MOLECULAR DIVERSITY OF HEPATITIS B VIRUS IN HIV INFECTED PATIENTS AT MBAGATHI DISTRICT HOSPITAL, NAIROBI CITY COUNTY.

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

To my children for being my enduring delight and to all the patients globally whose lives are afflicted by HBV and HIV infections.

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ABBREV`IATIONS AND ACRONYMS

3TC:	Lamivudine
ADV:	Adefovir Dipivoxil
AIDS:	Acquired Immunodeficiency Syndrome
AFP:	Alpha Fetoprotein
ALP:	Alkaline Phosphatase
ALT:	Alanine Aminotransferase
Anti- HBc:	Antibody to Hepatitis B Core Antigen
Anti-HBe:	Antibody to Hepatitis B Envelope Antigen
Anti-HBs:	Antibody to Hepatitis B Surface Antigen
APR:	Aspartate Aminotransferase to Platelet Ration Index
ART:	Anti-Retroviral Therapy
AST:	Aspartate Aminotransferase
CCC:	Comprehensive Care Centre
cccDNA:	Convalently closed circular DNA
CDC:	Centers for Disease Control and Prevention
CHB:	Chronic Hepatitis B Virus infection
CLIMA:	Chemoluminiscence Immunoassay
DNA:	Deoxyribonucleic Acid
dsDNA:	Double Stranded Deoxyribonucleic Acid
ECDC:	European Centre for Disease Control and Prevention
ESAL:	European Association for the Study of the Liver
ETV:	Entecavir
HAART:	Highly Active Antiretroviral Therapy
HBcAg:	Hepatitis B Core Antigen
HBeAg:	Hepatitis B Envelope Antigen

HBIG:	Hepatitis B immunoglobulin
HBsAg:	Hepatitis B Surface Antigen
HBV:	Hepatitis B Virus
HCC:	Hepatocellular Carcinoma
HIV:	Human Immunodeficiency Virus
I IFN-α:	Alpha interferon
IgM anti-HBc:	An Immunoglobin M antibody to hepatitis B core antigen
IVD:	In vitro diagnostic test
KEMRI:	Kenya Medical Research Institute
LdT:	Telbivudine
MEGA v4:	Molecular Evolutionary Genetics Analysis version 4
MMS:	Ministry of Medical Services
MPHS:	Ministry of Public Health and Sanitation
NAs:	Nucleos(t)ide analogues
NCBI:	National Center for Biotechnology Information
ORFs:	Open Reading Frames
PCR	Polymerase Chain Reaction
Pol:	Polymerase
PWID:	Persons Who Inject Drugs
RDT:	Rapid Diagnostic Test
RFLP:	Restriction Fragment Length Polymorphism
RT:	Reverse Transcriptase
TDF:	Tenofovir
WHO:	World Health Organization

ABSTRACT

With increasing access to antiretroviral therapy (ART) across Sub-Saharan Africa, HIV-infected individuals live longer and are frequently co-infected with HBV due to similar transmission routes. Persons with Hepatitis B Virus and HIV dual-infection advance more swiftly to end-stage hepatic disease. With 8-15% genomic divergence by DNA sequence, HBV is currently classified into 10 genetic variants, from A to J with numerous subtypes. HBV genotypes have been clarified as influencing the clinical outcome of the chronic disease in hosts. Considering paucity of data on HBV genotypes among HBV/HIV co-infected individuals in Kenya and the significance of HBV variants in antiretroviral therapy response, a cross-sectional study on the molecular diversity of Hepatitis B virus genotypes and the risk factors among HIV infected patients in Kenya was undertaken. The systematic random sampling was used to recruit 180 HIV seropositive male and female individuals attending routine CD4+ T-lymphocyte and viral load laboratory monitoring. A structured questionnaire was administered to capture socio-epidemiological data while an ELISA for qualitative testing of HBsAg in human serum was used and HBV DNA was extracted from 9 HBsAg seropositive samples and HBV genotypes established in 5 nucleic acids by nested-PCR of pre-S gene, direct sequencing and phylogenetic tree clustering. The HBV prevalence was found to be 5.0% serologically and 2.8% by PCR. Drug injection, heavy alcohol consumption, low use of protection during sexual intercourse and increased frequency of sexually transmitted infections were found to be direct risk factors for increased HBV infection. This study reveals that HBV genotype A1 (60%) and D (D6 and D7) were the most prevalent and they showed very low genetic diversity. In addition, these strains indicated very close phylogenetic relationship to those isolated from Sudan, S. Africa, Botswana and Tunisia. The five isolate genotypes were found to have susceptible mutations in S gene for Lamivudine, Adefovir, Entecavir and Tenofovir. In conclusion, the HBV/A1 was predominant variant and this imply the possibility of increased incidence of the HBV genotype A1 in Kenya and indeed across the region due to increased migration and regional interaction of human populations.

CHAPTER ONE: INTRODUCTION

1.1Background Information

Chronic HBV infection is a major cause of Hepatocellular carcinoma (HCC) in majority of Sub-Saharan and Asian regions and almost 25% of all HBV persistently infected persons ultimately progress to HCC. HBV carriers have 100-fold risk of HCC than that for non-carriers (Ganem & Prince, 2004). Africa suffers from the highest burden of HIV infection and, alongside Asia, are the leading reservoirs of chronic Hepatitis B Virus (CHB) (Modi & Feld, 2007).

With increasing access to antiretroviral therapy across Sub-Saharan Africa, HIV-infected individuals live longer and the effects of co-infection with chronic viral hepatitis is an emerging critical public health problem (Modi & Feld, 2007). Hepatic infection is among the major causes of illness and fatality among HIV-infected individuals (Soriano *et al*., 2008). The mortality likely hood from hepatic infection among HIV/HBV concurrently diseased persons is 13-fold more as compared to those infected with only one virus (Thio *et al*., 2002). HIV co-infection accelerates HBV-related liver damage, leading to earlier cirrhosis and end-stage liver disease. Conversely, the presence of HBV co-infection complicates the management of HIV and increases the morbidity and mortality of HIV-infected patients (Sheng *et al*., 2012). Patients infected with HIV have a high risk for HBV infection due to similar transmission routes for both HBV and HIV infections (Sulkowski, 2008). Although both HIV/HBV are transmissible at a countless frequency by parenteral, percutaneous and sexual contacts; Hepatitis B Virus is fifty to a hundred-fold highly

contagious as compared to HIV and up to 10% of HIV infected patients are also suffering from chronic HBV (CHB) infection (WHO, 2012; Soriano *et al*., 2010).

Hepatitis B Virus is currently categorized into ten genotypes, A-J with various subtypes and serotypes. The prevalence of these genotypes, subtypes and serotypes varies from region to region and ethnically worldwide (Rosas-Acosta, 2013). HBV genotypes have 8-15% genomic divergence by DNA sequence (Norder *et al*., 2004). HBV genetic variants are known to influence the clinical outcome of the chronic disease in hosts. Recent studies in Kenya have reported the presence of HBV genotype A1 and D3 variants among patients attending liver clinic with different clinical manifestation and disease outcome association (Ochwoto *et al*., 2013; Mutuma *et al*., 2011). Dually infected women with HIV/HBV have been described to be at a more risk for increased ALT levels in contrast to HIV mono-infected females (Day *et al*., 2013; Ochwoto *et al*., 2013). Based on HAART intervention for HIV patients that prolongs life and the co-infection with chronic HBV, it was essential to carry out a molecular diversity study on HBV genotypes in HIV infected patients in Kenya in comparison with those identified elsewhere.

1.2 Statement of the Problem

Hepatitis B viral disease is a common cause of hepatic impairment, liver cirrhosis and HCC; that are the major risk factors for illness and death among the HIV infected individuals. With increasing access to antiretroviral therapy across Sub-Saharan Africa, HIV-infected individuals have a longer life and are frequently co-infected with HBV due to similar transmission routes. Persons that are HBV/HIV co-infected advance more promptly to end-stage liver disease. Kenya is among the countries that are affected by

hepatitis B, and a high HIV burden, leading to frequent HIV/HBV co-infection. HBV is among the major infectious agents responsible for about 20% of all cancers in Kenya (MPHS & MMS, 2011). In Kenya, studies ((Harania *et al.,* 2008; Atina *et al*., 2004) have established 6-26.9% HBsAg in HIV sero-positive and patients presenting with jaundice, while studies (Ochwoto *et al.,* 2016; Ochwoto *et al.,* 2013; Mutuma *et al.,* 2011) on liver-related problems have reported between 44-75% HBsAg positivity. Hepatitis B Virus co-infection with HIV has serious implications including: higher rates of liver-associated morbidity and fatality, intensified HBV multiplication, immunity alteration to Hepatitis B virus within the antiretroviral therapy environment and liver toxicity from HAART medications complicating the effective management of HIV/AIDS. Considerable molecular variation occurs throughout the HBV genome and the genetic diversity is correlated with topographical distribution of genotypes. A previous Kenyan study strongly linked the occurrence of hepatoma in liver clinic patients to HBV (Mutuma *et al*., 2011). Variants of Hepatitis B sub-genotypes D6, D4, D3, E, A1 and A2 have been isolated in Kenyan blood donors and liver clinic clients, whereas A1 and D genotypic variants are the most dominant genotypes in patients with liver diseases (Ochwoto *et al.,* 2013).

Growing evidence confirms that HBV genotypic variants are significant determinants in Hepatitis B viral infection development, vaccination, response to ART and particular HBV mutants evolving during therapeutic pressure (either from vaccination or ART) complicate the management of Hepatitis B disease. HBV co-infection complicates the management of HIV, increases the morbidity and mortality of HIV-infected patients and; there are increased risk of Hepatitis flares as a result of immune alteration within HAART setting and increased risks of ART regimen resistance in people living with HIV.

1.3 The Rational of the study

There was scanty data on HBV genetic variants in HBV/HIV co-infected individuals in Kenya and the implication of HBV genotypes in ART response. HBV genotypes within HIV infected population that is concurrently suffering from Hepatitis B Virus that carry diagnostic and treatment escape mutants would avail valuable evidence that can advance novel management regimens for patients suffering from HBV and HIV.

1.4 Research Questions

i. What is the prevalence and risk factors of hepatitis B virus in HIV infected patients?

ii. What are the various HBV genotypes and drug mutations in HIV-HBV co-infected patients?

1.5 Objectives

1.5.1 Broad Objective

To determine the molecular diversity of hepatitis B genotypes in HIV infected patients at Mbagathi District Hospital, Nairobi.

1.5.2 Specific Objectives

i. To determine the prevalence and risk factors of hepatitis B virus in HIV infected patients.

ii. To establish the various HBV genotypes and drug mutations among the HIV-HBV co-infected patients.

1.6 Significance of the Study

This research has evidential outcomes that are valuable to public health and medical experts, patients, research scientists and the general public by expanding their understanding of HBV prevalence and genotypic variations among HIV infected patients. This enhances the knowledge of individual risk factors for liver disease, cancer development, HBV infection, HIV co-morbidities and form the basis for better disease management and; for designing improved preventive strategies.

CHAPTER TWO: LITERATURE REVIEW

2.1 Hepatitis B Virus Infection

Hepatitis B Virus is an incompletely double-stranded DNA virus, hepatotropic (Adoga, 2012) and a significant cause of progressive complications of liver cirrhosis, prolonged hepatic infection, HCC and liver failure among Southern European, Asian and African regions (Serviddio, 2013; Michielsen *et al*., 2005). Liver cancer is among the mostly occurring malignant tumors and is ranked among the top-five most occurring solid malignancies and 3rd major risk factor for malignancy-associated mortality globally among males (EASL, 2012; El-serag, 2012). Persons with chronic HBV risk serious illness and death, and risk infecting others. In 2015, HBV infection caused an approximately 887,220 mortalities globally and, HBV was a predominant causative agent for HCC among other significant risk factors for HCC in the highly endemic regions with CHB (WHO, 2017).

Approximately 45% of all hepatocellular carcinoma and 30% of liver cirrhosis incidences are caused by Hepatitis B Virus, with greater magnitudes in medium and low economic nations. The occurrence of Hepatocellular carcinoma and liver cirrhosis is lower below the ages of thirty five to forty years then increases exponentially above 40 years of age. Conversely, HCC incidences are high in Hepatitis B infected young male adults and children within the rural western Alaska, Amazon and Africa regions. Besides this, there is a substantial financial strain because of the life lost due HBV infection and it accounts for 5 to 10% of liver transplants (WHO, 2015). In Sub-Saharan Africa, liver malignancy is recounted as the second and fourth leading malignant cases in males and females respectively in Sub-Saharan Africa (ACS, 2011). Tumors are the 3rd risk factors for

mortality in Kenya, with annual estimate of 28,000 incidences and 22,000 mortalities. HBV is among the major infectious agents responsible for about 20% of all cancers (MPHS & MMS, 2011). Both HBV and HIV are considered to be hyper-endemic in Sub-Saharan continent, with 2% of all annual deaths in Africa resulting from clinical consequences of HBV infection and 25% of all annual deaths resulting from the clinical consequences of HIV infection (Kramvis & Kew, 2007).

2.2 Epidemiology of HBV

Globally, most persons infected with CHB acquire it perinatally or in infantile stages and HBV infection occurs all over the world (WHO, 2015). Over two billion are approximated to have acquired Hepatitis B Virus globally, with a third of the global population already exposed to HBV. Worldwide, the occurrence of Hepatitis B Virus in the overall population is 3.5% and approximately two hundred and fifty-seven million people are surviving with CHB. Hepatitis B Virus occurrence differs across the world from 0.1% to 20%, with the Western Pacific and African regions having the highest HBV prevalence (6.2%) (WHO, 2017). Annually, over six-hundred thousand persons die as a result of Hepatitis B Virus disease and approximately 4.5 million Hepatitis B Virus incidences happen globally, with 1/4 developing to hepatic infection (WHO, 2012).

The world can be divided into low, moderate and high endemic regions based on HBV carrier states (Franco *et al*., 2012). The occurrence of active Hepatitis B disease is determined by HBsAg serology in the general population of a specific geography. The high endemic areas have prevalence of 8% and above, intermediate prevalence has 2-7%

prevalence while low endemic areas have less than 2% prevalence (WHO, 2017). Low endemic regions of HBV prevalence include United States, Western Europe, New Zealand, and Canada and constitutes 12% of the world's population with a lifetime infection risk of less than 20%. Intermediate HBV prevalence geographical areas represent 43% of the global populace and include Middle East, Japan, Latin and South America, Mediterranean countries and Central Asia, with a life time infection risk of 20-60%. High endemic regions for HBV prevalence include Sub-Saharan continent, China and Southeast Asia with more than 60% lifetime risk of infection. In these high endemic regions, horizontal transmission among children and from mother to child vertical transmission are the most common HBV infection routes (Serviddio, 2013).

The stage of development at which one acquires HBV directly relates with HBV chronicity risks since the degree of advancement from acute to CHB decrease as one advances in oldness; and this results in diverse prevalence rates globally. It is less than 5% risk of chronicity for adults and about 90% risk of chronicity for perinatal and early childhood HBV infections (Mauss *et al.*, 2016). Asia and Africa constitute high Hepatitis B Virus endemic zones with high rates of chronicity due to early childhood and perinatal as the common routes of transmission (Hou *et al*., 2005). The HBV moderate endemic areas of Eastern Europe and the Mediterranean have common HBV routes of transmission through perinatal, household, sexual and nosocomial. In contrast, very low HBV endemic areas of Australia, Europe and North America have less than 1% HBV prevalence since sexual and IV drug usage consists the majority HBV contact routes (Alter, 2003).

Africa constitutes about 20% of the chronic HBV carriers (65 million) with Sub-Saharan sub-continent reported to have extremely high prevalence rates (Kramvis & Kew, 2007). In Kenya, HBsAg seems to vary in different population groups. Some studies have established HBsAg prevalence of 6-26.9% in HIV seropositive and patients presenting with jaundice (Harania *et al*., 2008; Atina *et al*., 2004), however surveys on liver-related problems have reported between 8.8-61.4% HBsAg positivity (Ochwoto *et al.*, 2016; Ochwoto *et al*., 2013; Mutuma *et al*., 2011).

2.3 The Hepatitis B Virus Family, Genome and Replication

HBV is a 42-nanometer, enveloped with an inner icosahedral nucleocapsid containing circularized and partially double stranded DNA (dsDNA) and is classified in the family *Hepadnaviridae,* genus *Orthohepadnavirus* (Adoga, 2012). Although other HBV variants exist in higher primates, mankind is the sole recognized reservoir for Hepatitis B Virus and HBV replication primarily takes place in hepatocytes. HBV is highly infectious, can remain in the environment for at least a week, is heat stable but is sensitive to solvents and detergents since they denature lipids from the viral envelop. (WHO, 2017).

The 3.2kb HBV DNA genome has four ORFs that encodes S, P, X and C genes and 7 proteins. The core protein is encrypted by C gene, Pre-C encodes the serum envelop antigen while the HBsAg three-related viral envelopes are encrypted by S gene, HBx protein is encoded by the X gene, and the viral DNA polymerase is coded by P gene (Schaefer, 2007; Kann & Gerlich, 2005). HBV envelop antigen (HBeAg) is a secreted dimeric protein and its presence in body fluids (including salivary, seminal and vaginal

secretions) shows active HBV replication, with the body fluids being highly infectious. HBV core antigen (HBcAg) is a viral capsid that occurs on HBV DNA enclosed by the assembled capsids. High levels of anti-HBc antibodies that are not protective appear during the infection. HBV polymerase (Pol/RT) is responsible for reverse transcriptase activity, while HBV x antigen (HBxAg) controls the transcription required to initiate infection (EASL, 2017). HBsAg (PreS1/PreS2) consists of a huge, average and minor surface envelope glycoprotein with three proteins: large surface proteins (LHBs), middle (MHBs), and small (SHBs). Besides these, non-contagious sub-viral particles (SVPs) are also formed and are released in the body fluids with a quotient of virus/particles: SVPs = 1:3000 (WHO, 2017).

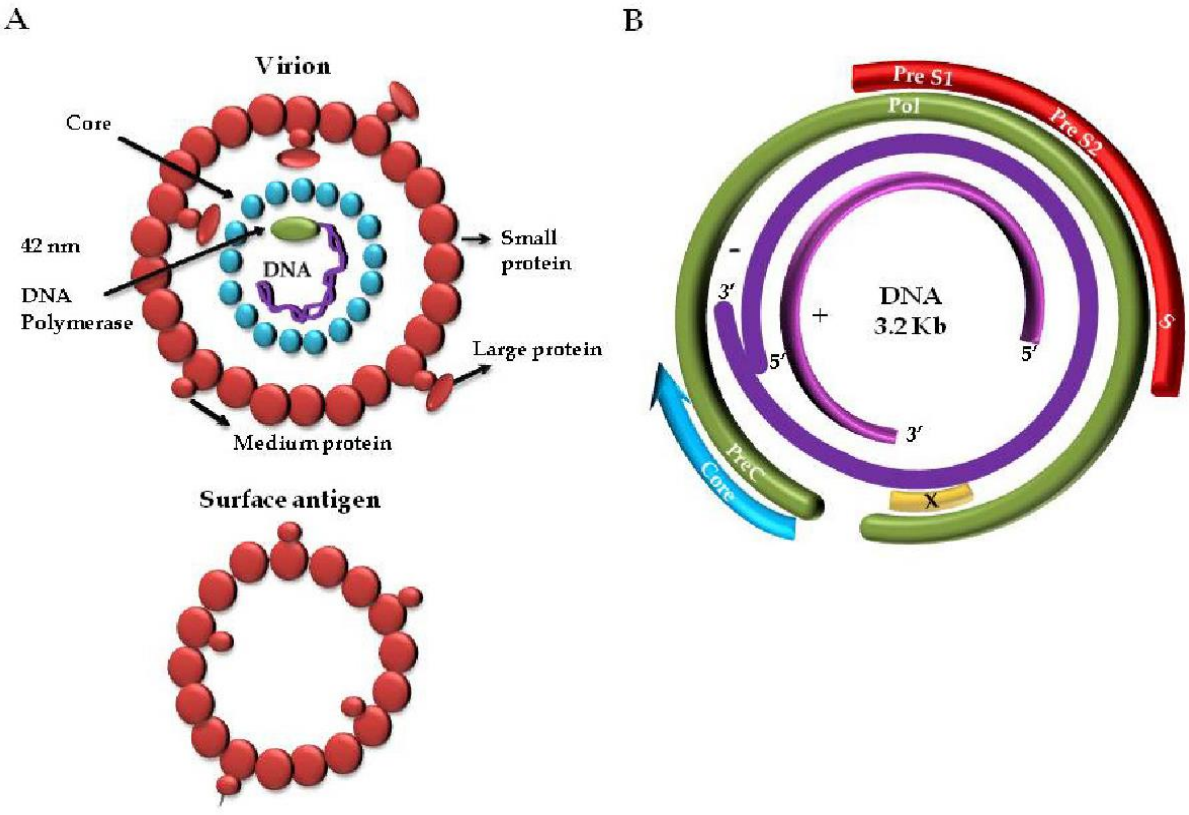

Figure 2. 1: HBV Virion and genome organization structure (Source: Adapted from Adoga, 2012)

HBV reproduces by reverse transcription through an RNA intermediary into pre-genomic and several sub-genomic messenger RNAs (Adoga, 2012), using virus-encrypted polymerase. The polymerase is devoid of proof reading mechanism and this leads to varied HBV sequence heterogeneity (Soriano *et al*., 2006). When HBV enters hepatocytes, the nucleocapsid is taken into the nucleus and HBV-relaxed circular DNA (rcDNA) is emitted. The rcDNA is changed to a covalently closed circular DNA (cccDNA) that is enclosed by histones into an episomal chromatinized structure. This acts as a transcript template for total viral copies which are transformed to various viral proteins. Besides to coding the viral polymerase and the capsid protein, the pre-genomic RNA is reverse transliterated to different rcDNA inside the viral capsid. The DNA comprising of nucleocapsids in the cytoplasm are either reused in the nucleus to sustain cccDNA pool, or enveloped and released via endoplasmic reticulum. Virions (42nm in diameter) realeased non-cytopathically through cellular secretory passageway are highly infectious. The infected cells produce enormous and further non-genomic, non-infectious 22nm sub-viral spherical/filamentous elements. Random integration of viral DNA in host genetic material ensues, though this is non-essential for viral reproduction and is one of the key modalities convoluted in hepatocyte transformation (EASL, 2017).

HBV genome may be assimilated in the infected host DNA liver cells, resulting into long term disease and the assimilated HBV genome fragment may aid in HCC development. HBx is a non-structural protein essential for encoding the viral DNA and may aid in oncogenicity of HBV (WHO, 2017). Viral cccDNA is the main genetic structure liable for persistence of infection in the hepatocytes of infected persons even subsequent to lasting

ART, seroconversion and HBsAg loss. The control of the intrahepatic pool of cccDNA comprise many elements such as the dynamics of septicity in the liver and the intrahepatic antiviral immune reaction. Viral and host factors regulate the cccDNA encoding (EASL, 2017).

2.4 HBV Genotypes and Distribution

2.4.1 HBV Genetic Variability

HBV genotypic distinction is shown by incomplete sequencing of its genome (Magnius & Norder, 1995). HBV S gene is more preserved as compared to pre-S region with a 1306bp fragment incompletely consisting of polymerase coding regions(S/POL) and HBsAg useful for genotyping (Serviddio, 2013). Genotypic diversity may be due genomic recombination, drifting of the virus genome through neutral evolution and continuous acclimatization of HBV to genomic-contributing factors to particular host populations (Adoga, 2012). The HBV reverse transcriptase is void of proof reading and this leads to recurrent genomic mutations with concurrence of hereditarily unique viral species (quasi-species) in patients which emerge depending on environmental pressure in the host. The interaction amongst the virus, liver cells and the immunological reaction or antiviral therapy is believed to cause genetic mutation that have the potential to escape host immune responses or antiviral therapy (EASL, 2017).

2.4.2 HBV Genotypes, Sub-genotypes and Geographic Distribution

HBsAg was serologically used to classify the HBV into 4 serotypes adr, ayr, ayw and adw with 9 insignificant subtypes (adrq, ayw1, adrq+, ayw4, adw4q, ayw2 ayr, adw2 and ayw3) (Franco *et al.,* 2012, Rosas-Acosta, 2013 and Norder *et al*., 2004). The current classification is founded on comparison in 8% divergence within complete genomic nucleotide sequence and is classified into ten genotypes; A- J with many HBV sub-genotypes existing that differ by >4% (Kurbanov *et al*., 2010; Serviddio, 2013; WHO, 2017).

Hepatitis B genotypes have distinctive geographical and cultural distributions: genotypes A (with subtypes A1-A6) and D (subtypes D1-D7) dominate in India, Africa and Europe; B (subtypes B1-B9) and genotypes C (subtypes C1-C19) predominate in Asia; genotype E predominates in Central and West Africa; and F (subtypes F1-F4) predominates in South and Central America. Genotype G is mainly found in Central and North America and Europe (Adoga, 2012). The most recent genotypes are H (US, Mexico and Central America), I (a recombinant type of A, C, & G) first determined in Vietnam (Tran et al., 2008) and J in Japan (Tatematsu *et al*., 2009). Genotype A (sub-genotype A1) mainly predominates in Eastern, Central and Southern Africa while sub-genotype A3 is dominant in western Africa. Genotype D and E are found mostly in Northern Africa and Western and Central Africa respectively (Kramvis & Kew, 2007). Studies in Kenya have reported HBV A1, A2, D1, D3, D4 and E genomic variants among sick persons with hepatic-related problems and in lifeblood contributors (Nyairo *et al*., 2016; Kilongosi *et al*., 2015; Kwange *et al*., 2013; Ochwoto *et al*., 2013; Mwangi *et al*., 2008).

2.5 HBV Genotypic Variants' Influence on Disease Advancement, Vaccination and Treatment

HBV genotypes are linked to different clinical outcomes and are important factors that determines infection progress and response to ART in HBV patients (WHO, 2017). Structural and functional distinction among HBV genetic variants influences the progression, severity and probability of HBV disease complexities, HBeAg seroconversion, treatment responses and probably HBV immunization (Franco et al., 2012, Chu & Lok, 2002). More proportions of Hepatocellular Carcinoma have been reported in patients suffering from genotypic variants F and C in comparison to clients with D or B genotypes; mostly in South African clients diseased with specific sub-genotypes of A. The proportion and common age for HBeAg clearance to anti-HBe differs by Hepatitis B genomic variant since patients with genotype C continue being HBeAg-positive for an elongated time in years than clients with genotypes A, B, D, or F (WHO, 2015). Evidence reported in India, in which HBV variants D & A are dominant, indicates clients who had genotypic variant D had comparatively greater infection severity and progress of HCC (Thakur *et al*., 2002). Persons infected with HBV genetic variants D & C had less reaction degree to interferon treatment as compared to those who had genetic variants B or A (Janssen *et al*., 2005; Kao *et al*., 2002). A research from Alaska determined that the clinical and virological characteristics of 5 HBV genetic variants had an average age seroconversion to anti-HBe from HB surface antigen considerably lesser amongst genotypic variants F, B, D and A as compared to C (Livingston *et al*., 2007). In a research to establish the effect of HBV genetic variants on liver disease in Vietnam established variant A to be more common in symptomatic clients than the asymptomatic ones while

genotypic variant C was regular in hepatocellular carcinoma clients. HBV Genetic variants were reported commonly in persons chronically infected with HBV as compared to those had acute hepatitis, hepatocellular carcinoma and liver cirrhosis. Additionally, viral loads in clients with mixed HBV genotypes were considerably more than in mono-HBV variants patients and were similarly linked to more in vitro HBV replication. The study concluded that HBV infection with mixtures of HBV genetic variants is common in Europe, Asia and Africa. The differences in the infection outcomes between mono-HBV genotype and mixed Hepatitis B genetic variants imply that co-infection with diverse HBV-genotypes is linked with altered infection progression and clinical consequences (Toan *et al*., 2006).

Various naturally arising pre-core gene alterations that hinder HBeAg synthesis, have been recognized in negative Hepatitis B e-antigen clients with CHB. Hepatitis B genetic variants impact the incidence of pre-core mutants, however, the functionality of this alteration in hepatic infection is indistinct. Hepatitis B envelop antigen is undetectable in immune escape-mutant patients since basal core and pre-core promoter region transformation of Hepatitis B genetic makeup gives rise to genotypes incapable of showing Hepatitis B envelop antigen (WHO, 2015). Seroconversion of HBeAg linked to the rise of HBV mutants (basal core promoter A1762T and G1764A or pre-core G1896A alterations) is commonly found in persons suffering from genetic variants D & B unlike individuals infected with HBV genetic variants A & C (Chu & Lok, 2002). The greater occurrence of pre-core G1896A genetic alteration in variants D & B unlike in genetic variants A and C may partially clarify why residual hepatic disease is regular among persons suffering from genotypes B & D (Chu *et al*., 2003). HBV genetic variant G seems defective, thus is

frequently found composed of other genotype, providing transcript elements required for reproduction. Viral quantification in persons with a mix of genetic variants is commonly greater as compared to those only infected with a specific variant (Rosas-Acosta, 2013). Genotype A is the leading variant in USA and more receptive to interferon than variant D Asia (Rosas-Acosta, 2013).

In dually HIV-HBV infected persons, HBV genetic variant A is reported to have greater baseline plasma HBV DNA loads (Soriano *et al*.,2010), greater incidence of HBeAg positivity, with enhanced HBV DNA subdual during anti-HBV treatment unlike persons infected with genotypes D, E, F, and G (Jain *et al*., 2007; Lacombe *et al*., 2006). Pre-core and Basal Core Promotor mutants have been seen more regularly in clients with genotypic variant D unlike among individuals infected with variant A (Ramos *et al*., 2007). HIV positive persons dually infected with Hepatitis B genetic variants B & C are more prone to having severe hepatic flares, HB envelop antigen seroconversion, Lamivudine resistance, and hepatic illness–associated mortality unlike among clients concurrently with genotypic variant C infection (Sheng *et al*., 2012). It is reported that the HBV recombinant vaccine formulated from genotype A does not offer immunity against other HBV genotypes (Norder *et al*., 2004). Likewise, a Kenyan study established that genomic mutation affects HBV hepato-carcinogenicity and HBeAg phenotypic expression in HBV genotypes A and D (Ochwoto *et al*., 2013).

