SERO-PREVALENCE, GENOTYPES AND RISK FACTORS ASSOCIATED WITH HEPATITIS B VIRUS INFECTIONS AMONG HIV INFECTED PATIENTS ATTENDING MALINDI SUB-COUNTY HOSPITAL IN KENYA

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Sero-prevalence, Genotypes and Risk Factors Associated with Hepatitis B Virus Infections among HIV Infected Patients Attending Malindi Sub-county Hospital in Kenya

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DECLARATION

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

Signature…………………………………….. Date……………………………

James Gitau Komu

This thesis has been submitted for examination with our approval as the University supervisors.

Signature…………………………………….. Date……………………………

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JKUAT, Kenya

Signature…………………………………….. Date……………………………

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KEMRI, Kenya

DEDICATION

I dedicate this work to my beloved wife Noel Mumbi, our son Cyprian Komu and my loving parents Mr. and Mrs. Michael Komu Gitau for their moral, spiritual support, and encouragement to keep me going during this study.

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ABBREVIATIONS AND ACRONYMS

3TC	Lamuvidine
ABI	Applied Bio-systems
Ag	Antigen
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
AZT	Zidovudine
CCC	Comprehensive care centre
cDNA	Complementary DNA
CD4	Cluster of Determination 4
CI	Confidence Interval
CSW	Commercial Sex Worker
CVR	Centre for Virus Research
DDBJ	DNA Data Base of Japan
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide Triphosphates
EFV	Efivarenz
ELISA	Enzyme linked immunosorbent assay
ERC	Ethical review committee
HBeAg	Hepatitis B E antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus

HCC	Hepatocellular carcinoma
HIV	Human immunodeficiency virus
HRP	Horse Radish Peroxidase
IDU	intravenous drug use
IgG	Immunoglobulin G
ITROMID	Institute of Tropical Medicine and Infectious diseases.
IQR	Inter Quartile Range
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
MEGA 4	Molecular Evolutionary Genetic Analysis 4
NVP	Nevirapine
OR	Odds Ratio
ORF	Open reading frame
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
SD	Standard deviation
SPSS	Statistical package for social sciences software
SSC	Scientific Steering Committee
TDF	Tenofovir
TMB	Tetramethylbenzidine

ABSTRACT

Human Immunodeficiency Virus (HIV) and Hepatitis B Virus (HBV) co-infections are common all over the world. Several studies have shown that HIV infection modifies the course of HBV infection by increasing rates of chronicity, prolonging the time the HBV stays in circulation and increasing liver-related morbidity. Factors such as intravenous drug use, multiple blood transfusions, presence of tattoos, unsafe sexual practices and being health workers have been implicated as drivers of infection & transmission of HBV & HIV. Determining the HBV genotypes and their diversity among HIV positive patients would help in their appropriate therapeutic management. This study was aimed at determining the sero-prevalence and genotypes of HBV isolated from HIV patients as well as the associated risk factors among patients attending the comprehensive care center at Malindi Sub-county Hospital in a descriptive cross-sectional study. Malindi was chosen as a suitable study site because of the high numbers of residents involved in sex tourism as well as intravenous drug use. A total of 446 HIV positive patients attending the CCC were recruited in this study upon consenting. Social demographic, clinical history and risk factors of the participants was obtained using a structured questionnaire. Five millilitres of whole blood was obtained from each participant, 50µl of which was used for CD4 cell counts using a flow cytometer. HBsAg serology was done using Hepanostika® HBsAg Ultra ELISA kit (BioMérieux SA) and HBV DNA was extracted from all HBsAg positive samples. Nested polymerise chain reaction (PCR) and sequencing of the Pre S1 region was done. Independent t test was used for comparison of means for the CD4 counts between the HIV mono-infected and HIV-HBV mono-infected. STATA was used to perform bivariate and multivariate analysis to determine factors associated with HBV infections using Poisson regression with significance set at $P \leq 0.05$ and odds ratio with corresponding 95% confidence interval. Sample sequences were compared with published HBV genotypes sequences from GenBank and Phylogenetic trees were constructed using the NJ Plot software using a PHB file created through the DNA Database of Japan (DDBJ) to determine the HBV genotypes. Out of the 446 HIV positive participants screened, 126 (28.3%) were males and 320 (71.7%) females. The mean age of males

was 44.93 years (SD ±10.16) while that of females was 42.22 years (SD ±10.88). Based on ELISA, 22/446 (4.93%) participants had HIV-HBV co-infection. Co-infection among males was 8.7% (11/126) compared to 3.4% (11/320) among females. Only 12 of the 22 positive samples by ELISA amplified via PCR. Out of the 12 HBV DNA positive samples 10 were successfully sequenced. Phylogenetic analysis of the Pre S1 region revealed that 9/10 (90%) samples belonged to genotype A while 1/10 (10%) belonged to genotype E. Males (p=0.028) and intravenous drug use (p= 0.008) were significantly associated HBV infections. The high prevalence (4.9%) of HBV among HIV patients attending Malindi Sub-county hospital is most likely highly driven by intravenous drug use and multiple sexual partners among the male gender. The distribution of HBV genotypes among HIV patients attending Malindi Sub-county hospital was similar to that previously reported in the general population. This study recommends for a continuous surveillance of HBV-HIV co-infections as well as analysis of HBV genotypes in different risk groups since they have an impact on disease management. Public awareness on the risk factors associated with the co-infection should also be emphasised.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The term hepatitis refers to a medical condition defined by the inflammation of the liver. Viruses cause most cases of hepatitis. The type of hepatitis is named for the virus that causes it. Hepatitis can also occur as a result of the liver being exposed to harmful substances such as alcohol (Haaheim *et al.,* 2002; Kudesia & Wreighitt, 2009).

Hepatitis B virus (HBV) infection is considered as one of the most important public health problems. Human immunodeficiency virus (HIV), positive patients are at a high risk of contracting HBV infection because the two viruses have shared transmission routes. (Pourkarim *et al.,* 2011). Infection with the HBV has several consequences, which may range from acute hepatitis, fulminant hepatitis, chronic asymptomatic carrier state, chronic hepatitis, cirrhosis or hepatocellular carcinoma (Haaheim *et al.,* 2002).

In the world, it's approximated that 33 million people are infected with HIV and among these about 3 million (10%) are co-infected with HBV (Kourtis *et al.,* 2012). In Kenya the prevalence of HBV-HIV co-infection has been reported to be as high as 6% and significantly higher in males 19.2% compared to 14.2% in females (Muriuki *et al.,* 2013).

HBV and hepatitis C virus (HCV) co-infections among HIV infected individuals are growing worldwide due to inaccessibility to effective vaccines, need for expensive treatment, chronicity of morbidity and associated mortality (Muriuki *et al.,* 2013). Individuals with HIV-HBV co-infection have higher HBV DNA levels (Dore *et al.,* 2010). Increased mortalities and morbidity in HIV patients as a result of liver disease are partly due to co-infection with HBV and HCV since these viruses increase intra-hepatic apoptosis which is a consequence of liver fibrosis (Diwe *et al.,* 2013).

HBV has a wide genetic variability which has resulted into the emergence of 10 genotypes with several sub-genotypes which are labelled alphabetically from A-J (Tangkijvanich *et al.,* 2013). There is remarkable evidence suggesting that HBV genetic variability is an important factor in determining disease progression and also response to antiviral treatment (Poukarim *et al.,* 2011).

HBV is more prevalent among HIV–infected individuals than in the general population which can be attributed to the shared risk factors for transmission and acquisition of these two life threatening viruses (Chen *et al.,* 2013). Some of these risk factors associated with the transmission of HIV and HBV include: involvement in intravenous drug use, scarification/tattooing, having several heterosexual partners/involvement in commercial sex, male having sex with men and occupational hazards for health workers (Alter, 2006; Chen *et al.,* 2013; Ranjbar *et al.,* 2013).

1.2 Statement of the Problem

Both HIV and HBV are some of the most chronic viral infections all over the world. Worldwide, there may be 3 to 6 million HIV-infected people living with chronic HBV (Kourtis *et al.,* 2012; Muriuki *et al.,* 2013). Co-infection with HIV and HBV exists due to the fact that both viruses share similar transmission routes including sexual, blood contact, and injecting drug usage making it more common among high risk groups that fall under the said transmission routes. HBV and HIV co-infection has been shown to increase mortality rates among HIV-infected persons. HBV infection can complicate the treatment of HIV patients on antiretroviral therapy causing toxicity as a result of the consumption of three times more antiretroviral drugs than in HIV infection alone (Muriuki *et al.,* 2013). Co-infection with the two viruses can be generally said to increase the severity of the negative effects, with the hazard of developing AIDS or death event being almost two times for those with chronic hepatitis compared with that of HIV alone (Athena *et al.,* 2012; Chun *et al.,* 2012). The HBV genotype is also a key factor since different genotypes have been shown to determine disease progression as well as response to antiviral treatment. Since the menace of HBV 50 years ago, despite the various successes achieved like highly sensitive and specific screening methods, vaccination programmes and

availability of antiviral therapy, there are problems still associated with the HBV infection. These include; approximately three-quarters of persons harbouring the virus aren't aware that they are infected, low screening uptake and diagnosis rates, low uptake and adherence to antiviral therapy including regular monitoring. This results to negative clinical outcomes for the majority of individuals chronically infected with HBV remaining sub-optimal (Lorcanini *et al.,* 2015).

1.3 Justification

HIV and HBV share very similar transmission routes, meaning that persons who are infected with either virus would be at a high risk of being co-infected with the other. Infection with HIV exacerbates the course and outcome of HBV infection leading accelerated progression to liver disease resulting in increased mortality and morbidity. On the other hand, HBV increases the risk of hepatic toxicity that results from the consumption of antiretroviral drugs used in managing HIV. The persons who participated in this study were recruited from a population of patients attending the Comprehensive Care Clinic at Malindi Sub-county Hospital. Malindi was selected as a suitable study site because it is located along the coastal strip of Kenya, a region that is popular with tourists both local and foreign. This region is on record for having a fairly large population of persons who engage in recreational intravenous substance abuse and in some cases they may share needles to inject themselves with the narcotics and/or engage in unprotected sexual contact. There is increasing evidence of high rates of HBV especially in the HIV patients and the fact that HBV is not routinely screened among HIV patients, clinicians tend to target HIV only during treatment. The diversity of countries of origin of the tourists to this area and their interaction with the local people would arouse the epidemiological curiosity about the introduction of new strains, their genetic diversity and the implications on the clinical presentation of disease in cases where patients are co-infected. There is therefore need to know the prevalence of HBV and to associate this to the socio-demographic and clinical characteristics associated with their co-infection. Characterization of HBV among the HIV patients will help in understanding the distribution of specific genotypes in the study setting. The genetic pattern of the virus will in turn help clinicians in making sound decisions during prescription of drugs to

these patients since different HBV genotypes have different responses to the antiretroviral drugs.

1.4 Research Questions

1. What is the prevalence of HBV among HIV patients attending comprehensive care centre at Malindi Sub-county Hospital?
2. **What is the phylogenetic relationship of the HBV variants isolated from HIV patients attending the comprehensive care centre at Malindi Sub-county Hospital?**
3. What are the socio-demographic, clinical characteristics and risk factors associated with HBV infections among HIV patients attending the comprehensive care centre at Malindi Sub-county Hospital?

1.6 Objectives

1.6.1 General Objective

To determine the sero-prevalence, HBV genetic diversity and risk factors associated with hepatitis B virus infections among human immunodeficiency virus infected patients attending the comprehensive care clinic in Malindi sub-county hospital.

1.6.2 Specific Objectives

1. To determine the prevalence of HBV among HIV patients attending comprehensive care Centre at Malindi Sub-county hospital.
2. To determine the genetic diversity of the HBV variants isolated from HIV patients attending the comprehensive care centre at Malindi Sub-county hospital.
3. To determine the socio-demographic, clinical characteristics and risk factors associated with HBV infections among HIV patients at the comprehensive care centre at Malindi Sub-county hospital.

CHAPTER TWO

LITERATURE REVIEW

2.1 Classification and structure of HBV

HBV belongs to the *Hepadnaviridae* family and contains a double-stranded circular DNA and a DNA polymerase that can repair the gap in the virion genome and also has reverse transcriptase activity. The viral particle is often referred to a Dane particle and has diameter size of 42 nm (Kudesia & Wreighitt, 2009; Haaheim *et al.,* 2002). The virus has an envelope with three surface glycoproteins, which are related to each other. Within the envelope, a viral nucleocapsid is found which is commonly referred to as the core (Ranjbar *et al.,* 2011).

2.2 HBV genome

HBV genome is a relaxed-circular, partially duplex DNA containing 3.2 kb, and a polymerase responsible for the synthesis of viral DNA in infected cells and is found within the core (Ranjbar *et al.,* 2011). The genome is made up of overlapping reading frames and the analysis of the coding potential of the genome reveals four open reading frames (ORF) (Fig 2-1) that are conserved between dozens of the virus isolates (Zuckerman, 1996).The first, ORF P, codes for a terminal protein on the minus strand as well as viral polymerase (reverse transcriptase, RNase H, and DNA polymerase activity). ORF C codes for nucleocapsid structural protein as well as HBV e antigen (HBeAg), which is responsible for immunomodulation and replication inhibition functions. ORF S/pre-S codes for viral surface glycoproteins (HBsAg; hepatitis B surface antigen) that bind to cell receptors and facilitate viral entry. Finally, ORF X codes for a transcriptional transactivator that codes for involved in the development of HCC (Guirgis *et al.,* 2010). HBV Genome replication occurs through an RNA intermediary phase (Harrison *et al.,* 2011)

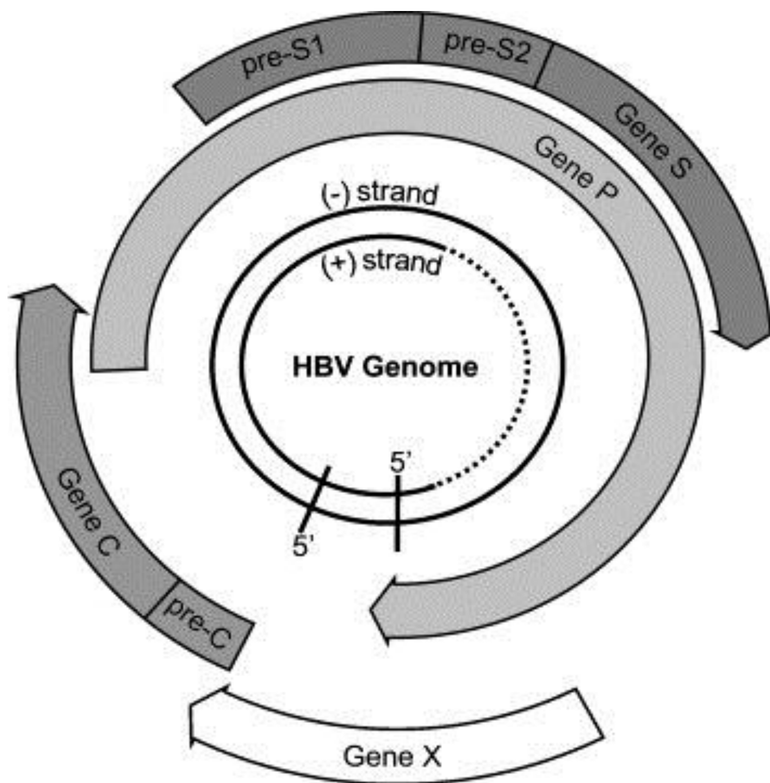

Figure 2.1: Schematic diagram of the HBV genome (Guirgis et al., 2010).

