THE PREVALENCE OF HEPATITIS B AND C VIRUSES AMONG HIV SERO-DISCORDANT COUPLES IN KISUMU, KENYA

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The Prevalence of Hepatitis B And C Viruses Among Hiv Sero-Discordant Couples in Kisumu, Kenya

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DECLARATION

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

Signature: …… Date: ……..

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

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DEDICATION

This work is dedicated to my loving wife Stellah Chepkorir Kagume, sons Adrian Njenge, Alvin Kabangi, Austin Wachira, my parents Mr. Stephen Njenge and Mrs. Mary Wambui Njenge and my brothers and sisters Immaculate Muringo Njenge, Phylis Mugure Njenge, Benjamin Kabangi Njenge and Augustine Victor Wachira Njenge for their support and motivation during this study.

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ABSTRACT

Human Immunodeficiency Virus-1 (HIV-1) sero-discordant couples are at risk of transmitting HIV, Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) to each other. Hepatitis C Virus and Hepatitis B Virus are common among HIV-infected persons in Africa. There are many HIV sero-discordant couples in Kenya who are at risk of transmitting to each other STIs including HIV, HCV and HBV due to shared modes of transmission. Although infection with HCV and HBV is common among HIV-infected persons, their prevalence and risk factors and relationship to HIV infection are not well characterized among HIV-discordant couples in Kenya. This cross-sectional study evaluated the prevalence of HBV and HCV infection and associated risk factors among 270 heterosexual HIV sero-discordant couples. Written informed consent was obtained prior to enrollment of study subjects. Serum samples and data from questionnaires at baseline were obtained from the Phase III randomized placebo-controlled trial of acyclovir for Herpes Simplex Virus-2 (HSV-2) suppression to prevent HIV transmission ssssamong HIV sero-discordant couples site in Kisumu County. Hepatitis B surface antibody and anti-HCV antibody testing was conducted using third generation HBV and HCV Enzyme linked immunosorbent assay (ELISAs). The serum samples were analyzed for Hepatitis B surface antigen, antibodies to Hepatitis B core antigen and HCV Immunoglobulin G (IgG) antibodies. STATA statistical software was used for analyzing the data. Chi-square was used to assess the association among categorical variables while independent t test was to assess the difference in groups with continuous outcome. The prevalence of HBV and HCV was not associated with age, gender, income, education, number of children or years lived together and use of contraception. Among the HIV-1 discordant couples studied, 397 (74.9%) of the couples did not have HBV or HCV infection, 88 (16.6%) had HCV mono-infection, 45 (8.5%) had HBV mono-infection and 9 (1.7%) had HCV/HBV/HIV co-infection. Hepatitis C Virus prevalence was associated with the HIV-1 infection. Since the prevalence of HBV was above that of the general population such as blood donors, HBV prevention measures

should be encouraged among HIV-1 sero-discordant couples to reduce HBV transmission rates among couples. All HIV-1 positive patients should be tested for both HBV and HCV, since HIV positive patients are likely to have HBV or HCV co-infection.

CHAPTER ONE

INTRODUCTION

1.1 Background

There are about 338,000 HIV-1 sero-discordant couples reported in Kenya (Kaiser *et al*, 2011). Infection with Hepatitis C Virus (HCV) and Hepatitis B Virus (HBV) is common among HIV infected persons due to their shared modes of transmission (Liu and Hou, 2006; Ashraf *et al*, 2010; Chen *et al*, 2011) and this represents an increasingly important public health problem (Maddrey, 2000).

Partners in HIV-1 sero-discordant couples are at risk of transmitting to each other STIs such as HBV and HCV which are common in HIV infected patients. Human Immuno-deficiency Virus negative individuals in sero-discordant relationships may be at increased risk of HIV sero-conversion following infection with other STIs including HCV and HBV (Rufagari *et al*, 2006). If a person living with HIV has an STI, then inflammation will activate and recruit more immune cells to the infected genitals or rectum. Some of the immune cells in a person living with HIV are already infected with HIV; therefore the inflammatory response brings more HIV (contained in the infected immune cells) to the site of the STI in the genitals or rectum. Consequently, more HIV enters the body fluids in that area, thus increasing the possibility of HIV being transmitted to the HIV negative person (James, 2012).

Human Immunodeficiency Virus positive individuals with chronic HBV and HCV co-infection tend to experience more rapid liver disease progression than HIV negative people (Highleyman, 2009) and treatment of viral hepatitis due to dual HBV/HCV infection represents a challenge (Liu and Hou, 2006). Some preliminary studies showed that patients with dual HBV and HCV infection had responded poorly to interferon monotherapy (Liu and Hou, 2006). Hepatitis C may affect the course and management of HIV infection (Mark and David, 2003). Co-infection also results in higher HBV

associated liver morbidity, faster progression to cirrhosis, and more frequent flares of hepatic transaminases which occur with immune reconstitution syndrome or due to interruption of Anti-retroviral therapy (Thio *et al,* 2002; Lacombe and Rockstroh, 2012).

