SUSTAINABILITY OF IMMUNE RESPONSE TO HEPATITIS B VIRUS VACCINATION THREE YEARS AFTER VACCINATION AMONG HIV-1 INFECTED AND UNINFECTED ADULTS IN KENYA

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Sustainability of Immune Response to Hepatitis B Virus Vaccination Three Years after Vaccination among HIV-1 Infected and Uninfected Adults in Kenya

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A thesis submitted in partial fulfillment of the Requirements for the degree of Master of Science in Medical Virology of the Jomo Kenyatta University of Agriculture and Technology

2021

DECLARATION

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

Signature...…… …… Date…….

Rose Wanjiku Kamoni

This thesis has been presented for examination with our approval as University supervisors

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DEDICATION

I would like to dedicate this project to my dear parents' Mr. and Mrs. Kamoni, my siblings Grace Kamoni, Kennedy Kamoni, and James Warui, for all the support that they have extended to me regarding my education journey. Also Similarly, I would like to express my special dedication to all my supervisors' who gave me guidance and support and allowed me to learn from them.

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I thank the Almighty God for the gift of life and health; it is by His grace that I have been able to not only carry out this project, but also to surmount the challenges I encountered in my education.

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ACRONYMS AND ABBREVIATIONS

ALT	Alanine Transferase
Anti-Hbs	Anti–Hepatitis B surface Antibody
CTRL	Clinical Trial Research laboratory
DNA	Deoxyrebonucleic acid
ELISA	Enzyme-linked immunosorbent assay
EQA	External Quality Assurance
ERC	Ethical Research Committee
HBcAg	Hepatitis B core antigen
HBeAg	Hepatitis B e Antigen
HBsAg	Hepatitis B surface Antigen
HBV	Hepatitis B Virus
HCC	Hepato Cellular Carcinoma
HIV 1 +	Human Immunodeficiency Virus positive
HIV 1-	Human Immunodeficiency Virus Negative
HIV-1	Human Immunodeficiency Virus type
Iu/ml	International unit per millilitre
IgG	Immunoglobulin G
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KNH	Kenyatta National Hospital

OD	Optical Density
PHRD	Partners in Health and Research Development (PHRD) Thika
PrEP	Pre-exposure prophylaxis
UON	University of Nairobi
WHO	World Health Organization

ABSTRACT

Hepatitis B virus (HBV) infection, a leading cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma, worldwide is preventable by vaccination. Following the completion of the recommended vaccination series over 90% of adults will develop protective immune levels of anti-HBS antibodies. However, there is a paucity of data on sustained protective immune levels of Anti-HBS antibodies, among HIV-infected African adults. This study aimed at assessing if there is an immune response sustained to hepatitis B virus vaccination three years after vaccination among HIV-1 infected and uninfected adults in Kenya. A retrospective study was carried out to analyse archived serum samples. The study was conducted at the Partners PrEP Study clinic in Thika which was among sites for a phase III, multi-site, randomized, double-blind, placebo controlled trial of daily oral tenofovir-based pre-exposure prophylaxis (PrEP) for prevention of HIV-1 acquisition. The samples were randomly selected and participant's demographic information was retrieved from case referral forms that were been filled every time participant's visited the clinic. A total of 336 serum samples retrieved were measured for Hepatitis B surface antibody (anti-HBs) titers using ELISA kit Murex DiaSorin LIAISON anti-HBs II assay (DiaSorin, Saluggia, Italy). Those serum samples that did not have protective anti-HBs titers were further tested for Hepatitis B surface antigen (HBsAg), a marker of infection with HBV DiaSorin Murex HBsAg Version 3 assay kit (DiaSorin, S.p.A. UK). Univariate logistic regression to determine factors associated with non-response to HBV vaccination was used. Of the 336 participants serum samples tested, 176 (52.4%) were from HIV-1 infected individuals, of whom 40 (22.7%) were male. 160 samples from HIV-1 uninfected individuals of whom 125 (78.1%) were male. The mean (standard deviation) age of the study population was 34.6 (8.5) years. Of the 62 (18%) individuals who did not have protective anti-HBs titers three years post- vaccination, 50 (81%) were HIV-1 infected. HIV infected persons were more likely to have less protective anti-HBs titers ($p<0.001$) compared to HIV uninfected persons. In addition, compared to men, women were more likely not to have protective anti-HBs levels (11.5% vs. 25.1%, $p=0.002$). Seven (11.3%) of the 62 samples that did not have a positive antibody response for anti-HBs also tested positive for HBV surface antigen (HBsAg), all of whom were HIV-1 positive individuals. In conclusion, more than a quarter of HIV-infected individuals vaccinated against HBV did not have protective anti-HBs titers three years post-vaccination, some of whom acquired HBV infection. Regular testing for the immune response to HBV vaccination among HIV infected persons should be considered and reviewed regularly, and those with waning antibodies be offered booster doses. Additional research is needed to evaluate the impact of HBV booster doses in this population. In addition, those persons infected with HIV are at a higher risk of being infected with HBV and efforts should be made to vaccinate them.

CHAPTER ONE
INTRODUCTION

1.1 Background information

Hepatitis B virus (HBV) infection is a leading cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma worldwide (Sun *et al.*, 2014), is more common among HIV–infected individuals due to shared risk factors for viral acquisition (Chun *et al.*, 2012). Current statistics estimate that 10% of 34 million HIV-infected patients have concurrent chronic HBV infection (Kourtis *et al.*, 2012). Different studies have shown that prevalence or incidence of HBV infection among HIV-infected patients vary widely based on the risks for HIV and HBV transmission, implementation of HBV vaccination programs, and the geographic regions with different levels of endemicity of HBV infection in the general population (Sun *et al.*, 2014; Whitaker *et al.*, 2012). Even so, Africa which has the highest HIV-1 disease burden globally has high endemicity for HBV infection with 50 million of the 360 million people infected with HBV worldwide living in Africa (Irungu *et al.*, 2012). According to the World Health Organization (WHO, 2014), the world can be divided into areas based on the levels of endemicity of HBV infection that are defined by the prevalence of chronic HBV infection: low endemicity of less than 2%; intermediate endemicity of 2%-8%; and high endemicity, of more than 8%. High rates of 25% have also been reported in countries where the virus is highly endemic WHO, 2014 . Countries in Asia and sub-Saharan Africa are among those with high HBV endemicity with vertical and early childhood exposure as the most common modes of transmission respectively; similarly, the prevalence of HBV among HIV-infected individuals in these areas is higher at an estimated 20-30% (Uneke *et al*., 2005). In the US, a study reported an incidence of acute HBV infection of 12.2 cases per 1000 person-years in a large HIV cohort and a chronic HBV prevalence of 7.6% (Chun *et al.*, 2010). Studies have reported that men infected with both HIV and HBV have liver-related mortality that is eight times higher than that in men with HIV alone and 17 times higher than in those with HBV alone (Whitaker *et al.*, 2012). This increases the risk of chronic infection, because HIV infection adversely affects all phases of hepatitis

B infection, due to this the rate of hepatitis B antigen clearance decreases and thus increases the risk for cirrhosis and hepatocellular carcinoma due to acceleration loss of hepatitis B surface antibody which causes an increase of virus replication (Thio, 2009b).

Vaccination is the best method of prevention against HBV and following complete immunization more than 95% of infants and children and more than 90% of adults achieve protective anti-HBS titers greater than 10 IU/mL (Bailey *et al.*, 2008). International guideline in the United States and the British HIV Association in Europe (EACS, 2012; Geretti *et al.*, 2008) recommends vaccination with the 3 doses primary vaccine series given during 6 months period to all individuals with HIV who are susceptible to HBV (Whitaker *et al.*, 2012). Studies done in high-income countries on HBV vaccination have suggested that compared to HIV-uninfected persons, HIV-infected persons have diminished responses to HBV vaccination. One study found that 20%–70% of HIV-infected persons developed an immune response to HBV vaccination (Kim *et al.*, 2008). A US-based HIV outpatient study reported that only 37% of eligible HIV-infected patients who completed the vaccination series achieved protective antibody titers (Tedaldi *et al.*, 2004). A study conducted among HIV-infected adults in Kenya found that nonresponse to HBV vaccine at one year post vaccination was higher among HIV- infected participants, compared to HIV-uninfected participants, and revaccination of initial non-responders resulted in a higher overall response to revaccination (Irungu *et al.*, 2012).

Data from low–income countries for determination of long-term immune protection of HBV Vaccine in adults are few. This study aimed to compare the levels of HBV antibody titers in both HIV infected and HIV uninfected adults in Kenya, 36-month post-HBV vaccination and also determine incident Hepatitis B infection, among individuals with low levels of protective antibodies post-vaccination. This information could be useful in designing HBV vaccination schedules and for developing public health policy with respect to the rationale for and timing of booster vaccinations, especially among the immunosuppressed adult population.

1.2 Statement Problem

Research has shown that vaccines are the greatest public health intervention in combating diseases. Even so, vaccination of HIV infected and other immunosuppressed people is done with caution requiring more studies to determine the level of response

Studies done in Kenya have not looked at the sustained immune response to HBV among HIV-1 infected persons one-year post- vaccination. A study done by Irungu *et al.* (2012), explored HBV vaccine immune responses one-year post-vaccination in HIV-1 infected persons, however, the study did not look at the sustained immune response to HBV among HIV-1 infected persons beyond one-year post- vaccination.

This study sought to examine the sustained immune response to the HBV vaccination 3 years post-vaccination in a subset of individuals who had immune responses one-year post-vaccination.

1.3 Justification

The third objective of the united nations sustainable development is to ensure healthy lives and promote wellbeing for all at all ages with objective 3b partly aiming to support the research and development of vaccines and medicines for communicable and non-communicable diseases that primarily affect developing countries while providing access to affordable essential medicines and vaccines for all. On the other hand, among the visions of the Kenyan government through the social pillar is to shift the bias of the national health bill from curative to preventive care with special attention being paid to lowering incidences of HIV/AIDS among other diseases. One of the ways to achieve these two objectives is to ensure that vaccines given to HIV infected persons are effective in preventing the diseases they are made against.

Irrespective of the fact that the majority of healthy persons respond positively to vaccines against hepatitis B (>100 IU/mL), there is a small proportion of persons whose response to HBV vaccine is poor or none with the titers of anti-HBs mounting to 10–100

IU/mL and <10 IU/mL, respectively. A person with anti–HBs titers below 10 IU/mL, following the vaccination series is defined as unresponsive. Consequently, the protection of such persons against infection may be contested (Madaliński *et al.*, 2015). Predictors of poor or nonresponse to HBV vaccination include low CD4+ T-cell count, male gender, high viral load, smoking, alcohol use among others (Irungu *et al.*, 2012).

Few studies have been conducted in sub-Sahara Africa on HBV vaccine response in HIV-1 negative and positive adults; hence, there is a paucity of information on the efficacy of the vaccines and the level of HBsAb response these persons have three years post- vaccination. It is for this reason that this study was conducted with the aims of determining the immune response to HBV vaccination three years after HBV vaccination and comparison of responses in HIV-1 infected and uninfected adults. Determination of the HBsAb response after vaccination of HIV-1-infected adults was aimed to provide information that would assist clinicians in the timely revaccination of non-responders with HBV vaccine hence significantly increasing the development of protective antibody titers.

1.2 Problem statement

Research has shown that vaccines are the greatest public health intervention in combating diseases. Even so, the HIV is known for its destruction of immune cells responsible for the protection of the body. As a result of this, vaccination of HIV infected persons and other immunosuppressed people is done with caution requiring more studies to determine the level of immune response and safety of the patients' post-vaccination. Due to limited studies, there is a paucity of data from low-income countries on whether HIV-1 infected persons have diminished responses to HBV vaccination, compared with HIV-1-uninfected persons, and for how long the vaccine gives protection in HIV-1 infected persons.

A study done by Irungu *et al.* (2012), explored HBV vaccine immune responses one-year post-vaccination in HIV-1 infected persons, however, the study did not look at the

sustained immune response to HBV among HIV-1 infected persons one-year post-vaccination. This study sought to answer this question by further examining the sustained immune response to the HBV vaccination 3 years post-vaccination in a subset of individuals who had immune responses one-year post-vaccination.

1.3 Justification

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determining the immune response to HBV vaccination three years after HBV vaccination and comparison of responses in HIV-1 infected and uninfected adults. Determination of the HBsAb response after vaccination of HIV-1-infected adults was hoped to provide information that would assist clinicians in the timely revaccination of non-responders with HBV vaccine hence significantly increasing the development of protective antibody titers.

1.4 Research questions

1. What is sustained immune response levels years post HBV vaccination among HIV-1 infected and HIV-1 uninfected adults in Kenya?
2. What factors are associated with a sustained immune response to HBV vaccination in HIV-infected and uninfected adults?
3. What were the incident positive HBsAg results among the HIV-1 infected and HIV-1 uninfected adults who received the HBV vaccine?

1.5 Objectives

1.5.1 Broad objective

To determine immune response to HBV vaccination three years after HBV vaccination three years after HBV vaccination among HIV-1 infected and uninfected adults in Kenya

1.5.2 Specific objectives

1. To determine the proportion of Hepatitis B virus (HBV) antibody response 36 months post-HBV vaccination among HIV-1 infected and HIV-1 uninfected adults in Kenya.

2. To determine the predictors of low HBV antibodies titers among HIV-1 infected and uninfected persons, 36-month post-vaccination with HBV vaccine.
3. To determine the incidence of HBV infections among HIV-1 positive and negative persons, 36-month post-vaccination with HBV vaccine.

1.6 Significance of the study

Findings will assist the health facility to help them advice those that are HIV infected persons who receive HBV vaccination should have their antibody responses reviewed regularly and those with waning antibodies be offered booster doses. In addition, those persons infected with HIV are at a higher risk of being infected with HBV and efforts should be made to vaccinate them.

CHAPTER TWO
LITERATURE REVIEW

2.1 Hepatitis B Virus

Hepatitis B Virus is a member of the *Hepadnaviridae* family of viruses and resembles retroviruses (Liang *et al.*, 2009) of DNA viruses. The virus particle consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. Hepatitis B surface antigen (HBsAg), a key indicator for notification of acute HBV infection is expressed on the surface of the virus particle (Hollinger *et al.*, 2001). The virus is hepatotoxic with a narrow host range and only naturally infects humans. Infection with this virus causes inflammation of the liver, even so, other causes of hepatitis are hepatitis A, C, D, and E viruses (Hollinger *et al.*, 2001).

2.2 Virology of HBV

Hepatitis B virus is a double-stranded DNA virus in the *Hepadnaviridae* family (CDC, 2017). The morphology of the hepatitis B virus; it's a dane particle 42 nm in diameter, which contains DNA genome, DNA-dependent DNA polymerase involved in replication, and has hepatitis B core antigen and hepatitis B e antigen. The genome is circular dsDNA 3.2 kb in size, DNA is double-stranded but one strand (short) has a gap about 700 nucleotides in length, the long strand has a nick near the 5` end. The virus replicates by attaching to a hepatocyte using the virion S protein and enters by endocytosis, virus nucleocapsid moves to the nucleus where transcription takes place, the minus strand is transcribed to give mRNAs plus a 3.4kb RNA transcript called the pregenome. The pregenome and the shorter mRNAs move to the cytoplasm and are translated. Replication of the genome is unique and entails a reverse transcription of DNA from an RNA. RNA is digested away and a plus DNA strand is transcribed, the newly synthesized virus cores congregate in the cytoplasm, associate with viral DNA, and bud through the endoplasmic reticulum at areas already containing S antigens. The new enveloped viruses emerge without cell lysis (Liang *et al.*, 2009). Main antigens:

HBsAg, HBcAg, and HBeAg each induce corresponding antibodies, except for HBcAg, all these antigens and antibodies, together with viral DNA polymerase, can be detected in the blood at various times after infection and are referred to as markers because their presence or absence in an individual patient marks the course of the disease and also gives a good idea of the degree of infectivity for others. HBcAg is detectable in hepatocyte (Liang *et al.,* 2009). Group-specific antigen associated with various combinations of subtype determinants d, y, w, and r, these combinations are grouped into six or more genotypes which have no clinical significance but are useful epidemiologically since their geographical distribution differ (Hollinger *et al.*, 2001; Hatton *et al.*, 1992). In combination with HBV DNA sequencing, they may also help decide whether a particular carrier is the source of infection of another person. The finding of identical subtypes would of course not confirm the possibility but differing subtypes would rule it out (Milich *et al.*, 2003; Zhang *et al.*, 2001; Liang, 2009).

