending : 14/July/2024.

License No: **NACOSTI/P/23/27249**

138995

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

See overleaf for conditions

THE SCIENCE, TECHNOLOGY AND INNOVATION ACT, 2013 (Rev. 2014)

Legal Notice No. 108: The Science, Technology and Innovation (Research Licensing) Regulations, 2014

The National Commission for Science, Technology and Innovation, hereafter referred to as the Commission, was the established under the Science, Technology and Innovation Act 2013 (Revised 2014) herein after referred to as the Act. The objective of the Commission shall be to regulate and assure quality in the science, technology and innovation sector and advise the Government in matters related thereto.

CONDITIONS OF THE RESEARCH LICENSE

1. The License is granted subject to provisions of the Constitution of Kenya, the Science, Technology and Innovation Act, and other relevant laws, policies and regulations. Accordingly, the licensee shall adhere to such procedures, standards, code of ethics and guidelines as may be prescribed by regulations made under the Act, or prescribed by provisions of International treaties of which Kenya is a signatory to
2. The research and its related activities as well as outcomes shall be beneficial to the country and shall not in any way;
    i. Endanger national security
    ii. Adversely affect the lives of Kenyans
    iii. Be in contravention of Kenya's international obligations including Biological Weapons Convention (BWC), Comprehensive Nuclear-Test-Ban Treaty Organization (CTBTO), Chemical, Biological, Radiological and Nuclear (CBRN).
    iv. Result in exploitation of intellectual property rights of communities in Kenya
    v. Adversely affect the environment
    vi. Adversely affect the rights of communities
    vii. Endanger public safety and national cohesion
    viii. Plagiarize someone else's work
3. The License is valid for the proposed research, location and specified period.
4. The license any rights thereunder are non-transferable
5. The Commission reserves the right to cancel the research at any time during the research period if in the opinion of the Commission the research is not implemented in conformity with the provisions of the Act or any other written law.
6. The Licensee shall inform the relevant County Director of Education, County Commissioner and County Governor before commencement of the research.
7. Excavation, filming, movement, and collection of specimens are subject to further necessary clearance from relevant Government Agencies.
8. The License does not give authority to transfer research materials.
9. The Commission may monitor and evaluate the licensed research project for the purpose of assessing and evaluating compliance with the conditions of the License.
10. The Licensee shall submit one hard copy, and upload a soft copy of their final report (thesis) onto a platform designated by the Commission within one year of completion of the research.
11. The Commission reserves the right to modify the conditions of the License including cancellation without prior notice.
12. Research, findings and information regarding research systems shall be stored or disseminated, utilized or applied in such a manner as may be prescribed by the Commission from time to time.
13. The Licensee shall disclose to the Commission, the relevant Institutional Scientific and Ethical Review Committee, and the relevant national agencies any inventions and discoveries that are of National strategic importance.
14. The Commission shall have powers to acquire from any person the right in, or to, any scientific innovation, invention or patent of strategic importance to the country.
15. Relevant Institutional Scientific and Ethical Review Committee shall monitor and evaluate the research periodically, and make a report of its findings to the Commission for necessary action.

APPENDIX III: Informed Consent Form

CONSENT TO PARTICIPATE IN A RESEARCH PROJECT
TITLE: PREVALENCE GENETIC DIVERGENCE AND DRUG RESISTANCE OF HEPATITIS B VIRUS AMONG HIV-INFECTED PATIENTS ATTENDING KISII LEVEL 5 HOSPITAL

Dear Participant

Explanation of Procedures:

You are hereby invited to voluntarily take part in a research project aimed at determining the prevalence, genetic divergence and drug resistance of Hepatitis B Virus among HIV-infected patients attending Kisii Level 5 Hospital, Kisii County, Kenya. The natural course of HBV infection is adversely affected by co-infection with HIV leading to faster progression of the disease to liver cirrhosis, immune suppression and may eventually cause death. Early detection of HBV/HIV co-infection could help to optimize the control of HBV/ HIV, prevent transmission and curb drug resistance associated with co-infection. A trained counselor will examine and advise you. Thereafter, 5ml blood specimen will be collected.

Risks:

No major risk will be expected from this event. However, a slight discomfort may be experienced during blood specimen collection by a phlebotomist.

Benefits:

Knowing your HBV status will be of paramount importance to you and your doctor for development of effective management which might include vaccination against HBV. There will be no financial benefits or rewards for participating in this research. Your

community will by large benefit by formulation of policies governing HBV HIV co-infection using the data that will be acquired.

Confidentiality:

Personal data containing your names shall not be revealed to unauthorized persons. Publication and reporting in the final research document shall omit any identity references that may disclose your private data.

Withdrawal from the study:

No penalty will be preffered against you for refusing to participate. You will continue receiving the best care possible from the research team without victimization.

Contact Persons:

Any participant can track the research progress though Mr. Phinehas Guthua Mugo Njeru (0722629490).

Concerns related to the rights of participants shall be raised though Dr. Anthony Kebira of Kenyatta University, Biochemisty, Microbiology and Biotechnology department or through the chairman of Kenyatta University Research and Ethics Review Committee; chairman.kuerec@ku.ac.ke.

Signatures:

I ……. do hereby confirm that I am comfortable with the explanation given about the study, as all of my queries have been satisfactorily addressed. I acknowledge that further questions about the survey should be referred to the investigators. Furthermore, I affirm that I have willingly accepted the terms cited above.

Age ……..

Sex ……...…..

Sub County …….

Location …….

Sub-location …….

Signature …… Date …../ …./ ..….

Witness Name ……

Signature …… Date ……./ …./ ….

Interviewer Signature …… Date ……./ …./ ….

APPENDIX IV: Questionnaire

1. Age……
2. Gender □ Male □ Female
3. Marital status □ Single □ Married □Widowed
4. Occupation □ Professional □ Casual □ Business
5. What is your education level?

   □ Primary □ Secondary □ Post secondary □ None

6. Accepted testing for HIV and HBV? □ Yes □ No
7. Do you have any blood transfusion history? □ Yes □ No
8. Do you have any history of HBV Vaccination?
9. Are you on any HAART treatment? □ Yes □ No

   If you ticked yes, how long have you been on treatment? ……

10. HIV Screening Results: □ Positive □Negative
11. HBV status: □ Positive □Negative

Your participation is highly appreciated.