HEPATITIS B VIRUS SEROPREVALENCE AMONG HIV INFECTED INDIVIDUALS SEEKING CARE AT SELECTED HOSPITALS IN KERICHO COUNTY, KENYA

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Hepatitis B virus Seroprevalence among HIV infected individuals seeking care at selected hospitals in Kericho County, Kenya

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A Thesis Submitted in Fulfillment for the Degree of Master of Science in Laboratory Management and Epidemiology in the Jomo Kenyatta University of Agriculture and Technology

2018
DECLARATION

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

Signature_________________________________ Date _____________________________

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This thesis has been submitted for examination with our approval as the university supervisors,

Signature_________________________________ Date _____________________________

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Signature_________________________________ Date _____________________________

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KEMRI, Kenya
DEDICATION

This thesis is dedicated to my husband, Richard Kirui, my sons Paul, Noah, Ng’ash, Mark and Allan. Mum Esther Lasoi and Teresia Mibei, who are a source of my encouragement and inspiration.
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God bless you all
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ABBREVIATIONS AND ACRONYMS

AIDS  Acquired Immune Deficiency Syndrome
ALP   Alkaline phosphatase
ALT   Alanine Transaminases
ART   Anti-Retroviral therapy
AST   Aspartate Transaminases
CCC   Comprehensive Care Clinic
CD4   Cluster of differentiation
DNA   Deoxyribonucleic Acid
dNTPs Deoxyribonucleotide Triphosphates
EDTA  Ethylene Diamine Tetra-acetatic Acid
ELISA Enzyme Linked Immunosorbent Assay
HAART Highly Active anti-retroviral therapy
HBV   Hepatitis B Virus
<table>
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<th>Abbreviation</th>
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<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<tr>
<td>HBsAg</td>
<td>Hepatitis B surface antigen</td>
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<td>HBeAg</td>
<td>Hepatitis B e antigen</td>
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<td>HBcAg</td>
<td>Hepatitis B core antigen</td>
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<td>HCC</td>
<td>Hepatocellular carcinoma</td>
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<tr>
<td>HCV</td>
<td>Hepatitis C virus</td>
</tr>
<tr>
<td>H_2SO_4</td>
<td>Sulphuric Acid</td>
</tr>
<tr>
<td>ITROMID</td>
<td>Institute of Tropical Medicine and Infectious Diseases</td>
</tr>
<tr>
<td>JKUAT</td>
<td>Jomo Kenyatta University of Agriculture and Tech</td>
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<tr>
<td>KNBS</td>
<td>Kenya National Bureau of Standards</td>
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<tr>
<td>MOH</td>
<td>Ministry of Health</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>RPM</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
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<tr>
<td>µl</td>
<td>Micro liter</td>
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<tr>
<td><strong>WHO</strong></td>
<td>World Health Organization</td>
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Hepatitis B virus (HBV) and human immunodeficiency virus (HIV) co-infection are common blood borne infections with common routes of transmission. The HIV infection however is associated with more rapid progression of viral hepatitis-related liver disease. The clinical benefits of antiretroviral therapy (ART) have been associated with an apparent increase in liver disease–related mortality in coinfected patients. Antiretroviral (ARV) drugs active against both HIV and HBV may prevent development of significant liver disease by directly suppressing HBV replication. Complications due to ART treatment failures may therefore jeopardize the clinical benefits of ART, especially in HIV/HBV co-infected patients. In Kenya currently, there is a paucity of data on the prevalence of HBV genotypes and its correlation with immunological and hepatological parameters among HIV infected patients. These data are vital in development of treatment strategies especially in HIV/HBV co-infection cases. This cross sectional study enrolled 303 HIV patients attending Kericho, Kapkatet and Londiani District Hospitals in the Kericho County to answer the above objectives. The HBV status was determined using ELISA while the genotypes identified using sequence analysis. Immunological and hepatic parameters were determined using flow cytometer and chemistry analyzer respectively. Factors associated with HBV/HIV co-infection were evaluated using structured questionnaire. Patients’ characteristics were analyzed by using Kruskal–Wallis test (noncategorical variables) and χ2-test or Fisher's exact test (categorical variables). The mean age of the 303 study patients was 34.4 (SD = 9.7; range 16-71) years. The patients’ mean CD4 was 259.5 (± SD 129.1) cells per ml. The mean Alanine aminotransferase (ALT) level was 32.9 (± SD 18.95) U/L, and the mean Aspartate aminotransferase (AST) level was 36.7 (± SD 14.7) U/L with (63.4%) of them having AST level within the normal range (0-37U/L). The mean alkaline phosphatase (ALP) level was 156.9 (± SD 59.6) U/L and there was no difference between the proportion of patients who had normal levels of <147 U/L and those who had abnormal levels of > 147 U/L (51.5% and 48.5%). Only 15 (5%) of the HIV patients were co-infected with Hepatitis B virus, majority (93%) of which were HBV genotype A. The
HBV/HIV co-infected patients had lower CD4 compared mono-infected patients (CD4 mean of 137.2 cells/ml versus 265.9 cells/ml) (P = 0.013). The HBV/HIV co-infected patients had a higher mean ALT (mean ALT of 62 U/L versus 31.4 U/L) (P = 0.001). Similarly the HBV/HIV co-infected patients had higher mean AST (mean AST of 65.5 U/L versus 35.2 U/L) (P = 0.001). This study, confirms the increasing number of HBV/HIV co-infection in Kenya with predominance of HBV genotype A. The male gender, increasing age, lower CD4 count, ART use, elevated liver enzymes are a key pointer for increased risk of HBV co-infection. Early detection of HBV/HIV co-infection are vital in instituting treatment strategies including immunization of susceptible individuals and revaccination of HIV-infected individuals who do not respond to the standard HBV vaccination schedule. Further, the community of HIV patients could benefit from clinical, molecular and immunological investigations to elucidate the interaction of HBV-HIV coinfection.
CHAPTER ONE

INTRODUCTION

1.1 Background

Hepatitis B virus (HBV) infection is a significant public health problem endemic to most parts in sub-Saharan Africa. The prevalence in Kenya is amongst the highest worldwide (Ott et al., 2012), with some regional differences. In Kenya, HBsAg prevalence in adults ranges from 3% to 25%, with the highest rates in HIV-infected adults (Hyams et al., 1989; Kibaya et al., 2015; Kerubo et al., 2015). Human Immunodeficiency Virus infection is associated with more rapid progression of viral hepatitis-related liver disease, including cirrhosis, end-stage liver disease, hepatocellular carcinoma, and fatal hepatic failure (Thio et al., 2002; Puoti et al., 2006). The pathogenesis of accelerated liver disease in HIV-infected patients has not been fully elucidated but HIV-related immunodeficiency and a direct interaction between HIV and hepatic stellate and Kupffer cells have been implicated (Rodriguez-Mendez et al., 2000; Chung et al., 2001).

Co-infection with hepatitis B virus (HBV) and HIV is common, 70-90% of HIV-infected individuals in the United States having evidence of past or active infection with HBV (Alter, 2006). Factors affecting the prevalence of chronic HBV include age at time of infection and mode of acquisition, which vary geographically. In the United States and Western Europe, HBV often is acquired in adolescence or adulthood through sexual
contact or injection drug use. Although spontaneous clearance of HBV acquired in adulthood occurs in >90% of immune-competent individuals, HIV-infected persons are half as likely as HIV-uninfected persons to spontaneously clear HBV (Alter, 2006). Therefore, chronic HBV infection occurs in 5-10% of HIV-infected individuals who are exposed to HBV, a rate 10 times higher than that for the general population (Alter, 2006). A study done in United States reported that HIV/HBV co-infection rates are highest among men who have sex with men (MSM) and injection drug users (Hoffman et al., 2007). In contrast, in Asia and sub-Saharan Africa, where vertical and early childhood exposure are the most common modes of transmission, respectively, and overall HBV prevalence is higher, the prevalence of HBV among HIV-infected individuals also is higher, at an estimated 20-30% (Hoffman et al., 2007).