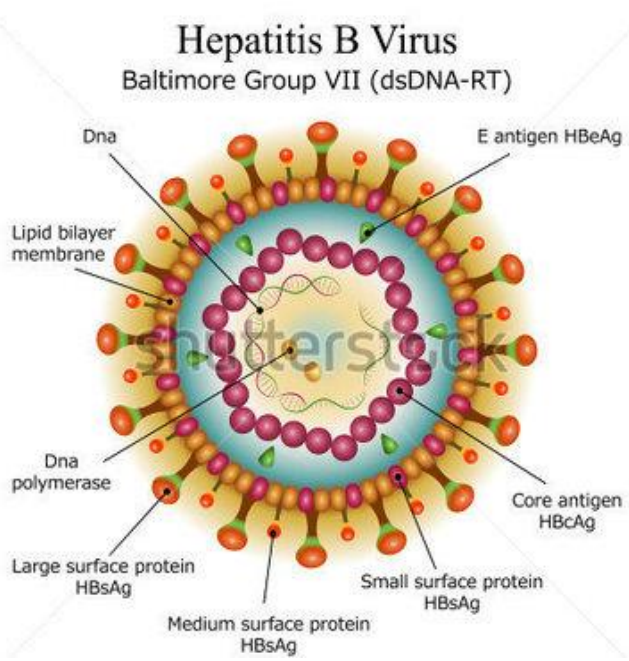

Figure 2.1: Schematic diagram of the hepatitis B virus (Strewblow *et al*., 2012)

2.3 Immunopathogenesis of HBV

Following HBV infection, there is initial hepatitis that is either symptomatic or non-symptomatic. Successful clearance and resolution of infection depend on the age and immune status of the individual with most infections of immunocompetent adults being self-limiting. Persistent or chronic infection is more likely to occur following vertical transmission (from mother to child) or horizontal transmission to children or immunocompromised adults (Starkman *et al.*, 2003). The immune determinants of successful clearance of HBV are not fully understood but both cellular and humoral immune responses are important (Rehermann *et al.*, 1995; Webster *et al.*, 2000). At the same time, however, liver inflammation and disease are also believed to be largely immune-mediated. Therefore, a complex interaction exists between HBV and the host in the initial clearance of HBV, the long-term persistence of HBV, and the pathogenesis of HBV liver disease.

2.4 Immunogenicity of HBV

Although the appearance of anti-HBs indicates recovery, it plays a little part if any in that process which is primarily affected by cytotoxic (CD8+) T cells. Hepatocytes are not well endowed with class 1 histocompatibility antigen but HBV infection stimulates the production of α-interferon which in turn increases the display of class 1 antigen on the liver cells and thus permits their lysis by the cytotoxic lymphocytes (Davis *et al.*, 1998). As with many other infections, the CMI response may enhance as well as cure the illness depending on the balance of forces involved. Immunopathological damage is a major component of the response to HBV and is diminished in those with defective immunity (Davis *et al.*, 1998).

2.5 HBV and HIV coinfection

Persons with HIV infection are at increased risk of co-infection with HBV as the routes of transmission are shared. Evidence suggests that HIV and HBV virus co-infection

poses important public health considerations in resource-limited settings (Thio, 2009a). In more resource-constrained settings liver transplantation is not an option, only a handful of the middle-income countries perform liver transplant (Chun *et al.*, 2012; Gilson *et al.*, 1997), but the procedure is not yet being performed in people with HIV in those countries, although it is under consideration in Brazil (Castellar *et al.*, 2007). HBV and HIV coinfection is common, with 6%–10% of HIV-positive individuals being co-infected with these viruses because of the common routes of transmission (Puoti *et al.*, 2002).

2.6 HBV Manifestation

Chronic HBV infection generally consists of two phases: an early replicative phase with active liver disease and a late/low replicative phase with remission of liver disease. There is an additional immune tolerance phase in which virus replication is not accompanied by active liver disease in patients who acquire HBV infection perinatally (Torok, 2012), although the exact mechanisms by which this occurs remain unclear. The transition from the immune tolerant to the immune clearance phase occurs during the 2nd and 3rd decades in most patients (Liang, 2009). During this phase, spontaneous HBeAg clearance increases to a rate of 10–20% yearly. Seroconversion of HBeAg is frequently, but not always, accompanied by increases in serum ALT. Exacerbations are due to the elevation of HBV DNA serum and a shift of HBcAg from nuclear to cytoplasmic sites within hepatocytes. In a minority, death occurs due to hepatic failure. There is significant damage to the liver parenchyma and raised transaminases on these patients are liable to repeated episodes of hepatitis (Lok *et al.*, 2009), are at risk of developing cirrhosis and hepatocellular carcinoma (Bisceglie, 2009). The initial phase of chronic HBV infection consists of virus replication (with HBeAg positivity and high serum HBV DNA levels) and active liver disease (elevated serum ALT and chronic hepatitis on liver biopsy). The prevalence of HBeAg among non-Asian adults with chronic HBV infection is lower than that seen in Asian adults, but the rate of spontaneous HBeAg clearance appears to be similar at 10–20% per year in geographical distribution.

2.7 Epidemiology of Hepatitis B Virus

HBV infects more than 350 million people worldwide with, an estimated 240 million persons being chronically infected which is equivalent to 5% of the world's population (Ly *et al.*, 2016). HIV infection significantly impacts the course of HBV infection, leading to accelerated liver disease and increased mortality up to eightfold compared to those infected by HIV-1 only. Adult vaccination of HIV-infected persons where HBV exposure uniformly occurs before adulthood may not be needed (Thio, 2009a). In a recent review of the global epidemiology of HBV infection (WHO, 2014) the prevalence of HBV infection is decreasing, particularly evident in central sub-Saharan Africa, tropical and central Latin America, Southeast Asia, and central Europe. Expanded programs of immunization against HBV have been proposed to significantly contribute to such an observation (WHO, 2012). In areas that implemented universal neonatal HBV vaccination program such as Taiwan and Alaska, the incidence of acute HBV infection prevalence of chronic HBV infection, and incidence of HCC in children have significantly declined (Thio *et al.*, 2002), so has mortality due to chronic liver disease as well as HCC in persons aged 5-29 years.

The world is divided into sections based on the levels of endemicity of HBV infection (WHO, 2014), that is defined by the prevalence of chronic HBV infection, these are low endemicity, < 2%; intermediate endemicity, 2%-8%; and high endemicity, >8%, with rates being as high as 25% in countries where the virus is highly endemic. African countries, including Kenya, have a prevalence of chronic hepatitis B infection (CHBI) in the higher intermediate (5–7%). In areas of high endemicity of chronic HBV infection, the transmission of HBV mainly occurs through perinatal transmission (predominantly in East and Southeast Asia) or in young children through close household contact or medical or traditional scarification procedures (predominantly in Africa) (WHO, 2012).

In high endemicity regions like Asia and Africa, most HBV infections have been found to occur within the first 5 years of life through perinatal and horizontal transmission. It is estimated that due to HBV-related chronic liver disease approximately 25% of infected

infants will die in their adulthood age (Thio *et al.*, 2002; Zimmerman *et al.*, 1997). Due to this, it is important to test pregnant mothers and adequate treatment be administered to avoid such occurrence.

2.8 Prevention of Hepatitis B Virus

Although the modes of transmission of HBV are the same as those for HIV, HBV is transmitted more efficiently than HIV(Alter, 2006). Other than the adoption of safe sex practices and avoidance of sharing needles and diluents, HBV vaccination remains the most effective approach to prevent HBV infection and its chronic consequences. According to the HIV treatment guidelines by the US Department of Health and Human Services (OARAC, 2013), pre-vaccination screening should include HBsAg, anti-HBs antibody (anti-HBs), and anti-HBc. Serological markers may be time-dependent variables in HIV-infected patients, which are associated with host immunity and viral activities; and, therefore, periodic measurements are recommended (Sun *et al.*, 2014). The presence of anti-HBs at levels of > 10 international units/L (IU/L) is consistent with seroprotection and at levels of > 100 IU/L is associated with long-term protection (Pasricha *et al.*, 2006). Anti-HBs antibody titers decrease over time and can decrease below protective concentrations. Hepatitis B vaccine was introduced in the early 1980s. The World Health Organization (WHO) recommended that all countries introduce a policy of universal hepatitis B vaccination to prevent and control HBV infection, that is, in 1991 and by the end of 2008, the hepatitis B vaccine for infants was introduced in 177 countries nationwide. As of now, the hepatitis B vaccine covers globally at an estimate of 69% (WHO, 2014).

In HIV-infected patients, those with CD4 cell counts ≥ 350 cells/μL had a higher seroconversion rate (anti- HBs ≥ 10 IU/L) compared to CD4 cell counts < 350 cells/μL (39.3% *vs.* 26.3%) (Wai *et al.*, 2002). Failure of anti-HBs seroconversion and lower anti-HBs titers after HBV vaccination in HIV-infected patients is reported to be associated with detectable plasma HIV RNA, lower CD4 cell counts (Sun *et al.*, 2014), age, HCV coinfection, occult HBV infection, alcohol abuse, and the general health status of the

host (Shire *et al.*, 2004). A favorable response to ART may improve serological response. Based on these data, early vaccination is recommended in HIV-infected patients before CD4 cell counts decline. These also strengthen the arguments for universal HBV vaccination of individuals at risk for HIV infection before they become HIV-infected and their immunosuppression worsens (Sun *et al.*, 2014).

In Kenya, hepatitis B vaccination was incorporated into the expanded program of immunization in the year 2002 (Ly *et al.*, 2016). The vaccine has a protection rate of approximately 95% in immunocompetent children and adolescents and 90% in adults (Assad *et al.*, 1999). Unfortunately by the time vaccine was introduced worldwide in the 1980's, those who were born then were not immunized and thus stood the danger of being chronic carriers and some later developed the chronic liver disease and hepatocellular carcinoma (MOH, 2014).

The first licensed hepatitis B vaccines were plasma-derived and composed of purified HBsAg; most currently available hepatitis B vaccines are produced by recombinant DNA technology. After vaccination antibody levels ranging between 10 and 100 mIU/ml is termed below protective level, recommendation is that the individual receives a single booster vaccination at this time, with no need for retesting (JCVI, 2007). At least one study showed that hepatitis B vaccination is less effective in patients with HIV (Pasricha *et al.*, 2006). According to the most recent World Health Organization estimate, two billion people worldwide have serologic evidence of past or present HBV infection, and 360 million are chronically infected and at risk for HBV-related liver disease. Approximately one-third of all cases of cirrhosis and half of all cases of hepatocellular carcinoma can be attributed to chronic HBV infection. HBV is estimated to be responsible for 500,000–700,000 deaths each year (WHO, 2012). The Knowledge gap to be filled is the duration of HBV antibody immune response waning.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study site

This study was conducted at the Partners PrEP Study at Partners in Health, Research Development, Thika which was among sites for a phase III, multi-site, randomized, double-blind, placebo-controlled trial of daily oral tenofovir-based pre-exposure prophylaxis (PrEP) for the prevention of HIV-1 acquisition (Baeten *et al.*, 2014).

3.2 Study design

This was a retrospective study conducted on archived serum samples collected from a prospective cohort of HIV-1 serodiscordant couple study in the Partners PrEP study (Baeten *et al.*, 2014).

3.3 Study population

The Partners PrEP study enrolled heterosexual HIV-1 serodiscordant couples. At the study entry, the HIV–1 seropositive were not receiving antiretroviral therapy and did not meet Kenyan guidelines for antiretroviral therapy initiation.

The HIV seronegative participants were not infected with the hepatitis B virus. At baseline, all participants were tested for HBV. Those testing negative were vaccinated and immune response to vaccination was determined six months after 3rd dose post-vaccination. For those not responding, revaccination was done and response to revaccination was determined. Archived serum samples from consenting participants of the Partners PrEP study were used in this study.

3.3.1 Inclusion criteria

- Participants in the parent study who had provided written informed consent allowing their samples to be used in future research.

- Participants who had a documented immune response to HBV vaccine at 12 months post-vaccination.

3.3.2 Exclusion criteria

- Participants who did not have archived serum samples collected 36 months post-HBV vaccination.
- Participants who were not included in the parent study,meaning they were not vaccinated

3.4 Study sample size

The sample size that was used in this calculation is the Fleiss formula (Fleiss, 1981)

$$n = \left(\frac{r+1}{r}\right)\frac{\sigma^2(Z_\beta + Z_{\alpha/2})^2}{(\mu_1 - \mu_2)^2}$$

Power = for 80% power, Z_β =.84

Alpha = 0.05 significance level, Z_α =1.96

Ratio = r=1 (equal number of cases and controls)

SD = σ=12.0

Effect size = Difference in means ($\mu_1 - \mu_2$) = 5.0

$$n = (2)\frac{12^2(7.84)}{(5)^2} = (2)(45.16) = 90.32$$

Hence n = 180 (90 HIV+ and 90 HIV-)

Thus, for this study, a total of 180 participant's samples were to be used. However,to increase the power of the study, all participants from the parent study who had archived samples and had consented to their samples being used in research had their samples included in the study. All participants included in this analysis, had sample collected 36 months after the HBV vaccination series started. In total 336 samples were used; 176 HIV-1 positive participants and 160 HIV-1 negative participants.

3.6 Sampling method and data collection

3.6.1 Sample retrieval from the archived system in Clinical Trial Research Lab(CTRL)

Simple random sampling was used to retrieve archived serum samples from CTRL laboratory where the samples had been archived at -80^0c which employs the laboratory information management system freezerworks in managing samples archiving in its freezers. The laboratory is situated at Kenyatta National Hospital at the University of Nairobi, Obstetrics, and Gynecology Department. Participant samples were identified using the Participant Identification number and the visit number of the participant in Freezerworks program. This information was extracted and recorded in an excel file for sample retrieval.

The retrieved serum samples were thawed at room temperature for 30 minutes; Aliquots of approximately 200 μL were placed in a separately labeled cryovial. The remaining sample was marked by a dot on the lid of the serum vial (using a VWR marker) to indicate the sample has been thawed once. A comment was also added in the Freezerworks comment column of each of the sample that had been retrieved to indicate it had been thawed on a specific date. The main sample was returned to its original location in the cryobox and returned to the -80°C freezer using the retrieval list as a reference.

3.6.2 Collection of social demographics information

Participant's social demographic information was retrieved from case referral forms that had been filled every time participant's visited the clinic. Participant socio-demographics that were of interest to this study included age, BMI, number of children, years of school, monthly income, weekly drinking, and CD4 count. Data on relevant participants was retrieved from the storage computer at the Thika clinic in excel files by using participant's identification number to match the case referral forms. The retrieved information was saved in an excel sheet. excel file and imported into SPSS version 23 where it was cleaned and coded for ease of analysis.

3.7 Laboratory Test procedures

The archived serum samples that were retrieved were removed from the -80°C freezer and allowed to thaw. Hepatitis B antibody and antigen status in archived serum samples was determined as follows:

3.7.1 Diagnosis of hepatitis B antibody virus IgM in serum by ELISA

Retrieved serum samples were tested for the presence of antibodies against HBV, using the ELISA kit Murex DiaSorin LIAISON anti-HBs II assay (DiaSorin, Saluggia, Italy), to determine the presence of antibodies against HBV. DiaSorin anti-HBs is an enzyme immunoassay for the detection of antibodies against hepatitis B surface antigen (anti-HBs) in serum or plasma. Anti-HBs is produced in the immunological response to hepatitis B surface antigen (HBsAg) either through exposure to the hepatitis B virus (HBV) or as the result of immunization with HBsAg.

The test was conducted following the manufacturer's instructions (DiaSorin HBsAb kit insert). Murex anti-HBs was based on microwells coated with HBsAg. Samples, incubation buffers, controls, and calibrators were incubated in the wells and any anti-HBs present binds to the antigen in the microwells. The microwells were then washed to remove excess anti-HBs before an enzyme tracer prepared from HBsAg labelled with

horseradish peroxidase (HRP) was added. The presence of anti-HBs present in samples or calibrators was proportional to the enzyme activity, which was measured after the addition of a colourless chromogen/substrate which enables the colour to develop to be measured with a photometer. The colour change and intensity were monitored using a spectrophotometer plate reader set at 450 nm with a correction filter between 620 and 650 nm. The presence of hepatitis B specific IgM was indicated by optical density values above the cut-off.

