PREVALENCE AND ASSOCIATED RISK FACTORS TO HEPATITIS B VIRUS INFECTIONS AMONG TREATED HIV INFECTED MOTHERS AND THEIR EXPOSED INFANTS VISTING HIV CARE CLINICS OF KENYATTA NATIONAL HOSPITAL, NAIROBI

JAMES MBURU KANG'ETHE

MASTER OF SCIENCE

(Medical Virology)

JOMO KENYATTA UNIVERSITY OF

AGRICULTURE AND TECHNOLOGY

2018

Prevalence and Associated Risk Factors to Hepatitis B Virus Infections among Treated HIV Infected Mothers and their Exposed Infants visting HIV Care Clinics of Kenyatta National Hospital, Nairobi

James Mburu Kang'ethe

A thesis submitted in partial fulfilment for the Degree of Master of Science in Medical Virology in the Jomo Kenyatta University of Agriculture and Technology

2018

DECLARATION

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

Signature.. ……. Date ……..

James Mburu Kang'ethe

This thesis has been submitted for examination with our approval as University Supervisors

Signature.. ……. Date ……..

Prof. Rebecca Waihenya, PhD

JKUAT, Kenya

Signature.. ……. Date ……..

Dr. Raphael Lwembe, PhD

KEMRI, Kenya

Signature.. ……. Date ……..

Dr. Onesmus W. Gachuno, PhD

UoN, Kenya

DEDICATION

This work is dedicated to my wife, son, daughter and parents for their tireless encouragement throughout my study period.

ACKNOWLEDGEMENTS

I would like to thank the Almighty God for all His mercies, my supervisors Prof. Rebecca Waihenya, Dr. Raphael Lwembe and Dr. Onesmus Gachuno for their professional guidance and tireless efforts to assist me during the course of my research. I would also like to thank all the lecturers at JKUAT and ITROMID; KEMRI for their immense support and dedication, my statistician Mr. Kenneth Mutai for his support in data cleaning and analysis, Dr. Musa Otieno of KEMRI, Mr. David Kibe and Mr. Brian Khasiwa both of University Nairobi, my collegues James Komu and Daniel Njenga as well as the entire Virology fraternity for giving me moral support throughout and my family for their unwavering support. Last but not least I would like to thank the participants who volunteered and provided me with data. God bless.

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ABBREVIATIONS AND ACRONYMS

3TC	Lamivudine
ALT	Alanine aminotransferase
ART	Antiretroviral therapy
AZT	Zidovudine
BCP	Base core promoter
CHB	Chronic Hepatitis B
CVR	Center for Virus Research
DNA	Deoxyribonucleic acid
EFV	Efavirenz
ELISA	Enzyme-linked immunosorbent assay
EME	Euromedi equip
ERC	Ethical Review Committee
FTC	Emtricitabine
HAART	Highly Active antiretroviral therapy
HAV	Hepatitis A Virus
HBcAb	Hepatitis B core antibodies

HBeAg	Hepatitis B envelope Antigen
HBSAG	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Heapatocellular carcinoma
HCV	Hepatitis C Virus
HEI	HIV exposed infants
HIV	Human immunodeficiency virus
HRP	Horseradish peroxidase
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IQR	Interquatile range
IUD	Injection drug use
JKUAT	Jomo Kenyatta University of Agriculture & Technology
JNK	c- Jun N- terminal kinase
Kb	Kilobase
KEMRI	Kenya Medical Research Institute
KNH	Kenyatta National Hospital

MAPK	Mitogenactivated protein kinase
MHR	Major Hydophillic region
ML	Milliter
MTCT	Mother to childhood transmission
NA	Nucleside analogues
NEF	Negative regulatory factor
NO	Nitric oxide
NVP	Nevirapine
PKB	Protein Kinase B
PMTCT	Prevention of mothers to childhood transmission of HIV
Rb	Retinoblastinoma gene
REV	Regulator of viral protein expression
RNA	Ribonucleic acid
RT-PCR	Real Time Polymerase Chain Reaction
SD	Standard deviation
SERU	Scientific and ethics review unit
SGPT	Serum glutamic pyruvic transaminase

SIV	Simian immunodeficiency virus
SPSS	Statistical Package for Social Sciences
SSA	Sub Saharan Africa
SSC	Scientific Steering Committee
STD	Negative regulatory factor
STD	Sexually transmitted diseases
TAT	Transactivator of transcription
TDF	Tenofovir
TMB	Tetramethylbenzidine
UON	University of Nairobi
Vif	Viral infectivity protein
VP	Viral protein
WHO	World Health Organization HBV
YMDD	Tyrosine- methionine aspartate

ABSTRACT

Hepatitis B virus (HBV) infection is a major public health problem affecting approximately 360 million people globally. Mother-to-child transmission (MTCT) is responsible for more than one third of chronic HBV infections worldwide. Mothers who are co- infected with HBV/ Human Immunodeficiency virus (HIV) and are antiretroviral therapy (ART) naïve have a high tendency of transmitting the two viruses during pregnancy, delivery or postnatally. This study aimed to determine the prevalence and associated risk factors of HBV infections among Highly Active antiretroviral therapy (HAART) receiving HIV-infected mothers and their exposed infants at the Kenyatta National Hospital (KNH) in Kenya. Eligible mothers and their exposed infants were recruited from a cohort enrolled in a Prevention of mother to child transmission of HIV (PMTCT) program in KNH. A structured questionnaire was used to capture the socio-demographic data of the participants and information on associated factors to HBV infections. Four milliliters (ml) sample of paired whole blood were obtained from HIV positive mothers and their exposed infants. Whole blood was separated into plasma and stored at -80^{o}C. HBV infection was determined using Euromedi Equipp (EME) rapid kit for Hepatitis B surface antigen (HBsAg) test and confirmed by a HBsAg Enzyme linked immune sorbent assay (ELISA). The HBsAg sero reactive samples were further screened for HBV envelope antigen (HBeAg) using ELISA (Accubiotech co.ltd). Samples which turned positive with ELISA and rapid tests were subjected to Polymerase-chain reaction (PCR) targeting the preS1 region using nested primers. HBV infection was presented as a proportion with 95% confidence interval and the associations tested using chi-square tests. A total of 534 HIV-infected mothers and their highly exposed infants were recruited. The mean age of the mothers was 31.2 years (SD 5.4 years) and the infants had a median of 6 months (IQR 3-10 months). Four hundred and thirty-three (81.1%) of the mothers were married, 272 (50.9%) having tertiary education and 113 (59.5%) were employed. One hundred and thirteen (21.2%) of the mothers were aware of HBV infection and HBV vaccination. Most of the mothers were currently receiving HAART

with 502 (94%) of the mothers taking TDF/3TC/NVP and 32 (6%) on AZT/3TC/ NVP or AZT/3TC/EFV. Out of 534 mothers, 19(3.6%) were positive for HBV. All the 19 samples that gave positive HBsAg results tested negative for HBeAg. Out of the 19 samples that tested positive with ELISA, also gave positive results with PCR targeting the preS1 gene. All exposed infants tested negative for HBV with the HBsAg rapid, ELISA and PCR tests. History of dental surgery was associated with increased rate of HBV infection among the HIV-infected mothers (OR 3.3 (95% CI 1.1-9.6). In conclusion, the results of this study suggest that the HAART regimen received by the HIV infected pregnant mothers may have prevented vertical transmission of HBV infections to exposed infants.

CHAPTER ONE

INTRODUCTION

1.1 Background

Chronic hepatitis B virus (HBV) infection is the leading cause of end-stage liver disease worldwide. In 2015, 3.61% of the population was infected with HBV globally and 8.83 % in the Sub Saharan African (SSA) region. Within the Southern and Eastern African region, the prevalence ranges between 2-8% (WHO, 2015).

A previous study in Ethiopia reported 3.8% of pregnant women were infected with HBV (Zenebe *et al.,* 2014). Studies in Kenya have shown HBsAg prevalence of 8.8% in the general population with a wider range in urban areas between 8-30% (WHO, 2015). Another study in pregnant women attending antenatal care in a tertiary hospital in Kenya showed that 4.2% were HBV-infected (Kilonzo *et al.,* 2014). Kenya is one of the HIV infection hotspots in SSA and high prevalence has been reported in women (Avert, 2015).

HIV/HBV co-infection is common in HIV type-1-infected individuals with a prevalence ranging from 5% to 20% (Nyirenda *et al.,* 2008; Alter, 2006). Studies have reported a wide range of HIV/HBV co-infection from 1.5% in Cameroon (Noubiap *et al.,* 2015) to 19% in Ethiopia (Zenebe *et al.,* 2014). Similar findings were reported in India where 4.6% of the HIV-infected pregnant women had HBV co-infection (Mave *et al.,* 2014).

History of blood transfusion is one of the risk factors associated with HBV infection. A study in Ethiopia found that pregnant women who had a previous history of blood transfusion were about four times more at risk of HBV infection (Zenebe *et al.,* 2014; Noubiap et *al.,* 2015). Injection drug users (IDUs), body piercing and tattooing have also been linked to HBV infection due to sharing of contaminated objects. Sharing of needles among IDUs has been shown to highly contribute to transmission of HIV and

HBV with a study showing a HBV prevalence of 9.6% among the IDUs. Also, body tattooing increased the risk of HBV infection six fold (Kilongosi *et al.,* 2015).

HBV screening in resource limited settings in HIV-infected mothers is rarely done despite the potential of transmission to their infants. A study in Australia found out HBV transmission rate of 2.9% in HBV-exposed infants (Wiseman *et al.,* 2009). This study was set to investigate the transmission of HBV infections from HIV-infected mothers receiving HIV HAART to their exposed infants.

1.2 The statement of the Problem

The menace of HIV among pregnant women has been highly intervened to ensure the well being of the infected mothers as well as prevention of transmitting the HIV virus to their infants during gestation period, delivery or postnally. However the same HIV-infected mothers may be co-infected with hepatitis B virus. Chronic Hepatitis B Virus is the leading cause of end stage liver disease worldwide. Mother to child transmission (MTCT) is responsible for more than one third of chronic HBV infections worldwide (WHO, 2015). Due to the limited resources in the health care settings, the HBV may end up undetected especially if there is no predominant signs and symptoms of hepatitis B virus infection. This poses a great risk of transmitting the HBV to the infant through the same routes as with HIV. The policy of testing HBV among all pregnant mothers, delivering mothers and breastfeeding mothers who turn out to be sero reactive for HIV has not been implemented in our routine care. These mothers are enrolled onto PMTCT care and initiated on HAART regimen containing some of the drugs meant to manage HBV such as lamivudine and tenofovir. Thus in presence of the Hepatitis B virus, HIV-infected mothers might not be totally suppressing the two viruses with their ART regimen and these could potentiate transmission of these two highly infectious viruses to their exposed infants. A study done in Malawi showed a 9.8% HBV transmission rate to the exposed infants from their HIV/ HBV co- infected mothers (Charsela *et al.,* 2014). There is limited data in Kenya and regionally on the HBV infections among HIV

infected mothers receiving HAART for the management of HIV and transmission of the two viruses to the exposed infants.

1.3 Justification of the study

The occurrence of new HBV infections among HIV-infected pregnant mothers and the exposed infant has scanty information documented in Kenya. There is also no routine HBV screening among HIV infected mothers who are initiated on HAART. Pregnant mothers have a right to access the right health care services and management. Co- infection with HIV and HBV among the pregnant mothers pose substantial risks to their infants if the infection is unknown or not early managed (Charsela *et al.*, 2014). The use of ARTs in the management of HIV might be on the other hand be complicating HBV infection if present. The study evaluated prevalence of HBV among HIV positive mothers and the factors associated with HBV infection. It also evaluated the presence of HBV in exposed infants. Reduction of new HBV viral infection reduces child mortality rates and HIV respectively which is one of the millennium development goals (no. 4, 5 and 6). The aim of this study was to determine the prevalence and factors associated with HBV infections among HIV-infected HAART receiving mothers and their exposed infants**.** Findings of this study will be useful in informing the national PMTCT programs, to initiate discussions on strategies of implementing routine HBV screening among all HIV infected pregnant, delivering and breastfeeding mothers and their exposed infants.

1.4 Research questions

1. What proportion of HIV infected mothers on HAART are co-infected with HBVamong HIV infected mothers at KNH?
2. What is the proportion of vertical transmission of HBV among HBV exposed infants at KNH?
3. What are the risk factors associated with HBV infection among HIV-infected HAART receiving mothers?

1.5 Objectives

1.5.1 General objective:

To determine the prevalence and the factors associated with HBV infections among HIV-infected HAART receiving mothers and their exposed infants.

1.5.2 Specific objectives:

1. To determine the proportion of HBV infection among HIV-infected HAART receiving mothers at KNH.
2. To determine the propotion of vertically transmitted HBV among the HBV exposed infants at KNH.
3. To determine the factors associated with HBV infection among HIV-infected HAART receiving mothers at KNH.

CHAPTER TWO

LITERATURE REVIEW

2.1 Mother to child transmission of Human Immunodeficiency Virus

Sub-Saharan Africa is most heavily affected by HIV compared to the rest of the world and women account for 60 % of all adult infections (UNAIDS, 2008). Women of childbearing age account for nearly half of those infected with HIV and without interventions to prevent mother-to-child transmission of HIV (PMTCT), 12% to 40% of HIV-infected women will transmit infection to their infants (Paintsil *et al.*, 2009). According to UNAIDS, MTCT accounted for majority of the 420,000 children newly infected with HIV in 2007 and 90 % were in sub-Saharan Africa (UNAIDS, 2007). By 2007, Kenya had an estimated number of births of 1.73 million per annum and HIV prevalence among pregnant mothers was 6.7 % with 163,800 births exposed to HIV infection (UNAIDS, 2007).

Several success stories on prevention of vertical transmission have been reported around the world including sub-Saharan Africa. This has been achieved mainly through programs offering interventions by use of antiretroviral therapy (ART) drugs (WHO, 2017).

2.2 Hepatitis B virus

Chronic viral hepatitis is highly prevalent and creates a substantial burden to healthcare systems globally. The World Health Organization (WHO) estimates that over 350 and 250 million people worldwide are chronic carrier of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection respectively. These two diseases are the cause of significant global mortality and morbidity with approximately 1 million deaths each year attributable to them and their sequelae, liver disease and primary liver cancer. Although the efforts have been met with the long-lasting level of success and holds the promise for large reductions in disease burden in spite of the huge numbers of HBV-infected population (WHO, 2010).

Hepatitis B virus has been considered to be one of the most serious and prevalent health problems, affecting more than 2 billion people worldwide. The availability of highly effective vaccine against HBV since 1982 could not avoid the current status of chronic carriers of the disease which has been reached to more than 350 million. HBV is carried in blood and in other body fluids including saliva, tears, semen and vaginal secretions and can be transmitted from person to person by a variety of means depending on the epidemiologic pattern within a geographic area. As suggested by WHO, the infection of HBV early in life is associated with the highest risk of chronic infection, and progression to liver cirrhosis and Hepatocellular carcinoma (HCC). About 90% of infants infected with hepatitis B virus around the time of birth, 30% of children infected in early childhood and 6% of those infected after five years of age will develop chronic hepatitis B virus infection. It has also been observed that the people with chronic hepatitis B (CHB) have a 15% to 25% risk of dying prematurely from HBV associated liver cirrhosis or HCC and 0.5 to 1.2 million people die annually from HBV infection (WHO, 2010).

Sustained reductions in Hepatitis B seroprevalence and Hepatitis B-related deaths have been observed in countries where universal infant vaccination against hepatitis B is in place. The benefits of infant immunization are most prominent in the countries, previously of high hepatitis B endemicity (Zainetti *et al.*, 2008).