In the last few years, there has been a substantial increase in the number of HIV-infected subjects receiving antiretroviral therapy (ART) in Kenya (Katabira and Oelrichs, 2007), with notable clinical benefits. In individuals coinfected with HBV, ART may attenuate liver disease progression by preserving or restoring immune function and reducing HIV-related immune activation and inflammation (Szczech et al., 2004; Reekie et al., 2011). Antiretroviral (ARV) drugs active against both HIV and HBV (such as tenofovir disoproxil fumarate [TDF], lamivudine [3TC], and emtricitabine [FTC]) also may prevent development of significant liver disease by directly suppressing HBV replication (Atta et al., 2006; Estrella et al., 2006).
The current HIV treatment success is jeopardized by drug resistance (Vella, 2002). Studies have shown that most ARV are prone to the development of drug resistance. The clinical significance of the development of resistance is still being debated. Clearly, in many HIV/HBV coinfected patients, resistance presages a return of higher level viremia, and in some of these patients further liver injury develops. Further, HIV-infected patients coinfected with HBV have an increased risk for antiretroviral therapy-related hepatotoxicity, particularly when HBV DNA levels exceed 10,000 copies/ml (Hoffmann et al., 2008). For all of these reasons, effective treatment of HBV in persons coinfected with HIV is clearly a high priority. Currently in Kenya, especially in Kericho County, there is a paucity of data on the prevalence of HIV-HBV co-infection and how this might impact ART treatment. The purpose of this study was therefore to determine the prevalence HIV-HBV co-infection and circulating HBV genotypes. The study also characterized immune-hepatological parameters associated with HIV-HBV coinfection in Kericho County in order to optimize therapeutic management of HIV/HBV co-infected patients in Kenya.

1.2 Statement of the problem

In HIV-HBV confections, HIV causes increased rates of persistent HBV infection, increased cirrhosis and liver related mortality and increased risk of hepatocellular carcinoma at lower CD4 T cell count (Thio, 2009). Studies have shown that HIV coinfection adversely impacts on the natural history of HBV by accelerating progression
to chronic liver disease due to drug related hepatotoxicity and hepatitis reactivation (Muriuki et al., 2013). The impact of HBV cannot be limited to liver hepatotoxicity but also results in failure in immunological recovery of HIV positive patients. Liver-related disease has emerged as the leading cause of non-HIV-related mortality in parts of the world where effective antiretroviral therapy (ART) is widely available (Bonacini et al., 2004). With increased access to antiretroviral drugs for HIV patients, cases of HBV co-infections has been on the rise coupled with dramatic rise of survival by these individuals (Lacombe et al., 2010). HBV/HIV co-infection leads to increased morbidity and mortality than HIV or HBV mono infections increasing concerns to the burden of these co-infections. Studies therefore evaluating the prevalence of HBV/HIV co-infection are urgent especially in Africa, currently categorized as high HBV endemic region (Harania et al., 2008; Thio, 2009; Bello & Olabode, 2011).

1.3 Justification of the study

The rates of HBV/ HIV co-infection are high (10–20%) in countries where HBV infection is either endemic or intermediate to high HBV cases such as Kenya (Thio, 2009). The HBV and HIV co-infections is a growing worldwide health problem characterized by lack of effective vaccines, need for expensive treatment, chronicity of morbidity and associated mortality. With increased access to antiretroviral drugs for HIV patients, migrating populations and social networking by intravenous drug use, cases of HIV and HBV co infections have been on the rise (Lacombe et al., 2010),
coupled with the dramatic rise in survival rates of these individuals (Sulkowski et al., 2000). As a result of these factors, cases of hepatic diseases have also been on the rise (Sulkowski et al., 2002). In Kenya, the HIV epidemic has been well documented; scanty data exist on the HIV/HBV co infection especially in Kericho County where the prevalence of HIV is on the rise.

1.4 Research Questions

1. What is the prevalence of HBV among HIV infected patients in Kericho County?
2. Which is the common HBV genotype in the study population?
3. What is the correlation of HBV co-infection on the immunological and hepatological parameters among HIV infected individuals in Kericho County?
4. What is the relationship between HBV/HIV co-infection and HIV infected individuals in Kericho County?

1.5 Objectives

1.5.1 Broad Objective

Determine the prevalence of HBV among HIV positive patients and its correlation with immunological and hepatological parameters among HIV infected individuals in Kericho County
1.5.2 Specific Objectives

1. Determine the prevalence of HBV among HIV infected patients in Kericho County
2. Determine the HBV genetic diversity among HIV infected patients in Kericho County
3. Evaluate the correlation between HBV co-infection on the immunological and hepatological parameters among HIV infected individuals in Kericho County
4. To compare the relationship between HBV/HIV co-infection and HIV monoinfected individuals in Kericho County
2.1 The Biology of HBV

The HBV belongs to the genus Orthohepadnavirus. The genus contains three other species: the Ground squirrel hepatitis virus, Woodchuck hepatitis virus, and the Woolly monkey hepatitis B virus. The HBV belongs to the Hepadnaviridae family (King et al., 2011). Generally, HBV is small (diameter of 42 nm) with three distinct morphological characteristic. The most abundant are small, spherical, noninfectious particles, containing HBsAg, that measure 17 to 25 nm in diameter and are composed of lipid particles (Hollinger and Liang, 2001). There is the tubular, filamentous form of various lengths, with a diameter comparable to that of the small particles. They also contain HBsAg polypeptides (Hollinger & Liang, 2001). The third morphological form is the 42 nm hepatitis B virion, which is a complex, spherical, double shelled particle. The structure consists of an outer envelope containing host-derived lipids and all S gene polypeptides, the large (L), middle (M), and small (S) surface proteins, also known as pre-S1, pre-S2 and HBsAg. The nucleocapsid contains core proteins HBcAg, a 3.2 kb, circular, partially double stranded viral DNA genome, an endogenous DNA polymerase (reverse transcriptase) enzyme, and protein kinase activity (Ganem et al., 2001).
The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes present on its envelope proteins, and into eight genotypes (A–H) according to overall nucleotide sequence variation of the genome. The genotypes have a distinct geographical distribution and are used in tracing the evolution and transmission of the virus. Differences between genotypes affect the disease severity, course and likelihood of complications, and response to treatment and vaccination (Kramvis et al., 2005).

2.2 Hepatitis B Virus Replication Cycle

Figure 2.1 shows the replication Cycle of HBV. The main feature of the hepadnavirus replication cycle is the cardinal feature which is the replication of the DNA genome by reverse transcription of RNA intermediate (Ganem & Prince, 2004). The incoming HBV virions are bound by cell-surface receptors, the identity of which remains unknown. After membrane fusion, cores are presented to the cytosol and transported to the nucleus. There, their DNA genomes are converted to a covalently closed circular (ccc) form, which serves as the transcriptional template for host RNA polymerase II. This enzyme generates a series of genomic and sub genomic transcripts (Ganem & Prince, 2004).

All viral RNA is transported to the cytoplasm, where its translation yields the viral envelope, core, and polymerase proteins, as well as the X and preC polypeptides. Next, nucleocapsids are assembled in the cytosol, and during this process a single molecule of genomic RNA is incorporated into the assembling viral core. Once the viral RNA is encapsidated, reverse transcription begins (Pollack & Ganem, 1994). The synthesis of
the two viral DNA strands is sequential. The first DNA strand is made from the encapsidated RNA template; during or after the synthesis of this strand, the RNA template is degraded and the synthesis of the second DNA strand proceeds, with the use of the newly made first DNA strand as a template (Wang & Seeger, 1993; Pollack & Ganem, 1994). Some cores bearing the mature genome are transported back to the nucleus, where their newly minted DNA genomes can be converted to cccDNA to maintain a stable intranuclear pool of transcriptional templates (Ganem & Prince, 2004). Most cores, however, bud into regions of intracellular membranes bearing the viral envelope proteins. In so doing, they acquire lipoprotein envelopes containing the viral L, M, and S surface antigens and are then exported from the cell.
Figure 2.1: Replication Cycle of HBV (adopted from Ganem and Prince, 2004)
2.3 Stages of HBV Chronic Infection

There are three phases of chronic HBV disease (Shi et al., 2009; Annie, 2010; **Immune Tolerant phase**: This involves an incubation period of 120 days and duration of less than 6 months after infection. No host immune response is mounted at this initial stage despite high serum viral DNA levels (Annie, 2010). At this stage the patients are considered to be at low risk of progressing to cirrhosis or hepatocellular carcinoma and no antiviral therapy is recommended for them. This phase is characterized by a high level of HBV replication with little or no evidence of active hepatic inflammation. Hepatic transaminases are normal and liver biopsy, if performed, would show little or no inflammation. Most children infected at birth or during early childhood will be immune tolerant and remain in the immune tolerant phase for years; however, most eventually will progress to immune active disease. Given the lack of immune response to HBV, treatment is not recommended during this period, but individuals should be monitored for transition to immune active disease as well as for hepatic fibrosis and hepatocellular carcinoma (HCC) as they progress. At this stage, the HBV DNA is detectable, as are HBeAg and hepatitis B surface antigen (HBsAg). Because of the high level of HBV viremia, this phase/stage is highly infectious (Annie, 2010)

**Immune Active phase**: This stage involves clearance of HBV by host immune system. There is sero-conversion of HbeAg to anti-HBe (Chu et al., 2004). Depending on the immune status of the infected individual, 90-95% is able to mount an immune response
to HBV and this confers immunity to the person, which is confirmed by the presence of anti-HBe. Unfortunately, 5-10% of those infected who fail to develop this immunity progress to the third stage and are likely to develop HCC. In Africa, 70-80% of the general population has been previously exposed to HBV infection (WHO, 2008) and there is risk of transmission to others (Anne, 2010).