2.3 HBV genotyping

At least 10 different methods, which can be applied in the HBV genotyping have been developed (Guirgis *et al.,* 2010). The method to be used in a given case is determined by several factors, which may include sensitivity, specificity and turnaround time of each method. Each method has its advantages and disadvantages. These methods may include: sequencing and phylogenetic analysis, real time PCR, INNO-LiPA (a reverse hybridization technique that has been developed by Innogenetics), restriction fragment polymorphism (RFLP), multiplex PCR, serological subtyping, Oligonucleotide microarray chips, flow through reverse dot blot (FT-RDB), reverse fragment mass polymorphism and PCR invader assay (Guirgis *et al.,* 2010). This study used sequencing and phylogenetic analysis which is the gold standard, most reliable as it does not depend on a small nucleotide or amino acid sequence as other methods do, can detect new genotypes and recombination

between genotypes. The method is however less efficient in detecting mixed genotypes, cumbersome (cannot be used when analysing many samples), expensive and requires expertise (Guirgis *et al.,* 2010).

2.4 HBV genetic diversity and its importance

The genetic diversity of HBV has resulted to the emergence of 10 distinct genotypes named alphabetically from A–J and several sub-genotypes, which vary in their geographic distributions. (Tangkijvanich *et al.,* 2013). Genotype A is prevalent in Europe, India, Africa and America. Genotypes B and C are predominant in China, Japan and Southern Asia whereas genotype D is widespread in the Mediterranean area and the Middle East region. Genotype E is found in patients from West Africa and genotype F in Central and South America. Genotype H has been described in Mexico and Central America. Genotype G has been first identified in France and the United States, and was recently detected in Mexico (Hannachi *et al.,* 2010).

In Kenya a study done in the general population showed genotype A (88%) is most prevalent followed by genotype E (8%) and then genotype D (4%) (Mwangi *et al.,* 2008). Studies in the country have shown the predominance of sub-genotypes A1, A2, A3, A4, D1, D4 and D6. (Ochwoto *et al*., 2013; Kwange *et al.,* 2013). Increasing evidence suggests that HBV genotype is an important factor in determining HBV disease progression and response to antiviral treatment (Pourkarim *et al.,* 2011). In addition it is possible for some mutations to emerge as a result of medical pressure, which may be due vaccine or antiviral therapy use, resulting in complications in the management of the HBV infection. It's also important to note that different HBV genotypes have varying impacts on the progression of HBV infection (Pourkarim *et al.,* 2011). Some studies have shown that the HBV genotype is responsible to responses to therapy against the virus. For example, HBeAg-positive genotype B patients are more responsive to IFN compared to genotype C patients (Guirgis *et al.,* 2010).

2.5 Transmission and risk factors for HBV and HIV

Both HIV and HBV share common routes of transmission. The main modes usually involve percutaneous and mucous membrane exposures to infectious blood and body fluids that contain blood (Alter, 2006). This spread may involve any of the following routes; parenteral (blood exposure), sexual, vertical (from mother to baby) routes with the infection developing from 6 weeks to 6 months after exposure to the virus (Kudesia & Wreighitt, 2009). For HBV the main mode of transmission varies geographically. For example, in areas where the prevalence is low such as Northern Europe and North America, the infection is mainly transmitted from one individual to the other through sexual contact or injecting drug use, whereas in areas with high prevalence such as Sub-Saharan Africa and South East Asia, the infection is most commonly acquired perinatally or in early childhood (Aspinall *et al.,* 2011). Despite the shared transmission routes, HBV is more efficiently transmitted than HIV (Otedo, 2004).

Intravenous drug users, individuals who have undergone multiple blood transfusions, individual with several sexual partners whether homosexuals or commercial sex workers, people with history of dental surgeries, tattooed/ people with body piercings, people working in a healthcare set-up are therefore at an increased risk of acquiring these co-infections (Lavachy, 2004; Ranjbar *et al.,* 2013; Zenebe *et al.,* 2014).

2.6. Pathogenesis of HBV

2.6.1. Pathogenesis of Hepatitis B Virus

When HBV infects an individual, both viral clearance and disease pathogenesis are largely mediated by the acquired immune response to the HBV (Chisari *et al.,* 2010). The HBV replication cycle is not directly cytotoxic to cells. This agrees with the observation that many HBV carriers are asymptomatic and have minimal liver injury, despite extensive and ongoing intrahepatic replication of the virus (Ganeem & Prince, 2004). Generally, it is acknowledged that the antibody mediated responses contributes to the clearance of circulating virus particles and the prevention of viral

spread within the host while the cellular immune response eliminates infected cells. (Chisari *et al.,* 2010). According to (Shepard *et al.,* 2006) several factors including person's age, viral factors and the host immune response which could lead to liver cirrhosis and/or Hepatocellular carcinoma (HCC). Approximately 15–40% of infected patients will develop cirrhosis, liver failure, or hepatocellular carcinoma (Lavanchy, 2004).

The immune responses to HBV and their role in the hepatitis B pathogenesis are still not completely understood. Some clinical studies have shown that in acute, self-limited hepatitis B, strong T-cell responses against many HBV antigens were proven to exist in the peripheral blood, including both major histocompatibility- complex (MHC) class II, CD4+ helper T cells and MHC class I, CD8+ cytotoxic T lymphocytes. (Ganeem & Prince, 2004; Ranjbar *et al.,* 2011).

2.6.2 Pathogenesis of Liver Disease in HIV-HBV co infections

HBV-related liver disease being an immune-mediated process and the fact that it is exacerbated by the immunodeficient state caused by HIV is contradictory. This paradoxical relationship can be explained by a number of theories. In HIV-infected persons, a rapidly progressive form of liver disease due to viral cytopathic effect (fibrosing cholestatic hepatitis) rather than the immune response has been described. It has also been hypothesized that HIV modulation of the HBV-specific immune response can alter the hepatic cytokine environment and subsequently affect liver disease. However, this hypothesis has not been studied to date. Another theory postulates that immune activation from HIV-related microbial translocation may be a mechanism for accelerating liver disease progression (Thio, 2009).

2.7 Laboratory Diagnosis in HBV Infections

HBV is a dynamic virus and its diagnosis becomes complicated from the fact that it can be co-infected with HIV. However, correct diagnosis and monitoring of the HBV/HIV co-infection in conjunction with elaborate understanding of the mechanisms the viruses use to cause drug resistance can help clinicians to manage

co-infection with more ease resulting to more productive results (Ranjbar *et al.,* 2011; Thio, 2009).

Diagnosis of HBV is mainly based upon the serological identification of the various HBV antigens or the antibodies produced in response to these antigens. Both antigens and antibodies can be detected in patient blood using a solid phase test (enzyme immunoassay). The individual components manifest in specific disease patterns as shown in table 2.1 (Kayser *et al.,* 2005).

Table 2.1: HBV Markers used in laboratory diagnostic of HBV infections **(Kayser et al., 2005).**

Status	**Diagnostic test**
Acute infection	HBc-IgM, HBs-Ag
Vaccine immunity	HBs-IgG
Recovered, healed	HBs-IgG, HBc-IgG
Chronic, patient infectious	HBe and HBs-Ag, PCR
Exclusion of HBV	HBc-IgG negative
Serology inconclusive, therapeutic monitoring	Quantitative PCR

2.8 HBV-HIV co infection

Approximately 40 million people in the world are infected with HIV and among these 2-4 million persons are infected chronically with HBV (Alter, 2006). This translates to a prevalence of 5-10%. These co-infection estimates are influenced by several factors which may include geographic differences in the prevalence of chronic infection by age, the efficiency of exposures that account for most transmission, and the prevalence of persons at high risk for infection (Alter, 2006).

It has been observed that HBV-HIV co-infection lead to increased morbidity and mortality as compared to HIV or HBV infections occurring singly (Muriuki *et al.,* 2013). HBV-HIV co-infection can complicate the treatment of HIV patients on antiretroviral therapy (ART). The risk of ART toxicity is increases three times, and this risk further increases when patients are under concomitant medications for tuberculosis (Firnhaber *et al.,* 2012). Other studies have shown that HIV co-infection adversely impacts on the natural history of HBV and HCV by accelerating progression to chronic liver disease due to drug-related toxicity of hepatocytes and hepatitis reactivation (Muriuki *et al.,* 2013).

There have also been suggestions of re-activation of HBV infection or new infections in HIV patients as shown by a study done in Kisumu district hospital in Kenya back in 2004. This study also indicated that HIV-HBV co-infected patients have lower CD4 counts than those with HBV mono-infection. All co-infected patients tested positive for IgM anti HBc (Otedo, 2004).

2.9 Treatment of Hepatitis B virus

The main goal towards treatment of HBV infected patients is to lower the level of viraemia as well as to improve hepatic dysfunction (Ganem & Prince 2004). Some of the antivirals used against HBV include interferon alfa-2b which is usually administered subcutaneously three times in a week for a duration of not less than three months, antiviral drugs including lamivudine, adefovir, entecavir, and peginterferon alfa-2a all are approved as initial therapy for chronic hepatitis B. (Lavanchy, 2004; Keeffe *et al.,* 2006). When choosing a drug of choice to administer to the patient, some of the considerations made are; efficacy, safety, incidence of resistance, method of administration, and cost (Keeffe *et al.,* 2006).

For cases of HBV-HIV co-infections in Kenya, the national guidelines for treatment of hepatitis B and C viral infections (2015) recommends that nucleos (t)ide analogues lamivudine, Tenofovir and Entecavir as well as pegylated interferon alfa 2a and 2b should be used. Oral drug therapy in this case should be used ensuring that triple drug combination is employed with at least two drugs being active against HBV.

Once therapy has been administered, the usual indicators of successful therapy include the loss of HBeAg, sero-conversion to anti-HBe antibodies and reduction of the circulating viral load. Complete cure of infection i.e. loss of HBsAg and complete disappearance of viremia is achieved only infrequently (in 1 to 5 percent of patients) with current regimens, although the increasing numbers of active antiviral drugs might lead to an upward revision of this figure in the future. (Ganem & Prince 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

The study was descriptive hospital-based cross-sectional study carried out between May 2015 and April 2017.

3.2 Study site

The study was carried out at Malindi Sub-County Hospital comprehensive care centre (CCC). Malindi is located 120km northeast of Mombasa town (Fig 3-1). Malindi is in Kilifi County and it is the biggest town in the county. Its coordinates are 3°13′25″S 40°7′48″E. The main economic activity of the area is tourism with many tourists from all over the world. The tourists interact with the local dwellers of this place increasing chances of transmission of some HBV genotypes currently not distributed in the region.

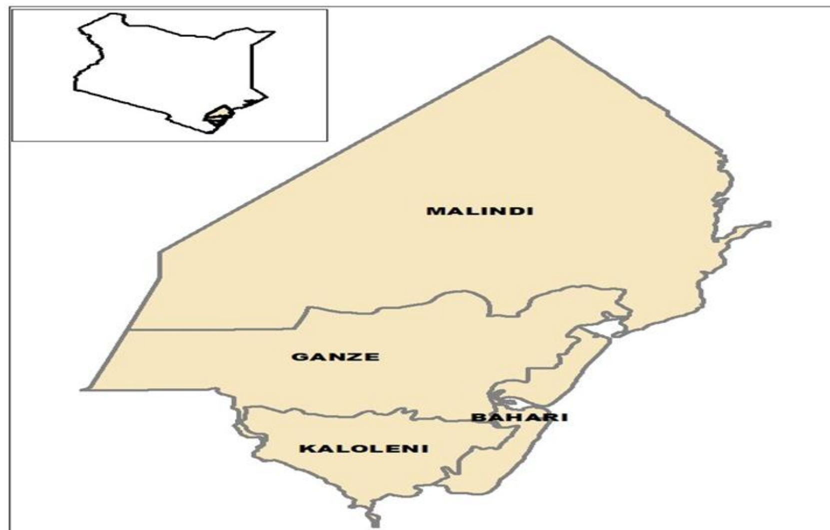

Figure 3.1: The map of Kilifi Sub-county. Malindi is located along the Indian Ocean along the Kenyan coastline.

The Malindi sub-county hospital CCC provides services to patients with HIV mainly from Malindi sub-county as well as other neighbouring sub-counties in Kilifi county including; Kaloleni, Ganze and Magarini. The clinic serves approximately 4500 patients. Some of the services offered include; Clinical evaluation and primary medical care, medical nutrition therapy, medical care management, medication counselling, early intervention services and pharmaceutical assistance. It also coordinates other services for the HIV patients out of the hospital setting such as home health, hospice and subspecialty referrals.

Analysis of samples was done at KEMRI's Centre for Virus Research (CVR) located along Mbagathi Way in Nairobi.

3.3 Study Population

Participants of this study were recruited from patients (both male and female of 18 years and above) attending the CCC at Malindi Sub-county Hospital.

3.3.1 The Inclusion Criteria

1. All HIV positive patients (both male and female) aged 18 years and above attending Malindi Sub-county Hospital CCC.
2. Those who voluntarily consented to participate in the study.

3.3.2 The Exclusion Criteria

i) Any vulnerable persons such as prisoners, mentally challenged etc.

3.4 Sample Size Determination

The minimum sample size was calculated based on the desired degree of precision and the anticipated prevalence according to Naing (2003)

$$N = \frac{Z^2 \, p \, (1-p)}{d^2}$$

Where; N = Minimum number of samples required

Z = the X-axis value of the normal curve that cuts off an area α at the tails of the normal curve. Where 1-α is equal to the desired confidence level.

p = prevalence of HIV co-infection in coastal Kenya

d = desired level of precision

The prevalence (p) of HIV in the coastal region is 5.9 % (Kimanga et al., 2014), Z is 1.96 and d is 0.05. Therefore;

$$N = \frac{1.96^2 \times 0.059 \times 0.941}{0.05^2}$$

N = 85 Patients.

However, a total of 446 HIV positive patient samples were used for this study higher above the required minimum sample size to increase the strength of results obtained.

3.5 Sampling Design

Consecutive sampling was used to recruit the study participants until the required sample size was attained. However those who were selected and did not meet the inclusion criteria or did not consent were not added to the study. Recruitment of the patients in the study was integrated within the day-to-day procedures of the clinic.

3.6 Collection of social demographic and clinical characteristics.

This started only after the recruited patients agreed to participate in the study. The consenting process was done in a confidential counseling room by a professional counselor who was recruited for this study. At this stage the professional counselor also counseled the patients and clearly indicated why it is important to test for HBV and give a clear and easy-to-understand explanation of what either HBV positive or

negative results would mean to a HIV positive person. The social demographic characteristics were obtained through administration of a structured questionnaire (appendix II) by the principal investigator. The clinical characteristics of the patients were obtained from the health records of the patients from the CCC since its part of the routine checkup upon visiting the clinic. The baseline CD4 count of the study participants was obtained after analysis of the patient samples flow cytometry using the BD Facs Caliber machine at the Malindi Sub-county Hospital main laboratory by the technologists working there. This was after seeking permission from the patients in the consent form. This information was then transferred to the questionnaire in the respective section.

3.7 Laboratory Procedures

3.7.1 Blood collection

Five millilitres of blood were drawn from each patient. This was strictly done by a well-trained and experienced phlebotomist from Malindi Sub-county hospital using accepted venepuncture standard operating procedures in the hospital. This first involved the inspection of both arms visually and selecting the one lacking bruises, abrasions or sites of infection. A tourniquet was applied on the selected arm a few inches above the expected puncture site. The patient was asked to make a fist to make the veins more prominent. The puncture site was then sterilized with a swab soaked in 70% alcohol. Using a disposable, sterile needle 5mls of blood were collected into a vacutainer bottle. Fifty microliters of the whole blood sample was used to analyse the CD4 count of each participant using the BD Facs Caliber Machine which uses flow cytometry (Appendix VII). The whole blood was then separated into plasma and peripheral blood mononuclear cells (PBMC). The PBMC fractions were discarded immediately after separation. The plasma samples were packaged using the basic triple packaging system according to the International Air Transport Association (IATA) regulations. This included primary receptacles (screw capped containers) which were leak proof covered with a layer of absorbent material, a secondary receptacle (zip lock bags) which were also leak proof enclosing the primary receptacle and finally a leak-proof outer packaging which was the cool

box with ice cubes. The cool box was then labelled with an easily noticeable biohazard symbol. These samples were transported from Malindi Sub-county hospital to the KEMRI- CVR-Hepatitis laboratory by a paid for courier service company. Upon arrival at the KEMRI, the samples were unpackaged and stored in 200ul aliquots at -80^{0}C in a freezer with controlled access.