Although rates of coinfection with at least two of HBV, HCV and HIV are well reported in numerous studies from Europe and America, there are few data on the prevalence of coinfection in African populations (Harania *et al*, 2008). Several studies have been conducted in Kenya to determine prevalence and risk factors for HBV and HCV in different populations such as volunteer blood donors and clients at Voluntary Counseling and Testing (VCT) centers (Karuru *et al*, 2005), Injection Drug Users (IDUs) (Mark *et al*, 2015, Muasya *et al*, 2008), HIV patients (Muriuki *et al*, 2013), jaundice patients (Ochwoto *et al*, 2016) and pregnant women Okoth *et al,* 2006) but none has been conducted among HIV Sero-discordant couples.

Although infection with HCV and HBV is common among HIV-infected persons, their prevalence, risk factors and relationship to HIV infection are not well characterized among HIV-discordant couples in Kenya. As a result, the study sought to evaluate the potential risk factors associated with coinfections and determine the prevalence of HCV and HBV among HIV sero-discordant couples in Kisumu County, Kenya.

1.2 Statement of the problem

Partners in HIV-1 sero-discordant couples are at risk of transmitting to each other HIV and other viral STIs such as HBV and HCV which are common in HIV infected patients and share the same modes of transmission. In addition, HBV prevalence is high in sub-Saharan Africa and HCV-related liver disease is becoming an important cause of morbidity and death among HIV infected patients. Majority of HBV, HCV studies conducted have focused on HIV patients, blood donors, general population and no study has been conducted focusing on HIV sero-discordant couples. There is need to establish

the prevalence and risk factors of HCV and HBV among HIV-1 sero-discordant couples since these arenot well characterized in Kenya.

In different parts of the world the rate of Chronic HBV infection varies from 0.1 to 20% and every year about 600,000 - 1,000,000 people die from hepatitis related disorders (Fattovich, 2003; Nice, 2013). The prevalence of HBV is particularly high in sub-Saharan Africa and accounts for over 80% of the adult patients with sporadic hepatitis. The World Health Organization (WHO) estimates that 257 million persons were chronic HBV carriers worldwide by July 2017 and in 2015 hepatitis B resulted in 887,000 deaths, mostly from complications (including cirrhosis and hepatocellular carcinoma) (WHO, 2017b). Hepatitis B prevalence is highest in the WHO Western Pacific region and the WHO African region, where 6.2% and 6.1% respectively of the adult population is infected (WHO, 2017b).

In Kenya, the prevalence of HBsAg ranges from between 8% - 30% (Bagshawe and Nganda, 1973; Bowry *et al*, 1985; Mutuma, 2011; Margolis *et al*, 1991, Kiire, 1996). Over 70% of patients with Hepatocellular carcinoma (HCC) are positive for HBsAg (Bowry *et al*, 1985). Hepatocellular carcinoma is the fifth commonest solid tumour among the Kenyan population.

Globally, an estimated 71 million people have chronic hepatitis C infection, and a significant number of this group will develop cirrhosis or liver cancer (WHO, 2017a). Hepatitis C Virus is one of the 10 leading causes of infectious disease deaths worldwide, approximately 399,000 people die each year from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma (WHO, 2017a). Recently HIV and HBV co-infection have added to the burden of disease leading to higher morbidity and mortality.

Hepatitis C is found worldwide and the most affected regions are WHO Eastern Mediterranean and European Regions, with the prevalence of 2.3% and 1.5% respectively. Prevalence of HCV in other WHO regions varies from 0.5% to 1.0% (WHO, 2017a). In Africa, HCV prevalence varies between 0.6-18% with countries in

North Africa having higher incidence rates that those in sub-Saharan Africa (http://apps.who.int/medicinedocs, 2017). Work done in Kenya shows the prevalence of HCV in blood donors is between 1.5-2.5% with higher values in IV drug users up to 30% (http://apps.who.int/medicinedocs, 2017). It is now estimated that intravenous drug use accounts for 80% of acute HCV infections (Nice, 2013).

Studies in Kenya report rates between 12 to 40% of HIV/HBV co-infection and low prevalence rates (between 0.5% to 1.5%) of HCV co-infection (http://apps.who.int/medicinedocs, 2017). HCV associated liver disease is a major cause of mortality in HIV/HCV co-infected individuals (Lacombe and Rockstroh, 2012).