3.7.2 Diagnosis of Hepatitis B surface antigen in serum by ELISA

Participants samples that tested negative to presence of antibodies i.e. less 10 IU/ml antibody against HBV three years post-vaccination were further tested for the presence of HBsAg as a marker of infection with HBV by ELISA immunological test using DiaSorin Murex HBsAg Version 3 assay kit (DiaSorin, S.p.A. UK) following the manufacturer's instructions (HBsAg kit insert). Murex HBsAg Version 3 is a rapid and sensitive immunoassay enzyme used to detect hepatitis B surface antigen in human serum or plasma. In Murex HBsAg Version 3, the samples were pre-incubated in microwells coated with a mixture of mouse monoclonals specific for different epitopes on the determinant of HBsAg. Affinity-purified goat antibody to HBsAg conjugated to horseradish peroxidase was then added to the sample in the well. During the two incubation steps, any HBsAg present in the sample was bound to the well in an antibody-antigen-antibody-enzyme complex. In the absence of HBsAg, no conjugate was bound. After washing to remove the sample and unbound Conjugate, a solution containing 3, 3', 5, 5'-tetramethylbenzidine (TMB) and hydrogen peroxide was added to the wells. Wells which contained HBsAg and hence bound peroxide was added to the wells. Wells which contained HBsAg and hence bound conjugate developed a purple colour that was converted to orange when the enzyme reaction was terminated with sulphuric acid. The amount of colour was determined spectrophotometrically and was directly proportional to the amount of Conjugate bound and hence the concentration of HBsAg in the sample. The presence of Hepatitis B specific IgM resulted in a colour change in the TMB from colourless to blue and then yellow on stopping the reaction

with 0.5M HCL. The colour change and intensity were monitored using a spectrophotometer plate reader set at 450nm with a correction filter between 620 and 650nm. The presence of hepatitis B specific IgM was indicated by optical density values above the cut-off in the antigen well compared to the control.

3.7.3 ELISA Validity and results interpretation

Assay validity

Results of an assay are valid if the following criteria for the calibrators were met:

The absorbance value for the blank well must range between 0.000 and 0.150.

The mean absorbance for the negative control must be less than 0.100.

The mean absorbance for calibrator 1 must range between 0.035 and 0.300.

The ratio of the mean absorbance for calibrator 1 to the mean absorbance for the negative control must be greater than or equal to 2.0.

The ratio of the absorbance for calibrator 2 to the mean absorbance for calibrator 1 must be greater than or equal to 4.5.

Interpretation of Results

a. **Non-Reactive Results**

Samples that gave an absorbance less than the mean absorbance of the 10 IU/ml Calibrator were considered non-reactive.

b. **Reactive Results**

Samples that gave an absorbance of equal to or greater than the mean absorbance of the 10 IU/ml Calibrator were considered reactive in the assay and were presumed to contain

anti-HBs at >10 IU/ml. However, the specific level of immune response could not be quantified by the used method.

For the Hepatitis B surface antigen, serum samples that gave an absorbance greater than cut off were termed positive while those which had absorbance less than cut off were termed negative.

3.8 Data Management, Analysis, and Presentation

3.8.1 Data management

Data on relevant participant's social demographics which included age, gender BMI among others were retrieved from storage computer at the Thika clinic in excel files. This was merged with HBV antibody and HBsAg test results that were done at the CTRL laboratory. The excel file containing the study data was then imported into SPSS version 23 where it was cleaned and coded for ease of analysis. The cleaned SPSS file was further imported into STATA Version 13 for analysis. Hard copy data was kept in cabinets under key and lock.

3.8.2 Data analysis and presentation

The proportion of the individual with the hepatitis B antibody was assessed with the accuracy of Enzyme Immuno Assay where ELISA reader took the optical density readings from the readings from each plate. The cut-off and validity of each plate were obtained from the kit protocol.

3.9 Internal and external quality control for ELISA Test

CTRL laboratory where laboratory testing was done was enrolled for External Quality Assurance (EQA) i.e. Human Quality Assessment Services (HUQAS) that was carried out quarterly.

3.10 Ethical considerations

For this study/research, approval from Kenyatta National Hospital (KNH); the University of Nairobi (UON) Ethical Research Committee (ERC) number P685/11/2014 was obtained. The institutional review boards of the University of Washington and Kenyatta National Hospital also had approved the prospective interventional PrEP study, which is the parent study. Serum samples from participants who provided written informed consent for future use of their samples were used. The study was done according to UON/ERC guidelines and regulations on human archived samples use and care and standard operating procedures as founded in WHO guidelines.

3.11 Study limitations

Hepatitis antibodies against hepatitis B core antigen (anti-HBc) was not measured, although persons with isolated core antibody positivity (i.e., those who were positive for anti-HBc, negative for HBsAg, and negative for HBsAb) may have had a past infection with waning HBsAb and may have been misclassified as susceptible to HBV.

3.12 Benefits of the study

Findings were presented to the health facility to help them advice those that are HIV infected persons who receive HBV vaccination should have their antibody responses reviewed regularly and those with waning antibodies be offered booster doses. In addition, those persons infected with HIV are at a higher risk of being infected with HBV and efforts should be made to vaccinate them.

CHAPTER FOUR
RESULTS

4.1 Proportion of HBV antibody response 3 years post-vaccination among HIV-1 infected and uninfected adults.

The study sought to assess the sustainability of immune response to hepatitis B virus vaccination among HIV infected and uninfected adults in Kenya. The first objective was to determine immune response to HBV vaccination three years after HBV vaccination and compare responses in HIV-1 infected and uninfected adults.

4.1.1 Comparison of sustained HBV antibody response

A total of 336 participants' archived serum samples were retrieved randomly and tested for HBV antibody. 274 (81.5%) participants had protective levels (>10 IU/ml) of hepatitis B surface antibodies in the serum three years post HBV vaccination while 62 (18.5%) had levels of anti-HBS antibody titers less than 10 IU/ml. Of the participants with greater than10 IU/ml titers, 148 (92.5%) were HIV-1 uninfected while 126(71.6%) were HIV-1 infected. (Figure 4.1).

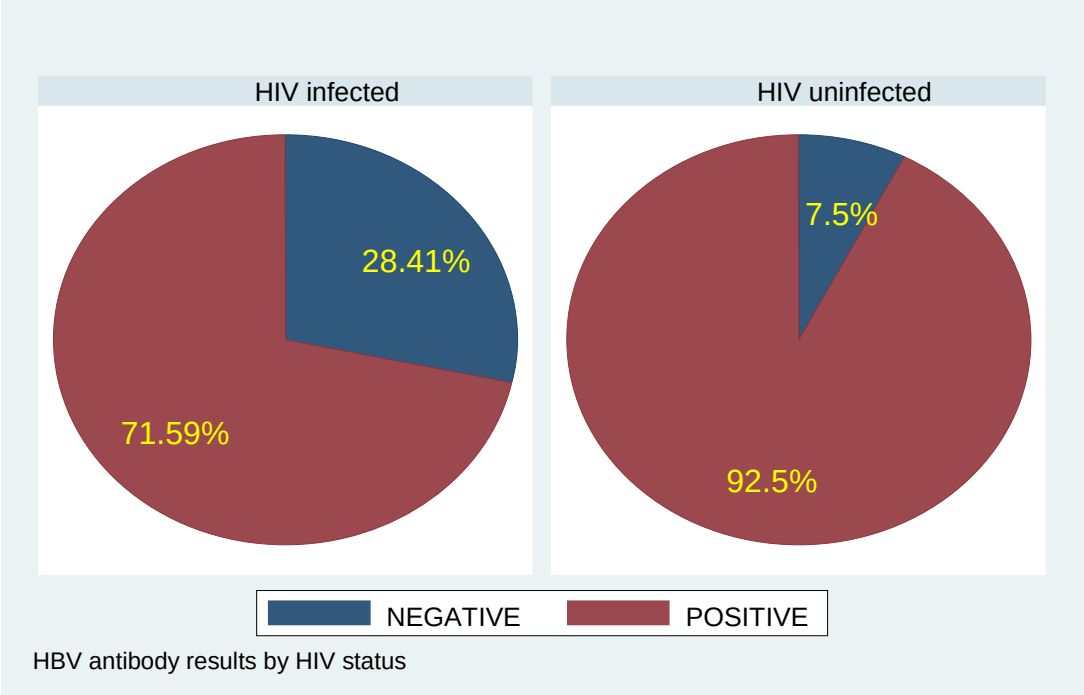

Figure 4.1: Hepatitis B surface antibody Results by HIV status

4.2 Factors associated with a sustained immune response to HBV vaccination in HIV-1 infected and uninfected adults

Of the 336 study participants, 274 (81.5%) tested positive to the presence of hepatitis B surface antibodies in circulation three years post-vaccination while 62 (18.5%) tested negative. A positive test was interpreted as having an antibody titer above 10 IU/ml. Further, among the individuals who tested negative for hepatitis B surface antibodies in circulation three years post-vaccination, 50 (80.6%) were HIV-infected while 12 (19.3%) were HIV-uninfected person. Additionally, 43(69%) were women and 19 (31%) were men.

The study population ranged between 19 years and 60 years with a mean of 34.55 ± 8.47 years. HIV-infected participants had a mean age of 33.3 ± 8.16 years while HIV uninfected participants had a mean age of 35.9 ± 8.61 years. The mean CD4 count among HIV-1 infected was 526 (±243 SD). There was no difference in BMI, age, the number of children, weekly drinks, and years of school between HIV-1 infected and HIV-1 uninfected participants. Table 4.1 summarizes the participants' baseline-

demographic characteristics,

Table 4.1: Participants baseline socio-demographic characteristics

Characteristics N (%) or mean (SD)	HIV infected (n = 176)	HIV uninfected (n=160)
Age	33.32 (8.17)	35.91 (8.61)
Sex, female	136 (77.27)	35 (21.88)
Number of children		
At least one child	161 (91.5)	136 (85)
Years of education		
Less than 8 years	58 (33)	65 (40.6)
>=8 years	118 (67)	95 (59.4)
Monthly income		
<=1000	81 (46)	38 (23.8)
Between 1001-5000	61 (34.7)	83 (51.9)
Between 5001-10000	21 (11.9)	24 (15)
>10000	13 (7.4)	15 (9.4)
Alcohol drinks per week		
No alcohol use	165 (93.8)	141 (88.1)
Characteristics N (%) or mean (SD)		
BMI		
<18.5	28 (8.3)	14 (4.2)
18.5 – 24.9	100 (29.8)	116 (34.5)
25.0 – 29.9	22 (6.5)	35 (10.4)
>30.0	10 (3.0)	11 (3.3)
CD4 count	526.24 (243.45)	

HIV infected persons compared to HIV uninfected were more likely to have a non-response to HBV vaccination at three years post-vaccination (28.4% vs 7.5%, OR = 4.9, 95% CI 2.5, 9.6 p <0.001) (Table 4.2). Similarly, compared to men, women were more likely to have low anti-HBS titers (11.5% vs 25.1%, OR 2.58, 95% CI 1.43, 4.66, p=0.002). Among HIV infected participants, those with CD4 count greater than 500

cells/µL were less likely to have low titers compared to those with less than 500 cells/µL (20.8% vs 34.3%, OR = 0.5, 95% CI 0.25, 0.99, p =0.048). This is shown in Table 4.2

Table 4.2: Factors associated with less anti–Hbs /in circulation 36 months' post-vaccination

Characteristics N(%) or mean (SD)	HBsAb positive (n=274)	HBsAb negative (n= 62)	OR (95% CI)	P value
HIV status				
Uninfected	148(92.5%)	12(7.5%)	Ref	
Infected	126(71.6%)	50(28.4%)	4.9(2.5,9.6)	<0.001
Sex				
Male	146(88.5%)	19(11.5%)	Ref	
Female	128(74.9%)	43(25.1%)	2.58(1.43,4.66)	0.002
Age (years)				
18-30 years	84(78.5%)	23(21.5%)	Ref	
>30 years	190(83.0%)	39(17.0%)	0.75(0.42,1.33)	0.33
BMI				
<18.5	35(83.3%)	7(16.7%)	Ref	
18.5-24.9	176(81.5%)	40(18.5%)	1.14(0.47,2.274)	0.78
25.0-29.9	47(82.5%)	10(17.5%)	1.06(0.37,3.07)	0.91
>30	16(76.2%)	5(23.8%)	1.56(0.43,5.68)	0.50
CD4 (cells/ml)				
<500	65(65.7%)	34(34.3%)	Ref	
>500	61(79.2%)	16(20.8%)	0.50(0.245,0.99)	0.048
Number of children				
None	34(87.2%)	5(12.8%)	Ref	
Any	240(80.8%)	57(19.2%)	1.62(0.60,4.31)	0.34
Monthly income				
None	59(74.7%)	20(25.3%)	Ref	
Any	215(83.7%)	42(16.3%)	0.58(0.31,1.06)	0.07
Alcohol use per week				
None	247(92.5%)	20(7.4%)	Ref	
Any	27(39.1%)	42(60.9%)	0.47(0.14,1.59)	0.22

˟CD4 count for HIV infected only

4.3 Incidence of HBV infection among HIV-1 infected and uninfected persons, 36-month post-vaccination with HBV vaccine.

Of the 62 serum samples that tested low for anti-HBs titers, they were tested for Hepatitis B surface antigen to check if there were any HBV infection incidences. Seven (11.3%) participants tested positive for the presence of HBsAg. All samples testing positive for HBsAg were for HIV infected participants, six were female and four were aged greater than 30 years. Participants with any monthly income, compared to those with none were less likely to test positive for HBsAg (4.8% vs 25.0%, OR 0.15, 95% CI 0.03, 0.86, p = 0.03). Baselines; CD4 count, gender, years of education, and age were not associated with increased risk of acquisition of Hepatitis B infection (Table 4.3).

Table 4.3: Factors associated with positive HBsAg among those with less than 10 lU/ml anti-Hbs titres 36 months' post vaccination

Characteristics N(%) or mean (SD)	HBsAg positive (n = 7)	HBsAg negative (n = 55)	OR (95% CI)	P-value
HIV status				
Uninfected	0 (0%)	12(100.0%)	Ref	
Infected	7 (14.0%)	43(86.0%)	-	-
Sex				
Male	1(5.3%)	18(94.7%)	Ref	
Female	6(14.0%)	37(86.0%)	2.92(0.33,26.10)	0.34
Age				
18-30 years	23(13.0%)	20(87.0%)	Ref	
>30 years	4(10.3%)	35(89.7%)	0.76(0.15,3.75)	0.74
CD4 count				
<500	6(17.6%)	28(82.4%)	Ref	
>500	1(6.3%)	15(93.7%)	0.31(0.03,2.83)	0.3
Number of children				
None	0 (0%)	5 (100.0%)	Ref	
Any	7 (12.3%)	50 (87.7%)	-	
				-
Years of education				
<8 years	2 (10.0%)	18 (90.0%)	Ref	
>8 years	5 (11.9%)	37 (88.1%)	1.22(0.21,6.89)	0.83
Monthly income				
None	5(25.0%)	15(75.0%)	Ref	
Any	2(4.8%)	40(95.2%)	0.15(0.03,0.86)	**0.03**
Alcohol use per week				
None	6 (10.2)	53(89.8)	Ref	
Any	1 (33.3)	2 (66.7)	4.42(0.35,56.26)	0.25

*CD4 count for HIV infected only

CHAPTER FIVE
DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

5.1.1 Proportion of HBV antibody response 3 years post-vaccination among HIV-1 infected and uninfected adults

This study found that, three years post HBV vaccination almost one in five of the study population did not have adequate protective levels of hepatitis B antibodies. It was found that eighty percent of those not having protecting anti-Hbs titers were HIV infected individuals. Among HIV-1 infected participants 71.59% had sustained immune response to hepatitis B antigen 36-month post-vaccination compared to 92.5% of HIV-1 uninfected participants. These findings are similar to others that reported better response to hepatitis B vaccination among HIV negative persons compared to HIV infected persons (Assad *et al.*, 1999; Irungu *et al.*, 2013; Uneke *et al.*, 2005). There was slightly lower response among HIV-1 negative participants compared to other studies that had reported a greater than 95% sustained mmune response after HBV vaccination among HIV uninfected persons (Fonseca *et al*.,2005). However, these studies assessed the immune response at one year.

It study also observed a higher proportion (72%) of HIV-infected individuals with protective antibody titers at three years compared to other studies (Fonseca *et al*., 2005; Rey *et al*., 2000) what is the prevalence. This is likely due to the booster HBV vaccine given to participants who did not respond to the initial 3-series of HBV vaccination. All participants that received repeat vaccination after having failed the initial series had anti-Hbs titers greater than 10 lU/ml (data not shown). Studies have reported improved immune responses and outcomes to hepatitis B vaccine among HIV infected persons who received booster vaccines compared to those who only received an initial 3-dose series of HBV vaccine (Assad *et al.*,1999; Whitaker *et al.*,2012; Irungu *et al.*,2012).

5.1.2 Predictors of HBV vaccine failure

The predictors or factors that were of interest to this study were Participant socio-demographics which included HIV status, age, gender, BMI, number of children, years of school, monthly income, weekly drinking, and CD4 count.