**Inactive carrier state:** During this phase, HBeAg is lost and HBV DNA declines, often to undetectable levels (Chu *et al.*, 2004). Hepatitis B e antibody (HBeAb) seroconversion can occur, followed by hepatitis B surface antibody (HBsAb) seroconversion, indicating immune control of HBV infection (Annie, 2010). A small proportion of people will continue to have detectable low-level HBV DNA, which may be intermittent and is referred to as "occult" HBV infection (Annie, 2010).

**2.4 Prevalence of HBV**

Hepatitis B Virus and HIV are the most common chronic viral infections all over the world. HBV occurs worldwide, the prevalence and transmission mode vary geographically, and it can be classified into three endemic patterns (Kowdley, 2004; Knipe and Howley, 2013; WHO, 2013). Approximately 45% of the world's population lives in regions of high endemicity, defined as areas where 8% or more of the population are positive for HBsAg such as Southeast Asia and Sub-Saharan Africa. The moderately endemic areas, such as Mediterranean countries and Japan, are defined as those areas where 2–7% of the population is HBsAg positive, and around 43% of the world's
population lives in regions of moderate endemicity. Western Europe and North America are considered as areas with low endemicity (<2% of the population is HBsAg positive) and it constitutes 12% of the world's population (Kowdley, 2004; Knipe and Howley, 2013; WHO, 2013) as shown in figure 2.4. In Kenya varied prevalence rates have been reported; in 1989 Okoth et al., reported a prevalence of 11.4% HBV infection among outpatients attending three hospitals in Mombasa, Kilifi, and Malindi. Muruiki et al., 2013 identified a prevalence of 6% HBV infection among HIV infected individuals in Nairobi, Kenya. Among the health workers, Suckling et al. (2006) reported 41% HBV positive while Mutuma et al. (2011) observed a prevalence of 8.8% HBsAg in an asymptomatic rural nomadic population in Kenya.
2.5 Prevalence of HBV-HIV co-infection

Divergence in the prevalence rates of HBV/HIV co-infection has been shown to vary according to the risk factors involved, socioeconomic status and initial burden of infectious markers in the community. It also varies from one country to another and even between different regions within the same country, sample size, test kit sensitivity and specificity (Taiwo et al., 2012). In Kenya varied HBV/HIV co-infection rates have been reported; Chepkurui et al., (2015), reported a prevalence of 5% of HBV/HIV coinfection in Kericho County, similar prevalence of co-infection has been reported in Kenya,
Kerubo et al. (2015) reported prevalence of 4.26% HBV/HIV co-infection in Nairobi, while Harania et al. (2008) and Muruiki et al. (2013) both reported a 6% co-infection prevalence in Nairobi at different time point. The HBV/HIV co-infection rates was also consistent with findings from other regions such as 9.9% HBV/HIV co-infection in Lusaka Zambia (Kapembwa et al., 2011), 9% in Abidjan Cote d’ Ivoire (Rouet et al., 2004), 4% among patients attending Royal Victoria Teaching Hospital in Malawi (Mboto et al., 2010), 9.2% in Nigeria (Lesi et al., 2007), 3.9% in Ethiopia (Shimelis et al., 2008) and 6% in South Africa (Lodenyo et al., 2000). However, higher prevalence of co-infection has been reported such as; 53% HBV/HIV in Kisumu Kenya (Otedo, 2004), 15% in Tanzania (Telatela et al., 2007), 20.4% in Malawi (Nyirenda et al., 2008), 22.2% in Nigeria (Sud et al., 2001), 14.5% in Argentina (Fainboim et al., 1999), and 19.7% in South Africa (Hoffmann et al., 2008). Much lower prevalence of 3.9% in Ethiopia is recorded (Belay et al., 2010), 4.8% in South Africa (Di Bisceglie et al., 2010), 4.1% in Uganda and 2.4% in Rwanda (Pirillo et al., 2007).

2.6 Reactivation

Hepatitis B virus DNA persists in the body after infection, and in some people the disease recurs—although rare, reactivation is seen most often following alcohol or drug use (Villa et al., 2011) or in people with impaired immunity (Katz et al., 2008). HBV goes through cycles of replication and non-replication. Approximately 50% of overt carriers experience acute reactivation (Mastroianni et al., 2011). Males with baseline
ALT of 200 UL/L are three times more likely to develop a reactivation than people with lower levels. Although reactivation can occur spontaneously, people who undergo chemotherapy have a higher risk (Mastroianni et al., 2011). The risk of reactivation varies depending on the serological profile; those with detectable HBsAg in their blood are at the greatest risk, but those with only antibodies to the core antigen are also at risk. The presence of antibodies to the surface antigen, which are considered to be a marker of immunity, does not preclude reactivation. Treatment with prophylactic antiviral drugs can prevent the serious morbidity associated with HBV disease reactivation (Mastroianni et al., 2011).

2.7 Synergetic effect of HBV and HIV

HIV infection may modify the course of acute HBV, with a lower incidence of icteric illness and lower rates of natural clearance of HBV. HIV positive people with chronic HBV coinfection have greater levels of HBV DNA and lower rates of clearance of HBeAg (Piroth et al., 2007). The levels of serum transaminases appear lower in HBV/HIV coinfected individuals than in HBV monoinfected individuals. However, normal levels of transaminases do not mean that there is no underlying hepatic fibrosis. The risk of end-stage liver disease and liver cirrhosis is increased in cases of HBV/HIV-1 coinfection. There is an emergence of liver related diseases as a major cause of morbidity and even mortality that is not associated to HIV-infection in regions where effective antiretroviral therapy is available. Several cohort studies have shown that the
risk of liver-related deaths is 2-3 times higher in HBV/HIV coinfected patients than in patients with HIV monoinfection (14% as compared with 16%) (Annie, 2010). Coinfection of HIV and HBV is associated with recurrent flares of hepatic transaminases which can occur with immune reconstitution inflammatory syndrome (IRIS) due to antiretroviral therapy, interruption of HBV/HIV treatment or the development of resistance to HBV/HIV treatment; they also can occur spontaneously (Chauvel et al., 2007). Past research has not shown any clear cut lines on the impact of HBV on the disease course of HIV infection. Some studies had earlier showed that there is an increased rate of developing AIDS among HIV infected individuals who have been exposed to HBV (Eskild et al., 1992). Other studies found no change in the progression of HIV disease or survival of patients (Gilson et al., 1997).
2.8 Diagnosis of HBV infection

Various methods are used to characterize HBV infection including: serological markers shown in table 2.8. Others includes; virological marker HBV DNA and genotype; biochemical markers such as alanine aminotransferase (ALT); and Histological marker such the degree of hepatic fibrosis and inflammation (Danta, 2014).

Table 2.1: Interpretation of HBV Immunologic Markers

(Adapted from Mast et al., 2006)

<table>
<thead>
<tr>
<th>HBsAg*</th>
<th>HBcAb†</th>
<th>HBsAb‡</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Susceptible to HBV infection (should be vaccinated)</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>+</td>
<td>Immune because of vaccination</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Immune because of natural HBV infection</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>–</td>
<td>Acute or chronic HBV infection</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Interpretation unclear; four possibilities:</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1. Resolved HBV infection (most common)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. False-positive HBcAb, thus susceptible</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3. “Low-level” chronic HBV infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4. Resolving acute HBV infection</td>
</tr>
</tbody>
</table>

HBcAb - hepatitis B core antibody; HBsAb - hepatitis B surface antibody; HBsAg - hepatitis B surface antigen; + - positive test result; – negative test result. * - The presence of HBsAg indicates that the person is infectious. †-HBcAb appears at the onset of acute HBV infection. Presence may also indicate chronic HBV infection or a false-positive test. ‡-The presence of HBsAb indicates recovery and immunity from HBV infection or successful immunization against HBV.
2.8.1 Serological assays

**Hepatitis B surface antigen (HBsAg):** HBsAg is the established serological marker used routinely for the diagnosis of acute or chronic HBV infection, the screening of blood or organ donors, and the surveillance of persons at risk of acquiring or transmitting HBV. The antigen is usually detectable between week 4 and week 10 in acute infection. Chronic HBV infection is defined by the persistence of HBsAg for more than six months (Chan *et al.*, 2011; Janssen *et al.*, 2012).