1.3 Justification of the study

Hepatitis C Virus and Hepatitis B Virus are common among HIV-infected persons in Africa. There are many HIV sero-discordant couples in Kenya who are at risk of transmitting to each other STIs including HIV, HCV and HBV due to shared modes of transmission. Although infection with HCV and HBV is common among HIV-infected persons, their prevalence and risk factors and relationship to HIV infection are not well characterized among HIV-discordant couples in Kenya. Previous studies in Kenya have not determined the prevalence and risk factors of HBV and HCV in HIV Sero-discordant couples; most have focused on prevalence and risk factors of HBV and HCV among HIV patients, general population, blood donors, IDUs and Hepatitis patients. There are no studies which have been conducted among HIV sero-discordant couples to determine the prevalence of HCV and HBV and the risk factors associated with these viruses. In a couple, one partner who is positive for either HCV or HBV may transmit the disease to the negative partner. Consequently, HIV-negative individuals in sero-discordant relationships may be at increased risk of HIV sero-conversion following infection with other STDs. The results of this study provide baseline evidence on the potential risk factors associated with coinfections and establish the prevalence of HCV and HBV among HIV sero-discordant couples in Kisumu County, Kenya.

1.4 Research question

In a population of HIV sero-discordant couples in Kisumu County, Kenya, what are the risk factors associated with infection with Hepatitis C Virus and Hepatitis B Virus?

1.5 Objectives

1.5.1 General objective

The general objective of this study was to determine the prevalence and risk factors associated with Hepatitis B Virus and Hepatitis C virus infection among HIV sero-discordant couples in Kisumu County, Kenya.

1.5.2 Specific objectives

1. To determine the prevalence of Hepatitis C Virus infection among HIV sero-discordant couples in Kisumu County, Kenya."
2. To determine the prevalence of Hepatitis B Virus infection among HIV sero-discordant couples in Kisumu County, Kenya."
3. To determine the risk factors associated with Hepatitis C Virus and Hepatitis B Virus infection among HIV sero-discordant couples in Kisumu County, Kenya."

CHAPTER TWO

LITERATURE REVIEW

Human Immunodeficiency Virus discordance or a "mixed-status" relationship is a sexual relationship between partners with different HIV statuses: one partner is HIV-positive and one is HIV-negative (aids.gov, 2011). The spouses of HIV-infected index patients are at increased risk for HIV infection (Kumarasamy *et al*, 2010). Discordancy is, thus, a major contributor to the spread of HIV/Acquired Immunodeficiency Syndrome (AIDS) in Africa (Guthrie *et al*, 2007). In addition, HIV discordance is common within couples in Africa, ranging from 3% to 20% in the general population (Zaba,2005) and 30% to 51% within couples in which one partner seeks HIV care services (Kabatesi *et al*,2002).

There are about 338,000 discordant couples in Kenya and factors independently associated with HIV-discordance include young age among women, increasing number of lifetime sexual partners among women, HSV-2 infection in either or both partners and the lack of male circumcision (Kaiser *et al*, 2011). In addition, Sexually transmitted infections (STIs), particularly genital ulcerative diseases; HIV viral load; condom use; and specific sexual practices, particularly high number of sexual partners and higher frequency of sexual contact, have also been identified as correlates of transmission of HIV among HIV discordant couples (Guthrie *et al*, 2007).

2.1 HEPATITIS C VIRUS

2.1.1 Hepatitis C Virus and burden of HCV co-infection

Hepatitis C is a liver disease caused by the hepatitis C virus: the virus can cause both acute and chronic hepatitis, ranging in severity from a mild illness lasting a few weeks to a serious, lifelong illness (WHO, 2017a). The patients with chronic HCV infection, the risk of cirrhosis of the liver is between 15–30% within 20 years (WHO, 2017a).

In a global systematic review and meta-analysis of the Prevalence and burden of HCV co-infection in people living with HIV, Platt and colleagues estimated that 2.3 million people have HIV and hepatitis C co-infection - globally, and approximately 6.2% of people living with HIV also have hepatitis C (Platt *et al*, 2016). In HIV-infected individuals, HIV–HCV co-infection was 2·4% within general population samples, 4·0% within pregnant or heterosexually exposed samples, 6·4% in men who have sex with men), and 82·4% in people who inject drugs (PWID). Consequently, the study noted a consistently higher HCV prevalence in HIV-infected individuals than HIV-negative individuals across all risk groups and regions, but especially in PWID (Platt *et al*, 2016). A study in Kenya had differing results where the prevalence of HCV infection among medical in-patients was observed to be similar in HIV positive and HIV negative group of patients (Karuru *et al*, 2005).