The study found out that those with low anti-Hbs titers were HIV infected, had low CD4 count, and of the female gender. This is similar to other studies conducted in the adult African population, which reported gender and low CD4 count as being predictors of the poor immune response to hepatitis vaccination (Irungu *et al.,*2012) and (Matthews *et al.,*2015). Interestingly, an earlier study conducted in this cohort had reported male gender as a predictor of non-response to hepatitis B immune response 6 months post initial HBV vaccination (Irungu *et al.,* 2012). Low HBV vaccine immune response rates among men have been reported elsewhere, although the mechanism for this poor response is not clear (Cruciani *et al*., 2008; De *et al*., 2009). The female gender might be explained briefly due to female being more than men in this study.

This study didn't examine any association between ART and response to vaccination, although few participants-initiated ART during the initial vaccination series. The role of ART in the immune response to HBV vaccination is unclear. While some studies have demonstrated that participants receiving ART have better immune responses to vaccines (Kim *et al*.,2008; Landrum *et al.,*2009; Psevdos *et al*.,2010), others have not (Cruciani *et al.,* 2009; De *et al*.,2008; Pettit *et al*.,2010). Prior studies have suggested that modification of the standard HBV vaccine regimen by using higher HBV vaccine doses, increasing the number of HBV vaccine injections, or both significantly improve HBsAb seroconversion rates among HIV-1–infected adults, (Psevdos *et al*., 2010). All HIV-1–infected persons in this study had relatively intact immune systems, with all having CD4+ T-cell counts of >250 cells/μL at the start of initial vaccination, which may in part explain the high rate of vaccine response; as reported in other studies (Landrum *et al*., 2009; Pasricha *et al*., 2006) provision of HBV vaccine to HIV-1–infected persons earlier in the course of HIV-1 infection, before the development of advanced

immunosuppression, is associated with a better HBV vaccine response. Among HIV-infected persons, post-vaccination testing, and revaccination of nonresponders with a full series is recommended (Mast *et al.,* 2006).

Malnutrition which can be connected with low income has been associated with the impaired immune response to vaccines, including HBV vaccine among children and adults (Fabrizi *et al.*,2012; Miyagawa *et al*., 2013), and some studies have found improved response rates with micronutrient supplementation (Langkamp-Henken *et al*.,2006). This study did not show any statistical significant difference between those with income or with none. The likely hypothesis is that having low income is associated with impaired immune response.

The age study population for this study ranged between 19 years and 60 years with a mean of 34.55 ± 8.47 years. HIV-infected participants had a mean age of 33.3 ± 8.16 years while HIV uninfected participants had a mean age of 35.9 ± 8.61 years. There was no statistical significant difference in terms of age between HIV-1 infected and HIV-1 uninfected participants, this agreed with (Morris *et al.,* 1989) who failed to find such an association. Although other studies done by (Ginaldi *et al*., 1999) suggested that age-associated changes in humoral and cellular immune function may result in decreased vaccine effectiveness in older individuals, compared with children or young adults. (Clements *et al*., 1994) suggested that older individuals are less likely to have a serological response to recombinant hepatitis B vaccine. The issue is of practical importance because the differential efficacy of the vaccine in different age groups could change the optimal vaccination policy. Also, there are those studies like (Leder *et al*., 2001) that found that the association between age and nonresponse to vaccine remained fairly constant, regardless of the age cutoff used. Even age cutoffs as low as 30 years predicted an increased risk of nonresponse among older individuals. Thus, the increased risk of nonresponse to the vaccine may apply to individuals young enough to be at risk for the long-term complications of chronic hepatitis B infection, including cirrhosis and hepatocellular carcinoma, and to individuals of childbearing age. The relevance of the latter point relates to the high degree of vertical transmissibility of the virus. More

generically, the implication that adults aged >30 years are immunologically different from younger individuals has important implications for the vaccination of adults for travel to the developing world and for vaccination to prevent occupationally acquired illnesses (Bolyard *et al*., 1998). While not definitive, these results suggest that revaccination should be initiated within 24-36 months of initial vaccination, for maximal results.

Low levels of response to hepatitis B vaccine in people with higher levels of BMI is maybe due to the distribution of vaccine in fat not in the muscle which decreases its absorption. Significant effects of age and BMI on the levels of response to hepatitis B vaccine have been reported from Belgium, (Van Damme *et al*., 2016) China, (Yang *et al*., 2017) Turkey (Asan *et al*.,2017), and Iran, (Aminian *et al*.,2017). In contrast, this differed from this study since it didn't show any statistical significance.This might be because majority approximately more than two third of participant's had normal BMI i.e between 18.5-29.9 Baba Mahmoodi *et al*., 2000 reported that smoking and obesity had negative effects on the production of antibodies after vaccination with hepatitis B. (Young *et al.*, 2013) reported that obese individuals (BMI $\geq$ 30 kg/m^2) were significantly more likely to be nonresponders following two recombinant hepatitis B vaccine doses which also differed with the findings of this study.

This study also did not show any statistical significance in years of education i.e. educational level and the number of children. This agreed with the study which was done in Yemen which revealed a considerable proportion of vaccinated children remains to be reconsidered for either revaccination or booster doses due to lack, inadequate or low response. The trend of decreasing the antibody level with increasing age suggests a need for careful monitoring of HBV vaccine efficacy in Yemen. Demographic factors such as gender number of inhabitants per room and the educational level of father did not significantly affect the immune response to the HBV vaccine (Talal *et al*., 2005).

In another study done by (Brian *et al*., 1990) there was no difference in the mean anti-HBs between alcoholics and controls younger than 45 years of age, but in persons greater than 45 years of age, alcoholics had a lower mean anti-HBs level than did non-alcoholics; this difference, however, was not significant and this agreed with this study.

5.1.3 Incidence of HBV infection after HBV vaccination

In this study, the socio-demographics that were considered and thought to be correlated with the cause of the incidence of HBV infection were HIV status, gender, age, CD4 count, and the number of children, years of education, monthly income, and weekly alcoholic drinks.

In this study, the high risk of incident HBV infection was found among HIV-infected individuals and those who had no income. This study agrees with the study done by (Saha *et al*., 2017) that showed that HBV replication and immune control in HIV/HBV confection in the natural course of HBV mono-infection, the formation of the episomal HBV covalently closed circular DNA (cccDNA) and the integration of the HBV DNA supported the production of HBsAg and HBV replication. HBsAg has an inhibitory impact on the adaptive immune response and on the production of anti-HBs antibodies, which allows definitive protection and recovery from HBV infection. Little is known about the outcome of the natural course of HBV infection in coinfected people. Accelerated liver disease progression and HCC evolution, which has been documented in HIV/HBV infected patients, is likely to be related to high HBV-DNA levels (Saha *et al*., 2017) and poor immunological control of HBV replication associated with the low CD4 positive cell counts, which are typical of HIV/HBV coinfected subjects. A greater tendency to synchronize HBV infection with poor HBeAg seroconversion has been demonstrated in coinfected Nigerian people before the onset of cART (Wandeler *et al.*, 2016) where higher HBV DNA and detectable HBeAg levels were independently associated with lower CD4+ T cell counts.

Low socioeconomic status has been associated with an increased risk of Hepatitis B infection (Whitaker *et al.*, 2012). This agreed with this study and also with research which was done on Chinese ethnic group which showed low socioeconomic status is a strong risk factor for HBsAg. Hepatitis B transmission was associated with low socioeconomic status and there was a moderate positive trend that large family size has more risk of developing hepatitis B. In this study, large family size which was termed as the number of children did not show any association with hepatitis B infection. Socio-economic status was also found to be associated with hepatitis B positivity in the study that was reported by (Ugwuja *et al.*, 2008). Others have reported varying findings showing that social class does not influence HBV infection in children (WHO 2006).

There was no association between alcohol use and incidence of HBV infection in this study, although the mechanisms underlying the complex interaction between alcohol and hepatitis virus infection in the progression of liver disease are not fully understood, possible explanations include effects on viral replication, increases in oxidative stress, and a weakening of the immune response (Gitto *et al.*,2014; Larkin *et al*., 2001) reported that in HBV transgenic C.B-17 SCID mice fed a standard Lieber-DeCarli ethanol liquid diet, elevated levels of HBV RNA as replicative intermediates, and increased expression of HBs, core and X antigens were observed in the liver. With ethanol, the level of HBsAg and viral DNA in serum increased by up to 7-fold compared with mice fed the control diet. These findings may provide a partial explanation for the effects of alcohol on viral replication and the high frequency of HBV markers observed among alcoholics. Similarly, Min *et al.,* 2013 showed that in human HepAD38 hepatoma cells infected with HBV, 100 mmol ethanol treatment approximately doubled the transcriptional activity of HBV promoters by increasing the expression of nuclear receptors such as hepatocyte nuclear factor-4α and peroxisome proliferator-activated receptor-α. In addition, CYP2E1-induced oxidative stress potentiates the ethanol-induced transactivation of HBV.

Although it is well documented that HCV-positive drinkers are 2 to 3 times more likely to develop HCC than abstinent individuals, (Novo-Veleiro *et al*., 2016; Punzalan *et al*., 2015; Siu *et al*., 2009; Fukushima *et al.,* 2006) whether HBV infection and alcohol consumption synergistically increase the incidence of HCC is still controversial.

However, certain factors, such as alcohol abuse, make HBV chronically persist which put patients at a higher risk for developing fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) (Terrault *et al.,* 2016; Suliman *et al*., 2019; Li *et al*., 2019).

Overall, the effect of alcohol metabolism on protein function, DNA, changes to the immune system, and oxidative stress affect both hepatocytes and other liver cells. They take place under both acute and chronic exposure to alcohol and induce significant functional impairments resulting in cell death, tumorigenesis, altered cell to cell communication, and become more prone to viral infection (Ganne-Carrié *et al*., 2019; Dolganiuc *et al*., 2015).

It has been reported that a combination of HCV infection and daily alcohol intake (> 80 g) increased the risk of HCC development > 100-fold (Serfaty *et al*., 2016). The incidence of HBV is higher among alcoholics than among the general population (Larkin *et al*., 2001, Wilkening *et al*., 2003). Studies have been conducted on the combined effect of alcohol and viral hepatitis in the progression of liver diseases, but the role of alcohol metabolism as risk factors in the pathogenesis of HBV infection has not been studied yet (Dolganiuc *et al*., 2015).

Alcohol abuse pattern has a wide geographical distribution depending on alcohol drinking habits in various parts of the world. As reported, about 50% of HBV carriers drink alcohol and more than 10% are heavy drinkers in the Korean population (Park *et al*., 2014).

This study demonstrated a reduced immune response to HBV vaccination among African HIV infected persons 36 months post-vaccination. These findings can guide policies to develop new vaccination strategies or provide booster vaccines, as well as the need for continuous HBV vaccine immune response monitoring among HIV infected people. A limitation of this study was the lack of HIV-1 viral load measurements among HIV infected persons which have also been associated with the immune response to HBV vaccination. In addition, antibodies against hepatitis B core antigen (anti-HBc) was not measured, although persons with isolated core antibody positivity (i.e., those who were positive for anti-HBc, negative for HBsAg, and negative for HBsAb) may have had a past infection with waning HBsAb and may have been misclassified as susceptible to HBV. The HBsAb assay used in this study did not quantify antibody titers, and thus it cannot describe differences in titers. Finally, the ultimate measure of success in vaccination would be to follow participants and determine the incidence of HBV infection in the vaccinated participants which were part of the third objective of this study, but this would be prohibitively expensive and time-consuming, and the development of HBsAb titers has been demonstrated in multiple populations to be a strong surrogate marker for protection from HBV infection (Mast *et al.,* 2006). To the best of our knowledge, this is one of the few studies to explore the immune response to HBV vaccination and revaccination and the first study to check the incidence of HBV infection among HIV-1–infected adults in Africa, where the HIV-1 prevalence is highest. These findings add to the body of research on HBV vaccine immune responses and may help guide policy on the best practices for revaccinating HIV-1–infected persons who do not respond to the standard HBV vaccination schedule. In summary, as has been seen in high-income settings, HIV-1–infected adults in Kenya had a suboptimal response to standard HBV vaccine, and HIV-1 uninfected adults had a high response. HIV-1–infected achieved a 71.59% response with revaccination with a standard vaccine series. A Study done by (François-Xavier & Lionel, 2017) showed that monitoring the anti-HBs titer remains necessary, as it has been reported that only less than half i.e. 42% of HIV infected responders kept their protective titers 13–18 months after their last dose of vaccine. This shows that the determination of the HBsAb response after vaccination

of HIV-1–infected adults and timely revaccination of nonresponders with 3 additional HBV vaccine doses can significantly increase the development of protective antibody titers.

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5.2 Conclusion

HBV antibody titers sustainability among HIV-1 infected and uninfected 36 months post- HBV vaccination.

In conclusion, the study showed that in a cohort of adults there was sustained immune response to hepatitis B vaccine 36-month post-vaccination among both HIV infected and uninfected participants. However, the reduction in immune response was greater among adults living with HIV infection and in some resulted in the acquisition of HBV infection. This could be partly attributed to the reduced immune responses in individuals living with HIV.

Predictors of HBV vaccine failure

Eleven percent of those with low titers were found to have HBV infection. The factors that correlated with failure were being HIV positive,low CD4 count and of female gender.

Incidence of HBV infection after HBV vaccination

It was identified 7 cases of new HBV infection among HIV infected individuals. Due to the waning immune response to the hepatitis B vaccine observed in this study, this advocate for regular evaluation of immune response to HBV especially among HIV infected persons, and administration of booster doses to those with reduced immunity.

5.3 Recommendation

Given the waning immune response to the hepatitis B vaccine observed in this study, it suggested that HIV infected persons who receive HBV vaccination should have their antibody responses reviewed regularly, and those with waning antibodies be offered booster doses.

Vaccination of HIV infected and other immunosuppressed should be done with caution requiring more studies to determine the level of immune response

HIV infected persons in this region are at a high risk of HBV infection and efforts should be made to vaccinate them.

Further work is required to measure IgG and antibodies against hepatitis B core antigen (anti-HBc).

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APPENDICES

Appendix I: Authorization letter to use archived samples from PrEP Study

Partners in Health and Research Development

Thika Section 9, OAU Road, Next to Thika Nursing Home
P.O. Box 19865 - 00202
NAIROBI
Tel: +254-67-22561 / 21305
Mobile: 0725-641110
E-mail: phrd@pipsthika.org

INVESTIGATORS
Dr. Nelly R. Mugo
Dr. Kenneth Ngure

COLLABORATING INSTITUTIONS
KEMRI
Kenyatta National Hospital

19th November 2014

Secretary
KNH-UoN Ethical Research Committee,
Kenyatta National Hospital,

REF:PERMISSION TO USE PARTNERS PrEP PARTICIPANTS' ARCHIVED SAMPLES .

Rose Kamoni has my permission to use repository samples from the recently concluded Partners PrEP study, from Thika site to conduct her research work. Specifically responding to the question of sustained immune response to Hepatitis B vaccination, three years post vaccine comparing HIV-infected and uninfected individuals. This work is complementary to a previous study conducted by Dr. Elizabeth Irungu, who will be one of Rose Kamoni's primary mentors for this project.

It is my expectation that Rose Kamoni, as a skilled and experienced laboratory technician, who has worked with the Partners in Health Research and Development will handle the repository samples with care and abide by Good Clinical Laboratory Practice. Only samples from participants who have written informed consent to long term storage will be used. A protocol has been developed to ensure that those samples are properly retrieved from storage, thawed, aliquoted and the remnant samples re-archived in appropriate temperature for future use.

Rose will write a standard operating procedure for handling the laboratory samples, which will be reviewed and approved by the laboratory director. She will use the samples to test hepatitis B antibody levels, she will also conduct additional testing for hepatitis B surface antigen.

I strongly commend Rose's efforts to advance her career through additional training and her initiative to conduct research. Part of our expectation from Rose Kamoni is that she will publish her study findings.

I congratulate Rose on her success in a getting a grant reward through a competitive process.

Thanking you in advance,

Sincerely,

NRMugo

Dr Nelly R. Mugo, MBChB, MMed (Obs/Gyn), MPH
Principal Investigator, Partners in Health Research and Development

rwamba@csrtkenya.org

Appendix II: KNH-UON ERC Approval Letter (P685/11/2014)

KENYATTA NATIONAL HOSPITAL APPROVED 16 APR 2015 KNH/UoN-ERC P.O Box 20723-00202 NAIROBI

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

KNH/UON-ERC
Email: uonkeh_erc@uonbi.ac.ke
Website: https://erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonkehserc
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/172

16th April, 2015

Kamoni Rose

JKUAT

Dear Rose

Research Proposal: Sustainability of Immune Response to Hepatitis B virus (HBV) vaccination three years after Vaccination among HIV-Infected and Uninfected Adults in Kenya (P685/11/2014)

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 16th April 2015 to 15th April 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 severe 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.