2.6 Transmission, Pathogenesis and Clinical Manifestation of HBV Infection

2.6.1 HBV Transmission

Hepatitis B virus is highly infectious and is easily spread through contact of mucosal membranes and broken skin of septic body fluids. HBV is spread through all body fluids including vaginal secretions, blood, tears, saliva and semen. HBV transmission depends on the epidemiology within a specific locality and is spread majorly through parenteral contact, sexual contact and perinatal during delivery. The parenteral contact routes include skin injuries from infected sharps such as tattoos, pierce, shaving, body lesions and exudates, brushing of the teeth, dental procedures, surgery, blood transfusion, organ and tissue transplants, bites, pre-mastication or contact from HBV contaminated surfaces (WHO, 2017). HBV is 50 to 100 folds more contagious than HIV, with survival ability on outer surfaces for 7 days at minimum. HBV is considered the utmost frequently spread blood-borne virus in hospital environments (WHO, 2012). Hepatitis B positive mothers for envelop and surface antigens have greater possibilities of transmitting the infection to their new born with up to 70 to 100% and up to 40% risks within Asian and African regions respectively as compared to babies delivered by HBeAg and HBsAg negative moms, having 5 to 30% and 5% risks in Asia and Africa respectively. Mothers having high active HBV replication posse the most perinatal transmission risks to newborns at the time of delivery and, without prophylaxis the HBeAg seropositive mothers transmit to their infants. The chances for mother to child spread are also higher when pregnant mom experiences acute hepatitis B from 1st or 2nd phase of the gestation or in two months' post-partum period. Though Hepatitis B infection to a fetus can occur utero, it occurs rarely and it is largely linked to ante-partum hemorrhage and placental tears. HBsAg Seropositive

mothers to HBsAg minimizes the risks of perinatal HBV infection to their infants when they deliver through surgery (WHO, 2017; WHO, 2015).

Horizontal transmission of HBV infection among family members mainly consisting of household, intra-familial and child to child contact is also common and fifty percent and above transmission in children is as a result of vertical transmission since HBV incidences reached the climax in seven-to-fourteen-year-olds before the advent of neonatal immunizations (WHO, 2015).

2.6.2 Pathogenesis and Clinical Manifestation of Hepatitis B Disease

Severe Hepatitis B is incubated at a mean of seventy-five days with a variation range duration of thirty-to-one hundred and eighty days. The hepatocyte damage throughout the HBV septic period is facilitated by reaction of the host's immunity. Consequences of Hepatitis B septicity hinge on host dynamics comprising of sex, hereditary factors of the host, age, extra simultaneous diseases and associated treatments, and on virus-related features comprising of the HBV genetic mutants, and viral DNA levels (WHO, 2017). Hepatitis B septicity might be either severe or chronic, and can vary from lack of symptoms/ minor illness to acute or infrequently fulminant hepatitis. Acute hepatitis B is typically a restricted illness manifested by severe inflammation and hepatocellular necrosis, with a 0.5-1% instance mortality degree. CHB septicity involves a range of syndrome, with a description as insistent Hepatitis B septicity through the occurrence of manifest blood/serum HBsAg for an elongated period of more than 6 months in the presence or lack of the accompanying active viral reproduction and indication of hepatocellular damage plus inflammation (WHO, 2015; Burnett *et al.,* 2005). Full-blown

hepatitis occurs in 0.1–0.6% of severe circumstances, and commonly resulting to critical hepatic failure accompanied by signs like jaundice, dark urine, great lethargy, queasiness plus belly agony (WHO, 2012).

The HBV range of infection plus its CHB natural history varies. In some instances, Chronic Hepatitis B is passive and results to insignificant hepatic illness; whereas in others, it leads to advanced hepatic fibrosis, resulting into cirrhosis with end-stage liver disease, besides a distinctly amplified possibility of HCC independent from the existence of cirrhosis, commonly several years subsequent to first HBV contagion. Chronic Hepatitis B septicity takes 8–20% aggregate risk of evolving into cirrhosis above 5 years. In individuals with cirrhosis, there is approximately twenty percent yearly possibility of hepatic non-restitution and yearly pronounced HBV-associated prevalence HCC, with an oscillation of one-to five percent. Clients without treatment with decompensated cirrhosis risk poor prospects of recovery, as fifteen-to-forty percent live for about 5 years. Numerous host and viral dynamics, particularly concomitance with HIV, HCV and HDV in combination with additional cofactors including alcoholism, aflatoxins can escalate the proportion of infection development and the danger of evolving to Hepatocellular Carcinoma (WHO, 2015).

The early HBV septicity can be without any signs, or might manifest as severe hepatitis accompanied or not with yellowish tints on eyes and skin, or else end in fulminant hepatitis. Severe hepatitis B, categorized by serious inflammation and hepatocellular necrosis, happens in roughly one percent of mother-to-child infection, ten percent of infancy stage

and thirty percent of late childhood and adulthood infections. Full-blown hepatitis rarely occurs in infancy and childhood; nevertheless, happens in 0.5%–1% of mature circumstances of severe HBV, with a mortality proportion of 20%–33%. The degree of progress of CHB is contrariwise associated with age at acquisition of the disease, arising in about 80%–90% in infant's septic during birth, 30%–50% of children infected below 6 years, and less than five percent septic happening in or else well grown-ups. Latest HBV septicity is shown by the existence of HBsAg and IgM in blood against HBcAg. Throughout the early very replicative stage of septicity, clients remain similarly seropositive for HBeAg (WHO, 2017).

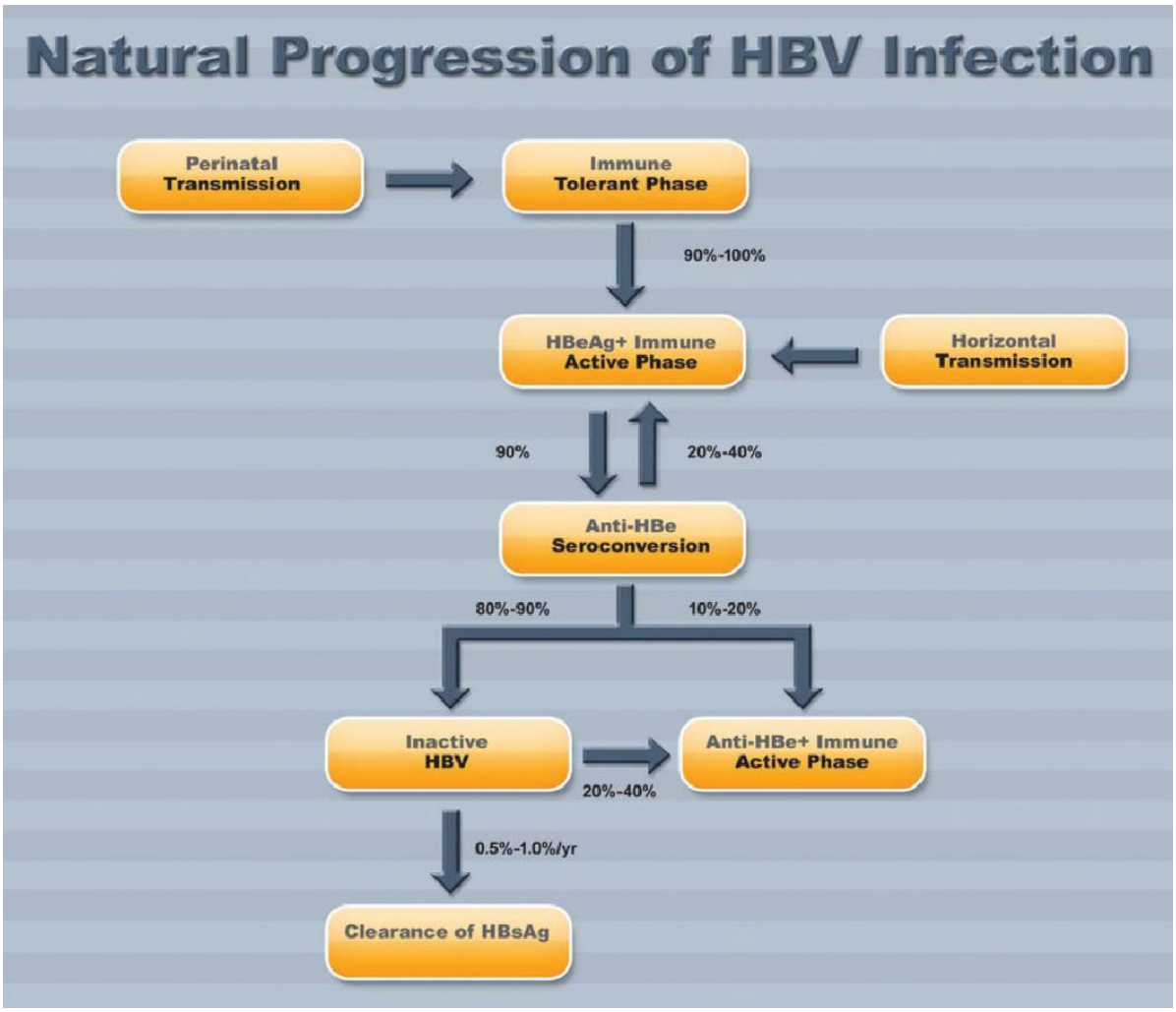

Figure 2. 2: Algorithm to display the natural history of chronic hepatitis B virus infection (Adopted from McMahon, 2009).

CHB septic clients face great danger of advancing to liver cirrhosis and HCC (WHO, 2017). It is projected that liver cancer epitomizes nearly 4% entirely novel malignancy incidences detected globally and above 2/3 of altogether incidences of liver cancer universally are instigated by Hepatitis B Virus. Up to ninety percent of newborns who acquire HBV within the 1st year of life while thirty-to- fifty percent of children acquiring the infection amongst 1-4 years of age develop CHB carriers. Approximately ten percent

of acquiring HBV in maturity develop chronic and up to twenty-five of all chronically disease-ridden patients ultimately perish from HBV-associated liver cancer or cirrhosis (Franco, et al., 2012; Ganem & Prince, 2004).

2.6.3 Host Immune Response to HBV

HBV infects the hepatocytes, resulting in hepatocellular necrosis and inflammation. The hepatocyte damage follows immune-mediated injury to infected hepatocytes and HBV is equally a known oncogenic agent that pose a greater danger of increasing HCC development (WHO, 2015). In serious determining disease, the reaction of the inborn and acquired immune coordination to HBV is effective and appropriate. Viral resolution encompasses the stimulation of a vigorous acquired T-cell response prompting both a cytolysis of dependent and independent antiviral effects through the countenance of antiviral cytokines, likewise inducing B cells generating neutralizing antibodies to impair extending the virus. Liver cell regeneration subsequent to the infected cell apoptosis result to cccDNA attenuation. When the severe hepatitis develops to long-lasting, there is a progressive decrease in HBV particular T-cell functioning. CHB septicity advances via clear infection stages that are intensely related to client's age. CHB might not be perpetuated by immune tolerance concept since kids and young adults with CHB septicity take an insusceptible profile that is less compromised than in grown-up individuals. Recent studies have reported that HBV lasts through virus-particular and universal T-cell malfunction intermediated by various controlling pathways, but deprived of distinctive T-cell founded resistant marks for medical phenotypes (EASL, 2017).

Majority of HBV infections are own-restrictive and succeeded by the progress of anti-HBs and anti-HBc in patients with cleared the HBV septicity. Resolution of noticeable HBsAg in body fluids typically proceeds numerous weeks/months. There is a great proportion of cross-immunity among HBsAg genotypes and immunity to HBV septicity after immunization which is shown via the attendance of anti-HBs (WHO, 2017).

2.7 The Hepatitis B Viral Infection Diagnosis

2.7.1 The Serological Diagnosis of HBV

The HBV genetic makeup codes for Hepatitis B Surface antigen(HBsAg), Hepatitis B core antigen (HBcAg), the viral polymerase and the Hepatitis B x protein (HBx). HBV is found in blood as a 42-nm, double-shelled particle, consisting of an external envelope component of HBsAg plus an internal nucleocapsid consprising HBcAg. HB Viral DNA is found in serum and is a marker of active HBV reproduction. HBeAg, in contrast to HBsAg and HBcAg, is not particulate, however it is found as a solvable protein in serum (WHO, 2015). Globally, accessibility to inexpensive and quality assured HBV testing is inadequate with only 9% of Hepatitis B infections having been estimated to have been tested through serological assays. Hepatitis B Virus infection's serological diagnosis is done by a quality-assured in vitro diagnostic test, through a laboratory-grounded enzyme immunoassay /chemo-luminescence or through a rapid diagnostic test (RDT) to identify anti-HBs (WHO, 2017). The Hepatitis B Virus might be identified thirty to sixty days' post infection and it continues for a varied duration (WHO, 2012). Hepatitis B serologic testing is an enzyme immunoassay-based and it may be done by detecting virally encrypted proteins and their equivalent immunological markers: HBsAg, anti-HBs, HBeAg, anti-HBe, and anti- HBc (Servoss & Friedman, 2006). HBsAg in blood appears throughout severe or long-lasting

HBV septicity. The occurrence of HBsAg shows the patient is contagious and persistence longer than six months signifies HBV chronicity. Occurrence of Anti-HBs is construed as signifying convalescence, protection against HBV disease and successful vaccination against hepatitis B. Total anti-HBc occurs during the inception of signs in severe hepatitis, continues for long term and defines past or current septicity with HBV in an indeterminate period. Occurrence of HBeAg shows patient's blood and other fluids being extremely infectious (WHO, 2012; Servoss & Friedman, 2006). The Positivity of IgM anti-HBc point to the latest severe HBV septicity below 6months (WHO, 2012).

Screening for high-risk clusters of individuals for HBsAg and HBV vaccination for populations at most risk and not immune should comprise family and intimate interactions of individuals with CHB, HIV clients, PWIDs, MSM, FSW, persons with multiple sex partners, native folks, individuals in prison, and transgender individuals. Prevention of Hepatitis B spread should need to include testing of organ and body tissue contributors for HBsAg. Besides, community-wide testing is commended for immigrants from HBV widespread regions (WHO, 2015). The recommended sample for HBV testing is serum. Immunoassays for viral proteins and immunoglobulins are characteristically employed during diagnosis and screening. Equally Hepatitis B antigens and antibody remain firm for days at room temperature, for months at 4°C, and for many years when frozen at –20°C to –70°C. Test choice need to be premised on the individual's risk dynamics, immunization antiquity and outcomes from past examinations (Krajden *et al.,* 2005). HBsAg is an important indicator for HBV testing and the 1st immunological indicator to occur

throughout the Hepatitis B disease course. HBsAg specificity and sensitivity is hinged on the testing threshold of serological tests (Cabezas-Fernandez & Cabeza-Barrera, 2012).

2.7.2 The Molecular Diagnosis of HBV

HBV is known to occur in serum of HbeAg positive persons approximately at 108–109 viral particles/mL^{-1} concentration. The DNA subsists in great titers in serum and body secretions in both severe and chronic HBV phases. Largely, modest viral titers are detectable in salivary, vaginal and seminal excretions (Kidd- Ljunggren *et al*., 2006). Furthermore, blood quantities of DNA generally are subject to HBV genetic variant, and HBeAg amount in blood, influencing the evolution of liver cirrhosis to carcinoma (Rosas-Acosta, 2013). Molecular biology methods include restriction fragment length polymorphism (RFLP), sequencing, PCR with genotype-specific primers, line probe assay or real-time PCR, viral load tests, drug resistance mutation tests, and core promoter/pre-core mutation assays (Vivekanandan & Singh, 2010) are usually utilized to identify, compute, sequence and explore HBV genotypes (Servoss & Friedman, 2006).

Numerous techniques for genotyping HBV are already established and these include: PCR with genotype-specific primers, post-PCR hybridization or line probe assay, melting curve analysis (MCA), enzyme-linked immune-sorbent assay-based methods and restriction fragment length polymorphism (RFLP). The PCR process offers an expedient means for prompt and economical testing in regions with diverse topographical HBV genetic variants in circulation (Liu *et al*., 2006). Hepatitis B variant determination by evolutionary/phylogenetic exploration premised on nucleotide series yields the greatest

dependable and quality genotyping outcomes. Nonetheless, it is unsuitable technique for large-scale molecular analysis. Additionally, a number of studies have described the HBV molecular analysis via RFLP process, with RFLP seemingly having less sensitivity and specificity. PCR molecular analysis technique is modest, faster, and has higher specificity for HBV molecular analysis with primer-specific as compared to RFLP (Naito *et al*., 2001).

2.8 HBV Prevention and Management

2.8.1 HBV Prevention

Hepatitis B disease risks may be minimized via an adjustment in behavior and increasing one's information of the disease. Screening of all organ and tissue donations, besides ensuring quality disinfection in hospital procedures greatly minimizes the Hepatitis B transmissibility hazards. Additionally, testing of all expectant mother's aids in preventing perinatal transmission during delivery. Immunization is the best efficient approach of avoiding Hepatitis B illness and its negative consequences on the liver health globally. A significant problem of CHB continues since the universal coverage through the natal dosage remains very small, approximate at 39% globally in 2015. Due to inadequate universal natal dosage or additional active interventions, the spread of Hepatitis B illness vertically is still the foremost basis for long-lasting hepatic disease when exposed children grows up (WHO, 2017).

A plasma-derived HBsAg vaccine was filtered from the blood of chronic clients in early 1980s.Though it was safe and effective, worries of the spread of active Hepatitis B Virus plus additional transmissible-blood infections made it to be rejected (CDC, 2012). The

endorsed recombinant HBV immunization has HBsAg prepared by inserting plasmids with already implanted HBsAg genes in mammalian/yeast cells. The inoculation is safe and prevents HBV infection and its long-lasting consequences (WHO, 2002; ECDC, 2010). It is administered in 3 intramuscular dosages, stimulating over 95% in 0-19 year olds and over 90% in healthy grown-up persons' sufficient immune protection (WHO, 2002). Hepatitis B immunoglobulin (HBIG), that is serum-derived from donors with great concentrates of anti-HBs, is utilized as an adjunct to HBV inoculation for post-exposure immune-prophylaxis (WHO, 2012).

HBV immunization is given in a 3 dosage sequences: 2 priming dosages given in one-30 days separately, is followed by a 3rd dosage given six months later. Immunogenicity descents to less than ninety percent in individuals forty years and above. HBV inoculation a pre-exposure efficiency of 80–100% and a post-exposure efficiency of 70–95%, subject to if HBIG is administered with the inoculation. The period of protection seems to be lifelong, and booster inoculations are not usually commended (WHO, 2017). HBIG gives inert and a three-to-six month's provisional anti-HBsAg immunity. Usage of immunoglobulin intents to offer inactive immunity for high risk individuals to HBV and for persons with insufficient immunization or unreactive to HBV vaccine in the past. HBIG given unaccompanied remains the main mode of immunity subsequent to Hepatitis contact for persons non-responsive to HBV immunization. Besides this, it is useful subsequent to liver transplantation in terminal HBV cases to avoid relapse of the infection in the transplanted liver (WHO, 2017). In instances of contact to HBV inadvertently, post-exposure prophylaxis is commended for all none immunized individuals, by passive-active

immunity. An initial dosage of inactive and lively vaccination ought to be given earlier. A Twelve-hour post the exposure to HBV is commonly taken the most recent period for maximum efficacy for post-exposure prophylaxis. A dosage of HBIG ought to be given concurrently, if the cause is identified to be HBsAg-positive. The additional 2- dosages of inoculation ought to be given afterwards from 4 and 12-24 weeks correspondingly. Immunized persons with a known reaction have no necessity for post-exposure prophylaxis. Persons without post-vaccination ought to be screened for anti-HBs titer immediately. Without being tested, or if the anti-HBs titer is inadequate with less than 100 IU/l of blood, individuals will need a 2^{nd} sequence of immunization. Persons recognized to be non-reactors needs twofold dosages of HBIG administered a month separately. The spread of Hepatitis B virus has been documented subsequent to transplanting of HBsAg-positive individuals' extra-liver tissues like kidney and cornea (Mauss *et al*., 2016).

HBV inoculation is given at anterolateral part of the thigh in newborns and at deltoid muscle in more mature and grownups. The immunization efficiency of HBV inoculation is dependent on occurrence of IgG antibodies to HBsAg (anti-HBs) post complete immunization. An anti-HBs antibody titers of ≥10 mIU/mL detection one-two months' post inoculation of the last dosage of the principal immunization sequences is taken to be reliable immunological indicator of lasting immunity against Hepatitis B septicity. There are negligible adversarial responses, including localized pain, myalgia and slight rise in body temperature, typically in twenty-four hours and minor responses are likely to be uncommon in kids unlike in grown-ups (WHO, 2017).

2.8.2 HBV Management

Determining the phase of hepatic infection in HBsAg-positive individuals is crucial in guiding incident treatment and specify the necessity for therapy. There is no particular therapy for severe hepatitis B illness. Medical administration is grounded upon palliative management and reprieve of signs, such as giving of sufficient diet and replenishing of liquids lost via queasiness and watery stool (WHO, 2017). The objectives of management of CHB are to attain continued regression of HBV reproduction and decrease in hepatic decompensation, thus minimizing morbidity and fatality due to advancement in hepatic infection. The eventual aim is to inhibit cirrhosis, hepatic failure and HCC. Factors useful in assessing management response consist of normalization ALT, reduction in serum HBV DNA level, and disappearance of HBeAg with or without detectable of anti-HBe, and reconstitution through hepatic histology (WHO, 2017; EASL, 2017).

Management of CHB infection is known to avoid or defer advancement to cirrhosis, diminish the occurrence of Hepatocellular carcinoma and increase existence via lasting viral suppression, although it is not curative (WHO, 2017). CHB is manageable with alpha Interferon (IFN-α) and 6 nucleoside/nucleotide analogues (NAs); telbivudine (LdT), lamivudine (3TC), entecavir (ETV), tenofovir (TDF) and adefovir dipivoxil (ADV) (ESAL, 2012). Though all NAs act on Hepatitis B polymerase, in general the medications impede viral nucleocapsid formation and halts viral DNA development by immature chain termination and their mechanisms of action differs; adefovir hinders the priming of reverse transcription; lamivudine, emtricitabine and tenofovir impede the formation of the viral (-) strand DNA; and entecavir prevents 3-main phases of HBV reproduction (WHO, 2015;

Rosas- Acosta, 2013). Tenofovir and entecavir antiviral medications are the favorite first-line therapy for all persons aged twelve years and above since they have a higher barrier to drug resistance. Entecavir is recommended in 2–11-year-olds. Lamivudine, Adefovir and Telbivudine have a lower barrier to resistance, may result to drug resistance plus are not commended for CHB treatment. In HBV/HIV co-infected persons aged 3 years and above, tenofovir + lamivudine (or emtricitabine) + efavirenz as a fixed-dosage amalgamation is acclaimed as the favorite choice to start the treatment. In HIV-infected expectant and lactating mothers, a single-daily fixed-dosage amalgamation of tenofovir + lamivudine (or emtricitabine) + efavirenz is acclaimed as first-line ART lifetime management and it also applies to ART started for PMTCT (WHO, 2015).

The 2nd regimen treatment in individuals with established or alleged antiviral resistance such as the previous experience or major non-reactive to lamivudine, entecavir, adefovir or telbivudine, it is recommended a change to tenofovir. Although tenofovir is favored for HBV mono-infected expectant mothers, there are no ARTs commended for the regular usage for antiviral therapy to prevent vertical Hepatitis B transmission. Though NAs are effective inhibitors of HBV reproduction, they rarely cure, and clearance of HBsAg is seldom, thus necessitating lifelong NA treatment for CHB disease (WHO, 2015). The advantage of NA treatment above Interferons (IFN) includes less adverse effects and an oral single dose that is taken daily. The major benefits of IFN above NAs is lack of resistance, and accomplishment of greater levels of HBeAg and HBsAg clearance. Nevertheless, interferons are not recommended in cases of decompensated cirrhosis and hypersplenism, thyroid disease, autoimmune diseases, acute heart artery illness, kidney

transplant malady, gestation, seizures and psychiatric sickness, simultaneous use of specific medications, retinopathy, thrombocytopenia and leucopenia. Likewise, Interferons cannot be administered in newborns below one year and in expectant mothers, below 50% of individuals managed with IFN will react, it is expensive and IFN are given by inoculation (WHO, 2015).

2.8.3 Monitoring for HBV Disease Progression and Treatment Response

Assessment of infection evolution and management response in individuals with CHB prior to, during and after-therapy ought to be done yearly by measuring HBsAg, ALT level (and AST for APRI), HBeAg, and Hepatitis B DNA rates. Non-invasive tests (APRI score or Fibro Scan) to evaluate the occurrence of cirrhosis, in patients devoid of cirrhosis from the start and when on therapy, adherence ought to be checked frequently. In addition to this, assessment of baseline kidney function and calculation of initial kidney malfunction ought to be checked in all patients before introducing the ART. Renal function ought to be assessed yearly in clients using long standing tenofovir or entecavir therapy, and development observed judiciously in children for some drug toxicity. In patients with cirrhosis despite the age and health determinants such as family history of HCC, individuals above forty-years of age with no clinical indication of cirrhosis and with HBV DNA level >2000 IU/mL, it is commended by WHO for regular observation for HCC with abdominal ultrasound and alpha-fetoprotein screening after every six months (WHO, 2015).

According to EASL, 2017 clinical guidelines, Hepatitis B treatment progress need to be monitored via virological, serological, biochemical, and histological indicators. Virological reaction throughout NA management is by untraceable HBV DNA by means of an accurate PCR reaction with a maximum discovery of 10 IU/ml. Immunological response is assessed by testing HBeAg and anti-HBs occurrence. Biochemical response is determined by a normalization of ALT levels while histological reaction is well-defined as a reduction in necro-inflammatory action without deteriorating in fibrosis as compared to prior treatment histological outcomes.

2.8.4 Occult HBV Infection

Occult HBV infection is described as continuous occurrence of Hepatitis B DNA in the hepatocytes in individuals whose HBsAg is not found in serum and majority are as well anti-HBc positive. Occult HBV could be activated by extended chemo- or immunosuppressive treatment and individuals with occult infection can similarly show a significant source of fresh infections in blood transfusion amenities in HBV-endemic poor regions where HBsAg is utilized as the only indicator of septicity in donor peoples. Individuals with lost HBsAg and without HBV DNA yet anti-HBc positive might reactivate when exposed to strong immunosuppressive medications (WHO, 2015). The nonexistence of HBsAg in HBV occult septicity is interpreted to be as a result of several mechanisms such as little rate of HBV reproduction as result of host's immune reaction or concurrent infection with other pathogens, relationship of HBsAg to anti-HBs occasioning the development of immunity complexes which diminish the circulation of free antigen, and as a result of genetic alterations, that impede HBsAg manifestation or modification of

HBsAg antigenicity, thus inhibiting testing by commercial tests (Mello *et al*., 2011). According to Chemin *et al*., (2011), Occult HBV could occur due to diverse mechanisms comprising of faulty HBsAg expression such as S gene mutation in structural or regulatory features and prevention of HBV replication consequent to concurrent HCV infection. The discovery of occult HBV septicity is done by computing HBV DNA in the hepatocytes persisting for 6 months and above with discreet HBsAg in the serum and anti-HBc positivity. Recent surveys (Jepkemei *et al.,* 2020 and Aluora *et al.,* 2020) have shown presence of ocuult HBV in Kenya at 18.7% and 2.4% respectively.

2.8.5 Hepatitis B disease virological evaluation and Hepatic severity assessment

Monitoring of further HBV immunological indicators and assessing aminotransferase quantities help in establishing hepatocyte inflammation; measurement of HBV DNA levels; and phase of hepatocyte cirrhosis by non-invasive tests (NITs) including aspartate aminotransferase (AST) to platelet ratio index (APRI), transient elastography (FibroScan) or FibroTest ought to be utilized regularly to guide and stipulate HBV treatment. Blood Hepatitis B DNA titers taken via real-time PCR in comparison to infection evolution are important in distinguishing impassive HBeAg-negative illness from passive CHB, besides gauging the efficiency of ART (WHO, 2015).

Consequently, WHO (2015) recommends comprehensive evaluation of HBV progressive hepatic sickness for medical assessment of cirrhosis characteristics and indication of decompensation. Liver enzyme quantification of serum bilirubin, albumin, ALT/AST ratio, alkaline phosphatase (ALP), and prothrombin time; besides complete blood count, as well

as platelet computation are essential for effective CHB management. Assessments of hepatic synthetic function and/or portal hypertension comprise serum albumin, bilirubin, platelet count and prothrombin time. Continuous diminishing of serum albumin titers, increase in bilirubin and extension of the prothrombin time are typical indications of decompensated cirrhosis. Additional regular examinations include ultrasonography and alpha-fetoprotein (AFP) quantity for intermittent surveillance for HCC, and endoscopy in individuals with cirrhosis (WHO, 2015).

Hepatic biopsy and scoring of histology are useful in confirming the rate of necro-inflammation and fibrosis. The clinical characteristics of CHB on hepatic biopsy are dependent on the phase of the infection, host protective immunity and the rate of HBV replication. Blood indicators for fibrosis, including APRI and FIB-4, Fibro Test and FibroScan done to exclude progressive fibrosis are non-invasive approaches for monitoring the stage of hepatic infection (WHO, 2015).

2.8.6 HBV and Hepatocellular Carcinoma (HCC)

Although HBV hepatic-carcinogenesis is not clearly understood, it is advanced that the continuous inflammation and hepatic injury result to an amplified hepatic turnover, leading to the buildup of genetic modifications. These can as well be triggered by chromosomal integrations of Hepatitis B virus that might be detected up to 80% of all HBV-associated Hepatocellular carcinoma. Moreover, some HBV proteins such as HBV-X protein may trans-activate tumor elements and proto-oncogenes such as c-MYC (Reeves et al., 2013). Other multiple and complex mechanisms of how HBV cause liver cancer are documented

as encompassing 3 main distinguished mechanisms; At the beginning, long-lasting HBV septicity prompts inflammation and deregulation of the physiological equilibrium amongst hepatic proliferation, differentiation and apoptosis. This upset form frequently result to cirrhosis, a precursor of Hepatocellular carcinoma. Subsequently, in the initial carcinogenic course, Hepatitis B DNA is incorporated into cell genome of the host, potentially becoming an insertional mutagen that deregulate contiguous oncogenes or tumor suppressors. Lastly, Hepatitis B articulates HBx proteins which interacts with various cellular elements, upsetting numerous features of transcription, proliferation, or existence. The influence of each of the above mechanisms is contingent upon the host immunity reaction, synergic influence of environmental aspects, in addition to molecular physiognomies of HBV genotypes involved (Chemin *et al*., 2011).

Precise transmutations in the Hepatitis B genetic makeup, specifically those that cause the amino acid variations in the viral peptides shown in HBV genome, can impact the successive risks of emerging Hepatocellular carcinoma or cirrhosis. The dual impact replacements including A1762T and G1764A in BCP portion of Hepatitis B remain independent risk factors for the progressive hepatic disease and Hepatocellular carcinoma among individuals' ill with A2, B, C, and D genotypes unlike among those with F1 variant. Pre-core transmutations are linked to hepatic inflammation, mainly anti-HBe–positive immunity impassive Hepatitis B, and with growth of Hepatocellular carcinoma (McMahon, 2009).

2.8.7 HBV and Drug Resistant

HBV RT-polymerase is devoid of proofreading actions and this result to regular alterations in the genetic make-up of the virus, resulting to coexistence of viral quasi species that are genetically unique in infected persons that emerge depending on the host environmental pressure. The interaction amongst the virus, hepatocyte, protective reaction and ART is believed to drive the evolution of HBV genetic variants with the potential to escape host immunological reactions or resistance to ART (EASL, 2017 and Rana *et al.,* 2011).