2.3 Structure of Hepatitis B Virus genome

The genome of HBV is made of circular DNA, but it is unusual because the DNA is not fully double-stranded. One end of the full length strand is linked to the viral DNA polymerase. The genome is 3020–3320 nucleotides long (for the full-length strand) and 1700–2800 nucleotides long (for the short length-strand). The negative-sense (non-coding) is complementary to the viral mRNA. The viral DNA is found in the nucleus soon after infection of the cell. The partially double-stranded DNA is rendered fully double-stranded by completion of the (+) sense strand and removal of a protein molecule from the (-) sense strand and a short sequence of RNA from the (+) sense strand. Non-coding bases are removed from the ends of

the (-) sense strand and the ends are rejoined. There are four known genes encoded by the genome, called C, X, P, and S. The core protein is coded for by gene C (HBcAg), and its start codon is preceded by an upstream in-frame AUG start codon from which the pre-core protein is produced. HBeAg is produced by proteolytic processing of the pre-core protein. The DNA polymerase is encoded by gene P. Gene S is the gene that codes for the surface antigen (HBsAg). The HBsAg gene is one long open reading frame but contains three in-frame "start" (ATG) codons that divide the gene into three sections, pre-S1, pre-S2, and S. Because of the multiple start codons, polypeptides of three different sizes called large, middle, and small (pre-S1 + pre-S2 + S, pre-S2 + S, or S) are produced. The function of the protein coded for by gene X is not fully understood but it is associated with the development of liver cancer. It stimulates genes that promote cell growth and inactivates growth regulating molecules (Sung *et al.*, 2001).

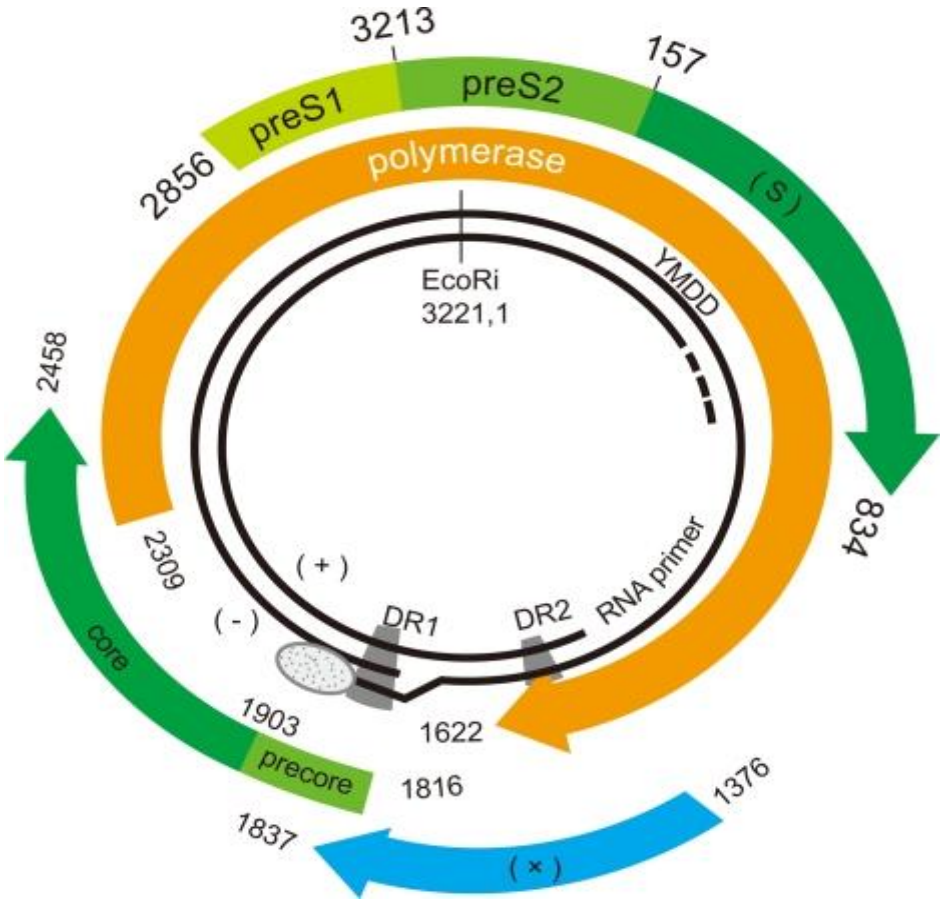

Figure 2.1: Structure and organization of hepatitis B virus genome (Lin & Kao, 2011)

The pol gene (P) has four main domains namely:

i. The polymerase / reverse transcriptase domain which has the reverse transcription activity,
ii. The terminal protein (TP) - contains the tyrosine residue that primes DNA synthesis and covalently links P to the viral DNA.
iii. RNase domain containing two enzymatic active sites catalyzing the reverse transcription of the RNA template and the degradation of the RNA template.
iv. The spacer domain with no known function other than to connect the terminal protein domain with the rest of the P. The spacer domain, however, habours at aa position 320 a thrombin cleavage site. This generates the possibility that not a full length protein, but a truncated polymerase is linked to the HBV genome due to intracellular proteolytic processing.

The transcript encoding the HBV polymerase (Pol) works as a replication intermediate, namely, as pregenomic RNA. HBV replicates through reverse transcription with the pregenomic RNA. Initiation of replication occurs via a priming reaction in which a nucleotide becomes covalently linked to the tyrosine residue within the terminal-protein domain of *HBV Pol*. In this step, the 5′ epsilon stem-loop region is recognized by *HBV Pol*, and this process has a preference for *cis* pregenomic RNA, which appears to be cotranslational. After the coupling of 3 or 4 nucleotides that are linked to a tyrosine residue of *HBV Pol*, these oligonucleotides are translocated to a complementary sequence in the 3′ copy of DR1 and extended by *HBV Pol*. The RNA template is degraded by the RNase H activity of *HBV Pol*. The synthesis of minus-strand DNA terminates at the 5′ end of pregenomic RNA, and plus-strand DNA is synthesized by *HBV Pol*. In this replication step, *HBV Pol* functions as a DNA-dependent *DNA Pol*. (Refer to figure i). Although the 5′ epsilon stem-loop region of pregenomic RNA is used in priming, many other studies have

reported that the 3′ epsilon stem-loop region of pregenomic RNA is enough for priming (Sung *et al.,* 2001).

2.4 Transmission and pathogenesis of HBV

The main carrier for HBV infection is blood. Body fluids like semen and saliva have also been found to play role in viral transmission (Hou *et al.,* 2005). In high endemic areas, vertical transmission is the most common route with horizontal routes being common route during the schooling age. Sharing of syringes between injections by drug users and unprotected sex are the main routes of transmission in areas with low prevalence (Lavanchy, 2004). HBV infection can also be transmitted through the transfusion of unscreened blood, usage of non-sterilized instruments, tattooing, baber cuts, dental surgery, hemodialysis and multiple-dose vials in health care settings predisposes one to HBV infection. The outcome of HBV infection is not the same in all patients and may be either asymptomatic, self-limited infection or fulminant Hepatitis and chronic disease.

2.4.1 Natural history of HBV infection

Upon an infection with HBV the incubation period takes approximately 8 to 12 weeks. (Shepard *et al.,* 2006). The progression of the infectection is determined by the individuals' age, viral factors and response by the host immune leading to either liver cirrhosis and/or Hepatocellular carcinoma (HCC) (Shepard *et al.,* 2006). Most of the perinatal and childhood infections have been shown to be asymptomatic with high chances of progressing to chronicity as opposed to adult infections thar are usually acute and self resolving (Rodes *et al.,* 2003).

2.4.2 Acute hepatitis

After six months of infection, the acute phase of HBV infection is observed. The clinical symptoms associated with acute infection include; jaundice, nausea, weight loss and flu-like illness. However, patients may also suffer from fever, urticaria and arthralgia. These symptoms generally subside within a few weeks along with

disappearance of HBV DNA and seroconversion from HBeAg to anti-HBe. A large proportion of the cases are asymptomatic and the infection may pass without notice (Blackberg & Kidd-Ljunggren, 2000). Most patients with acute hepatitis B are HBsAg positive at presentation, but the critical test is IgM anti-HBc, which confirms acute HBV infection. If HBsAg is detected after six months of infection, the patient is considered to be a chronic carrier.

In adults the risk for chronic disease is usually low. Acute hepatitis may in some cases progress to fulminant hepatitis leading to liver failure (with high mortality reported. Use of lamivudine or other nucleoside analogues of choice is recommended in such cases, though liver transplantation would still be recommended (Wang & Tang, 2009).

2.4.3 Chronic hepatitis

Persistence of HBsAg for more than 6 months in the serum of an infected person leads to chronic HBV infection, with no anti-HBc-IgM (Shepard *et al..,* 2006). The hosts' immune response determines the progression of chronic infection from acute or subclinical, though age of the patient is also a factor. Chronically infected patients with HBV and with no detectable anti-HBs, develop HBeAg, which is a marker of HBV replication and correlates with greater infectivity. Seroconversion to anti-HBe is usually associated with resolution of the infection and/or the development of BCP/PC mutations in the virus (Zhang *et al.,* 2016). Chronic HBV infections can be divided into the following four stages;

i) *Immune tolerance phase*: Active viral replication and immune system tolerance are the characteristics seen in this phase. Initially, HBV DNA replicates at a high level and the HBs and HBe antigens are produced and detectable with very high detectable antibodies. For the first 10years, ALT levels are normal, which may last for 30 years, an indication of no liver inflammation. This has been observer among infants who acquire the infection at birth or early days of life (Yim & Lok, 2006).

ii) *Immune clearance phase*: In this phase, the immunologic response that causes inflammation and hepatic injury are observed. There is seroconversion from HBeAg-positivity to anti-HBe, followed by moderate/severe necroinflammation of hepatocytes and elevated ALT levels resulting from viral clearance. This mostly occurs in the second to third decade of life especially in patients with perinatally acquired disease (Yim *et al.,* 2006).

iii) *Inactive carrier state*: The third phase is which is accompanied by seroconversion of HBeAg, with low levels of HBV DNA and ALT. At this point of undetectable HBV DNA, no inflammation occurs though anti-HBe persists. (Yim *et al.,* 2006).

iv) *Reactivation stage.* Theres the presence of HBV DNA, high ALT levels and icreased tendancy of liver damage leading to cirrhosis and fibrosis. (Yim *et al.*, 2006). The antigen produced in this stage is used as a replication marker. Occurrence of seroconversion is indicativeof remission of liver disease and viral clearance (Yim *et al.,* 2006).

2.4.4 Occult hepatitis

Occult hepatitis is the presence of HBV DNA in the liver where HBsAg test was negative by currently modernized assays (Zhang *et al.,* 2015). Negative HBsAg patients, with serum HBV DNA levels < 200 IU/ml, are considered to have true occult Hepatitis (OBI). OBI reflects that HBV persists life-long in a small proportion of the hepatocytes. It has been suggested that the molecular basis of OBI is related to the long term persistence of viral cccDNA in the nuclei of hepatocytes. Occult Hepatitis B has been associated with reactivation among immunocompromised individuals, increased risk for liver cancer, Hepatitis C infection treatment interference. It has also been linked to increases risks of transmission during blood transfusion and organ transplant (Schmeltzer & Sherman, 2010).

Five clinical contexts of OBI have been documented: (i) recovery from acute infection leading to seroconversion from HBsAg to anti-HBs, (ii) chronic HBV infection with mutant strains that have a mutation in the S region and this could

result in diagnostic failure of HBsAg by routinely used assays, (iii) chronic infection without any marker except HBV DNA, (iv) chronic infection with HBsAg levels too low to be detected by serological assays the most common type occurring in endemic areas , (v) OBI in HIV-infected individuals. OBI has many impacts on different clinical aspects, including the possible transmission of infection, risk of reactivation and enhancing liver disease progression that can lead to HCC. The clinical importance of OBI remains controversial, which is the main reason for the growing interest in this topic (Raimondo *et al.,* 2013).

2.4.5 Liver cirrhosis

This is the advanced stage of liver fibrosis that is accompanied by distortion of the hepatic vasculature leading to portal hypertension, liver function impairment and HCC. Therefore, patients chronically infected with HBV can develop cirrhosis. Alcohol consumption, viral hepatitis (HAV/ HCV have also being highly associated with liver cirrhosis. (Schuppan & Afdhal, 2008).

2.4.6 Hepatocellular carcinoma

Chronic infection witg HBV results to hepatocellular carcinoma (HCC). Globally more than 50% of HCC cases have been reported where in endemic areas the prevalence ranges between 70-80%. (Nguyen *et al.,* 2009). Given that HBV is hepatotrophic virus then it posses the ability to cause both acute and chronic hepatitis infections. Therefore during infection, innate and adaptive immunity are established and activated in response in order to eliminate infection. An acute inflammation is a short process that is usually protective for the host with the purpose of eliminating the pathogen. By passing of the immune system by the virus results to chronic active hepatitis with high rate of persistence thus triggering the liver cell to carcinogenesis (De Visser & Coussens**,** 2005).

Polymorphonuclear cells which are inflammatory cellsand other phagocytes are activated during this process, ending up releasing cytokines, chemokines and nitric oxide (NO) particularly, an inducible isoform of nitric oxide synthase (iNOS), and

NO-derived reactive nitrogen species (RNS) (Szabo *et al.,* 1997). DNA damage by the free oxygen radicals targets p53 gene (tumor suppressor gene) and retinoblastoma disrupting their roles on control of apoptotic mechanisms resulting in cancer. On the other hand HBV DNA integration may cause chromosomal deletions, as found at the chromosomal region 17p11.2-12 causing the loss of the p53 gene. HBV DNA integration may cause disruptions or translocations, resulting in genetic instability The HBV genome has itself some oncogenic activities, expressing from integrated HBV the X gene (HBx), which may contribute indirectly to carcinogenesis by activating pathways such as mitogenactivated protein kinase (MAPK), c-jun N-terminal kinase (JNK), protein kinase C (PKC), phosphatidylinositol 3-kinase (PI 3-kinase), protein kinase B (PKB/Akt) and JAK/STAT signaling cascades (Diao *et al.,* 2001). It disrupts p53 pathway and alters the expression level of the retinoblastoma gene (Rb), affecting cell cycle progression (Edamoto *et al.,* 2003). HBx is not considered a direct transforming gene and therefore, the tumor induction mechanism of HBV is believed to be indirect (Carrillo *et al.,* 2007).

2.5 Human Immunodeficiency Virus and Hepatitis B Virus among pregnant women

Mothers' with elevated HBV viraemia, high HBV DNA, and HBeAg positivity have been shown to pose high risk of perinatal transmission of HBV to their exposed infants. Majority of infants born to HBsAg positive and HBeAg positive mothers get infected with HBV in the perinatal period when no interventions are made (Wiseman *et al.,* 2009). Most of these infections occur during delivery due to contact with maternal blood and vaginal secretions. In utero (Trans placental) HBV transmission occurs in 10% - 16% of infants of HBV carrier mothers when no intervention is initiated. In utero HBV infection is thought to occur through placental breach as in threatened preterm labour, placental infection or through ascend of infected vaginal fluids into the uterus. Amniocentesis in hepatitis B carrier mothers rarely causes in utero HBV infection (Ronald *et al.,* 2010). HBV transmission has been shown in 5 - 10% of infants of HBsAg positive HBeAg positive mothers despite passive active vaccination at birth (Hill *et al.,* 2002). Mothers who are HBV carriers pose a high

risk to their exposed infants after delivery due to continued contact with an infected household contact.

HBV and HIV screening among pregnant mothers are recommended during their first antenatal visits infections on the first antenatal visit (ACOG, 2007). Elevated serum glutamic pyruvic transminase (SGPT) accompanied by thrombocytopenia are signs of renal fibrosis.This features should be followed with radiological diagnosis and additional serological analysis such as Hepatitis B virus Core antigen (HBcAg) (Ronald *et al.,* 2010).Treatment of chronic hepatitis B mono-infection in pregnancy is mainly supportive. Viral load HBV DNA levels are estimated in the gestation period of 22weeks and above and if above 10 6 log copies per milliliter ARVs are started for prevention of mother to childhood transmission of HBV (WHO, 2011). Lamivudine (3TC), emtricitabine (FTC) and telbivudine monotherapy have been shown to be effective for prevention of mother child transmission of hepatitis B. Tenofovir is considered a better choice due to its efficacy and less likelihood of resistance as well as a better safety profile in pregnancy. Lamivudine and telbivudine have been reported to have a higher incidence of ARV resistance with long term therapy. Mothers with advanced HBV associated liver disease but DNA levels less than 10 log copies/mL are started on antiviral therapy to slow disease progression but not for prevention of perinatal transmission. ARV monotherapy is not recommended in HIV positive mothers (Ronald *et al.*, 2010).

HIV/HBV Co-infected mothers should be started on HAART effective for the treatment of both HIV and HBV. The preferred first line of treatment is TDF + (3TC or FTC) + EFV while the alternative first line is the use of 3 nucleoside reverse transcriptase inhibitors (NRTIs): AZT+ (3TC or FTC) + TDF. There is a high risk of developing lamivudine resistance (3TC) if 3TC is used without combination with a second ARV effective against HBV. Up to 90% of patients on Lamivudine monotherapy for hepatitis B treatment will develop lamivudine resistance in 4 years.