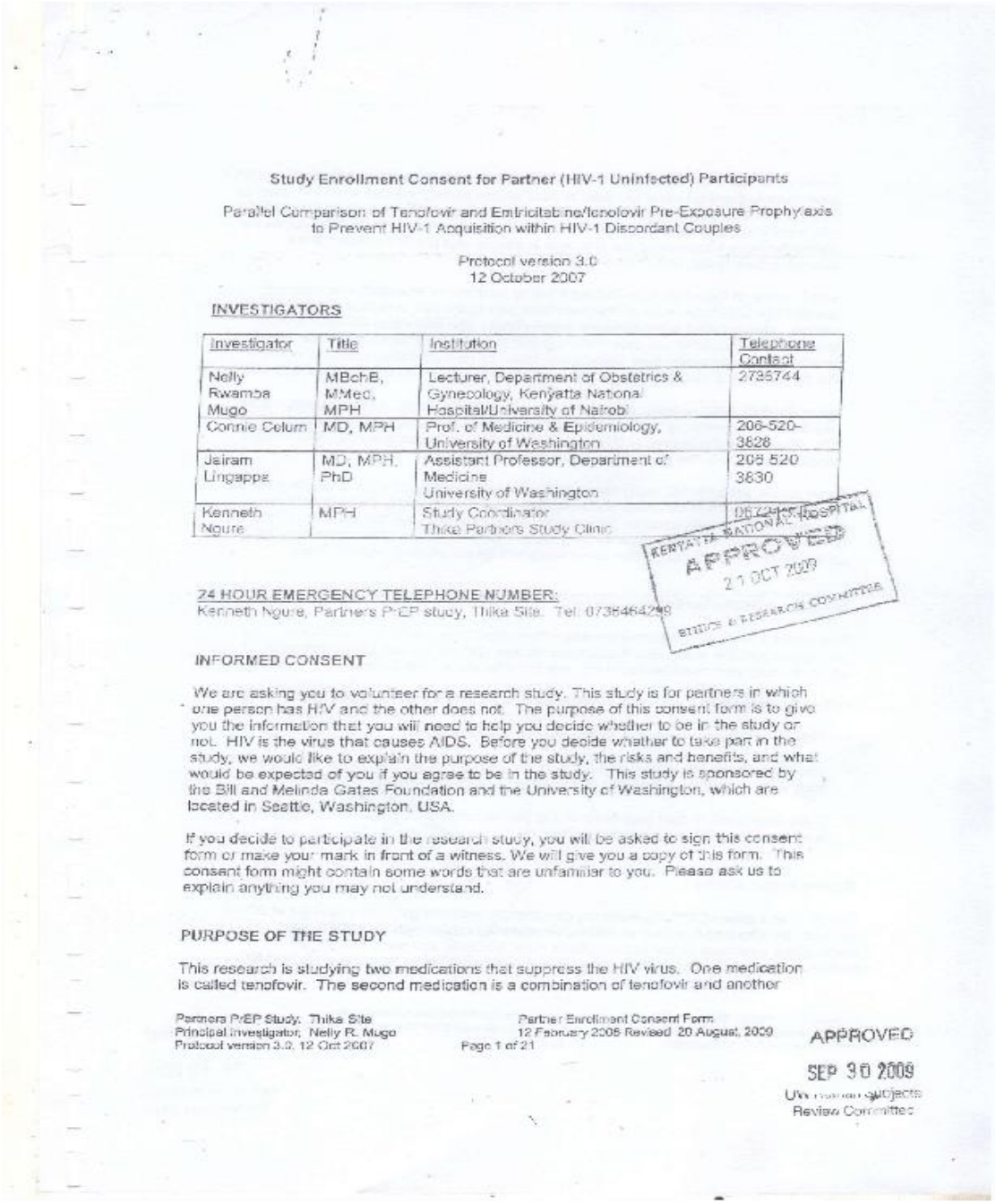
For more details consult the KNH/UoN ERC website **www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Chair, KNH/UoN-ERC
Supervisors: Dr. Bhavna Chohan
Co-Investigators: Dr. Nelly Mugo, Dr. Kenneth Ngure, Dr. Elizabeth Irungu, Dr. Harrison Tamoh, Dr. F.A. Okoth

Appendix III: KNH-UON ERC / UW Human Subjects Review Committee Approval Letter for PrEP Study



Study Enrollment Consent for Partner (HIV-1 Uninfected) Participants

Parallel Comparison of Tenofovir and Emtricitabine/tenofovir Pre-Exposure Prophylaxis to Prevent HIV-1 Acquisition within HIV-1 Discordant Couples

Protocol version 3.0
12 October 2007

INVESTIGATORS

Investigator	Title	Institution	Telephone Contact
Nelly Rwamba Mugo	MBchB, MMed, MPH	Lecturer, Department of Obstetrics & Gynecology, Kenyatta National Hospital/University of Nairobi	2725744
Connie Celum	MD, MPH	Prof. of Medicine & Epidemiology, University of Washington	206-520-3828
Jairam Lingappa	MD, MPH, PhD	Assistant Professor, Department of Medicine University of Washington	206 520 3830
Kenneth Ngure	MPH	Study Coordinator Thika Partners Study Clinic	0722167

KENYATTA NATIONAL HOSPITAL
APPROVED
21 OCT 2009
ETHICS & RESEARCH COMMITTEE

24 HOUR EMERGENCY TELEPHONE NUMBER:
Kenneth Ngure, Partners PrEP study, Thika Site. Tel: 0736464299

INFORMED CONSENT

We are asking you to volunteer for a research study. This study is for partners in which one person has HIV and the other does not. The purpose of this consent form is to give you the information that you will need to help you decide whether to be in the study or not. HIV is the virus that causes AIDS. Before you decide whether to take part in the study, we would like to explain the purpose of the study, the risks and benefits, and what would be expected of you if you agree to be in the study. This study is sponsored by the Bill and Melinda Gates Foundation and the University of Washington, which are located in Seattle, Washington, USA.

If you decide to participate in the research study, you will be asked to sign this consent form or make your mark in front of a witness. We will give you a copy of this form. This consent form might contain some words that are unfamiliar to you. Please ask us to explain anything you may not understand.

PURPOSE OF THE STUDY

This research is studying two medications that suppress the HIV virus. One medication is called tenofovir. The second medication is a combination of tenofovir and another

Partners PrEP Study, Thika Site
Principal Investigator, Nelly R. Mugo
Protocol version 3.0, 12 Oct 2007

Partner Enrollment Consent Form
12 February 2006 Revised 20 August, 2009
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medication called emtricitabine. This combination is known as Truvada®. Both tenofovir and Truvada® are pills that are used to treat people already infected with HIV. Studies have shown that these medications are safe when used once per day as treatment of HIV by HIV-infected people. Tenofovir and Truvada® are not cures for HIV, but they are very effective in suppressing the HIV virus in people who are HIV-infected and in improving their health and immune function.

The purpose of this study is to find out if taking tenofovir or Truvada® every day can prevent HIV-uninfected men and women from getting the HIV virus from their infected partner. This is called **pre-exposure prophylaxis**. The study also will learn whether tenofovir and Truvada® are safe (meaning that they do not produce significant health problems in persons who take them) when taken by HIV-uninfected men and women. We do not know if taking tenofovir or Truvada® will prevent HIV infection in a safe way.

Approximately 3900 couples, all from Africa, will be in the study. All the couples will be HIV-discordant, meaning that one person has HIV and the other person does not have HIV. This will be the largest of this kind of study ever. Approximately 500-800 couples are planned to be in the study here at the Thika study clinic.

Each couple will be in the study for a minimum of 24 months and up to a maximum of 36 months. The length of the study will depend on how long the study takes to meet its objectives. Those who become HIV infected during the study will be asked to continue for one year after infection, regardless of when during the 36 months of maximum follow-up the HIV infection occurs. The whole study will take at about 5 years to finish.

YOUR PARTICIPATION IS VOLUNTARY

This consent form gives information about the study that we will discuss with you. Once you understand the study, and if you agree to take part, we will ask you to sign your name or make your mark on this form. We will offer you a copy to keep.

Before you learn about the study procedures, it is important that you know the following:

- You do not have to be in this study if you do not want to join.
- You may decide not to take part in the study, or to withdraw from the study at any time, without losing the benefits of your or your partner's routine medical care.
- If you decide not to take part in the study, you can still be told about research study later, if one is available and you qualify.

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STUDY MEDICATION

Tenofovir at a dose of 300 mg once per day and the combination of emtricitabine at 200 mg once per day and tenofovir at 300 mg once per day (known as Truvada®) are medications approved by the United States Food and Drug Administration for the treatment of HIV infection. In this study, we will evaluate if taking tenofovir or Truvada®

every day prevents men and women who are not infected with HIV from getting the HIV virus.

Tenofovir and Truvada® were chosen for this study for several reasons:

- We know that they have low levels of side effects compared to other medications used to treat HIV.
- They can be taken once a day.
- HIV does not easily become resistant to tenofovir or Truvada®.
- Studies done in animals have shown that tenofovir and Truvada® can sometimes prevent infection with a virus similar to HIV.

Tenofovir and Truvada® have not been approved by the US FDA for the prevention of HIV. Gilead Sciences is the pharmaceutical company that produces tenofovir and Truvada®, and Gilead Sciences will supply the medications for this study.

We do not know if taking tenofovir or Truvada® can prevent you from getting HIV. That is why we are conducting the study.

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STUDY GROUPS

In this research study, you will be assigned to one of three study groups – one group will receive tenofovir, one group will receive Truvada®, and one group will receive placebo-placebo. You will take two pills of study medication each day. The placebo-group will receive pills that have the same physical appearance and have the same taste as tenofovir and Truvada® but the pills will not contain a medication that is used to treat HIV infection or any other medication.

The two pills that you will take each day will look different. Everyone in the study will receive pills that look the same as the pills you will receive. You will get the same type of study medication throughout the study. A computer program will randomly decide which type of study medication you receive. You will have an equal chance of being assigned to the tenofovir group, the Truvada® group, or the placebo group. Neither you nor any of the research staff in this clinic will know who is taking tenofovir, who is taking Truvada®, and who is taking the placebo. You cannot be told which group you are in until 6-24 months after the end of the study. This means you may have to wait up to 7 years to find out which pills you were randomly assigned to take.

All three groups are very important to this study. Couples in all three groups will have the same study visits. All couples will get condoms, treatment for other STDs, and counseling on how to avoid HIV and other infections passed during sex.

No matter what study group you are in, you must remember that we do not know if tenofovir or Truvada® work to prevent passing HIV from one person to another. The only known way to protect against getting HIV during sex is to use a condom every time you have sex.

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It is very important that you do not share the study medication with anyone, including your partner. If the drugs that are used in this study are used alone in someone who has HIV, the HIV virus can become resistant to these medications. If you share the study medications with your partner, the HIV virus your partner has could become resistant to these medications, which could limit your partner's treatment options for the future.

STUDY PROCEDURES

If you decide to take part in the study, your first visit will continue today, after you read, discuss, and sign or make your mark on this form. At today's visit, several things will happen:

- The study staff will ask you questions about your medical history and your sexual practices
- You will undergo a physical exam, including a genital exam to look for sexually transmitted infections.
    - For women, the study staff will collect swab samples during the genital exam from your genital area to test for sexually transmitted infections. These will be collected using soft, sterile swabs. A Pap smear may also be performed.
- We will ask your permission to obtain a blood sample (up to 80.5 ml / less than 17 teaspoons). We will obtain the sample using a sterile instrument (needle) inserted into a vein in your arm. The blood sample will be used for tests at the clinic and by study researchers at the University of Washington.
    - The blood for testing for HIV will be obtained by pricking your finger. When we do HIV testing for this study, we first do a test that gives results in about 20 minutes. You will get the result of that test when it is available, on the same day you give blood and have the test. If the test shows that you may have HIV infection, we will do another different test to confirm this result. This test takes about 1-2 weeks, so you will have to come back here at that time to get the results. You will talk with the study staff about the meaning of your results and how you feel about them. Sometimes HIV tests are not clearly positive but also not negative. In that case, we will do more tests until we know the result for sure. You must receive your HIV test results to stay in the study. If the HIV test is positive today, you will not be able to enroll in the study.
    - Some of the blood will be used to test for syphilis.
    - Some of the blood may be used to test for infection with herpes simplex virus, another sexually transmitted infection.
    - Some of the blood will be used to confirm the results of the HIV tests that we do here and will look for other genetic, infectious, and immune factors that affect the chances of becoming HIV-infected.

- You will be asked to provide a urine sample.
    - For men, the urine sample will be tested for sexually transmitted diseases by researchers at the University of Washington.
    - For women, the urine sample will be used for a pregnancy test. You are not eligible for this study if you are pregnant.
- If your blood sample from the Screening Visit showed no evidence of hepatitis B infection, and no evidence of immunity to hepatitis B, you will be given information about the disease and also offered to begin the hepatitis B vaccination series. This will include vaccine injections also at 3 and 6 months.
- You will then be randomly assigned to receive study medication.
    - The study pharmacy staff will give you two bottles of study medication pills. You should take one pill from each bottle, once every day, by mouth, until your next scheduled visit. The study staff will give you information on the possible side effects and will teach you methods to not forget to take the pills every day.

After today's visit, you will have scheduled study follow-up every month. You will take the study medications every day. Your partner will also have regular follow-up visits scheduled, for every 3 months. During the months when you and your partner both have a visit, you should come to the clinic together, for your convenience and so that we can do counseling together as a couple. If that is not possible, you can come for your visits by yourself if you wish. Each visit will take about 60 minutes.

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At each of your monthly visits you will:

- Be asked questions about your health and medical history, including whether you have any clinical symptoms or side effects from the study drug.
- Be asked questions about your sexual practices.
- Talk with study staff about ways to avoid HIV and other infections passed during sex. We encourage you to have this counseling with your partner, but you can have it by yourself if you wish.
- Talk with study staff about the HIV test and give blood [up to 4 ml / less than 1 teaspoon], either from a finger stick and/or from your arm, for the test.
    - If the HIV test in the clinic is positive or not clear positive or negative, you will stop study medication until more tests are done to know for sure. If further testing demonstrates that you are HIV negative, you will be allowed to resume study medication.

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- Some of the blood may be used to confirm the results of the HIV tests that we do here and will look for other genetic, infectious, and immune factors that affect the chances of becoming HIV-infected.

- Get condoms.

- Get medical care or referrals for medical care and other services if you need them.

- Give updated information on where you live and how to keep in contact with you. The study staff will use this information to remind you of scheduled visits. If you miss a visit, the study staff will try to contact you by telephone. They also may visit your home to find you. They will try to reach you through the contact people that you list. If they talk to these people, they will not tell them why they are trying to reach you.

- Receive study medication. At your first monthly visit you will return the bottles of study medication pills that were dispensed at your Enrollment Visit. You will be asked to answer questions about the pills you took during the previous month and be counseled about methods for not forgetting to take your pills during the following month. The pharmacy staff will provide you with new bottles with pills for the following month. This process will be repeated each month.

- You will be counseled about the importance of not sharing the study medication with anyone, including your partner.

- Women will be asked to provide a urine sample. This will be used for a pregnancy test.

Some of your monthly visits may take place at your home. The study staff will talk with you about whether this will be an option.

At the first month and every 3 months:

- You will undergo a physical exam.

- Give more blood [up to a total of 97.5 ml / less than 20 teaspoons]. This will be used:

  - To test the function of your kidneys, your liver, your pancreas, and your blood counts. This is to check the safety of the study drug. The study staff will provide you with the results of these laboratory tests. If at any time you have a result of a test that is abnormal, we will contact you so that you will know. If you have an abnormal result of a laboratory, you will be evaluated at the study clinic and appropriate treatment will be provided to you. If you need treatment that is not available at the study clinic, the staff will refer you for additional care.
  - For an HIV test your finger will be pricked, the same as at each monthly visit.
  - Some of the blood will be sent to study researchers at the University of

Washington to look for genetic, infectious and immune factors that affect the chances of becoming HIV-infected.

Every 12 months:

- You will undergo a genital exam to look for sexually transmitted infections.
    - For women, the study staff will collect swab samples during the genital exam from your genital area to test for sexually transmitted infections. These will be collected using soft-tipped, sterile swabs. A Pap smear may also be performed to look for pre-cancerous or cancer cells from the cervix.
    - For men, you will be asked to provide a urine sample that will be tested for sexually transmitted diseases by researchers at the University of Washington.
- A blood sample will also be checked for syphilis.