Studies have shown that rates of liver disease are higher in persons who are co-infected with HIV and HCV than they are in persons with HCV alone. Furthermore, people who do not receive HIV treatment are less likely to spontaneously clear their hepatitis infection; they also have higher hepatitis viral loads and experience more rapid hepatitis disease progression than HIV-negative people (Graham *et al*, 2001).

Studies by Rufagari and colleagues indicated that HIV-negative individuals in sero-discordant relationships may be at increased risk of HIV sero-conversion following infection with other STDs including HCV and HBV (Rufagari *et al*, 2006). As a result, early diagnosis and treatment of STIs may significantly reduce HIV transmission among HIV discordant couples (Rufagari *et al*, 2006). Some reports suggest a higher rate of sexual transmission of HCV in partners of subjects who are HIV positive (Filippini *et al*, 2001). However, other studies have produced results that are consistent with a low or null transmissibility of HCV in heterosexual relations or general population (Norah, 2002), even when the index case is HIV co-infected (Marincovich *et al*, 2003).

2.2 HEPATITIS B VIRUS

2.2.1 Hepatitis B Virus Markers and burden of HBV co-infection

Hepatitis B is a viral infection that attacks the liver and can cause both acute and chronic disease; HBV is transmitted through contact with the blood or other body fluids of an infected person (WHO, 2017b). In addition, Hepatitis B is a potentially life-threatening liver infection caused by the HBV and is a major global health problem. It can cause chronic infection and puts people at high risk of death from cirrhosis and liver cancer (WHO, 2017b).

The main serum markers of Hepatitis B are Hepatitis B Virus surface antigen (HBsAg), HBV surface antibody (HBsAb), HBV e antigen (HBeAg), HBV e antibody (HBeAb), and HBV c antibody (HBcAb) (Lee, 1997). Hepatitis B surface antigen (HBsAg) is present in acute or chronic infection. Hepatitis B surface antibody (anti-HBs) is a marker of immunity acquired through natural HBV infection, vaccination, or passive antibody transfer (immune globulin) (Lee, 1997). Hepatitis B core antibody (anti-HBc) is an Immunoglobulin M (IgM) which is indicative of infection in the previous six months. Immunoglobulin G (IgG) is indicative of more distant HBV infection that may have been cleared by the immune system or that may persist (Lee, 1997). Positive HBsAg and anti-HBc IgG are indicative of persistent chronic HBV infection. Hepatitis B e antigen (HBeAg) correlates with a high level of viral replication and is often called a "marker of infectivity". Hepatitis B e antibody (anti-HBe) correlates with low rates of viral replication. HBV DNA correlates with active replication and is useful in monitoring response to treatment of HBV infection, especially in HBeAg-negative mutants (Lee, 1997).

Relatively few studies have evaluated the burden of HIV and HBV co-infection, and the effect that the increased prevalence of HIV/AIDS in the region may be having on the epidemiology of HBV infection (Burnett *et al*, 2005). Although HBV infection is

endemic in sub-Saharan Africa (Mphahlele *et al*, 2002), the association between HIV and HBV infection in the same region is not expected to be as strong (Nakwagala and Kagimu, 2002; Burnett *et al*, 2003) as in non-African countries of low HBV endemicity like in the United States (Herrero, 2001; Rogers *et al*, 2000). Therefore, there has been no massive increase in HBV prevalence in HIV-positive adults in sub-Saharan Africa (Nakwagala and Kagimu, 2002) and it is expected that a large number (53-78%) of HIV sero-positive patients have evidence of past exposure to HBV (Otedo, 2004)).

In addition, there is an increased risk of Hepatitis B virus transmission in HIV discordant couples. For example, among women, a prior history of a sexually transmitted infection, injecting drug use (IDU), having had more than five lifetime sex partners, and having exchanged sex-for-goods were significantly associated with HCV infection, whereas an IDU history, syringe sharing, and having exchanged sex-for-goods were found to be associated with HBV infection (Mari´a De Los, *et al*, 2004).

2.3 HIV, HBV and HCV Co infection

About 1% of persons living with HBV infection (2.7 million people) are also infected with HIV. Conversely, the global prevalence of HBV infection in HIV-infected persons is 7.4% (WHO, 2017a). Although rates of coinfection with at least two of HBV, HCV and HIV are well reported in numerous studies from Europe and America, there are few data on the prevalence of coinfection in African populations (Harania *et al*, 2008).

Worldwide, there may be about 25 million to 50 million people co-infected with HBV and HCV (Mendizabal *et al*, 2009). Co-infection with the HBV and HCV is not uncommon, especially in areas with a high prevalence of HBV infection and among people at high risk for parenteral infection (Liu and Hou, 2006). Generally, the prevalence of HCV is around 10-20% in patients with chronic HBV-infection, while 2-10% of anti-HCV positive patients have markers of HBV (Liu and Hou, 2006).