HBV ART resistance is described by the onset of resistance giving rise to alterations and compensatory transmutations, frequently involving the harboring of numerous transformations in the RT polymerase and conforming modifications in HBsAg. HBV mutation develops normally in the course of antiviral therapy with the nucleoside analogue lamivudine (Neumann-Fraune *et al*., 2013). Entecavir (ETV) and tenofovir (TDF) are the latest antiviral nucleos(t)ide analogues with seldom drug resistant. The most common Lamivudine (3TC) resistance transmutations are rtM204V or rtM204I (WHO, 2017). Genomic resistance evolving in ETV barrier is decreased among Hepatitis B variants with 3TC resistance transmutations rtM204V and rtM204I. Owing to the overlying reading frames of the RT polymerase and HBsAg, alterations in the Hepatitis B genetic makeup regularly impact the amino acid arrangement for RT polymerase and HBsAg proteins. Hepatitis B surface antigen is a viral trans-membrane protein and is a major target for the immunological response in patients. Children born to CHB infected mothers have been reported with HBsAg genetic alterations that failed to illicit adequate immune response after HBV immunization. This insusceptible evasion is bestowed by transmutations in

Hepatitis B surface antigen inhibiting bonding of the counteracting antibodies. Besides, modifications in HBsAg are linked to infection advancement and increase incidences of HCC. During such circumstances, immature stop codons result in the manifestation of truncated HBsAg, resulting in emission of a cluster of virions plus subviral fragments from the diseased hepatocytes (Neumann-Fraune *et al.,* 2013).

2.9 HBV and Co-infection with other Viral Hepatitis

Chronic Hepatitis B viral co-infections with HCV and/or HDV are described by a reciprocal inhibition of viral reproduction and by an additional serious clinical appearance as equated with either chronic HCV or CHB disease alone. HAV superinfection subverts equally HBV and HCV reproduction in persons with an already-existing CHB or Chronic HCV (Coppola *et al.,* 2014).

2.9.1 HBV and HDV Co-infection

Hepatitis D Virus (HDV) is a minor deficient RNA virus requiring HBV for it to be communicable. The modes of HDV spread are similar to those for HBV although mother-to-child transmission is seldom and HDV prevalence is global. Endemic regions for HDV are the Asian, West and Horn of Africa, Mediterranean and the Amazon Basin; with low presence in Eastern Asia, Southern Africa, North America and Northern America (WHO, 2015). An estimated fifteen million (five percent) of Hepatitis B patients are also concurrently suffering from Hepatitis D Virus. The Hepatitis D viral septicity happens solely in HBV-infected persons since HDV is defective that needs HBV surface proteins

in order to develop its envelope in Hepatitis B and Hepatitis D dual infected liver cells (WHO, 2017). Acute and fulminant hepatitis is more often witnessed in HDV and HBV dual infections unlike HBV mono-infection. Immunization against HBV averts severe HDV co-infection (WHO, 2015). Hepatitis D septicity may happen in 2 fashions. One fashion is initiated by the dual infection of HBV plus Hepatitis D Virus; resulting to serious acute hepatitis with greater fatality proportion as compared to severe hepatitis in mono-infected CHB individuals, although it infrequently leads to long-term disease. The other fashion of HDV occurs when HDV super-infects HBV carriers. This is distinguished by more serious severe hepatitis in formerly symptom-free Hepatitis B carriers or as an escalation of primary CHB. In contrast to dual infection form, super-infection by Hepatitis D in Hepatitis B carriers nearly and continually results into long-term infections. A greater number of people suffering from long term Hepatitis B and Hepatitis D dual infection progress to hepatic decompensation, cirrhosis and hepatocellular carcinoma unlike in CHB only (McMahon, 2009).

2.9.2 HBV and HCV Co-infection

An estimate of 15% of HBV chronically sick persons also suffer from Hepatitis C Virus (HCV). Similarly, up to 25% of individuals suffering from Hepatitis C Virus may correspondingly be infected with HBV. Hepatitis B and Hepatitis C dual infections frequently occur in HBV high prevalence areas in South America, sub-Saharan Africa and Asia. Patients with dual HBV/HCV infection stand greater risk of advancing to Hepatocellular carcinoma at an earlier age and with more aggressive HCC (WHO, 2015).

HCV super-infection hinders Hepatitis B reproduction in persons with already CHB. HCV/HBV acute co-infection can reduce the period of HBsAg antigenemia and similarly lessen peak of serum aminotransferase titers in comparison to critical hepatitis B only. Conversely, HBV/HCV acute dual infections and similarly acute Hepatitis C on superimposed on CHB have both been described as intensifying the risks to more serious hepatitis and fulminant liver failure. Besides these, individuals with twin Hepatitis B and C septicity face more increased degrees of liver cirrhosis and advancing to HCC than either virus mono-infection. Thus, HCV seems more domineering virus and suppresses Hepatitis B DNA levels in dually HCV-HBV infected persons (McMahon, 2009).

2.10 The HBV and HIV Co-infection

Hepatic disease is a key source of illness and death among HIV positive clients (Soriano *et al*., 2008). Africa carries the highest HIV disease burden and alongside Asia, are the leading reservoirs of CHB (Modi and Feld, 2007). CHB is a notable causative agent of hepatocellular carcinoma in majority of Asian and Sub-Saharan regions and approximately 25% of all CHB persons ultimately progress to HCC. Chronically infected patients with HBV are at 100 times increased dangers for advancing to HCC unlike in none infected individuals (Ganem & Prince, 2004). With increasing access to antiretroviral therapy across Sub-Saharan Africa, HIV-infected individuals live longer predisposing them to the effects of co-infection with chronic viral hepatitis, an emerging critical public health problem (Modi & Feld, 2007). Patients infected with HIV are at particular risk for HBV infection due to similar transmission routes for both HBV and HIV infections (Sulkowski, 2008). Although both HBV and HIV are spread at countless incidences by parenteral,

percutaneous and sexual contacts, Hepatitis B Virus is fifty-to-hundred-fold highly contagious as compared to HIV (WHO, 2012; Soriano *et al*., 2010). The mortality risk due to hepatic complications in HBV/HIV dually-infected individuals is 13-fold more as compared to those infected with only one virus (Thio *et al*., 2002). HIV co-infection accelerates HBV-related liver damage, leading to earlier cirrhosis and end-stage liver disease. Conversely, the presence of HBV co-infection complicates the management of HIV and increases the morbidity and mortality of HIV-infected patients (Sheng *et al*., 2012). Cases of hepatitis flare up have been reported among HIV-HBV dually ill individuals that have little CD4 T-lymphocyte amounts but encounter immunity reconstruction following introduction of the HAART (McMahon, 2009).

Approximately 1% of chronically infected Hepatitis B persons are similarly living with HIV while the Hepatitis B incidence among HIV positive individuals is about 7.4% that comprise around 2.7 million out of 36.7 million HIV patients globally (WHO, 2017; Dao, *et al*., 2011). Whereas mortality due to TB, Malaria and HIV is decreasing worldwide, there is an increase in the number of mortalities caused by viral hepatitis over time. Hepatic infections are a key source of indisposition and fatality amongst viral hepatitis/HIV dually sick individuals (WHO, 2017). Between the HIV/HBV dual infected, individual-characteristic dynamics such as, age, CD4+ cell count, sex, viral load, ART category and period, plus AIDS-defining type of ailment influence the reaction to HBV inoculation (WH O, 2017).

HIV co-infection has a major effect on nearly all facet of the HBV natural history septicity. This impact comprises greater proportions of chronicity after critical HBV septicity, increased degrees of Hepatitis B multiplication and reactivation, lesser rates of spontaneous remission, greater incidences of occult HBV, more prompt development to cirrhosis and Hepatocellular carcinoma, more hepatic-associated death, and diminished therapeutic response in comparison to people with HBV mono-infection (WHO, 2015). An amplified likelihood of death as a result of progressive hepatic sickness among HBV-HIV dual septicity has been established in contrast to individuals with mono-infections. Fibrosis or cirrhosis of the liver is more regular in the dual infection with HBV/HIV. Hepatitis B - particular CD8+ T-lymphocyte reaction is diminished in individuals concurrently with HBV/HIV infection (Dao *et al*., 2011).

In HIV/AIDS setting, HBV is not considered an opportunistic infection since it is a common dual infection found among HIV clients due to related modes of transmission (Franco *et al*., 2012). Liver disease is an evolving significant cause of death in HBV-HIV concurrently infected individuals as other HIV-related illnesses have diminished following the universal initiation and enhanced access to antiretroviral treatment and management. HBV co-existence with HIV in individuals results to an aggravated progress to AIDS-related outcomes and all-lead to death (WHO, 2015). HIV patients, specifically those with compromised immunity, are unlikely to respond to vaccination for Hepatitis B, face greater danger of ART failure, and have higher prospects of progressing to long term sickness following exposure to Hepatitis B virus. Besides, persons with HIV/HBV dual infection more often have insistent immunological indicators, have advanced HBV-DNA levels, and

suffer from more serious hepatic complications due to long term Hepatitis B septicity. Furthermore, HIV/HBV concurrent septicity escalates the risk of advancing hepatic impairments, including hepatic failure and hepatocellular carcinoma (Soriano *et al*., 2010). The resultant effects of HBV-HIV dual infection also comprise of higher rates of liver-associated morbidity and death, amplified HBV multiplication, immunity restoration to Hepatitis B virus within antiretroviral therapy settings, and liver toxicity from HAART medications (Hoffmann & Thio, 2007; Mayaphi *et al*., 2012).

HIV infection leads to higher HBV viral loads, increased occult HBV infection and leads to higher likely hood of transmitting Hepatitis B virus to HIV patient's close contacts. The increased HBV replication due to profound immunosuppression and loss of immunity reaction to HBV may cause reactivation of HBV, as well as exposure to novel Hepatitis B infections since persons' infected with HIV clear immune antibody quantities more rapidly (Mayaphi *et al*.,2012; Brook, 2006). Early initiation of combined treatment with emtricitabine/lamivudine and tenofovir, is commended treatment for HBV-HIV dually infected individuals in order to prevent HBV drug resistance, especially to Lamivudine, that has a small resistance barrier (Mayaphi *et al*.,2012; WHO, 2010). Every HIV-infected person with Hepatitis B co-infection are recommended to be put on ART treatment regardless of CD4 T-lymphocyte count (EASL, 2017).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Area

The blood specimens were drawn from HIV seropositive individuals visiting Mbagathi District Hospital, Nairobi, for routine $CD4^+$ monitoring in the laboratory. Mbagathi District Hospital is situated in Nairobi County, a cosmopolitan city with people from all parts of the country. The choice of Mbagathi hospital was based on the fact that it was an infectious disease hospital with proximity to KEMRI where it could be easy to collaborate with the KEMRI Hepatitis Laboratory for storage of the samples and processing of the data.

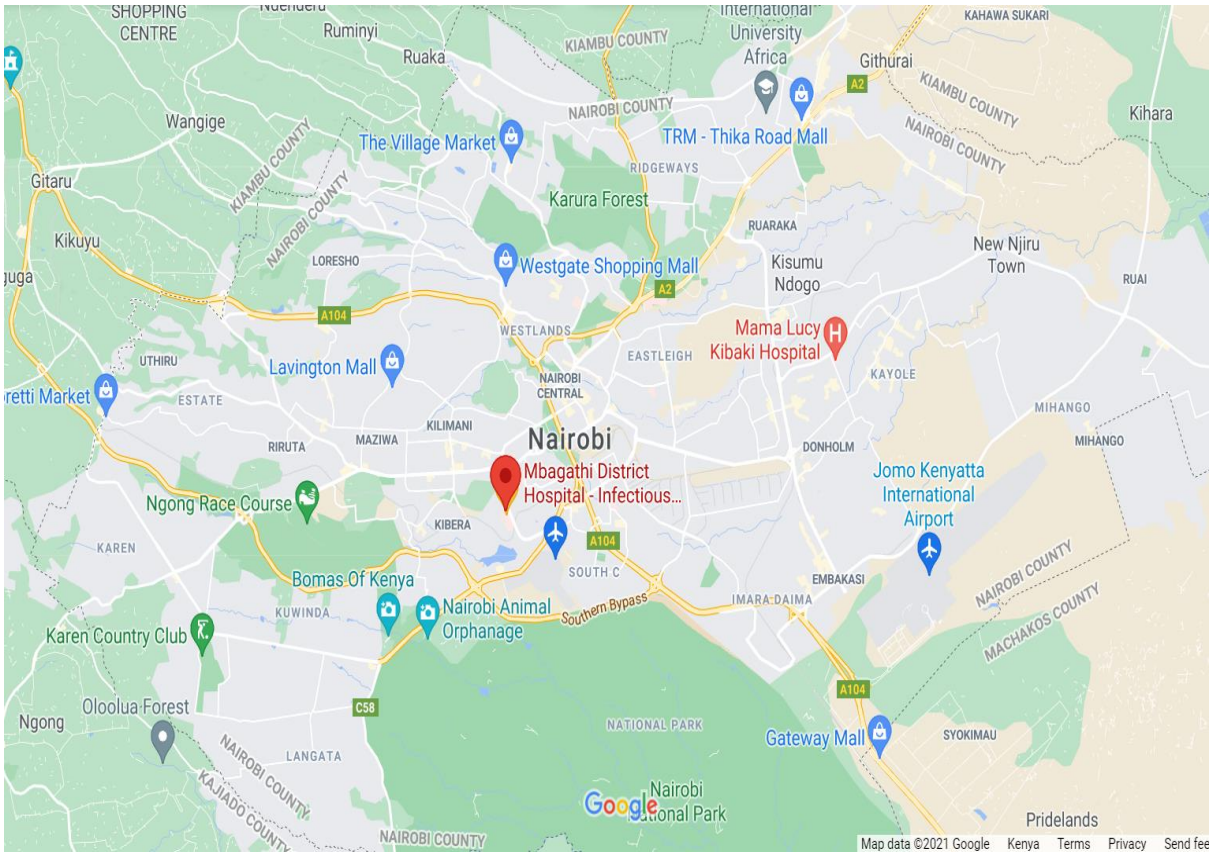

Figure 3. 1: Google map of Nairobi County showing Mbagathi District Hospital and its Catchment areas

3.2 Study Population

The research population comprised 180 male and female persons who had been diagnosed with HIV. The participants were patients who were attending Mbagathi District Hospital CCC clinic for routine CD4+ T-lymphocyte and viral load count for management with HIV antiretroviral therapy (ART) and who consented to the research.

3.3 Study Design and Recruitment of Participants

This was a cross-sectional survey conducted between April 2014 and March 2015. The HIV-infected patients were recruited while seeking laboratory services at Mbagathi District Hospital for routine CD4+ T-lymphocyte and HIV viral load count. In order to ensure unbiased and equal opportunity among study subjects, a systemic random sampling was used whereby every 10^{th} patient who consented to the study was recruited consecutively until the required sample size was obtained. Patients who visited the CCC site were numbered systematically and asked for consent for every 10^{th} patient on the CCC clinic visit list on a daily basis. The overall findings of this study group are assumed to be typical of the entire HIV patients' populace who were visiting the hospital laboratory for CD4+ T-lymphocyte and HIV viral count services.

3.4 Exclusion and Inclusion Criteria

Only patients diagnosed with HIV, seeking laboratory services for HIV viral load and CD4+ T-lymphocyte count, above18 years and who consented to the study were included. The guardians or next of kin accompanying the patients consented on behalf of the very

sick HIV-infected patients. Patients below 18 years, those who could not consent to the study and the very sick patients unaccompanied by either their guardians or next of kin were excluded. The study population ages were generally categorized into youthful (18-35 years), adulthood (36-57 years) and elderly (above 58 years) in order to generally compare the risk factors attributable to each age group.

3.5 Sample Size Determination

HIV and HBV have similar transmission routes that occur in high proportions in Kenya, with a high HBV incidence in HIV co-infected individuals (Day *et al*., 2013). Using an average of 10% of the postulated prevalence as the most recent point and periodic HBV prevalence (Day *et al.,* 2013; WHO, 2012; Soriano *et al*., 2010) in HIV-HBV dual infection, the sampling population was established as follows:

$N = Z^2P\ (1\text{-}P)/D^2$ (Fischer *et al*., 1998)

Where N = Minimum sample size required,

Z = 1.96 standard error (at 95% confidence interval)

P = postulated prevalence (0.1) in the general population

D = 0.05 the inverse of 95% confidence limit (the allowable error)

$N = 1.96^2 \times 0.1\ (1\text{-}0.1) / 0.05^2 = 138$ samples, this was rounded off to 180 samples were considered from both males and females.

3.6 Ethical Consideration

Ethical approval was given by the Kenyatta University Ethical Review Committee (Appendices III-VII) and hospital approval was sought from Mbagathi District Hospital Ethical Committee (Appendix VIII). All research participants were informed on the research verbally and each of them singed a consent form that authenticated that they had all information regarding potential benefits and risks, and assurance of confidentiality of any information given as well as test results. To ensure confidentiality, the questionnaires (Appendix II) and each sample collected were given a unique identification code.

3.7 Sample Collection, Transportation and Protection from Viral Infection

The socio-epidemiological data was collected from patients using a structured questionnaire. The 5ml whole blood specimen was collected consecutively in EDTA vacutainer and divided into two aliquots of 2.5ml each with the assistance of the medical laboratory technician until the required number was attained. Aliquots for HBV serology were stored at -2 to 7 ^{0}C and the second aliquots of HBV seropositive samples for HBV genotyping were deep frozen at -80^{0}C. Upon the completion of data collection, the blood samples for the two aliquots were sealed securely in ice-bag cool boxes, labeled as biohazard human blood samples and transported to KEMRI Hepatitis Laboratory by laboratory technicians. The respective CD4+ T-lymphocyte and HIV viral count of the patients were obtained from the Mbagathi Hospital laboratory records.

The research samples were handled with caution, always wearing protective clothing, washing hands with soap and antiseptic, disinfecting and cleaning all working surfaces,

equipment and apparatus. All disposal materials and the blood samples were decontaminated before disposal into respective waste bins.

3.8 HBsAg Assay, DNA Extraction, Amplification and Sequencing

3.8.1 HBsAg Enzyme-Linked Immune-Absorbent Test

The Hepatitis B surface antigen enzyme-linked immune-absorbent (ELISA Test), is a solid phase for qualitative detection of HBsAg in Human serum and it was carried out in duplicate following the procedure outlined in CTK Biotech laboratory manual (HBsAg Elisa Kit, CTK Biotech, Inc. USA). The blood cells were removed by centrifugation and a Pasteur pipette was used to remove 10ml supernatant plasma from the cell pellet.

HBsAg ELISA Test is premised on antibody sandwich method for identifying Hepatitis B surface antigen in blood. The assay comprises compact microwells pre-coated with monoclonal anti-HBsAg and liquefied conjugates comprising polyclonal anti-HBsAg complexes with horse reddish peroxide (HRP-HBsAb conjugates). In the test, the serum sample and HRP-HBsAb were incubated concurrently in the pre-coated microwells. HBsAg, when existing in the sample, reacted against anti-HBsAg antibody pre-coated microwell surface as well as the HRP-HBsAb, forming sandwich conjugates. The unbound complexes were subsequently washed off. The appearance of the complexed conjugate was displayed by a blue colour when incubated with TMB (3,3′,5,5′-Tetramethylbenzidine) substrate for fifteen minutes at 37^0C. The assay was ended by stop solution (0.16M sulfuric acid) and absorbance read using micro plate reader at 450nm against a filter of 620-690nm reference wave length.

3.8.2 Extraction of HBV DNA

Hepatitis B DNA was mined from serum by use of a High Pure Viral Nucleic Acid extraction kit from Roche (Roche Diagnostics International AG, Rotkreuz, Switzerland.) as per manufactures guidelines. Briefly, 200µl of binding buffer (comprising of 6 M guanidine HCl) complemented with poly (A) and 50µl of Proteinase K was added to 200µl of specimen serum. After incubating at 72^{o}C for 10 minutes another 100µl of binding buffer was added and mixed thoroughly. High pure filter tube and the collection tube were combined and the mixture transferred into the upper reservoir of the two tubes. After centrifugation at 8500g for one minute, the collection tube was discarded. Immediately, 500µl of inhibition buffer was added and centrifuged as above. The flow through was discarded and 450µl of wash buffer added twice and centrifuged as above. In order to completely remove the wash buffer, the high pure filter tube was centrifuged at maximum speed (13000rpm, at 6^{0}C) for ten seconds. Finally, the DNA was eluted in 50µl of elution buffer (10 mM Tris-Cl, pH 8.5) and then DNA collected in a sterile Eppendorf tube and stored at -20^{0}C.

3.8.3 Amplification of HBV DNA

Five µL of the extracted viral DNA was used as a PCR template in each assay to amplify S genes (Mizokami *et al.*, 1999). The PCRs were performed in a nested approach using S gene specific primers: S1-LLr; (5'-CGTTGACATACTTTCCAATCAA-3') and S1-LLf; (5'-TCCTGCTGGTGGCTCCAG-3') in the first round with, S2-nLLr; (5'-CAACTCCCAATTACATARCCCA-3') and S2-nLLf (5'-ACCCTGYRCCGAACATGGA-3') in the second round (Mizokami *et al.*, 1999).

A 25 µL master mix reaction was prepared for both assays separately consisting of 1X PCR buffer, 1.5 mM $MgCl_2$, 1U/ µL Taq polymerase (KemTaq®), 200 µM of each dNTP (Invitrogen, Carlsbad, CA), 5µL of template DNA and 0.5 µM of primer pair both in the first and second round. Both the first and second round PCR amplification conditions were an initial denaturation of 94°C for 4 minutes followed by 40 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 1 minute, with a final extension of 72°C for 7 minutes. The PCR products were resolved in 1.5% ethidium bromide-stained agarose gel and, and visualized under ultraviolet light. The presence of 300 bp and 800 bp fragments indicated successful amplification of HBV S-genes respectively.

3.8.4 HBV PCR Product purification and DNA sequencing

Before sequencing, the subsequent PCR products were purified with QIAquick PCR purification kit (Qiagen, Valencia, CA) following manufacturer's instructions. Sequencing was carried out in both forward and reverse directions separately by use the second round primers (S2-nLLr and S2-nLLf) for S-gene with Big Dye Terminator sequencing chemistry (Stuyver *et al.*, 2000, Amini-Bavil-Olyaee *et al.*, 2008). The sequencing was done on an ABI PRISM® 3100 Genetic Analyzer and DNA base calling performed using the DNA Sequencing Analysis software V3.7. The sequence data was then imported into the Genetyx-Windows computer software version 9.1.0 (Genetyx Corporation, Tokyo, Japan) to assemble the two sequence segments into a single contiguous sequence.

3.9 Data Management and Analysis

3.9.1 Socio-epidemiological Data Analysis

Socio-epidemiological data recorded was entered, cleaned and analyzed using SPSS version 20.0. The sero-prevalence of HBsAg was expressed in percentages with their corresponding 95% confidence intervals for entire study group and by use of age and sex. Pearson Chi-square test of independence was used to test for correlation between socio-epidemiological variables and HBV prevalence. A p value of 0.05 or less was considered significant.

3.9.2 Genotypic analysis

To determine HBV genotypes, the generated contiguous sequence encoding the Pre-S gene were aligned with complete HBV genotypes A-J reference sequences from Genbank using Basic Sequence Alignment Tool (BLAST) available at http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome (Hardie and Williamson, 1997). Phylogenetic Tree was constructed using the neighbor joining program and analyzed using MEGA6 (Tamura *et al.*, 2013). The generated trees were visualized using the Fig Tree software (http://tree.bio.ed.ac.uk/software/figtree/) (Hardie and Williamson, 1997). Genetic distance matrix generated by MEGA 6 was used to approximate genetic distances among the isolates and their GenBank counter parts. The molecular diversity of Hepatitis B Virus genotypes amongst HIV infected patients in Kenya was compared with those identified elsewhere in order to show genetic evolution and epidemiology.

Further, HBV drug resistance mutations were assigned by analyzing the generated S-gene contiguous sequences using the HBV drug resistance database at: http://hivdb.stanford.edu/HBV/HBVseq/development/HBVseq.html that automatically detected the drug mutations based on the subject HBV sequences.

CHAPTER FOUR: RESULTS

4.1 Prevalence and Risk Factors of HBV in HIV Infected Patients by Socio-demographic and Epidemiological Characteristics

This research involved both male and female respondents. A total of 180 respondents participated with the mean age of 34.5 years with the female respondents accounting for 56.1% (n=101) while the male respondents accounted for 43.9% (n=79). Over two thirds (69.5%) of the respondents were youth (18 – 35 years), almost a quarter (23.3%) of the respondents were adult (36-57 years) and minority (7.2%) were the elderly (58 years and above). From a sample size of 180, 9 (5.0%) samples tested HBsAg positive by serology and 5 (2.8%) tested HBV positive by PCR.

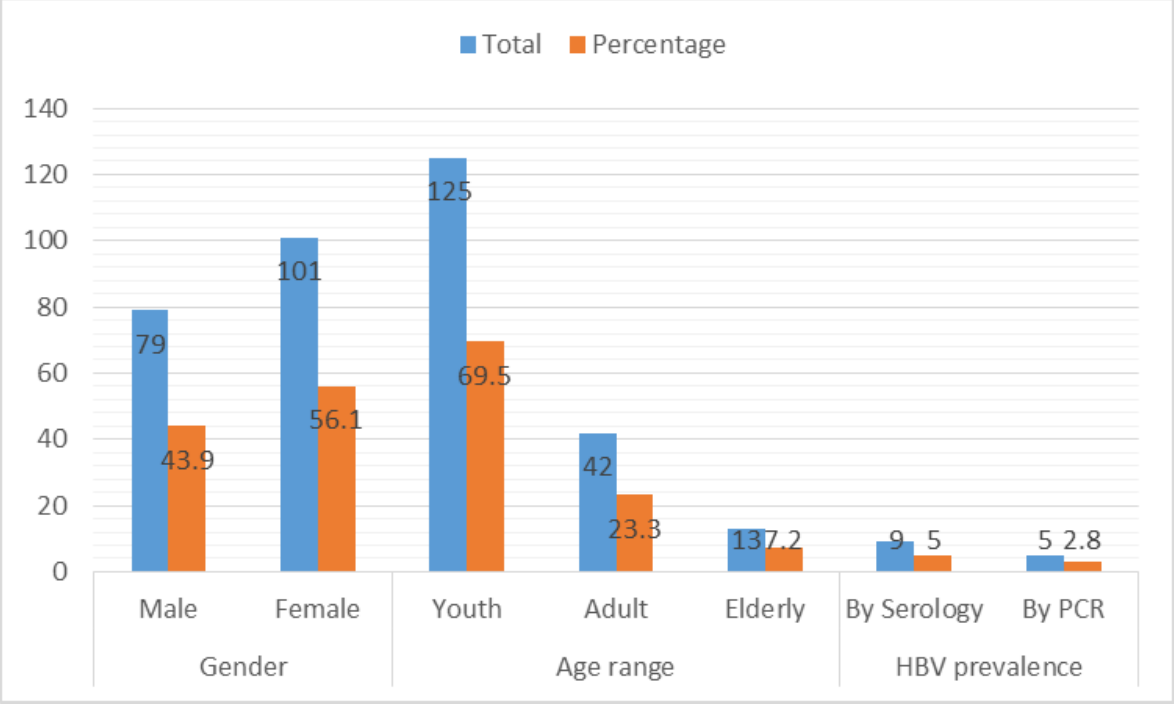

Figure 4. 1: Study sample size by gender, age group and HBV prevalence

4.1.1 HBV Prevalence risk factors in HIV Infected patients by Marital, Education and Employment Status

From the study population, half (50.0%) of the participants were married, followed by the singles (35.0%), widowed (8.3%) and then the divorced/separated (6.7%). Majority (38.0%) of singles were male and youthful (46.4%) and most of the married (51.5%) were females and youthful (46.4%). Similarly, a higher proportion of the separated/divorced (6.9%) were adult females while most of the widowed (8.9%) were females and elderly (69.2%). The age of the respondents had substantial correlation with marital status ($\chi 2$=86.744, df=6, P=0.001). The outcomes revealed a greater Hepatitis B prevalence among the males (3.8%) than among the females (2.0%) and higher (3.2%) in the youth group than in the adults (2.4%). Nonetheless, age of the respondents had no key HBV prevalence variation as correlated with the gender. The HBV prevalence was higher (6.7%) in the widowed respondents followed by singles (3.2%) and married (2.2%).

A higher (36.7%) proportion of respondents had primary education, almost a third (28.9%) had college education, a quarter (25.6%) had secondary education, minority (8.9%) of the respondents had no formal education while two thirds (66.1%) of the respondents were unemployed and only a third (32.9%) were employed. A higher proportion of participants with no formal education were male (10.1%) and elderly (38.5%), most of the participants with primary education were females (38.6%) and elderly (53.8%), a slightly higher proportion of respondents with secondary education were females (25.7%) and adults (28.6%), while majority of those with college education were males (30.4%) and youthful (32.0%); Table 4.1.

Table 4. 1: Prevalence of HBV in HIV infected patients by Marital, education and employment status against Gender

Variable		Gender			HBV Prevalence
		Male	Female	P-Value	
Marital status	Single	30(38.0%)	33(32.7%		2(3.2%)
	Married	38(48.1%)	52(51.5%)	χ^2= 0.574	2(2.2%)
	Separated/ divorced	5(6.3%)	7(6.9%)	P = 0.902	0
	Widowed	6(7.6%)	9(8.9%)		1(6.7%)
	Total (180)	**79(43.9%)**	**101(56.1%)**		**5(2.8%)**
Formal education	None	8(10.1%)	8(7.9%)		0
	Primary	27(34.2%)	39(38.6%)	χ2=0.592	1(1.6%)
	Secondary	20(25.3%)	26(25.7%)	P = 0.898	0
	College	24(30.4%)	28(27.7%)		4(7.7%)
Employment Status	Employed	20(25.6%	39(38.6%)	χ2= 4.447	1(1.7%)
	Unemployed	57(73.1%)	62(61.4%)	P = 0.108	4(3.4%)

There was a substantial relationship between the level of formal education and age (χ2=21.680, df=6, P=0.001). Most of employed respondents were females (38.6%) and elderly (69.2%) while majority of unemployed respondents were males (73.1%) and adults (78.6%) followed by youths (66.1%). There was substantial correlation regarding employment status and age (χ2=10.747, df=4, P=0.030). The occurrence of HBV was found to be greater in respondents with college education (7.7%) and unemployed (4.3%) (Table 4.2).