**Antibody to core antigen (anti-HBc):** The anti-HBc appears at the onset of symptoms in acute hepatitis B and persists for life. The presence of anti-HBc indicates previous or ongoing infection with hepatitis B virus in an undefined time frame. In acute infection, anti-HBc immunoglobulin M (IgM) is found in high concentrations which gradually decline, complementing the corresponding increase in anti-HBc IgG over a three to six month period. Elevation of anti-HBc IgM usually signifies acute infection, but low elevations may also occur during the reactivation of chronic HBV. Anti-HBc IgG remains positive for life following exposure to HBV, however, unlike anti-HBs; anti-HBc is not a protective antibody.

**Antibody to e antigen (anti-HBe):** The HBeAg) is produced during active viral replication and may act as an immunogen or a tolerogen, leading to persistent infection (Milich & Liang, 2003). The loss of HBeAg and the development of anti-HBe is termed as HBeAg seroconversion, and has been used as an end-point for treatment in HBeAg-
positive people, as it has been shown that seroconversion is associated with a lower risk of disease progression (Milich & Liang, 2003).

2.8.2 Virological assays

**HBV DNA:** Polymerase chain reaction (PCR) and other molecular amplification technology have made it possible to directly quantify the level of HBV replication. Detection of HBV DNA is currently an integral part of HBV diagnosis and management (Lindh & Hannoun, 2005). The introduction of real-time PCR has improved performance of quantification of HBV DNA (Weiss *et al*., 2004).

**HBV Genotyping:** Involves sequencing the HBV genome. Currently about eight genotypes (A-H) have been identified. These genotypes vary geographically, with the four most common genotypes being A–D. The most prominent genotypes in the Asia-Pacific region are B and C (Fung & Lok, 2004). Currently, genotyping is only a research tool; patients are not routinely genotyped in Australia. However, it may become a relevant test in future clinical practice, to identify patients at greater risk for disease progression (Wai *et al*., 2002).

**Biochemical Assays:** The biochemical markers assessing the severity of the liver disease include: aspartate aminotransferase (AST) and alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase, bilirubin, and serum albumin and globulins, blood counts and prothrombin time, and hepatic ultrasound
(EASL, 2012). ALT level is the main biochemical marker used in viral hepatitis which is used as a surrogate marker for necroinflammation in the liver (Danta, 2014). Usually, ALT levels are higher than those of AST. However, when the disease progresses to cirrhosis, the ratio may be reversed. A progressive decline in serum albumin concentrations and/or increase of (gamma) globulins and prolongation of the prothrombin time, often accompanied by declining platelet counts, are characteristically observed after cirrhosis has developed. Studies show that between 12% and 43% of patients with chronic HBV and normal ALT levels have significant hepatic fibrosis (stage 2 fibrosis or greater) (Lai et al., 2005; EASL, 2012).

2.9 Prevention and Control of HBV

The prevention of chronic HBV infection has become a high priority in the global community (Abigael et al., 2010). Strategies include; (i) Preventing new HBV infections through immunization; (ii) Reducing morbidity and mortality from chronic HBV infections by providing appropriate medical referral, evaluation and management of chronically-infected persons. (iii) Assess the type and quality of data needed from state and local viral hepatitis surveillance systems to guide and evaluate prevention services (WHO, 2008).
CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

This was a cross sectional study that involved HIV positive individuals to determine the HBV prevalence, circulating genotypes, and immune-hepatic parameters in Kericho County.

3.2 Sampling Method

Systematic sampling was involved whereby every third patient attending the clinic was enrolled until the sample size was reached.

3.3 Study Site

The study was conducted in Kericho County. The County has a population of 752,396 (KNBS, 2009) and covers an area of 2,111km$^2$. The county has five sub county hospitals that offer CCC services. Study sites were selected based on the capacity to do all the baseline tests within the hospital. The three namely Kericho, Kapkatet and Londiani sub county hospital were used as the study sites.
3.4 Study Population

The study population was HIV patients attending comprehensive care clinic (CCC) at the three sub county hospitals in Kericho County.

3.4 Eligibility Criteria

3.4.1 Inclusion Criteria

HIV positive patients were recruited and enrolled into the study if they were; 15 years and above attending CCC of the three district hospital in Kericho, willing to give informed consent, willing to give blood samples for HIV and HBV testing and willing to secure time for a 20 minutes for interview.

3.4.2 Exclusion Criteria

Those patients who were excluded were; aged below 15 years, unwilling to give informed consent, unwilling to give blood samples for HIV and HBV testing and unwilling to secure time for a 20 minutes for interview.

3.5 Sample Size Determination

Sample size was determined using Lemeshow et al. (1990).

\[ n = \left( \frac{z}{m} \right)^2 p(1-p) \]
Where,

- $z$ is the critical value based on the desired confidence level (e.g., $z = 1.96$ for 95% confidence level);
- $m$ is the margin of error or precision of the estimate in this case $m = 0.05$.
- $p$ is the estimated value of the proportion. In this study, $p$ is the proportion of 0.07 (assumed prevalence of HIV/HBV, 7%), Day et al., (2013).

Thus $= 1.96^2 \times 0.07 \times 0.93 / 0.05^2 = 101$. A minimum of 101 HIV patients were recruited per each of the three district hospital giving a total of 303 HIV patients recruited in this study.

### 3.6 Sampling Procedure

The program officers of the three District hospital were briefed of the study and permission obtained from them to allow this study be nested in their HIV treatment programs. The program head provided us with the list of patients meeting recruitment criteria. The schedule of these patients were noted which was used to guide consenting and recruiting subjects in the study. The study thereafter conveniently recruited all accessible and consenting HIV patients from the three hospitals till the target was reached.
3.7 Recruitment Procedure

Study participation was purely on voluntarily basis. Before recruitment patients gave written informed consent (Appendix 1). The counselor explained to them the details of the study, including potential risks and benefits of participation. The participants were then assigned a participant identification number (PIN). This was to ensure anonymity for the study participants. The counselor then administered the structured questionnaire (Appendix 2) and thereafter the participant went to the laboratory with a PIN for blood collection.

3.8 Collection of Blood

Blood samples were drawn in the laboratory from consenting patients. About 10mls of venous blood was collected into commercially available anticoagulant (EDTA) treated tubes and plain tubes at equal volumes. The blood in EDTA tubes were used for immunological testing such as CD4 and for HBV serology. The blood in plain tubes was used for chemistry analysis (ALT, AST, and ALP). Upon collection blood in plain tubes were centrifuged after clotting to separate serum.
3.9 Laboratory Analysis

3.9.1 Immunological and chemistry analysis

The CD4 count was conducted with a FACSCount TM flow cytometer (Becton Dickinson, BD Biosciences, San Jose, USA). Blood chemistry was conducted with neurolizer (Diagnostics, Massy, France). All the immunological and chemistry were done according to manufacturer’s instructions.

3.9.2 HBsAg Detection

Serological testing to detect the HBsAg from HIV positive plasma was carried out using Hepanostika ELISA kit at the satellite blood transfusion laboratory in Kericho. This was done according to manufacturer’s instructions. Briefly, 0.1 ml of plasma sample was dispensed into a micro-ELISA plate, coated with anti-HBs antibody, and incubated at 37°C for 2 hours or let to stand at room temperature overnight. The wells were then washed four times with 0.3 ml of wash buffer. Labeled antibody (0.1 ml) was then added, incubated at 37°C for 2 hours and washed four times. Substrate solution (0.1 ml) was added, and incubated at room temperature for 50 minutes. Finally, 50 ul of H₂SO₄, 2M, was added to stop the reaction. The plates were read using a spectrophotometer.

(KCO,SOPs Hep001/2015)
3.9.3 DNA Extraction from Plasma

Viral genome extraction from plasma was carried out according to manufacturer’s instructions (SMITEST R&D RNA/DNA, Genome science Co. Ltd, Tokyo, Japan). Briefly, Enzyme solution, precipitation solution and sample diluent were put in a 1.5 ml tube. About 100 ul of plasma (virus) was added into the same tube. This was vortexed to mix and incubated at 55 °C for 30 minutes by dry thermo unit. Nucleic acid purification, protein lyses buffer were then added, mixed gently and incubated at 55°C. About 600ul cold 2-Propanol was then be added and put in ice for 15 minutes. After centrifugation (12,000g/ 4 °C/3mins), the supernatant was discarded and pellet washed with 70% alcohol by centrifugation at 12,000g/4 °C/3mins. The clear pellet obtained were air dried at room temperature for 15 min, resuspended with RNAse/DNAse free water and kept at –80°C until use.