Human Immunodeficiency Virus - 1 and Hepatitis B and C viruses' coinfection is common in Sub-Saharan Africa due to similar routes of transmission. However, prevalence of triple infection with HIV, HBV and HCV among HIV positive inpatients, outpatients and injecting drug users is usually low (Borkakoty *et al*, 2007; Harania *et al*, 2008). For instance, in a study conducted in Nairobi among 300 patients attending HIV clinic, low levels of co-infection with HIV, HBV, HCV was reported; 15.3% (46/300) were HIV-1 co-infected with either HBV or HCV or both, 10.3% (31/300) with HIV-1 and HCV and 6% (18/300) with HIV-1 and HBV infections. However, only three individuals (1%) were co-infected with the three viruses (HIV/HBV/HCV) (Harania *et al*, 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site

The study site for this study was Kisumu County. Kisumu County is located in western Kenya (Appendix I). Kisumu County borders Vihiga County to the North, Nandi County to the North East, Kericho County to the East, Nyamira County to the South, Homa Bay County to the South West and Siaya County to the West (Softkenya.com, 2017; Commons.wikimedia.org, 2017). Kisumu County headquarters are in Kisumu City. The county has a shoreline on Lake Victoria. The vast majority of the people belong to the Luo ethnic community, the third largest tribe in Kenya. The County is relatively densely populated, with a population of 968,909 within an area of 2, 407.0 km² (Kenya National Bureau of Statistics, 2014). The study participants for this study were recruited from Kisumu County since the parent study was interested in HIV sero-discordant couples and there was a high population of HIV sero-discordant couples in Kisumu County at the onset of the parent study in 2004.

3.2 Study population

The study population consisted of heterosexual HIV sero-discordant couples. In this study, heterosexual couples were defined as sexual partners of the opposite gender who are married, have been living together, or otherwise considered each other a primary partner, one partner was HIV-1 positive, and the other partner was HIV-1 negative.

3.2.1 Inclusion criteria

Index and partner participants who met the following criteria were included in the study:

a) They should be 18 years and above.

b) Able and willing to provide written informed consent to be screened for infectious markers and to take part in the study.

c) Are partners who are sexually active (defined as having had vaginal intercourse with the partner participant at least three times in the last three months)?

d) Index participant was HIV-infected based on positive Enzyme ImmunoAssay (EIA).

e) Partner participant was HIV negative based on negative EIA.

3.2.2 Exclusion criteria

During the recruitment process, any participants who responded positive to any question indicating exposure to any of the following risk factors was not included in the study: history of blood transfusion, history of injectable drug use, surgery perinatal transmission and familial exposures to hepatitis.

3.3 Study design

This was a retrospective study nested within a completed Phase III randomized placebo-controlled trial (Lingappa *et al,* 2008 and 2009). The parent protocol at the Center for Microbiology Research was Ssc No. 861. Serum samples and data from completed epidemiological questionnaires were obtained from the completed study in 2004 (Lingappa *et al,* 2008 and 2009).

3.4 Study setting

Participants were recruited from Kisumu County (Lingappa *et al,* 2008 and 2009). Participants were recruited from a variety of sources including HIV VCT centers, antenatal clinics and programs for prevention of mother-to-child HIV transmission, referral from HIV care providers, and community promotion activities for couples' VCT

and other HIV-related prevention and care activities. Participants were also referred to the study from other local research projects and health and social service providers serving the target study population (Lingappa *et al*, 2008 and 2009).

3.5 Sample size

The sample size formula below was used to estimate the sample size for the descriptive study.

$$n = \frac{z^2 \hat{p}(1-\hat{p})}{m^2}$$

Where:

p = expected prevalence or estimated proportion

m= degree of precision or a tolerance error margin or width of the

Confidence Interval (CI) where m=0.05

z= Z statistic for a level of confidence where z=1.96.

For this study, the confidence level of 95%, and the error margin of ±5% were considered acceptable and from past studies it was expected that;

Twenty percent of HIV patients are HCV positive (Ockenga *et al*, 1997; Spengler and Rockstroh 1998), and 53% of medical outpatients with jaundice and low CD4 cell counts will have HBV and HIV co-infection (Otedo, 2004). Using this information in the sample size formula above, a sample size of 223 couples with HCV co-infection was estimated. The sample size is expected to achieve the required sufficient precision for the estimated prevalence of HCV and HBV in HIV discordant couples at the same time being able to meet the cost of processing the samples. Analyzed sample size was 270 couples since some of the study participants had inadequate specimen amounts and

missing questionnaire data. Couples whose sexual behavior information was complete in the questionnaires were selected from the pool of HIV sero-discordant couples in the completed parent study (Lingappa *et al,* 2008 and 2009).