3.7.2 Serological methods for determination of HBV status

The plasma from the HIV patients was screened for the presence of HBV surface antigen (HBsAg) by sandwich enzyme-linked immunosorbent assay (ELISA) using Hepanostika® HBsAg Ultra ELISA kit (BioMérieux SA Diagnostics) according to the manufacturer's instructions. Briefly, 25 µl of the plasma was pipetted into respective well of a micro-ELISA strip and incubated at 37° C for 1 hour after which 50 µl of HRP-labelled anti-HBs conjugate was added into each well followed by a 1 hour incubation at 37° C. Each well was washed and soaked six times with phosphate buffer followed by addition of 100 µl TMB substrate. After 15 minutes the reaction was stopped by addition of 100 µl sulfuric acid. Human serum non-reactive for HBsAg was used as the negative control while the kit HBsAg was used as a positive control. Upon completion of the assay the development of colour indicates the presence of HBsAg, while no or low colour development suggests the absence of HBsAg

3.7.3 DNA extraction

HBV genomic DNA was extracted from plasma of seropositive individuals using the Qiagen® DNA extraction kit (QIAGEN) following the manufacturer's instructions (Appendix VI). Briefly, this involved digestion of 200µl of plasma using 20µl of proitenase K and precipitation of proteins using absolute alcohol. This was followed by binding and elution of the DNA through a spin protocol using provided buffers. The extracted viral DNA was stored at -20°C awaiting further analysis.

3.7.4 Amplification of HBV Surface region

HBV DNA extracted from all the serologically positive samples for HBsAg were used as template in a PCR to amplify the Pre S1 (681bp) region by nested PCR using a set of primers. Briefly, the master mix for the first amplification included 2.5µl of 10x PCR buffer, 2.0µl of 10mMdNTPs, 1µl of 25mM $MgCl_2$, 0.3µl of 10µM first set of primer (S1F and S1R) each, S1F: 5´- TCCTGCTGGTGGCTCCAG -3´ (55-72) and S1R: 5´- CGTTGACATACTTTCCAATCAA -3´ (995-974), 0.2µl of 5U/µL Taq polymerase and 5µl of extracted DNA. The final reaction volume were 25µl. The PCR conditions started at 94^0C for 5 minutes, followed by 40 cycles of 94^0C for 40 seconds, 46^0C for 30 seconds and 72^0C for 2 minutes and final extension for 72^0C for 10 minutes (Ochwoto *et al*., 2016)

The second master mix included 5µl of 10x PCR buffer, 1.5µl $MgCl_2$, and 4.0µl of dNTPs, 0.25µl of second set of primer (S2F and S2R) each whose sequences are, S2F: 5´- ACCCTGYRCCGAACATGGA -3´ (155-173) and S2R: 5´- CAACTCCCAATTACATARCCCA -3´ (835-814) and 2µl Taq polymerase and 10ul of the 1st PCR products. The conditions for the 2nd PCR were similar to the 1st PCR except that annealing was done at 50° C (Ochwoto *et al.,* 2016). A confirmed HBV positive sample by both ELISA and PCR was used as the positive control while the master mix with no sample added was used as the negative control.

3.7.5 Analysis of PCR products by Gel Electrophoresis

Visualization of amplified DNA were done on 1.2% agarose gel in 1x TAE buffer stained with ethidium bromide. Briefly, the amplicons were stained with ethidium bromide (0.05%) and loaded in an already prepared 1.2% agarose gel. They were allowed to separate for 45minutes in an electrophoretic tank set at 100 volts. The PCR amplicons were visualized using a UV trans-illuminator (UVP, San Gabriel, A, USA). In this study, an average molecular weight for the PreS1 gene (681bp) was expected.

3.7.6 Sequencing and viral genotyping

3.7.6.1 Sequencing PCR

A sequencing PCR were performed on the cleaned amplified amplicons using the BigDye terminator v3.0 in a Sequencing Ready Reaction Kit outsourced from Macrogen, Netherlands. To describe this briefly, sequencing PCR was carried with a total reaction mixture of 20 µl. This contained; 3 µl of DNA, 3 µl of 5X sequence buffer, 2.0 µl Big Dye, 10.5 µl of distilled water, and 1.5 µl of primer. For each sample, two reactions were prepared S2f 5'- ACCCTGYRCCGAACATGGA-3'as sense primer and S2r (5'- CAACTCCCAATTACATARCCCA -3' as anti-sense primer. The amplification was performed as follows; denaturation for 5 minutes at 96°C, and again for 10 seconds at 96°C, annealing at 50˚C for 5 seconds and final extension 60˚C for 4 minutes for 25cycles.

3.7.6.2 Purification of Amplicons and loading to DNA genetic Analyser

The products were then purified. Briefly, 2µl sodium acetate, 50µl of absolute ethanol, 20µl of sequencing PCR product and were mixed in a 200µl tube. This was vortexed and incubated at room temperature (RT) for 15 minutes in dark. The amplicons were centrifuged at 14000 revolutions per minute (rpm) for 30 minutes at RT. A five hundred (500ml) of 70% ethanol was added and centrifuge at 1500 rpm for 5 minutes and supernatant discarded. The above two steps were repeated once and DNA pellet was air dried for a minimum 45 minutes. After drying, 20µl of highly deionized (HiDi) formamide was added and heated at 95˚C for 3 minutes, chilled on ice for 3 minutes and finally transferred into sequencing tube and loaded on the automated ABI 310 genetic analyzer (Applied Biosystems, Foster City, CA).

3.7.6.3 Sequence Editing and Analysis

The sequences were read and edited using Sequence Scanner Software v1.0 which is compatible with the Genetic Analyzer. The complete DNA sequences and published reference HBV genotypes sequences from GenBank were assembled and aligned

using GENETYX version 9 software and then fed into the MEGA4 software for trimming. Phylogenetic analysis and genotyping was done by creating a PHB file using DNA Data Bank of Japan (DDBJ) using the trimmed sequences and references and opening the PHB file using NJplot computer software (http://pbil.univ-lyon1.fr/software/njplot.htm)

3.8 Data Management, Analysis and Presentation

3.8.1 Data management

Data collected from the patients attending Malindi Sub-county Hospital CCC was transferred to SPSS version 21.0 database. This data was kept out of reach of any unauthorized personnel using passwords. A backup of the same data was stored in Ms Excel software with password protection. All the hard copies of the data sheets were stored in locked cabinets only restricted to the access of the principal investigator

3.8.2 Data analysis

Data was entered, cleaned and analysed using Statistical package for social sciences (SPSS) version 21. The prevalence of co-infection with HIV and HBV was calculated by dividing the number of occurrences of co-infection during this study by the total number HIV positive patients sampled and was expressed by percentages. Poisson regression was used to perform Univariate and multivariate analyses (significance set at $P \leq 0.05$) and odds ratio with corresponding 95% confidence interval was used to determine the association between the HBsAg status and Age, gender, occupation, employment status, level of education, marital status, intravenous drug use, blood transfusion history, tattooing/scarification and the sexual history. Independent sample T-test was used to determine the association between the HBsAg and the CD4 count. A PHB file created through the DDBJ was used for inferring a phylogenetic tree using NJplot computer software.

3.9 Ethical Considerations

The study started after approval of the proposal by the KEMRI's Ethical Review Committee (ERC) (SCC protocol 2915) (Appendix IV) with all the methods used clearly indicated. The study was also approved by the Kilifi County Research committee (Ref:DOH/KLF/RESCH/VOL.I/21) (Appendix V). No participant was recruited or handled for this study before the approval had been granted by these committees. The participants of this research project were be human beings who only participated after having the aspects of the study being clearly explained to them and after voluntarily accepting to consent. The samples were to be discarded via incineration at KEMRI immediately after completion of the work, as was a requirement for ethical consideration that the samples would not be used for any other study apart from the current one.

CHAPTER FOUR

RESULTS

4.1 Descriptive characteristics of study participants

A total of 446 participants consisting of 126 (28.3%) males and 320 (71.7%) females were recruited for this study. Their mean age was 42.98 years (SD±10.75) and a median (IQR) of 43 (34.75-50). The mean age of males was 44.93 years (SD ±10.16) while that of females was 42.22 years (SD ±10.88). The largest group (35.4%) had participants aged between 41-50 years while the smallest group comprised persons aged less than 30 years of age. Those aged between 31-40 years were 28.1% while those above 50 years 22.6%.

Slightly more than half of the participants had primary education (53.8%) followed by 24.2% of participants who had no formal education while those with secondary education were 17.3%. Only 4.7% of the participants had tertiary education. Based on employment status, 55.2% of the participants were either employed/self-employed while 44.8% were unemployed. Slightly above half of the participants were married (53%) while 36% were either divorced or widowed. Only 11% of the participants were single. Table 4.1 summarises the descriptive characteristics of the study participants.

Table 4.1: Demographic characteristics of the study participants

Variable	Sample size (N = 446)	
	No	%
Age (Years)		
Mean 42.98 (SD ± 10.75)		
Median 43 (IQR 34.75 - 50)		
Range 58 (18 - 76)		
≤30	62	13.9
31-40	125	28.1
41-50	158	35.4
≥50	101	22.6
Gender		
Males	126	28.3
Females	320	71.7
Level of Education		
Primary	240	53.8
Secondary	77	17.3
Tertiary	21	4.7
none	108	24.2
Employment status		
employed/self employed	246	55.2
unemployed	200	44.8
Marital status		
Single	48	11
Married	236	53
widow/divorced	162	36

Key: N - Number; % - Percentage; SD- standard deviation; IQR-Interquartile range

4.2 Prevalence of HBV among study participants in relation to their social demographic characteristics.

Table 4.2 summarises the prevalence of HBV of the study participants in relation to their social demographic characteristics.

Table 4.2: Prevalence of HBV among study participants in relation demographics

Variable	Sample size (n = 446)	HBV Positive (N=22)	
	No	No	%
Gender			
Male	126	11	8.7
Female	320	11	3.4
Age (Years)			
≤30	62	2	3.2
31-40	125	4	3.2
41-50	158	9	5.7
>50	101	7	6.9
Employment status			
employed/self employed	246	14	5.7
unemployed	200	8	4
Level of Education			
Primary	240	14	5.6
Secondary	77	1	1.3
Tertiary	21	1	4.8
Non formal	108	6	5.6
Marital status			
Single	48	1	2.1
Married	236	12	5.1
Widowed/Divorced	162	9	5.6

Key: N - Number; % - Percentage

4.3 Analysis of HBV Pre S1 region by gel electrophoresis

The HBV Pre S1 region with an expected size of 681bp was amplified using a nested PCR and the product was visualized by a UV trans-illuminator (UVP, San Gabriel, A, USA) (Fig 4.1). Twelve out of the 22 (54.5%) sample showed bands which was enough evidence for HBV DNA in the samples.

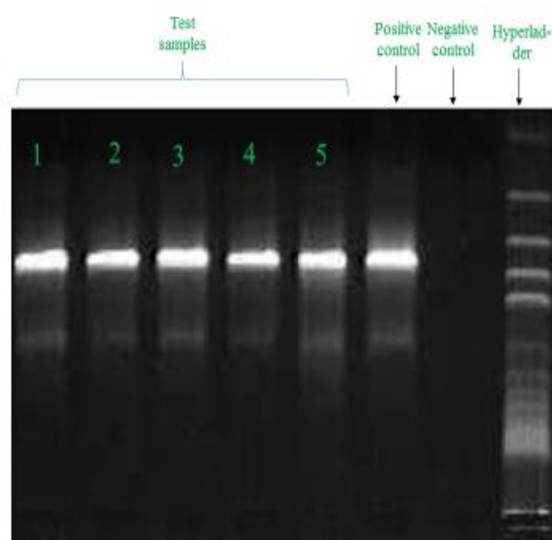

Figure 4.1: Agarose gel picture for HBV Pre S1 region

4.4 Phylogenetic analysis and genotypes

Twelve out of 22 (54.5%) samples showed bands indicating presence HBV DNA. Ten of these samples were successfully sequenced using the Pre S1 forward and reverse primers and analysed phylogenetically. Phylogenetic analysis revealed that 9/10 (90%) belonged to genotype A which were all categorised into sub-genotype A1 while 1/10 (10%) belonged to genotype E as shown in fig 4-1.

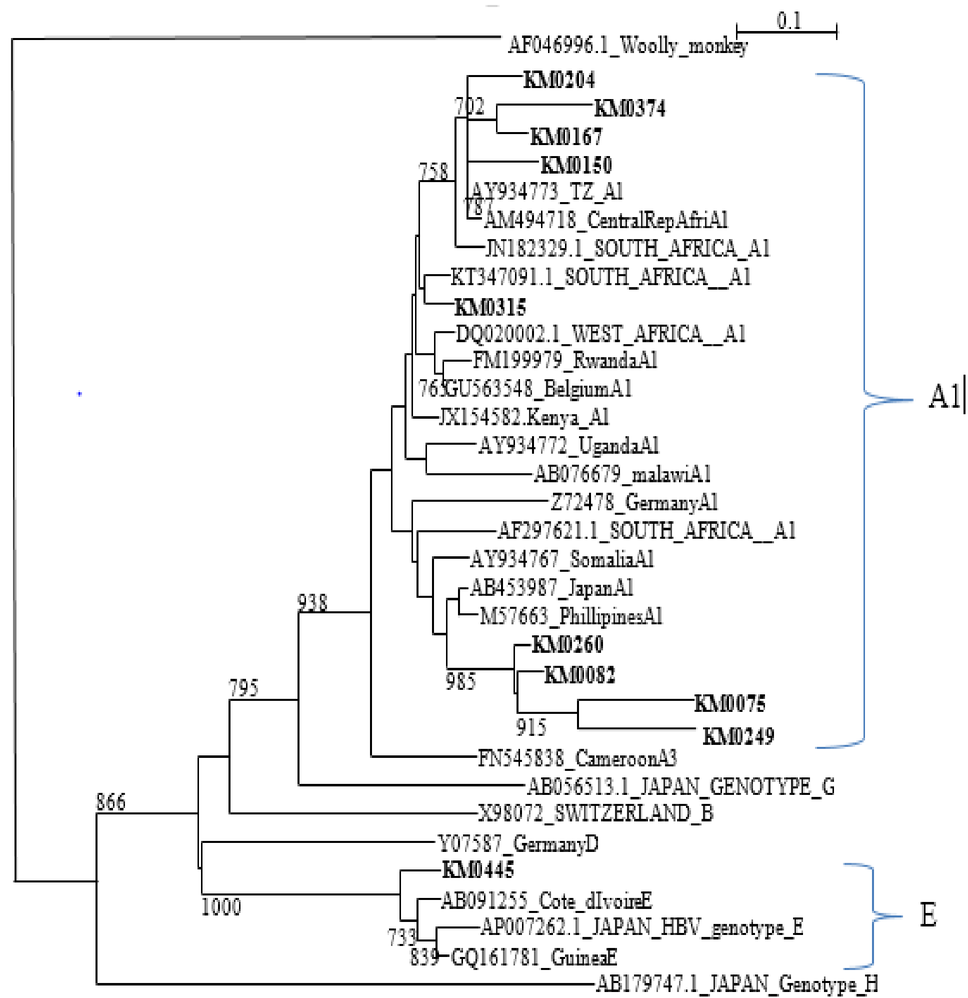

Figure 4.2: Phylogenetic analysis based on the Pre-S region from 10 HBV strains from HBV-HIV co-infected patient samples and selected reference sequences representing some of the known HBV genotypes/Sub-genotypes from the GeneBank. The tree is rooted from the Woolley Monkey virus genome sequence.