3.9.4 Genotyping of HBV.

HBV genotypes were identified by PCR restriction fragment-length polymorphism of the surface gene of HBV. Briefly, DNA was extracted from 200 µL of plasma samples by using the QIAamp blood kit (Qiagen), and the fragment of the HBV genome between nucleotide positions 256 and 796 was then amplified. The PCR products were subsequently treated with restriction enzymes. After incubation, the samples were run on a 2% agarose gel and stained by ethidium bromide. Six genotypes (A-F) of HBV could be identified by the restriction patterns of DNA fragments. Unclassified genotype was
defined as an unpredicted or atypical restriction pattern. To avoid false-positive results, instructions to prevent cross-contamination were strictly followed, and results were considered valid only when they were obtained in duplicate. (Chih-lin Lin et al., 2005)

3.9.5 Amplification and sequencing of the pre-S gene.

The entire pre-S gene (564 nucleotides; nucleotide positions 2828-176) was further amplified and sequenced. This region was chosen because the pre-S gene is one of the most variable regions of the HBV genome (Uy et al., 2002). The first round primers were HBPr1 (GGGTACCATATTGCTGG) and HBPr135 (CA (A/G) AGACAAAAGAAATTGG) outer PCR while second round or Inner primers were HBPr2 (GAACAAGAGCTACAGGATGCATGG) and HBPr3 (CCACTGCTGCTGCTGAGGATG) sense and antisense primers consecutively (Stuyver et al., 2000). The master mix contained; DNA grade water -9ul, 10x PCR buffer -2ul, dNTPs- 2ul, first round primer pair-0.4ul and Taq polymerase 0.2 ul. Template DNA, (4.0ul) reaction was used for amplification. The cycling condition included over 40 cycles, of denaturation at 94°C for 30s, annealing at 50°C for 30s and elongation at 72°C for 30. Samples in first-round PCR were then amplified with nested PCR primers for 35 cycles with the same thermal profile.
3.9.6 Gel Electrophoresis of PCR Products

Amplified products were detected by electrophoretic analysis on 2% agarose gel containing 0.005% ethidium bromide and the results adjacent to DNA ladder (50 bp) were looked into under UV light. (Sambrook et al., 2001). After using ethidium bromide gels, contaminated gloves and other equipment (DRY wastes) are managed just as chemical hazardous waste are managed. The gels are dried under the hood in an open container before bringing to the waste room for disposal. (NOTE: The gels must not have running liquid in the bottom of the bag), but this does not apply to other chemical waste. Environmental Health and Safety (EHS) prefers the use of sealable, disposable plastic containers to store ethidium bromide gel waste, or wide mouth jars. Please note that these containers will not be given back to you (they are for one time use), nor does EHS supply these containers. Sturdy bags can also be used as well. In either case, there should be no free flowing liquids in these containers when they are brought to the waste room for subsequent disposal. (Guidelines for Ethidium Bromide Waste Management & Disposal, University of Tennessee-Knoxville)

3.10 Sequence Analysis

Sequences obtained were compared with published sequences from the same genomic region of 8 HBV genotypes available in National centre for Biotechnology Information (NCBI) Genbank. Alignment was performed using clustal w in MEGA 5 software
3.11 Data Management

All subjects were assigned a subject identification number (SID). The SID contained the patient’s initials and a serial number based on their enrollment. All data was entered into the study databases in Microsoft Excel (Microsoft, USA). The study maintained a double entry system for the data. All paper research records were kept in locked filing cabinet located in a restricted-access room at the research location.

3.12 Data Analysis

All statistical analyses were performed using STATA version 12 (Stata Corp LP, Texas, USA) at a significant level of \( p \leq 0.05 \). Data were presented in frequencies and percentages using tables and charts. Baseline characteristics were analyzed by using Kruskal–Wallis test (noncategorical variables) and \( \chi^2 \)-test or Fisher's exact test (categorical variables). Factors associated with HBV coinfection were determined using bivariate and multivariate analyses.

3.13 Ethical Considerations

The study was conducted according to the Declaration of Helsinki and International Conference on Harmonization Guideline on Good Clinical Practice (ICH-GCP). The protocol and informed consent form were reviewed and approved by the Jaramogi Oginga Odinga Teaching and Referral Hospital (JOORTH) research and ethics committee and the respective District Hospitals administration prior to any protocol-
related procedures being conducted. Written informed consent was obtained from each participant prior to any protocol-specified procedures being conducted. To maintain confidentiality, initials and coded numbers were used to identify the participants’ source documents and study reports. All study records were maintained in a secured location within the research location in Kericho. Participant information were not obtained or released without written permission from the participant/participant’s legally authorized representative except as necessary for monitoring of the study. Participation in this study was completely voluntary and the participants had a choice to withdraw even after accepting to participate.
CHAPTER FOUR

RESULTS

4.1 Subjects socio-demographic characteristics

A total of 303 HIV patients seeking ART treatment and care in Kericho County were recruited in the study. The patient’s socio-demographic characteristics are presented below.

4.1.1 Age

The mean age of the study participants was 34.4 (SD = 9.7; range 16-71) years. There were two age group peaks; 39.3% of the patients were aged 31 to 40 years and 32.7% aged between 21 to 30 years. Other age categories included 16.8% aged between 41 to 50 years and 6.9% aged above 51 years while the least 4.3% were aged ≤ 20 .(Figure 4.1.2)

4.1.2 Gender

In this study, 209 (69%) were female patients while less than a quarter (n = 94; 31%) were males patients. Female were significantly more than males ($\chi^2 = 43.647; df = 1; P = 0.001$). Across all age groups majority of the participants were females ($\chi^2 = 17.133; df = 4; P = 0.001$). Among those aged ≤ 20 years female were (12; 92.3%) while males were(1; 7.7%); those aged 21 to 30 years female were (76; 76.8%) while males were (23;
Among those aged 31 to 40 years (83; 69.7%) were females while (36; 30.3%) were males. For those aged 41 to 50 years (30; 58.8%) were females while (21; 41.2%) were male. Those aged ≥ 51 years males were more than female (13; 61.9%) while females were (8; 13%) as shown in figure 4.1.2.

Figure 4.1: Distribution of gender according to age

4.1.3 Education level

Majority (48.5%) of the patients had secondary level of education followed by 30% and 21.5% patients who had primary and tertiary level education respectively. Education levels was significantly different ($\chi^2 = 34.772; \text{df} = 2; P = 0.001$).
4.1.4 Marital status

More than half of the patients (62%) were currently married, 24.5% were single (never married before) while only 13.5% were separated, divorced or widowed. Marital status was significantly different ($\chi^2 = 117.8; \text{df} = 2; P = 0.001$).

4.1.5 Occupation

About 47.5% of the patients were not engaged in any form of employment, 25.1% were engaged either in formal or informal employment followed by 23.8% and 3.6% patients who were farmers and business persons respectively ($\chi^2 = 117.02; \text{df} = 3; P = 0.001$).

4.1.6 Region of origin

Most of the patients originated from rural areas compared to the urban 64.4% versus 35.6% ($\chi^2 = 24.98; \text{df} = 1; P = 0.001$).

4.1.7 Duration for HIV

There was near distribution of patients and the duration for which they have lived with HIV; About 35.6% had lived with HIV for a duration of more than 12 months followed by 33.7% and 30.7% who had lived with HIV for between 6 to 12 months and below 6 months respectively. Duration with HIV was not statistically significant ($\chi^2 = 1.129; \text{df} = 2; P = 0.581$).
4.1.8 ART treatment

Patients who were already receiving ART treatment were almost equal to those who were currently not on ART 54.5% with 45.5% respectively ($\chi^2 = 2.406; df = 1; P = 0.135$).

4.1.9 HBV immunization

Almost all of the patients (94.4%) had not taken the HBV immunization and only 5.6% had been immunized ($\chi^2 = 238.815; df = 1; P = 0.001$).

4.2 Patients’ immuno-hepatic characteristics

Table 4.2 shows the immuno-hepatic parameters of the study participants. The mean CD4 was 259.5 (± SD 129.1) cells per ml. The median was 234 ranging from 20 to 852 cells per ml. About three quarters (75.6%) of the patients had CD4 < 350 cells/ml considered immunological failure while only 24.4% had immunological suppression (CD4 count above 350 cells/ml) at the time of enrolment ($\chi^2 = 79.29; df = 1; P = 0.001$).