Table 4. 2: Prevalence of HBV in HIV infected patients by Marital, education and employment status against Age

Variable	Age (years)				HBV Prevalence
	18-35	36-57	>58	P-Value	
Marital status Single Married Separated/ divorced Widowed **Total (180)**	58(46.4%) 58(46.4%) 7(5.6%) 2(1.6%) **125(69.5%)**	5(11.9%) 28(66.7%) 5(11.9%) 4(9.5%) **42(23.3%)**	0 4(30.8%) 0 9(69.2%) **13(7.2%)**	$\chi2$=86.744 P=0.001	2(3.2%) 2(2.2%) 0 1(6.7%) **5(2.8%)**
Formal education None Primary Secondary College	9(7.2%) 42(35.6%) 34(27.2%) 40(32.0%)	2(4.8%) 17(40.4%) 12(28.6%) 11(26.2%)	5(38.5%) 7(53.8%) 0 1(7.7%)	$\chi2$=21.680 P=0.001	0 1(1.6%) 0 4(7.7%)
Employment Status Employed Unemployed	41(33.1%) 82(66.1)	9(21.4%) 33(78.6%)	9(69.2%) 4(30.8%)	$\chi2$=10.747 P=0.030	1(1.7%) 4(3.4%)

4.1.2 HBV Prevalence risk factors in HIV Infected patients by Sexual Contact

Most (78.9%) of respondents had one sexual partner and 20% of the respondents had two and more sexual partners. A higher (79.7%) proportion of respondents with one sexual partner were male and youthful (84.0%) and most (13.9%) of respondents with two sexual partners were female and elderly (7.7%) (Table 4.3). Similarly, most (13.9%) of the respondents who had multiple (more than two) sexual partners were males and elderly (46.2%) (Table 4.3). There was a substantial relationship amongst the number of sexual partners and age of the respondents ($\chi2$=49.936, df=8, P=0.001). Majority (91.7%) of the

respondents had regular sex partners and minority (3.3%) of the respondents had commercial sex partners. Majority (96.2%) of respondents who had regular sex partners were males and youthful (94.4%) while more (4.0%) respondents who had commercial sex partners were females and elderly (7.7%) (Table 4.3). HBV prevalence was found to be 3.0% in respondents who had regular sex partners and none with those who had commercial sex partners. Almost all (97.8%) sampled respondents were heterosexual while the minority, 1.7% and 0.6% of the respondents, were bisexual and homosexual respectively (Table 4.3). Heterosexual practice was higher (98.7%) in male and elderly (100.0%) respondents while both bisexual and homosexual practices were higher (2.0% and 1.0% respectively) in female and adult respondents (2.4%). The HBV prevalence was found to be 2.8% in the heterosexual respondents.

Table 4. 3: Prevalence of HBV in HIV infected patients by sexual contact against Gender

Variable		Gender			HBV Prevalence
		Male	Female	P-Value	
No. of sexual partners	One	63(79.7%)	79(78.2%)		4(2.8%)
	Two	4(5.1%)	14(13.9%)	$\chi2$= 5.429	1(9.1%)
	More than two	11(13.9%)	7(6.9%)	P = 0.246	0
Type of sex any sexual partner partners	**Without**	1(1.3%)	8(7.9%)	$\chi2$= 4.514	0
	Regular	76(96.2%)	89(88.1%)	p= 0.105	5(3.0%)
	Commercial	2(2.5%)	4(4.0%)		0
Type of sex one practices	Heterosexual	78(98.7%)	98(97.0%)		5((2.8%)
	Homosexual	0	1(1.0%)	$\chi2$= 1.608	0
	Bisexual	1(1.3%)	2(2.0%)	P = 0.658	0
Frequency of using protection during sex	None	6(7.6%)	30(29.7%)		1(2.7%)
	Always	45(57.0%)	47(46.5%)	$\chi2$=13.869	1(1.1%)
	Sometimes	28(35.4%)	24(23.8%)	P = 0.001	3(5.8%)
Suffered from any STI	Yes	12(15.2%)	18(15.8%)	$\chi2$= 2.588	2(7.1%)
	No	65(82.3%)	85(84.2%)	P = 0.274	3(2.0%)

Half (51.1%) of the respondents reported always using sexual protection (condoms) during sexual intercourse, almost a third (28.9%) sometimes used sexual protection and a fifth (20%) did not use any protection during sexual intercourse (Table 4.3). Most (57.0%) of the respondents who reported always using sexual protection were males and youthful (89.6%), those who sometimes used protection during sexual intercourse mostly (35.4%) were males and elderly (61.5%), while majority (29.7%) of those who did not use any protection during sexual intercourse were females and adults (2.4%). There was a significant association between the respondents' frequency of using protection during sexual intercourse and gender ($\chi2$=13.869, df=4, P = 0.001) and age ($\chi2$=28.622, df= 4, P = 0.001). The occurrence of HBV was highest (5.8%) among the respondents who always used protection during sexual intercourse, lower (2.7%) for respondents who used no protection during sexual intercourse and lowest (1.1%) among the respondents who

sometimes used protection (Table 4.3). In the sampled population, 16.7% of the participants reported to have a history of suffering from sexually transmitted infections (STIs), mainly syphilis, gonorrhea and candidiasis. The history of suffering from STIs was slightly higher (15.8%) in females and in adults (26.1%) (Table 4.3). The prevalence of HBV was established to be highest (7.1%) among the participants who reported to have suffered from STIs and lowest (2.0%) among the participants who reported not to have suffered from any STIs. However, there was no substantial correlation in HBV prevalence among the respondents who had suffered from STIs and those who did not have any history of suffering from STIs, ($\chi 2=2.623$, df=4, P=0.623).

Table 4. 4: Prevalence of HBV in HIV infected patients by sexual contact against Age

Variable	Age (years)				HBV Prevalence
	18-35	36-57	>58	P-Value	
No. of sexual One **partners** Two More than two	105(84.0%) 8(6.4%) 12((9.6%)	32(76.2%) 2(4.8%) 7(16.6%)	5(38.5%) 1(7.7%) 6(46.2%)	χ2=49.936 P=0.001	4(2.8%) 1(9.1%) 0
Type of sex None **partners** Regular Commercial	4(3.4%) 118(94.4%) 3(2.4%)	3(7.1%) 37(88.1%) 2(2.8%)	2(15.4%) 10(76.9%) 1(7.7%)	χ^2=12.067 P = 0.148	0 5(3.0%) 0
Type of Heterosexual **sex one** Homosexual **practices** Bisexual	123(98.4%) 0 2(1.6%)	40(95.4%) 1(2.4%) 1(2.4%)	13(100.0%) 0 0	χ^2=7.491 P = 0. 278	5((2.8%) 0 0
Frequency of None **using protection** **Always** **during** Sometimes **sexual intercourse**	2(1.6%) 112(89.6%) 11(8.8%)	1(2.4%) 36(85.7%) 5(11.9%)	0 5(38.5%) 8(61.5%)	χ^2=28.622 P = 0.001	1(2.7%) 1(1.1%) 3(5.8%)
Suffered from Yes **any STI** No	16(12.8%) 107(85.6%)	11(26.1%) 31(73.9%)	1(7.7%) 12(92.3%)	χ^2= 7.823 P = 0.451	2(7.1%) 3(2.0%)

4.1.3 HBV prevalence risk factors in HIV Infected Patients by Past HBV infection History

Majority of the respondents (68.3%) did not know their HBV family history and only a third (31.1%) confirmed had no family history. More (31.6%) males in the adult age group

(40.5%) reported to have had no HBV family history while most of the respondents who reported that they did not know their HBV family history were females (69.3%) and elderly (84.6%) (Table 4.5). All the HBV positive results were among the participants who did not know their HBV family History with a HBV prevalence of 4.1%. Minority (6.7%) of the respondents had previously been tested for HBV, with 83.3% (n=10) of them turning out to be positive for HBV.

Table 4. 5: Prevalence of HBV in HIV infected patients by HBV infection History against Gender

Variable		Gender			HBV Prevalence
		Male	Female	P-Value	
HBV Family History	Yes	1(1.3%)	0		0
	No	25(31.6%)	31(30.7%)	$\chi2$= 1.323	0
	Don't know	53(67.1%)	70(69.3%)	P = 0.516	5(4.1%)
Past HBV testing results	tested +ve	5(%)6.3	5(5.0%)	$\chi2$= 0.161	0
	Never tested	74(97.7%)	96(95.0%)	P = 0.689	5(2.9%)
Vaccinated against HBV	yes	3(3.8%)	7(6.9%)	$\chi2$= 0.829	0
	No	76(96.2%)	94(93.1%)	P = 0.362	5(2.9%)
Diagnosed with any liver Problem	Yes	2(2.5%)	3(3.0%)	$\chi2$= 1.321	1(20.0%)
	No	77(97.5%)	93(97.0%)	P = 0.519	4(2.6%)

Previous HBV testing results indicated that there was higher HBV prevalence in males (6.3%) and in adults (7.1%) (Table 4.5). Out of 10 respondents that had been previously been tested HBsAg positive, only one respondent (0.6%) had received HBV treatment but did not complete the treatment (Table 4.5). Majority of the respondents (94.4%) had not been vaccinated against HBV, with a higher (6.9%) HBV vaccination history in females and elderly (7.7%) (Table 4.5). Similarly, the molecular results of this study established a HBV prevalence of 2.9% in those who had previous negative HBV testing results. Only 5.6% of respondents reported to have had HBV vaccination and all the HBV positive

results (2.9%) were among those who did not have HBV vaccination history (Table 4.5). Only 2.8% of the respondents reported to have had liver problems, although none of the respondents was able to specify the nature of the liver problem.

Table 4. 6: Prevalence of HBV in HIV infected patients by HBV infection history against age

Variable		Age (years)				HBV Prevalence
		18-35	36-57	>58	P-Value	
HBV Family History	yes	0	1(2.4%)	0	χ^2=13.741 P = 0.089	0
	No	37(29.6%)	17(40.5 %)	2(15.4%		0
	Don't know	88(70.4%)	24(57.1 %)	11(84.6 %)		5(4.1%)
Past HBV testing results	Tested +ve	7(5.6%)	3(7.1%)	0	χ2=0.967 P=0.617	0
	Never tested	118(94.4%)	39(92.9 %)	13(100%)		5(2.9%)
Vaccinated against HBV	yes	7(5.6%)	2(4.8%)	1(7.7%)	χ2= 0.981 P= 0.913	0
	No	118(94.4%)	40(95.2 %)	12(92.3 %)		5(2.9%)
Diagnosed with any liver Problem	Yes	4(3.2%)	1(2.4%)	0	χ2= 0.930 P = 0.368	1(20.0%)
	No	120(96.0%)	41(97.6 %)	13(100%)		4(2.6%)

The history of HBV liver problems was higher (3.0%) in males and in the youthful (3.2%) respondents. The HBV prevalence was higher (20.0%) in the respondents who reported to have a history of liver problem and lower (2.6%) in those who did not report any history of a liver problem history. The results indicated a substantial correlation between Hepatitis B prevalence and the history of liver problems in the participants (χ2=16.778, df=4, P=0.002). Conversely, there was no important relationship amongst gender, age and HBV prevalence with HBV family history, history of HBV testing and vaccination.

4.1.4 HBV Prevalence risk factors in HIV infected patients by Blood Transfusion, IDU and Alcohol Use

Minorities (2.2%) of the respondents reported to have been transfused with blood in the past and were mostly males (2.5%) and adults (4.8%). None of the respondents reported to have been a blood donor in the past. The prevalence of HBV was found to be 2.8% in participants who had not been transfused with or donated blood in the past. On drug abuse, minority (3.8%) of respondents reported to be injection drug users (IDUs), and a higher (2.5%) proportion of the IDUs' respondents were males and adults (4.8%). The tables 4.7 and 4.8 below show cross tabulation of HBV risk factors by blood transfusion, IDU and alcohol use against gender and age in HIV positive respondents.

Table 4. 7: Prevalence of HBV in HIV infected patients by blood transfusion, IDU and alcohol use by gender

Variable		Gender			HBV Prevalence
		Male	Female	P-Value	
Transfused with blood	Yes	2(2.5%)	2(2.0%)	$\chi2$= 0.062	0
	No	77(97.5%)	99(98.0%)	P = 0.803	5(2.8%)
Use of IDU	Yes	3(3.8%)	4(4.0%)	$\chi2$= 1.287	2(28.6%)
	No	75(94.9%)	97(96.0%)	P = 0.525	3(17.4%)
Alcoholism	Yes	9(11.4%)	14(13.9%)	$\chi2$= 0.242	1(4.3%)
	No	70(88.6%)	87(86.1%)	P = 0.622	4 (2.5%)

This study established a higher (28.6%) HBV prevalence among the IDUs and a lower (17.4%) HBV prevalence in non-injection drug users. There was an important connotation between HBV prevalence and the injection drug abuse ($\chi2$=18.846, df=4, P=0.001). Only 12.8% of the respondents reported to use alcohol heavily and a higher (13.9%) proportion of this were females and adults (16.7%). The incidence of HBV was established to be greater (4.3%) in participants who used alcohol heavily and lower (2.5%) in non-alcoholic

respondents. Nevertheless, there was no substantial variation in HBV occurrence among the alcoholic and non-alcoholic respondents ($\chi 2$= 3.719, df=2, P=0.156).

Table 4. 8: Prevalence of HBV in HIV infected patients by blood transfusion, IDU and alcohol use by age

Variable	Age (years)				HBV Prevalence
	18-35	36-57	>58	P-Value	
Transfused	2(1.6%)	1(4.8%)	0	χ^2= 4.782	0
Yes	123(98.4%)	40(95.2%)	13(100%)	P = 0.310	5(2.8%)
with blood					
No					
Use of IDU	6(4.8%)	1(2,4%)	0	$\chi 2$=4.333	2(28.6%)
Yes	119(95.2%)	40(95.2%)	13(100.0%)	P=0.343	3(17.4%)
No					
Alcoholism	14(11.2%)	7(16.7%)	2(15.4%)	$\chi 2$=0.928	1(4.3%)
Yes	111(88.8%)	35(83.3%)	11(84.6%)	P=0.629	4(2.5%)
No					

4.1.5 HBV Prevalence in HIV infected patients by ARV Treatment and CD4 Counts

Two-thirds (65.0%) of the respondents were on first line HIV drug regimen, 11.7% were on second line HIV drug regimen while 23.3% were not on any HIV drug regimen. Generally, more (84.8%) male and elderly (92.3%) respondents were on ART than female (70.3%) and youthful (71.2%) respondents. The use of first line HIV drug regimen was higher (72.2%) in male and elderly (86.6%) respondents. Similarly, most (12.7%) of the respondents on second line HIV drug regimen were males and adults (16.7%), while majority (29.7%) of the respondents that were not on any HIV drug regimen were females and youthful (28.8%). The HBV prevalence was found to be higher (4.8%) among the respondents on second line HIV drug regimen than among those on first line (1.8%) HIV

drug regimen. Nevertheless, there was no substantial variation in Hepatitis B prevalence among the respondents on first line and second line HIV drug regimens ($\chi 2$= 3.032, df=4, P=0.552).

Most (60.0%) of the respondents reported to have been on ART for a long duration (4 years and above) and minority (16.7%) reported to have been on ARVs for a short duration (0-3years). Among the respondents that had been on ARV for a long duration, a higher (62.0%) proportion of the respondents were among the males and elderly (86.6%). Likewise, among the respondents that had been on ARV for a short duration, a higher (22.8%) proportion of respondents were males but youthful (19.2%). There were significant associations between the duration of being on HIV drug treatment and gender ($\chi 2$= 7.260, df=4, P = 0.027) and, age ($\chi 2$=11.477, df=4, P = 0.022). Subsequently, majority (74.4%) of respondents on first HIV drug regimen and all (100%) respondents on second line HIV drug regimen had been on ART for a long duration. There was substantial relationship between the kind of HIV therapy regimen and the time period of respondents being on ARVs ($\chi 2$= 188.974, df=4, P = 0.001). There was no major dissimilarity in HBV prevalence amongst the respondents who had been on ART treatment for a shorter duration and a longer duration ($\chi 2$= 3.136, df=4, P = 0.535).

The table 4.9 below shows cross tabulation of HBV Prevalence in HIV infected patients by gender and age against HIV drug regimen, duration of ARV treatment and the level of CD4 counts.

Table 4. 9: Prevalence of HBV in HIV infected patients by Gender and Age against ARV Drug Regimen, Duration of being on ARV, CD4 Counts and the HBV Genotypes.

Variable	Gender			Age(years)				HBV Prevalence
	Male	Female	P-Value	18-35	36-57	58 and above	P-Value	
HIV Drug Regimen								
None	12(15.2%)	30(29.7%)	$\chi 2$= 5.228 P = 0.073	36(28.8%)	5(11.9%)	1(7.7%)	χ^2=7.939 P=0.094	0
First line	57(72.2%)	60(59.4%)		76(60.8%)	30(71.4%)	11(86.6%)		4(3.4%)
Second Line	10(12.7%)	11(10.9%)		13(10.4%)	7(16.7%)	1(7.7%)		1(4.8%)
HIV Drug Use(years)								
None	12(15.2%)	30(29.7%)	$\chi 2$= 7.260 P = 0.027	36(28.8%)	5(11.9%)	1(7.7%)	χ^2=11.477 P = 0.022	0
0-3	18(22.8%)	12(11.9%)		24(19.2%)	5(11.9%)	1(7.7%)		2(6.7%)
More than 3	49(62.0%)	59(58.4%)		65(52.0%)	32(76.2%)	11(86.6%)		3(2.8%)
CD4 Count levels								
1-250	8(10.1%)	21(20.8%)	$\chi 2$= 4.254 P = 0.119	22(17.6%)	6(14.3%)	1(7.7%)	$\chi 2$= 2.031 P = 0.730	2(6.9%)
251-500	34(43.0%)	43(42.6%)		50(40.0%)	21(50.0%)	6(42.2%)		1(1.3%)
501 and above	37(46.8%)	37(36.6%)		53(42.4%)	15(35.7%)	6(42.2%)		2(2.7%)

Majority (43.3%) of the respondents had 251-500 CD4 counts, followed (41.1%) by respondents with 501 and above CD4 counts, and minority (15.6%) of the respondents had 250 and below CD4 counts. Among the respondents with 250 and below CD4 counts, a higher (20.8%) proportion was among female and youthful (17.6%) respondents. Almost equal proportion among males (43.0%) and females (42.6%) had 251-500 CD4 counts, with majority (50.0%) of these respondents being in adult age group (36- 57 years). A

higher (46.8%) proportion among male respondents and almost equal proportion among the youthful (42.4%) and elderly (42.2%) respondents had 501 and above CD4 counts. The HBV prevalence was highest (6.9%) among the respondents with 250 and below CD4 counts, followed by respondents with 501 and above CD4 counts (2.7%), and lowest (1.8%) among respondents with 251-500 CD4 counts. Comparing CD4 counts with HIV drug regimen and ARV treatment duration, most (54.8%) of respondents who were not on any ARV treatment had 501 and above CD4 counts. Majority (44.4%) of respondents on first line HIV drug regimen and respondents who had been on ARV treatment for a shorter duration (53.3%) had CD4 counts between 251 and 500. Most (47.6%) of the respondents on second line HIV drug regimen had CD4 counts of 501 and above, while respondents who had been on ARV treatment for a longer duration had almost equal proportion of CD4 counts of 251-500 (40.7%) and 501 and above (39.8%). There were no significant associations in respondents' CD4 counts with gender, age, HIV drug regimens, ARV treatment duration and HBV prevalence.

4.2 The HBV Genotypes in HIV infected Patients

4.2.1 HBV DNA Amplification and Sequencing

The ELISA was used to test for HBsAg for all the 180 blood specimens obtained from HIV positive clients. 9(5.0%) samples (male were five and female were 4) turned out to be HBsAg positive and HBV genotypes were tested using the S gene in all the 9 samples that were positive for HBsAg ELISA. Nested PCR amplifications were successful in 5 (2.8%) samples (where male were three while female were two) for S gene (Table 4.10). The rest

of the samples that failed to be amplified were taken to have undetectable low DNA levels or were simply false-positives.

The table 4.10 below shows the HBsAg ELISA and PCR test prevalence by positivity rate and gender

Table 4. 10: HBV prevalence by HBsAg ELISA, PCR and by gender

Test	Positive	Negative	HBV Prevalence by gender	
			Male	Female
HBsAg ELISA (n=180)	9(5.0%)	181(95.0%)	5(2.8%)	4(2.2%)
PCR (n=9/180)	5(2.8%)	4(2.2%)	3(1.7%)	2(1.1%)

The Hepatitis B S transcribing region sequences effectively amplified from the 5 DNA positive specimen. Figure 4.2 below shows 700bp amplified PCR fragment size.

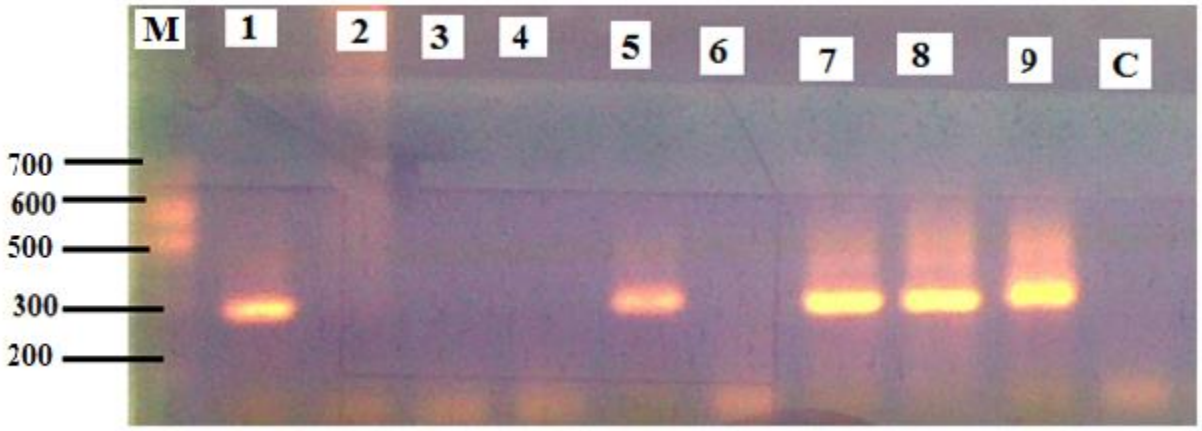

Figure 4. 2: 1.5% Ethidium Bromide-Stained Agarose Gel for HBV S gene nested PCR

Gel photo showing nested PCR products of nine samples (1-9) and a control C of HBV S gene. M is 100 base pair ladder; 1.5% ethidium bromide stained agarose gel using S1 and S2 Primers for HBV S gene. Five Samples were positive. Samples 1, 5, 7, 8 and 9 were positive while samples 2, 3, 4, 6, and C were negative for HBV S gene.

4.2.2 Genetic diversity

The alignment of S gene sequences was carried out using ClustalW and viewed in MEGA 6.0 (Appendix X). This alignment showed that HBV S gene is highly conserved. Phylogenetic tree of the five sequence isolates and 16 homologue sequences from GenBank with S gene HBV sequence from the monkey as outgroup put the isolates into four independent clusters as shown in Figure 4.3 below.

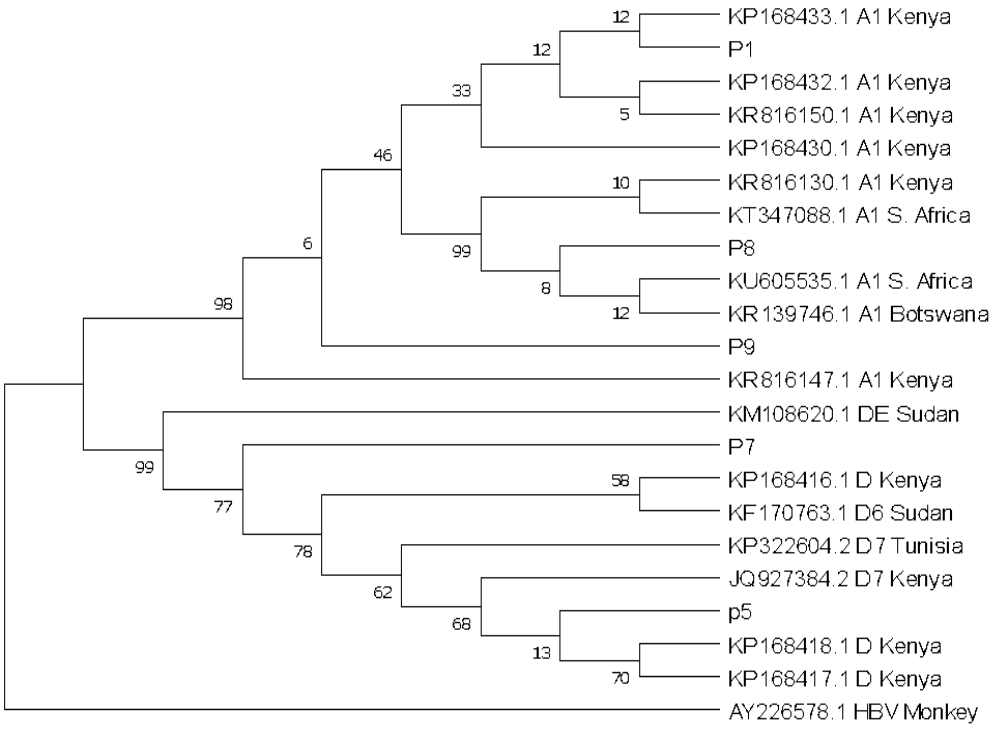

Figure 4. 3: Maximum Likelihood phylogeny of S gene sequences (710nt) of HBV.

GenBank accession numbers of comparative sequences are given together with their country of origin. The tree with the highest log likelihood (-1605.24) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model (1993), and then selecting the topology with superior log likelihood value. This analysis involved 22 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There was a total of 674 positions in the final dataset. Evolutionary analyses were conducted in MEGA X

Isolate P1 clustered with KP168433.1 (sub-genotype A1), KP168432.1 (sub-genotype A1), KR816150.1 (sub-genotype A1) and KP168430.1 (genotype A), all of which were isolated

in Kenya. This branch was supported by 33% clustering association. Isolate P5 clustered together with GenBank JQ927384.2 (sub-genotype D7), KP168418.1 (sub-genotype D), and KP168417 (sub-genotype D) all of which were isolated from Kenya and KP322604.2 (sub-genotype D7) from Tunisia. Isolate P7 clustered with KP168416.1 (genotype D) from Kenya and KF170763.1 (sub-genotype D6) and KM108620.1 (sub-genotype DE) both from Sudan. Isolate P8 clustered with KR816130.1 (genotype A) from Kenya, KU605535.1 (sub-genotype A1) from South Africa, KT347088.1 (sub-genotype A1) from South Africa and KR139746.1 (genotype A1) from Botswana. The branch having these isolates was supported by 98% clustering association. Isolate P9 clustered with GenBank KR816147.1 (genotype A1) isolated from Kenya.

Genetic distance matrix on Figure 4.4 generated by MEGA 6 was used to approximate genetic distances among the isolates and their GenBank counter parts. The larger the value for the organism, the higher the genetic divergence and the lower the value, the higher the similarity. Genetic distances among isolates are shown in Red, genetic distances between isolates and close GenBank homologues are shown in yellow while the distances between GenBank isolates deemed to be exactly the same strain are shown in blue (Figure 4.4 below).

1		P1	3	4	5	6	P5	8	9	10	11	P7	13	14	15	P8	17	18	19	20	P9	22
2	P1 (A1)																					
3	KP168433.1_A1_Kenya	0.0015																				
4	KR816150.1_A_Kenya	0.0029	0.0015																			
5	KP168430.1_A1_Kenya	0.0015	0.0000	0.0015																		
6	KP168432.1_A1_Kenya	0.0029	0.0015	0.0029	0.0015																	
7	p5 (D7)	0.0502	0.0487	0.0502	0.0487	0.0502																
8	JQ927384.2_D7_Kenya	0.0502	0.0486	0.0502	0.0486	0.0502	0.0029															
9	KP168418.1_D_Kenya	0.0502	0.0487	0.0502	0.0487	0.0502	0.0029	0.0029														
10	KP168417.1_D_Kenya	0.0502	0.0487	0.0502	0.0487	0.0502	0.0029	0.0029	0.0000													
11	KP168416.1_D_Kenya	0.0455	0.0439	0.0455	0.0439	0.0455	0.0044	0.0044	0.0044	0.0044												
12	P7 (D6)	0.0486	0.0471	0.0455	0.0471	0.0486	0.0134	0.0134	0.0134	0.0134	0.0089											
13	KM108620.1_DE_Sudan	0.0424	0.0408	0.0424	0.0408	0.0424	0.0089	0.0089	0.0089	0.0089	0.0044	0.0104										
14	KF170763.1_D6_Sudan	0.0455	0.0439	0.0455	0.0439	0.0455	0.0044	0.0044	0.0044	0.0044	0.0000	0.0089	0.0044									
15	KP322604.1_D7_Tunisia	0.0471	0.0455	0.0471	0.0455	0.0471	0.0029	0.0029	0.0029	0.0029	0.0015	0.0104	0.0059	0.0015								
16	P8 (A1)	0.0194	0.0179	0.0194	0.0179	0.0194	0.0549	0.0549	0.0549	0.0549	0.0534	0.0565	0.0502	0.0534	0.0518							
17	KR816130.1_A1_Kenya	0.0119	0.0104	0.0119	0.0104	0.0119	0.0471	0.0470	0.0471	0.0471	0.0455	0.0486	0.0424	0.0455	0.0439	0.0074						
18	KU605535.1_A1_S._Africa	0.0119	0.0104	0.0119	0.0104	0.0119	0.0471	0.0470	0.0471	0.0471	0.0455	0.0486	0.0424	0.0455	0.0439	0.0074	0.0000					
19	KT347088.1_A1_S._Africa	0.0119	0.0104	0.0119	0.0104	0.0119	0.0471	0.0470	0.0471	0.0471	0.0455	0.0486	0.0424	0.0455	0.0439	0.0074	0.0000	0.0000				
20	KR139746.1_A1_Botswana	0.0119	0.0104	0.0119	0.0104	0.0119	0.0471	0.0470	0.0471	0.0471	0.0455	0.0486	0.0424	0.0455	0.0439	0.0074	0.0000	0.0000	0.0000			
21	P9 (A1)	0.0179	0.0164	0.0179	0.0164	0.0179	0.0629	0.0629	0.0629	0.0629	0.0581	0.0613	0.0549	0.0581	0.0597	0.0316	0.0270	0.0270	0.0270	0.0270		
22	KR816147.1_A1_Kenya	0.0029	0.0015	0.0029	0.0015	0.0029	0.0471	0.0471	0.0471	0.0471	0.0424	0.0455	0.0393	0.0424	0.0439	0.0194	0.0119	0.0119	0.0119	0.0119	0.0149	
23	AY226578.1_HBV_Monkey	0.1390	0.1372	0.1354	0.1372	0.1390	0.1263	0.1298	0.1298	0.1298	0.1281	0.1316	0.1316	0.1281	0.1263	0.1424	0.1335	0.1335	0.1335	0.1335	0.1533	0.1354

Figure 4. 4: Genetic distances among isolates and their GenBank counterparts

Further genetic distance analysis using MEGA 6.0 as shown in Table 4.11 below identified isolates as follows: sample P1 as sub-genotype A1, sample P5 as sub-genotype D7, sample P7 as sub-genotype D6, sample P8 as sub-genotype A1 and sample P9 as sub-genotype A1. Genetic distances between isolates P1, P8 and P9 and their homologue GenBank isolates ranged from 0.15% and 1.49%, genetic distances between GenBank isolates and P5 was 0.29% and the distance between P7 and GenBank close homologue was 0.89%.