4.5 Socio-demographic factors associated with HBV among HIV participants

Male gender was significantly associated with HBV infection among the study participants both in bivariate (OR 2.2, 95% CI 1.1 to 5.9) and univariate analysis (OR 3.2, 95% CI 1.3 to 8.1). Age, employment status, level of education and marital status were not statistically significant. Table 4.3 summarises the socio-demographic associated with HBV among HIV patients attending Malindi sub-county hospital

Table 4.5: Socio-demographic factors associated with HBV among HIV positive participants

Variable	Sample size (N = 446) No	HBV positive No	%	P - value	Bivariate OR (95% CI)	P – value	Multivariate OR (95% CI)
Gender							
Male	**126**	**11**	**8.7**	**0.029**	**2.2(1.1-5.9)**	**0.013**	**3.2(1.3-8.1)**
Female	320	11	3.4	Referent	Referent	Referent	Referent
Age (Years)							
≤30	62	2	3.2	0.34	0.5(0.1-2.2)	0.542	0.6(0.1-3.0)
31-40	125	4	3.2	0.218	0.5(0.1-1.5)	0.384	0.6(0.2-2.0)
41-50	158	9	5.7	0.697	0.8(0.3-2.2)	0.961	1.0(0.4-2.7)
>50	101	7	6.9	Referent	Referent	Referent	Referent
Employment status							
employed/self employed	246	14	5.7	0.426	1.4(0.6-3.4)	0.378	1.5(0.6-3.8)
Unemployed	200	8	4	Referent	Referent	Referent	Referent
Level of Education							
Primary	240	14	5.6	0.92	1.1(0.4-2.7)	0.606	0.8(0.3-2.2)
Secondary	77	1	1.3	0.178	0.2(0-1.9)	0.056	0.1(0-1)
Tertiary	21	1	4.8	0.887	0.9(0.1-7.1)	0.511	0.5(0.1-4.3)
Non formal	108	6	5.6	Referent	Referent	Referent	Referent
Marital status							
Single	48	1	2.1	0.352	0.4(0.0-3.0)	0.532	0.5(0.1-4.2)
Married	236	12	5.1	0.841	0.9(0.4-2.1)	0.514	0.7(0.3-1.8)
Widow/Widower/Divorced	162	9	5.6	Referent	Referent	Referent	Referent

Key: N-number; %-Percentage; CI- Confidence interval, OR- Odds Ratio

4.6 Clinical factors associated with HBV among HIV positive participants

Among the four clinical history related factors analysed i.e. the ARV drug been used, CD4 count, duration since HIV diagnosis and duration since initiation of ARVs none was associated with the risk to HBV infection. Although not statistically significant (p = 0.429) those who were HIV-HBV co-infected had a lower CD4 count (378 cell/µl) compared to those who were HIV mono-infected (419.5 cell/µl) (Table 4.4)

Table 4.4: Clinical factors associated with HBV among HIV positive participants

Variable	**Sample size (N = 446)**	**HBV positive**		**P - value**	**Bivariate**	**P - value**	**Multivariate**
	No	No	%		OR (95% CI)		OR (95% CI)
ARV drug currently on use							
3TC, TDF, EFV	120.0	8	6.7	0.15	4.6(0.6-36.8)	0.995	0
AZT, 3TC, NVP	149	10	6.7	0.144	4.6(0.6-36.2)	0.995	0
TDF,3TC, NVP	108	3	2.8	0.573	1.9(0.2-18.4)	0.995	0
Not started	69	1	1.4	Referent	Referent	Referent	Referent
CD4 count							
Less than 500	292	16	5.5	0.476	1.4(0.6-3.6)	0.433	1.5(0.6-3.9)
Greater than 500	154	6	3.9	Referent	Referent	Referent	Referent
Duration since HIV diagnosis							
0-5yrs	227	9	4	0.995	0	1	0
6-10yrs	204	13	6.4	0.965	0	1	0
>10yrs	15	0	0	Referent	Referent	Referent	Referent
Duration since ARV initiation							
0-5yrs	264	11	4.2	0.996	0	0.999	0
6-10yrs	175	11	6.3	0.996	0	0.999	0
>10yrs	7	0	0	Referent	Referent	Referent	Referent

Key: N-number; %-Percentage; CI- Confidence interval, OR- Odds Ratio

4.7 Risk/behavioural factors associated with HBV infection among HIV positive participants attending Malindi sub-county hospital.

The participants who were involved in intravenous drug use were significantly associated with HBV infection both in bivariate (OR 5.3, 95% CI 1.6 to 17.8) and multivariate analysis (OR 4.1 95% CI 1.0 to 16.6) Other analysed factors in this study including history of blood transfusion, scarification/tattooing, commercial sex work, sexuality and number of partners were not significantly associated with HBV infection. Table 4.5 summarises the risk factors associated with HBV infections among HIV positive participants attending Malindi sub-county hospital.

Table 4.5: Risk factors associated with HBV infections among HIV positive participants

Variable	Sample size (N = 446)	HBV positive		P - value	Bivariate	P – value	Multivariate
	No	No	%		OR (95% CI)		OR (95% CI)
Intravenous drug use							
Yes	**13**	**3**	**23.1**	**0.008**	**5.3(1.6-17.8)**	**0.05**	**4.1(1.0-16.6**
no	433	19	4.4	Referent	Referent	Referent	Referent
History of blood transfusion							
Yes	36	0	0	1	0	0.995	0
No	410	22	5.4	Referent	Referent	Referent	Referent
Scarification/Tatooing							
Yes	9	0	0	0.999	0	0.997	0
No	437	22	5	Referent	Referent	Referent	Referent
CSW							
Yes	23	1	4.3	0.897	0.9(0.1-6.5)	0.813	0.8(0.1-6.0)
No	423	21	5	Referent	Referent	Referent	Referent
Sexuality							
Homosexual	1	0	0	1	0	0.999	0
Bisexual	4	0	0	1	0	0.998	0
Heterosexual	441	22	5	Referent	Referent	Referent	Referent
No. of patners in past six months							
One	329	14	4.3	0.161	0.5(0.2-1.3)	0.398	0.7(0.2-1.7)
2 to 3	24	1	4.2	0.531	0.5(0.1-4.2)	0.846	0.8(0.1-8.8)
≥4	7	0	0	0.994	0	0.998	0
None	86	7	8.1	Referent	Referent	Referent	Referent

Key: N-number; %-Percentage; CI- Confidence interval, CSW- Commercial sex worker; OR- Odds Ratio

CHAPTER FIVE

DISCUSSION

5.1 Discussions

Several studies have been carried out in Sub-Saharan Africa including Kenya to access the burden of hepatitis B virus among HIV infected individuals. However majority of this studies focus on populations that form risk sub-populations for HIV and HBV transmission or newly diagnosed HIV patients.

Generally, the prevalence of HBV is higher among HIV–infected individuals than in the general population which can be attributed to the shared risk factors for transmission and acquisition of these two life threatening viruses (Chun *et al.,* 2012). The present study reported a HBV sero-prevalence of 4.9% (22/446) among HIV positive participants based on laboratory analysis of HBsAg through ELISA. The rates of HIV-HBV co-infections have previously been reported to be as high as 10–20% in countries where HBV infection is either endemic or intermediate to high HBV cases (Muriuki *et al.,* 2013). In Kenya, a study by Muriuki *et al.,* 2013 on the prevalence of HBV and HIV co-infection showed a prevalence of 6% with co-infection rates significantly higher among males (19.2%) compared to females (14.3%). This result was also in agreement with other studies carried out in Kenya including; 5.7% (Wambani *et al.,* 2015), 4.26 % (Kerubo *et al.,* 2015) and 3.6 % as reported by Webale *et al.,* (2015) among HIV positive non-IDUs in Mombasa, Coastal Kenya. The results were also similar to those reported in other parts of the world including 5.2 % in Morocco (Rebbani *et al.,* 2013) and 3.8 % in Brazil (Brandaoa *et al.,* 2015). In South Africa between 5% and 17% of HIV-infected patients in the country were found to be co-infected with Hepatitis B virus (Chun *et al.,* 2012).

The results of this study were found to be lower than those of other studies carried including in Kenya which reported 53 % (Otedo, 2004) and 50.6 % (Ochwoto *et al.,* 2016), 25.5 % among HIV patients in Morocco (Magoro *et al.,* 2016), 19.2 % among HAART naïve patients in China (Chen *et al.,* 2013), 12 % among HIV patients in

Columbia (Bautista *et al.,* 2014), 10.5 % among HIV patients in Lesotho (Mugomeri *et al.,* 2015), 9.6 % among HIV IDUs in Mombasa, Kenya (Webale *et al.,* 2015). The higher HBV prevalence rates in this studies compare to the current study could have been as a result of using sub-populations that have evident signs associated with HBV such as the jaundiced patients who were attending clinics due to liver related complication, being selected from HBV high risk sub-populations such as the intravenous drug users or newly diagnosed patients who had not started ART.

On the other hand, the results reported in this study were about two times higher compared to reports from Nigeria as reported by Dore *et al.,* (2010) and Diwe *et al.,* (2013) indicating HBV/HIV prevalence rates of 2.4% and 2.2% respectively, 2.2 % in Malawi (Varo *et al.,* 2016) and 2.5 % in Brazil (Freitas *et al.,* 2014). The higher prevalence rate in the present study could be as a result the samples being collected from a HBV prevalence intermediate (2-7%) area as classified by WHO (Zenebe *et al.,* 2014). As reported by Mukami *et al*., (2013) other factors that might have resulted in disparities in the prevalence rates between this studies result and those conducted in other places could include but not limited to sample size used, specificity and sensitivity of kits used and diversity of behavioural characteristics of each population.

Phylogenetic analysis of the HBV genotypes in the current study revealed the presence of genotype A (90%) and E (10%) as would be expected of the Kenyan population. This observation is in agreement with other studies done in Kenyan blood donor set-up that reported a prevalence of 88% (Mwangi *et al.,* 2008), 90.3 % (Ochwoto *et al.,* 2016), 100 % (Webale *et al.,* 2015) of genotype A1. Mwangi et al., (2008) had also reported genotype E although at a much lower prevalence than genotype A. All the genotype A sequences belonged to the HBV genotype A1 sub-genotypes. Five of the genotype A and the genotype E sequences clustered with sequences from countries in sub Saharan Africa including Tanzania, central Africa republic, South Africa Ivory coast and Guinea. This could be attributed to trade relationships between these African countries. The rest four sub-genotype A1 samples clustered with samples from Somalia and the Asian clade (Phillipines and

Japan). This could be as a result of immigrants from Somalia who access the Kenyan coast and tourists from the Asian countries.

The current study looked for association between the social demographic, clinical history and risk/behavioral factors with HBV among HIV positive participants. Male gender was found to be significantly associated with HBV infection among the HIV positive participants. This finding agrees to other studies carried out in Lesotho, Brazil and Nigeria (Mugomeri *et al.,* 2015, Freitas *et al.,* 2014, Balogun *et al.,* 2012). Contrary to the findings of this study, Kerubo *et al.,* (2014) in a study involving participants from two informal settlements in Nairobi, Kenya did not find any association between HIV/HBV co-infection with gender. The fact that male gender is associated with HBV in the current study can be attributed to risky behaviours related to HBV transmission such as multiple sexual partners without use of protective measures and involvement in intravenous drug use more than their female counterparts.

Although not statistically significant ($p = 0.429$) those who were HIV-HBV co-infected had a lower CD4 count (378 cell/µl) compared to those who were HIV mono-infected (419.5 cell/µl). This observation is in agreement with other studies done in Kenya (Otedo, 2004) and Ghana in a similar set-up (Olawumi *et al.,* 2014). Low CD4 counts among HBV-HIV co-infected patients implies a relatively longer or severe HIV infection and immune compromise that may enhance re-activation of HBV infection in the presence of HIV (Otedo, 2004).

Involvement in intravenous drug use was also found to be significantly associated with HBV infection OR, 5.3 (95% CI 1.6-17.8) among HIV participants of this study. Zenebe *et al.,* (2014) reported that individuals who used injectable drugs were 5 times more likely to have HBV infections which is in tandem with the current study. This is a clear evidence of the efficiency of intravenous drug use in transmission of blood borne viral infections (Garfein *et al.,* 1996). Contrary to what would be expected, the co-infection rates seemed to decrease as the number of partners' increases. This would be attributed to the fact that those with more than one sexual partner are aware of the risk they have for contracting sexually transmitted

infections and hence use condoms as compared to those with a single sexual partner who under normal circumstances would not use protection. To add on this, those who were having multiple sexual partners in this study were majorly aged below 40 years meaning they were young hence higher chances of being educated on ways of protecting themselves against STIs.

Other studies have documented that factors such as increasing age (Freitas *et al.,* 2014), blood transfusion (Zenebe *et al.,* 2014, Alhuraiji *et al.,* 2014) tattooing/scarification (Zenebe *et al.,* 2014) homosexuality (Freitas *et al.,* 2014, Brandaoa *et al.,* 2015) to be associated with HBV infections among HIV positive patients. This did not however agree with the present study findings probably due to low numbers of participants who had the above characteristics.

Some of the limitations of this study included the use of a single serological marker. Not all serological markers of HBV were used to for diagnosis of HBV therefore chances of leaving out possible occult cases positive for HBV DNA extraction. In the risk factors analysis, majority of participants who are at higher risk of HBV acquisition such as the known men who have sex with men and intravenous drug users could not participate in the study as they have their special clinics where they receive similar services as those offered in the comprehensive care centre where this study was done. Finally the current study was not able to analyse liver function enzymes (Aspartate transferase and Alanine transaminase) and viral load which would have shown relationship between HBV infection and clinical variables among those who are HIV-HBV co-infected.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The following conclusions can be made based on the findings of this study:

1. The study found an intermediate HBV-HIV prevalence of 4.9 % among patients attending Malindi Sub-county hospital CCC which is agrees with results from other studies done in the country.
2. Phylogenetic analysis of the Pre S1 region revealed that 9/10 (90%) samples belonged to genotype A while 1/10 (10%) belonged to genotype E.
3. Gender and intravenous drug use were found to be significantly associated with HBV infection.

6.2 Recommendations

1. There should be continuous surveillance of HBV-HIV co-infections as they share similar routes of transmission to ensure early detection and management of cases using ARV drug regimens that counter both.
2. Continued analysis of HBV genotypes among HIV patients should be continued to study their circulation trends and diversity among different risk groups.
3. Public awareness on the role of intravenous drug use should be thoroughly made to those who are involved and ensure one person one needle policy is implemented. Efforts should be made to ensure counselling and methadone therapy reaches out to all persons using injectable drugs. Emphasis on safe sexual practices among males and females should continue relentlessly. More studies on risk factors associated with the HBV-HIV co-infections should be done among specific sub-populations e.g. persons having tattoos or CSWs to ensure a big population of person sharing a behaviour is sampled for strong results.

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APPENDICES

Appendix I: Informed Consent Form

Study title: Molecular epidemiology and risk factors associated with HBV infection among human immunodeficiency virus (HIV) patients attending comprehensive care clinic (CCC) at Malindi Sub-county Hospital, Kenya.

Principle investigators

James G Komu- Student JKUAT-ITROMID

Co-investigators

Prof. Juliette Ongus- Senior lecturer department of Medical Laboratory Sciences, JKUAT

Dr. Raphael Lwembe- Research scientist, KEMRI

Dear participant,

My name is... a student from JKUAT working on the above mentioned project.

The purpose of this study is to determine the various types of HBV, relate the HBV-HIV co-infection with demographic aspects of the patients as well as, determining the prevalence of HBV/HIV co-infection among HIV patients attending the CCC at Malindi Sub-county Hospital.

Introduction

Hepatitis B Virus is one of the viruses associated with hepatitis a disease that affects the liver. The virus is spread through the same means as HIV and so HIV patients are at increased risk of being infected by the virus even without their knowledge since it is not part of routine medical checkup for the HIV patients. Individual co-infected with the two viruses are more likely to develop long-lasting hepatitis which result to

liver disease, associated with high mortality and morbidity rates as compared to HIV or HBV mono-infections. Again different genotypes for the HBV exist which have different geographical distributions in the world. Some genotypes have been associated with more specific antiretroviral drugs resistance than others hence determination of the genotype helps determine the appropriate drug to use.

So that I can be sure you are informed about this project, you will/a nurse will read with you this consent Form. This form will inform you why this research study is being done, what will happen in the research study, and possible risks and benefits to you. If there is anything you do not understand, please ask questions.