The mean Alanine aminotransferase (ALT) level was 32.9 (± SD 18.95) U/L and a median of 28 U/L ranging from 8 to 225 U/L. Majority (83.5%) of the patients’ ALT level was within the normal range of 0 to 45 U/L compared to only 16.5% who had elevated ALT levels of >45 U/L associated with hepatotoxicity ($\chi^2 = 136.003; df = 1; P = 0.001$).
The mean Aspartate aminotransferase (AST) level was 36.7 (± SD 14.7) U/L and a median of 34 U/L ranging between 14 to 131 U/L. Slightly above half (63.4%) of the patients’ AST level were within the normal range of <37U/L while 36.6% had elevated AST levels of >37.1 U/L ($\chi^2 = 21.653; df = 1; P = 0.001$).

The mean alkaline phosphatase (ALP) level was 156.9 (± SD 59.6) U/L and a median of 143.6 U/L ranging from 38.3 to 316.4 U/L. There was no difference between the proportion of patients who had normal levels of <147 U/L and those who had abnormal levels of > 147 U/L ($\chi^2 = 0.267; df = 1; P = 0.641$).
Table 4.1: Patients’ immune-hepatic parameters

<table>
<thead>
<tr>
<th>Immuno Hepatic Characteristic</th>
<th>Sample size</th>
<th>$\chi^2$</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td></td>
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<tr>
<td>CD4 (cells/ml)</td>
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<tr>
<td>Mean (± SD 129.1) = 259.5</td>
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<tr>
<td>Median = 234</td>
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<tr>
<td>Range = 832 (20 - 852)</td>
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<tr>
<td>≤350</td>
<td>229</td>
<td>75.6</td>
<td>79.29</td>
<td>1</td>
</tr>
<tr>
<td>&gt;350</td>
<td>74</td>
<td>24.4</td>
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<tr>
<td>ALT (U/L)</td>
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<tr>
<td>Mean (± SD 18.95) = 32.9</td>
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<tr>
<td>Median = 28</td>
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<tr>
<td>Range = 217 (8-225)</td>
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<tr>
<td>≤45</td>
<td>253</td>
<td>83.5</td>
<td>136.003</td>
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<td>&gt;45</td>
<td>50</td>
<td>16.5</td>
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<tr>
<td>AST (U/L)</td>
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<tr>
<td>Mean (± SD 14.7) = 36.7</td>
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<tr>
<td>Median = 34</td>
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<tr>
<td>Range = 117 (14 - 131)</td>
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<tr>
<td>≤37</td>
<td>192</td>
<td>63.4</td>
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<tr>
<td>&gt;37</td>
<td>111</td>
<td>36.6</td>
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<tr>
<td>ALP (U/L)</td>
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<tr>
<td>Mean (± SD 59.6) = 156.9</td>
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<tr>
<td>Median = 143.6</td>
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<td></td>
</tr>
<tr>
<td>Range = 278 (38.34-316.4)</td>
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<td></td>
</tr>
<tr>
<td>≤147 U/L</td>
<td>156</td>
<td>51.5</td>
<td>0.267</td>
<td>1</td>
</tr>
<tr>
<td>&gt;147 U/L</td>
<td>147</td>
<td>48.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ml - milliliters; L - Liters; U - Units; No - Number; % - Percentage; $\chi^2$ - Chi square; df - Degree of freedom; P - Level of significance; P ≤ 0.05 indicates the relationship is significant
4.3 Prevalence of hepatitis B virus among HIV patients in Kericho County

Among the 303 HIV patients, only 15 (5%) of them were positive for Hepatitis B virus compared to 288 (95%) who were sero-negative as shown in figure 4.3.

![Figure 4.2: Seroprevalence of HBV among study patients](image)

4.4 Prevalence of hepatitis B genotypes among HIV patients in Kericho County

Among the 15 HIV patients found co-infected with HBV, majority 14 (93%) were HBV genotype A and only 1 (7%) was HBV genotype D as shown in figure 4.3. Sequences
obtained were compared with published sequences from the same genomic region of 8 HBV genotypes available in National centre for Biotechnology Information (NCBI) Genbank. Alignment was performed using clustal w in MEGA 5 software.

**Figure 4.3: Prevalence of HBV genotypes among study patients**

**Table 4.5 socio-demographic characteristics of HBV/HIV co-infection and HIV mono-infection**

Table 4.5 highlights the socio-demographic characteristics of patients with HBV/HIV co-infection and those with HIV mono-infection. Most of the HBV/HIV co-infected patients were males 66.7% while most HIV mono-infection was among the female 70.8% (P = 0.004).
Around 80% of the HBV/HIV co-infected patients were aged 31 to 40 years versus 100% aged >51 years for HIV mono-infection (P = 0.024).

Patients who were currently married were most affected in both cases 66.7% HBV/HIV co-infection and 61.8% HIV mono-infection. There was no differences between those HBV/HIV co-infected and those HIV-mono-infected and marital status (P = 0.513).

There was no differences between patients HBV/HIV co-infected and those HIV-mono-infected and across the hospital of treatment (P = 0.413).

The distribution of both HBV/HIV co-infection and HIV-mono-infection was almost similar among different occupation type (P = 0.851).

Most of the HBV/HIV co-infected patients (60%) were from the urban settings while more than half (65.6%) of the HIV-mono-infection were from the rural area (P = 0.043).

There was no significant differences in the distribution of patients HBV/HIV co-infected and those HIV-mono-infected with the duration of HIV infection (P = 0.413).

Most of the HBV/HIV co-infection (86.7%) occurred among patients who were on ART; similarly half (52.8%) of the HIV-mono-infection occurred among patients on ART (P = 0.008).
Table 4.2: Socio-demographic characteristics of HBV/HIV co-infection and HIV mono-infection

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample size</th>
<th>HBV/HIV co-infection</th>
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4.6 Immuno-hepatic characteristics of HBV/HIV co-infection and HIV mono-infection

Table 4.6 shows the immunological and hepatic parameters of the study participants. The HBV/HIV co-infected patients had a lower mean CD4 - immunological failure (lower CD4 mean of 137.2 cells/ml and range of 20 to 260 cells/ml) compared to HIV mono-infected individual who had higher mean CD4 (lower CD4 mean of 265.9 cells/ml and range of 33 to 852 cells/ml). Further, all (100%) the HBV/HIV co-infected had their CD4 below 350 cells/ml, the level considered low and warranting ART initiation and about 74% of the HIV mono-infected individuals (P = 0.013).

The HBV/HIV co-infected patients had a higher mean ALT – levels considered hepatotoxic (mean ALT of 62 U/L) while for the HIV mono-infected patients the mean ALT was 31.4 U/L – levels considered normal. Almost all (93.3%) of the HBV/HIV co-infected had ALT levels above >45 U/L, the level considered hepatotoxic and only 12.5% of the HIV mono-infected patients had ALT above 45U/L (P = 0.001).

The HBV/HIV co-infected patients had a higher mean AST – levels considered hepatotoxic (mean AST of 65.5 U/L) and mean AST of 35.2 U/L – levels considered normal for the HIV mono-infected patients. Almost all (93.3%) of the HBV/HIV co-infected had AST levels above >37 U/L, the level considered hepatotoxic while 33.7% of the HIV mono-infected patients had AST above 37 U/L (P = 0.001).
The HBV/HIV co-infected patients had a higher mean ALP – levels considered hepatotoxic (mean ALP of 175.2 U/L) while the HIV mono-infected patients had a mean ALP of 155.9 U/L, levels considered close to normal. Over half (66.7%) of the HBV/HIV co-infected had ALP levels above >147 U/L, the level considered hepatotoxic and 47.6% of the HIV mono-infected patients had ALP above 147 U/L. The ALP levels was not significantly different between the HBV/HIV co-infected and those with HIV mono-infection (P = 0.119).
Table 4.3: Immuno-hepatic characteristics of HBV/HIV co-infection and HIV mono-infection

<table>
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<th>HIV mono-infection</th>
<th>P value</th>
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<td>217 (8 - 225)</td>
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4.7 Factors associated with HBV infection

Table 4.7 highlights factors associated with hepatitis B infection among HIV patients attending various hospitals in Kericho County. In bivariate analysis, female patients were less likely to be infected with HBV compared to male patients OR 0.22 (95% CI 0.08 to 0.7). Patients who had normal ALT (≤ 45 U/L) were less likely to be infected...
with HBV compared to patients with elevated ALT levels of > 45 U/L OR 0.01 (95% CI 0.001 to 0.1). Further, patients who had normal AST (≤ 37 U/L) were less likely to be infected with HBV compared to patients with elevated AST levels of > 37 UL OR 0.04 (95% CI 0.005 to 0.3). On the other hand patients who had initiated ART treatment were more likely to be infected with HBV compared to the ART naïve patients OR 5.4 (95% CI 1.2 to 24.1).