Table 4. 11: Isolate identification by BLASTn at GenBank and genetic distance in MEGA V6

Sample name	NCBI identity	% identity	Country	Genetic distance	NCBI genotype	Possible isolate genotype
P1	KP168433.1	99	Kenya	0.001473	A1	**A1**
P5	JQ927384.2	98	Kenya	0.002947	D7	**D7**
P7	KF170763.1	99	Sudan	0.00889	D6	**D6**
P8	KR816130.1	98	Kenya	0.00739	A1	**A1**
P9	KR816147.1	99	Kenya	0.00739	A1	**A1**

The resultant isolates were deposited in the NCBI GenBank with the HBV Genotypes Accession Numbers MG845879-MG845886 (Appendix XI). Subsequent analysis revealed that there was a slightly greater HBV incidence among the male (3.8%) and youthful (3.2%) HIV infected patients as compared to the females and adult respondents of 36 years and above ($\chi 2$= 2.708, df=4, P = 0.258 for gender and $\chi 2$= 1.242, df=4, P = 0.871 for age), as shown in the table 4.12 below.

Table 4. 12: Distribution of HBV Sub Genotypes by Gender and Age.

Genotype	Gender		Age (years)			Total	
	Male	**Female**	**Youth (18-35)**	**Adult (36-57)**	**Elderly (58 and above)**		
None	76(96.2 %)	99(98.0 %)	121(96.8 %)	41(97.6 %)	13(100.0 %)	**175(97.2%)**	
A1	1(1.3%)	2(2.0%)	2(1.6%)	1(2.4%)	0	**3(1.8 %)**	**5(2.8 %)**
D(D6&D7)	2(2.5%)	0	2(1.6%)	0	0	**2(1.0 %)**	
χ2	$\chi 2$=2.708			$\chi 2$= 1.242,		**180(100.0%)**	
P-value	P =0.258			P = 0.871			

Majority (60%) of Hepatitis B genotypes were obtained from HIV positive clients who admitted to be on IDU and had normal CD4 level (250 and above); while most of the HBV genotypes were obtained from the clients on second line ARV use (80%) and on long term duration (60%), as shown in table 4.13 below.

Table 4. 13: Distribution of HBV Sub Genotypes against IDU use, and ARV Use, Duration and CD4 Levels

Sample	Genotype	IDU Use	HIV Drug Regimen	ARV Drug Use Duration	CD4 Level
P1	A1	no	Second line	Short-term (0-3 years)	251-500
P5	D7	yes	Second line	Long-term(4 and above years)	501 and above
P7	D6	yes	First line	Long-term	1-250
P8	A1	yes	Second line	Long-term	1-250
P9	A1	no	Second line	Short-term	1-250

4.2.3 HBV Drug Mutations and Resistance Prediction

The five genotypes identified in the study had susceptible mutations in S gene for Lamivudine, Adefovir, Entecavir and Tenofovir (Table 4.13 and Appendix XII). Owing to increased mutational proportions in the HBV genome, it can be categorized into numerous genotypes with an 8% genomic divergence as a normal cut-off. The most common alteration involves replacement of methionine for valine or isoleucine rtM204V/I (Table 4.13 and Appendix XII).

Table 4. 14: Mutations and their effect on HBV drugs

Sample	Mutation	N	N(t)RTI Treated			R/S/U
			L-Nuc. and/or ETV	ANPs	L-Nuc. and/or ETV + ANPs	
P1(A1)	R110G	Y	Y	Y	Y	S
	N122H	Y	Y	Y	Y	S
	Y126H	Y	Y	Y	Y	S
	M129L	Y	Y	Y	Y	S
	V163I	Y	Y	Y	Y	S
	R217L	Y	Y	Y	Y	S
P5 (D7)	P237T	Y	Y	Y	Y	S
P7(D6)	C6P	N	N	N	N	U
	E8P	N	N	N	N	U
	H9G	N	N	N	N	U
	R120G	Y	Y	N	N	S
	Q130P	Y	Y	Y	Y	S
	Q149K	Y	Y	Y	Y	S
	P237T	Y	Y	Y	Y	S
	W243T	N	N	N	N	U
	Y245C	Y	N	N	N	S
	S246A	Y	N	N	N	S
	M250L	N	N	N	Y	R
P8 (A1)	C6E	N	N	N	N	U
	D7H	Y	Y	N	N	S
	E8R	N	N	N	N	U
	R18K	Y	Y	N	Y	S
	I53L	Y	Y	N	N	S
	S109P	Y	Y	Y	Y	S
	N122H	Y	Y	Y	Y	S
	T128S	Y	N	N	Y	S
	M129L	Y	Y	Y	Y	S
	R242K	Y	Y	N	N	S
P9 (A1)	N122H	Y	Y	Y	Y	S
	Y126H	Y	Y	Y	Y	S
	M129L	Y	Y	Y	Y	S
	V163I	Y	Y	Y	Y	S
	R217L	Y	Y	Y	Y	S
	M250W	N	N	N	N	R
	Y252V	N	N	N	N	R
	I253V	Y	Y	Y	Y	S
	I254V	Y	N	N	N	S
	S256G	Y	Y	N	Y	S
	W257E	N	N	N	N	U

Key: N - non-treated, R-drug resistance, S means HBV susceptibility, U means Unknown effect, N(t)RTI means nucleos(t)ide RT inhibitors, RT means reverse transcript, L-Nuc. Means L-nucleoside analogs (lamivudine, emtricitabine, telbivudine); ETV - entecavir; ANPs - acyclic nucleoside phosphonates (adefovir, tenofovir), Y means from treated individuals, mutants in red causes drug resistance and * means stop codon.

Isolate P1 had six mutations on its S gene from individuals treated with nucleos(t)ide analogs. Isolate P5 had developed a single substitution from amino acid proline to methionine at position 237 (Table 4.13 and Appendix XII). Isolate P7 had developed 12 RT mutations. Sample P8 had accumulated 11 mutations on the S gene. However, two mutations at position 6 and 7 had not been documented from either treated or non-treated individuals and therefore their effects are not known. Isolate P9 had accumulated 11 mutations on the S gene, 8 of which had been previously observed in non-treated patients (Table 4.13 and Appendix XII).

CHAPTER FIVE: DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1. Discussion

This study was conducted in HIV seropositive individuals who were attending Mbagathi District Hospital for routine CD4+ T-lymphocyte and viral load count for management with antiretroviral therapy (ART) and who consented to the study. Mbagathi District Hospital is situated in Nairobi County, a cosmopolitan city with people from all parts of the country. It was cross-sectional research carried out between April 2014 and March 2015. Although the 180 (Male=79) with mean age of 34.5 years, cases sampled in this study do not represent the national registries, the result of this research contributes to the knowledge of HBV epidemiology in HIV positive individuals in Kenya.

In this study, the 5.0% HBV incidence is comparable to majority of the HBV referenced prevalence that are based on serological testing. This is in conformity with WHO establishment that occurrence of active Hepatitis B disease is determined by HBsAg serology in the general population of a specific geography (WHO, 2017; Serviddio, 2013 and Franco *et al*., 2012). This mirrors other local research by Kilongosi *et al*., (2015), Kerubo *et al*., 2014; Day *et al.,* (2013*),* Muriuki *et al*., 2013, Kim *et al*., (2011) and Hariana, (2008) which established 5.1-5.7%, 4.26%, 7.0%, 5.68%, 6.9%, and 6.2% HBV prevalence respectively in HIV positive individuals. A recent systematic review survey in Kenya has confirmed the mean of 6.14% HBV prevalence among HIV population, which concurs with the result of this study (Downs *et al.,* 2023). From regional countries, similar studies have reported concurring HBV prevalence in HIV populations. Surveys in Ethiopia

established HIV-HBV prevalence of 5.6% and 5.5% that mirrored previous studies that had established 3-5.9% prevalence in Ethiopia (Wondimeneh *et al.,* 2013 and Deressa *et al*., 2017), Hawkins *et al*., (2013) from Tanzania has reported 6.2% prevalence among HIV-infected population, a survey from HIV clinic patients reported HBV prevalence of 7.9% in Uganda (Chiesa *et al*., 2020), while a similar study from South Africa reported 5% prevalence among patients receiving ART for HIV disease (Bisceglie *et al.,* 2010). Other global studies have reported similar results. A survey reviewing HIV/HBV concurrent infections reported a median prevalence of 7.8% in sub-Saharan Africa, 11.5% in West African Countries, 5.4% in Southern African Countries and 4.1% in East African countries with wide variability within countries and regions (Stabinski *et al.,* 2015). The global prevalence of HBV in the general population is 3.5%, with the African region having the prevalence of 6.2% (WHO, 2017) while 7.4% of all HIV individuals are also concurrently infected with HBV (Soriano *et al*., 2010).

Previous studies have suggested that in Kenya, HBsAg prevalence seems to vary in different population groups. In contrast to this research that established HBV occurrence at 5% in the HIV positive individuals, other local surveys have reported higher HBV prevalence in the general population. Similar surveys by Jepkemei *et al*., (2020) has reported HBsAg positivity of 10.1%, 18.7% and 58.8% for MSM-SM, occult HBV infection and HIV-HBV dual infection respectively; Harania *et al*., (2008) reported 17% HBsAg positivity among HIV patients and Atina *et al.,* (2004) established HBsAg prevalence 26.9% in patients presenting with jaundice while surveys on liver-related problems and blood donors have reported between 2.4-61.4% HBsAg positivity (Aluora *et*

al., 2020; Nyairo *et al.,* 2016; Ochwoto *et al*., 2013 and Mutuma *et al*., 2011). A recent systematic review by Downs *et al.,* (2023) reported that HBV in general population as a low-risk group is 3.36% while the pooled occurrence in high-risk groups is 6.18-29.9% for high and very high risk individuals. Although Kenya and Africa region have been classified as high endemic regions with 8% and above HBV prevalence with more than 60% lifetime risk of infection (Serviddio, 2013; Franco *et al.*, 2012), this study established an intermediate prevalence of 2-7% as per WHO HBV epidemiological classification (WHO, 2017). The prevalence differences might be due to variations in the study populations and the related risk factors as reported elsewhere (Wondimeneh *et al.,* 2013, Deressa *et al*., 2017). The low reported HBsAg sero-prevalence in this study maybe due to dual effect of antiretroviral therapy that have effect on HBV disease since all HBeAg positive individuals reported to have been on ART for long duration of at least 4 years and above similar to a previous study (Deressa *et al*., 2017). Worldwide, an estimated 5-10% of HIV infected persons are also con-infected with HBV (Stabinski *et al.,* 2015). For instance, a prospective survey in Malawi found 12% HBsAg incidences among PLHIV as an attestation of the variability in the region (Aoudjane *et al*., 2014). This study further confirms that HBV is a regular co-infection in Kenyan HIV positive populace due to similar routes of transmission and predisposing risk factors as reported in previous studies (Sulkowski, 2008; Kramvis & Kew, 2007). Besides, this coincides with other previous surveys that with increasing access to antiretroviral therapy in Kenya and the rest of Sub-Saharan Africa, HIV-infected individuals are living longer and the effects of co-infection with chronic viral hepatitis is an emerging critical public health problem (Modi & Feld, 2007).

The prevalence of HBV was first determined serologically followed by molecular assays using PCR and sequencing. The HBV prevalence was found to be 5.0% (n=9) serologically and 2.8% (n=5) by PCR. The rest of the samples that failed to be amplified were either taken to have undetectable low DNA levels or were simply false-positives. This is compatible with other reported surveys where in Mwangi *et al*., (2008) out of 80 HBsAg positive blood samples, only 52 were successfully amplified molecularly; in Kwange *et al.*, (2013) only 21 samples could be amplified out of 32 HBsAg positive plasma specimen while in Kilongosi *et al*., (2015) only 23 out of 33 samples were amplified. Similarly in Khamduang *et al*., (2012) a Thailand study, out of 44 HBsAg positive samples that were selected for molecular analysis, 8 samples were found to have insufficient DNA levels for analysis while 2 samples were found to be false positives. This supports Quint *et al*. (1995) study that concluded that serological testing techniques have a severe problem of incorrect results and cannot proficiently establish genetic variances among the strains. PCR with direct sequencing is a precise procedure for the identification of genetic variability of HBV. However, from Villar *et al*., (2015) each detection technique offers advantages and limitations. Immunological assays are useful in Hepatitis B Viral detection since they are simple, automated and convenient. Yet, they may consume more time, and might be costlier, hence rapid tests have been advanced in order to overcome the limitations in serological testing. Molecular methods are more expedient in detecting prolonged HBV septicity; to detect HBV occult disease; to assess the progression of infection; to aid in management decisions and evaluate ART effectiveness; and to detect resistance transformations to antiretroviral therapy. Molecular approaches have greater specificity and sensitivity; as well as higher dynamic levels of detection in contrast to other diagnostic

evaluations such as serological tests. HBsAg serological assays' specificity and sensitivity is hinged on the testing threshold of serological tests (Cabezas-Fernandez & Cabeza-Barrera, 2012). Cabezas-Fernandez & Cabeza-Barrera (2012) reported that because HBV is heterogeneous due to genotype and sub-genotype diversity, the sensitivity of HBsAg tests might as well rely on HBsAg antigenic difference and some HBsAg transformations that arise subsequent to immunological-selection pressure may escape discovery by commercial HBsAg tests. Although this study was limited in that it did not screen for other serological, biochemical and virological panels of HBV infection, the difference in HBV prevalence means that population-based HBV screening studies by serological markers may tend to establish higher HBV prevalence than by molecular screening. This confirms to other similar studies that have concluded that serological tests have low sensitivity with prognosis significance when pooled with clinical case definition while DNA detection are more sensitive (Cota *et al*., 2012). Several other comparative studies on HBV molecular detection versus serological test have confirmed the proficiency of PCR procedure in determining HBV variants. The PCR process offers an expedient means for prompt and economical testing in regions with diverse topographical HBV genetic variants' in circulation (Liu *et al*., 2006). Although PCR technique is unsuitable for large-scale molecular analysis, Hepatitis B variant determination by evolutionary/phylogenetic exploration grounded on nucleotide sequences yields the greatest dependable and assured genotyping outcomes. PCR molecular analysis technique is modest, faster, and has higher specificity for HBV molecular analysis with primer-specific as compared to RFLP (Naito *et al*., 2001). The results of this study are contrary to the recently reported survey by Aluora *et al*., (2020), which had only 2 HBsAg positive samples while 9 HBV DNA detected

samples. This plausible explanation is due to the occult HBV which cannot be detected by HBsAg serology. The fact that this study was carried out among individuals exposed to ART therapy, it could be one of the limitations since occult HBV was not investigated.

Although there was no significant HBV prevalence by gender and age similar to a previous study in Nigeria (Mustapha and Jibrim 2004), the results revealed that the HBV prevalence was greater (3.8%) in males than females (2.0%) and higher (3.2%) in the youth group than in the adults (2.4%). This concurs with Aluora *et al.,* (2020) survey which reported that youth group (19-28 years) had higher probability of being HBsAg positive with significant association. Previous HBV testing results history in the study population also confirmed that there was higher HBV prevalence in males (6.3%) though with higher prevalence in adults (7.1%) than in the youth age group (18-35 years). This mirrors an earlier Kenyan study by Mutuma *et al.,* (2011) that reported greater male HBV carrier state and HCC dispositions than in females. Similarly, Muruiki *et al*., (2013) reported higher HBV prevelance in males than in females. Likewise, a previous Tanzanian study had comparable conclusion that unlike the HIV positive individuals, HBV-HIV co-infected individuals had higher probability of being male and younger (Hawkins *et al.,* 2013). Likewise, a recent Ethiopian study also had comparable results where it reported slightly higher HBV occurrence in males (7.8%) than in females at 4.1%. (Deressa *et al.,* 2017). Other studies have also predominantly concluded that the male gender has increased risk factor of having HBV/HIV co-infection than in female (Niramon, L. & Wathanachai, S. 2010; Nakwagala & Gakimu 2002; Choy *et al.,* 2019, and Spradling *et al.,* 2009). Similarly, another population-wide survey observed that HCC frequencies were high in Hepatitis B infected

young male adults and children in Africa, rural western Alaska and the Amazon (Kramvis & Kew, 2007). This is in contrast to observation reported in a West African study where HBV prevalence was higher (41.6%) in age-group 40-49 years (Mustapha & Jibrim 2004). A longitudinal analysis in CHB trends among the heterogeneous HIV positive individuals in USA reported a statistically higher prevalence of HBV in men than women (Spradling *et al.,* 2009). The differences may be associated with more risk factors predisposing more male and a particular age group to more HBV infection than to females. Risk factors to HBV infection such as IDU, multiple sexual partners and alcoholism are more associated with males than females (Choy *et al.,* 2019 and Deressa *et al.,* 2017), hence predisposing more males to HBV infection. In addition, another survey also concluded similarly that the higher percentage of HBsAg positive males harboring HBV chronic infection may be the result of differences in tribal and sexual behaviors between males and females (Zampino *et al.,* 2015).

Socioeconomic status also tends to contribute to the prevalence of HBV from this study. The HBV prevalence was higher (6.7%) in the widowed respondents followed by singles (3.2%) and married (2.2%) with a significant association (P=0.001). Mustapha & Jibrim (2004) in Nigeria, had similar result where they reported that in the widowed and divorced had 53%, followed by the unmarried at 32.5% and lastly those married had 21.6% HIV/HBV co-infections. McQuillan *et al*., (1999) also made similar observations that being divorced or separated from marriage significantly increased predisposition to HBV among the non-Hispanic White population. The reason advanced for this is that being single probably predisposes one to more multiple sexual relationships and commercial

sexual transactions. The prevalence of HBV was found to be higher in respondents with college education (7.7%) and unemployed (4.3%) with a substantial relationship between the level of formal education, employment and age ($\chi2$=21.680, df=6, P=0.001 and $\chi2$=10.747, df=4, P=0.030 for education and employment respectively). This is in contrast to a study in USA where it was observed that those who had less than a high school level and some college education had increased predisposition to HBV infection across all races (McQuillan e *et al.,* 1999). Unlike the USA study, the results in this study is surprising since it is expected that those with increased level of education have better knowledge and prevention on HBV predisposing risk factors. There was higher frequency of unemployed HBV patients as compared to the employed ones. It is a surprising correlation because it is naturally expected that individuals with higher socioeconomic status like education tend have better health care access opportunities, including vaccination. In a study to understand interracial disparity of liver diseases in the United States, Nguyen & Thuluvath, (2008) found that the burden of prolonged hepatic disease, specifically HBV septicity, was higher in Hispanics and African Americans. The study concluded that race was completely intertwined with socioeconomic, cultural, and genetic determinants of health outcomes, and can be an alternate indicator to other health factors predictors. Consequently, ethnic disproportions could show disparities in social class, access to medical care, and quality of care. This problem is additionally supported by high HBV prevalence among the widows, probably due to lack of support and better health opportunities for them due to poverty and burden of HIV treatments. A thorough comprehension on how socioeconomic status impact on HBV health outcomes will certainly enhance the innovation of better policies

and interventions to diminish socioeconomic gaps on the burden of hepatic infection among the HIV positive individuals.

Drug injection, heavy alcohol consumption, low use of protection during sexual intercourse and increased frequency of sexually transmitted infections were found to be direct risk factors for increased HBV infection similar to what is reported elsewhere (Deressa *et al.*, 2017). In this study, injection drug abuse significantly contributed to high prevalence (28.6%) of HBV (P=0.001). This is similar to other Kenyan studies by Webale *et al*., (2015) who reported a high HBsAb positivity of 8.3% in injection drug use in HIV patients and by Kilongosi *et al*., (2015) who reported higher detections of 5.1-5.7% HBsAg in HIV positive injection drug users, with 15.5-29.0% regular exposures to HBV contacts. From Kilongosi *et al.,* (2015), it was concluded that higher degrees of HBsAg positivity in HIV positive IDUs pointed to secondary contacts of Hepatitis B virus after HIV infection due to higher dangers from injection and sexual behaviors that were rampant among HIV positive IDUs along the coastal Kenya. Downs *et al.,* (2023) recent survey in Kenya is also in congruent with this study where it reported IDUs are among the very high risk with 29.9% HBV prevalence. Besides this, a Canadian study in HIV/HBV dual infected persons reported a 28% of study subjects who had a history of drug injection with diminished virological suppression (Rana *et al*., 2021). Other previous studies have also reported similar trends. Krajden *et al.,* (2005) reported that injection drug usage is a major risk factor of contacting HBV infection among the youth. Garfein *et al*., (1996) reported 77.4% prevalence among the people who had undergone drug abuse for about six years. They also established that within the first 6 years, sero-prevalence proportions for HBV-HIV increased with cumulative period of injecting that could not be expounded by the

distributions of education, marital status, race, sexual orientation, age, income and gender among cohorts distinguished by length of time for IDU. From their data, it was concluded that the majority of new infections transpired immediately the injection drugs were initiated. It is therefore possible that patients got infected with HBV before being infected with HIV or they were infected at the same time.

Although not significant, this study also found that most of the patients infected with HBV were heavy alcohol consumers (4.3% prevalence). These results were similar to Ndako *et al*. (2013) who indicated that the HBV incidence among the alcoholics in the community was greater as compared to non-alcoholics. Jerrells *et al*. (2002) likewise established that drunkenness contributes to viral hepatitis, since the HBV frequency was higher in key subjects that may have consumed alcohol than in controls (Dunn et *al.*, 2005), that may lead to viral hepatitis. A study by Bedogni *et al*. (2008), also established that HBV infection in the alcoholics was related to earlier advancement of liver damage with a raised risk of developing cirrhosis. Alcohol intake of greater than 40 g/day has been linked to elevated aminotransferases and an amplified likely hood of advancing to hepatic impairment (Krajden *et al.,* 2005). In addition, Laskus *et al*. (1992) study found that HBV prevalence in alcoholics was about 4 times more than in controls. Perhaps this is why HBV frequency was greater among males than females and young population than in adults as these are the groups that mostly indulge in alcoholism.

This study established significant association (P=001) between the respondents' frequency of not using protection during sexual intercourse and with a higher history of suffering

from STIs in females (15.8%) and in adults (26.1%). A high percentage of patients who reported to have suffered from STI's in the past were HBV sero-positive. This was similar to Risbud *et al*., (2002) study which found a correlation between STDs and HBV among the patients and STI clinic attendees. HBV disease was related to a positive serologic result for syphilis (McQuillan *et al*., 1999). A study in Tanzania by Bart *et al*., (1997) linked HBV septicity to recent syphilis infection in males while clients with active STDs were directly linked to *Trichomonas vaginalis* in women and recent syphilis in men. The Tanzanian survey concluded that both Hepatitis B and STIs might have been acquired concurrently or within a short time duration since both syphilis and *trichomoniasis* presented high possibilities of direct interactions of blood and genital fluids, all of which were effective "vehicles" for Hepatitis B viral spread. These studies clearly points out the significance of STDs in accelerating the rate at which one can get infected with HBV. This positive correlation between STI's and HBV prevalence further supports W.H.O, (2002) that sexual contact is the most frequent route of HBV transmission. Krajden *et al.,* (2005) also reported that sexual transmission is a common risk factor for spreading HBV septicity among the youth. A Study in Uganda mirroring this among HIV patients also reported that higher past exposure to HBV infection was greater among the PLHIV than HIV seronegative individuals and it was directly correlated with more sexual partners (Nakwagla & Gakimu 2002). A similar result was observed in Ethiopia among HIV patients (Deressa *et al.,* 2017). Hepatitis B disease risks may be minimized via an adjustment in behavior and increasing one's information of the disease, especially to all immuno-compromised HIV patients in order to lower the risk factors.

Majority of the HIV respondents (68.3%) did not know their HBV family history and all HBV positive results were among the participants who did not know their HBV family History with a HBV prevalence of 4.1%. Minority (6.7%) of the respondents reported to have previously been tested for HBV, with 83.3% (n=10) of them turning out to be positive for HBV. The results of this study concurs with previous survey which concluded that regular Hepatitis B screening is not practically accessible within the majority African health care settings to the great length where Hepatitis B co-infection and resistance are not known (Kim *et al.,* 2011). This points to the fact that there is low HBV testing access globally and thus majority of populations are ignorant of their HBV status and risk factors. This means that majority of perinatal HBV transmission and horizontal HBV acquisition cases could be perpetuated by ignorance. This is similar to what WHO, (2017) has affirmed that globally, accessibility to inexpensive and quality assured HBV testing is inadequate with only 9% of Hepatitis B infections having been estimated to have been tested through serological assays. Indeed, access to HBV testing in Kenya remains limited as reported previously (Downs *et al.,* 2023; Mwangi *et al.,* 2008). Organ and body tissue contributors ought to be tested for HBsAg to avoid HBV spread. Besides, community-wide testing is commended for immigrants from HBV widespread regions (WHO, 2015). Screening of all organ and tissue donations, besides ensuring quality disinfection in hospital procedures greatly minimizes the Hepatitis B transmissibility hazards. Additionally, testing of all expectant mother's aids in preventing perinatal transmission during delivery ((WHO, 2015). Screening of all organ and tissue donations, besides ensuring quality disinfection in hospital procedures greatly minimizes the Hepatitis B transmissibility hazards.

Additionally, testing of all expectant mother's aids in preventing perinatal transmission during delivery (Franco et al., 2012).

Interestingly, none of the participants who had responded to have been tested HBV positive was found to be HBV positive by this study. This could be either due to false positive HBV results (Quint *et al.* 1995) or clearance of the HBV infection through the immune system or ART therapy. It could also be typical cases of occult HBV that need further molecular studies. This closely collaborates with a recent study in Kenya (Jepkemei *et al.,* 2020) that reported 18.1% occult Hepatitis Virus among HBsAg negative individuals with indications that Occult HBV risks maybe be greatly influenced by HIV therapy, HIV dual septicity, test specificity and sensitivity as well as HBsAg transcribing region mutations that in turn influence diagnostic detection and population prevalence. According to Chemin *et al.*, *(*2011), Occult HBV could occur due to diverse mechanisms comprising of faulty HBsAg expression such as S gene mutation in structural or regulatory features and prevention of HBV replication as a result of concurrent HCV infection. The nonexistence of HBsAg in HBV occult septicity is interpreted to be as a result of several mechanisms such as little rate of HBV reproduction as result of host's immune reaction or concurrent infection with other pathogens, relationship of HBsAg to anti-HBs resulting in the development of immune complexes that diminish the circulation of free antigen, and as a result of genetic alterations, that impede HBsAg manifestation or modification of HBsAg antigenicity, thus inhibiting testing by commercial tests (Mello *et al.,* 2011). Among the 10 participants who reported to have previously tested HBV positive, only one was initiated on HBV treatment but did not complete the treatment course, pointing to the need for enhanced ART treatment

adherence and support for effective HBV treatment outcomes among PLHIV. This is supported by previous studies that reported CHB clients without treatment with decompensated cirrhosis risk poor prospects of recovery, as fifteen-to-forty percent live for about 5 years. Numerous host and viral dynamics, particularly concurrent infection with HIV, HCV and HDV in combination with additional cofactors including alcoholism and aflatoxins can escalate the proportion of infection development and the danger of evolving to Hepatocellular Carcinoma (WHO, 2015).

Only 5.6% of respondents reported to have had HBV vaccination and all the HBV positive results (2.8%) were among those who did not have HBV vaccination history. This confirms the importance of HBV vaccination that confers lifelong protection against HBV. This mirror previous results in a population wide survey in China that found only 3.8% of adults 20 years and above vaccinated as compared to 85.9% for those below 20 years. This was attributed to 20 years of universal HBV vaccination programmes that benefited the younger generation as compared to the adults (Shen *et al.,* 2010). Although this study did not obtain other HBV serological and immunological markers such as anti-HBsAg and anti-HBc that could help understand the innate or vaccine-induced immunity, universal HBV immunization was initiated in Kenya in 2001, at time when all study participants were all indisposition for postnatal vaccine coverage in childhood, yet the same HBV vaccine remains not easily accessible beyond childhood stages as reported elsewhere in African region (Deressa *et al*., 2017). This is the most plausible explanation for low vaccination prevalence in the current sampled population. In contrast, a study among health workers in Nigeria reported a 36.2% vaccination rate as reported in other previous studies (Ogoina

et al., 2014). The low vaccination rates in the general population as compared to higher vaccination rates in health care workers is mostly influenced by knowledge gaps in different population cohorts. Whereas health care workers have more knowledge on HBV and the importance of vaccination, the general population may not have such. Moreover, health care workers in most cases are compelled by health care work-related polices to be screened for HBV and take vaccine as work place safety precautions unlike in the general population. A significant problem of CHB continues since the universal coverage through the natal vaccination remains very small, approximate at 39% globally in 2015. Screening for high-risk clusters of individuals for HBsAg and HBV vaccination for populations at most risk and not immune should comprise family and intimate interactions of individuals with CHB, HIV clients, PWIDs, MSM, FSW, persons with multiple sex partners, native folks, individuals in prison, and transgender individuals (WHO, 2015). Immunization is the best efficient approach of avoiding Hepatitis B illness and its negative consequences on the liver health globally (Franco *et al.*, 2012). A significant problem of CHB continues since the universal coverage through the natal dosage remains very small, approximate at 39% globally in 2015. Due to inadequate universal natal dosage or additional active interventions, the spread of Hepatitis B illness vertically is still the foremost basis for long-lasting hepatic disease when exposed children grows up (WHO, 2017). This confirms the importance of HBV vaccination that confers lifelong protection against HBV and booster inoculations are not routinely commended (WHO, 2017).