Procedures

Once you have agreed to enroll in the study, you were asked some personal questions concerning the study. This will done by a professional nurse counselor and will only take about 10 minutes. The study team will also access your health records to obtain any other information they may need. Five milliliters of blood were drawn using methods to protect infection by disease causing organisms by a well trained and experienced individual from this institution. This will take about one minute. There were no attachment of names to the blood samples, but an identification number assigned to you were used to label the sample. This is to ensure complete confidentiality of the test results. The sample were taken to the laboratory, analyzed and final report concluded in less than one year. This work were done at the KEMRI HIV and Hepatitis laboratories.

Potential risks, discomforts or inconveniencies

Blood collection will not expose you to any health risks except minimum discomfort associated with puncturing of your skin. The discomfort will last only for few minutes. There might also be some possibility of minimum swelling during withdraw of blood.

Potential benefits

This study will enable you as the participant to know your HBV infection status and the genotype that is infecting you because the results were given back. This information will enable the clinicians to make wise decision in prescribing antiretroviral drugs for you which will increase your life expectancy. The study may also assist the government when planning management of problems resulting from viruses to include all viral co infections. No monetary benefits were offered by this study.

Participation and voluntarism

Your participation in this project is free and voluntary. You have a right to decline participation in the project at this time by failing to sign this form. You may also stop at any time. Failure to participate will not affect the services you receive from this facility now or in future.

Confidentiality

The information you give in the questionnaire were kept confidential and will only be shared by the project staff for analysis. Results of this study may be presented in public talks or written articles, but no information were presented that identifies you since I will use a special identification codes other than individual names

PARTICIPANT'S CONSENT

For participants who informed consent was read for them.

By signing my name below, I confirm the following:

This entire consent document has been read to me. All of my questions have been answered to my satisfaction.

The study's purpose, procedures, risks and possible benefits have been explained to me.

I voluntarily agree to participate in this research study. I agree to follow the study procedures as directed. I have been told that I can stop at any time.

Name of participant...Signature…………..............

Date…………………….........................

Name of witness……………………………………….Signature………………….

For participants who read the informed consent on their own

By signing my name below, I confirm the following:

I have read the entire consent document. All of my questions have been answered to my satisfaction.

I have understood the study's purpose, procedures, risks and possible benefits.

I voluntarily agree to participate in this research study. I agree to follow the study procedures as directed. I have understood that I can stop at any time.

Name of participant...Signature…………..............

Date……………………...............................

Project contacts

If you have any questions about this project you may contact us through mobile number 0712043355 (texts and calls) or email jamsgitau@yahoo.com Or the Kenya Medical Research Institute ethical committee on the below address.

The Secretary

KEMRI Ethics and Research committee

P.O. BOX 54840-00200 Nairobi

Tel. 020- 2722541 or 0722 205901 or 0733 400003.

Email address: **ercadmin@kemri.org**

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CONSENT AGREEMENT FORM

I participated in consent process and Acknowledge enrolment of this participant into the study.

Name of Principal investigator/ Assistant…………………………………………….

Signature……………………………………………Date……………………………

Appendix II: Fomu Fahamisho ya Kibali.

Mada ya uchunguzi: Sifa za Molekula na hatari zinazohuishwa na maambukizi ya virusi vya hepatitis B (HBV) Miongoni mwa wagonjwa wa virusi vya ukimwi (VVU) wanaohudhuria kliniki ya huduma kina (CCC) katika Hospitali ya kaunti-ndogo ya Malindi.

Mchunguzi mkuu

James G Komu- Mwanafunzi JKUAT-ITROMID

Wachunguzi-wenza

Prof. Juliette Ongus- mhadhiri mkuu idara ya maabara matibabu ya kisayansi, JKUAT

Dr. Raphael Lwembe- mwanasayansia mtafiti, KEMRI

Mpendwa Mshiriki,

Jina langu ni …………………….. Mwanafunzi kutoka JKUAT anayeshughulikia mradi huu uliotajwa hapo juu.

Kusudio la uchunguzi huu ni kuweza kutafuta aina mbalimbali za HBV, kisha kuhusisha maambukizi-saidizi ya HBV-VVU na dhana za kidemografia za wagonjwa pamoja na, kuamua uwepo wa maambukizi-saidizi ya HBV/VVU miongoni mwa wagonjwa wa VVU wanaotembelea hospitali ya wilaya ya Malindi katika CCC.

Utangulizi

Virusi vya Hepatitis B ni mojawapo ya virusi vinavyohusishwa na Hepatitis ambao ni ugonjwa unaoathiri maini.Virusi huenea kupitia kwa njia sawa na ile ya VVU na

hivyo basi wagonjwa wa VVU wako katika hatari iliyoongezeka ya kuwa na virusi hivi hata bila ya ufahamu wao kwa sababu si mojawapo wa ukaguzi wa kimatibabu wa kimazoea kwa wagonjwa hawa wa VVU. Mtu aliyeambukizwa tena na virusi viwili anao uwezekano mkubwa zaidi wa kupata Hepatitis kali zaidi ambayo itasababisha ugonjwa wa maini, unaohusishwa na vima vya kiwango cha juu vya kufa vikilinganishwa na maambukizo-pekee ya VVU au HBV. Vilevile jinotaipu tofauti za jinotaipu za HBV zinapatikana na ambazo zinao uenezi tofauti wa kijiografia kote ulimwenguni. Baadhi ya jinotaipu zimehusishwa na dawa mahususi zaidi za kupunguza makali ya maambukizi haya kuliko nyingine na hivyo basi uamuzi wa jinotaipu hizi husaidia kuamua ni dawa ipi inayofaa kutumiwa. Ili niwe na hakika kuwa umefahamishwa kuhusu mradi huu, utasoma/mtasoma na muuguzi fomu hii ya kibali. Fomu hii itakufahamisha kwa nini uchunguzi huu wa utafiti unafanywa, ni nini kitafanyika katika uchunguzi huu wa utafiti na hatari pamoja na manufaa yanayowezekana kwako. Kama kunalo lolote usilolielewa, tafadhali uliza maswali.

Taratibu

Pindi utakapokuwa umekubali kujiunga katika uchunguzi huu, utaulizwa baadhi ya maswali yanayohusiana na uchunguzi wenyewe. Hii itafanyiwa na mshauri muuguzi mtaalamu na itachukua muda wa dakika kumi hivi.Timu ya utafiti itakubaliwa kutumia kumbukumbu za afya za washiriki. Mililita tano za damu zitatolewa kwa njia ya kitaalamu na mtaalamu mwenye tajiriba na mafunzo kutoka katika taasisi hii. Hii itachukua karibu dakika moja. Hakutakuweko na vibandiko vya majina katika sampuli hizo za damu, lakini nambari ya kitambulisho itakayohusishwa na wewe itaweza kutumika katika kutambulisha sampuli hiyo. Hii ni kuhakikisha kuwa ufaragha kamilifu wa matokeo ya kipimo hiki unafikiwa. Sampuli hii itapelekwa kwenye maabara, kwa uchunguzi na ripoti kamilifu kutolewa ndani ya muda usiozidi mwaka mmoja. Kazi hii itafanyiwa katika maabara ya KEMRI VVU na yale ya Hepatitis.

Hatari,usumbufu tarajiwa

Ukusanyaji wa damu hautakuathirii katika hatari yoyote ya kiafya isipokuwa tu maumivu kidogo yanayohusiana na kule kudugwa kwenye ngozi yako. Maumivu haya yatadumu kwa dakika chache tu. Huenda pia kukawa pia na baadhi ya uwezekano wa uvimbe mdogo wakati uondoaji wa damu.

Faida-tarajiwa

Uchunguzi huu huenda ukakuwezesha ukiwa mshiriki kujua hali yako ya maambukizi ya HBV na jinotaipu ambayo imekuambukiza. Taarifa hii itawezesha wanakliniki kuweza kutoa uamuzi wa busara katika kukushauri ni dawa zipi za kupunguza makali ya ugonjwa na hivyo basi kuzidisha uhai wako. Uchunguzi huu huenda ukasaidia pia serikali wakati inapopangilia usimamizi wa janga la virusi ili kuweza kujumuisha maambukizi saidizi yote ya virusi. Hakuna manufaa yoyote ya kifedha yatakayokuwepo kwako katika uchunguzi huu.

Kushiriki na Kujitolea mhanga

Kushiriki kwako katika mradi huu ni bila malipo na kwa kujitolea mhanga. Unayo haki ya kukataa kushiriki katika mradi huu wakati huu kwa kukataa kupiga saini fomu hii. Unaweza pia kusita kuendelea na mradi huu wakati wowote. Kushindwa kushiriki hakutaathiri huduma zozote unazopokea kutoka katika huduma hii sasa au hata katika siku za usoni.

Ufaragha

Taarifa unayotoa katika hojaji (questionnaire) hii itahifadhiwa kwa ufaragha na itaonyeshwa tu wafanyikazi wa mradi huu kwa kusudio la uchambuzi. Matokeo ya uchunguzi huu huenda yakawasilishwa katika mikutano ya umma ua makala yaliyoandikwa, lakini hakuna taarifa yoyote itakayowasilishwa itakutambulisha

KIBALI CHA KUSHIRIKI

Kwa washiriki ambao fomu fahamishi ya kibali imesomwa kwao

Kwa kupiga saini na kuliandika jina langu hapa chini, nathibitisha yafuatayo:

Waraka huu wote wa kibali umesomwa kwangu. Maswali yangu yote yameweza kujibiwa na nikatosheka.

Kusudio, taratibu, hatari na manufaa yanayowezekana katika uchunguzi huu yameweza kuelezewa kwangu.

Ninajitolea kwa hiari yangu kukubali kushiriki katika uchunguzi wa utafiti. Nakubali kufuata taratibu za uchunguzi huu kama nilivyoelekezwa. Nimeelezewa pia kuwa naweza kusitisha kushiriki kwangu wakati wowote.

Jina la mshiriki…………………………………..... Saini…………………………

Tarehe…………………………………..

Jina la shahidi……………………………………. Saini…………………………

Kwa washiriki ambao wamejisomea fomu Fahamisho ya kibali

Kwa kupiga saini na kuliandika jina langu hapa chini, nathibitisha yafuatayo:

Nimeusoma waraka huu wote wa kibali. Maswali yangu yote yameweza kujibiwa na nikatosheka.

Nimejisomea kusudio, taratibu, hatari na manufaa yanayowezekana katika uchunguzi huu.

Ninajitolea kwa hiari yangu kukubali kushiriki katika uchunguzi wa utafiti. Nakubali kufuata taratibu za uchunguzi huu kama nilivyoelekezwa. Nimeelezewa pia kuwa naweza kusitisha kushiriki kwangu wakati wowote.

Jina la mshiriki…………………………………..... Saini…………………………

Tarehe…………………………………..

Anwani za mradi

Kama una swali lolote unaweza kuwasiliana nasi katika namba ya mkononi 0712043355 (arafa au hata kupiga simu) au baruapepe *jamsgitau@yahoo.com* Au Kamati ya Maadili ya Kimatibabu ya Taasisi ya Kemri kwa anwani ifuatayo.

Katibu

Kamati ya Utafiti na Maadili ya KEMRI

S.L. Posta 54840-00200 Nairobi

Simu. 020- 2722541 au 0722 205901 au 0733 400003.

Barua pepe: ercadmin@kemri.org

FOMU YA MKATABA WA KIBALI

Nilishiriki katika mchakato huu wa kibali na nina dhibitisha kujiunga kwa mshiriki huyu katika uchunguzi huu.

Jina la Mchunguzi mkuu/Msaidizi…………………………………………………

Saini……………………………………………Tarehe…………………………….

Appendix III: Questionnaire

Date of hospital visit …………………………

Unique study number...

A: Socio-Demographic Characteristics

1. Date of birth..
2. Sex: Male ( ) Female ( )
3. Employment status: Employed_____Unemployed____ Self employed_____
4. Occupation……………………………………………
5. Which is your country of origin? ....
6. Level of education

    a. None [ ]
    b. primary [ ]
    c. secondary [ ]
    d. tertiary [ ]

7. Marital status

    a. Single [ ]
    b. Married [ ]
    c. Divorced [ ]
    d. Widow/widower [ ]
    e. Co-habiting [ ]

8. a. Have you travelled to other countries other than Kenya in the past 12 months?

    YES [ ] **NO** [ ]

    b. If yes which countries did you visit...?

B: Clinical Characteristics

9. When were you diagnosed with HIV…………………………………………………...

10. List of symptoms

    a. ……………………………….
    b. ……………………………….
    c. ……………………………….

11. Are you on antiretroviral therapy? ______________ Since when? ______________

12. Indicate the ART drug history ____________

    a. Have you changed the drug regimen during treatment......................................?
    b. Reason for changing regimen...

13. a. Have you ever been tested for HBV **YES** [ ] **NO** [ ]

   b. If yes what were the results...
   c. Current baseline CD4 {cell/mm^3}...
   d. Liver function tests: SGPT…………….. SGOT…………………..

14. Have you been involved in any of the following activities in the past 12 months?

   a. Intravenous drug use **YES [ ]** **NO [ ]**

   b. Blood transfusion **YES [ ]** **NO [ ]**

   c. scarification/tattooing **YES [ ]** **NO [ ]**

15. Sexual history

a. i) Homosexual [ ] ii) Heterosexual [ ] iii) Bisexual [ ]

b. Number of partners for the past six months

i) One [ ] ii) 2-3 [ ] iii) more than 4 [ ]

c. i) Have you ever been involved in commercial sex activities: YES ______ NO: _________

d. If YES **are your sexual partners**

i) Male [ ] ii) Female [ ] iii) Both Male and female [ ]

Appendix IV: Questionnaire (Kiswahili Version) .

Tarehe…………………………

Nambari ya usajili…………………………

A: Dhana Za Kidemographia

1. **Tarehe ya kuzaliwa…………………………**
2. **Jinsia Mme ( ) Mke ( )**
3. Hali ya Ajira: Nimeajiriwa_____Sijaajiriwa ____ Nimejiajiri_____
4. Kazi Halisi…………………………
5. Nchi ya kuzaliwa…………………………
6. Kiwango cha elimu

   e. **Hujawahi soma ( )**
   f. Shule ya msingi ( )
   g. Shule ya upili( )
   h. Uliendeleza masomo baada ya shule ya upili ( )

7. **Hali ya ndoa**

   f. Sijaolewa/Sijaoa [ ]
   g. Nimeolewa/Nimeoa [ ]
   h. **Umetalakiwa [ ]**
   i. Umefiwa na bibi/bwana [ ]
   j. Kiushi pamoja bila kufunga ndoa

8. Kwa muda wa miezi 12 iliyopita, ushawahi safari kwenye nchi nyingine isipokuwa Kenya?

Ndio [ ] **La** [ ]

Ni nchi gani ulizo safari …………………………

B: Dhana Za Kikliniki

9. **Ulijua hali yako ya HIV lini? …………………………**
10. Dalili za Maradhi

    d. ……………………………….
    e. ……………………………….
    f. ……………………………….

11. **Unameza dawa za kupunguza makali mwilini? ______________ Kwa mda upi?______________**
12. Historia ya matumizi ya dawa za kupunguza makali mwilini …………………………

    c. Umeshawahi kubadilisha aina ya dawa za kupunguza makali mwilini? ....
    d. Sababu ya kubadilisha aina ya dawa za kupunguza makali mwilini? ....

13. **Ushawahi pimwa virusi vya HBV Ndio [ ] La[ ]**

    e. Iwapo ndio, majibu kutokana na upimaji wa virusi vya HBV yalikua yapi?
    f. Kiwango cha CD4 {cell/mm^3}kwa wakati huu..
    g. Uchunguzi wa hali ya ini: SGPT…………….. SGOT…………………..
14. Ushawahi husika na yafuatayo?

    a. Kujidunga dawa za kulevya kwenye mishipa Ndio [ ] La[ ]
    b. Kuongezwa damu mwilini Ndio [ ] La[ ]
    c. Kuchanja ngozi/tattoo Ndio [ ] La[ ]

15. Historia ya Ngono

16. i)Shoga [ ] ii) Ngono baina ya mke na mume [ ] iii)Ngono baina ya mke (mume/mke) mume (mume/mke) [ ]

17. Umeshiriki ngon na watu wangapi kwa muda wa mieza sita iliopita

ii) mmoja [ ] ii) 2-3 [ ] iii) zaidi ya 4 [ ]

18. Ushawahi husika kwenye usherati Ndio ______ La: _________

19. Kama ndio unashiriki na

ii) Wanaume [ ] ii) Wanawake [ ] iii) Wanaume na wanawake [ ]

Appendix V: Ethical approval from KEMRI'S ethical Review committee

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **April 01, 2015**

TO: **JAMES GITAU KOMU,**
PRINCIPAL INVESTIGATOR

THROUGH: **DR. GEORGE NAKITARE,**
THE DIRECTOR, CVR,
NAIROBI

April 7th 2015

DIRECTOR
CENTRE FOR VIRUS RESEARCH
P.O. BOX 54628
NAIROBI.