3.6 Sampling

3.6.1 Recruitment

Participant recruitment was done according to the completed study protocol (Lingappa *et al*, 2008 and 2009; Ngure *et al*, 2010). The participants were asked to consent to the ongoing study and any other ancillary study (Lingappa *et al*, 2008 and 2009). The consent process and administering of the questionnaire was done by a trained interviewer (Lingappa *et al,* 2008 and 2009; Ngure *et al*, 2010).

3.6.2 Serum sample collection and Transportation

Serum samples were obtained from the parent study repository at KEMRI-CUSF laboratories in Kisumu, Kenya. All samples were appropriately labeled with the participants study identification numbers. The samples were packaged and shipped according to IATA Packing Instruction 650 (www.iata.org, 2017). The samples were shipped by courier services from Kisumu to the Kenya National Blood Transfusion Services (KNBTS).

3.6.3 Laboratory Methods

All laboratory tests were conducted at the KNBTS. HBsAg and anti-HCV antibody testing was conducted on coded frozen serum samples from each participant using third generation HBV and HCV Enzyme linked immunosorbent assay (ELISAs). Murex HBsAg version 3 and Murex Anti-HCV version 4 were used for the detection of Hepatitis B surface antigen (HBsAg) and anti-HCV IgG antibodies respectively. All positive samples were retested in duplicate to rule out false positive cases.

Samples that were positive for HBsAg and anti-HCV IgG antibodies were confirmed using the ANILAB SYSTEMS HBsAg EIA PLUS kit and HUMAN anti-HCV kit respectively.

HBsAg reactive samples were tested further for antibody to Hepatitis B core antigen (anti-HBc-IgG/IgM) using DRG Diagnostics Anti HBC ELISA, to discriminate possible window phase HBV cases from chronic carrier cases. Window phase cases were defined as samples that tested negative for anti-HBc-IgG/IgM, while chronic carrier cases were defined as samples that tested positive for anti-HBc-IgG/IgM.

3.6.3.1 Murex HBsAg Version 3 Abbott Murex

3.6.3.1.1 Assay Principle

In Murex HBsAg version 3, the samples were pre-incubated in microwells coated with a mixture of mouse monoclonal specific for different epitopes on the 'a' determinant of HBsAg. Affinity purified goat antibody to HBsAg conjugated to horseradish peroxidase was then added to the sample well. During the two incubation steps any HBsAg present in the samples were bound to the well in an antibody-antigen-antibody-enzyme complex. In the absence of HBsAg no conjugate was bound. After washing to remove 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 Conjugate developed a purple color which was converted to orange when the enzyme reaction was terminated with sulphuric acid.

3.6.3.1.2 Assay Procedure

In this assay, a Substrate solution and Wash fluid were prepared. Twenty five microliter of sample diluents was added to each well. A total of 672 wells were used for this test. Seventy five microliter of samples and controls were added to the designated wells. The plates were then covered with a lid and incubated for 60 minutes at 37± 1°C. After

incubation, 50µl of conjugate was added to each well. After incubation, the plates were placed in a shaker for 10 seconds and then covered with a lid and incubated for 30 minutes at 37± 1°C. After this incubation, the plates were washed 5 times. After washing, the plates were inverted to tap out any residual Wash Fluid onto absorbent paper. Immediately after washing the plates, 100µl of substrate solution was added to each well. The plates were then covered with a lid and incubated for 30 minutes at 37± 1°C for color to develop. Fifty microliter of stop solution was then added to each well. After 15 minutes, the absorbance of each well was read at 450 nm using 620 nm to 690 nm as the reference wavelength.

3.6.3.1.3 WASH Procedures

An automated microplate strip washer was utilized during all assays in this study. Five wash cycles were conducted using working strength Wash Fluid (Glycine/Borate Wash Fluid). During washing the following was ensured;

a) Flow -through washing with a fill volume of 500 µl/well.
b) The dispense height was set to completely fill the well with a slight positive meniscus, without causing an overflow.
c) The time taken to complete one aspirate/wash/soak cycle was approximately 30 seconds.
d) No liquid was left in the well (by use of a double aspirate step in the final cycle where possible).
e) After washing was completed, the plates were inverted and tapped to remove any residual Wash Fluid onto absorbent paper.

3.6.3.1.4 Calculation of results

Each plate was considered separately when calculating and interpreting results of the assay. In this study, each plate used contained 96 wells. The plate lay-out in the entire sample analysis was as shown below with Red plates indicating a reactive sample, NC-

Negative control, PC- Positive control, KNC- Known Negative Control and KPC- Known Positive Control.

Negative Control

The mean absorbance of the replicates of the negative control was calculated. If one of the negative control wells had an absorbance more than 0.03 above the other, then higher value was discarded.