At end of follow-up, which will occur at a minimum of 24 months and up to a maximum of 36 months, depending on how quickly the overall study meets its objectives:

- You will be finished with taking study drug.
- You will undergo the same study procedures as at a usual monthly visit, including counseling, a symptom questionnaire, condoms, medical care or referral, and collection of information on where you live.
- A blood sample [up to 46.5 cc / less than 10 teaspoons] will be requested. Some of this blood will be sent to study researchers at the University of Washington to look for genetic, infectious and immune factors that affect the chances of becoming HIV-infected. Your finger will be pricked for rapid HIV testing.
- You will be asked to continue monthly follow-up for an additional 2 months to monitor you for HIV and health status after stopping the study medication. At these visits after stopping study medication, you will be asked to provide a blood sample [up to 29.5 cc / about 6 teaspoons]. Your finger will be pricked for rapid HIV testing. Some of this blood will be sent to study researchers at the University of Washington to look for genetic, infectious and immune factors that affect the chances of becoming HIV-infected. You will undergo the same study procedures as at a usual monthly visit, including counseling, a symptom questionnaire, condoms, medical care or referral, and collection of information on where you live.

At any time in the study:

- If you or the study staff think you may be having any health problems, you may need

to undergo a physical exam and may need to provide blood or other samples for testing.

- If you are having health problems that may be due to a sexually transmitted infection, you will have a genital exam and receive medicine to treat the infections as needed.
- You can have extra counseling and testing for HIV if needed between scheduled visits, either with your partner or by yourself.
- If you and your partner end your relationship before your last scheduled study visit, we will ask you to stay in the study as originally scheduled and continue the study medication (for a minimum of 24 months, or until the study has met its objectives).
- If you decide to leave the study before your last scheduled study visit, we will ask you to have a final study visit with all the exams and tests listed above.

PREGNANCY (women only)

Although infants born to HIV-infected women taking tenofovir or Truvada® during pregnancy have not been found to have a greater chance of having birth defects, we do not know for sure if these drugs are safe to the fetus in women who become pregnant.

During this study, you will receive counseling at each visit about the potential that you may become pregnant. You will also receive counseling about your options for contraception. You can receive some forms of contraception from the study clinic. You may choose whether or not you want to receive contraception.

If you become pregnant at any time during the study, your study medication will be stopped and the study staff will counsel you. You will continue to be followed in the research clinic. You will also receive, or be referred for, antenatal care. If you do not carry the pregnancy successfully to term, you will be allowed to resume study drug once you are no longer pregnant. If you become pregnant and give birth, we will ask that you bring the infant to the clinic within the first month after birth and then every 3 months for the first year. At each visit, we will measure the weight and growth of the child. At two of those visits, we will ask to obtain a small blood sample from the child, to check the function of the kidneys.

If you are breastfeeding, you will not be allowed to enroll in the study or take the study medication. If you begin breastfeeding during the study, your study medication will be stopped. You will continue to be followed in the research clinic. You will be allowed to resume the study medication when you are no longer breastfeeding.

The effect on infants of tenofovir or Truvada® when taken during pregnancy and breastfeeding by HIV-uninfected women is unknown.

It will be important that we monitor infants whose mothers have taken the study medications during early pregnancy, so we can learn if taking tenofovir or Truvada® during pregnancy is safe.

IF YOU BECOME HIV INFECTED

During the course of the study we will provide you with condoms and other materials to help prevent you from getting HIV. However it is possible that you can become HIV infected.

If you have a positive HIV test during the study:

- The study staff will talk with you about this test result and what this means for you.
- You will stop the study medication.
- The staff will ask your permission to obtain a second blood test [up to 95.5ml / less than 20 teaspoons] that will be used to confirm the initial positive test.
    - Some of the blood will be used to test the function of your kidneys, your liver, your pancreas, and your blood counts.
    - Some of the blood will be used to test for syphilis.
    - If you were not immune to hepatitis B at study enrollment and you declined the hepatitis B vaccine, some of the blood will be used to test for hepatitis B.
    - Some of the blood will be used to perform a CD4 count, a measure of your immune function.
    - Some of the blood will be sent to study researchers at the University of Washington to look for genetic, infectious and immune factors that affect the chances of becoming HIV-infected.
- You will undergo a physical exam, including a genital exam to look for sexually transmitted infections.
    - For women, the study staff will collect swab samples during the genital exam from your genital area to test for sexually transmitted infections. These will be collected using soft-tipped, sterile swabs.
    - For women, an additional swab will be obtained from your genital area. This will be sent to study researchers at the University of Washington to test for HIV in the genital area.
- You will be asked to provide a urine sample.
    - For men, the urine sample will be tested for sexually transmitted diseases by researchers at the University of Washington.
    - For women, the urine sample will be used for a pregnancy test.

- You will then be asked to return for another visit after about 2 weeks. At that visit, results from the confirmatory HIV test will be available. If those results confirm that you have become infected with HIV, we will ask that you continue follow-up at this clinic every 3 months until the study is finished, or for at least 12 months.
    - Note that if you become infected with HIV, depending on when that occurs, your total duration of follow-up in this clinic may be longer than 36 months.
    - For example, if you are found to be infected with HIV at your 32 month visit, you will be requested to continue your follow-up for an additional 12 months. In that case, the maximum follow-up in this clinic may be 44 months.
- At each of the visits after HIV infection is confirmed:
    - You will undergo a physical examination.
    - You will be asked to provide a blood sample [up to 17 – 80.5 ml / less than 17 teaspoons].

Some of the blood will be sent to study researchers at the University of Washington to look for genetic, infectious and immune factors that affect the chances of becoming HIV-infected. Tests will also be done to look for resistance to HIV medications and other characteristics of early HIV infection. If we find that you have laboratory results that might be useful to your medical care, those results will be provided to you and your doctor.

- Some of the blood will be used to perform a CD4 count, a measure of your immune system.

- For women, we will perform a genital exam and obtain a swab from your genital area. These will be sent to study researchers at the University of Washington to test for HIV in the genital area.
- For men, we will ask you to provide a semen sample once, 3 months or later after HIV infection is detected.

- We will also request samples from your partner to help us understand HIV transmission from one person to another.

IMPORTANCE OF NOT SHARING THE STUDY MEDICATION

In this research study, you will take study medication each day. Your partner will not take any study medication. Your partner will come for follow-up visits every three months, for a total of 2-4 years. At your partner's visits to the research clinic, the study staff will ask your partner questions about his/her health and will collect samples of his/her blood and genital secretions. These samples will be used to investigate whether

there are factors that either increase or decrease the chances that HIV is transmitted from one person to another. They will also be used to determine whether some factors about the HIV infection your partner has increase or decrease the chance that the study medication you are taking works to prevent you from acquiring HIV infection.

It is very important that you do not share your study medication with your partner or with anyone else. Although tenofovir and Truvada® are used to treat HIV infection, they are only effective for treating people who already HIV infection if they are used in combination with other medications used for treating HIV infection. If your partner takes your study medication, he/she may be exposed to tenofovir or Truvada®. If he/she are exposed to tenofovir or Truvada®, his/her HIV could become resistant to tenofovir, emtricitabine (one of the components of Truvada®), or lamivudine (a medication similar to emtricitabine). Resistance to these antiretroviral medications reduce the effectiveness of HIV treatment for your partner and may limit your partner's HIV treatment options. If your partner requires HIV treatment during the course of this research study, we will refer him/her for appropriate treatment or, if treatment is not available elsewhere, provide treatment at the research clinic.

Hepatitis B infection

Hepatitis B is an infection that affects the liver. It can be transmitted sexually. Many people who become infected with hepatitis B clear the infection. Some fail to clear the infection and develop chronic hepatitis B infection.

If the results of your tests done at the Screening Visit show no evidence of chronic hepatitis B infection, you may have been previously infected but do not have persistent infection, you may have been previously immunized, or you may not be immune. If you are not immune, we will offer you hepatitis B vaccination as part of this study to protect you from getting hepatitis B in the future. If you do not want the vaccine or do not complete the three injections required for the vaccine to work completely, we will test you for hepatitis B later in the study and may ask you to provide additional blood samples (up to 8.5 cc/ less than 2 teaspoons) each month for 2 months.

SPECIMEN STORAGE AND USE OF SAMPLES AND DATA FOR FUTURE STUDIES

We would like to save data from this study and samples of your blood, urine, semen, and genital secretions at the Thika site's laboratory in Nairobi and at the University of Washington for future research by us and by other researchers. We will use these samples only for research to better understand how people get HIV, HIV-related diseases, and sexually-transmitted infections. This will include testing for genes which may affect whether a person is more or less likely to get these infections, have more severe infection, or how people respond to the medications used in this study. Gene studies may test for a specific gene to understand these effects or help researchers find new genes that may have these effects. This research is experimental and these tests are not useful for your clinical care. Before your samples leave the clinic, they will be assigned a code and your name will not be on them. Your name will be linked to the code only at this clinic and only for five years after the study is completed. After that time, the link between your name and the code on your data and samples will be

destroyed. An Institutional Review Board or Independent Ethics Committee, which watches over the safety and rights of research participants, must approve any future research studies using your data and samples. If you agree to store your samples, we will keep them for as long as there is sample that can be used for future research. If you do not want to have your samples saved for future research, you can still be in this study and your samples will be destroyed once testing for this study is completed. If you agree to store your samples now, but change your mind before the end of the study, let the study staff know and we will make sure that your samples do not get stored for future research. We will not sell your data or samples. Tests done on your samples may lead to a new invention or discovery. We have no plans to share any money or other benefits resulting from this invention or discovery with you.

RISKS AND/OR DISCOMFORTS

You may feel discomfort or pain when your blood is drawn. You may feel dizzy or faint. You may have a bruise where the needle goes into your arm. You may feel discomfort during genital exams.

You may become embarrassed, worried, or anxious when talking about your sexual practices, ways to protect against HIV and other infections passed during sex, and your HIV test results. You may become worried or anxious while waiting for your test results. If become infected with HIV, knowing this could make you worried or anxious. Talking about HIV and finding out your test results could cause problems between you and your partner. Trained counselors will help you and your partner deal with any feelings or questions you may have.

The study staff will make every effort to protect your privacy and confidentiality while you are having the study procedures. However, it is possible that others may learn of your participation here and, because of this, may treat you unfairly or discriminate against you. For example, you could have problems getting or keeping a job, or being accepted by your family or community.

Risks potentially related to the study medication

You may have symptoms or adverse effects while participating in the study. These symptoms or adverse effects may be due to participation in the study or due to illnesses that have no relation to the study, like a cold or flu. All persons who participate in this study will be watched carefully to monitor their health. The medical team in charge of your health care may give you medicines to treat the adverse effects. Many adverse effects disappear as soon after you stop taking the study pills. In some cases, the adverse effects can be serious, long-lasting, or may never disappear.

You should tell the doctor of the study clinic about any symptoms that you feel while you are participating in the study. If you have any symptoms, especially frequent vomiting, swollen feet, or abnormal shortness of breath, you should visit the clinic immediately and not wait for your next scheduled visit. You will be given a telephone number where the study doctors will be available 24 hours a day, 7 days a week. You should call them if you experience any serious symptoms.

The adverse effects that can occur in a small proportion of people taking tenofovir or

Truvada®® are well known because the medicationhas been used by many people. The following adverse events have been associated with taking tenofovir: fever, achiness and a general feeling of illness associated with allergic reaction. The following adverse effects have been associated with emtricitabine (one of the two medicines contained in Truvada®): headache, tiredness, difficulty sleeping, unusual dreams, skin darkening (palms and/or soles of the feet), incressed cough, runny nose and increased triglycerides in the blood (a substance derived from the metabolism of fat in your body).

Some mild adverse effects are expected to occur in up to 1 in 10 persons who take tenofovir or Truvada®. Other adverse effects are more serious, but are expected to occur in less than 1 in 100 persons who take tenofovir or Truvada®.

Occasional adverse effects (expected to occur in 1-10% of the participants in this study)

- Mild problems of kidney function that are only detected by laboratory tests (changes in creatinine and phosphate in the blood that do not cause you to feel different in any way)
- Lack of energy/general body weakness
- Upset stomach, vomiting, soft or liquid stools
- Abdominal pain
- More intestinal gas than normal
- Dizziness

Rare adverse effects (expected to occur in less than 1% of the participants in this study)

These adverse effects have been rarely observed in persons with HIV infection who are receiving tenofovir or Truvada®, plus other medications. It is not known whether these adverse effects will also occur in people like you, who do not have HIV infection and who are not taking other drugs for the treatment of HIV.

- Rash
- Liver function problems
- Serious kidney damage or failure
- Low phosphate levels (which is a chemical in the blood) or protein or sugar in the urine
- Inflammation or swelling and possible damage to the pancreas. The pancreas is an organ located in the abdomen that produces insulin and substances that aid in digestion.
- Allergic reaction
- Small changes in the mineral density of bones were observed in studies of HIV-infected people who were given tenofovir or Truvada®, plus other drugs used for HIV treatment. The changes in the mineral density of the bones did not cause any fractures, or other problems that bothered the patients. It is unknown if tenofovir or Truvada® can decrease the strength of bones in HIV-uninfected persons like you.
- Lactic acidosis has occurred in HIV-infected persons taking tenofovir or Truvada®, in combination with other drugs. Lactic acidosis is a condition that can produce shortness of breath, nausea, and liver failure. This is a serious adverse effect of some treatments used for HIV infection.
- **You should call or come to the study clinic if you have unexplained urination, weight loss, cramps, muscle pain, dizziness, excessive fatigue, nausea, vomiting, or shortness of breath. If you have these symptoms, or**

any other symptoms that concern you, the study staff will evaluate your symptoms and determine whether you should stop your study pills.

Tenofovir and Truvada® have been studied in many people so the possible adverse effects are well known. Still, it is possible that tenofovir or Truvada® may have rare adverse effects that are not yet recognized. The researchers will let you know if they learn anything that might make you change your mind about participating in the study. You should notify the study doctors if you feel that the study medication is causing you to have any symptoms.

Other medications

Many medications can be taken in parallel with the study medication used in this research study. However, some medications should not be taken while you are taking the study medication. If you take other medications during your participation in the study, there is a chance that we will need to obtain additional blood, undergo medical evaluations and laboratory tests. While taking some medications, you will need to temporarily stop taking the study medication. If so, you would continue to participate in the study visits in order to monitor your health. In addition, you should tell the team of researchers if you plan to enroll in any other clinical trial while you are participating in this study. When you visit the study clinic, we will provide you with a list of medications that you should not take while you are participating in this study, and if you authorize us to, we will send this information to any doctor that you consider necessary to be informed. Please ask your study doctor about these medications if you have any questions.

Unknown risks

The experimental treatments may have side effects that no one knows about yet. The researchers will let you know if they learn anything that might make you change your mind about continuing to participate in the study.

Risk of acquiring HIV infection and drug resistance

If you are receiving tenofovir or Truvada® during the study, we do not know if it will protect you from becoming infected with HIV. If you are receiving the placebo, we definitely know that this pill will not protect you from becoming infected with HIV. You may become infected with HIV during this study from your HIV-infected partner or from other sexual partners you may have. It is very important to use all the known risk reduction strategies to prevent the acquisition of HIV, like using condoms for all sexual relations and keeping your number of sexual partners low.

If you are receiving tenofovir or Truvada® and you become infected with HIV during the study, you could become infected with a strain of the HIV virus that could be resistant to tenofovir, emtricitabine (one of the components of Truvada®), or lamivudine (a medication similar to emtricitabine). These three medications are used for HIV treatment. Resistance to antiretroviral medications may make effective HIV treatment more difficult and may limit your treatment options. Resistance to antiretroviral medications can affect the response of the virus in such a way that the virus may

become resistant to tenofovir, emtricitabine, or to both. You will be able to discuss treatment and the generation of resistance to medications with the study doctor.

For more information about risks of this study, ask your study doctor.

BENEFITS

You may get no direct benefit from being in this study. **We do not know if tenofovir or Truvada® help HIV-uninfected individuals who have HIV-infected partners from getting HIV.** Plus, you may receive placebo, and not the tenofovir or Truvada® pills. Study staff will remind you of the importance of using condoms to protect against HIV.

You or others may benefit in the future from information learned in this study. You also may get some personal satisfaction from being part of research on HIV. This is true no matter what study group you are in.