Dear Sir,

RE: SSC PROTOCOL NO. 2913-(*RESUBMISSION 2 OF INITIAL SUBMISSION*): MOLECULAR EPIDEMIOLOGY AND RISK FACTORS ASSOCIATED WITH HEPATITIS B VIRUS INFECTIONS AMONG HUMAN IMMUNODEFICIENCY VIRUS INFECTED ATTENDING COMPREHENSIVE CARE CLINIC IN MALINDI SUB-COUNTY HOSPITAL IN KENYA.

Reference is made to your letter dated 25th March, 2015 and the revised documents received at the KEMRI/Scientific and Ethics Review Unit (SERU) on 27th March, 2015.

This is to inform you that the Committee notes that the issues raised at the 235th meeting of the KEMRI/Ethics Review Unit (ERC) held on 20th January, 2015 have been adequately addressed.

Consequently, the study is granted approval for implementation effective this day **1st April, 2015** for a period of one year. Please note that authorization to conduct this study will automatically expire on **March 31, 2016.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **February 17, 2016**.

You are required to submit any proposed changes to this study to SERU for review and the changes should not be initiated until written approval from SERU is received. Please note that any unanticipated problems resulting from the implementation of this study should be brought to the attention of SERU and you should advise SERU when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

EAB

PROF. ELIZABETH BUKUSI,
ACTING HEAD,
KEMRI/SCIENTIFIC AND ETHICS REVIEW UNIT

In Search of Better Health

Appendix VI: Ethical approval from Kilifi County Health Research Committee

THE COUNTY GOVERNMENT OF KILIFI
RESEARCH COORDINATING UNIT, DEPARTMENT OF THE HEALTH SERVICES

Telephone: 0721627306
0721843015
0721359983
Email: langat.eva@gmail.com
aceobonyo@gmail.com
kazunguwilfred@hotmail.com

P.O. BOX 519-10808
KILIFI, KENYA

Date 3 June 2015

14/7/2015
Noted.
Attention DASCO & CCC I/C

When Replying/Telephoning quote
REF: DOH/KLF/RESCH/VOL.I/33

James Gitau Komu M.Sc. Medical Virology, B.Sc.
Institute of Tropical Medicine and Infectious Diseases
Jomo Kenyatta University of Agriculture and Technology
P.O. Box 62000-00200 Nairobi
Email: jamsgitau@yahoo.com

14/7/15
For. NURSING OFFICER INCHARGE
MALINDI DISTRICT HOSPITAL
P.O. Box 4, MALINDI

Dear Sir,

RE: AUTHORIZATION TO CARRY OUT A STUDY IN KILIFI COUNTY

After going through your revised proposal, the committee is glad to grant you an institutional authorization to proceed with your research on **"Molecular Epidemiology And Risk Factors Associated With Hepatitis B Virus Infections Among Human Immunodeficiency Virus Infected Patients Attending Comprehensive Care Clinic In Malindi Sub-County Hospital In Kenya".**

The study should be conducted within the expiry date of your ethical approval and upon completion of you research, you are required to submit a written report to the Kilifi County Research Committee (KCRC) detailing the findings, conclusion and recommendations of your study.

We wish you the very best as you conduct your research.

Regards,

Evaline Langat
Research Coordinator
KILIFI COUNTY HEALTH RESEARCH COMMITTEE

Appendix VII: DNA extraction protocol from blood

QIAamp® DNA Mini and

Blood Mini Handbook

For DNA purification from whole blood, plasma, serum, buffy coat, lymphocytes, dried blood spots (QIAamp DNA Mini Kit only), body fluids,

Third Edition **June 2012**

cultured cells, swabs, and tissue (QIAamp DNA Mini Kit only)

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our

Sample& AssayTechnologies

advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

Purification of DNA, RNA, and proteins

Nucleic acid and protein assays

microRNA research and RNAi

Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

Elution volume	Yield (µg)	Yield (%)	DNA concentration (ng/µl)
200	6.80	100	34.0
150	6.51	95	43.4
100	6.25	92	62.5
50	5.84	86	116.8

DNA was purified with QIAamp Kits following standard protocols. Average values obtained from 20 preparations are shown.

Protocol: DNA Purification from Blood or Body Fluids (Spin Protocol)

This protocol is for purification of total (genomic, mitochondrial, and viral) DNA from whole blood, plasma, serum, buffy coat, lymphocytes, and body fluids using a microcentrifuge. For total DNA purification using a vacuum manifold, see “Protocol:

DNA Purification from Blood or Body Fluids (Vacuum Protocol)” on page 29.

Important points before starting

All centrifugation steps are carried out at room temperature (15–25°C).

Use carrier DNA if the sample contains <10,000 genome equivalents (see page 17).

200 µl of whole blood yields 3–12 µg of DNA. Preparation of buffy coat (see page 18) is recommended if a higher yield is required.

Things to do before starting

Equilibrate samples to room temperature (15–25°C).

Heat a water bath or heating block to 56°C for use in step 4.

Equilibrate Buffer AE or distilled water to room temperature for elution in step 11.

Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to the instructions on page 16.
If a precipitate has formed in Buffer AL, dissolve by incubating at 56°C.

Procedure

Pipet 20 µl QIAGEN Protease (or proteinase K) into the bottom of a 1.5 ml microcentrifuge tube.

Add 200 µl sample to the microcentrifuge tube. Use up to 200 µl whole blood, plasma, serum, buffy coat, or body fluids, or up to 5 x 10^6 lymphocytes in 200 µl PBS.

If the sample volume is less than 200 µl, add the appropriate volume of PBS.

QIAamp Mini spin columns copurify RNA and DNA when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not PCR. If RNA-free genomic DNA is required, 4 µl of an RNase A stock solution (100 mg/ml) should be added to the sample before addition of Buffer AL.

Note: It is possible to add QIAGEN Protease (or proteinase K) to samples that have already been dispensed into microcentrifuge tubes. In this case, it is important to ensure proper mixing after adding the enzyme.

Add 200 µl Buffer AL to the sample. Mix by pulse-vortexing for 15 s.

To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution.

If the sample volume is larger than 200 µl, increase the amount of QIAGEN Protease (or proteinase K) and Buffer AL proportionally; for example, a 400 µl sample will require 40 µl QIAGEN Protease (or proteinase K) and 400 µl Buffer AL. If sample volumes larger than 400 µl are required, use of QIAamp DNA Blood Midi or Maxi Kits is recommended; these can process up to 2 ml or up to 10 ml of sample, respectively.

Note: Do not add QIAGEN Protease or proteinase K directly to Buffer AL.

Incubate at 56°C for 10 min.

DNA yield reaches a maximum after lysis for 10 min at 56°C. Longer incubation times have no effect on yield or quality of the purified DNA.

Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

Add 200 µl ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

If the sample volume is greater than 200 µl, increase the amount of ethanol proportionally; for example, a 400 µl sample will require 400 µl of ethanol.

Carefully apply the mixture from step 6 to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.*

Close each spin column to avoid aerosol formation during centrifugation.

Centrifugation is performed at 6000 x *g* (8000 rpm) to reduce noise. Centrifugation at full speed will not affect the yield or purity of the DNA. If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the QIAamp Mini spin column is empty.

Note: When preparing DNA from buffy coat or lymphocytes, centrifugation at full speed is recommended to avoid clogging.

Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x *g* (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.*

It is not necessary to increase the volume of Buffer AW1 if the original sample volume is larger than 200 µl.

* Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach. See page 6 for safety information.

Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min.

Recommended: Place the QIAamp Mini spin column in a new 2 ml collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.

This step helps to eliminate the chance of possible Buffer AW2 carryover.

Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 200 µl Buffer AE or distilled water. Incubate at room temperature (15–25°C) for 1 min, and then centrifuge at 6000 x *g* (8000 rpm) for 1 min.

Incubating the QIAamp Mini spin column loaded with Buffer AE or water for 5 min at room temperature before centrifugation generally increases DNA yield.

A second elution step with a further 200 µl Buffer AE will increase yields by up to 15%.

Volumes of more than 200 µl should not be eluted into a 1.5 ml microcentrifuge tube because the spin column will come into contact with the eluate, leading to possible aerosol formation during centrifugation.

Elution with volumes of less than 200 µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield (see Table 5, page 25). For samples containing less than 1 µg of DNA, elution in 50 µl Buffer AE or water is recommended. Eluting with 2 x 100 µl instead of 1 x 200 µl does not increase elution efficiency.

For long-term storage of DNA, eluting in Buffer AE and storing at –20°C is recommended, since DNA stored in water is subject to acid hydrolysis.

A 200 µl sample of whole human blood (approximately 5 x 10^6 leukocytes/ml) typically yields 6 µg of DNA in 200 µl water (30 ng/µl) with an A_{260}/A_{280} ratio of 1.7–1.9.

For more information about elution and how to determine DNA yield, purity, and length, refer to pages 24–25 and Appendix A, page 50.

Appendix VIII: BD FACS Count CD4 Protocol

BD FACSCount™ CD4

For enumerating absolute counts and determining percentages of CD4 T lymphocytes in unlysed whole blood

50 Tests—Catalog No. 339010

2/2015 23-8777-03

IVD CE

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1. INTENDED USE

BD FACSCount™ CD4 reagents are used to enumerate the absolute counts of CD4 T lymphocytes and determine the percentage of lymphocytes that are CD4 T lymphocytes in unlysed whole blood (CD4 counts and CD4 percentages). The reagents are intended for in vitro diagnostic use on a BD FACSCount™ instrument.

Clinical Applications

CD4 counts and CD4 percentages have been used to evaluate the immune status of patients with, or suspected of developing, immune deficiencies such as acquired immune deficiency syndrome (AIDS).[1,2]

The CD4 antigen is the receptor for the human immunodeficiency virus (HIV).[3] The absolute number and percentage of CD4 T lymphocytes are the cellular parameters most closely associated with HIV disease progression and patient prognosis.[4] The number of CD4 T lymphocytes declines in HIV infection.[5-7]

2. PRINCIPLES OF THE PROCEDURE

A single test requires one ready-to-use reagent tube.

When whole blood is added to the reagent tube, fluorochrome-labeled antibodies in the reagents bind specifically to white blood cell surface antigens, and a fluorescent nuclear dye binds to the nucleated blood cells. After a fixative solution is added, the sample is run on the instrument. During sample acquisition, the cells pass through the laser light, which causes the labeled cells to fluoresce. This fluorescent light provides the information necessary for the instrument to identify and count the lymphocytes and CD4 T lymphocytes.

Reagents or Materials Required but Not Provided

- BD Vacutainer® EDTA blood collection tubes or equivalent
- Disposable pipet tips (Catalog No. 340292) or equivalent
- Vortex mixer (See Recommended Brands of Materials in the *BD FACSCount System User's Guide For Use with BD FACSCount CD4 Reagents*.)
- BD FACSFlow™ sheath fluid (Catalog No. 342003) or equivalent
- BD FACSCount™ controls (Catalog No. 340166)
- BD FACSCount system

4. INSTRUMENT

BD FACSCount CD4 reagents are designed for use on a BD FACSCount instrument. We recommend running BD FACSCount controls daily. Be sure to use the BD FACSCount CD4 protocol disk with the most recent control data when running samples stained with CD4 reagents on the BD FACSCount instrument. See

the *BD FACSCount System User's Guide For Use with BD FACSCount CD4 Reagents* for detailed instructions.

5. PROCEDURE Collecting Blood

Collect blood aseptically by venipuncture, using EDTA blood collection tubes.[19] A minimum of 100 μL of whole blood is required for this procedure. Follow the collection tube manufacturer's guidelines for the minimum volume of blood to be collected to ensure proper specimen dilution, especially when determining absolute counts with reference beads.

Anticoagulated blood stored at room temperature (20°C–25°C) must be stained within 24 hours of draw and must be analyzed within 48 hours of staining.

NOTE Do not use previously fixed and stored patient samples. Whole blood samples refrigerated before staining can give aberrant results. Specimens obtained from patients taking immunosuppressive drugs can yield poor resolution.[20] Blast cells can interfere with test results.
Hemolyzed specimens should not be used.

Performing Quality Control

We recommend performing a control run using BD FACSCount controls to check system accuracy and linearity. Run controls each day before you run patient samples or whenever you open a new reagent lot. See the *BD FACSCount System User's Guide For Use with BD FACSCount CD4 Reagents* for detailed information on performing a BD FACSCount control run.

Preparing Tubes

NOTE We recommend that you prepare no more than 15 reagent tubes at one time.

1. Label the tab of each reagent tube with the patient accession number or number that identifies the tube of blood.
2. Vortex each tube upside down for 6 seconds and upright for 6 seconds.

   NOTESet the vortex speed to a
   setting that causes the liquid to rise to the top of the tube.
3. Open each reagent tube with the coring station.

Adding Blood

1. Invert the EDTA tube 5 to 10 times to make sure that the whole blood is adequately mixed.
2. Pipette 50 μL of whole blood into the reagent tube labeled with the corresponding patient accession number.

   Reverse pipetting is critical to accuracy. We recommend using the BD FACSCount pipet that is provided with the BD FACSCount system.

   Pipette whole blood onto the side of the tube just above the liquid reagent.

If an electronic pipet is not available, follow these instructions for manual reverse pipetting.

- Depress the button to the second stop. When you release the button, excess sample is drawn up into the tip.
- Depress the button to the first stop to expel a precise volume of blood. This leaves excess blood in the tip.

Always change to a new tip between tubes. Discard tips in an appropriate biohazard container.

3. Cap the tube and vortex upright for 6 seconds.
4. Repeat steps 1 through 3 to prepare a sample tube for each patient specimen.
5. Incubate the tubes for 30 minutes at room temperature (20°C–25°C) in the workstation. Close the cover to protect the reagents from light.

NOTE Correct incubation time is critical and must be at least 30 minutes but no longer than 40 minutes for each sample tube.

Adding Fixative

1. Uncap each sample tube and pipette 50 μL of fixative solution into each tube.

   Always change to a new tip between tubes. Discard tips in an appropriate biohazard container.

2. Recap each tube and vortex upright for 6 seconds.

Run the sample tubes on the
BD FACSCount instrument within
48 hours of adding fixative. Store samples at room temperature, protected from light, until they are run on the instrument.

Running Patient Samples

See the *BD FACSCount System User's Guide For Use with BD FACSCount CD4 Reagents* for detailed information on running patient samples.

Make sure you enter the patient accession number in the software before you begin.

1. Vortex the CD4 tube upright for 6 seconds.

WARNINGInadequate suspension of
white blood cells can result in inaccurate results.

2. Uncap the tube and set the cap aside.
3. Place the sample tube in the sample holder and press Run.

   A software message will indicate when the analysis is complete.

4. Remove the sample tube and recap it.

   Discard the sample tube in an appropriate biohazard container.

5. Repeat steps 1 through 4 for the remaining samples.

6. EXPECTED RESULTS Reference Ranges

The reference ranges for BD FACSCount CD4 reagents shown in Table 1 were determined at BD Biosciences in San Jose, CA. Subjects were healthy adults between the ages of 18 and 65 years.

Table 1 Representative reference ranges for BD FACSCount CD4 reagents

Parameters	**na**	**Mean**	**95% Reference Range**
Absolute CD4 (cells/µL)	141	906.65	380–1,704
Percent CD4	141	44.90	30.13–60.23

a. n=sample size

7. PERFORMANCE CHARACTERISTICS

Performance of the reagents was established by testing at BD Biosciences in San Jose, CA and at three clinical laboratories in the US.