The HBV prevalence was higher (20.0%) in the respondents who reported to have a history of liver problem. The results indicated a significant association (P=0.002) between HBV

prevalence and the history of liver problems. This concurs with similar previous (Ochwoto *et al.,* 2013; Ochwoto *et al*., 2016) clinical studies in Kenya that have directly correlated HBV with hepatic problems in which HBV/HIV dually infected individuals with HBV alterations that were associated with CHB and HCC in the clinic clients. Similarly, Mutuma *et al*., (2011) reported greater HBV carrier state dispositions among the males in liver clinic patients that directly correlated with high HCC incidences in the liver biopsies. This is dangerous in the sense that HBV infection is likely the cause most reported cases of liver disorders. Liver related deaths is the most frequent cause of non-AIDS-related mortality among HIV infected persons. Active HBV infection in HIV setting is one of the predictors of liver-related deaths, contributing to 16.9% of deaths in HIV patients (Sheng *et al.,* 2012; WHO, 2017). Dual infection with HBV complicates the clinical progression of HIV among infected individuals, and can as well adversely affect management of HIV septicity (Chandra *et al*., 2013). In one of earlier studies, Bréchot *et al*. (1981) found HBV virus in tumour cells of hepatocellular carcinoma patients. These authors therefore concluded that HBV is the causative agent of hepatocellular carcinoma. Development of hepatoma in HIV positive individuals further complicates HIV management in individuals. In the investigation of causes of mortality in HIV infected individuals, Bica *et al*. (2001), established that terminal liver disease was the prominent cause of decease among HIV positive patients. During this examination, 55% of the patients' mortality due to terminal hepatic dysfunction had either CD4+ T-lymphocyte counts or more than 200 cells/mm^3 or undetectable plasma HIV RNA levels with a year prior death, signifying that only liver disease could have led to their death. As a consequence, to universal increase in HAART initiation and coverage to more HBV endemic and poor regions, it is also paramount to

scale up the assessment of HBV influence in patients co-infected with HIV-HBV that are receiving antiretroviral therapy (Ndako *et al*., 2013). Besides this, only 2.8% of the respondents reported to have had liver problems, although none of the respondents was able to specify the nature of the liver problem. This further confirms that majority of the individuals may have low knowledge of underlying liver problems, predisposing them to late diagnosis especially in the settings of HAART regimens for HIV disease. Minorities (2.2%) of the respondents reported to have been transfused with blood in the past with zero HBV prevalence among those with the history of blood transfusion. This is similar to a previous study that established that blood transfusion was not a major risk predisposing to HBV infection (Nakwagala & Gakimu, 2002).

Two-thirds (65.0%) of the respondents were on first line HIV drug regimen, 11.7% were on second line HIV drug regimen while 23.3% were not on any HIV drug regimen. Although not significant, the HBV prevalence was found to be higher (4.8%) among the respondents on second line HIV drug regimen than among those on first line (1.8%). There was substantial relationship between the type of HIV drug regimen and the duration of respondents being on ARVs ($\chi 2$= 188.974, df=4, P = 0.001). Previous studies have described the impact of ART regimen in HIV patients over time with reciprocal effect on HBV replication. Hepatitis flare up cases have been described in HIV/HBV dual infected individuals that have low CD4 T-lymphocyte counts but experience immune reconstitution following initiation of HAART (McMahon, 2009). A long-term HBV/HIV cohort survey in Tanzania found similar results where the cohort had correlated low CD4 + counts and that Hepatitis B co-infection substantively had negative effect on therapy outcomes among the PLHIV. The explanation advanced for this was due to Hepatitis B septicity led to CD4

+ cell-mediated destruction via T-lymphocyte activation or splenic sequestration from progressive hepatic impairment. (Hawkins *et al.,* 2013). HIV infection leads to higher HBV viral loads, increased occult HBV infection and leads to higher likely hood of transmitting Hepatitis B virus to HIV patient's close contacts. The increased HBV replication due to profound immunosuppression and loss of immune response to Hepatitis B can result in reactivation of HBV, as well as exposure to novel Hepatitis B infections since persons infected with HIV clear immune antibody quantities more rapidly (Mayaphi *et al.,* 2012; Brook, 2006). Interestingly, no HBV was detected in HIV patients who were not on any ART regimen. This may be due to the fact that they had not been exposed to the long-term toxicity that may result in reactivation of occult HBV, or that they had stronger immunity that could clear HBV exposure as compared to individuals who had an already negative compounding effects of HAART.

Majority (43.3%) of the respondents had 251-500 CD4 counts, followed (41.1%) by respondents with 501 and above CD4 counts, and minority (15.6%) of the respondents had 250 and below CD4 counts. Among the respondents with 250 and below CD4 counts, a higher (20.8%) proportion was among female and youthful (17.6%) respondents. This is in contrast to a similar study that found males having lower mean CD4+ measures than in females and in elderly above 50 years and above (Wondimeneh *et al.,* 2013). The explanation for this difference in gender immune status was that individuals have unique immunity and viral hepatitis may adapt differently in individuals, with high dual HIV/HBV replication rates, thus impairing immune response in patients. The lower CD4+ counts in males associated men with being more muscular than females, thus engaging in more

manual activities daily with more resultant mental stress that could impact negatively on their immune status. However, in the current study lower CD4+ counts in females than males were probably related to more observed (84.8%) male and elderly (92.3%) respondents being on ART earlier and longer than in females (70.3%) and youthful (71.2%) respondents. The major explanation for this maybe that at the time of this study, being initiated on ART was based on CD4+ levels in a patient and hence more females and youths (18-35 years) may have been delayed to be initiated on ART owing to their naturally having better immune status as compared to the males and elderly in general. Various studies have documented that both HIV and HIV suppress immunity of patients. Hepatitis B -particular CD8+ T-lymphocyte reaction is diminished in individuals concurrently with HBV/HIV infection (Dao *et al*., 2011). Thus, if the clients were not being initiated on ART immediately, then it is expected their immunity would decline over time. Therefore, the current world-wide adopted approach that every HIV-infected person with Hepatitis B co-infection are recommended to be put on ART treatment regardless of CD4 T-lymphocyte count (EASL, 2017) is judicial to help all HIV and HBV clients at the earliest opportunity.

Although not substantive, slightly more (43.3%) respondents had moderate immunity (251-500 CD4 + counts) while higher proportion (6.9%) of HBV incidence was found among the respondents with low immunity (250 and below CD4 + counts). Furthermore, this study reveals higher (6.7%) HBV occurrence among the respondents who had been on ARVs for a shorter (0-3years) and lower (2.8%) among respondents who had used ARV for a longer duration (4 years and above). This can therefore mean that most patients are infected with HBV early before ART treatment begins and perhaps these treatments have been playing

an important role in clearing HBV virus from their system. This reasoning is supported by Ndako et al. (2013), where they demonstrated Hepatitis B viral clearance in response to antiretroviral therapy subsequent to twenty-week period of treatment in persons with HBeAg positivity. This is similar to a recent Ethiopian study in which majority (94%) of the participants were on ART for at least 6 months and concluded that with immune restitution following the initiation of HAAT, the respondents might have automatically eliminated HBsAg following convalesce from severe Hepatitis B and thus could be carriers of occult Hepatitis B septicity (Deressa *et al.,* 2017).

Generally, more (84.8%) male and elderly (92.3%) respondents were on ART than female (70.3%) and youthful. The HBV prevalence was highest (6.9%) among the respondents with 250 and below CD4+ counts. This study demonstrates that persons with HIV and HBV co-infection had lower CD4 T-lymphocyte tallies than those with HIV mono-infection. This is not the only study reporting such findings. A similar study has established that persons with Hepatitis B mono-infection had lower CD4 cell amounts as compared non-HBV infected patients (Chen *et al.,* 2006). Liver disease is an evolving significant cause of mortality in HIV/HBV dually-infected patients as other HIV-related illnesses have diminished following the universal initiation and enhanced access to antiretroviral treatment and management. HBV co-existence with HIV in individuals results to an aggravated progress to AIDS-related outcomes and all-lead to death (WHO, 2015). From Yang *et al*. (2014), management procedures for HBV-HIV concurrently infected individuals acclaimed that patients that require therapy for HBV be given ART in advance. Most probably the strict adherence to these guidelines is the reason behind this observation. Other previous studies in Tanzania and Ethiopia have demonstrated the HIV-HBV

reciprocal effect on immunological response. Hawkins *et al.*, (2013) reported that concurrent co-infections of HBV and HIV were related to lower CD4+ counts during immune restoration with an amplified hepatic toxicity rate after initiating the ART and greater risk of death. This study concluded that HBV co-infection had negative ART outcomes in HIV patients. This result concurs with earlier studies that hepatic disease is a major cause of morbidity and death in HIV patients (Soriano et al., 2008). Although this study had no comparison with HIV mono-infection clients, CD4+ cell counts had a lower mean value as compared to HIV infected persons only from a similar study in Ethiopia (Wondimeneh *et al*., 2013). A recent study in Canada has also presented similar concurring observation that patients with HIV and HBV dual septicity have less average baseline CD4+ cell measures (Rana *et al*., 2021). Similarly, Bisceglie *et al*., (2010) from South Africa observed that lower CD4+ count were directly related to HBeAg positivity. These results confirm that HBV infection negatively impacts on immune status, confounding higher risks of immune failure in already vulnerable HIV patients. As reported previously, loss of immunity due to HIV disease may cause HIBV septicity and recurrence leading to increased occurrence of Hepatitis B among PLHIV who had previous HBV remission (Makondo *et al*., 2012).

This study established the presence of HBV genotype A1 (60%) and D (40%) with sub-genotypes D6 and D7. This coincides with other previous surveys in Kenya (Mwangi *et al.,* 2008; Ochwoto *et al.,* 2013 and Kilongosi et al., 2016) that also established the presence of HBV genotypes A1 and D. Although the HBV genotype distribution pattern in Kenya is heterogeneous, similar to other documented earlier studies in Kenya, HBV geneotype

A1 was found to be predominant than HBV/D (Aluora *et al.*, 2020; Nyairo *et al*., 2016; Kilongosi *et al.*, 2015; Ochwoto *et al.,* 2013 and Mwangi *et al*., 2008). Other studies that mirror this from neighboring countries have shown that HBV/A is predominant in the region. Deressa *et al*., (2017) also reported HBV genotypes A and D with HBV/A being predominant in Ethiopia, with additional genotypes E, C, and G also reported. A recent HBV review survey in Tanzania has confirmed similar results with HBV/A1 being the most common at 86.1% and followed by HBV/D at 12.3% in the study cohort, in addition to HBV/E also observed (Kilonzo *et al.,* 2018). Forbi *et al., (*2017) also reported similar results with HBV/A1 being dominant at 86.9% followed by HBV/D in Tanzania. Similarly, Kafeero *et al., (*2022) has observed concurring results where they reported HBV/A1 (46%) and HBV/D4 with mixed recombinant D/E in Uganda. Equally, a survey in Malawi found HBV/A1 to be domineering and attested that HBV/A1 is the majority variant that is also the mostly reported in South Africa, Kenya and Zambia (Aoudjane *et al.,* 2014). A similar study in Sudan although in Liver disease patients, identified HBV/D6 confirming that D6 is geographically prevalent in the Maghreb and Madagascar (Yousif *et al.,* 2013). Studies have documented that HBV/D is more prevalent in Northern Africa, especially sub-genotypes D1 and D7 (Zampino *et al.,* 2015). The difference of prevalence of HBV genotypes confirms the possibility of increased prevalence of the HBV genotype A1 in Kenya and this is similar to other studies reported in Sub-Saharan Africa where HBV genotype A majorly belong to sub-genotype A1 (Kramvis & Kew, 2007). The increase in the prevalence of HBV/A1 across the region maybe due to increased migration and regional interaction of human populations while the evolution of HBV/D has been previously

documented as possibly due to intensive trade routes and regular travels from Eastern Africa (Zampino *et al.,* 2015).

Apart from this survey reporting HBV/A1 is the dominant variant, the occurrence of HBV/D6 and HBV/D7 seems to be new among the HIV populace since previous studies have not reported the same. In contrast to previous Kenyan studies that apart from reporting HBV/A1 is the most common genotype, they also have established other Hepatitis B genotypes A (sub-geneotypes A2), genotype D (sub-genotype D1, D3, D4 & D6) plus E in patients with liver-related problems, HIV positive clients and blood contributors (Nyairo *et al.,* 2016; Kilongosi *et al*., 2015; Kwange *et al.,* 2013; Ochwoto *et al*., 2013; Mwangi *et al*., 2008). This confirms that HBV is still evolving, especially due to the fact that the majority of respondents were on ART, hence predisposing HBV to higher probability of mutation as an adaptation strategy among HIV positive cohorts as reported previously (Adoga, 2012). The circulation of HBV genotypes A1 and D were more predominant in males and in the youth. This mirrors previous studies that have observed more HBV prevalence in males than in females (Niramon, L. & Wathanachai, S. 2010; Nakwagala & Gakimu 2002; Hawkins *et al.,* 2013; Choy *et al.,* 2019, and Spradling *et al*., 2009). This is in contrast to an earlier study which found HBV/A and HBV/D similarly distributed in all age groups (Biswas, *et al.,* 2009). The discovery of HBV/D7 further adds to genotypic pool of HBV in Kenya. An increased diversity of HBV genotypes in HIV patients in Kenya imply an increased HBV evolutionary pressure and transmission within populations considering that the study was within a cosmopolitan city with representative individuals from all over Kenya. Genotypic diversity may be due genomic recombination,

drifting of the virus genome through neutral evolution and continuous acclimatization of HBV to genomic determinants of particular host populations (Adoga, 2012).

The present survey presents 5 sequences of S regions of HBV. As expected from previous studies by others (Ochwoto *et al*., 2016; Nyairo *et al*., 2016 and Kilongosi *et al.,* 2015), these sequences were of genotype A and Genotype D. It seems then HBV genotype A1 and D are the major dominant in Kenya. The 5 isolates of genotypes of both A and D sequences showed very low genetic diversity as previously reported (Aluora *et al.,* 2020). These results therefore support earlier reports (Ochwoto *et al*., 2016) which showed that S gene isolate are not genetically diverse while Kilongosi *et al.,* (2015) found little HBV/A1 genetic variability and concluded that it could have due to recent introduction among HIV positive IDUs. The overall mean distance of 3 A1 sequences was 2.0% or 1.04 % when all S gene sequences from GenBank were included. This diversity is low considering that diversity index of $\geq 4.0\%$ is regarded as minimum threshold for new sub-genotypes (Yousif *et al*., 2014). Most of GenBank sequences earlier isolated from Kenya or elsewhere in Africa were highly related, for example, genetic distances between KU605535.1, KT347088.1 from South Africa, KT347088.1 from Kenya and KR139746.1 from Botswana were 0.00%. This indicates that all of them were actually of the same strain. They were identified as Genotype A sub-genotype A1. KP168430.1 and KP168433.1, both isolated in Kenya are of the same strain identified as A1. KP168417.1 and KP168418.1 both isolates from Kenya and identified as sub-genotype D7 are of the same strain as genetic distances between them was exactly 0.0%. This is evidence that most of the HBV isolates earlier isolated in Kenya are very similar genetically. This concurs with previous observations that HBV S gene is more conserved than other portions of HBV genome

(Croagh *et al.*, 2015) and that low genetic diversity suggests a short evolutionary history of HBV in the region (Zampino *et al.*, 2015). This is in contrary to a study in rural cohort that observed high diversity in HBV/A and D variants, indicating high emigration and immigration in the Southern Africa region. High mobility and interaction of populations with surrounding countries can result to greater risk of STIs including Hepatitis B, thus additionally increasing genomic variability of HBV in a region. The predominance of HBV/A1 in Eastern and Southern Africa coincides with an earlier proposition that it has been prevalent in African populations for lifelong (Makondo *et al.*, 2012).

Further analysis for genetic distances revealed the isolated HBV genotypes had little variability. This therefore further indicates that isolates in Kenya and their neighbouring Sudan are genetically similar. However, though small but these genetic distances are enough to indicate that organisms are mutating at a higher rate considering that the time of their collection in years was not much. Further evidence of diversity is indicated by phylogenetic tree clustering. Even most of the strains in the same cluster presented branches of unequal length showing that they were genetically different. Contrary to Ochwoto *et al*. (2016) study that found some strains having 0.0% genetic distances with other strains in the GenBank isolated from Tunisia, these did not exactly match the GenBank genotypes. This maybe as a result of different study setup, whereas former study isolated strains from patients showing jaundice who might not have ever been exposed to HBV drugs, this study isolated the strains from HIV patients most of who had already exposed to ARV drugs that have an effect on HBV (Ejele *et al.*, 2003) providing selection pressure for evolution. This is also supported by a previous study which noted that

immunosuppression in HIV clients may vary the evolution rate of HBV (Makondo *et al*., 2012). The establishment of sub-genotypes A1, D6 and D7 as HBV-HIV co-infection patients in Kenya greatly adds to other global studies that revealed that Hepatitis B genetic variants have discrete topographical and racial distributions with prevailing HBV/A and HBV/D (sub-genotypes A1-A6 and D1-D7 respectively) in India, Africa, and Europe (Adoga, 2012). Genotype A (sub-genotype A1) mainly predominates in Eastern, Central and Southern Africa while sub-genotype A3 is dominant in western Africa. Genotype D and E are found mostly in Northern Africa and Western and Central Africa respectively (Kramvis & Kew, 2007).

Considering that most (68.3%) HIV clients did not know there HBV history and status in this study, the observed HBV genotypes A and D may have serious implication among HIV patients since HBV genotypes A and D have been reported to have higher rates of HBV chronicity than genotypes B and C (Lin C. & Kao J., 2010). Besides this, in low income countries like Kenya where only 5.6% respondents confirmed to have HBV vaccination history and the fact all the HBV positive results (2.8%) were among those who did not have HBV vaccination history in this study, acute infection with HBV/A may substantially increase redistribution of HBV variants among individuals with existing chronic HBV. As reported elsewhere, the prevailing CHB in a population after acute HBV may be attributable to vaccination status, host-viral factors, routes of transmission and various levels of HBV variant distribution in a topographical location (Lin C. & Kao J., 2010). Hence, HBV genotyping may enhance the identification of patents with a greater risk of disease progression and establish optimum ART management regimens. HBeAg

seroconversion and HBsAg seroclearance are known key events whereby early HBeAg seroconversion confers a favorable HBV disease outcome and this too is also affected by genotypes. In a prospective Spanish study, it was observed that HBV genotype A had higher rate of sustained seroconversion than patients with HBV/D. Similarly, genotypes A and B had greater rates of spontaneous HBsAg seroclearance than genotypes D/C (Lin C. & Kao J., 2010). Likewise, the same study also observed that HBV/D was associated with higher progressive hepatic disease and more frequencies of A1762T/G1764A base-core promotor (BCP) mutants than in HBV/A. Dual infection with HBV complicates the clinical progression of HIV among infected individuals, and can as well adversely affect management of HIV disease (Chandra *et al*., 2013). Since HBeAg seroconversion phenotype varies between HBV/A and D, it may be interesting to have further studies in Kenyan PLHIV population since genotypes A and D have become dominant.

This study established that all HBV genotypes (A1, D6 and D7) were susceptible to drug mutations with most common transformations containing exchange of methionine for valine or isoleucine rtM204V/I comparable to earlier surveys in Kenya that have reported 3TC drug mutants (Aluora et al., 2020; Jepkemei et al., 2020, Ocwhwoto *et al*., 2013 and Kim *et al.*, 2011). This is also comparable to a French study where more than half of the respondents had 3TC drug resistance in HBV/HIV populace and the major drug alteration reported. The same survey also established that rtM180L occurred in 87.5% incidences linked to rtM204V or V (Thibault *et al*., 2013). Similarly, another survey also reported substantively more HBV Lamivudine therapy resistant mutations at rtL180M, rtM204V/I and rtV173L positions in HBV/HIV co-infected individuals unlike among persons with

Hepatitis B infection alone (Belyhun *et al*., 2017). A long-term Thailand survey also reported almost all patients had 3TC resistance involving rtM204I/V along with rtL180M (Khamduang *et al.,* 2012). In the same breath, an Ethiopian survey among HIV/HBV clients reported more than half the clients had rtV173L, rtL180M, and rtM204V drug resistance related to 3TC (Deressa *et al.,* 2017). An earlier study in Malawi in similar population found M204I as a universal occurrence in patients and the same had diminished susceptibility to other nucleotides as well (emtricitabine, telbivudine, and entecavir), confirming the prevalence of the M204I as the most prevalent mutation for 3TC (Aoudjane *et al*., 2014). In this study, all the isolates were found in respondents who were on ART and majority had been on therapy for a long duration (4 years and above). Hence, the identification of M204I only unlike the other studies that had other 3TC mutants most likely because the participants had not been exposed to ART. This is supported by Aoudjane *et al.,* (2014) study that found M204I Lamivudine resistance evolved fast within a short period after 6 months in all respondents with high HBV DNA proportions, followed by other more resistance drug mutants. Khamduang *et al.,* (2012) also found similar results and reported that 3TC evolution happens at a degree of 15–20%/year, while their study found the same at 3%, comparable to Kim *et al.,* (2011) that had 7%. The result of this study are similar to an earlier survey in Kenya which also found 3TC resistance among the subjects and recommended that long term follow up observations among HIV/HBV dually infected subjects with increased HBV screening strategies are urgently needed to better understand the ART outcomes (Day *et al*., 2013).

The outcome of this research also established 3 drug resistance alterations at HBV S gene (M250W and Y252V for HBV/A1 while M250L for HBV/D6) unlike in the similar studies among blood donors and patients presenting with liver problems in Kenya: Aluora *et al.*, (2020) recently found several alterations (S109P, T114P, N122P, N122H,Y126H, M129L, M133T and T143M): Jepkemei *et al.*, (2020) has recently reported T116N, T118A and D144E alterations; Nyairo *et al*., (2016) found mutations T143M and M133I; Kwange *et al.*, (2013) reported F20S, Y200F, and P203R while Ochwoto *et al*., (2013) found A1762T/G1764A and G1896A all of which were majorly reported to carry immune, diagnostic and vaccine escape mutants. The differences in the mutants is mainly thought to be due to HBV population and genetic-based variability. Similar to the study by Aluora *et al*., (2020), it is possible the increase in HBV mutants prevalent to the epitopes of B-cell and T-cell alternations could have occurred in the host populations or could have been transmitted with already mutated forms HBV.

This study identified several mutations from all the isolates. However, these mutations might not have occurred as a result of treatment because they had been identified in untreated patients previously. Previous studies have reported that most frequent drug mutations encompass replacement of methionine for valine or isoleucine rtM204V/I. In a Kenyan survey by Kim *et al*., (2011), three subjects had pre-treatment mutations correlated to diminished in vitro predisposition to adefovir (rtI233V, rtA181S) and lamivudine (rtV207I). Six of the 12 RT mutations in isolate HBV/D6 had already been isolated in untreated patients and therefore might not have resulted from drug exposure. Four of them, C6P, E8P, H9G and W243T have not been documented before. At position 10 of the S

gene in isolate HBV/A1, a mutation led to the introduction of a stop codon TGA that causes a premature, non-functional HBeAg. This study established a mutation that had previously led to the resistance in individuals managed with an amalgamation of L-Nuc., ETV and ANPs in HBV/D6. This mutation involved the substitution of methionine with leucine at position 250 of the S gene. This is similar to another study that reported that major significant alterations in the HBsAg gene are amino acid exchanges at positions 145,141, and 131 in the main ‘‘a’’ determinant and inserts among amino acids 122 and 123 (Cabezas-Fernandez & Cabeza-Barrera, 2012).This results also mirror a previous survey in Kenya where 37.9% alterations were found in the 124-147 range of “a” determinant (Nyairo *et al.,* 2016). Substitution of amino acid tryptophan with glutamic acid at position 257 in isolate P9 had not been documented before and therefore its effect on drug resistance remains unknown. Positions 250 and 252 in HBV S gene are highly conserved and it is understood that mutations on these bases would likely result in drug resistance (Margeridon-Thermet & Shafer, 2010). Isolate HBV/A1 had a substitution of methionine with tryptophan at position 250 while tyrosine was substituted with valine at position 252. Substitution of methionine with either leucine, isoleucine or valine in position 250 had been observed earlier (Fu *et al.,* 2016) and have all been associated with drug resistance in patients. However, substitution of methionine with tryptophan had not been observed before. Another novel mutation observed in this study is substitution of tyrosine with valine at position 252. Methionine and tryptophan and tyrosine and valine have different physicochemical properties (Betts & Russell, 2003).

This study concurs with several local studies that have affirmed increased HBV drug mutations especially in HIV HAART context. As reported elsewhere (Downs *et al.,* 2023), there are no vigorous HBV screening interventions in Kenya and vaccination is mainly limited to postnatal clinics, thus predisposing majority of HBV active cases to continuous cyclic transmissions in risky populations like the current HIV positive study cohort. The implications for this are serious since majority of individuals remain unaware of their HBV status and are most likely to find out until during clinical manifestations of hepatic complications in hospitals. Apart from HBV variants A1 and D6 that has been previously documented in Kenya, the establishment of HBV/D7 is new among the study cohort and this augments the affirmation that HBV evolution is mutating a high rate. HBV RT-polymerase is devoid of proofreading actions and this result to regular alterations in the genetic make-up of the virus. It leads to coexistence of viral quasi species that are hereditarily unique viral species in infected persons that emerge dependent on the host environmental pressure. The interaction amongst the virus, hepatocyte, protective reaction and ART is believed to drive the evolution of HBV genetic variants with the potential to escape host immunological reactions or resistance to ART (EASL, 2017).

The continuous use of 3TC as a combination therapy with tenofovir and emtricitabine in the HIV positive ART regimen consistently may continue to contribute to the wide 3TC drug resistance commonly reported in other studies in Kenya (Downs *et al.,* 2023). Presently, the 5 NAs analogs for medical uses anti-hepatitis B management comprise of lamivudine, telbivudine, adefovir, entecavir, and tenofovir. Studies have established that every nucleoside analog has most common drug-resistant sites. Alterations in these sites

lead to the emergence of clinical drug resistance (Tipples et al., 1996; Allen at al., 1998; Suppiah et al., 2014). The ultimate management goal of chronic hepatitis B is to prevent HBV replication and achieve sustained viral clearance as much as possible (Fu et al., 2016). Even though nucleos(t)ide analogs perform a particular function to HBV management, prolonged use of NAs analogs as ART might lead to Hepatitis B drug-resistant strains and decrease anti-viral efficiency, hence triggering disease recurrence (Stein & Loomba, 2009). Therefore, exploring the HBV drug-resistant mechanism is significant to Hepatitis B management among the HIV positive cohort that are already face a lifetime risks to ART hepatoxicity. HIV infection leads to higher HBV viral loads, increased occult HBV infection and leads to higher likely hood of transmitting Hepatitis B virus to HIV patient's close contacts. The increased HBV replication due to profound immunosuppression and loss of immunity reaction to HBV may cause reactivation of HBV, as well as exposure to novel Hepatitis B infections since persons' infected with HIV clear immune antibody quantities more rapidly (Mayaphi *et al*.,2012; Brook, 2006). Early initiation of combined treatment with emtricitabine/lamivudine and tenofovir, is commended treatment for HBV-HIV dually infected individuals in order to prevent HBV drug resistance, especially to Lamivudine, that has a small resistance barrier (Mayaphi *et al*.,2012; WHO, 2010). Every HIV-infected person with Hepatitis B co-infection is recommended to be put on ART treatment regardless of CD4 T-lymphocyte count (EASL, 2017).

5.2 Conclusions

The difference in HBV prevalence by ELISA (5%) and by PCR (2.8%) means that population-based HBV screening studies by serological markers may tend to establish higher HBV prevalence than by molecular screening.

Drug injection, heavy alcohol consumption, low use of protection during sexual intercourse and increased frequency of sexually transmitted infections were found to be direct risk factors for increased HBV infection

There was low HBV testing among the respondents and thus majority of HIV populations are ignorant of their HBV status and risk factors and thus majority of perinatal HBV transmission and horizontal HBV acquisition cases could be perpetuated by ignorance.

Majority of the individuals may have low knowledge of underlying liver problems, predisposing them to late diagnosis especially in the settings of HAART regimens for HBV/HIV disease contexts.

The establishment of Hepatitis B sub-genotypes A1, D6 and D7 in HBV-HIV co-infected patients greatly augments other local and global studies that have reported that Hepatitis B genotypic variants have distinctive topographical and ethnic circulations with genotypes HBV/A and HBV/D with subtypes A1-A6 and D1-D7 respectively dominating in Africa.

An increased diversity of HBV genotypes in HIV patients imply an increased HBV evolutionary pressure and transmission within populations considering that the study was within a cosmopolitan city with representative individuals from all over Kenya.

This study isolated the strains from HIV patients most of who had already been exposed to ARV drugs that have an effect on HBV providing selection pressure for evolution.

Considering that most (68.3%) HIV clients did not know there HBV history and status in this study, the observed HBV genotypes A and D may have serious implication among HIV patients since HBV genotypes A and D have been reported to have higher rates of HBV chronicity than genotypes B and C.

All HBV genotypes (A1, D6 and D7) were susceptible to drug mutations with most common transformations containing substitution of methionine for valine or isoleucine rtM204V/I..

The HIV co-infected patients with HBV were not aware of their co-infection status, thus were only on HIV therapy. This lack of HBV co-management in HIV/HBV co-infected persons is likely to lead to the increased evolution and distribution of HBV mutants of interest to public health management.

Limitation of the study

This study was limited in that it did not screen for other serological, biochemical and virological panels of HBV infection and there was no control study populations in order to help elucidate various comparative observations. The final samples for molecular analysis were few (n=5) and this limited the amount of information that could be elicited on the same as compared to other studies that had larger genomic samples. The socio-demographic data was collected through a questionnaire; hence it is likely to have had some memory prejudices. The study also took long to be completed due to limited financial

resources and thus the HBV individuals found to have been positive there were no interventions to reach them.

5.3 Recommendations

5.3.1 Recommendations from the Study

There is need for enhanced preventive HBV awareness creation and increased access to routine HBV screening, molecular studies and vaccination among the HIV infected population.

A thorough comprehension on how socioeconomic status impact on HBV health outcomes will certainly enhance the innovation of better policies and interventions to diminish socioeconomic gaps on the burden of liver disease among the HIV patients.

Only 5.6% of respondents reported to have had HBV vaccination and all the HBV positive results (2.8%) were among those who did not have HBV vaccination history. This coupled with an aging HIV positive population that is already immune-compromised, it is critical to increase HBV vaccination coverage among the adult HIV population.

Among those found to be HBV positive, there is need for enhanced ART treatment adherence and support for effective HBV treatment outcomes.

As a consequence, to universal increase in HAART initiation and coverage to more HBV endemic and poor regions, it is also paramount to scale up the assessment of HBV influence in patients co-infected with HIV-HBV that are receiving antiretroviral therapy.

There should be intervention programs that enable researchers to follow up and link HBV positive individuals for enhances management of their HBV status.

5.3.2 Recommendations for further research

1. Design prospective evaluations that will intentionally correlate the significance of socioeconomic status and healthcare access, quality and outcomes in HBV/HIV dual infections
2. Four RT mutations C6P, E8P, H9G and W243T in HBV/D6 and substitution of amino acid tryptophan with glutamic acid at position 257 in HBV/A1 had not been documented before and therefore further research to determine if these amino acids can play the same roles in the resultant polypeptide and the effect on the drugs that target them.
3. More surveys need to be carried out determine exactly the causes of higher HBV prevalence in males than in females.
4. Since HBeAg seroconversion phenotype varies between HBV/A and D, it may be interesting to have further studies in Kenyan PLHIV population since genotypes A and D have become dominant.
5. Substitutions of methionine with tryptophan at position 250 and substitution of tyrosine with valine at position 252 were observed in HBV/A1. Both substitutions are known to have different physicochemical properties and the same need further investigations in local HBV/HIV co-infected population.