Cut-off Value

The Cut-off value (COV) was calculated by adding 0.05 to the mean of the Negative Control replicates.

3.6.3.1.5 Quality Control

The results of the assay were valid if the following criteria for the controls were met.

Negative Control

The mean A450/ref of the negative control was less than 0.15 or the mean A450 of the negative control was less than 0.2.

Positive Control

The A450/ref or A450 of the positive control was more than 0.8 above the mean A450/ref or A450 of the negative control.

3.6.3.1.6 Interpretation of results

Non-Reactive Results

Samples that gave an absorbance less than the Cut-off value were considered non-reactive in Murex HBsAg Version 3.

Reactive Results

Samples that gave an absorbance equal to or greater than the Cut-off value were considered reactive in the assay. Such samples were retested in duplicate using the original sample source. Samples that were reactive in at least one of the re-tests were presumed to contain HBsAg and were confirmed by using the Murex HBsAg Confirmatory Version 3 kit (2G27-01) and tests for other HBV markers. Samples that were non-reactive in both wells on retests were considered non-reactive.

3.6.3.2 DRG Anti-Hepatitis B Core Antigen ELISA

3.6.3.2.1 Assay Principle

The purified recombinant HBcAg was coated on the solid phase of multi-wells, each plate comprised of 96 wells. Serum sample and Horseradish peroxidase labeled with Anti-HBc (conjugated) were added to coated wells, and form competitive combination. After incubation, if Anti-HBc content was high in the sample, a complex of HBcAg-Anti-HBc was formed, and little complex of HBcAg-Anti-HBc labeled with HRP was formed. The wells were washed to remove this complex, and then incubated with substrates (TMB); subsequently there was little color change. If Anti-HBc was not present in the sample, there was significant color change.

3.6.3.2.2 Assay Procedure

In this assay, one blank, two positive and two negative controls for each test were set. A total of 69 wells were used for this test. Then 0.05ml serum samples, positive and negative control serum were added into the coated wells. One drop (approximately 0.05ml) of enzyme conjugant was added into the same coated wells. The wells were mixed thoroughly and incubated for 60 minutes at 37°C. Each well was then washed automatically 5 times and brought to dry after the operation. One drop (approximately 0.05ml) of substrate A and B were added respectively to each well and incubated for 10

minutes at 37°C. One drop (approximately 0.05ml) of stop solution was then added to each well and the wells mixed thoroughly and the absorbance measured at 450nm against the blank.

3.6.3.2.3 Interpretation of results

Colorimetric Method

Cut Off value calculation:

COV = the average Optical Density (OD) of negative controls x 0.5

Positive OD450 of sample < COV

Negative OD450 of sample >/ COV

Invalid if the OD of negative is below or equal 1.000 the result is invalid.

3.5.3.3 Human Anti-HCV ELISA

3.6.3.3.1 Assay Principle

The test utilized specific HCV recombinant antigens (core, NS 3, NS 4, NS 5) coated on microtiter wells. Specimen's antibodies, if present or controls binded to the HCV antigen (1. Step) at the solid phase. After incubation unbound specimen components were removed by washing. For the second incubation step anti-human IgG HRP conjugate was added, which bond specifically to the immobilized human anti-HCV IgG antibodies, and formed a sandwich immune-complex. After a second washing step to remove excess conjugate, TMB/Substrate was added (Step 3). A blue color developed changing to yellow after stopping the reaction. The intensity of the colors was directly proportional to the HCV-IgG-Ab concentration in the specimen. The absorbance of controls and specimen was determined by using ELISA micro plate readers or

automated ELISA systems. Results for patient samples were obtained by comparison with the cut-off value.

3.6.3.3.2 Assay Procedure

In this assay, 200µl of diluent was dispensed into each well. Then 10µl of Negative control and Positive control samples were added to the respective wells. Ten microliter of specimen was added to each of the remaining wells. The wells were then covered with adhesive strips and incubated for 60 minutes at 37°C. The plates were then washed 8 times. Fifty microliter of substrate reagent A was then dispensed into each well, and then 50µl of substrate reagent B was added to each well including A1 and mixed. The plates were then incubated for 30 minutes at 17°C to 25°C. After incubation, stop solution was added to all the wells. The absorbance was measured at 450nm within 15 minutes after terminating the reaction, using a reference wavelength of 620-690nm.