Co-infected patients requiring change to second line due to HIV treatment failure or development of resistance but whose HBV DNA is well suppressed, should retain TDF +(3TC or FTC) in addition to the second line HAART (Ronald *et al.,* 2010).

2.6 Hepatitis B Virus resistance in Human Immunodeficiency Virus infected mothers on antiretroviral therapy

Lamivudine monotherapy is associated with the emergence of drug resistance in a significant proportion of treated patients. According to WHO (2015), the HBV/HIV co infected patients are recommended for commencement of treatment immediately regardless of their CD4 count where they are given pegylated interferon for period of up to 48 weeks under monitoring. Based on the liver biochemistry and viral loads, two active HBV drugs; entecavir and tenofovir are then initiated. In case of HIV co-infections, tenofovir plus emtricitabine or lamivudine are also given. Unless contraindicated, the drug of choice is Pegylated interferon as recommended by WHO. This intervention is aimed at reducing the risk of progressive chronic liver diseases, transmission to others individuals and prevention of long term complications such as liver cirrhosis and hepatocellular carcinoma and death (WHO, 2015). Besides the difficulty to obtain tenofovir in many countries, patients are very rarely screened for HBV infection and, when screened, the determination of HBV-DNA to assess the need of treatment is not feasible in general. Access to antiretroviral therapy is growing at increased speed in resource-limited settings and a high proportion of HIV-infected women is also attending the antenatal clinics and receiving treatment and care for the prevention of mother-to-infant transmission (Clementina *et al.,* 2012). Genotypic HBV lamivudine resistance has been found in 39% of HIV–HBV co-infected individuals treated with lamivudine as part of highly active antiretroviral therapy. These patients exhibited significantly elevated HBV viral loads and serum ALT, and were infected with a lamivudine-resistant HBV strain that was potentially transmissible to HBV-vaccinated individuals. (Clawick *et al.,* 2012). Lamivudine, an oral nucleoside analogue, is effective against both HIV and HBV replication. Lamivudine has promptly inhibited HBV replication in more than 80% of cases in both HIV-and non–HIV-infected patients. However, HBV

resistance to lamivudine caused by HBV-DNA polymerase gene mutations has been reported in both liver transplanted and immunocompetent patients. Incidence of HBV resistance to lamivudine is of 14% to 27% after 1 year of treatment in non–HIV-infected patients (Lok *et al.,* 2003). Therefore, with frequent usage of lamivudine as guided by WHO treatment guideline, has led to increased number of patients with lamivudine-resistant mutations (YMDD).

For instance, drug resistance mutation like rtM204V/I, developed up to 23% of patients within one year and 70% after five years of treatment (Lai *et al.,* 2003; Lok *et al.,* 2003). Due to this challenge, entecavir and tenofovir have replaced lamivudine as a preferred treatment due to the lower risk for resistance development. This effect is due to their high genetic barrier compared to lamivudine hence requiring more than one mutation to result in drug resistance.

2.7 The HBV genotypes

Due to HBV unique life cycle which requires an error-prone reverse transcriptase for replication, HBV constantly evolves, resulting to a genetic variation in the form of genotypes, sub-genotypes, and mutations (Zhang *et al.,* 2016). Ten genotypes of HBV exists; A–J, with each genotype differing in sequence by more than 8% at the nucleotide level when compared to each other and less than 4% intragenotype divergence. The distribution and pattern of HBV genotypes are mostly as a result of human migration and behaviours. However there is still distinst geographical distributions on these genotypes (Santos *et al.,* 2010).

Genotype A for instance is distributed globally and is the main genotype found in Europe, North America, Africa and India whereas genotypes B and C are predominant in East and Southeast Asia. Genotype A is divided into seven sub genotypes A1-A7 (Zhang *et al.,* 2016). Among these HBV Sub genotypes; A1 circulates in Africa, Sub genotype A2 in Europe while sub genotype A3 has been detected in Central and West Africa. Sub genotype A4 has been reported in Gambia, sub genotype A5 in Nigeria and also among African descendants in Haiti. Sub genotype A6 has been detected in Belgium but among African-Belgian patients of

Congo and Rwanda origin (and A7 has been detected in Rwanda and Cameroon (Hubschen *et al.,* 2010).

Hepatitis B virus (HBV) sub genotype B1 is dominant in Japan, B2 is common in China and Vietnam, B3 is confined to Indonesia, and B4 is confined to Vietnam. B7, B8, and B9 have been found in an island in Southeast Asia (Huy *et al.,* 2004). HBV/C1 (Cs) is found mainly in Southeast Asia, whereas C2 (Ce) is predominant in East Asia (Lusida *et al.,* 2008). HBV/C3 was confined to Oceania, while C4 was exclusively found in Australia and regarded as the most divergent sub-genotype within HBV/C (Davies *et al.,* 2013). Sub-genotypes C5 and C7 were found in Philippines, while C6 and C8 to C16 were isolated from Indonesia (Sugauchi *et al.,* 2002). Genotype D which was previous divided into 4 sub genotypes (D1-D4) is mainly found in the Middle East and Mediterranean countries in Asia but it has been reported in Africa and Europe (Thedja *et al.,* 2011). However, new genotypes D5-D7 have been described in India, Indonesia and Mediterranean basin. HBV genotype E seems to be predominant in western-sub-Saharan Africa (Mahtab *et al.,* 2008). This genotype has not been detected outside Africa, except for a few rare cases mostly in individuals with an African background. Nevertheless, presence of this genotype has been detected in India and also in certain specific community in Colombia (Alvarado *et al.,* 2010).

HBV genotype G has been characterized in samples from USA, Mexico and France and appears primarily to be present as a coinfection with another HBV genotypes, most commonly genotype A. Genotypes F and H are found almost exclusively in Central and South America (Jutavijittum *et al.,* 2007). Recently, HBV genotype I and J were described in Northwestern China, Vietnam, Laos and Japan (Arankalle *et al.,* 2010). However, genotype F is divided into 4 sub genotypes: F1-F4. Subgenotypes F1 and F2 have been further divided in F1a, F1b, F2a and F2b. Morever, intergenotype recombination which plays an important role in the evolutionary history of HBV has also been described (Zhang *et al.,* 2016). For instance, B/C recombinants have been reported in Southeast Asia and East Asia where it is also prevalent (Shi *et al.,* 2012). Other inter-genotype recombinants such as A/D, A/E,

C/D and G/C recombinants have also been observed in different geographical regions (Yang *et al.,* 2006).

In Kenya, like other sub Saharan Africa countries, HBV genotypes A, D and E have been detected with genotype A being the most predominant. Irrespective of Kenya being considered as one of the most endemic fregion for HBV, the current circulating genotypes of HBV have not been fully identified (Mwangi *et al.,* 2008).

2.8 Drug resistance in HBV

Antiviral resistance has become an increasingly common problem during long-term treatment with nucleoside analogues (NA) including patients who receive sequential treatment with NA monotherapy. When lamivudine-resistant HBV variants emerge, viral load and liver enzyme levels may increase, clinical hepatitis may occur, and HBV infection can be fatal in a minority of patients. Thus, the clinical effectiveness of lamivudine monotherapy is limited by the frequent emergence of resistant HBV variants. Monotherapy with Lamivudine for HBV regiment is therefore not recommended (Wongprasit *et al.,* 2010). WHO recommends combination therapy of tenofovir plus lamivudine or emtricitabine in HBV/HIV-1–coinfected patients in order to prevent the emergence of resistant HBV variants (WHO, 2011). It further recommends that treatment should be initiated regardless of the CD4 counts in HIV/HBV coinfections to prevent faster progression to liver cirrhosis and liver related death (Thio *et al.,* 2002).

Drug resistance testing is currently examined in all HIV individuals in Western countries before initiating antiretroviral therapy, based on a reported prevalence of transmission of drug-resistant HIV of around 10% and the demonstration of impaired treatment response when antiretroviral drugs are used empirically in patients with acquired drug-resistant trains. Similar information for HBV infection is still scarce, but recent reports have emphasized that the rate of primary drug resistance mutations among drug-naive chronic hepatitis B-mono infected patients may be around 8% in Western countries and that patients infected with HBV resistant strains may be prone to subsequent treatment failure (Fung *et al.,* 2008). In almost all cases, lamivudine

resistance mutations are the ones recognized. However, it should be noted that lamivudine-resistant HBV strains display cross-resistance to emtricitabine, telbivudine, and to a lesser extent entecavir (Thio *et al.,* 2007).

Little is known about the rate of transmission of HBV resistant strains among HIV patients in Kenya where treatment with lamivudine has been widely used for more than a decade. Baseline drug resistance testing in HBV might be warranted in newly diagnosed HIV/HBV coinfected patients. Surveillance studies assessing the rate of primary HBV drug resistance in populations of HIV/HBV coinfected patients is therefore invertible. Moreover, a cost-benefit assessment of baseline HBV drug resistance testing in clinical practice is the implication this poses in selection of the first-line antiretroviral therapy in HIV infections (Trevin˜o *et al.,* 2009).

Antiviral resistance is one of the important factors that lead to treatment failure. In HBV where nucleoside analogues are used, emergence of drug resistance based on HBV polymerase are complex. However, eight codons are associated with primary resistance end up with predictable five major pathways. These pathways may include;

i) The L-nucleoside pathway (rtM204V/I), whereby lamivudine, emtricitabine, telbivudine, and clevudine treatment select out the rtM204V/I. This pathway includes entecavir in lamivudine-experienced patients.

ii) The acyclic phosphonate pathway (rtN236T), in which adefovir and tenofovir treatment select out and/or consolidate the rtN236T HBV quasispecies (Angus *et al.,* 2003).

iii) Through a shared pathway (rtA181T/V), whereby treatment with either L-nucleosides or acyclic phosphonates can result in selection of HBV quasispecies with rtA181T/V. This pathway is seen in about 40% of adefovir failure and less than 5% of lamivudine failure.

iv) Naive entecavir resistance pathway (rtL180M + rtM204V with one of rtT184, S202, or M250 codon changes). In this pathway, three mutations are required to appear simultaneously accounting for the very low resistance profile of entecavir (Suzuki *et al.,* 2007).

v) Multidrug resistance pathway. Complex patterns and clusters of specific mutations in HBV polymerase associated with multidrug failure. A recent example includes rtA181T + rtI233V + rtN236T + rtM250L. It is important to note that the rtI233V and M250L substitutions in isolation do not confer significant drug resistance nor significantly reduce replication capacity in the absence of selection pressure, but appear to act to compensate for the replication defects associated with acquisition of multidrug resistance (Locarnini, 2008).

However, some of these broad clusters of compensatory mutations, especially those acquired during lamivudine therapy, are compromising future salvage of therapy options (Zhang *et al.,* 2016). Nevertheless, in other genes of HBV, deletion mutations for instance in the PreS gene region and/or some point mutations in the major hydrophilic region (MHR) of S gene can lead to immune escape and occult HBV infection. It has also been shown that reverse transcriptase (RT) could also lead to an altered viral envelope due to the overlap between the envelope and polymerase (Zhang *et al.,* 2016).

Due to this effect, the mutation occurring at position A181T/V on RT region could cause a stop codon mutation (W172*), W172L and L173F mutations in the S region. In addition, occurrence of RT mutation at M204V/I position could also result into stop codon mutation at (I195M), (W196*) (W196S) and (W196L) positions in the S region; A1762T and G1764A mutations in the base core promoter (BCP) or G1896A mutation in Pre C, leading to decrease HBeAg expression or reduced replication fitness.(Zhang *et al.,* 2016).

Some mutations in the CTL epitope of HBV core gene have also been found to lead to reduced T cell immune escape. For the HBV X gene, occurrence of some point

mutations or truncated mutants in this gene have also been found to lead to tumorigenesis or other end-stage liver disease (Zhang *et al.,* 2016).

2.9 Human Immunodeficiency Virus

The human immunodeficiency viruses 1 and 2 (HIV-1, HIV-2) originated from the simian immunodeficiency viruses (SIVs) of primates. The two human immunodeficiency viruses, HIV-1 and HIV-2, are members of the family of Retroviruses, in the genus of Lentiviruses. In particular, retroviruses have been found to be associated with malignancies, autoimmune diseases, immunodeficiency syndromes, aplastic and haemolytic anaemias, bone and joint disease and diseases of the nervous system (Weiss *et al.,* 2004).

The many different strains of HIV-1 have been separated into major (M), new (N) and outlier (O) groups. Groups N and O are mainly confined to West and Central Africa (Gabon and Cameroon), though cases of Group O have been found world-wide due to international travel, after contact with infected individuals from these areas. The HIV strains in Group M are the ones mainly responsible for the HIV/AIDS pandemic, and they are so diverse that they have been subclassified into subtypes (or clades) A-K. This huge diversity of HIV-1 is important when diagnostic testing, treatment and monitoring are applied as the results may differ between different subtypes or clades The diversity of HIV-2 is much less, but subtypes A-H have been proposed (Damond *et al.,* 2004).

The human immunodeficiency viruses are approximately 100 nm in diameter. It has a lipid envelope, in which are embedded the trimeric transmembrane glycoprotein gp41 to which the surface glycoprotein gp120 is attached. These two viral proteins are responsible for attachment to the host cell and are encoded by the *env* gene of the viral RNA genome. Beneath the envelope, is the matrix protein p17, the core proteins p24 and p6 and the nucleocapsid protein p7 (bound to the RNA), all encoded by the viral *gag* gene. Within the viral core, lies 2 copies of the ~10 kilobase (kb) positive-sense, viral RNA genome (i.e. it has a diploid RNA genome), together with the protease, integrase and reverse transcriptase enzymes. These three enzymes are

encoded by the viral *pol* gene. There are several other proteins coded for by both HIV-1 and HIV-2, with various regulatory or immuno-modulatory functions, including *vif* (viral infectivity protein), *vpr* (viral protein R), *tat* (transactivator of transcription), *rev* (regulator of viral protein expression) and *nef* (Negative regulatory factor) (cleghorn *et al.,* 2005).

2.10 Risk factors associated with Hepatitis B Virus infection

HBV is transmitted through perinatal, sexual and parenteral/percutaneous exposure to infected blood or other body fluids. Perinatal transmission from carrier mothers to their babies is the most important factor in determining the prevalence of the infection in high endemic areas. It is estimated that approximately 90% of HBeAg-seropositive mothers (with high viral load) transmit HBV infection to their babies, compared with 10–20% of HBeAg-seronegative carrier mothers (Sorell *et al.*, 2009). The data also suggests that the incidence of HBeAg is higher in Asian than in African HBsAg carrier mothers (40 *vs* 15%), so perinatal transmission is greater in Asians, but mainly horizontal in Africans (Qirbi *et al.*, 2001).

In low endemic areas such as North America, the major source of HBV infection is sexual transmission which makes HBV to be considered as sexually transmitted disease (STD). The important mode of parentral transmission is sharing and/or reusing of syringes between injection drug users for drug with HBsAg-positive people. Parenteral/ percutaneous transmission can occur during surgery, after needle-stick injuries, intravenous drug use, and the procedures such as ear piercing, tattooing, acupuncture, circumcision and scarification. As with additional modes of transmission, working or residing in a health-care setting, living in a correctional facility, tattooing, renal dialysis and others who are likely to come into contact with potentially infected blood and blood products have been related to risk of transmission (Zenebe *et al.,* 2014).

2.10.1 Body piercing

Body piercing and tattooing have been reported in studies to be significantly

associated with HBV infection with participants having 3 and 5.7 times the chance of HBV infection (Zenebe *et al.,* 2014). In Bamako, Mali a study conducted identified that body tattooing has a significant association with HBV infection (Sidibe, 2001). Unsafe injection like traditional healers' practice is another factor for HBV transmission. Zenebe et al (2014) found that pregnant women who had a history of unsafe injection or abortion were at high risk of HBV infection. The practice of tattooing is also common in our country where different cultural application on unsafe injection is practiced.

2.10.2 Blood transfusion

Blood and blood products can transmit HBV to the recipients if not proprrly screened before transfusion. Approximately 6.6% prevanlence was reported among HBV-infected patients having history of blood transfusion compared to 2.2% in those who were HBV negative (Kamal *et al.,* 2011). Mothers receive blood and blood products while hospitalized ar during delivery and end up receiving donated blood that may be the source of HBV infection. In Ethiopia pregnant women who had a history of blood transfusion were 3.7 times more likely to be HBV-infected (Zenebe *et al.,* 2014).