Accuracy (Agreement)

CD4 absolute counts were enumerated and percentages were determined with BD FACSCount CD4 reagents on the BD FACSCount instrument using BD FACSCount CD4 software v1.0. Results were compared with results from the BD Tritest™ CD3 FITC/CD4 PE/ CD45 PerCP reagent in BD Trucount™ tubes on the BD FACSCalibur™ flow cytometer using BD Multiset™ software.

Whole blood samples were collected at random at three clinical laboratories. Regression statistics are reported in Table 2.

Table 2 Regression analysis of test versus predicate for CD4 absolute counts and percentages

Parameters	**n**	**R2**	**Slope**	**Intercept**	**Range**
Absolute CD4 (cells/µL)	101	0.981	0.971	12.695	59–3,405
Percent CD4	99	0.99	0.999	–0.391	5.51–64.69

Figure 1 Regression plot of test versus predicate for CD4 absolute counts (x-axis = BD FACSCount CD4 Absolute Counts, y-axis = BD Tritest CD4 Absolute Counts)

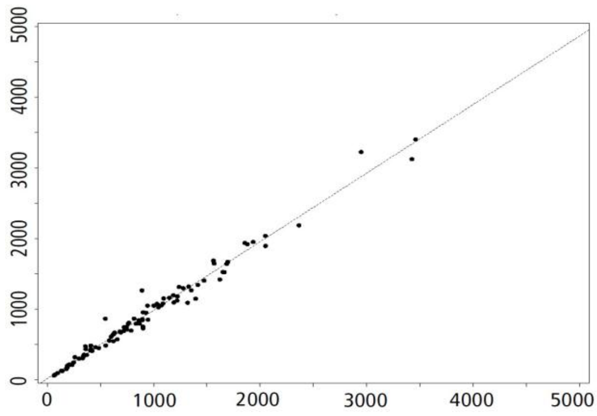

Figure 2 Regression plot of test versus predicate for CD4 percentages (x-axis = BD FACSCount CD4 Percentages, y-axis = BD Tritest CD4 Percentages)

Appendix IX: Reprint of publication

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Prevalence and Associated Risk Factors of Hepatitis B Virus Infections Among HIV-1 Infected Patients Attending the Comprehensive Care Clinic in Malindi Sub-County Hospital in Kenya

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Abstract

Human Immunodeficiency Virus (HIV) and Hepatitis B Virus (HBV) co-infections are common all over the world. Infection with HIV increases rates of HBV chronicity, prolong the time the HBV stays in circulation and increase liver-related morbidity. Factors such as intravenous drug use, multiple blood transfusions, presence of tattoos, unsafe sexual practices and being health workers have been implicated as drivers of infection & transmission of HBV & HIV. This study aimed to determine the prevalence and genotypes of HBV associated risk factors among HIV infected patients in a descriptive cross-sectional study. Malindi was chosen as a suitable study site because of the high numbers of residents involved in sex tourism as well as intravenous drug use. A structured questionnaire was used to capture social demographic data such as age, gender, employment status, occupation, the level of education and marital status, clinical history information such as duration since diagnosis with HIV, ART drug history, duration taking ARVs and baseline CD4 count and risk factors associated with HBV infections such as intravenous drug use, history of blood transfusion, tattooing/scarification, and the sexual history from 446 consenting randomly selected HIV infected participants. Five millilitres of whole blood was obtained from each participant, 50µl of which was used for CD4 cell counts using a flow cytometer. HBsAg serology was done using Diaspot® rapid diagnostic test and confirmed by Hepanostika® HBsAg Ultra ELISA kit (BioMérieux SA) and HBV DNA was extracted from all HBsAg positive samples. Nested polymerise chain (PCR) reaction and sequencing of the Pre S1 region was done. Sample sequences were compared with published HBV genotypes sequences from GenBank and Phylogenetic trees were constructed using the NJ Plot software using a PHB file created through DNA Database of Japan (DDBJ) to determine the HBV genotypes. Out of the 446 HIV positive participants, 126 (28.3%) were males and 320 (71.7%) females. Only 19/446 (4.26%) participants were positive for HBV based on rapid strip test while 22/446 (4.93%) participants had HBV based on ELISA. Twelve of the 22 ELISA positive samples were successfully amplified by PCR. Out of the 12 PCR positive samples 10 were successfully sequenced. Phylogenetic analysis revealed that 9/10 (90%) samples belonged to genotype A while 1/10 (10%) belonged to genotype E. Males (p=0.028) and intravenous drug use (p= 0.08) were significantly associated HBV infections. The high prevalence (4.9%) of HBV among HIV patients attending Malindi Sub-county hospital is most likely highly driven by intravenous drug use and multiple sexual partners among the male gender and is predominantly genotypes A and E which is similar to the general population.

Keywords: Hepatitis B virus, HIV, Co-infection, HBsAg, genotypes, intravenous drug use

Introduction

Hepatitis B virus (HBV) and Human Immunodeficiency Virus (HIV) are major public health problems and are among the 10 highest causes of infectious deaths in the world (Alter, 2006). The two viruses share similar routes of transmission (Alter, 2006, Otedo, 2004) with HBV having a hundred fold efficiency in transmission compared to HIV (Otedo, 2004). Individuals with HIV-HBV co-infection have higher HBV DNA levels (Dore *et al.,* 2010) which is associated with the immune deficiency due to HIV which in turn translates to increased HBV disease progression (Diwe *et al.,* 2013). On top of this, increased mortalities and morbidity in HIV patients as a result of liver disease are partly due to co-infection with HBV and HCV since these viruses increase intra-hepatic apoptosis which is a consequence of liver fibrosis (Diwe *et al.,* 2013).

In the world, it's approximated that 33 million people are infected with HIV and among these about 3 million (10%) are co-infected with HBV (Kourtis *et al.,* 2012). In Kenya the prevalence of HBV-HIV co-infection has been reported to be as high as 6% and significantly higher in males 19.2% compared to 14.2% in females in a study done among several Nairobi based health centres. (Muriuki *et al.,* 2013).

HBV has a wide genetic variability which has resulted into the emergence of 10 genotypes with several sub-genotypes which are labelled alphabetically from A-J (Tangkijvanich *et al.,* 2013). There is remarkable evidence suggesting that HBV genetic variability is an important factor in determining disease progression and also response to antiviral treatment (Poukarim *et al.,* 2011). In East Africa where Kenya resides, genotype A is the most

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prevalent (88%) followed by genotype E (8%) and then Genotype D (4%) (Mwangi *et al.,* 2008). Webale et. (2015) reported 100% genotype A1 among HIV-1 infected and non-infected injecting and non-injecting drug use in coastal Kenya. Genotype A1 had also been reported exclusively among HIV infected IDUs from Mombasa, region along the Kenyan coast. (Kibaya et al., 2015)

HBV is more prevalent among HIV–infected individuals than in the general population which can b attributed to the shared risk factors for transmission and acquisition of these two life threatening viruses (Chen *al.,* 2013). Some of these risk factors associated with the transmission of HIV and HBV include: involvement intravenous drug use, scarification/tattooing, having several heterosexual partners/involvement in commercial se male having sex with men and occupational hazards for health workers (Alter, 2006, Chen *et al.,* 2013, Ranjbar *al.,* 2013).

The primary aim of this study was to determine the prevalence of HBV and circulating genotypes among HI positive participants attending the comprehensive care clinic at Malindi Sub-county hospital. The diversity countries of origin of the tourists to this area and their interaction with the local people would arouse th epidemiological curiosity about the introduction of new strains, their genetic diversity and the implications on th clinical presentation of disease in cases where patients are co-infected. Characterization of HBV among the HI patients will help in understanding the distribution of specific genotypes in the study setting. The genetic patter of the virus will in turn help policy makers and clinicians in making sound decisions during prescription of drug to these patients since different HBV genotypes have different responses to the antiretroviral drugs.

Methods

Study site and patients recruitment

This was a descriptive hospital based cross sectional study carried out between May 2015 and April 2017 at th Comprehensive Care Centre (CCC) at the Malindi sub-county hospital. Malindi was selected as a suitable stud site because it is located along the coastal strip of Kenya, a region that is popular with tourists both local an foreign. Foreign tourist in Malindi come from all over the world with the Italians, Germans and those from th United Kingdom topping the list. This region is on record for having a fairly large population of persons wh engage in sex tourism as well as recreational intravenous substance abuse and in some cases they may share needle to inject themselves with the narcotics and/or engage in unprotected sexual contact. Simple random sampling wa used to recruit patients aged 18 years (due to ethical issues) and only those from the CCC. Any vulnerable person such prisoners and the mentally challenged were not recruited for the study. Participation was upon providing written informed consent. Those who were randomly selected and did not consent were not recruited and henc replaced.

Data on socio-demographic, clinical and associated risk factors

A study questionnaire was designed to obtain information from patients and also from the patient's hospital recor The information captured in the questionnaire included: social demographic characteristics including Age, gende employment status, the level of education and marital status, Clinical history of patients including Duration sinc diagnosis with HIV, ART drug history, duration taking ARVs and baseline CD4 count. The questionnaire als sought to obtain information on risk factors associated with transmission of HIV and HBV including Intravenou drug use, history of blood transfusion, tattooing/scarification, and the sexual history. The questionnaire wa administered in a confidential counselling room by a professional counsellor who was recruited for this study.

Sample collection, processing, shipping and storage

Five millilitres of blood was collected aseptically into EDTA vacutainer bottles (BD New Jersey, USA) usin venepuncture. Fifty microliters (50µl) of the sample were analysed for CD4 counts within 24 hours of collectio through flow cytometry using the BD Facs Caliber machine. The samples were then separated using centrifugatio at 3000 RPM for 10 minutes and the plasma stored at -20 C. The samples were then packaged using the bas triple packaging system according to the International Air Transport Association (IATA) regulations. Th included primary receptacles (screw capped containers) which were leak proof covered with a layer of absorbe material, a secondary receptacle (zip lock bags) which was also leak proof enclosing the primary receptacle an finally a leak-proof outer packaging i.e. cool box labelled with an easily noticeable biohazard symbol. Th samples were then transported to KEMRI under a cold chain through a paid for courier services. After un packaging they were stored at -80 C until serological and molecular tests were done.

HBV serology.

The samples were screened for HBsAg using the DiaSpot® HBsAg test kit which is a rapid one step test for th qualitative detection of HBsAg and Hepanostika® HBsAg Ultra ELISA kit (BioMérieux SA) which is based on "sandwich" principle. The tests were done according to the manufacturer's instructions.

DNA extraction and PCR amplification of HBV PRE S1 region
DNA from the 22 samples that tested positive for HBsAg was extracted from 50µl of plasma using Qiagen® DNA extraction kit (Qiagen) using the manufactures instructions. Extracted DNA was used as template in a PCR to amplify the Pre S1 (681bp) region by nested PCR using a set of primers (table1). To describe this briefly, the master mix for the first amplification included 2.5µl of 10x PCR buffer, 2.0µl of 10mM dNTPs, 0.3µl of first set of primer S1F and S1R (Table 1), 1µl Taq polymerase and 5µl of extracted DNA.

Table 1: Primers used for HBV PCR amplification and sequencing

Primers	Nucleotide position	Sequences	Expected fragment size region (bp)
PS1 forward PS1 Reverse	55-72 995-974	TCCTGCTGGTGGCTCCAG CGTTGACATACTTTCCAATCAA	940
PS2 Forward PS2 Reverse	155-173 835-814	ACCCTGYRCCGAACATGGA CAACTCCCAATTACATARCCCA	680

The PCR conditions started at 94°C for 7 minutes, followed by 38 cycles of 94°C for 40 seconds, 60° C for 1 minute and 72° C for 2 minutes and final extension for 72° C for 15 minutes. The second master mix will include 5µl of 10x PCR buffer, 1.5µl magnesium chloride, and 4.0µl of dNTPs, 0.25µl of second set of primer S2F and S2R (table 1) and 2µl Taq polymerase. Visualization of amplified DNA was done on 1.2% agarose gel in 1x TAE buffer stained with ethidium bromide.

Sequencing and viral genotyping
Purified PCR products were directly sequenced using BigDye terminator v3.0 in a Sequencing Ready Reaction Kit (Macrogen Inc, Korea) using the 2nd PCR primers S2F and S2R (table 1). The sequencing reactions were performed using ABI3730 automated sequencer (Applied Bio systems). The resulting forward and reverse sequences from each sample were assembled using the GENETYX® Ver.9 software. The sequences were aligned with references sequences from gene bank and trimmed with MEGA 7.0.25 software. Phylogenetic relationship were inferred by creating a PHB file through the DNA Data bank of Japan (DDBJ) and opening the file through NJ plot software.

Ethical consideration
The study was ethically approved by the KEMRI scientific steering committee (SSC) and the Ethical review committee (ERC) (SSC Protocol number 2913) before implementation. The study was also approved by the Kilifi County Research committee (Ref:DOH/KLF/RESCH/VOL.I/21) and the management of Malindi Sub-county Hospital.

Data analysis
Social demographic, clinical and risk factors data was entered and cleaned using Ms Excel and transferred to Statistical package for social sciences (SPSS) version 21. SPSS was used to analyse the frequencies, means and percentages for the social demographic data, clinical data and the risk factors against the HBV sero-positivity through cross-tabulation. Independent t test was used for comparison of means for the CD4 counts between the HIV mono-infected and HIV-HBV mono-infected. STATA was used to perform bivariate and multivariate analysis to determine factors associated with HBV infections using Poisson regression with significance set at $P \leq 0.05$ and odds ratio with corresponding 95% confidence interval.

Results
Baseline characteristics of study participants
A total of 446 HIV positive participants including 126 (28.3%) males and 321(71.8%) females were enrolled for the study. Their ages ranged from 18-76 years with a mean age of 42.98 +/-10.750 years. The mean age of males was 44.93 years (SD ±10.16) while that of females was 42.22 years (SD ±10.88). Other baseline characteristics of the study participants have been summarised in table 2

At the time of sample collection 69 (15.5%) individuals had not started ARVS. Those who were taking AZT, 3TC, NVP regimen were the majority (33.4%) while 26.9% were on 3TC, TDF, EFV. The rest (24.2%) were on TDF, 3TC, NVP

Table 2- Baseline characteristics among HIV positive patients attending Malindi sub-county Hospital

Variable	Sample size (N = 446)	
	No	%
Age (Years)		
Mean (± SD)	42.98	(± 10.75)
Median (IQR)	43	(34.75 - 50)
Range	58	(18 - 76)
≤30	62	13.9
31-40	125	28.1
41-50	158	35.4
≥50	101	22.6
Gender		
Males	126	28.3
Females	320	71.7
Level of Education		
Primary	240	53.8
Secondary	77	17.3
Tertiary	21	4.7
none	108	24.2
Employment status		
employed/self employed	246	55.2
unemployed	200	44.8
Marital status		
Single	48	11
Married	236	53
widow/divorced	162	36
ARV drug currently on use		
3TC, TDF, EFV	120.0	26.9
AZT, 3TC, NVP	149	33.4
TDF,3TC, NVP	108	24.2
Not started	69	15.5
CD4 count		
Mean (± SD)	417.49.	(±239.643)
Median (IQR)	403.5	(247.5-562)
Range	1331	0-1331
CD4 less 500	292	65.5
CD4 ≥500	154	34.5
Duration Since HIV diagnosis		
0-5 yrs	227	50.9
6-10 yrs	204	45.7
>10yrs	13	3.4
Duration since ART initiation		
0-5 yrs	264	59.2
6-10 yrs	175	39.2
>10yrs	7	1.6
Intravenous Drug Use		
Yes	13	2.7
no	433	97.3
History of blood transfusion		
Yes	36	8.1
No	410	91.9
Scarification/Tatooing		
Yes	9	2
No	437	98
Involvement in CSW		
Yes	23	5.2
No	423	94.8
Sexuality		
Heterosexual	441	98.9
Homosexual/Bisexual	5	1.1
Number of partners in the past 6 months		
None	86	19.3
One	329	73.8
2 to 3	24	5.4
≥4	7	1.6

Laboratory results

A total of 19 (4.26%) participants were HBsAg positive based on rapid strip test while 22 (4.94%) participants were positive for HBsAg based on ELISA. All the 19 samples that were positive via the rapid test were confirmed positive via ELISA. Males had the highest prevalence of the HIV-HBV co-infection 11 out of 126 (8.7%) compared to 11 out of 320 females (3.4%). HBV DNA was successfully amplified in 12 out of 22 (54.5%) samples positive for HBsAg ELISA. 10 out of the 12 HBV-DNA positive samples were successfully sequenced. This has been summarised in table 3

Table 3: A summary of the laboratory tests done and their results

	HBsAg serology (n=446)		HBV DNA PCR positive (n=22)	HBV Sequencing (n=12)
	Rapid	ELISA		
Positive	19 (4.26%)	22 (4.94%)	12 (54.5%)	10 (83.3%)
Negative	427 (95.74%)	424 (95.06%)	10 (45.5%)	2 (16.7%)

The mean CD4 count for the study population was 417.5 (SD = ±240.3 cell/µl). Although not statistically significant (p = 0.429) those who were HIV-HBV co-infected had a lower CD4 count (378 cell/µl) compared to those who were HIV mono-infected (419.5 cell/µl)..