You will get counseling and testing for HIV. You will get free condoms. If you are not immune to hepatitis B, you will be offered hepatitis B vaccination. If you or your partner have health problems that may be due to infections passed during sex, you will get medicine to treat them, if needed. For other health problems, the study staff will give you care and treatment that is available at the clinic. For care and treatment that is not available at the clinic, study staff will tell you about other places where care and treatment may be available.

The Thika site clinic will provide health care for you and your partner, while participating in this study. The study clinicians will provide contraceptives and medical treatments for common ailments such as malaria TB and others. However, for health care not available at the study clinic you will be referred to Thika district hospital, Kenyatta National Hospital or to a facility of your preference. Your partner will receive care and support related to his/her HIV infection while this study is ongoing. Depending on when you and your partner join the study, this care will be available to your partner for up to 2-4 years. Medications used to treat HIV will be given per national guidelines, and if needed this care will be provided either by the study clinic or by a referral clinic if needed.

If you become infected with HIV while in this study, you will be offered counseling and clinical services for HIV while the study is ongoing. After the study is over, the study staff will no longer be able to provide this care to you or your partner. You and your partner will be referred to other HIV care programs that are available to you.

NEW FINDINGS

You will be told any new information learned during this study that is important for your health or might cause you to change your mind about staying in the study. You will be told when the results of the study may be available, and how to learn about them.

COSTS TO YOU

There is no cost to you for being in this study. Treatments available to you from the study will be given free of charge.

REASONS WHY YOU MAY BE WITHDRAWN FROM THE STUDY

You may be removed from the study without your consent for the following reasons:

- The study is stopped or canceled.
- The study staff feel that staying in the study would be harmful to you.
- You are not able to attend study visits or complete the study procedures.

ALTERNATIVES TO PARTICIPATION

We do not know if tenofovir or Truvada® work to prevent HIV-uninfected persons from getting HIV from their HIV-infected partners. The only way to prevent getting HIV is to use a condom every time you have sex.

There may be other studies going on here or in the community that you or your partner may be eligible for. If you wish, we will tell you about other studies that we know about. There also may be other places where you can go for HIV counseling and testing. We will tell you about those places if you wish.

REIMBURSEMENT

You will receive money to cover your transport costs for traveling to the study clinic for each scheduled study appointment and an additional 200 Ksh for your time and effort spent at the clinic.

CONFIDENTIALITY

Efforts will be made to keep your personal information confidential. However, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law. Any sample from you or information about you will be identified only by code. The link between your name and code will be kept in a secure location at the clinic only. We will not discuss any information about you with your partner unless you give written permission, and will encourage you to be present during the discussion. Any publication of this study will not use your name or identify you personally.

Your study records may be reviewed by study staff and representatives of:

- the University of Washington, including study monitors
- the Bill and Melinda Gates Foundation
- the United States Food and Drug Administration
- Kenyatta National Hospital Ethics and Research Committee(KNH ERC)
- National Council of Science and Technology (NCST)
- Pharmacy and Poisons Board of the Ministry of Health (PPB)

RESEARCH-RELATED INJURY

The study staff will monitor your health and the health of your partner while you are in this study.

You will have a study visit every month. Your partner will have a visit every 3 months. If you or your partner have any health problems between visits, please contact the study staff. If you have a medical emergency that requires immediate care, please visit the nearest health facility and contact the Thika study clinic as soon as possible on, Tel: 067 21305 or the 24-hour emergency number: 0736464299.

If you are injured from participating in this study, you will be offered care at the study clinic, free of charge. It is important that you tell the members of team of researchers at this clinic if you feel that you have been injured because of taking part in this study.

There is not a program of monetary compensation through this institution. If you require medical care that that the study clinic cannot provide, the study doctors will refer you to the appropriate services or organizations that can provide care for the injury. If the study doctors determine that the injury is a consequence of your participation in the study, study funds will be used to pay for the medical care that you need.

You do not give up any legal rights by signing this consent form.

PROBLEMS OR QUESTIONS

If you ever have any questions about this study, you should contact Kenneth Ngure at Thika Partners in Prevention Study clinic, Tel 06721305/22561. If you have a research related injury you should call the 24-hour emergency number: 0736464299.

If you have questions about your rights as a research participant, you should contact Prof. Bhatt the chair of the Kenyatta National Hospital Ethics and Research Committee,. at 2726300 Ext. 43524

STATEMENT OF CONSENT AND SIGNATURES

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

Participant Name (print)	Participant Signature/Thumbprint	Date
Study Staff Conducting Consent Discussion (print)	Study Staff Signature	Date
Witness Name (print)	Witness Signature	Date

CONSENT FOR OFF-SITE STUDY PROCEDURES.

It may be necessary for the members of the team of researchers of this clinic to visit you at your home or another location as part of the study. Some of the scheduled study visits may take place at your home.

The study personnel will explain in greater detail the requirements to do these visits (like the conditions of the place, the type of visit and the duration of it) and the procedures to maintain your information in a confidential manner. However it is important that you know that home visits may eventually affect your confidentiality even if the study staff take precautions not to disclose the purpose of the visits.

To do this we will need you to authorize us to do so, please read carefully the following statement and initial and date one option. Choosing not to be visited outside of the study clinic will not affect your participation in this study.

Please make your mark

_____ I DO agree to be visited at a site other than the study clinic

_____ I DO NOT agree to be visited at a site other than the study clinic

Participant Name (print)	Participant Signature/Thumb print	Date
Study Staff Conducting Consent Discussion (print)	Study Staff Signature	Date
Witness Name (print)	Witness Signature	Date

SPECIMEN STORAGE AND USE OF YOUR DATA AND SAMPLES FOR FUTURE STUDIES:

Please make your mark

_____ I DO agree to store my data and samples for future research into HIV, HIV related diseases and other sexually transmitted diseases

_____ I DO NOT agree to store my data and samples for future research into HIV, HIV related diseases and other sexually transmitted diseases

Participant Name (print)	Participant Signature/Thumb print	Date
Study Staff Conducting Consent Discussion (print)	Study Staff Signature	Date
Witness Name (print)	Witness Signature	Date

UNIVERSITY OF NAIROBI/KENYATTA NATIONAL HOSPITAL ETHICS AND RESEARCH COMMITTTE
HOSPITAL ROAD ALONG NGONG ROAD
P. O. BOX 20723 -00202
NAIROBI
TELEPHONE 2726300 Extension 43524
CHAIRPERSON: PROFESSOR K.M. BHATT

UNIVERSITY OF WASHINGTON HUMAN SUBJECTS DIVISION
3045 15TH AVE. NE
SEATTLE WA
BOX 35142
TELEPHONE 011-1-206 543-0098

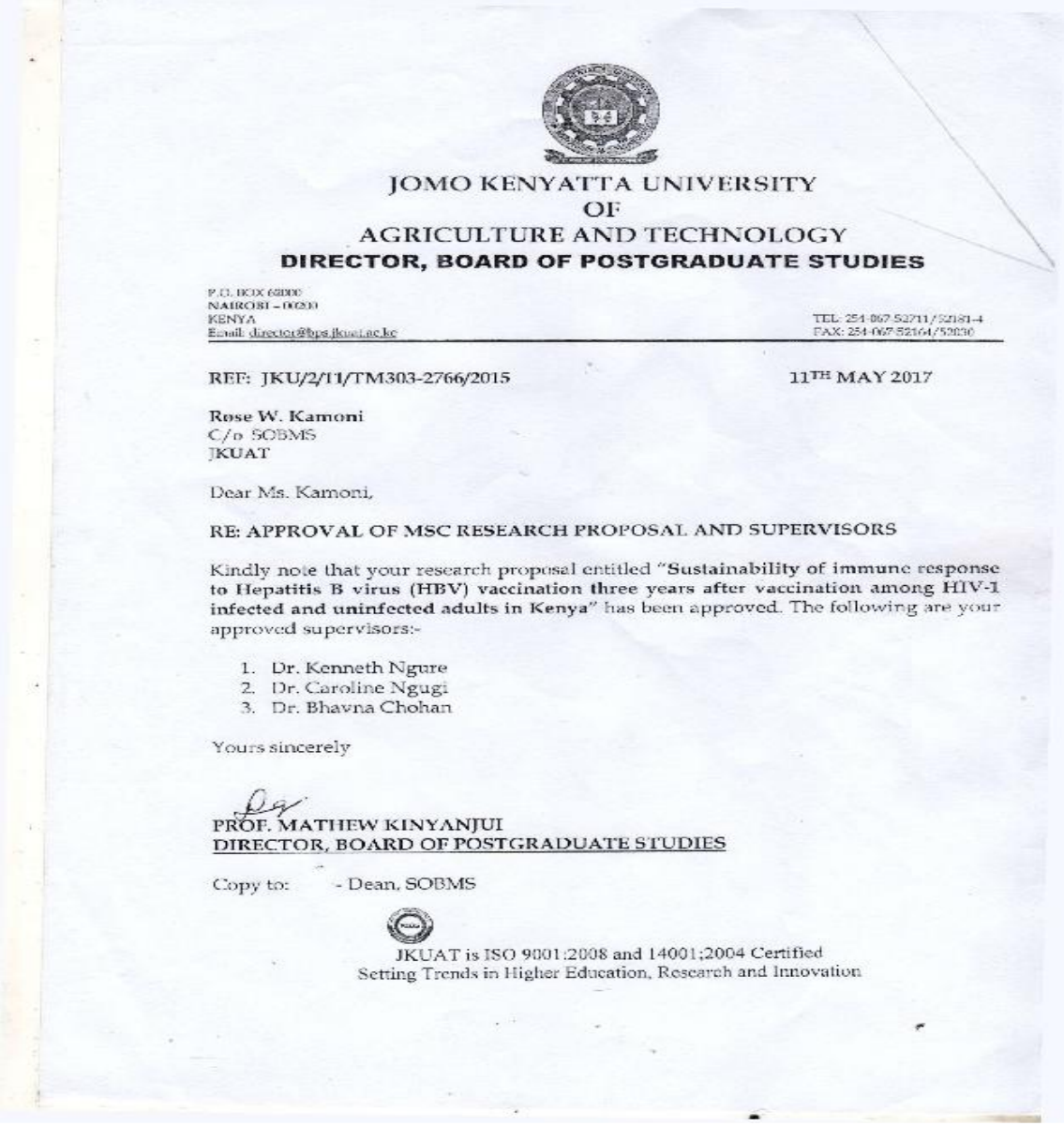
Copies to: 1. Investigators
2. Study participant

APPROVED
SEP 30 2009
UW Human Subjects
Review Committee

Study Enrollment Comprehension Questionnaire for Partner (HIV-1 Uninfected) Participants

		True	False	Don't know
1	The study seeks to find out if taking 2 pills daily can prevent the acquisition of HIV.			
2	This study also seeks to find out if it is safe for HIV-uninfected men and women to take a pill daily to prevent the acquisition of HIV.			
3	Once you initiate your participation in the study, you will be assigned to take either tenofovir, Truvada, or placebo.			
4	Tenofovir and Truvada have been authorized for use in the treatment of HIV and not for the prevention of HIV.			
5	You and the study staff will not be able to tell whether you have been assigned tenofovir, Truvada, or placebo.			
6	Once you begin your participation in the study, we will ask that you come to our clinic for monthly visits, for the next approximately 24 to 48 months.			
7	(women only) If you become pregnant during the study, you will stop taking the study medication. We do not know the effects of tenofovir and Truvada taken during pregnancy.			
8	(women only) If you are breastfeeding now, you will not be allowed in the study. If you decide to start breastfeeding during the study, you will stop taking the study medication. We do not know the effects of tenofovir and Truvada taken during breastfeeding.			
9	It is very important that you do not share your study drug with anyone, including your partner.			
10	Participating in this study will prevent you from getting HIV infection.			
11	There are many possible side effects that could occur as a result of taking the study medications. One of these is that the study medications may affect the function of the kidneys.			
12	You can come to this clinic at any time if you feel that you have a health problem. If you need more specialized care, the study staff will arrange for you to receive the needed medical attention.			
13	If you develop a health problem potentially related to the study medication, the study staff may instruct you to stop the medication, either temporarily or permanently.			
14	If during the study you have a positive test result for HIV, you will cease using the study medication. You will continue to receive medical attention.			
15	If you do not wish to participate in this study, you will not lose any benefit offered by this institution.			
16	You can go to the Ethical Review Committee to provide any comment or complaint that you have regarding your participation in the study.			
17	The study can be terminated at any moment if the investigators and authorities that regulate the conduct of this study decide this.			
18	If you need, there is a telephone number available 24 hours a day for you to call and contact a study physician.			
19	You cannot withdraw from the study once you have agreed to participate in it.			
20	Using a condom every time you have sex will reduce your chances of getting HIV.			

Appendix IV: Approval letter of research proposal and of supervisors from Director, Board of Post- graduate, JKUAT.



JOMO KENYATTA UNIVERSITY
OF
AGRICULTURE AND TECHNOLOGY
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

P.O. BOX 62000
NAIROBI – 00200
KENYA
Email: director@bps.jkuat.ac.ke

TEL: 254-067-52711/52181-4
FAX: 254-067-52164/52030

REF: JKU/2/11/TM303-2766/2015

11TH MAY 2017

Rose W. Kamoni
C/o SOBMS
JKUAT

Dear Ms. Kamoni,

RE: APPROVAL OF MSC RESEARCH PROPOSAL AND SUPERVISORS

Kindly note that your research proposal entitled "**Sustainability of immune response to Hepatitis B virus (HBV) vaccination three years after vaccination among HIV-1 infected and uninfected adults in Kenya**" has been approved. The following are your approved supervisors:-

1. Dr. Kenneth Ngure
2. Dr. Caroline Ngugi
3. Dr. Bhavna Chohan

Yours sincerely

PROF. MATHEW KINYANJUI
DIRECTOR, BOARD OF POSTGRADUATE STUDIES

Copy to: - Dean, SOBMS

JKUAT is ISO 9001:2008 and 14001:2004 Certified
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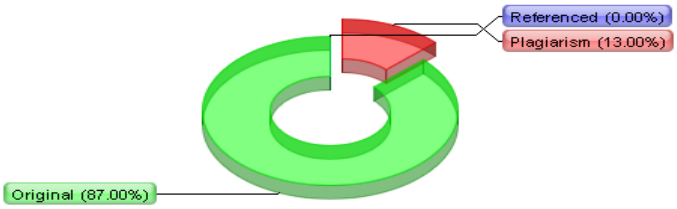
Appendix V: Plagiarism Report

Plagiarism Detector v. 1605 - Originality Report 26-Nov-20 13:28:55

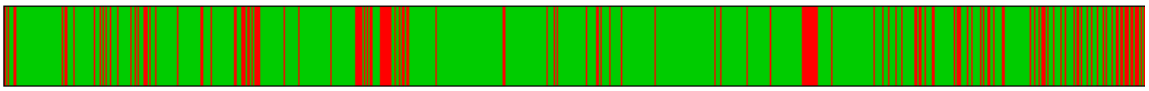
Analyzed document: ROSE WANJIKU KAMONI MSc Final.doc Licensed to: Jomo Kenyatta University of Agriculture and Technology

Comparison Preset: Word-to-Word. Detected language: English

Relation chart:



Distribution graph:



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No URLs detected

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AppendixVI: Published Manuscript

JMSCR·Vol||07||Issue||08||Page·798-801||August¶ 2019¶

http://jmscr.igmpublication.org/home/
ISSN (e)-2347-176x ISSN (p) 2455-0450

Research Article

crossref **DOI:** https://dx.doi.org/10.18535/jmscr/v7i8.136

IGM Publication

Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Sustainability of immune response to Hepatitis B Virus vaccination 3 years post vaccination among HIV-1 infected and uninfected adults in Kenya

Authors

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Abstract

Background: Hepatitis B virus (HBV) infection, a leading cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma worldwide is preventable by vaccination. Completion of recommended vaccination over 90% of adults develops protective anti-Hbs antibodies levels. However, there's paucity of data on sustained immune response to HBV vaccine among HIV infected African adults. A retrospective study was conducted and analysed 336 archived serum samples collected 3-years post HBV

vaccination from participants enrolled in the Partners PrEP study, for Hepatitis B surface antibody (anti-Hbs) using ELISA.Samples that didn't have protective anti-Hbs titers were further tested for Hepatitis B surface antigen (HBsAg). Univariate logistic regression was used to determine factors associated with non-response. Of the 336 samples tested, 176 (52.4%) were from HIV-1 infected, 40 (22.7%) were male. 160 samples from HIV-1 uninfected, 125 (78.1%) were male. The mean (standard deviation) age of the study population was 34.6 (8.5) years. Of the 62 (18%) who didn't have protective anti-Hbs titers, 50 (81%) were HIV-1 infected. HIV infected were more likely to have less protective anti-Hbs titers ($p<0.001$) compared to HIV uninfected. Compared to men, women were more likely not to have protective anti-Hbs levels (11.5% vs. 25.1%, $p=0.002$). Seven (11.3%) of the 62 samples that didn't have protective anti-Hbs titres, also tested positive for HBsAg, all were HIV-1 infected. More than a quarter of HIV infected vaccinated against HBV didn't have protective anti-Hbs titres, some acquired HBV infection. Regular testing for immune response to HBV vaccination among HIV infected should be considered.