2.10.3 Dental Surgery

Pregnant mothers with a history of dental procedure have been shown to be at high risk of HBV infection (Awole *et al.,* 2005). Zenebe et al (2014) found a prevalence of 12.3% among pregnant mothers who had a history of tooth extraction as compared to 1.9% HBV infectivity among preagnant women who have never had a tooth extraction. Poor sterilizertion of dental surgical kits and sub optimal hygienic standards highly facilitates transmission of HBV to dental patients.

2.10.4 Sexual transmission of HBV

Among adults, Hepatitis B transmission occurs primarily among unvaccinated adults with risk behaviors for Hepatitis B transmission, including having multiple sex partners and sex partners of people with chronic Hepatitis B infection. Hepatitis B is

easily transmitted through sexual activity. Sexual contact is the most common way Hepatitis B is spread in the United States.

Among adults seeking treatment in STD clinics, as many as 10%–40% have evidence of past or current Hepatitis B virus infection. Many of these infections could have been prevented through universal vaccination during delivery of STD prevention or treatment services. A study of adults diagnosed with acute Hepatitis B found that 39% had sought care or been screened for an STD before they were infected with Hepatitis B, indicating a significant missed opportunity to vaccinate at-risk persons when they first access STD prevention or treatment services (CDC, 2012).

2.10.5 Risk of transmission by breastfeeding

Breastfeeding has been suggested as an additional mechanism by which infants may acquire HBV infection, because small amounts of Hepatitis B surface antigen (HBsAg) have been detected in some samples of breastmilk. However, there is no evidence that breastfeeding increases the risk of mother to child transmission. A follow up study of 147 infants born to mothers known to be carriers of HBV in Taiwan (WHO, 1996) found similar rates of HBV infection in 92 children who were breastfed compared to 55 who were bottle fed. A study in Britain, involving 126 subjects, also showed no additional risk for breastfed versus non breastfed infants of carrier mothers (WHO, 1996). This study included the measurement of HBeAg status of the mothers, but found no association between maternal e-antigen status and transmission rates. These findings suggest strongly that any risk of transmission associated with breastmilk is negligible compared to the high risk of exposure to maternal blood and body fluids at birth. Experts on hepatitis, however, do have concerns that breast pathology such as cracked or bleeding nipples or lesions with serous exudates could expose the infant to infectious doses of HBV.

2.10.6 HBV transmission at birth

Transmission from infected mothers to their infants takes place primarily at the time of birth. A newborn infant has a 10% to 90% chance of becoming infected at the time

of delivery if its mother has chronic hepatitis B infection. The probability of transmission increases substantially if the mother is positive for both HBsAg and HBeAg, indicating active viral replication. It is estimated that 20% to 40% of HBsAg-positive mothers may be positive for HBeAg as well. Evidence suggest that in utero transmission is relatively rare, accounting for less than 2% of all infections transmitted from mother to infant. Instead, transmission occurs during the birth process, when contact with blood always occurs (WHO, 2006). Exposure occurs through micro-transfusion or hematologic leaks of mother's blood to the fetus during contractions, or through inoculation of mucosal membranes or breaks in the skin (eg, scalp electrodes). Detection of HBV DNA in cord blood might indicate MTCT, but HBV DNA detection could represent maternal-fetal transfusion during labor and delivery or contamination of cord blood sample (Zou *et al*., 2012).

2.11 Prevention of Hepatitis B Virus

Keeping the routes and modes of HBV transmission into consideration, the prevalence can be interrupted. Obviously, routine serological screening of all antenatal mothers and donor blood ensures considerable decline in transfusion-associated HBV. It is also suggestible to run the syringe-exchange program making contact with hard-to-reach population at fixed sites and on mobile van routes to deliver social and medical services, such as testing for HBV, counseling, and vaccination.

The implementation of national programs for infection control management have substantial challenges and regional efforts are needed to promote in the development of guidelines, training materials and a “health systems” approach to ensure high quality care.

Following the introduction of effective vaccine for HBV in early 1980s, the addition of HBV vaccination was recommended to all national immunization programs by WHO. The application of HBV vaccine at birth is very important where the prevalence of HBsAg is high though it is recommended by EPI in all countries (WHO, 2012).

2.12 Laboratory diagnosis of Hepatitis B Virus

Serologic tests for hepatitis B surface antigen (HBsAg) and hepatitis B core antibody (anti-HBc) immunoglobulin M (IgM) are required for the diagnosis of acute hepatitis B virus (HBV). HBsAg is positive in both acute and chronic HBV infection; however, the presence of IgM anti-HBc is diagnostic of acute or recently acquired infection. Antibody to HBsAg (anti-HBs) is produced after a resolved infection and is the only HBV antibody marker present after vaccination. The presence of HBsAg and total anti-HBc, with a negative test for IgM anti-HBc, indicates chronic HBV infection; the absence of IgM anti-HBc or the persistence of HBsAg for 6 months indicates chronic HBV infection. The presence of anti-HBc alone might indicate acute, resolved, or chronic infection or a false-positive result. To evaluate the patient's level of infectivity, quantification of hepatitis (HBV) DNA is essential, and the presence of hepatitis B e antigen (HBeAg) should be determined. Indeed, the best indication of active viral replication is the presence of HBV DNA in the serum. Hybridization or more sensitive polymerase chain reaction (PCR) assay techniques are used to detect the viral genome in the serum, as well as specific genotypes, mutants resistant to oral nucleoside and nucleotide analogues, and core and precore mutations. A positive result suggests not only the likelihood of active hepatitis but also that the disease is much more infectious, as the virus is actively replicating. HBV DNA testing is also recommended when occult HBV is suspected (positive anti-HBc and negative antibody to anti-HBs and HBsAg) or in cases in which all of the serologic tests are negative (CDC, 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was carried out at the Kenyatta National Hospital PMTCT program, to determine the hospital based prevalence of HBV. Kenyatta National Hospital is situated in Nairobi's South-western zone approximately 5 km from the Nairobi City Centre. KNH was considered a suitable site for this study due the availability of large numbers of HIV positive mothers, as well as accessibility to quality laboratory services at the University of Nairobi in KNH. It also had appropriate personnel including Obstetricians, physicians and gastro-enterologists and neonatologists at hand to manage mothers and neonates depending on the HBV test results.

HIV testing is offered routinely in KNH to all pregnant women on the first antenatal visit and subsequently on each visit for mothers who decline testing on the first visit or were not screened on the first visit. Couple counselling and testing for HIV is offered for all mothers who are able to bring their spouses/partners to the clinic. The HIV tests are done using Determine and First response by qualified HIV counselors and intervention done at the PMTCT laboratory incase of discrepancies. Mothers who test HIV positive are worked up for initiation of HAART for PMTCT. Baseline tests that are done include a HIV I RNA viral load, total blood count, serum creatinine, alanine aminotransferase (ALT) and a CD4 count. The mothers are then initiated on HAART regimen, which is usually continued for life after initiation in pregnancy. Mothers are advised for delivery by elective caesarean section if a viral loads done at 36 weeks gestation is above 1000 copies per microliter of blood. Mothers with viral load below 1000 copies per microliter are delivered by caesarean section for other obstetric indications. Mothers are enrolled in a postpartum high risk clinic for follow up after delivery for eighteen months (18) and eventually discharged to a comprehensive care centre for follow up and management of HIV. No routine HBV testing is done in the KNH antenatal clinic or postnatal clinic, even for mothers who are HIV positive. HBsAg and other HBV markers are however available on

request by the clinician for diagnostic purposes for mothers suspected to have HBV infection such as those with jaundice.

Sample analysis was done at the University of Nairobi (UON); Immunology department and Kenya Medical Research Institute (KEMRI), centre for virus research (CVR) respectively.

3.2 Study design

This was a cross sectional study conducted through consecutive enrollment of HIV infected Mothers and their exposed infants, and laboratory based study involving serological and molecular techniques. Data was collected through a face to face interview using a structured questionnaire to capture the socio-demographic data of the participants and information on predisposing factors for HBV infections such as the age, marital status, HAART use and history of blood transfusion and HBV.

3.3 Study population

Approximately 400 mothers aged between 9 years to 45years (reproductive age), who are HIV positive, are normally enrolled into a PMTCT program for HIV care and treatment every month. HIV-infected women attending PMTCT post partum care at KNH were included in this study. All the enrolled mothers were on HAART and had undetectable HIV 1 viral load. A total of 19 infants whose mothers were HBsAg positive were included. The women included those who delivered in KNH and those who delivered elsewhere and opted to seek postnatal services in the hospital. The services sought by the HIV positive mothers included PMTCT follow up for the HIV exposed babies. Postnatal PMTCT services include review of the mother's adherence to highly active antiretroviral therapy (HAART) treatment, use and adherence to Nevirapine (NVP) and Zidovudine (AZT) for the baby, compliance to the recommended infant feeding option and infant HIV testing at 2 weeks, 6 weeks, 6 months, 12 months and 18 months.

3.3.1 Inclusion criteria

1. Mother infant pair where the mother was HIV positive at the high risk clinic.
2. HBV exposed infants aged between 6wks to 18 months.

3.3.2 Exclusion criteria

Mothers and infants pairs with medical conditions such as hypertension, fever, or mulnutritioned, septic caesarian section wounds (for the mother) and difficulty in breathing signs and symptoms that required immediate medical attention and management.

3.4 Sample size calculation

Sample size was calculated using the Fisher's formula for cross sectional studies. The calculation was as follows:

$$n = \frac{Z^2 \times P\,(1-P)}{d^2}$$

Where

n = minimum sample size

Z = Z statistic for 95% level of confidence = 1.96

P = Estimated prevalence of HBV infection among HIV positive and HIV negative pregnant mothers = 4.2% *(*Kilonzo, 2013)

d = margin of error = 2%

$$\frac{1.96^2 \times 0.042 \times 0.958}{0.02^2}$$

Thus

$$= 386$$

A minimum of 386 mothers-infant pairs in post partum PMTCT follow up were required to meet the objectives of this study. However, 534 mothers-infant pairs were recruited to increase the power of the study and to take care of the fall outs. This was also meant to avoid wastage of the reagents for HBV ELISA.

3.5 Sampling design

Once the KEMRI Scientific and Ethics Review Unit (SERU) and the KNH/ UON Ethical Review Committee (ERC) approvals were in place a census of all mothers coming to the clinic for postnatal care in the PMTCT clinic was done during the study period. Sampling frame was drawn from the HIV-exposed infants (HEI) register where a list of all mother-baby pairs attending clinic were recorded. A 2-year cohort of approximately 600 HIV exposed infants are recorded in the register. The appointments diary also was used to identify the mothers who were expected in the clinic that day and it helped the researcher to minimize the chances of missing the eligible mothers attending clinic. Out of these sources, a list of mothers were drawn for each day and distributed to the clinician designated to see all HIV positive mothers in the PMTCT. The clinician would identify the mothers and refer them to the investigator after they had received all the services sought in the hospital. Once the postnatal mothers were identified, they were approached to seek their participation in the study.

3.6 Data collection procedures

Data collection was done by the investigator. The women enrolled in the study were interviewed through face to face interview using a structured questionnaire to capture the socio-demographic data of the participants and information on predisposing factors for HBV infections such as the age, marital status, HAART use

and history of blood transfusion and HBV.

Every file of the postnatal mother was marked with a unique identifier after interviews to ensure there were no repeat interviews on the same mothers in the subsequent visit. A green sticker with study number was used for unique identification of patients after the interview. The sticker was placed inside the top cover of the file. Also, a daily log of out-patient number, the name and the study number assigned to each mother enrolled was kept to countercheck when doing subsequent interviews. The investigator ensured the data collected was of high quality by checking through the questionnaire immediately after every interview, before the study participant left the interview room. Any missing or unclear response on the questions was corrected by requesting the mother for additional time to clarify the responses.

3.7 Laboratory procedures

Blood for HBV testing was collected in a phlebotomy room. The samples were then taken to an Immunology level II laboratory where they were centrifuged at 10,000 g for 10 minutes and plasma separated in a biosafety cabinet. Good laboratory practice was adhered to in handling the samples.

3.7.1 Blood sample collection`

From each of the subjects enrolled, 4 mL single draw whole blood sample was collected into EDTA tubes by veno-puncture. The same amount of blood (4ml) was drawn from the HBV exposed infants by the researcher.

3.7.2 Hepatitis B surface antigen rapid test

HBV screening was done using a point of care HBsAg rapid EME kit (Euromedi Equip ltd. UK). The dipstick rapid strip was dipped into serum/ plasma for 10 seconds and then laid flat on a clean, dry, non- absorbent surface as per the manufacturer's instructions. The results were then read after 15 minutes.

3.7.3 Hepatitis B surface antigen Enzyme Linked Immunosorbent Assay

Further screening for HBV was done with an ELISA. Samples from the HBV exposed infants were also screened using the Hepanostika HBsAg Utra, ELISA kit (France). In this, a sandwich ELISA involving primary and secondary anti-HBV were used to detect HBV surface antigen in plasma. Twenty five (25) µL of specimen diluents was assigned into micro elisa wells. 100 µL of undiluted sample was added and incubated at 37 °C for 60 minutes. 50 µL of the conjugate solution was added into each well and incubated at 37 °C for 60 minutes. Washing was done using the phosphate buffer for six times and 100 µL Tetramethylbenzidine (TMB) substrate added into each well. The plate at this point was incubated at 15 to 30 °C for 30 minutes in the dark. Thereafter the reaction was stopped by adding sulfuric acid into each well and the plates were read at 450nm wavelength.

3.7.4 Hepatitis B 'e' antigen Enzyme Linked Immunosorbent Assay

HBsAg positive samples were subjected to HBeAg ELISA that was performed using HB'e'Ag Accubiotech Co.ltd ELISA kit. HBeAg serology was done to determine if the infection was chronic and if there was the presence of continuous viral shedding thus the probability of high HBV DNA viral loads. 50 µL of sample was added onto the micro-well plates followed by addition of 50 µL Horseradish peroxidase (HRP) - conjugate which were mixed gently by tapping as per the manufacturer's instructions. The ELISA plate was then incubated at 37 °C, then washed five times using wash buffer. Fifty (50) µL chromogen was added, incubated in the dark for 15 minutes and 50 µL stop solution added. The plate was read at 450 nm wavelength.

3.7.5 Hepatitis B virus DNA qualitative PCR for detection of HBV

All the HBsAg ELISA positive samples from the mothers and the samples from the HBV exposed infants were subjected to Polymerized chain reaction (PCR) to increase the sensitivity of detection. HBV DNA was extracted from 100 µL of plasma sample using the QIAamp DNA Mini Kit extraction kit (Qiagen) according to manufacturer's instruction. Briefly, 0.5 ml of DNAzol was added and mixed

completely. This was then centrifuged at 13000 rpm for 1 minute. After discarding the supernatant 1ml absolute alcohol was added to dissolve pellet by gentle mixing. This was then centrifuged at 13000 rpm in a microfuge at 4 ^{O}C for 15 minutes. The supernatant was discarded and the pellet washed by 70% ethanol, air dried and then re-suspended in 1 ml water free DNase and RNase. (Stuyver *et al*., 1999)

The PCR was performed using a 96 well cycler (Gene AMP PCR system 9700, applied Biosystems).The preS1 region of the HBV gene was amplified using primers HBPr1 (GGGTCACCATATTCTTGGG) and HBPr135 (CA (A}G)AGACAAAAGAAAATTGG) for the first round PCR followed by a second nested reaction using HBPr2 (GAACAAGAGCTACAGCATGGG) and HBPr3 (CCACTGCATGGCCTGAGGATG) (Stuyver *et al*., 1999). The first and second PCR reaction was performed with a cycle at 94^{O}C for 10 min, followed by 40 cycles at 94 ^{O}C for 30 sec, 50 ^{O}C for 30 sec and 72 ^{O}C for 1 min, with a final extension of 72 ^{O}C for 10 minutes (Stuyver *et al*., 1999).

Amplicons (1 μL) were analyzed by electrophoresis on 1.5% agarose gels, stained with ethidium bromide and visualized on a UV transilluminator. The resulting DNA fragments were visible as clearly defined bands. A DNA standard ladder was used for the determination of the sizes of the sample bands. The agarose gel, gloves, centrifuge tubes, towels (contaminated with ethidium bromide) were placed in a sealable zip lock bag. The plastic zip lock bag was sealed when it was 75% full and placed into a second zip lock platic bag and labelled outside as ‘Ethidium bromide contaminated solids’. This was then taken to the disposal room.