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APPENDICES

Appendix I: Consent Form

Title: Molecular Diversity of Hepatitis B Virus in HIV infected Patients at Mbagathi District Hospital, Nairobi

INSTITUTIONS: Kenyatta University, KEMRI and Mbagathi District Hospital, Nairobi

Principle Supervisor: Dr. Washington Arodi, Kenyatta University, Kenya

Co-Principle Supervisor: Dr. Marion Warigia, Kenyatta University, Kenya

Researcher: Samuel Barasa Khaemba, Kenyatta University, Kenya

Investigator's Statement

I am Samuel Khaemba, a postgraduate student at Kenyatta University undertaking a research as part of my requirement of the degree of Master of Science in Infectious Diseases.

Purpose of the Study

This study is aimed at determining the extent and genotypes of HBV infection in HIV-positive people seeking CD4/ CD8 T-Lymphocyte testing at Mbagathi District Hospital.

Procedure

If you consent, the sample that you / your patient give for CD4 test will be used for testing the presence of Hepatitis B virus in your blood. You or your patient will not need to give any additional blood sample apart from the one you / your patient will already have given for the CD4+ test.

Questionnaire

You will also be given a written up questionnaire to assist in gathering socio-epidemiological information on Hepatitis B infection.

Assurance of Confidentiality

Your blood sample and the questionnaire will be given a unique code to conceal your / your patient's identity. The test results and information given will be treated confidentially and your name shall not be mentioned anywhere in our reports. Additionally, you will be asked whether you would like to get the results of the HBV test. Depending on the answer you give you shall / shall not be given the test result.

Basis of participation

You are free to accept or refuse the use of your/ your patient's blood sample for HBV testing. Your choice to consent or not to consent to this study will not affect the CD4 testing services you seek from this hospital.

Benefits of participation in the study

The tests carried out for HBV will enable you to know whether you/ your patient is infected with this virus. You can seek appropriate treatment without delay. This study will also help us to know whether Hepatitis B Virus and HIV co-infections rates are high and the HBV genotypes in HIV positive persons. If this is the case, then the information may help in designing appropriate interventions that involve the management of HIV and HBV infections in Kenya. Otherwise, there will be no other additional benefits to you.

Risks

As you give the blood sample for CD4+ T-lymphocyte count, you will be pricked and you will feel pain. There are no other risks associated with this study to you.

Obtaining additional information

You are free to seek clarity or ask any question. If you desire to get more information concerning the study, you are free to contact the principal investigator or the co-principal investigator.

Subject Statement and Signature

I have read the information stated above and have the opportunity to ask any questions regarding the above mentioned study. I have therefore consented for my sample/ sample of my patient to be tested for Hepatitis B Virus.

I wish to be informed of Hepatitis B Virus test results: Yes ( ) No ( )

Signature of the participant …….Date…….

Appendix II: Questionnaire to determine Molecular Diversity of Hepatitis B Virus in HIV Infected Patients at Mbagathi District Hospital, Nairobi

Questionnaire code no. ---------- Date of Interview----------

A. SOCIAL DEMOGRAPHICS

1. Gender : Male ( ) Female ( )
2. Age (Years) :18-27 ( ) 28-37 ( ) 38-47 ( ) 48-57 ( ) Above 58 ( )
3. Marital status : Single ( ) Married ( ) Separated/ divorced ( ) Widowed ( )
4. Occupation : ----------
5. Residence : ----------
6. Nationality : ----------
7. Highest education attained: Primary ( ) Secondary ( ) College ( ) None ( )

B. EPIDEMIOLOGICAL INFORMATION

8. Have you been tested for Hepatitis B virus before? Yes ( ) No ( )
9. If yes, what were the results? Positive ( ) Negative ( )
10. If the results were positive, did you receive any treatment? Yes ( ) No ( )
11. If yes, did you complete the treatment? Yes ( ) No ( )
12. Have you been vaccinated against HBV? Yes ( ) No ( )
13. If yes, which did you receive the vaccination? **……**
14. Has any of your family member suffered from Hepatitis B Virus infection?

    Yes ( ) No ( ) Don't know ( )

15. Have you been diagnosed with any liver problem? Yes ( ) No ( )
16. If yes, which liver problem have you been diagnosed with? ----------
17. How many sexual partners do you have? 1 ( ) 2 ( ) more than 2 ( )
18. What type of sex do you practice? Homosexual ( ) Heterosexual ( )
19. What type are your sex partners? Regular ( ) Commercial ( )

20. Do you use any protection measure during sexual intercourse? Yes ( ) No ( )

21. If yes, which ones? **…….**

22. How often do you use protective measure? Always ( ) sometimes ( ) Never ( )

23. If no, why? ----------

24. Do you use drug injection? Yes ( ) No ( )

25. Do you use alcohol heavily? Yes ( ) No ( )

26. Have you suffered from any STDs? Yes ( ) No ( )

27. If yes, which one? ----------

28. Have you ever donated blood? Yes ( ) No ( )

29. Have you ever been transfused with blood? Yes ( ) No ( )

30. If yes, which year was blood transfusion take place? **…….**

31. When first did you test HIV positive? ----------

32. Are you on ARV treatment for HIV infection? Yes ( ) No ( )

33. Which drug regimen are you on? First line ( ) Second line ( ) Third line ( )

34. If yes, how long have you been on ARV treatment? Less than 1 year ( )

1-2 years ( ) 3-4 years ( ) 5-6 years ( ) 7 years and above ( )

Thank you.

Appendix III: Protocol Materials used in the study

3. Add 50 µL of the HRP- HBsAb conjugates to each well, except the blank well.
4. Gently rock the wells for twenty second, then cover the wells.
5. Incubate the wells at 37℃ for to 60 minutes.
6. Carefully remove the incubation mixture by empting the solution into a waste container. Fill each well with diluted wash buffer and shake gently for 20-30 second. Discard the wash solution completely and tapping the plate on absorbent paper. Repeat above procedure 4 more times.
7. Add 50 µL (or 1 drop) of TMB substrate A and 50 µL (or 1 drop) of TMB substrate B into each well including the blank well.
8. Incubate at 37℃ in dark for 15 minutes.
9. Stop the reaction by adding 50 µL (1 drop) of stop buffer to each well. Gently mix for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
10. Set the microplate reader wavelength at 450 nm and measure the absorbance (OD) of each well against the blank well within 15 minutes after adding Stop Solution. A filter of 620 –690 nm can be used as a reference wavelength to optimize the assay result.

INTERPRETATION OF RESULTS

A. Set up the cut-off value

The cutoff value = N x 2.1

N: Mean OD of the negative control. Use 0.05 for calculation of the cut-off value if the mean OD is less than 0.05.

B. Calculation of specimen OD ratio

Calculate an OD ratio for each specimen by dividing its OD value by the Cut-off Value as follows:

$$\text{Specimen OD ratio} = \frac{\text{Specimen OD}}{\text{Cut-off Value}}$$

C. Assay validation

The mean OD value of the HBsAg positive controls should be ≥ 0.50.
The mean OD value of the HBsAg negative controls should be ≤ 0.10.

Check the procedure and repeat assay if above conditions are not met.

D. Interpretation of the results

Specimen OD ratio

Negative	< 1.00
Positive	≥ 1.00

1. The negative result indicates that there is no detectable HBsAg in the specimen.
2. Results just below the cut-off value (Lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to retest in duplicate the corresponding specimens when it is applicable).
3. Specimens with cut-off ≥ 1.00 are initially considered to be positive by the HBsAg ELISA Test. They should be retested in duplicate before final interpretation.

If after re-testing of a specimen, the absorbance value of the 2 duplicates are less than the cut-off value, the initial result is non repeatable and the specimen is considered to be negative with the HBsAg ELISA Test.

Non repeatable reactions are often caused by:

- Inadequate microwell washing,
- Contamination of negative specimens by serum or plasma with a high antibody titer,
- Contamination of the substrate solution by oxidizing agents (bleach, metal ions, etc.)
- Contamination of the stopping solution

If after retesting the absorbance of one of the duplicates is equal or greater than the cut-off value, the initial result is repeatable and the specimen is considered to be positive with the HBsAg ELISA Test, subject to the limitation of the procedure, described below.

PERFORMANCE CHARACTERISTICS

Clinical Performance

A total of 300 specimens from susceptible subjects were tested by a Chinese State Drug Administration (SDA) licensed reference EIA.. Comparison for all subjects is showed in the following table:

	HBsAg ELISA Test		
Ref. HBsAg EIA	**Positive**	**Negative**	**Total**
Positive	53	0	53
Negative	0	247	247
Total	53	247	300

Relative Sensitivity: 100%, Relative Specificity: 100%, Overall Agreement:100%

LIMITATION OF THE TEST

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of HBsAg in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The HBsAg ELISA Test is limited to the qualitative detection of HBsAg at a sensitivity level of 0. 2 ng/mL in human serum or plasma. The intensity of color does not have linear correlation with the antigen titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable HBsAg. However, a negative test result does not preclude the possibility of exposure to or infection with HBV.
4. A negative result can occur if the quantity of HBsAg present in the specimen is below the detection limits of the assay (below 0.2 ng/mL), or the HBsAg that are detected are not present during the stage of disease in which a specimen is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

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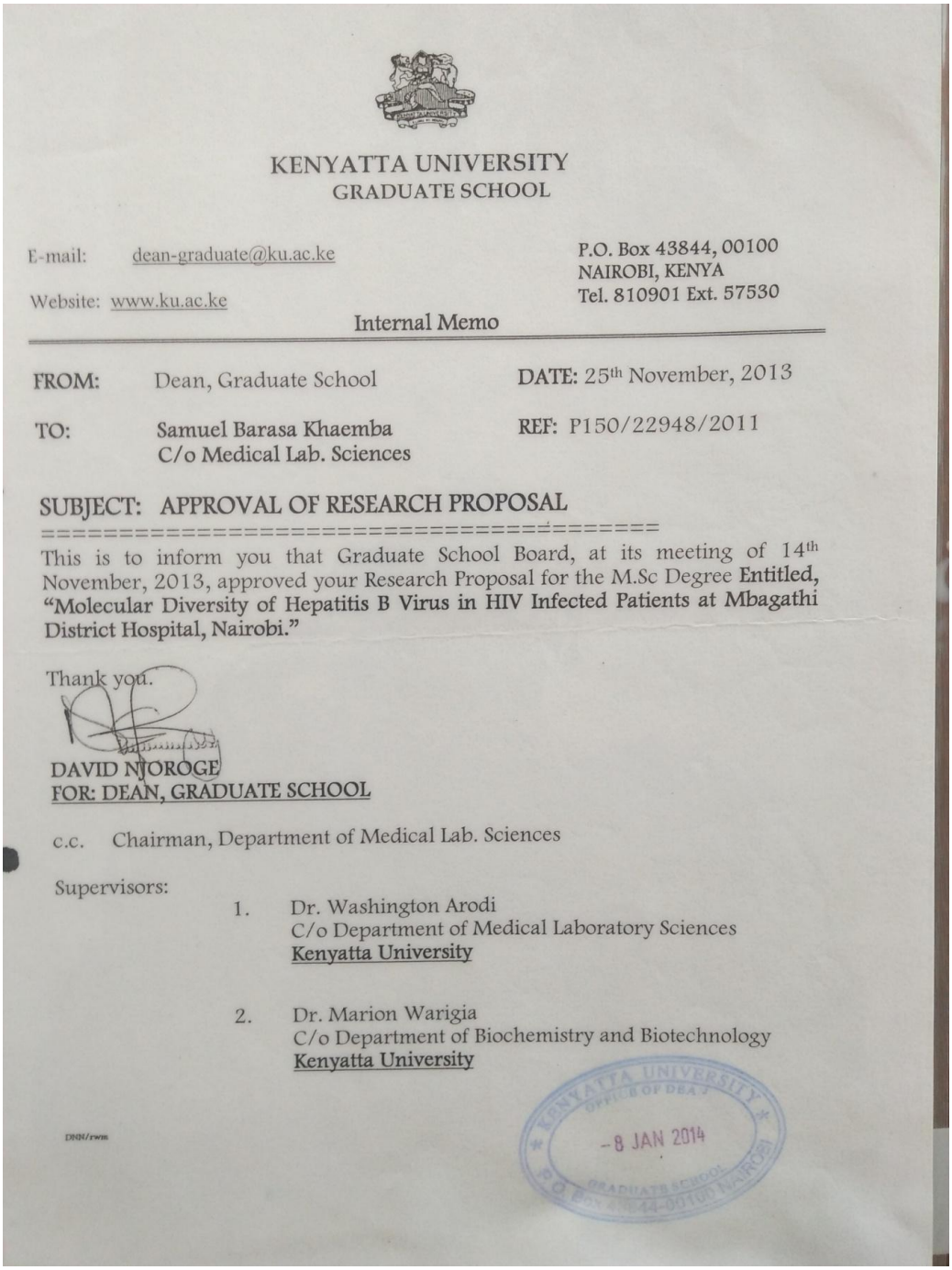
10110 Mesa Rim Road
San Diego, CA 92121,USA
Tel: 858-457-8698
Fax: 858-535-1739,
E-mail: info@ctkbiotech.com

PI-E0710. Rev. E.
Effective date: 2011-05-10

Index of Symbols

	consult see instructions for use
IVD	For *in vitro* diagnostic use only
REF	Catalog #
LOT	Lot Number
	Use by
N	Tests per kit
2-8°C	Store between 2-8°C
	Do not reuse
	Manufacturer
	Date of manufacture

Appendix IV: Kenyatta University Research Proposal Approval



KENYATTA UNIVERSITY
GRADUATE SCHOOL

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Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School

DATE: 25th November, 2013

TO: Samuel Barasa Khaemba
C/o Medical Lab. Sciences

REF: P150/22948/2011

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting of 14th November, 2013, approved your Research Proposal for the M.Sc Degree **Entitled, "Molecular Diversity of Hepatitis B Virus in HIV Infected Patients at Mbagathi District Hospital, Nairobi."**

Thank you.

DAVID NJOROGE
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Department of Medical Lab. Sciences

Supervisors:

1. Dr. Washington Arodi
   C/o Department of Medical Laboratory Sciences
   Kenyatta University

2. Dr. Marion Warigia
   C/o Department of Biochemistry and Biotechnology
   Kenyatta University

DNJ/rwm

KENYATTA UNIVERSITY OFFICE OF DEAN GRADUATE SCHOOL P.O. Box 43844-00100 NAIROBI
-8 JAN 2014

Appendix V: The Ethical Research Authorization

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

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

P. O. Box 43844
Nairobi, 00100
Tel: 8710901/12
Tel: 8710901/12

Our Ref: KU/R/COMM/51/292

Date: 28th February, 2014

Samuel Barasa Khaemba,
Department of Medical Laboratory Sciences,
Kenyatta University,
P.o Box 43844

RE: APPLICATION NUMBER PKU/180/I 157 – "MOLECULAR DIVERSITY OF HEPATITIS B VIRUS IN HIV INFECTED PATIENTS AT MBAGATHI DISTRICT HOSPITAL, NAIROBI." - Version2

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic "Molecular diversity of hepatitis B virus in HIV infected patients at Mbagathi district hospital, Nairobi" dated 27th February, 2014.

2. DECISION

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines **AND APPROVED that the research may proceed for a period of ONE year from 28th February, 2014.**

3. ADVICE/CONDITIONS

i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.
ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.
iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above

KENYATTA UNIVERSITY OFFICE OF THE CHAIRMAN 28 FEB 2014 ETHICS REVIEW COMMITTEE P.O. BOX 43844 - 00100 NAIROBI

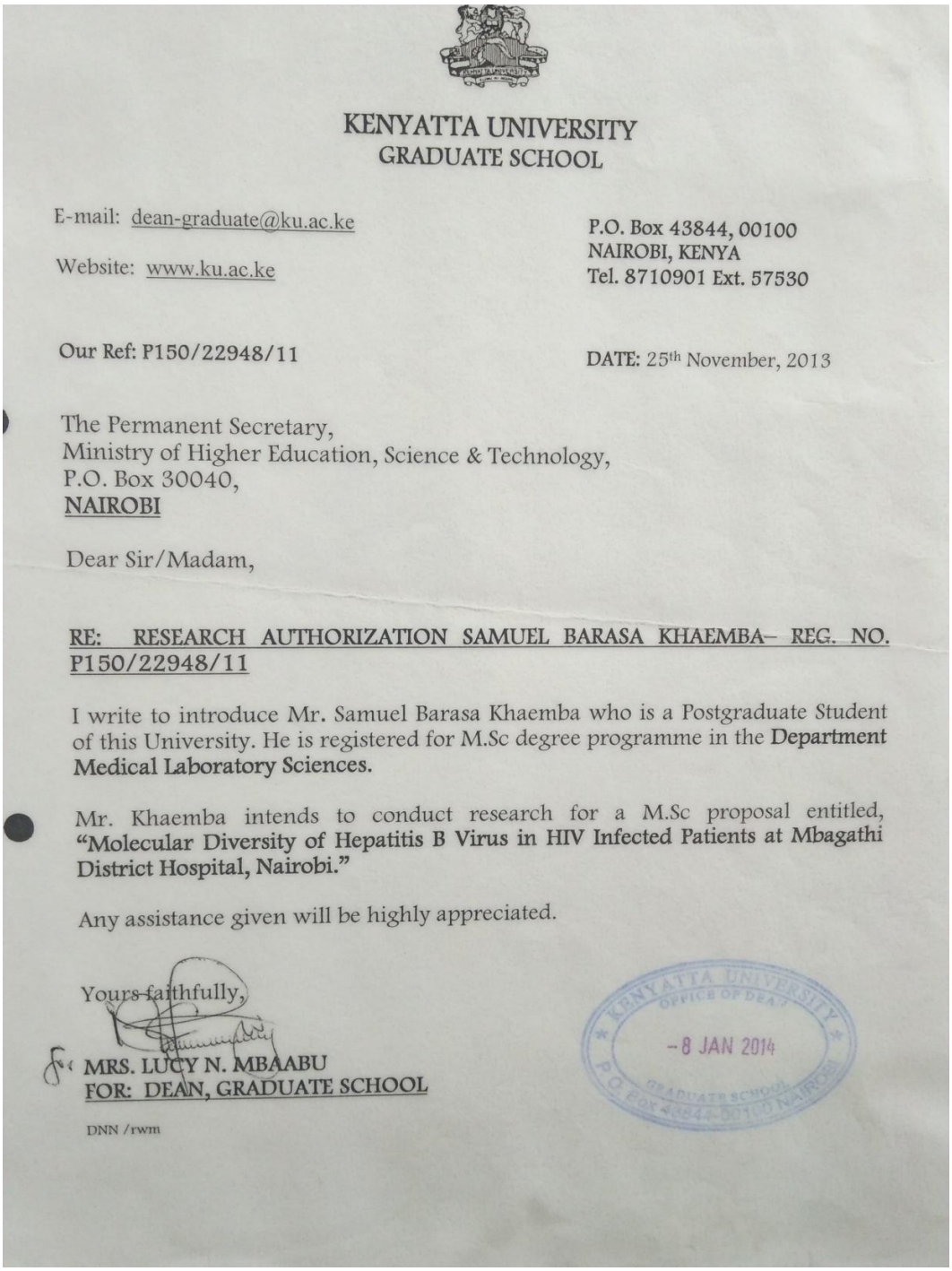
PROF. NICHOLAS K. GIKONYO
CHAIRMAN ETHICS REVIEW COMMITTEE

I Samuel Barasa Khaemba accept the advice given and will fulfill the conditions therein.

Signature..... Dated this day of 28th February, 2014.

cc. Vice-Chancellor
Director: Institute for Research Science and Technology

Appendix VI: Kenyatta University Research Authorization Introduction



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Our Ref: P150/22948/11

DATE: 25th November, 2013

The Permanent Secretary,
Ministry of Higher Education, Science & Technology,
P.O. Box 30040,
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION SAMUEL BARASA KHAEMBA– REG. NO. P150/22948/11

I write to introduce Mr. Samuel Barasa Khaemba who is a Postgraduate Student of this University. He is registered for M.Sc degree programme in the **Department Medical Laboratory Sciences.**

Mr. Khaemba intends to conduct research for a M.Sc proposal entitled, **"Molecular Diversity of Hepatitis B Virus in HIV Infected Patients at Mbagathi District Hospital, Nairobi."**

Any assistance given will be highly appreciated.

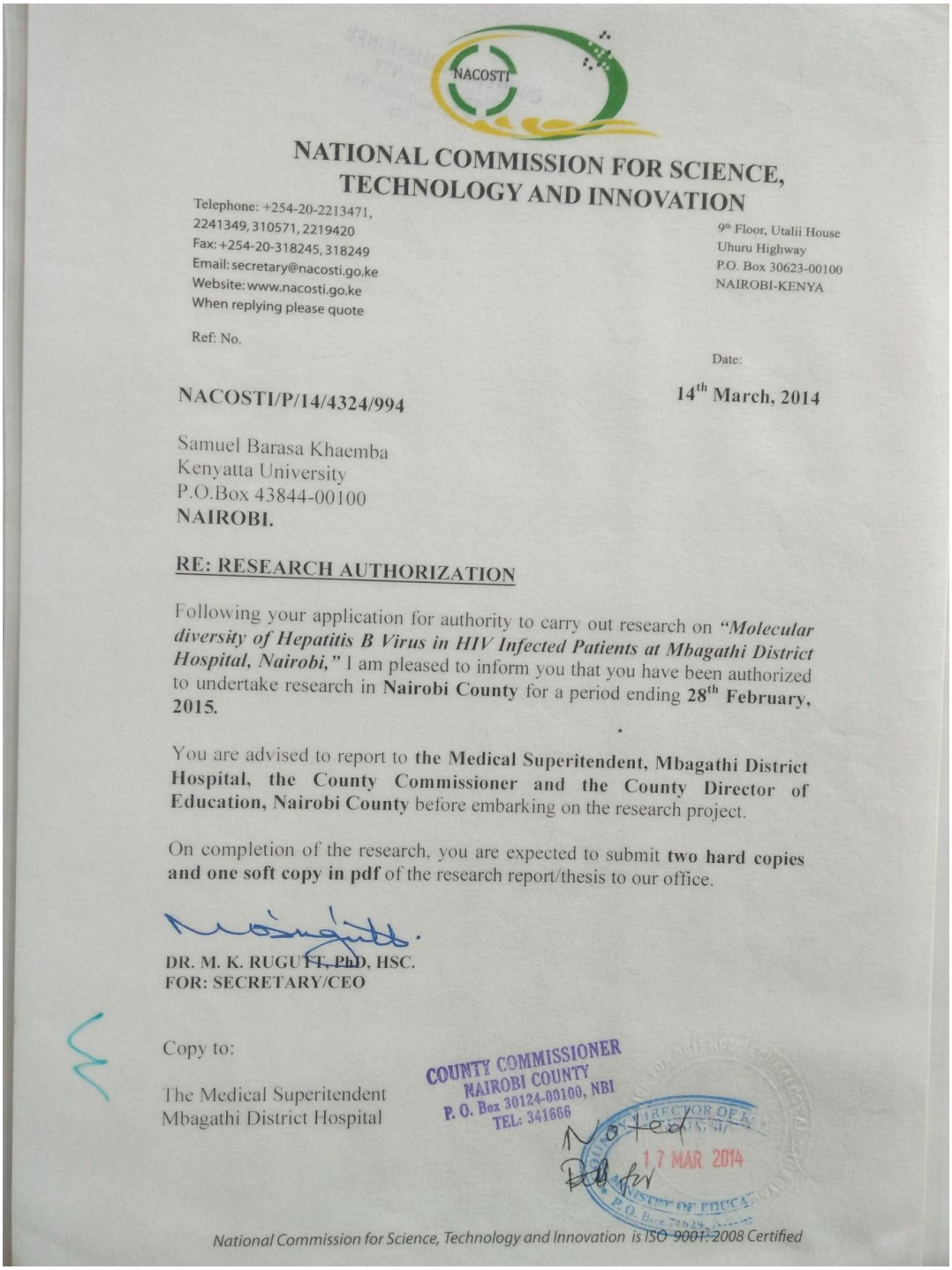
Yours faithfully,

MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

DNN /rwm

KENYATTA UNIVERSITY
OFFICE OF DEAN
- 8 JAN 2014
GRADUATE SCHOOL
P.O. Box 43844-00100 NAIROBI

Appendix VII: NACOSTI Research Authorization



NACOSTI

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

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NAIROBI-KENYA

Ref: No.

Date:

NACOSTI/P/14/4324/994

14th March, 2014

Samuel Barasa Khaemba
Kenyatta University
P.O.Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Molecular diversity of Hepatitis B Virus in HIV Infected Patients at Mbagathi District Hospital, Nairobi,"*** I am pleased to inform you that you have been authorized to undertake research in **Nairobi County** for a period ending **28th February, 2015.**

You are advised to report to **the Medical Superitendent, Mbagathi District Hospital, the County Commissioner and the County Director of Education, Nairobi County** before embarking on the research project.

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

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

Copy to:

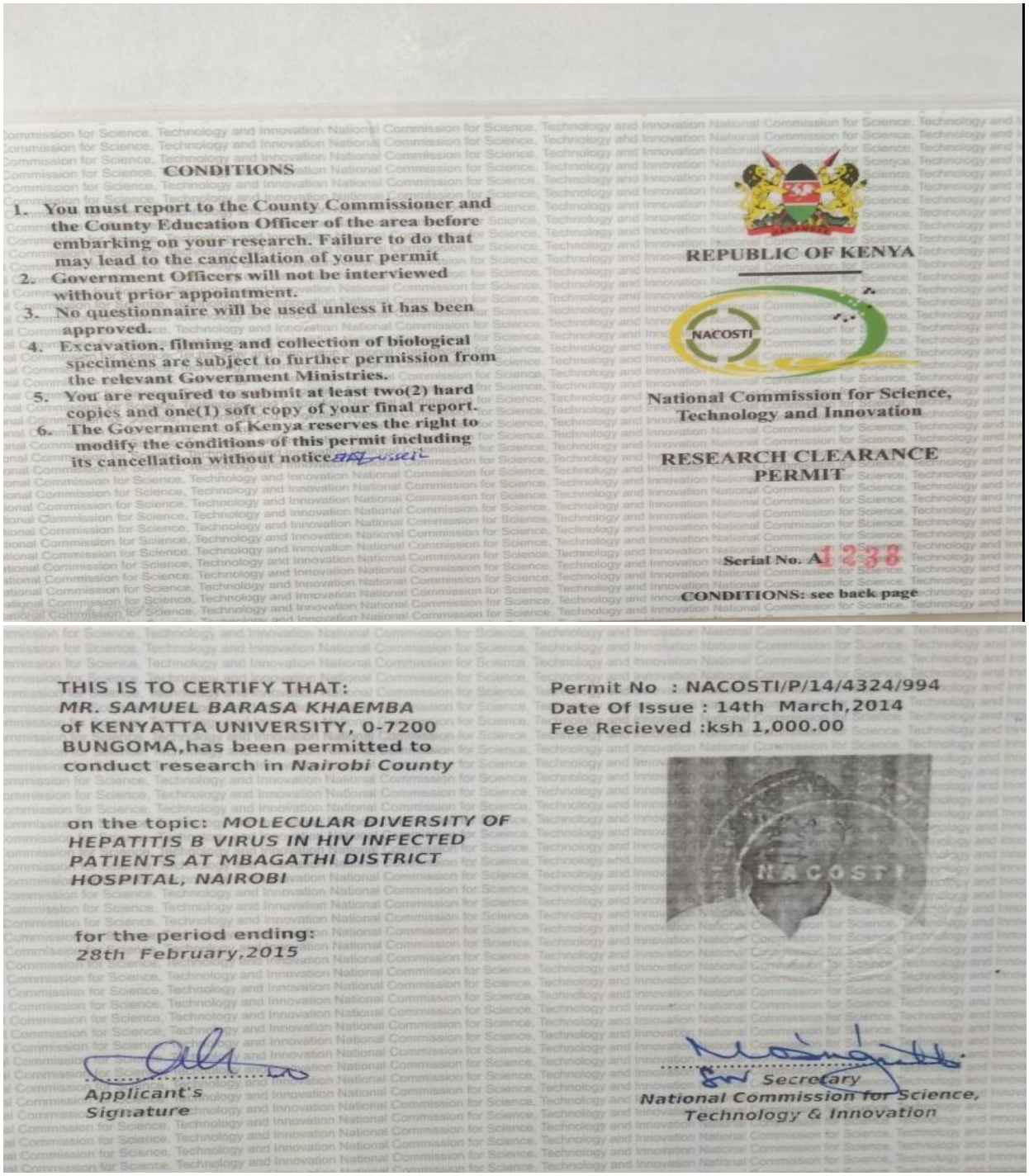
The Medical Superitendent
Mbagathi District Hospital

COUNTY COMMISSIONER
NAIROBI COUNTY
P. O. Box 30124-00100, NBI
TEL: 341666

17 MAR 2014

National Commission for Science, Technology and Innovation is ISO 9001:2008 Certified

Appendix VIII: NACOSTI Research Permit



CONDITIONS

1. You must report to the County Commissioner and the County Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit
2. Government Officers will not be interviewed without prior appointment.
3. No questionnaire will be used unless it has been approved.
4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.
5. You are required to submit at least two(2) hard copies and one(1) soft copy of your final report.
6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice

REPUBLIC OF KENYA

NACOSTI

National Commission for Science, Technology and Innovation

RESEARCH CLEARANCE PERMIT

Serial No. A 1238

CONDITIONS: see back page

THIS IS TO CERTIFY THAT:
MR. SAMUEL BARASA KHAEMBA
of KENYATTA UNIVERSITY, 0-7200
BUNGOMA,has been permitted to
conduct research in *Nairobi County*

on the topic: *MOLECULAR DIVERSITY OF HEPATITIS B VIRUS IN HIV INFECTED PATIENTS AT MBAGATHI DISTRICT HOSPITAL, NAIROBI*

for the period ending:
28th February,2015

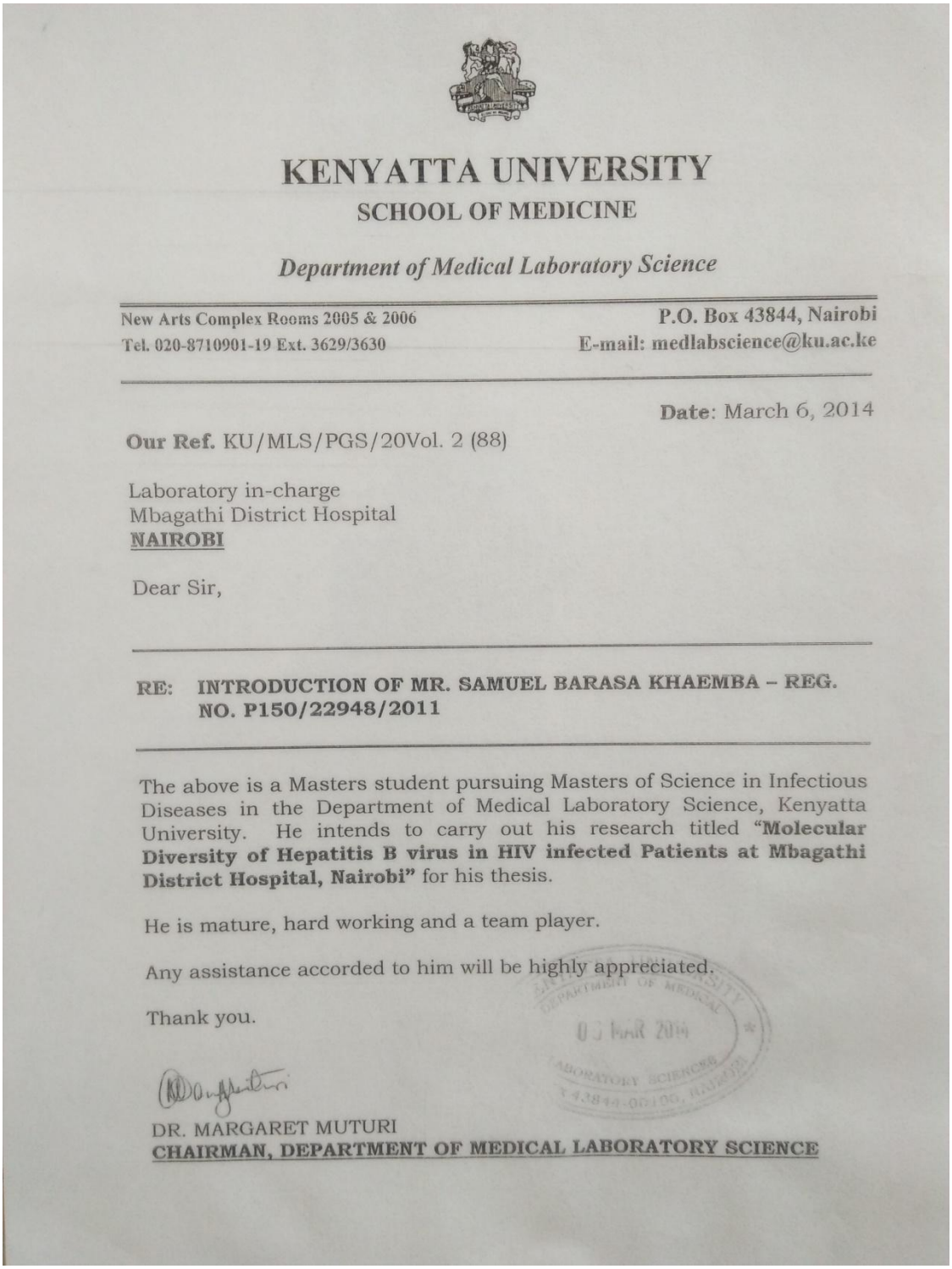
Permit No : NACOSTI/P/14/4324/994
Date Of Issue : 14th March,2014
Fee Recieved :ksh 1,000.00

NACOSTI

Applicant's Signature

Secretary
National Commission for Science, Technology & Innovation

Appendix IX: Letter of Introduction to Mbagathi District Hospital



KENYATTA UNIVERSITY

SCHOOL OF MEDICINE

Department of Medical Laboratory Science

New Arts Complex Rooms 2005 & 2006
Tel. 020-8710901-19 Ext. 3629/3630

P.O. Box 43844, Nairobi
E-mail: medlabscience@ku.ac.ke

Date: March 6, 2014

Our Ref. KU/MLS/PGS/20Vol. 2 (88)

Laboratory in-charge
Mbagathi District Hospital
NAIROBI

Dear Sir,

RE: INTRODUCTION OF MR. SAMUEL BARASA KHAEMBA – REG. NO. P150/22948/2011

The above is a Masters student pursuing Masters of Science in Infectious Diseases in the Department of Medical Laboratory Science, Kenyatta University. He intends to carry out his research titled **"Molecular Diversity of Hepatitis B virus in HIV infected Patients at Mbagathi District Hospital, Nairobi"** for his thesis.