In multivariate analysis, patients who had initiated ART treatment were independently more likely to be infected with HBV OR 12 (95% CI 1.5 to 99). On the contrary, patients who had normal ALT were almost 2% less likely to be infected with HBV OR 0.02 (95% CI 0.003 to 4).
Table 4.4: highlights factors associated with hepatitis B infection among HIV patients

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No - Number; % - Percentage; PR - Prevalence ratio; CI - confidence interval; ND - Not done
CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1. Prevalence of HBV-HIV co-infection

Our study found a prevalence of 5% HBV/ HIV co-infection. Similar prevalence of co-infection has been reported in Kenya example, Kerubo et al. (2015) reported prevalence of 4.26% HBV/HIV co-infection in Nairobi, while Harania et al., (2008) and Muruiki et al., (2013) both reported a 6% co-infection prevalence in Nairobi at different time point. The HBV/HIV co-infection rates was also consistent with findings from other regions such as 9.9% HBV/HIV co-infection in Lusaka Zambia (Kapembwa et al., 2011), 9% in Abidjan Cote d’ Ivore (Rouet et al., 2004), 4% among patients attending Royal Victoria Teaching Hospital in Malawi (Mboto et al., 2010), 9.2% in Nigeria (Lesi et al., 2007), 3.9% in Ethiopia (Shimelis et al., 2008) and 6% in South Africa (Lodenyo et al., 2000). However, these findings were low compared to those previous studies such as; 53% HBV/HIV co-infection obtained in Kisumu Kenya (Otedo, 2004), 15% in Tanzania (Telatela et al., 2007), 20.4% in Malawi (Nyirenda et al., 2008), 22.2% in Nigeria (Sud et al., 2001), 14.5% in Argentina (Fainboim et al., 1999), and 19.7% in South Africa (Hoffmann et al., 2008). The prevalence in our study was found to be higher than those obtained 3.9% in Ethiopia (Belay et al., 2010), 4.8% in South Africa (Di Bisceglie et al., 2010), 4.1% in Uganda and 2.4% in Rwanda (Pirillo et al., 2007). The observed diverse prevalence rates of HBV/HIV co-infection vary according to the risk factors involved,
socioeconomic status, initial burden of infectious markers in the community, which vary from one country to another and even between different regions within the same country, sample size, test kit sensitivity and specificity (Taiwo et al., 2012).

5.1.1. HBV genotype

In the current study HBV genotype A constituted 93% of all the genotypes identified while only 7% being genotype D. This is not surprising given that Genotype A has been shown to be widespread in sub-Saharan Africa, Northern Europe, and Western Africa while genotype D is dominant in Africa, Europe, Mediterranean countries, and India (Sugauchi et al., 2003). Previous studies in Kenya shows consistent pattern with our data, HBV variants among blood donor in Kenya indicates the predominance of genotype A (88%) followed by E (8%) and D (4%) (Mwangi et al., 2008). Among the HBV/HIV-co-Infected Injecting Drug Users from Mombasa, the HBV genotype A was the most common (Kibaya et al., 2015). Ochwoto et al., (2013) also reported similar trend as our studyof predominance of HBV genotype A and a few genotype D. Consistently studies have shown genotype A as the most common in blood donors, commercial sex workers and HIV infected patients on antiretroviral treatment (ART) in Kenya while genotypes D and E occur less frequently (Mwangi et al., 2008; Day et al., 2013; Kwange et al., 2013). In other parts of Africa consistency of genotype A predominance is reported such as in South Africa (Kimbi et al., 2004; Kramvis and Kew, 2007; Firnhabera et al., 2012).
Lodenyo et al., (2000) on the contrary in a study among liver patients in Kenya identified predominance of D. Studies identifying the circulating HBV genotypes in Kenya is key given that HBV genotypes have been shown to influence disease severity and response to antiviral treatment (Araujo et al., 2011; Gerlich et al., 2013;).

5.1.2. Consequences of HBV/HIV co-infection on immune hepatic parameters

The HBV/HIV co-infected patients had a lower mean CD4 137.2 cells/ml compared to mean of 265.9 cells/ml for those who HIV mono-infection. This observation was similar with other studies which showed a lower mean CD4 for HBV/HIV positive group than that of the HBV DNA-negative HIV patients in South Africa (Adrian et al., 2010; Firnhabera et al., 2012), in Nigeria (Tripathi et al., 2007), in Ethiopia (Yitayih et al., 2013), In Ghana (Olawumi et al., 2014) and in India (Olufemi et al., 2009). This observation is worrying since the progression of hepatic complications from HBV infection is accelerated in patients co-infected with HIV (Puoti et al., 2006; Thio, 2009). This is particularly so in HIV infected men with very low CD4 count (Bodsworth, 1991). In addition, HIV infected individuals are more likely to lose previously developed protective anti-HBs antibody and develop acute hepatitis B infection; this risk is also associated with lower CD4 counts (Biggar et al., 1987). The risk of hepatocellular carcinoma (HCC) in HIV infected patients who are co-infected with hepatitis B virus is also increased in individuals with lower CD4 counts (Clifford et al., 2008).
Liver enzymes alanine transaminase (ALT); aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in HBV-HIV co-infected were elevated than the HIV mono-infected patients. This is consistent with a previous study done in Ghana (Olawumi et al., 2014), in Nigeria (Otegbayo et al., 2008) in South Tamil Nadu (Ganesh et al., 2010), in Ethiopia (Yitayih et al., 2013) and in South Africa (Adrian et al., 2010; Firnhabera et al., 2012). These liver enzyme levels difference between different studies may be due to difference in study design, duration of the viral hepatitis infection as well as the patient’s condition like having chronic alcoholism or other drug induced hepatotoxicity. In addition, HIV can also infect the hepatic or kupffer cells that may further contribute for the development of liver fibrosis and raised liver enzyme levels (Cao et al., 1992).

5.1.3 Factors for HBV/HIV co-infection

The finding that the mono and co-infected patients shared similar demographic characteristics with respect to education level, marital status, occupation and duration with HIV was also reported by other authors (Teltela et al., 2007; Sadoh et al., 2011). Further, studies have reported positive associations between HIV/HBV co-infection and such factors as sex, age, education level, intravenous drug use and homosexual activity (Mohammadi et al., 2009; Freitas et al., 2014). The mean age of the mono-infected patients, however, was higher in this study. Those co-infected in this study were slightly older in age. This may be attributed to the fact that this age group was exposed before the current prevention strategies were available (inclusion of HBV in the NPI schedule
in 2004). According to Sadoh et al., (2011) immunization did not make any significant difference in the prevalence of co-infection. This conclusion, in our view is presumptuous because the study analyzed only the hepatitis B immunization history of the under-five instead of the whole study population.

In our study females were less likely to be HBV/HIV co-infected. In Brazil Freitas et al., (2014) showed that HBV infection was significantly associated with male gender. A predominance of the male gender has also been reported previously in other studies possibly due to high-risk behaviors for HBV infection increasing sexual and percutaneous exposure (Brag et al., 2006; Cortés et al., 2009).

The HBV/HIV infection occurred most frequently among the patients aged beyond 31 years of age. Freitas et al., (2014) also showed that of HBV infection was significantly associated with age over 35 years. Further, this finding was in line with previous studies, which reported that older age was associated with a higher risk of HBV exposure (Brag et al., 2006; Cortés et al., 2009). Family history of hepatitis, use of illicit drug and homosexual HBV infection within this age group is attributed to being male, a history of operations, a family member who is HBsAg positive, and not being immunized (CDC, 2006; Lin et al., 2007).

In this study all the HBV/HIV co-infection occurred among patients who recorded not having received HBV immunization. This is in line with other studies performed in southern Brazil between 2009 and 2010 showed that the vaccination coverage of
children and adolescents who were born after the hepatitis B vaccination was introduced was over 92% and that the HBsAg and anti-HBc prevalence were less than 1% and 10%, respectively (Tonial et al., 2011). Hepatitis B virus vaccination began in 1992 for children younger than five years of age and subsequently expanded to health professionals, students, firefighters, police and the military in 1994 and to adults under 20 years old in 2011; Livramento et al., 2011 Considering that the marked reduction in the prevalence of HBV infection markers among the general population is primarily a result of immunization against hepatitis B, vaccination may have also contributed to the decreased prevalence of HBsAg and anti-HBc (Scaraveli et al., 2011).