3.8 Statistical analysis

Statistical analysis was conducted using SPSS version 21. The prevalence of HBV in mothers was presented as a proportion with 95% CI. HBV transmission to infants was calculated as the proportion of infants of HBV-infected mothers with HBV infection. Factors associated with HBV infection were analyzed using independent t test to compare mean age between those mothers infected and those not infected. In addition categorical variables such as marital status, previous HBV testing, number

of sexual partners, mode of delivery, history of blood transfusion, dental surgery, body piercing, use of HAART, adherence to antiretroviral therapy (ART) drug and substance use, HBV vaccination and mother's job occupation were associated with HBV infection using Chi-square test of association. All statistical tests were interpreted at 5% level of significance (95% CI).

3.9 Ethical considerations

The study protocol and the informed consent were reviewed and approved by the Kenya Medical Research Institute Scientific and Ethics Review Unit (SERU; SSC protocol no. 2921), and the University of Nairobi/ Kenyatta National Hospital Ethics and Review Committee (ERC; ERC no. P329/05/2015).

3.10 Consent/ assent from participating mothers

The investigator introduced himself to the mother and gave an overview of the study before administering eligibility criteria to determine their eligibility into the study. Upon ascertaining that a mother was eligible in the study, the researcher also confirmed if she was willing and comfortable to participate in the study. Informed consent was administered to the women who met the inclusion criteria of the study. An assent was also given by the mother where the infant was exposed for HBV. The consenting process included giving general information on the study, the risks and benefits associated with the study, confidentiality and the women's freedom to decline to participate in the study. Those who agreed to participate in the study were enrolled after signing an informed consent form. The women who consented to participate were enrolled into the study until the desired sample size was achieved.

CHAPTER FOUR

RESULTS

4.1 Social demographic factors of the study population

As shown in Table 4.1, the children had a median age of 6 months (1.5 -18 months) and 61.2% (327) of them were females. The mean age of the mothers was 31.2 years (SD 5.4 years) ranging between 18 and 45 years. Four hundred and thirty-three (81.1%) were married, 272 (50.9%) had tertiary level of education, 318 (59.5%) were employed.

Among the mothers interviewed 12.4% (66) disclosed substance use; 12% (64) used alcohol, 1.1% (6) cigarettes and 2 mothers (0.4%) reported using hard drugs. The women had a mean age at first sexual intercourse of 17.5 years (SD 1.9 years). Condom use was very high at 99.8% (533) and 92.7% (495) of the mothers had a single sexual partner. Some of the mothers (4.9%) reported a history of blood transfusion and 5.2% (26) had ever received body piercing. History of dental surgery was reported in 10.3% (55) of the cases.

Table 4.1: Baseline characteristics of study participants attending PMTCT care and treatment clinic at KNH

Variable	Frequency (%)
Age of the mothers in years	
Mean (SD)	31.2 (5.4)
Min-max	18.0-45.0
Age of infant in months	
Median (IQR)	6.0 (3.0-10.0)
Min-max	1.5-18.0
Gender of infants	
Male	207 (38.8)
Female	327 (61.2)
Marital status	
Not married	101 (18.9)
Married	433 (81.1)
Education	
Primary and below	74 (13.9)
Secondary	188 (35.2)
Tertiary	272 (50.9)
Occupation	
Employed	318 (59.5)
Unemployed	216 (40.5)
Religion	
Christian	529 (99.1)
Muslim	5 (0.9)
Substance use	66 (12.4)
Alcohol	64 (12.0)
Cigarettes	6 (1.1)
Hard drugs (marijuana and cocaine).	2 (0.4)
Age at first sexual intercourse	
Mean (SD)	17.5 (1.9)
Min-max	14.0-25.0
Use of condoms	533 (99.8)
Ever received blood transfusion	26 (4.9)
Body piercing	28 (5.2)
Dental surgery	55 (10.3)
Number of sexual partners	
1	495 (92.7)
2	26 (4.9)
3	12 (2.2)
6	1 (0.2)
Use of HAART	534 (100.0)
TDF/3TC/NVP	502 (94.0)
AZT/3TC/NVP(or EFV)	32 (6.0)

As shown in figure 4.1, out of the total 534 mothers, 113 (21.2%) of the mothers were aware of HBV infection and only 2 mothers (0.4%) had ever been vaccinated for HBV. Mothers who had ever been talked to by a doctor about HBV were 21.3%.

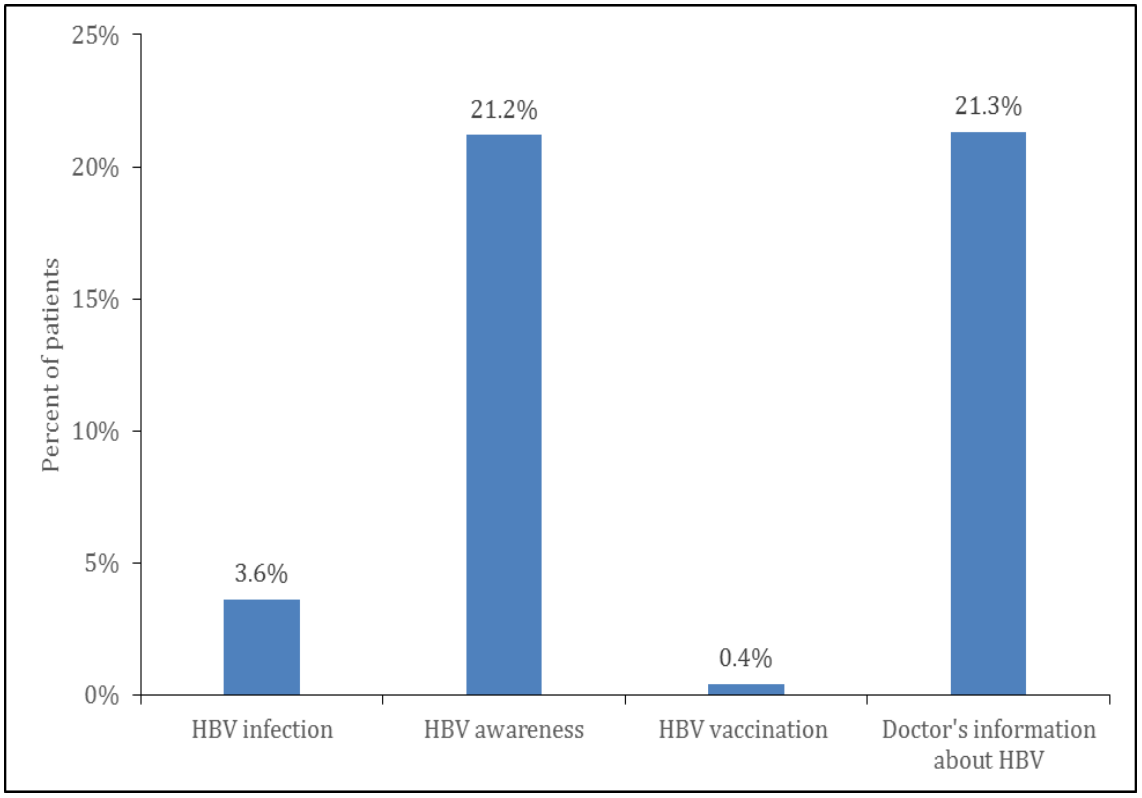

Figure 4.1: HBV prevalence, vaccination and level of awareness among study participants attending PMTCT care and treatment clinic at KNH.

4.2 HBV prevalence

As shown in table 4.2 below, 19 of the total 534mothers had a positive HBsAg results with both rapid, ELISA techniques and PCR targeting the preS1 region. All the HBV positive samples were negative for HBeAg. All the 19 HBV exposed infants were negative for HBsAg by rapid, ELISA and PCR techniques.

Table 4.2: HBV laboratory results

	HBsAg positive		HBV DNA PCR positive	HBeAg ELISA positive
	Rapid	ELISA		
Mothers (n=534)	13 (2.4%)	19 (3.6%)	19 (3.6%)	0
Infants (n=19)	0	0	0	-

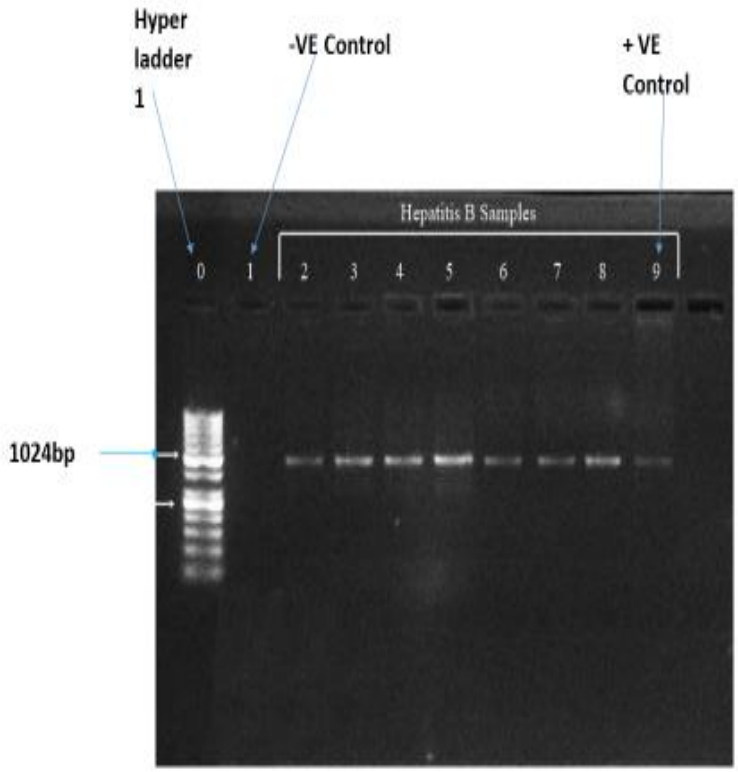

Figure 4.1: Bands showing amplification of HBV Pre S1 region by PCR among HBV infected study participants. The target amplified nucleic material was of 1024 bp.

4.3 Factors associated with HBV infection among study participants attending PMTCT care and treatment clinic at KNH

As shown in table 4.3, the prevalence of HBV was 9.1% (5) in patients who had undergone dental surgery compared to 2.9% (14) in those with no history of dental surgery, OR 3.3 (95% CI 1.1-9.6), p=0.036. Though not statistically significant, mothers with a history of blood transfusion had a prevalence of 7.7% (2) as compared to 3.3% (17) in those who have never been transfused. Mothers with primary level of education and business women had the highest HBV prevalence of 4.1% (3) and 5.8% (9) respectively. All the HBV infected mothers have never been vaccinated. Mothers with more than one partner had a higher HBV prevalence of 3.8%.

Table 4.3: Factors associated with HBV infection among study participants attending PMTCT care and treatment clinic at KNH.

Variable	HBV infection		OR (95% CI)	P value
	Positive (n=19) n (%)	Negative (n=515) n (%)		
Median age of infants in months (IQR)	4 (2-9)	6 (3-10)	-	0.309
Gender for HIV exposed infants				
Male	5 (2.4)	202 (97.6)	0.6 (0.2-1.6)	0.257
Female	14 (4.3)	313 (95.7)	1.0	
Mean age of the mother (SD)	31.4 (5.2)	31.2 (5.4)	-	0.891
Marital status				
Single	0 (0.0%)	82 (100.0%)	-	0.068
Married	17 (3.9%)	416 (96.1%)	1.0	
Unmarried	2 (10.5%)	17 (89.5%)	2.9 (0.6-13.5)	0.161
Religion				
Christian	19 (3.6%)	510 (96.4%)	-	1.000
Muslim	0 (0.0%)	5 (100.0%)		
Education				
None	0 (0.0%)	1 (100.0%)	-	1.000
Primary	3 (4.1%)	70 (95.9%)	1.3 (0.3-4.7)	0.740
Secondary	7 (3.7%)	181 (96.3%)	1.1 (0.4-3.1)	0.811
Tertiary	9 (3.3%)	263 (96.7%)	1.0	
Occupation				
Employed	4 (2.5%)	158 (97.5%)	1.0	
Business	9 (5.8%)	147 (94.2%)	2.4 (0.7-8.0)	0.137
Unemployed	5 (2.7%)	177 (97.3%)	1.1 (0.3-4.2)	0.872
Student	1 (2.9%)	33 (97.1%)	1.2 (0.1-11.1)	0.874
Substance use				
Yes	5 (7.6%)	61 (92.4%)	2.7 (0.9-7.6)	0.072
No	14 (3.0%)	454 (97.0%)	1.0	
Transfusion				
Yes	2 (7.7%)	24 (92.3%)	2.4 (0.5-11.0)	0.235
No	17 (3.3%)	491 (96.7%)	1.0	
Body piercing				
Yes	1 (3.6%)	27 (96.4%)	1.0 (0.1-7.8)	1.000
No	18 (3.6%)	488 (96.4%)	1.0	
HBV awareness				
Yes	5 (4.4%)	108 (95.6%)	1.3 (0.5-3.8)	0.570
No	14 (3.3%)	407 (96.7%)	1.0	
HBV vaccination				
Yes	0 (0.0%)	2 (100.0%)	1.0 (1.0-1.1)	1.000
No	19 (3.6%)	513 (96.4%)	1.0	
Partners				
1	18 (3.6%)	477 (96.4%)	0.7 (0.1-5.4)	1.000
2 or more	1 (3.8%)	38 (96.2%)	1.0	
Dental surgery				
Yes	5 (9.1%)	50 (90.9%)	3.3 (1.1-9.6)	0.036
No	14 (2.9%)	465 (97.1%)	1.0	

CHAPTER FIVE

DISCUSSION

5.1 HBV infections among HIV infected HAART receiving mothers

This study revealed an HBV infection rate of 3.6% among HIV-infected women enrolled for PMTCT and receiving effective HIV HAART at the KNH in Nairobi, Kenya. WHO classifies this region as an intermediate endemicity (with HBV prevalence ranging between 2 and 7%) (Abebe *et al.,* 2003; WHO, 2009). This study results agree with results from similar studies which reported HBV infection prevalence of 2.8% among HIV positive pregnant women (Kilonzo *et al*., 2014) and 4% HBV infection prevalence among HIV-infected delivering women in Malawi (Charsela *et al.,* 2014). A similar HBV prevalence of 2.4% among HIV infected pregnant mothers was reported in Rwanda close to a prevalence of 4.9% in Uganda (Pirillo *et al.,* 2007). This similarity may be due to the urban populations sampled or it may imply similar modes of transmission of HBV in the four regions which also have similar healthcare policies. Studies in Nigeria and Ethiopia reported higher prevalence of HBV-HIV co-infection at 9.5% (Pennap *et al.,* 2011) and 19% (Zennebe *et al.*, 2014) respectively. A study from Southern Ethiopia reported HBV infection prevalence of 0.6% among HIV-infected pregnant women (Ramos *et al.,* 2011). A prevalence of HBV among HIV negative pregnant women in Abidjan, Cote d'Ivoire was 8% and that among HIV positive women was 9.8%. Statistically the difference was not shown to be significant (Rouet *et al.,* 2004). However, HBV was of high endemicity in this region among the HIV negative and HIV positive pregnant women in comparison to the findings of the KNH study site. Simpore et al (2006) found an overall HBV prevalence in pregnant women of 9.8% in Burkina Faso, where the HBV prevalence was higher in HIV positive women at 11.6% than in HIV negative women at 7% though this difference was not significant. Cultural practices of Western Africa and Eastern Africa differ. This may explain this difference in the prevalence of HBV in HIV positive pregnant women.

The 3.6% proportion of HBV/ HIV infection in this study suggest a lower HBV prevalence compared to the 4.2 % by Kilonzo *et al.* (2014) in an overall cohort of HIV infected and HIV non-infected pregnant mothers at the Kenyatta National Hospital. In a multicentre cross-sectional study on 2241 pregnant women, Okoth et al reported the prevalence of HBsAg seropositivity in pregnant women in Kenya to be 9.3% in Kenya (Okoth *et al.,* 2006). Similar studies conducted in the Eastern Africa region also showed higher prevalence of HBV among pregnant women irrespective of their HIV status. The studies reported a HBV prevalence of 5.6% in Sudan and a 6.3% in Tanzania (Rasha *et al.,* 2007).