Keywords: *Hepatitis B Virus, Vaccine, HIV-1 infection, Kenya.*

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titis B virus (HBV) infection, a leading cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma worldwide, is more common among HIV–infected individuals due to shared risk factors for viral acquisition[1] . Current statistics estimate that 10% of 34 million HIV-infected patients have concurrent chronic HBV infection[3]. Prevalence and incidence of HBV infection among HIV-infected patients varies widely based on the risks for HIV and HBV transmission, implementation of HBV vaccination programs, and the geographic regions with different levels of endemicity of HBV infection in the general population[1,4]. Even so, Africa which has the highest HIV-1 disease burden globally has high endemicity for HBV infection with 50 million of the 360 million people infected with HBV worldwide living in Africa[5].

Countries in Asia and sub-Saharan Africa have high HBV endemicity with vertical and early childhood exposure being the most common modes of transmission respectively; similarly the prevalence of HBV among HIV-infected individuals in these areas is higher at an estimated 20-30%[5]. Studies have reported that men infected with both HIV and HBV have liver-related mortality that is eight times higher than that in men with HIV alone and 17 times higher than in those with HBV alone[4]. This is because HIV infection adversely affects all phases of HBV infection by increasing the risk of chronic infection, decreasing the rate of hepatitis B e-antigen clearance, increasing virus replication, accelerating the loss of HBsAb, and increasing the risk for cirrhosis and hepatocellular carcinoma [8].Vaccination is the best method of prevention against HBV and following complete immunization more than 95% of infants and children and more than 90% of adults achieve protective anti-Hbs titres greater than 10 mIU/mL[9]. International guidelines released by the National Institutes of Health (NIH), Centers for Disease Control and Prevention (CDC), HIV Medical Association and the US Advisory Committee on Immunization Practices in the United states and the European AIDS Clinical Society and the British HIV Association in Europe[10,11] recommend that all individuals with HIV who are susceptible to HBV should be vaccinated with the 3 dose primary vaccine series given during a 6 month period[4,13]. Studies done in high income countries on

HBV vaccination have suggested that compared to HIV uninfected persons, HIV infected persons have diminished responses to HBV vaccination. One study found that 20%–70% of HIV-infected persons developed an immune response to HBV vaccination[14]. A US-based HIV outpatient study reported that only 37% of eligible HIV infected patients who completed the vaccination series achieved protective antibody titers[12]. A study conducted among HIV infected adults in Kenya found that non response to HBV vaccine at one year post vaccination was higher among HIV infected participants, compared to HIV uninfected participants and revaccination of initial non-responders resulted in a higher overall response to revaccination[5]. Data from low–income countries for determination of long-term immune protection of HBV vaccine in adults are few. This study aimed to compare the levels of HBV antibody titers in both HIV infected and HIV uninfected adults in Kenya, 36-month post HBV vaccination and also determine incident Hepatitis B infection, among individuals with low-levels of protective antibodies post-vaccination.

Materials and Methods

Study population

This was a retrospective study conducted among HIV infected and HIV uninfected men and women attending a research clinic in Thika, Kenya who took part in the Partners PrEP Study[15]. In brief, the Partners PrEP Study was a phase III, multisite, randomized, double blind, and placebo controlled trial of daily oral tenofovir-based pre-exposure prophylaxis (PrEP) for the prevention of HIV acquisition. All participants were members of heterosexual HIV serodiscordant couples (i.e. couples in which one member was HIV seropositive and the other seronegative) in which the HIV infected partner did not meet national guidelines for initiation of antiretroviral therapy and the HIV uninfected partners were healthy, with normal renal, liver and haematological function. They were randomized to daily oral tenofovir, emtricitabine-tenofovir, or matching placebo with monthly follow-up for 36 months[15].

Sampling procedures

Stored serum samples obtained 36 months post vaccination from participants, who had developed a positive antibody response either during initial vaccination and/or after revaccination were retrieved and thawed[5]

Hepatitis B surface antibody and antigen ELISA

We analysed 336 archived serum samples. As part of screening procedures for the Partners PrEP Study, all potential trial participants were assessed for HBV by use of HBV surface antigen (HBsAg; Murex Abbot Murex, Dartford). Study participants who tested negative to presence of HBsAg and HBsAb were offered vaccination with 3 doses of 20 µg recombinant HBsAg (Euvax B [LG Life Sciences, Seoul, Korea], Revacc B [Bharat Biotech International, India], or Shanvac B [Shantha Biotech, India], depending on supplier availability) at 0, 1 to 3, and 6 months.

All the 336 samples were tested for presence of anti-Hbs titre using enzyme linked immune absorbent assay (ELISA) (DiaSorin LIAISON anti-HBs II assay, Italy) kit. In this, a sandwich ELISA involving primary and secondary anti-HBV were used to detect antibodies against hepatitis B surface antigen (anti-Hbs) in serum. Hundred (100) micro liters (µl) of incubation buffer was assigned into micro Elisa wells except for the blank well. 100 µl of undiluted samples were added into to the designated wells after dispensing the Control and Calibrators and incubated at 37 degrees Celsius for 120 minutes. Washing was done using the phosphate buffer for five times and hundred (100)µl of the working enzyme tracer solution was added into each well except the blank well and incubated at 37 degrees Celsius for 60 minutes. Second washing was done using the phosphate buffer for five times and 100 µl chromogen/substrate solutions was added into each well. The plate at this point was incubated at 37 degree Celsius for 30 minutes at room temperature, away from direct light. Thereafter the reaction was stopped by adding sulfuric acid into each well and the plates were read at 450 nm wavelength. Sixty two samples that had levels of anti-Hbs antibody titres less than 10 mIU/ml, were further tested for Hepatitis Surface antigen (HBsAg). It was done with an ELISA technique, using the HBsAg; Murex Abbot Murex, Dartford, (United Kingdom), kit. In this, a sandwich ELISA involving primary and secondary anti-HBV were used to detect HBV surface antigen in serum. Twenty five (25) micro liters (µl) of specimen

diluents was assigned into micro Elisa wells. 75 µl of undiluted sample was added and incubated at 37 degrees Celsius for 60 minutes. Fifty (50)µl of the conjugate solution was added into each well and incubated at 37 degrees Celsius for 30 minutes. Washing was done using the phosphate buffer for five times and 100 µl substrate solution added into each well. The plate at this point was incubated at 37 degree Celsius for 30 minutes. Thereafter the reaction was stopped by adding sulfuric acid into each well and the plates were read at 450 nm wavelength.

Antibody titers were reported as dichotomous positive/negative with titers <10mlU/ml being considered negative. Similarly, HBsAg results were reported as dichotomous positive/negative with those that tested positive for presence of HBsAg being interpreted as HBV infected.

Statistical analysis

Baseline factors associated with non response to HBV vaccination were assessed using univariate logistic regression methods. The variables evaluated included sex, education, age, income, BMI and alcohol use. Data analysis was done using STATA® version 13 software. All statistical tests were interpreted at 5% level of significance.

Ethical considerations

Approval to conduct the study was granted by the Kenyatta National Hospital-University of Nairobi Ethical Review Committee, approval no.P685/11/2014. The Partners PrEP study was approved by the institutional review boards of the University of Washington and Kenyatta National Hospital, approval no. P322/10/2007.

Results

Of the 336 serum samples analysed, 176 (52.4%) were from HIV infected participants and of those 136 (40.5%) were female. The mean (standard deviation [SD]) age of the study participants was 34.6 (8.5) years. The mean (SD) CD4 cell count for the HIV infected participants was 526 (243) cells/µL (Table 1).

Factors associated with less anti–Hbs titres in circulation 36 months' post vaccination

At 36-months post HBV vaccination, 274 (81.5%) participants had protective levels (>10 mIU/ml) of anti-Hbs antibody in the blood, while 62 (18.5%) had levels of anti-Hbs antibody titres less than 10 mIU/ml. Of the participants with <10 mIU/ml titres, 50 (80.7%) were HIV infected and 43 (69.4%) were female. HIV infected persons compared to HIV uninfected were more likely to have a non-response to HBV vaccination at 3 years post vaccination (28.4% vs 7.5%, OR = 4.9, 95% CI 2.5, 9.6 p <0.001). (Table 2). Similarly, compared to men, women were more likely to have low anti-Hbs titres (11.5% vs 25.1%, OR 2.58, 95% CI 1.43, 4.66, p=0.002). Among HIV infected participants, those with CD4 count greater than 500 cells/µL were less likely to have low titres compared to those with less than 500 cells/µL (20.8% vs 34.3%, OR = 0.5, 95% CI 0.25, 0.99, p =0.048).

Factors associated with positive HBsAg

Of the 62 serum samples that tested low for anti-Hbs titers, seven (11.3%) participants tested positive for presence of HBsAg. All samples testing positive for HBsAg were for HIV infected participants, six were female and four were aged greater than 30 years. Participants with any monthly income, compared to those with none were less likely to test positive for HBsAg (4.8% vs 25.0%, OR 0.15, 95% CI 0.03, 0.86, p = 0.03). (table 3). Baselines, CD4 count, gender, years of education and age were not associated with increased risk of acquisition of Hepatitis B infection.

Table 1: Participants baseline demographic characteristics

Characteristics N (%) or mean (SD)	HIV infected(n = 176)	HIV uninfected (n=160)
Age	33.32 (8.17)7).17)	35.91 (8.61)
Sex, female	136 (77.27)	35 (21.88)
Number of children At least one child	161 (91.5)	136 (85)
Years of education Less than 8 years	58 (33)	65 (40.6)
>=8 years	118 (67)	95 (59.4)

Monthly income <=1000 Between 1001-5000 Between 5001-10000 >10000	81 (46) 61 (34.7) 21 (11.9) 13 (7.4)	38 (23.8) 83 (51.9) 24 (15) 15 (9.4)
Alcoholic drinks per week No alcohol use	165 (93.8)	141 (88.1)
BMI		
<18.5	28 (8.3)	14 (4.2)
18.5 – 24.9	100 (29.8)	116 (34.5)
25.0 – 29.9	22 (6.5)	35 (10.4)
>30.0	10 (3.0)	11 (3.3)
CD4 count	526.24 (243.45)	

Table 2: Factors associated with less anti–Hbs titres in circulation 36 months' post vaccination

Characteristics N(%) or mean (SD)	HBsAb positive (n=274)	HbsAb Negative (n = 62)	OR (95% CI)	P value
HIV status			Ref	
Uninfected	148(92.5%)	12(7.5%)	4.9(2.5,9	**<0.001**
Infected	126(71.6%)	50(28.4%)	.6)	
Sex			Ref	
Male	146(88.5%)	19(11.5%)	2.58(1.4	**0.002**
Female	128(74.9%)	43(25.1%)	3,4.66)	
Age (years)			Ref	0.33
18-30 years	84(78.5%)	23(21.5%)	0.75(0.4	
>30 years	190(83.0%)	39(17.0%)	2,1.33)	
BMI			Ref	
<18.5	35(83.3%)	7(16.7%)	1.14(0.4	0.78
18.5-24.9	176(81.5%)	40(18.5%)	7,2.274)	0.91
25.0-29.9	47(82.5%)	10(17.5%)	1.06(0.3	0.50
>30	16(76.2%)	5(23.8%)	7,3.07)	
			1.56(0.4	
			3,5.68)	
CD4 (cells/ml)			Ref	
<500	65(65.7%)	34(34.3%)	0.50(0.2	**0.048**
>500	61(79.2%)	16(20.8%)	45,0.99)	
Number of children			Ref	
None	34(87.2%)	5(12.8%)	1.62(0.6	0.34
Any	240(80.8%)	57(19.2%)	0,4.31)	
Years of education			Ref	
<8 years	103(83.7%)	20(16.3%)	1.26(0.7	
>8 years	171(80.3%)	42(19.7%)	0,2.27)	0.43
Monthly income			Ref	
None	59(74.7%)	20(25.3%)	0.58(0.3	0.07
Any	215(83.7%)	42(16.3%)	1,1.06)	
Alcohol use per week			Ref	
None	247(92.5%)	20(7.4%)	0.47(0.1	0.22
Any	27(39.1%)	42(60.9%)	4,1.59)	

*CD4 count for HIV infected only

Table 3: Factors associated with positive HBsAg among those with less than 10mlU/ml anti-Hbs titres 36 months' post vaccination (N = 62)

Characteristics N(%) or mean (SD)	HBsAg positive (n = 7)	HBsAg negative (n = 55)	OR (95% CI)	P value
HIV status				
Uninfected	0 (0%)	12(100.0%)	Ref	
Infected	7 (14.0%)	43(86.0%)	-	-
Sex				
Male	1(5.3%)	18(94.7%)	Ref	
Female	6(14.0%)	37(86.0%)	2.92 (0.33, 26.10)	0.34
Age				
18-30 years	23(13.0%)	20(87.0%)	Ref	
>30 years	4(10.3%)	35(89.7%)	0.76 (0.15, 3.75)	0.74
CD4 count				
<500	6(17.6%)	28(82.4%)	Ref	
>500	1(6.3%)	15(93.7%)	0.31 (0.03, 2.83)	0.3
Number of children				-
None	0 (0%)	5 (100.0%)	Ref	
Any	7 (12.3%)	50 (87.7%)	-	
Years of education				
<8 years	2 (10.0%)	18 (90.0%)	Ref	
>8 years	5 (11.9%)	37 (88.1%)	1.22 (0.21, 6.89)	0.83
Monthly income	5(25.0%)	15(75.0%)	Ref	
None	2(4.8%)	40(95.2%)	0.15 (0.03 ,0.86)	
Any				**0.03**
Alcohol use per week				
None	6 (10.2)	53(89.8)	Ref	
Any	1 (33.3)	2 (66.7)	4.42 (0.35, 56.26)	0.25

*CD4 count for HIV infected only

Discussion

Three years post HBV vaccination almost one in five of the samples from study population did not have adequate protective levels of anti-Hbs titres. We found that eighty percent of those not having protecting anti-Hbs titres were HIV infected individuals. Our findings are similar to others that reported better response to hepatitis B vaccination among HIV negative persons compared to HIV infected persons[7,13,17]. We found a slightly lower response among HIV-1 negative participants compared to other studies that had reported a greater than 95% sustained of immune response after HBV vaccination among HIV uninfected persons[14]. However, these studies assessed immune response at one year. We also observed a higher proportion (72%) of HIV-infected individuals with protective antibody titres at 3 years compared to other studies [14,16]. This is likely due to the booster HBV vaccine given to participants who did not respond to the initial 3-series of HBV vaccination. All participants that received repeat vaccination after having failed the initial series had anti-Hbs titres greater than 10mlU/ml (data not shown). Studies have reported improved immune responses and outcomes to hepatitis B vaccine among HIV infected persons who received booster vaccines compared to those who only received an initial 3-dose series of HBV vaccine[4,5,13]. Our study found those with low anti-Hbs titres were HIV infected, had low CD4 count and of female gender. This is similar to other studies conducted in adult African population, which reported gender and low CD4 count as being predictors of poor immune response to hepatitis vaccination[5,16]. Interestingly, an earlier study conducted in this cohort, had reported male gender as a predictor of non-response to hepatitis B immune response 6 months post initial HBV vaccination[5]. The high risk of incident HBV infection was found among HIV infected individuals and those who had no income. Low socio-economic status has been associated with increased risk of Hepatitis B infection[2]. Our study has demonstrated reduced immune response to HBV vaccination among African HIV infected persons 36 months post vaccination. Our findings can guide policies to develop new vaccination strategies or provide booster vaccines, as well as need for continuous HBV vaccine immune response monitoring among HIV infected people.

Limitations

A limitation of this study was lack of HIV-1 viral load measurements among HIV infected persons which have also been associated with immune response to HBV vaccination[5].

In conclusion, we showed that in a cohort of adults there was sustained immune response to hepatitis B vaccine 36-month post vaccination among both HIV infected and uninfected participants. However, the reduction in immune response was greater among adults living with HIV infection. Fourteen percent of those with low titres were found to have HBV infection. Due to the waning immune response to hepatitis B vaccine observed in this study, regular evaluation of immune response to HBV vaccination especially among HIV infected persons and administration of booster doses to those with reduced immunity is recommended.

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Potential conflict of interest. All authors report no conflict

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