Phylogenetic analysis and genotypes

Phylogenetic analysis revealed that 9/10 (90%) belonged to genotype A which were all categorised into sub-genotype A1 while 1/10 (10%) belonged to genotype E. A phylogenetic tree was constructed by NJ plot software using a phb file generated from DNA Data Bank of Japan (DDBJ) as shown in fig 1.

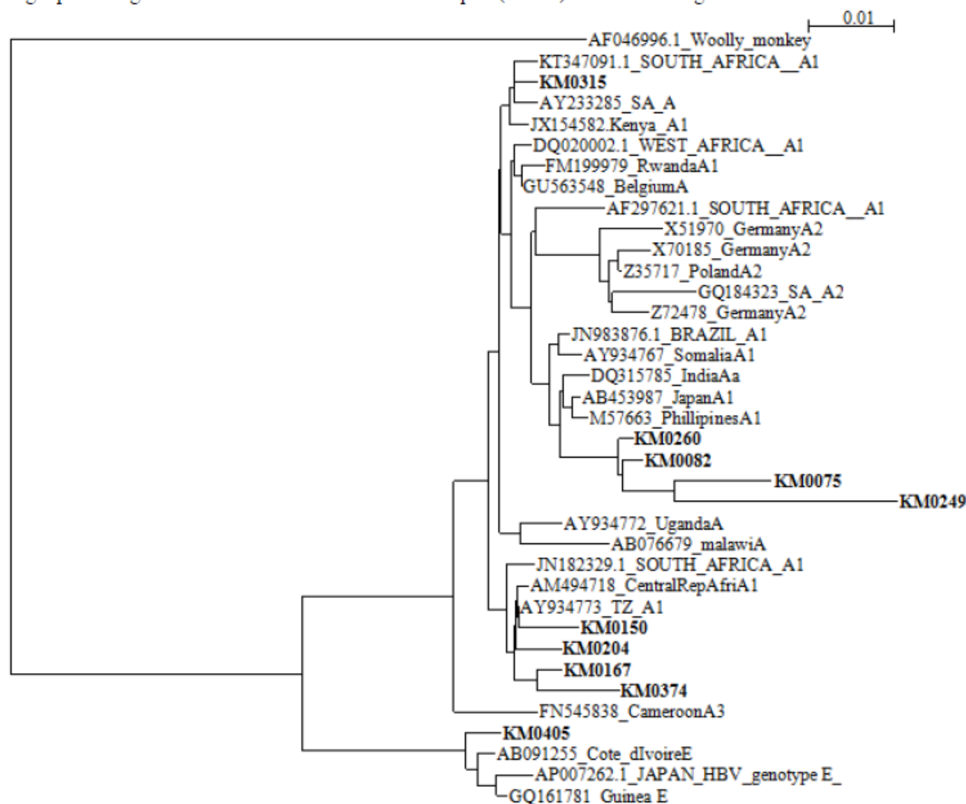

Fig 1: Phylogenetic analysis based on the Pre-S region from 10 HBV strains from HBV-HIV co-infected patients attending the Malindi Sub-County Hospital and selected reference sequences representing some of the known HBV genotypes/Sub-genotypes from the Genebank. The 10 HBV characterised in this study are indicated in bold and 9/10 fall under genotype A1 while 1/10 fall under Genotype E. The references are labelled with their accession numbers, country of origin and the genotype they belong to. The tree is rooted from the Woolley Monkey virus genome sequence.

Factors associated with HBV infections

Univariate analysis showed that gender (p = 0.029) and Intravenous drug use (p = 0.008) were significantly associated with HBV infection whereas all other factors were not statistically significant. In Multivariate analysis gender (p = 0.013) and Intravenous drug use (p = 0.05) were also statistically significant (Table 4)

Table 4: Univariate and Multivariate analysis of factors associated with HBV infection among HIV patients attending CCC at Malindi Sub-County Hospital. (N=446)

Variable	Sample size (N = 446)				Bivariate		Multivariate
	No.	HBV No. (n=22)	HBV %	P - value	OR (95% CI)	P - value	OR (95% CI)
Gender							
Male	**126**	**11**	**8.7**	**0.029**	**2.2(1.1-5.9)**	**0.013**	**3.2(1.3-8.1)**
Female	320	11	3.4	Referent	Referent	Referent	Referent
Age (Years)							
≤30	62	2	3.2	0.34	0.5(0.1-2.2)	0.542	0.6(0.1-3.0)
31-40	125	4	3.2	0.218	0.5(0.1-1.5)	0.384	0.6(0.2-2.0)
41-50	158	9	5.7	0.697	0.8(0.3-2.2)	0.961	1.0(0.4-2.7)
>50	101	7	6.9	Referent	Referent	Referent	Referent
Employment status							
employed/self employed	246	14	5.7	0.426	1.4(0.6-3.4)	0.378	1.5(0.6-3.8)
unemployed	200	8	4	Referent	Referent	Referent	Referent
Level of Education							
Primary	240	14	5.6	0.92	1.1(0.4-2.7)	0.606	0.8(0.3-2.2)
Secondary	77	1	1.3	0.178	0.2(0-1.9)	0.056	0.1(0-1)
Tertiary	21	1	4.8	0.887	0.9(0.1-7.1)	0.511	0.5(0.1-4.3)
Non formal	108	6	5.6	Referent	Referent	Referent	Referent
Marital status							
Single	48	1	2.1	0.352	0.4(0.0-3.0)	0.532	0.5(0.1-4.2)
Married	236	12	5.1	0.841	0.9(0.4-2.1)	0.514	0.7(0.3-1.8)
Widow/Widower/Divorced	162	9	5.6	Referent	Referent	Referent	Referent
ARV drug currently on use							
3TC, TDF, EFV	120.0	8	6.7	0.15	4.6(0.6-36.8)	0.995	0
AZT, 3TC, NVP	149	10	6.7	0.144	4.6(0.6-36.2)	0.995	0
TDF,3TC, NVP	108	3	2.8	0.573	1.9(0.2-18.4)	0.995	0
Not started	69	1	1.4	Referent	Referent	Referent	Referent
CD4 count							
Less than 500	292	16	5.5	0.476	1.4(0.6-3.6)	0.433	1.5(0.6-3.9)
Greater than 500	154	6	3.9	Referent	Referent	Referent	Referent
Duration since HIV diagnosis							
0-5yrs	227	9	4	0.995	0	1	0
6-10yrs	204	13	6.4	0.965	0	1	0
>10yrs	15	0	0	Referent	Referent	Referent	Referent
Duration since ARV initiation							
0-5yrs	264	11	4.2	0.996	0	0.999	0
6-10yrs	175	11	6.3	0.996	0	0.999	0
>10yrs	7	0	0	Referent	Referent	Referent	Referent
Intraveneous drug use							
Yes	**13**	**3**	**23.1**	**0.008**	**5.3(1.6-17.8)**	**0.05**	**4.1(1.0-16.6**
no	433	19	4.4	Referent	Referent	Referent	Referent
History of blood transfusion							
Yes	36	0	0	1	0	0.995	0
No	410	22	5.4	Referent	Referent	Referent	Referent
Scarification/Tatooing							
Yes	9	0	0	0.999	0	0.997	0
No	437	22	5	Referent	Referent	Referent	Referent
CSW							
Yes	23	1	4.3	0.897	0.9(0.1-6.5)	0.813	0.8(0.1-6.0)
No	423	21	5	Referent	Referent	Referent	Referent
Sexuality							
Homosexual	1	0	0	1	0	0.999	0
Bisexual	4	0	0	1	0	0.998	0
Heterosexual	441	22	5	Referent	Referent	Referent	Referent
No. of patners in past six months							
One	329	14	4.3	0.161	0.5(0.2-1.3)	0.398	0.7(0.2-1.7)
2 to 3	24	1	4.2	0.531	0.5(0.1-4.2)	0.846	0.8(0.1-8.8)
≥4	7	0	0	0.994	0	0.998	0
None	86	7	8.1	Referent	Referent	Referent	Referent

Discussion

Several studies have been carried out in Sub-Saharan Africa including Kenya to access the burden of hepatitis B virus among HIV infected individuals. However majority of this studies focus on populations that form risk sub-

populations for HIV and HBV transmission or newly diagnosed HIV patients. The present study focused prevalence, risk factors and genotypes of HBV in a generalized HIV infected patients population receiving medical care in one of government hospital set up in Kilifi County.

Generally, the prevalence of HBV is higher among HIV–infected individuals than in the general population which can be attributed to the shared risk factors for transmission and acquisition of these two life threatening viruses (Chun *et al.,* 2012). The present study reported a HBV prevalence of 4.9% (22/446) among HIV positive participants based on laboratory analysis of HBsAg through ELISA. The rates of HIV-HBV co-infections have previously been reported to be as high as 10–20% in countries where HBV infection is either endemic or intermediate to high HBV cases (Muriuki *et al.,* 2013). In Kenya, a study by Muriuki *et al.,* 2013 on the prevalence of HBV and HIV co-infection showed a prevalence of 6% with co-infection rates significantly higher among males (19.2%) compared to females (14.3%). This result was also in agreement with other studies carried out in Kenya including; 5.7% (Wambani *et al.,* 2015), 4.26 % (Kerubo *et al.,* 2015) and 3.6 % as reported by Webale *et al.,* (2015) among HIV positive non-IDUs in Mombasa, Coastal Kenya. The results were also similar to those reported in other parts of the world including 5.2 % in Morocco (Rebbani *et al.,* 2013) and 3.8 % in Brazil (Brandaoa *et al.,* 2015). In South Africa between 5% and 17% of HIV-infected patients in the country were found to be co-infected with Hepatitis B virus (Chun *et al.,* 2012).

The results of this study were found to be lower than those of other studies carried out in other studies including jaundiced patients in Kenya which reported 53 % (Otedo, 2004) and 50.6 % (Ochwoto *et al.,* 2016), 25.5 % among HIV patients in Morocco (Magoro *et al.,* 2016), 19.2 % among HAART naïve patients in China (Chen *et al.,* 2013), 12 % among HIV patients in Columbia (Bautista *et al.,* 2014), 10.5 % among HIV patients in Lesotho (Mugomeri *et al.,* 2015), 9.6 % among HIV IDUs in Mombasa, Kenya (Webale *et al.,* 2015). The higher HBV prevalence rates in this studies compare to the current study could have been as a result of using sub-populations that have evident signs associated with HBV such as the jaundiced patients who were attending clinics due to liver related complication, being selected from HBV high risk sub-populations such as the intravenous drug users or newly diagnosed patients who had not started ART.

On the other hand, the results reported in this study were contrarily higher compared to reports from Nigeria as reported by Dore *et al.,* (2010) and Diwe *et al.,* (2013) indicating HBV/HIV prevalence rates of 2.4% and 2.2% respectively, 2.2 % in Malawi (Varo *et al.,* 2016) and 2.5 % in Brazil (Freitas *et al.,* 2014). The higher prevalence rate in the present study could be as a result the samples being collected from a HBV prevalence intermediate (2-7%) area as classified by WHO (Zenebe *et al.,* 2014). As reported by Mukami *et al*., (2013) other factors that might have resulted in disparities in the prevalence rates between this studies result and those conducted in other places could include but not limited to sample size used, specificity and sensitivity of kits used and diversity of behavioural characteristics of each population.

Phylogenetic analysis of the HBV genotypes in the current study revealed the presence of genotype A (90%) and E (10%) as would be expected of the Kenyan population. This observation is in agreement with other studies done in Kenya that reported a prevalence of 88% (Mwangi *et al.,* 2008), 90.3 % (Ochwoto *et al.,* 2016), 100 % (Webale *et al.,*2015) of genotype A1. Mwangi et al., (2008) had also reported genotype E although at a much lower prevalence than genotype A. All the genotype A sequences belonged to the HBV genotype A1 sub-genotypes. Five of the genotype A and the genotype E sequences clustered with sequences from countries in sub Saharan Africa including Tanzania, central Africa republic, South Africa Ivory coast and Guinea. This could be attributed to trade relationships between these African countries. The rest four sub-genotype A1 samples clustered with samples from Somalia and the Asian clade (Phillipines and Japan). This could be as a result of immigrants from Somalia who access the Kenyan coast and tourists from the Asian countries.

The current study looked for association between the social demographic, clinical history and risk/behavioral factors with HBV among HIV positive participants. Male gender was found to be significantly associated with HBV infection among the HIV positive participants. This finding agrees to other studies carried out in Lesotho, Brazil and Nigeria (Mugomeri *et al.,* 2015, Freitas *et al.,* 2014, Balogun *et al.,* 2012). Contrary to the findings of this study, Kerubo *et al.,* (2014) in a study involving participants from two informal settlements in Nairobi, Kenya did not find any association between HIV/HBV co-infection with gender. The fact that male gender is associated with HBV in the current study can be attributed to risky behaviours related to HBV transmission such as multiple sexual partners without use of protective measures and involvement in intravenous drug use more than their female counterparts.

Involvement in intravenous drug use was also found to be significantly associated with HBV infection OR, 5.3 (95% CI 1.6-17.8) among HIV participants of this study. Zenebe *et al.,* (2014) reported that individuals who used injectable drugs were 5 times more likely to have HBV infections which is in tandem with the current study. This is a clear evidence of the efficiency of intravenous drug use in transmission of blood borne viral infections. Other studies have documented that factors such as increasing age (Freitas *et al.,* 2014), blood transfusion (Zenebe *et al.,* 2014, Alhuraiji et al., 2014) tattooing/scarification (Zenebe *et al.,* 2014) homosexuality (Freitas *et al.,* 2014, Brandaoa *et al.,* 2015) to be associated with HBV infections among HIV positive patients. This did not however

agree with the present study findings probably due to low numbers of participants who had the above characteristics.

Some of the limitations of this study included the use of a single serological marker. Not all serological markers of HBV were used to for diagnosis of HBV therefore chances of leaving out possible occult cases positive for HBV DNA extraction. In the risk factors analysis, majority of participants who are at higher risk of HBV acquisition such as the known men who have sex with men and intravenous drug users could not participate in the study as they have their special clinics where they receive similar services as those offered in the comprehensive care centre where this study was done. Finally the current study was not able to analyse liver function enzymes (Aspartate transferase and Alanine transaminase) and viral load which would have shown relationship between HBV infection and clinical variables among those who are HIV-HBV co-infected.

Conclusion

The high HBV-HIV prevalence of 4.9 % among patients attending Malindi Sub-county hospital Comprehensive Care Centre is most likely driven by intravenous drug use and multiple sexual partners among the male gender. Genotype A was the most prevalent 9/10 (90%) while 1/10 (10%) belonged to genotype E an observation that relates well with the distribution of HBV genotypes among the Kenyan general population. There is therefore need to routinely diagnose HBV among new comprehensive care centres recruits to ensure proper therapeutic management using antiretroviral drugs.

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