HBV prevalence among HIV negative pregnant women was reported at 5.6% (Kilonzo *et al.,* 2014) which was also higher than the prevalence reported in this study. It is notable that a higher HBV prevalence has been reported in the general population with studies in Kenya showing up to 8.8% prevalence with urban areas reporting ranges between 8 and 30% (WHO, 2015). Variations in the prevalence of the HBV across studies reflect the demographic and possibly exposure differences within HIV-infected populations who had better access to free antiretroviral therapy that leads to prolonged life period hence a like hood of HBV infection (Muriuki *et al.,* 2013). In addition, by virtual of HIV infection, low immune response could also have increased vulnerability to HBV infection.

5.2 Vertical transmission of HBV among HBV exposed infants

Mothers who are HBV positive pose a high risk to their exposed infants after delivery due to continued contact with an infected household contact. This study at the KNH reported a zero transmission of HBV among the HBV exposed infants. This was contrary to the results of a study conducted in Australia by Wiseman et al (2009) where they found a 3% perinatal transmission of HBV among HBV exposed infants. Alarmingly high rates of transmission ranging from 23%–28% have been reported in other countries such as China, despite passive and active immunoprophylaxis. Explanations to the transmission irrespective of the active immunoprophylaxis may reflect variation in adherence to

immunization protocols or possibly different prevalence of vaccine escape mutations (Xiao *et al.,* 2007; Zonneveld *et al.,* 2003).

All mothers sampled at the KNH were on effective first line HAART for managing HIV and had undetectable levels of HIV RNA viral loads. Mothers who were HBV-infected were on tenofovir (TDF) and lamivudine (3TC) based regimen; which are also recommended by WHO for management of HBV infections (WHO, 2012). The use of TDF and 3TC regimen in this population could have potentially caused the negative status of HBeAg and hence the possible suppression of HBV DNA viral load. This could have minimized the transmission of HBV to the exposed infants.

Vertical transmission of HBV infections has been confirmed in other studies to be influenced by hepatitis B 'e' antigen status (HBeAg) which is associated with high HBV DNA viral load (Kfutwah *et al.,* 2012). In this study all samples positive for HBsAg were negative for HBeAg, revealing a possibility of low HBV viral loads in the samples, and thus also contributing to zero transmission. Majority of infants born to HBsAg positive and HBeAg positive mothers get infected with HBV in the perinatal period when no interventions are made (Wiseman *et al.,* 2009). Most of these infections occur during delivery due to contact with maternal blood and vaginal secretions. In utero (Trans placental) HBV transmission occurs in 10% - 16% of infants of HBV carrier mothers when no intervention is initiated. In utero HBV infection is thought to occur through placental breach as in threatened preterm labor, placental infection or through ascend of infected vaginal fluids into the uterus. A study conducted by Hill et al (2002) reported that HBV transmission have been shown in 5 - 10% of infants of HBsAg positive HBeAg positive mothers despite passive active vaccination at birth.

The present Kenyan prevention program regarding HBV consists of vaccination against the virus. The primary hepatitis B immunization series conventionally consists of three doses of vaccine which include one mono-valent dose at 6 weeks followed by two mono-valent or combined vaccine doses at 10 and 14 weeks

respectively (WHO, 1996). All the HBV exposed infants had received at least one, two or all of the doses and this could have attributed to the zero transmission due to the passive immunity.

5.3 Factors associated with HBV infection

In this study, mothers who had undergone dental surgery had a higher prevalence of HBV infection. Thus dental surgery was significantly associated with HBV infection (P value 0.036) in this cohort. This group was 3.3 times more likely to be HBV positive compared to the mothers who did not have history of dental surgery. Similar findings were reported in Ethiopia where pregnant mothers who had undergone dental procedure were 2 times as likely to be HBV-infected (Awole *et al.,* 2005). Poor sterilization and re use of dental equipments during the procedure has been shown to be playing the major role in transmission of HBV to uninfected individuals (Awole *et al.,* 2005). Though statistically not significant, mothers with a history of blood transfusion had a higher prevalence (7.7%) compared to 3.3% in those who had no transfusion. Significant associations have been reported in other studies where one study reported a higher proportion of HBV-infected patients having history of blood transfusion (6.6%) compared to 2.2% in those who were HBV negative (Kamal *et al.,* 2010). Also, a study in Ethiopia found out that pregnant women who had a history of blood transfusion were 3.7 times more likely to be HBV-infected (Zennebe *et al.,* 2014). Body piercing and tattooing have been reported in studies to be significantly associated with HBV infection with participants having 3 and 5.7 times respectively the chance of HBV infection (Zennebe *et al.,* 2014). Similarly, intravenous drug users (IDUs) were reported in a study to have a higher risk of HBV infection with HBsAg positivity of 9.6% in HIV-infected IDUs compared 3.6% in HIV-infected non-IDUs (Kilongosi *et al.,* 2015). However, this study did not find any significant association between substance use and HBV infection. Due to the similar modes of transmission for HBV and HIV, the number of partners was also considered though it was not statistically significant in this study.

A marked reduction in HBV prevalence can explained by the fact that public health interventions such as health education on safe sex practices, the risks of having multiple sexual partners and avoidance of contact with blood and body fluids, which have been put in place to curb the transmission of HIV, reinfection of HIV and spread of other infectious pathogens such as HBV. Kenyans are well informed about these risks of HIV transmission and prevention to their HIV exposed partners and infants as is evidenced by the 2008-09 KDHS which estimated that 75% of women knew that the chance of acquiring and transmitting HIV can be reduced by using condoms whereas 92% of women interviewed knew that being faithful to one partner reduces chances of acquiring and transmitting HIV as well as getting re infected (superinfection) (KNBS, 2010). HIV and HBV share risk factors, modes of transmission and even antiviral treatment and therefore care, management and treatment of HIV generally prevents the spread of HBV as well (Francis *et al.,* 1999).

There was no association between age, education level or form of employment with HBV infection among the HIV positive women. A study conducted in Ethiopia among pregnant mothers attending antenatal care reported a HBV prevalence of 5.3% with no statistical significance among the various age groups (Fisseha *et al.,* 2008). This study did not show relationship between education and HBV infection. However, illiteracy has been shown to be associated with HBV infection indicating lack of public health awareness/ education amongst mothers (Awole *et al.,* 2005). Buseri F et al (2010) conducted their study in Niger where they found a higher HBV prevalence among pregnant women with no formal education and those aged between 20-24 years. In a different study conducted in Nigeria, Lar *et al.* (2009) found an increased HBV prevalence among pregnant women who reported to have at least secondary education and found that business ladies were significantly more likely to be HBV infected. This study did not find any association between the marital status and HBV/ HIV infection. Only two of the HBV infected mothers were unmarried. However in a study carried out in Nigeria, HBV/HIV co-infection rates were highest among widowed/divorced groups followed closely by the unmarried (Christy *et al.,* 2002-03).

The rate of immunization against hepatitis B was only 0.4% among all HIV infected sampled women. This hepatitis B vaccination uptake and implementation in this risk group is lower. Formula *et al.* (2013) reported a HBV vaccination rate of 2.7% among pregnant women in Cameroon which is higher than the vaccination rate among the HIV infected mothers in KNH. Developed countries have higher levels of HBV vaccination among groups at high risk of HBV and this has highly contributed to a lower HBV prevalence (Advisory Committee on Immunization Practices, 2013). The level of awareness of HBV among the HIV infected mothers was at 21.2%. In the background of an intermediate endemicity of HBV, there is need for massive awareness campaigns to educate pregnant women and the risk groups on HBV routine screening and vaccination. The 0.4% HBV vaccination rate among the HIV infected mothers in KNH is also well below the rate of HBV vaccination in adults below 49years years in the United States of America which was estimated to be 35.9% (Advisory Committee on Immunization Practices, 2013). The lack of a policy on hepatitis B vaccination for adults including those who are HIV positive in the public sector in Kenya is partially liable for the low hepatitis B vaccination rates among pregnant women. In Kenya there is no catch up immunization program for non-immunized infants over the age of 12 months. Nevertheless, Kenya as a country has never launched a campaign on HBV vaccination for adults. An approach which if implemented, could have led to high numbers of HBV respondents. The vaccine is available in private health facilities but ends up being inaccessible due to the high cost implications.

An intervention strategy needs to be implemented urgently to curb the spread of this infectious virus especially in the risk groups and antenatal mothers. Of the 2 mothers who had been vaccinated for HBV, none of them tested positive for HBV. Among the 19 mothers who were HBV infected, none of them was aware of HBV and had never been vaccinated against HBV.

Cultural practices that predispose to infection with HBV such as female genital mutilation and traditional scarification account for most of the regional differences in HBV prevalence (Okoth *et al.,* 2006). This risk factors for HBV infection were not assessed in this study.

5.4 Study limitations

HBV sequencing was not done to analyze for drug resistance that could have been caused by HIV ART intake. The HBV genetic diversity among the HBV/HIV infected mothers was also not done due to logistical issues.

This cross-sectional study did not determine HBV viral load that could have guided on the confirmation of occult blood. It did not utilize hepatitis B core antibody (IgG/ IgM) to assess previous HBV exposure.

Amplifaction was done only on one region of the HBV genome due to logistics. The primers used in the PCR protocol targeted the Pre S1 region and there could have been sensitivity non-correlation (low sensitivity on Pre S1 amplifaction) as compared to extraction, amplifaction and detection of other regions such as the *pol* gene (Locarni and Zoulim, 2010). The antiviral treatment that the patients were subjected to can induce mutations in the Pre S1 region and consequently the primers targeting the region did not bind with no amplification will taking place. Viral variants on the Pre S1 region also appear as a result of exogenous (immune-propylaxis and antiviral therapy factors) and/ or endogenous factors; host immunity (Locarni and Zoulim, 2010). The high spontaneous error rate in the reverse transcriptase (with no proof reading) also induces mutations in the virus, such as the Pre s/s variants making the amplifaction of the region to be challenging (Pollicino *et al.,* 2012).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The proportion of HIV infected HAART receiving mothers who were HBV infected was 3.6% at KNH, thus the HBV prevalence in this setting is of intermediate endemicity.

2. There was no HBV transmission detected between mothers and their children. In this population of HIV-infected pregnant mothers, our observations suggest that the HAART regimen received by them may have prevented vertical transmission of HBV infections to exposed infants.

3. The history of dental surgery was associated with HBV infection among the HIV infected mothers. There was no association between the age, marital status, educational level or form of employment with HBV infection.

4. Given that this was a cross sectional study, then it was not possible to follow the HBV exposed infants over time and establish if they acquired HBV from their infected mothers later because of the mother- infant close relation as well as failed or non-adherence to the recommended interventions. The effectiveness of the passive and active HBV immunization by HBV antibodies titration among the HBV exposed infants after some time was not determined as well.

6.2 Recommendations

1. HBV screening and immunization should be implemented in our PMTCT programs on routine basis.

2. Given that the report of this study suggested that HAART use among HBV/HIV infected mothers prevents HBV transmission to the exposed infants, then studies should be carried out among HIV negative mothers of the reproductive age who have

increased chances of transmitting HBV if infected since they are not on ARVs to suppress HBV if present.

3. HBV/ HIV infected mothers should be initiated on first line HAART and counselled for adherence to prevent HBV and HIV transmission to their exposed infants.

4. An intervention strategy to create awareness on HBV screening and vaccination among members of the public and public health workers such as dentists on infection control should be implemented.

6. Further research on occult HBV among HIV-infected mothers should also be carried out.

7. Further studies to amplify other HBV regions such as the pol gene should be conducted to determine if sensitivity is high as compared to amplifaction of Pre S1 region

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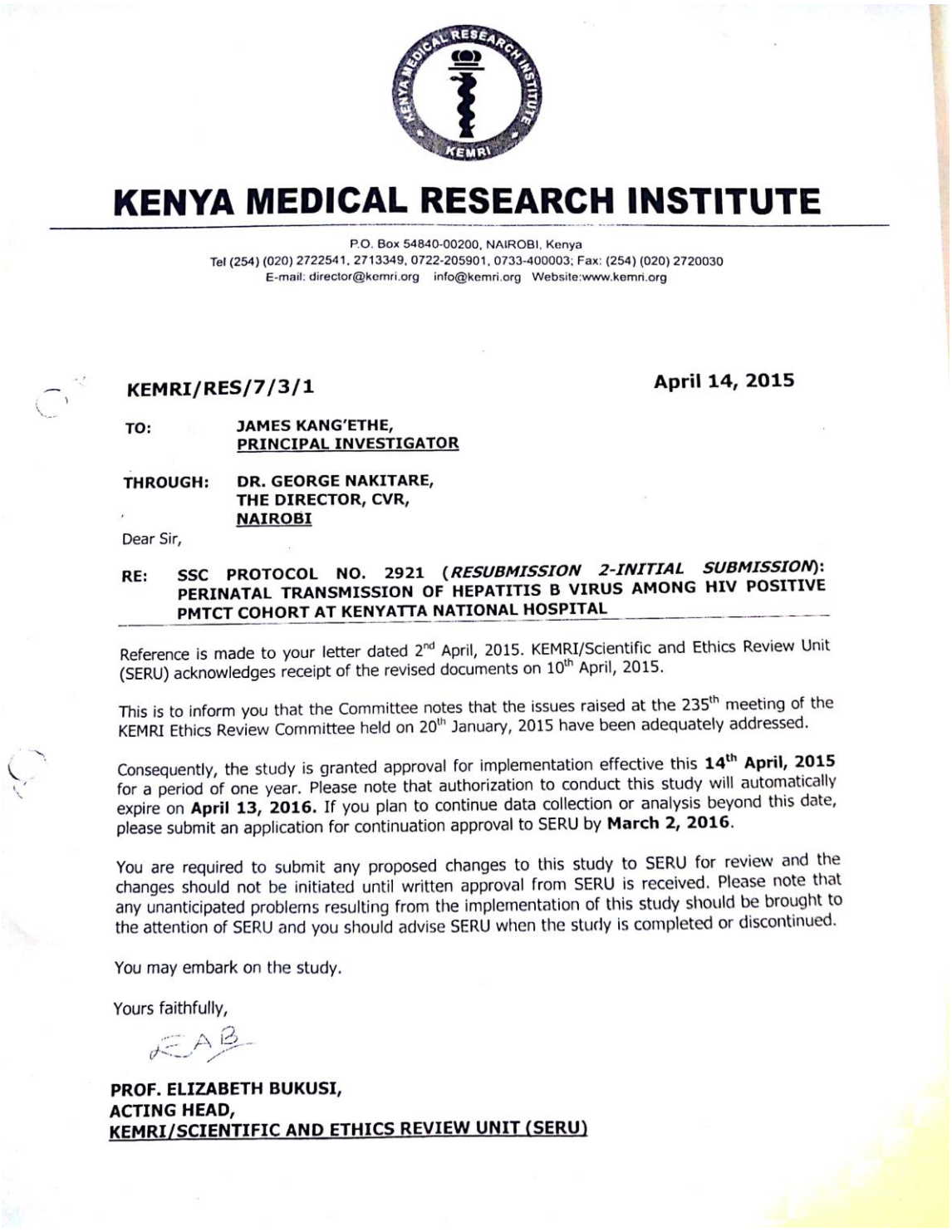
APPENDICES

Appendix I: KEMRI Seru Approval



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 14, 2015**

TO: **JAMES KANG'ETHE,**
PRINCIPAL INVESTIGATOR

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

Dear Sir,

RE: **SSC PROTOCOL NO. 2921 (*RESUBMISSION 2-INITIAL SUBMISSION*): PERINATAL TRANSMISSION OF HEPATITIS B VIRUS AMONG HIV POSITIVE PMTCT COHORT AT KENYATTA NATIONAL HOSPITAL**

Reference is made to your letter dated 2nd April, 2015. KEMRI/Scientific and Ethics Review Unit (SERU) acknowledges receipt of the revised documents on 10th April, 2015.