Those who were already on ART treatment had more cases of HBV/HIV co-infection. Studies have shown that most ARV is prone to the development of drug resistance. In many HIV/HBV co-infected patients, resistance presages a return of higher level viremia and in some of these patients further liver injury develops. In addition, HIV-infected patients co-infected with HBV have an increased risk for antiretroviral therapy-related hepatotoxicity, particularly when HBV DNA levels exceed 10,000 copies/ml (Hoffmann et al., 2008).

Patients with HBV/HIV co-infection were likely to have elevated liver enzymes (ALT, AST and ALP). Studies have shown that an individual patient’s liver enzymes levels are an important factor in the decision to initiate the treatment of chronic HBV. The elevated liver enzymes such as ALT levels indicate immune-mediated inflammation to eliminate
HBV-infected hepatocytes and a higher rate of hepatitis B virus e antigen (HBeAg) seroconversion (Liaw et al., 2005; Lok and McMahon, 2007).

Other factors that have been shown to be associated with HBV/HIV co-infection that we either did not find or measure included family history of hepatitis, use of illicit drug and homosexual behavior (Freitas et al., 2014).
5.2. Conclusions

Based on the findings of this study the following conclusions can be drawn

1. The results of this study suggest that the prevalence of HIV-HBV co infection in Kericho County is 5%.

2. This study re-affirms the predominance of HBV genotype A and to a less extent HBV genotype D. This is not surprising given that Genotype A has been shown to be widespread in sub-Saharan Africa, Northern Europe, and Western Africa while genotype D is dominant in Africa, Europe, Mediterranean countries, and India (Sugauchi et al. 2003).

3. The HBV/HIV co-infected patients were associated with lower mean CD4 compared to HIV mono-infection patients.

4. Liver enzymes alanine transaminase (ALT); aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in HBV-HIV co-infected were elevated than the HIV mono-infected patients. Though consistent with other studies, the minor difference in the actual mean may be due to difference in the duration of the viral hepatitis infection as well as the patient’s condition like having chronic alcoholism or other drug induced hepatotoxicity.
5.3 Recommendations

This and similar studies brings to point an important aspect to consider when designing management for HIV infected patients.

1. Standard HBV vaccination schedule is an important strategy for lowering the incidence of HBV infection among HIV-infected individuals. Also detecting and immunizing susceptible individuals.

2. More research should be done on Hepatitis B genotyping to differentiate the clinical outcomes of different genotypes and the same should be accessible.

3. Hepatitis B screening should be included as a baseline test together with CD4 and liver enzymes tests among HIV infected individuals.

4. Epidemiological studies would be instrumental to better understand the risk factors and mechanism of infection, and the prevailing genotypes. This would also provide avenues for preventive efforts.
REFERENCES


Cao, YZ, Dieterich, D, Thomas, PA, Huang, YX, Mirabile, M, & Ho, DD. (1992). Identification and quantitation of HIV-1 in the liver of patients with AIDS. *AIDS*, 6, 65-70.


Fung, S, Mazzulli, T, Sherman, M, & Popovic, V. (2009). Tenofovir (TDF) is effective in lamivudine (LAM)-resistant chronic hepatitis B patients who harbor rtA194T at baseline. 60th Annual Meeting of the American Association for the Study of Liver Diseases. Boston, U.S.A.


Gerlich, WH. (2013). Medical virology of hepatitis B: how it began and where we are now. *Virol J.* 10, 239.


Wai, CT, Chu, CJ, Hussain, M, & Lok AS. (2002). HBV genotype B is associated with better response to interferon therapy in HBeAg (+) chronic hepatitis than genotype C. *Hepatology. 36*(6), 1425-30.


Yitayih, W, Meseret, A, Fanaye, A & Yeshambel, B. (2013). *HBV and HCV seroprevalence and their correlation with CD4 cells and liver enzymes among HIV positive individuals at University of Gondar Teaching Hospital, Northwest Ethiopia*: University of Gondar.
Appendix 1: Informed Consent Form

Project title: Hepatitis B seroprevalence among HIV positive individuals in selected health facilities in Kericho County, 2014

Investigator: Chepkurui Lily Kirui

Introduction: I’m a researcher from JKUAT and am doing a study on seroprevalence of hepatitis B among HIV positive patients. The research will help identify the strains of circulating HBV and also determine the prevalence of HBV among infected individuals, and forms a basis on future monitoring of HIV/HBV co infection.

Confidentiality: In this study, all participants are given code numbers and not participant names thus nobody can track the results to you. The findings of this study will be kept confidential in coded records that will not be traced to individuals. In case you want to contact the principle investigator of this research project for whatever reasons you are free to do so any time on the following telephone number 0722978547.

Risks involved: There are no risks involved in the study.

Benefits: The benefit involved in this study is that when you are found co-infected with HBV you will receive further management in terms of drugs and also care.
**Reimbursement:** There is no direct compensation for participation in the study.

**Requirements:** 10 mls of blood will be drawn from you for liver profile and Hepatitis B virus testing. The procedure has minimal pain when drawing blood but will fade off after the injection. Your names will not be used when labeling the specimens but only special codes.

**Signature:** I confirm that I understand the information provided to me for the above study and that I have had the opportunity to ask questions. I understand that my participation is voluntary and that I am free to withdraw from the study at any time without any consequences.

I __________________________________________ agree to participate in the study.

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<th>Participant Name</th>
<th>Participant Signature/ Thumb print</th>
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<th>Study Staff Conducting</th>
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Appendix 2: Questionnaire

Date of interview: Day [ ] Month [ ] Year [ ]

1. Code ______________________

2. Age ______________________

3. Sex

   1) Male [ ]

   2) Female [ ]

4. Residence ___________________________

5. Level of Education

   1) Primary [ ]

   2) Secondary [ ]

   3) Tertiary [ ]

   4) None [ ]

6. Marital Status

   1) Married [ ] a) Monogamous [ ] b) polygamous [ ]

   2) Single [ ]

   3) Divorced [ ]

   4) Widowed [ ]

7. Occupation

   1) Farmer [ ]

   2) Civil servant [ ]
3) None [   ]
4) Other [   ] specify …………………….

8. Have you been immunized against Hepatitis B?
   1) Yes [   ]
   2) No [   ]

9. How long have you known your status?
   1) Less than 6 months [   ]
   2) 6 months—1 year [   ]
   3) More than 1 year [   ]

**Laboratory Results**

1) Hepatitis.
   1) Negative [   ]
   2) Positive [   ] if positive, indicate strain________________

2) Liver function test
   1) ALT ___________________________
   2) AST ___________________________
   3) ALP___________________________

3) CD4 Count ___________________________
Appendix 3: Ethical Approval

MINISTRY OF HEALTH
JARAMOGI ONGINGA ONGINGA TEACHING &
REFERRAL HOSPITAL
P.O. BOX 849
KISUMU

Date  20th May, 2014

Ref: ERC1B/VOL1/113

Chepkurui Lily Kirui,
JKUAT.

Dear Lily,

RE: FORMAL APPROVAL TO CONDUCT RESEARCH TITLED: “HEPATITIS B SEROPREVALENCE AMONG HIV INFECTED INDIVIDUALS SEEKING CARE AT SELECTED HOSPITALS IN KERICHO COUNTY, KENYA

The JOOTRH ERC (ACCREDITATION NO. 01713) has reviewed your protocol and found it ethically satisfactory. You are, therefore, permitted to commence your study immediately. Note that this approval is granted for a period of one year (20th May, 2014 to 21st May, 2015). If it is necessary to proceed with this research beyond the approved period, you will be required to apply for further extension.

Also note that you will be required to notify the committee of any protocol amendment(s), serious or unexpected outcomes related to the conduct of the study or termination for any reason.

Finally, note that you will also be required to share the findings of the study in both hard and soft copies upon completion.

The JOOTRH ERC takes this opportunity to thank you for choosing this institution and wishes you the best in your endeavours.

Yours sincerely,

FRED O. AKWATTA,
SECRETARY – ERC,
JOOTRH – KISUMU.
Appendix 4: Publication

Hepatitis B seroprevalence among HIV infected individuals seeking care at selected hospitals in Kericho County, Kenya, 2014

Lily Chepkurui
Zainab Gura
Fredrick Odhiambo
Andrew Nyerere