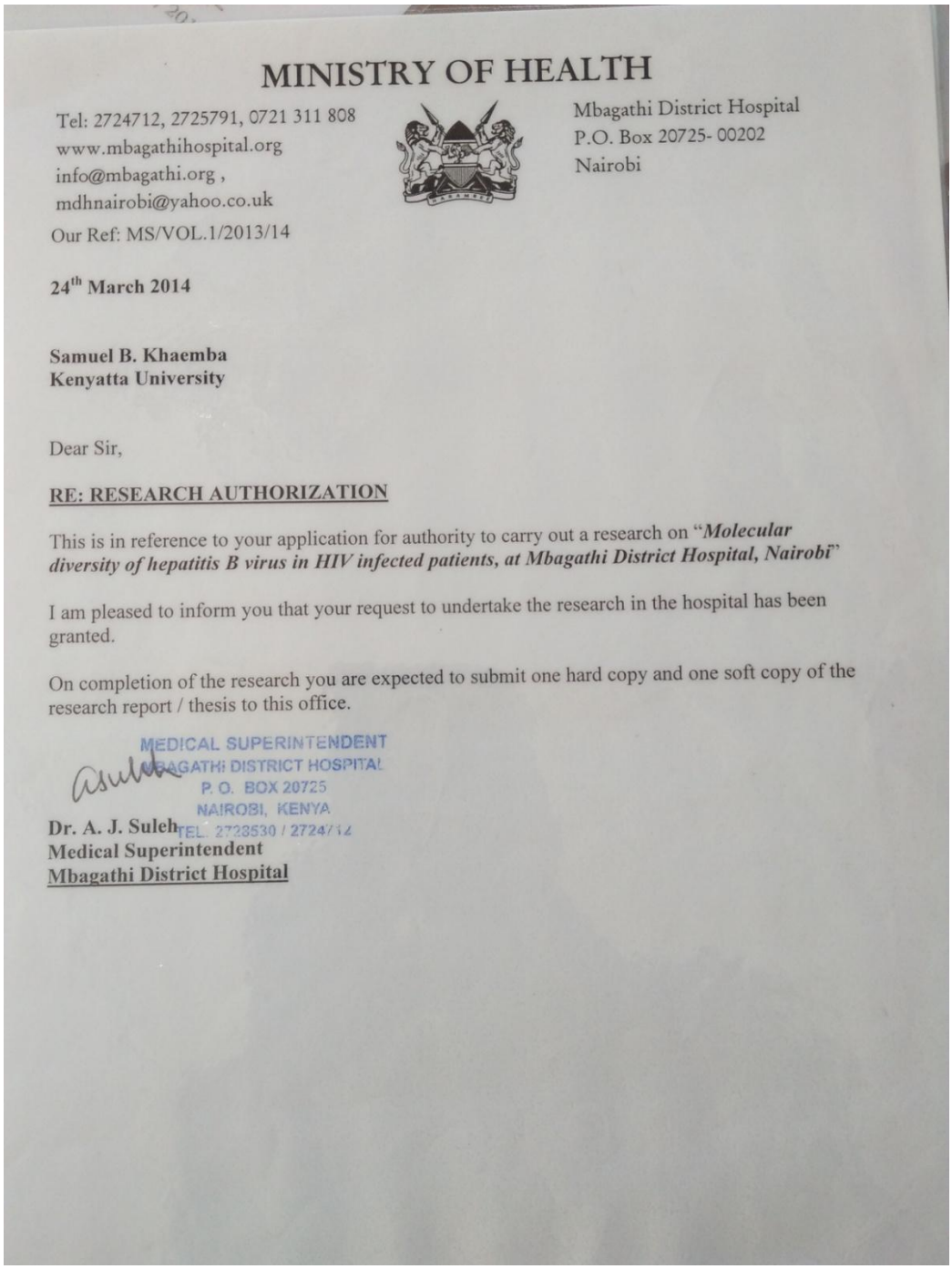
He is mature, hard working and a team player.

Any assistance accorded to him will be highly appreciated.

Thank you.

DR. MARGARET MUTURI
CHAIRMAN, DEPARTMENT OF MEDICAL LABORATORY SCIENCE

Appendix X: Mbagathi District Hospital Data Collection Authorization



MINISTRY OF HEALTH

Tel: 2724712, 2725791, 0721 311 808
www.mbagathihospital.org
info@mbagathi.org ,
mdhnairobi@yahoo.co.uk

Mbagathi District Hospital
P.O. Box 20725- 00202
Nairobi

Our Ref: MS/VOL.1/2013/14

24th March 2014

Samuel B. Khaemba
Kenyatta University

Dear Sir,

RE: RESEARCH AUTHORIZATION

This is in reference to your application for authority to carry out a research on "***Molecular diversity of hepatitis B virus in HIV infected patients, at Mbagathi District Hospital, Nairobi***"

I am pleased to inform you that your request to undertake the research in the hospital has been granted.

On completion of the research you are expected to submit one hard copy and one soft copy of the research report / thesis to this office.

MEDICAL SUPERINTENDENT
MBAGATHI DISTRICT HOSPITAL
P. O. BOX 20725
NAIROBI, KENYA
TEL: 2723530 / 2724712

Dr. A. J. Suleh
Medical Superintendent
Mbagathi District Hospital

Appendix XI: The HBV S Gene Sequenced Results

>P1

TTTCCCCCGGCCGCGGAATGAGAACATCACATCAGGATTCCTAGGACCC
CTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAGAATCCTCACAAT
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TCACCCGTGTGTCTTGGCCAAAATTCGCAGTCCCCAACCTCCAATCACTC
ACCAACCTCCTGTCCTCCAATTTGTCCTGGTTATCGCTGGATGTGTCTGC
GGCGTTTTATCATATTCCTCTTCATCCTGCTGCTATGCCTCATCTTCTTAT
TGGTTCTTCTGGATTATCAGGGTATGTTGCCCGTTTGTCCTCTAATTCCA
GGATCCACAACAACCAGCACGGGACCCTGCAAAACCTGCACGACTCCTG
CTCAAGGCAACTCTATGTTTCCCTCATGTTGCTGTACAAAACCTACGGAT
GGAAATTGCACCTGTATTCCCATCCCATCATCTTGGGCTTTCGCAAAATA
CCTATGGGAGTGGGCCTCAGTCCGTTTCTCTTGGCTCAGTTTACTAGTGC
CATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTCAGCT
ATATGGATGATGTGGTACTGGGGGCCAAGTCTGTACAACATCTTGAGTC
CCTTTATACCGCTGTTACCAATTTTCTTTTGTCTTTGGGTATACATTTAAA
CCCTAACAAAACAAAGAGGTGGGGGTATTCCCTGAATTCATGGTAGTTT
GAGTGTGTGTA

>p5

TTTAATCCCTGGCCGCCCAGATGAGAACATCACATCAGGATTCCTAGGA
CCCCTGCTCGTGTTACAGGCGGGGTTTTTCTTGTTGACAAAAATCCTCAC
AATACCGCAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTTCTAGGG
GGAACTACCGTGTGTCTTGGCCAAAATTCGCAGTCCCCAACCTCCAATC
ACTCACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGTTGGATGTGT
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CGGATGGAAACTGCACCTGTATTCCCATCCCATCATCCTGGGCTTTCGG
AAAATTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCCTGGCTCAGTTTAC
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TCAGTTATATGGATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCT
TGAGTCCCTTTTTACCGCTGTTACCAATTTTCTTTTGTCTTTGGGTATACA
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GTTATTATTTTTGGGAA

>P7

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CACCAACCTCCTGTCCTCCAACTTGTCCTGGTTATCGCTGGATGTGTCTG
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GTTGGTTCTTCTGGATTATCAAGGTATGTTGCCCGTTTGTCCTCTAATTC
CGGGATCTTCAACCACCAGCACGGGACCATGCCGAACCTGCACGACTCC
TGCTCAAGGAACCTCTATGTATCCCTCCTGTTGCTGTACAAAACCTTCGG
ACGGAAACTGCACCTGTATTCCCATCCCATCATCCTGGGCTTTCGGAAAA
TTCCTATGGGAGTGGGCCTCAGCCCGTTTCTCTTGGCTCAGTTTACTAGT
GCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCACTGTTTGGCTTTTAG
TTATATGGATGATGTGGTATTGGGGGCCAAGTCTGTACAGCATCTTGAG
TCCCTTTTTACCGCTGTTACCAATTTTCTTTTGTCTTTGGGTATACATTTA
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TGCAGGGGGGGGAATGTAGAGTTGA

>P8

CCTGAACACCGACATGGAGAACATCACATCAGGATTCCTAAGACCCCTG
CTCGTGTTACAGGCGGGGTTTTCCTTGTTGACAAGAATCCTCACAATACC
GCAGAGTCTAGACTCGTGGTGGACTTCTCTCAATTTTCTAGGGGGCTCA
CCCGTGTGTCTTGGCCAAAATTCGCAGTCCCCAACCTCCAATCACTCACC
AACCTCCTGTCCTCCAATTTGTCCTGGTTATCGCTGGATGTGTCAGCGGC
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TTCTTCTGGATTACCAAGGTATGTTGCCCGTTTGTCCTCTAATTCCAGGA
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>P9

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AATTTTCTAGGGGGATCACCCGTGTGTCTTGGCCAAAATTCGCAGTCCC

CAACCTCCAATCACTCACCAACCTCCTGTCCTCCAATTTGTCCTGGTTAT
CGCTGGATGTATCTGCGGCGTTTTATCATATTCCTCTTCATCCTGCTGCT
ATGCCTCATCTTCTTATTGGTTCTTCTGGATTATCAAGGTATGTTGCCCG
TTTGTCCTCTAATTCCAGGATCCACAACAACCAGCACGGGACCCTGCAA
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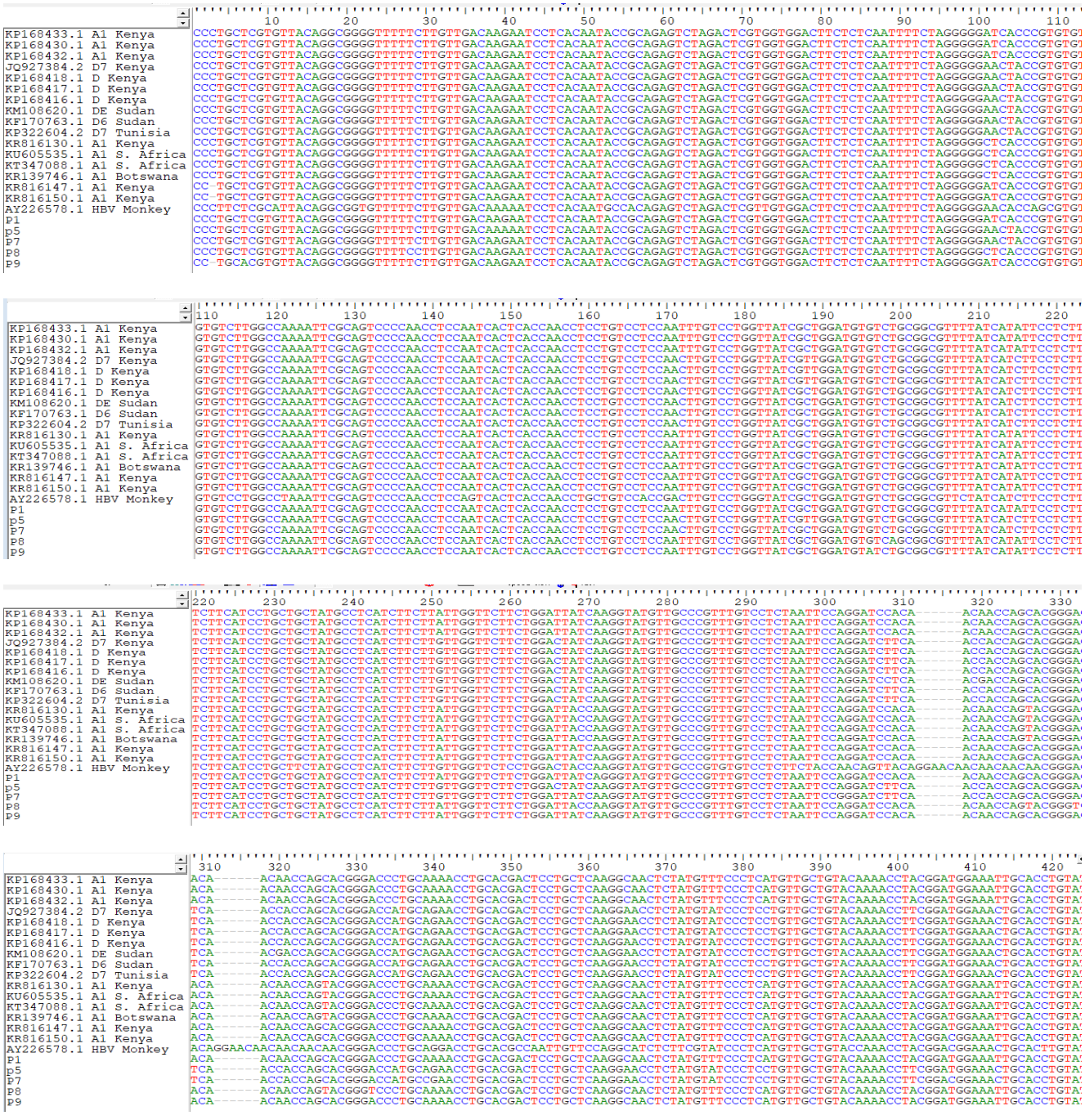
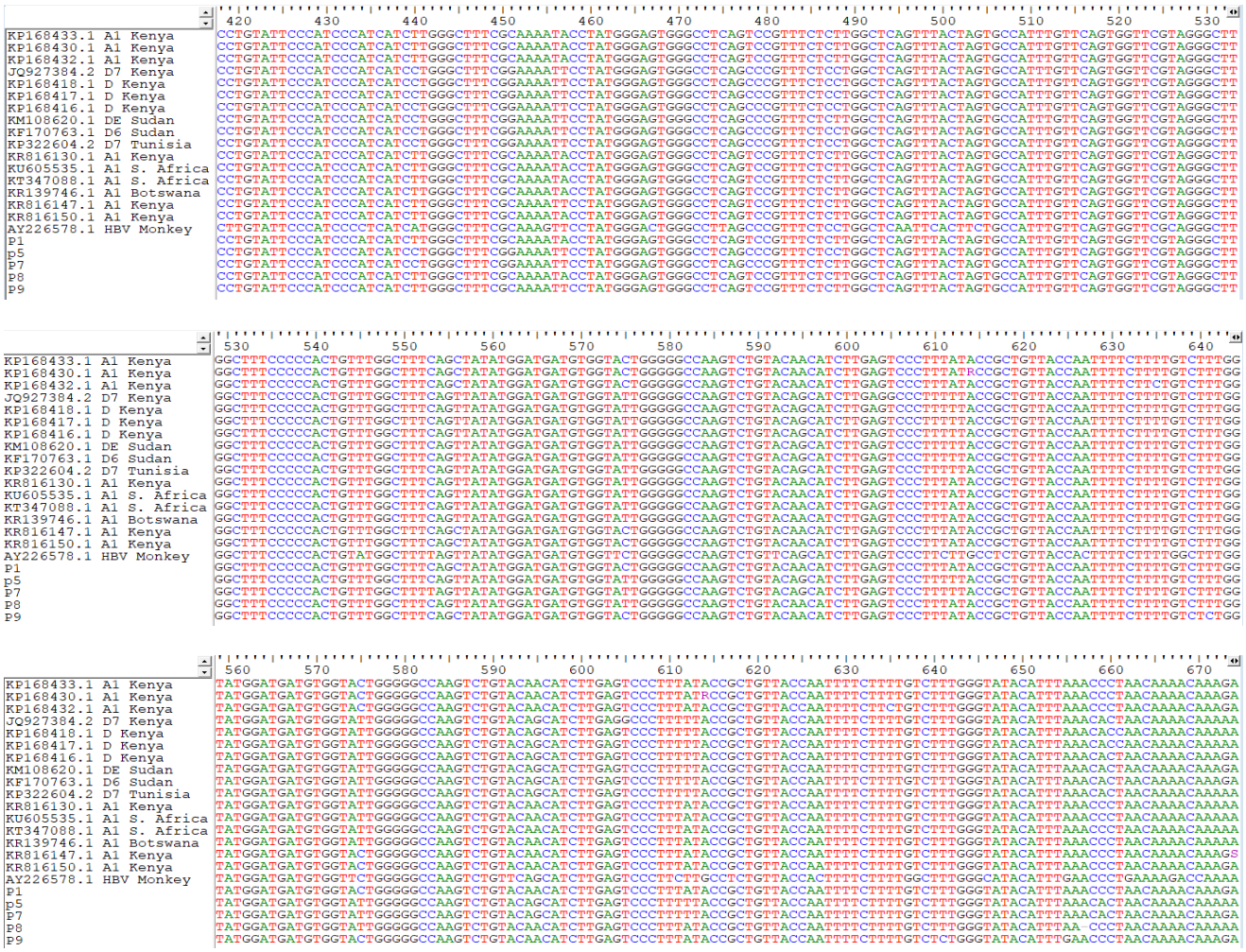
GTACAAAACCTACGGATGGAAATTGCACCTGTATTCCCATCCCATCATCT
TGGGCTTTCGCAAAATTCCTATGGGAGTGGGCCTCAGTCCGTTTCTCTTG
GCTCAGTTTACTAGTGCCATTTGTTCAGTGGTTCGTAGGGCTTTCCCCCA
CTGTTTGGCTTTCAGCTATATGGATGATGTGGTACTGGGGGCCAAGTCT
GTACAACATCTTGAGTCCCTTTATACCGCTGTTACCAATTTTCTTTTGTCT
CTGGGTATACATTTGAACCCTAACAAAACAAAGAGGTGGGGGTATTCCC
TGAATTTCTGGTTAGTGGTGGGGGGGGAGGGGGAAAAA

Appendix XII: The HBV S Gene Sequence Alignment as viewed in Bio Edit V7.0





A section of S gene sequence alignment: Similar nucleotides along the columns indicate sequence conservation while different nucleotides indicate sequence divergence. The sequences obtained were approximately 710bp

Appendix XIII: HBV Genotypes GenBank Accession Numbers MG845879-MG845886

BankIt2080975 HBV_S-gene_P1	MG845879
BankIt2080975 HBV_S-gene_p5	MG845880
BankIt2080975 HBV_S-gene_P7	MG845881
BankIt2080975 HBV_S-gene_P8	MG845882
BankIt2080975 HBV_S-gene_P9	MG845883
BankIt2081044 precore-gene_C5	MG845884
BankIt2081044 precore-gene_C7	MG845885
BankIt2081044 precore-gene_C8	MG845886

Appendix XIV: HBV Drug Mutations' Cross Tabulation Results

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HBV resistance prediction from genotype (version 2.0)

Sequence Information		Resistance Prediction	
Sample ID:	khaemba - P1	Lamivudine:	susceptible
Genotype:	A (A1)	Adefovir:	susceptible
RT Mutations:	R110G, N122H, Y126H, M129L, V163I, F249S, M250W, G251*, Y252F, I253E, I254C, G255V	Entecavir:	unknown mutation on rated position
SHB Mutations:	S207N	Tenofovir:	susceptible
Escape Mutations:		Telbivudine:	susceptible

Sequence Information		Resistance Prediction	
Sample ID:	khaemba - p5	Lamivudine:	susceptible
Genotype:	D (D7)	Adefovir:	susceptible
RT Mutations:	Y135S, Q149K, R153W, P237T, N248H, V253Y, I254F, G255W, C256E	Entecavir:	susceptible
SHB Mutations:	R24K, T127P	Tenofovir:	susceptible
Escape Mutations:		Telbivudine:	susceptible

Sequence Information		Resistance Prediction	
Sample ID:	khaemba - P7	Lamivudine:	susceptible
Genotype:	D (D6)	Adefovir:	susceptible
RT Mutations:	R120G, Q130P, Y135S, Q149K, P237T, W243T, Y245C, S246A, N248H, M250L, G251C, Y252L, V253Q, I254G, C256E, Y257C, G258R, S259V	Entecavir:	unknown mutation on rated position
SHB Mutations:	T127P, S193L	Tenofovir:	susceptible
Escape Mutations:		Telbivudine:	susceptible

Sequence Information	Resistance Prediction

Sample ID:	khaemba - P8	Lamivudine:	susceptible
Genotype:	A (A1)	Adefovir:	susceptible
RT Mutations:	R18K, I53L, S109P, N122H, T128S, M129L, V163I, N236[n.d.], R242K	Entecavir:	susceptible
SHB Mutations:	G10R, F20S, L77Q, A194V, S207N	Tenofovir:	susceptible
Escape Mutations:		Telbivudine:	susceptible
Sequence Information		Resistance Prediction	
Sample ID:	khaemba - P9	Lamivudine:	susceptible
Genotype:	A (A1)	Adefovir:	susceptible
RT Mutations:	N122H, Y126H, M129L, V163I, M250W, G251L, Y252V, I253V, I254G, S256E, W257G, G258E, T259K	Entecavir:	unknown mutation on rated position
SHB Mutations:	C76Y, Y161F, S207N	Tenofovir:	susceptible
Escape Mutations:		Telbivudine:	susceptible

APPENDIX XV: Abstract Presented at International AIDS Conference (IAS) 18-21st 2021

This study results were presented at International AIDS Conference (IAS) 18-21st 2021, **PEB087, page 119**/455 (AIS 2021 Abstract Book) through poster.

The link references are:

https://www.ias2021.org/wp-content/uploads/2021/07/IAS2021_Abstracts_web.pdf

https://theprogramme.ias2021.org/Abstract/Abstract/2227

https://theprogramme.ias2021.org/PAGMaterial/PPT/1493_4206/Samuel_Khaemba-IAS-2021_e-poster_presentation.pdf

https://theprogramme.ias2021.org/Programme/Session/380

MOLECULAR diversity of Hepatitis B Virus in HIV infected patients at Mbagathi District Hospital, Nairobi

PRESENTER; SAMUEL BARASA KHAEMBA

AUTHORS: SB. Khaemba[1], M. Warigia[1], W. Arodi[1], CS. Wekesa[2], FM. Ogollah[3]
[1]Kenyatta University, Nairobi, Kenya, [2]Friedrich-Schiller-Universität Jena, Germany, [3]Maseno University, Kisumu, Kenya

Introduction

With increasing access to ART, HIV-infected individuals live longer and HIV/HBV co-infection is an emerging critical public health problem.[1] Approximately 1% of people living with HBV are also infected with HIV and the prevalence of HBV in HIV infected people is 7.4%.[2] HBV genotypes have been clarified as influencing the clinical outcome of the chronic disease in hosts.[3]

HBV co-infection complicates the management of HIV, increases the morbidity and mortality of HIV-infected patients and; there are increased risk of Hepatitis flares due to immune reconstitution in the HAART setting and increased risks of ART regimen resistance in people living with HIV. [4]

Objectives

Due to paucity of data on HBV-HIV confection in Kenya, this study sought to establish:

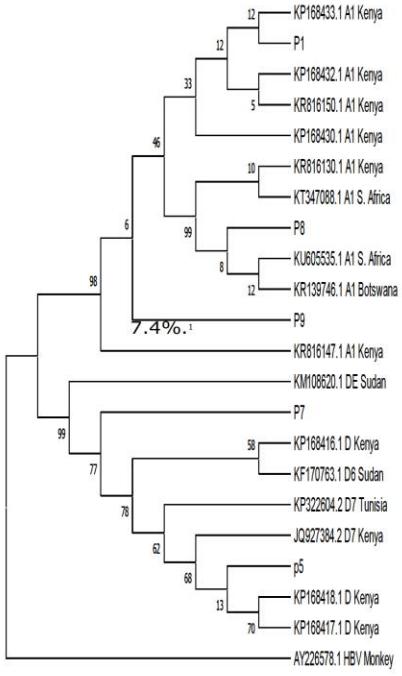
- ❑What are the various HBV genotypes in HIV-infected patients?
- ❑What are the HBV drug mutations found in HIV-HBV co-infected patients?

Methods

This was a cross-sectional study consisting of 180 HIV seropositive male and female individuals attending routine CD4+ T-lymphocyte and Viral load laboratory monitoring. An ELISA for qualitative detection of HBsAg in Human serum was used and HBV DNA was extracted from 9 HBsAg seropositive samples.

HBV genotypes established in 5 nucleic acids by nested-PCR of pre-S gene were directly sequenced and clustered by phylogenetic tree. HBV drug resistance mutations were assigned by analysing the generated S-gene contigoius sequences using the HBV drug resistance database.

Inadequate HBV co-management in HIV-HBV co-infected patients is likely to lead to the emergence and circulation of HBV escape mutants of interest which might lead to high HBV drug resistance strains to available ART options in Kenya.



Maximum Likelihood phylogeny of S gene sequences (710nt) of HBV. GenBank accession numbers of comparative sequences are given together with their country of origin.

Table of Isolate identification by BLASTn at GenBank and genetic distance in MEGA V6

Sample name	NCBI identity	% identity	Country	Genetic distance	NCBI genotype	Possible isolate genotype	GenBank Accession Numbers
P1	KP168433.1	99	Kenya	0.001473	A1	A1	MG845879
P5	JQ927384.2	98	Kenya	0.002947	D7	D7	MG845880
P7	KF170763.1	99	Sudan	0.00889	D6	D6	MG845881
P8	KR816130.1	98	Kenya	0.00739	A1	A1	MG845882
P9	KR816147.1	99	Kenya	0.00739	A1	A1	MG845883

Results

The HBV prevalence was found to be 2.8% by PCR. HBV genotype A1 and D are the most prevalent in Kenya and in this study, they showed very low genetic diversity. In addition, these strains showed very close phylogenetic relationship to those isolated from Sudan, S. Africa, Botswana and Tunisia. The five isolate genotypes were found to have susceptible mutations in S gene for Lamivudine, Adefovir, Entecavir and Tenofovir.

Conclusion

The HIV co-infected patients with HBV were not aware of their co-infection status, thus were only on HIV therapy. This lack of HBV co-management in HIV-HBV co-infected patients is likely to lead to the emergence and circulation of HBV mutants of interest to public health management.

Reference

1. Modi, A. and Feld, J. (2007). Viral Hepatitis and HIV in Africa. AIDS Reviews; 9: 25-39.
2. WHO, (2020). Hepatitis B, Key Facts. https://www.who.int/news-room/fact-sheets/detail/hepatitis-b (Accessed on 20th May 2021)
3. WHO, (2017). Global Hepatitis Report, 2017. https://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/. ISBN: 978-92-4-156545-5
4. Sheng,W., Hung,C., Chang,Y., Liu, C., Chen, M., Hsieh,S., Kao, J., Chen, P. and Chang, S. (2012). Differential Clinical and Virologic Impact of Hepatitis B Virus Genotypes B and C on HIV-Coinfected Patients Receiving Lamivudine-Containing Highly Active Antiretroviral Therapy.*Clinical Infectious Diseases*; **54** (4): 548–55.

Disclosures

The author and co-authors have no conflict of interest.

Contact Information: Samuel.baemba@gmail.com