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

Consequently, the study is granted approval for implementation effective this **14th April, 2015** for a period of one year. Please note that authorization to conduct this study will automatically expire on **April 13, 2016.** If you plan to continue data collection or analysis beyond this date, please submit an application for continuation approval to SERU by **March 2, 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,

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

Appendix ii: UoN/ KNH ERC Approval

ENDIX II: UON/ KNH ERC APPROVAL

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/325

20th July 2015

James M. Kang'ethe
Principal investigator
JKUAT

Dear Mr. Kang'ethe

RESEARCH PROPOSAL – PERINATAL TRANSMISISON OF HEPATITIS B VIRUS AMONG HIV POSITIVE PMTCT COHORT ATKENYATTA NATIONAL HOSPITAL (P329/05/2015)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 20th July 2015 – 19th July 2016.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **www.erc.uonbi.ac.ke**

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Appendix III: Published Article

Prevalence and Associated Factors of HBV Infections among HIV-Infected HAART Receiving Mothers and their Exposed Infants in Nairobi, Kenya

Abstract

Mother-to-child transmission (MTCT) of Hepatitis B virus (HBV) is responsible for more than one third of chronic HBV infections worldwide. Antiretroviral therapy (ART) naïve HBV/HIV co-infected mothers have a high tendency of transmitting the two viruses. This study aimed to determine prevalence & predisposing factors of HBV infections among HAART-receiving HIV-infected mothers and their exposed infants. A structured questionnaire was used to capture socio-demographic data and factors associated with HBV infections. As 4_ml sample of paired whole blood obtained from HIV positive mothers & their exposed infants was analyzed for Hepatitis B surface antigen (HBsAg) using both rapid and Enzyme-linked immuno sorbent assay (ELISA) tests. HBsAg positive samples were further screened for HBV envelope antigen (HBeAg) using ELISA. HBsAg positive samples with both ELISA and rapid tests were subjected to a nested Polymerase chain reaction (PCR) targeting the preS1 region. A total of 534 HIV-infected mothers - infant pairs were recruited. Mean age of mothers was 31.2 years (SD 5.4 years) and infants' median age of 6 months (IQR 3-10 months). 502 (94%) of the mothers were taking TDF/3TC/ NVP and 32(6%) were on AZT/3TC/ NVP or EFV. 19 of 534 (3.6%) mothers were HBV positive by both HBsAg rapid and ELISA tests. All 19 HBsAg positive samples tested HBeAg negative. 12 of the 19 HBsAg positive samples also tested positive on PCR targeting the preS1 gene. All infants' samples tested HBV negative with all tests. History of dental surgery was associated with increased rate of HBV infection (OR 3.3 (95% CI 1.1-9.6). In this population of HIV-infected pregnant mothers, our observations suggest that the

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James Mburu Kang'ethe[7], Rebecca Waihenya[2], Onesmus Gachuno[3], Kenneth Mutai[4], Musa Otieno[5], James Gitau Komu[6], Hilda Mesa[7] and Raphael M wamtsi Lwembe[1]*

[1]*Kenya Medical Research Institute, Center for Virus Research,*

Kenya

[2]*Department of Zoology, Jomo Kenyatta University of Agriculture and Technology Nairobi, Kenya* [3]*Department of Obstetrics and Gynecology, University of Nairobi, Nairobi, Kenya*

*rlwembe@yahoo.com, rlwembe@kemri.org

[4]*Kenyatta National Hospital, Comprehensive Care Center, Kenya*

Abbreviations: MTCT: Mother to Child Transmission; PMTCT: Prevention of Mother-to-Child Transmission; HBV: Hepatitis B virus; HIV: Human Immunodeficiency Virus; ART: Antiretroviral Therapy; HAART: Highly Active Antiretroviral Therapy; ELISA: Enzyme-Linked Immunosorbent Assay; HBsAg: Hepatitis B surface Antigen; HBeAg: Hepatitis B Envelope Antigen; PCR: Polymerase Chain Reaction; TDF: Tenofovir; 3TC: Lamivudine; NVP: Nevirapine; AZT: Zidovudine; EFV: Efavirenz; SSA: Sub Saharan Africa; IDU: Injection Drug Users; KNH: Kenyatta National Hospital; KEMRI: Kenya Medical Research Institute; EDTA: Ethylamine Tetra Acetic Acid; NAAT: Nucleic Acid Amplification Technique

Introduction

Chronic hepatitis B virus (HBV) infection is the leading cause of end-stage liver disease worldwide. In 2015, 3.61% of the population was infected with HBV globally and 8.83 % in the Sub Saharan African (SSA) region [1]. Within the Southern and Eastern African region, the prevalence ranges between 2-8% [1]. A previous study in Ethiopia reported 3.8% of pregnant women were infected with HBV [2]. Studies in Kenya have shown HBsAg prevalence of 8.8% in the general population with a wider range in urban areas between 8-30% [1]. Another study in pregnant women attending antenatal care in a tertiary hospital in Kenya showed that 4.2% were HBV-infected [3]. Kenya is one of the HIV infection hotspots in SSA and high prevalence has been reported in women [4]. HIV/HBV co-infection is common in HIV type-1- infected individuals with a prevalence ranging from 6% to 20% [5,6]. Studies have reported a wide range of HIV/HBV co-infection from 1.5% in Cameroon [7] to 19% in Ethiopia [2]. Similar findings were reported in India where 4.6% of the HIV-infected pregnant women had HBV co-infection[8].

History of blood transfusion is one of the risk factors associated with HBV infection. A study in Ethiopia found that pregnant women who had a previous history of blood transfusion were about four times more at risk of HBV infection [2,7]. Injection drug users (IDUs), body piercing and tattooing have also been linked to HBV infection due to sharing of contaminated objects. Sharing of needles among IDUs has been shown to highly contribute to transmission of HIV and HBV with a study showing

a HBV prevalence of 9.6% among the IDUs. Also, body tattooing increased the risk of HBV infection six fold [9]. HBV screening in resource limited settings in HIV-infected mothers is rarely done despite the potential of transmission to their infants. A study in Australia found out HBV transmission rate of 2.9% in HBV-exposed infants [10]. This study was set to investigate the transmission of HBV infections from HIV-infected mothers receiving HIV HAART to their exposed infants.

Materials and Methods

Study area

The study was carried out at the Kenyatta National Hospital (KNH). KNH is situated in Nairobi's South-western zone approximately 5 km from the Nairobi City Centre. Sample analysis was done at the University of Nairobi; Pediatrics department and Kenya Medical Research Institute (KEMRI) centre for virus research respectively.

Study population

Approximately 400 mothers of reproductive age, who are HIV positive, are normally enrolled into a PMTCT program for HIV care and treatment every month. A total of 534 HIV-infected women attending PMTCT care at KNH were included in this study. All the enrolled mothers were on HAART and had undetectable HIV viral load. A total of 19 infants whose mothers were HBsAg positive were included. The women included those who delivered in KNH and those who delivered elsewhere and opted to seek postnatal services in the hospital. The services sought by the HIV positive mothers included PMTCT follow up for the HIV exposed babies. Mothers and infants pairs with medical conditions such as hypertension, fever, or malnourished, septic caesarian section wounds (for the mother) were excluded

from the study due to their need for higher level care.

Sampling procedures

Consecutive sampling procedure was used to select participants. In this technique, all mothers-infant pairs who were registered in the clinic and met the recruitment criteria were approached by the research assistant at the triage in the clinic, and had the study explained to them. Upon ascertaining that a mother was eligible in the study, the research assistant also confirmed if she was willing and comfortable to participate in the study by taking her through the informed consent. The women who consented were enrolled into the study until the desired sample size was achieved. Data collection was done through face to face interview using a structured and pre-tested questionnaire.

Blood sample collection

From each of the participants enrolled, about 4_mL single draw whole blood sample was collected into ethylamine diamine tetra-acetic acid (EDTA) tubes by venom-puncture during their normal visit to the clinic. The same amount of blood (4_ml) was drawn from the HBV-exposed infants by a trained phlebotomist.

Sample separation and storage: This whole blood sample was centrifuged at 10,000g for 10 minutes where plasma was separated from the cells and stored at -80 °C.

Hepatitis B surface antigen rapid test: Initial screening for HBV in all the 534 mothers and the 19 HBV exposed infants was done using HBsAg rapid EME kit (Euromedi Equip ltd. UK). The dipstick rapid strip was dipped into serum/ plasma for 10 seconds and then laid flat on a clean, dry, non- absorbent surface as per the manufacturer's instructions. The results were then read after 15 minutes.

Hepatitis B surface antigen ELISA: Further screening for HBV in all the 534 mothers' samples was done with an ELISA. 19 samples from the HBV exposed infants were also screened using the Hepanostika HBsAg Ultra (France), ELISA kit. In this, a sandwich ELISA involving primary and secondary anti-HBV were used to detect HBV surface antigen in plasma. 25 micro liters (µl) of specimen diluents was assigned into micro Elisa wells. 100 µl of undiluted sample was added and incubated at 37 degrees Celsius for 60 minutes. 50 µl of the conjugate solution was added into each well and incubated at 37 degrees Celsius for 60 minutes. Washing was done using the phosphate buffer for six times and 100 µl TMB substrate added into each well. The plate at this point was incubated at 15 to 30 degree Celsius for 30 minutes in the dark. Thereafter the reaction was stopped by adding sulfuric acid into each well and the plates were read at 450 nm wavelength.

Hepatitis B 'e' antigen ELISA: All the 19 mothers' samples that were HBsAg positive were subjected to HBeAg ELISA that was performed using HB'e'Ag Accubiotech Co. Ltd (Beijing, China) ELISA kit. HBeAg indicates chronic HBV infection with continuous viral shedding thus probability of high HBV DNA viral loads. 50 µl of sample was added onto the micro-well plates followed by addition of 50 µl HRP-conjugate which were mixed gently by tapping as per the manufacturer's instructions. The ELISA plate was then incubated at 37 degrees Celsius, then washed five times using wash buffer. 50 µl chromate was added, incubated in the dark for 15 minutes and 50 micro liter stop solution added. The plate was read at 450nm.

Hepatitis B virus DNA qualitative PCR for detection of HBV: All the 19 HBsAg ELISA positive samples from the mothers and the 19 samples from the HBV exposed infants were subjected to nucleic acid amplification test (NAAT) to increase the sensitivity. HBV DNA was extracted from 100 µl of plasma sample using the QIAamp DNA Mini Kit extraction kit (Qiagen) according to manufacturer's instruction. The PCR was performed using a 96 well cycler (Gene AMP PCR system 9700, applied Biosystems). The preS1 region of the HVB gene was amplified using primers HBPr1 (GGGTCACCATATTCTTGGG) and HBPr135 (CA (A}G)

GACAAAAGAAAATTGG)forthefirstroundPCRfollowedbyasecond nested reaction using HBPr2 (GAACAAGAGCTACAGCATGGG) and HBPr3 (CCACTGCATGGCCTGAGGATG) (Stuyver et al. 1999). The

first and second PCR reaction was performed with a cycle at 94OC for 10 min, followed by 40 cycles at 94^{O}C for 30 sec, 50^{O}C for 30 sec and 72 ^{O}C for 1 min, with a final extension of 72 ^{O}C for 10 min. Amplicons (1microlitre) were analyzed by electrophoresis on 1.5% agarose gels, stained with Ethidium bromide and visualized on a UV trans illuminator. The resulting DNA fragments were visible as clearly defined bands. A DNA standard ladder was used for the determination of the sizes of the sample bands.

Statistical analysis

Statistical analysis was conducted using SPSS version 21. The prevalence of HBV in mothers was presented as a proportion with 95% CI. HBV transmission to infants was calculated as the proportion of infants of HBV-infected mothers with HBV infection. Factors associated with HBV infection were analyzed using independent t test to compare mean age between those mothers infected and those not infected. In addition categorical variables such as marital status, previous HBV testing, number of sexual partners, mode of delivery, history of blood transfusion, dental surgery, body piercing, use of HAART, adherence to antiretroviral therapy (ART) drug and substance use, HBV vaccination and mother's job occupation were associated with HBV infection using Chi-square test of association. All statistical tests were interpreted at 5% level of significance (95% CI).

Ethical considerations

The study protocol and the informed consent were reviewed and approved by the Kenya Medical Research Institute Scientific and Ethics Review Unit (SERU), and the University of Nairobi/ Kenyatta National Hospital Ethics and Review Committee (ERC).

Results

Five hundred and thirty-four (534) HIV positive mothers who were enrolled in PMTCT and 19 HBV exposed infants were enrolled in this study. The children had a median age of 6 months and 61.2% of them were females. The mean age of the mothers was 31.2 years (SD 5.4 years) ranging between 18 and 45 years? 433 (81.1%) were married, 272 (50.9%) had tertiary level of education, 318 (59.5%) were employed and 99.1% were Christians.

Risk profile of HBV

Among the mothers interviewed, 12.4% disclosed substance use, 12% used alcohol, 1.1% cigarettes and 2 mothers (0.4%) reported using hard drugs. The women had a mean age at first sexual intercourse of 17.5 years (SD 1.9 years). Condom use was very high at 99.8% and 92.7% of the mothers had a single sexual partner. Some of the mothers (4.9%) reported a history of blood transfusion and 5.2% had ever received body piercing. History of dental surgery was reported in 10.3% of the cases (Tables 1-4).

Table 1: Baseline Characteristics.

Variable	Frequency (%)
Age of the mothers	
Mean (SD)	31.2 (5.4)
Min-max	18.0-45.0
Age of child in months	
Median (IQR)	6.0 (3.0-10.0)
Min-max	1.5-18.0
Gender of children	
Male	207 (38.8)
Female	327 (61.2)
Marital status	
Not married	101 (18.9)
Married	433 (81.1)
Education	
Primary and below	74 (13.9)
Secondary	188 (35.2)
Tertiary	272 (50.9)
Occupation	
Employed	318 (59.5)
Unemployed	216 (40.5)
Religion	
Christian	529 (99.1)
Muslim	5 (0.9)
Substance use	

Alcohol	64 (12.0)
Cigarettes	6 (1.1)
Hard drugs	2 (0.4)
Age at first sexual intercourse	
Mean (SD)	17.5 (1.9)
Min-max	14.0-25.0
Use of condoms	533 (99.8)
Ever received blood transfusion	26 (4.9)
Body piercing	28 (5.2)
Dental surgery	55 (10.3)
Number of sexual partners	
1	495 (92.7)
2	26 (4.9)
3	12 (2.2)
6	1 (0.2)
Use of HAART	534 (100.0)
TDF/3TC/NVP	502 (94.0)
AZT/3TC/NVP(or EFV)	32 (6.0)

HBV laboratory results

Out of the total 534 mothers, 19 had a positive HBsAg results with both rapid and ELISA techniques. 12 of the 19 samples that tested positive with ELISA also gave positive results on PCR. All the HBsAg positive samples by ELISA were negative for HBeAg. All the 19 HBV exposed infants were negative for HBsAg by rapid ELISA and PCR techniques.

Table 2: HBV Laboratory Results.

	HBsAg positive			
	Rapid	**ELISA**	**HBV DNA PCR positive (n=19)**	**HBeAg ELISA positive (n=19)**
Mothers (n=534)	13 (2.4%)	19 (3.6%)	12 (63.2%)	0
Infants (n=19)	0	0	0	0

HBV prevalence and the level of awareness among mothers

The prevalence of HBV was 3.6% (95% CI 2.1-5.2%). 113 (21.2%) of the mothers were aware of HBV infection and only 2 mothers (0.4%) had ever been vaccinated for HBV. Mothers who had ever been talked to by a doctor about HBV were 21.3%.

Table 3: HBV Prevalence and Level of Awareness.

Variable	**Frequency (%)**
HBV infection	19 (3.6)
HBV awareness	113 (21.2)
HBV vaccination	2 (0.4)
Doctor ever talked about HBV	114 (21.3)

Factors associated with HBV infection

Prevalence of HBV was 9.1% in patients who had undergone dental surgery compared to 2.9% in those with no history of dental surgery, OR 3.3 (95% CI 1.1-9.6), p=0.036.

Table 4: Factors associated with HBV infection.

Variable	HBV infection		OR (95% CI)	P value
	Positive (n=19) (%)	Negative (n=515) n (%)		
Median age of infants in months				
(IQR)	4 (2-9)	6 (3-10)	-	0.309
Gender for infants				
Male	5 (2.4)	202 (97.6)	0.6 (0.2-1.6)	0.257
Female	14 (4.3)	313 (95.7)	1	
Mean age of the mother (SD)	31.4 (5.2)	31.2 (5.4)	-	0.891
Marital status				
Single	0 (0.0%)	82 (100.0%)	-	0.068
Married	17 (3.9%)	416 (96.1%)	1	
Divorced/separated/Widowed	2 (10.5%)	17 (89.5%)	2.9 (0.6-13.5)	0.161
Religion				
Christian	19 (3.6%)	510 (96.4%)	-	1
Muslim	0 (0.0%)	5 (100.0%)		
Education				
None	0 (0.0%)	1 (100.0%)	-	1
Primary	3 (4.1%)	70 (95.9%)	1.3 (0.3-4.7)	0.74
Secondary	7 (3.7%)	181 (96.3%)	1.1 (0.4-3.1)	0.811
Tertiary	9 (3.3%)	263 (96.7%)	1	
Occupation				
Employed	4 (2.5%)	158 (97.5%)	1	
Business	9 (5.8%)	147 (94.2%)	2.4 (0.7-8.0)	0.137
Unemployed	5 (2.7%)	177 (97.3%)	1.1 (0.3-4.2)	0.872
Student	1 (2.9%)	33 (97.1%)	1.2 (0.1-11.1)	0.874
Substance use				
Yes	5 (7.6%)	61 (92.4%)	2.7 (0.9-7.6)	0.072
No	14 (3.0%)	454 (97.0%)	1	
Transfusion				
Yes	2 (7.7%)	24 (92.3%)	2.4 (0.5-11.0)	0.235
No	17 (3.3%)	491 (96.7%)	1	
Body piercing				
Yes	1 (3.6%)	27 (96.4%)	1.0 (0.1-7.8)	1
No	18 (3.6%)	488 (96.4%)	1	
HBV awareness				
Yes	5 (4.4%)	108 (95.6%)	1.3 (0.5-3.8)	0.57
No	14 (3.3%)	407 (96.7%)	1	
HBV vaccination				
Yes	0 (0.0%)	2 (100.0%)	1.0 (1.0-1.1)	1
No	19 (3.6%)	513 (96.4%)	1	
Partners				
1	18 (3.6%)	477 (96.4%)	0.7 (0.1-5.4)	1
2 or more	1 (3.8%)	38 (96.2%)	1	
Dental surgery				
Yes	5 (9.1%)	50 (90.9%)	3.3 (1.1-9.6)	0.036
No	14 (2.9%)	465 (97.1%)	1	

Discussion

Our study revealed an HBV infections rate of 3.6% among HIV-infected women enrolled for PMTCT and receiving effective HIV HAART at the KNH in Nairobi, Kenya. WHO classifies this region as intermediate endemicity (with HBV prevalence ranging between 2 and 7%) [11,12]. Our results agree with results from similar studies which reported HBV infection prevalence of 2.8% among HIV positive pregnant women [3]. And 4% HBV infection prevalence among HIV-infected delivering women in Malawi [13]. Studies in Nigeria and Ethiopia reported higher prevalence of HBV-HIV co-infection at 9.5% [14], and 19% [2]. Respectively A study from Southern Ethiopia reported HBV infection prevalence of 0.6% among HIV-infected pregnant women [15]. It is notable that a higher HBV prevalence has been reported in the general population with studies in Kenya showing up to 8.8% prevalence with urban areas reporting ranges between 8 and 30% [1]. HBV prevalence among HIV negative pregnant women was reported at 5.6% [3]. This was also higher than the prevalence reported in this study. Variations in the prevalence of the HBsAg across studies reflect the demographic and possibly exposure differences within HIV-infected populations.

Vertical transmission of HBV infections has been confirmed in other studies to be influenced by hepatitis B 'e' antigen status (HBeAg) which is associated with high HBV DNA viral load [16]. In our study all samples positive for HBsAg were negative for HBeAg, revealing a possibility of low HBV viral loads in the samples, and thus also contributing to zero transmission. All mothers were on effective first line HAART for managing HIV and had undetectable levels of HIV RNA viral loads. Mothers who were HBV-infected were on Tenofovir (TDF) and Lamivudine (3TC) based regimen; which are also recommended by WHO for management of HBV infections [17]. The use of TDF and 3TC regimen in this population could have potentially caused the negative status of HBeAg and hence the possible suppression of HBV DNA viral load. This could have minimized the transmission of HBV to the exposed infants.

The present Kenyan prevention program regarding HBV consists of vaccination against the virus [18]. The primary hepatitis B immunization series conventionally consists of three doses of vaccine which include one mono-valent dose at 6 weeks followed by two mono-valets or combined vaccine doses at 10 and 14 weeks respectively [18]. All the HBV exposed infants had received at least one or all of the doses and this could have attributed to the zero transmission due to the passive immunity. In this study, mothers who had undergone dental surgery had a higher prevalence of HBV infection. This group was 3.3 times more likely to be HBsAg positive compared to the mothers who did not have history of dental surgery. Similar findings were reported in Ethiopia where pregnant mothers who had undergone dental procedure were 2 times as likely to be HBV- infected [19]. Though statistically not significant, mothers with a history of blood transfusion had a higher prevalence (7.7%) compared to 3.3% in those who had no transfusion. Significant associations have been reported in other studies where one study reported a higher proportion of HBV-infected patients having history of blood transfusion (6.6%) compared to 2.2% in those who were HBV negative [20]. Also, a study in Ethiopia found out that pregnant women who had a history of blood transfusion were 3.7 times more likely to be HBV-infected [2]. Body piercing and tattooing have been reported in studies to be significantly associated with HBV infection with participants having 3 and 5.7 times respectively the chance of HBV infection [2]. Our study did not show relationship between education and HBV infection. However, illiteracy has been shown to be associated HBV infection indicating lack of public health awareness/education amongst mothers [19]. Similarly, intravenous drug users (IDUs) were reported in a study to have a higher risk of HBV infection with HBsAg positivity of 9.6% in HIV-infected IDUs compared 3.6% in HIV-infected non-IDUs [9]. However, our study did not find any significant association between substance use and HBV infection. Due to the similar modes of transmission for HBV and HIV, the number of partners was also considered though it was not statistically significant in this study.

Conclusion

HBV infection was detected in mothers on HIV postnatal follow up. However there was no HBV transmission detected between mothers and their children. In this population of HIV- infected pregnant mothers, our observations suggest that the HAART regimen received by them may have prevented vertical transmission of HBV infections to exposed infants.

Study limitations

Quantitative HBV DNA viral load among the infected mothers was not determined and viral load status was only based on mothers HBeAg status due to lack of resources. Absence of vital information regarding which viral infection did the mother acquired first between HBV and HIV was also a limitation.

Recommendations

HBV screening should be implemented in our PMTCT programs on routine basis. HBV vaccination and immunization among HBV negative mother infant pair should be carried out. We also recommend further studies on occult HBV among HIV- infected mothers.

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Appendix IV: Patient Consent Seeking Form (English Version)

Prevalence and associated factors of Hepatitis B virus infections among Human Immunodeficiency Virus -infected Highly Active Antiretroviral Therapy receiving mothers and their exposed infants in Kenyatta National Hospital.

My name is James M. Kagethe. I am conducting a study entitled "Prevalence and associated factors of Hepatitis B virus infections among Human Immunodeficiency Virus -infected Highly Active Antiretroviral Therapy receiving mothers and their exposed infants in Kenyatta National Hospital''.

Purpose of study: This study intends to find out how many HIV-infected mothers and their exposed infants are infected with Hepatitis B virus. By assessing the mother and their infant together we would like to find out if the hepatitis infection of the child is from their mothers. The findings of this study will advise health programs on whether or not to incorporate HBV treatment among mothers who are currently being treated for HIV to prevent them from infecting their infants.

Procedure: Mothers who are currently receiving treatment for HIV to prevent them from infecting their infants who attend the PMTCT high risk clinic will be approached by their nurse or doctor and informed about the current study. Those consenting will then be recruited to participate in this study. Those who agree to participate in this study will be asked to give a single blood sample about 4 mls from veins in their arm. This will take about a minute and done using clean and safe items by a trained phlebotomist. This blood will be tested to find out if you are infected with hepatitis B virus. We will also test to find out if the virus from the mother is similar to that of the infant using more advanced methods. These tests will take about three months and we will inform you through your doctor of the outcome. Your doctor during your routine visit to the clinic will advice you on how to manage this diease if you are found to have it. We will also ask your nurse or doctor to give us a little information from your health records about your health status.

Privacy and confidentiality: We will use unique names to identify you and not by your real names

Risks: One potential risk of being in the study is the loss of privacy. However, we will do our best to make sure that the personal information gathered during this study is kept private. Also, you might feel a little discomfort at the time we will be taking your blood sample. The discomfort will last only for few minutes. The vein puncturing activity will be done by a well-qualified phlebotomist.

Benefits in participating: This study may enable the participant to know her HBV infection status and that of the infants. Also, the participant may know whether he/she is infected with virus strains untreatable by current drugs. These findings will enable the clinicians to provide you with better management. The mothers infected with hepatitis will be refered to the KNH medical out patient clinic (MOPC) for further management and followup whereas the infants will be linked to pediatrics clinic. You will however, receive no monetary incetive for your participation. Your decision whether or not to participate in this study will not affect your current enrollment in the PMTCT program.

Time involvement: This study will be part of your routine visit to PMTCT clinic and the general time involved in the clinic will not be extended.

Data storage: Data generated will be securely stored and accessible to the authorized personel only. The biological samples will be destroyed three years after the study.

Sample shipment and storage: The collected samples will be transported to KEMRI; Hepatitis Laboratory for analysis.

Subject's rights: If you have read this form and have decided to participate in this project, please understand your participation is voluntary and you have the right to withdraw your consent or discontinue participating at any time without penalty. You have the right to refuse to answer particular questions. Your individual privacy will be maintained in all published and written data resulting from the study.

Contact information

If you have questions about your rights as a study participant, or are dissatisfied at any time with any aspect of this study, you may contact - anonymously, if you wish

1. The secretary, KEMRI Ethical Review Committee, PO Box 54840 – 00200 Nairobi, Kenya; Tel: 020-2722541, 0722205901, 0733400003; Email address: ercadmin@kemri.org

AND/ OR

2. The secretary, KNH/UON- ERC, P.O.BOX 20723-00202, Tel 020-726300-9; Email: uonknh_erc@uonbi.ac.ke

I have read this form or had it read to me in a language that I understand. I have discussed the information with study staff. My questions have been answered. My decision whether or not to take part in the study is voluntary. If I decide to join the study I may withdraw at any time. By signing this form I do not give up any rights that I have as a research participant. I also sign to allow my samples to be stored at KEMRI for the duration of the study.

_____	_____	
Participant Name	Participant Signature/ Thumb print	Date
_____	_____	
Study Staff Conducting	Study Staff Signature	Date
……	…….	
Witness Name	Witness Signature/ Thumb print	Date

Appendix V: Parental Assent form for Infants Participation

Prevalence and associated factors of Hepatitis B virus infections among Human Immunodeficiency Virus -infected Highly Active Antiretroviral Therapy receiving mothers and their exposed infants in Kenyatta National Hospital

Description: Your infant aged below 18 years is being invited to participate in a research study to find out how many HIV-infected mothers and their infants are infected with Hepatitis B virus. By assessing the mother and their infant together we would like to find out if the hepatis B infection of the child is from their mothers.

Procedure: If you consent to have your infant participate, will ask you to allow a trained phlebotomist to obtain a single blood sample about 4 mls from veins of your infant. This blood will be tested to find out if your child is also infected with hepatitis B virus. We will also test to find out if the hepatitis B virus from the mother is similar to that of the infant using more advanced methods. These tests will take about three months and we will inform you through your doctor of the outcome. Your doctor during your routine visit to the clinic will advice you on how to manage this diease if you are found to have it.

Privacy and confidentiality. We will use unique names to identify your infant and not by their real names.

Risks: One potential risk of being in the study is the loss of privacy. However, we will do our best to make sure that the personal information gathered during this study is kept private. Also, your child might feel a little discomfort at the time we will be taking its blood sample. The discomfort will last only for few minutes. The vein puncturing activity will be done by a well-qualified phlebotomist.

Benefits in participating: This study will enable us find out if your child is also infected with hepatitis B virus. We will also find out if your child is infected with hepatitis B virus strains untreatable by current drugs. These findings will enable the clinicians to

provide your child with better management options. We will refer your child to the pediatrics clinic for treatment. You will however, receive no monetary incetive for your participation. Your decision whether or not to allow your child participate in this study will not affect their current enrollment in the PMTCT program.

Time involvement: This study will be part of your routine visit to PMTCT clinic and the general time involved in the clinic will not be extended.

Data storage: Data generated will be securely stored and accessible to the authorized personel only. The biological samples will be destroyed three years after the study.

Sample shipment storage: The collected samples will be transported to KEMRI; Hepatitis Laboratory for analysis and storage.

Subject's rights: If you have read this form and have decided to participate in this project, please understand you allowing your child to participat is voluntary and you have the right to withdraw your assent or discontinue child's participation at any time without penalty. You have the right to refuse to answer particular questions. Your individual privacy will be maintained in all published and written data resulting from the study.

Contact information

If you have questions about your rights as a study participant or are dissatisfied at any time with any aspect of this study, you may contact - anonymously, if you wish – The secretary, KEMRI Ethical Review Committee, PO Box 54840 – 00200 Nairobi, Kenya; Tel: 020-2722541, 0722205901, 0733400003; Email address: ercadmin@kemri.org.

I have read this form or had it read to me in a language that I understand. I have discussed the information with study staff. My questions have been answered. My decision whether or not to allow my child to take part in the study is voluntary. If I

decide to join the study I may withdraw at any time. By signing this form I do not give up any rights that I have as a research participant.

Signature

I _____ (Being the parent) of the infant _____I have read/understood the contents in this form. My questions have been answered. I agree to allow my child to participate in this study. I also sign to allow the samples of my child to be stored at KEMRI for the duration of the study.

_____	_____	_____
Participant Name	Participant Signature/ Thumb print	Date
_____	_____	_____
Study Staff Conducting	Study Staff Signature	Date
……	……..	……
Witness Name	Witness Signature/ Thumb print	Date

Appendix XI: Questionnaire

Prevalence and associated factors of Hepatitis B virus infections among Human Immunodeficiency Virus -infected Highly Active Antiretroviral Therapy receiving mothers and their exposed infants in Kenyatta National Hospital

1. Patient identification number _____

2. Date of visit (dd/mm/yr) ___/___/___

3. Gender Male ☐ Female ☐

4. Age ____ years

5. What is your marital status?

1. Single (never married) 2. Married 3. Divorced/Separated 4. Widowed 5. Other (specify)

6. Religion a. Christian b. Muslim c. Hindu

7. Education level of the respondent

a. None b. Primary c. Secondary d. Tertiary

8. Occupation of the respondent

a. Employed b. Business c. Unemployed d. student

9. Approximately how much do you earn per month? _____(Ksh)

10. Do you drink alcohol?

a. Yes b. No

11. If yes for how long have you been consuming alcohol? _____

12. Have you ever smoked cigarettes? _____

13. Do you currently smoke cigarettes? _____

14. How long have you been smoking cigarettes? _____

15. Have you ever used hard drugs (bhang, cocaine, etc)? _____

16. How long have you used these drugs? _____

17. How old were you when you first had sexual intercourse? _____

18. Have you ever used condoms? _____

19. Have you ever been blood transfused? _____

20. Tattoo are fashioned used by many, do you have any tattoo in your body? _____

21. If yes, when did you receive the tattoo? _____

22. Where did you receive the tattoo? _____

23. Have you heard of hepatitis B? _____

24. Have you ever received vaccination for hepatitis B? _____

25. If yes in 5 above, when

a. Within the last 5 years

b. > 5-10 years ago

c. In childhood

d. Don't know

26. Have you been advised by your doctor to take a hepatitis test? _____

27. How about a member of your household? _____

28. What is the age of your child (Months)? _____

29. What is the gender of your child? _____

30. How do you feed your infant?

a. Breastfeenig

b. Non breastfeeding

31. When were you diagonized with HIV? _____

32. Are you currently taking ARVs

a. Yes

b. No

33. What ARV drugs are you currently taking? _____

34. When was the last time you took *current ARVs*? _____

35. Did you ever miss taking current ARVs for a whole day or more? _____

36. How many different days did you miss taking current ARVs? _____

37. Given that you are taking ARVs which regimen among the following are you taking?

a. Single dose nevirapine

b. b. AZT only

c. HAART

38. Have you ever been involved in itravenous drug use?

a. yes

b. No

39. Have you ever done a dental surgery procedure?

a. Yes

b. No

40. Have you ever done hemo dialysis?

a. Yes

b. No

41. How many sexual partners do you have? ……