3.6.3.3.3 Interpretation of results

Calculation of Control values, Cut-off and Cut-off index

Mean absorbance value of Negative control in wells B1 and C1 (MNC) and Positive control in wells D1, E1 and F1 (MPC) were calculated according to:

$$MNC = \frac{A450\ (B1) + A450\ (C1)}{2}$$

$$MPC = \frac{A450\ (D1) + A450\ (E1) + A450\ (F1)}{3}$$

Cut-off value COV= (0.25XMPC) + MNC

Cut-off index S/Co=Aspecimen/COV

The test run was considered valid when:

a) Color in A1: colorless or light yellow, otherwise the test was invalid and was repeated.

b) MNC $\leq$ 0.20

c) MPC – MNC $\geq$ 0.40.

Table 3.1: Interpretation of assay results

Result	Interpretation
A450 (specimen) < COV	anti-HCV ab-**nonreactive**
A450 (specimen) ≥ COV	anti-HCV ab- **initially reactive: retest**
Retest in duplicate:	**Anti-HCV ab-**
Both results: **S/Co > 1.5**	**Reactive:** perform confirmatory test
Both results: **S/Co < 1.0**	**Nonreactive**
Both results: **1.0 < S/Co < 1.5**	**Equivocal:** monitoring of subsequent specimen; perform confirmatory test
Result 1: **S/Co < 1.0**	**Invalid,** (procedural error): retest
Result 2: **S/Co > 1.5**	

3.6.4 Research Variables

3.6.4.1 Risk factors of index and partner participants at enrollment

A detailed epidemiological questionnaire was administered to the study participants by a trained interviewer at enrolment to gather information on the predictor variables (Appendix II) (Lingappa *et al*, 2008 and 2009; Ngure *et al*, 2010).

3.6.4.2 Variables

The following sexual behavior information was extracted from questionnaires from the completed study: presence of STIs, condom use, number of sexual acts during the past month and use of herbs to dry the vagina/reduce secretions (Appendix II). The presence of Hepatitis B surface antigen (HBsAg) and anti-HCV antibodies in serum samples obtained from the study population was also a key variable in this study.

3.6.4.5 Strategies to reduce random and systemic error in measurements

1. **Site initiation training was done and the site reviewed prior to initiation.** All staff to be involved in the main study was oriented about the different project components including site visit where study participants will be recruited.

2. **Training and certifying the observers.** The interviewers were trained on how to administer the questionnaire. A preliminary study was conducted on some subjects to identify whether the interviewers had mastered the techniques that were required when administering the questionnaire.

3. **Refining the instruments.** Prior to commencement of the study, all the ELISA machines were serviced and calibrated to reduce variability and reduce instrument bias.

4. **Repetition.** An alternate ELISA was conducted to confirm positive samples.

3.6.5 Data management and analysis

3.6.5.1 Data collection

Demographic, behavioral and clinical data were collected from the completed study. The data was collected from the enrolled participants through interviewer-administered questions using forms translated into local languages and back translated to ensure

appropriate content (Lingappa *et al,* 2009). Data forms were scanned and entered using intelligent character recognition (ICR) DataFax software (DataFax, ver 3.7-004, Clinical DataFax Systems Inc., Hamilton, Ontario, Canada) and centrally double-verified by independent data technicians (Lingappa *et al*, 2009).

3.6.5.2 Data analysis

STATA statistical software was used for analyzing the data. Chi-square was used to assess the association among categorical variables while independent t test was to assess the difference in groups with continuous outcome. As part of multivariable analysis, logistic regression was used to describe relationship between HCV and HBV infection status as outcome variables and HIV status and demographic factors as predictable variables in HIV sero-discordant couples. The two outcome variables each was assessed independently but using the same predictor variables $P<0.05$ or confident Interval (CI) of odd ratio (OR) or Adjusted Odd ratio AOR which does not include 1 is considered as having significant association. HCV and HBV had binary outcome i.e. having or not having infection. The odds of a given characteristic of a predictable variable were assessed as compared to another would lead to testing positive for HBV and HCV.

3.6.6 Ethical considerations

The ethical committee of Kenya Medical Research Institution (KEMRI) approved the current study protocol; KEMRI SSC Protocol number 1350 (Appendix III). Written informed consent was obtained prior to enrollment of study subjects in the completed parent study. The participants were asked to consent to the completed study and other ancillary study in the future on stored specimens. The following information was given during participants' education sessions to ensure that they had the information needed to make an informed choice: a complete description of the aims of the study; a list of infectious agents being screened; potential benefits and risks; blood collection procedures and assurance of confidentiality of any information given as well as test

results. Participants who tested positive for HCV or HBV were followed in the completed study, and referred to KEMRI where additional investigations and appropriate management and follow-up were given. Participants in the parent study were refunded their transport costs. All participants' information and test results were confidentially kept. Results were for a thesis for masters in Epidemiology, and will be disseminated by preparation of a manuscript for publication in a peer reviewed journal.

CHAPTER FOUR

RESULTS

4.1. Demographic Characteristics of Enrolled Couples

The study population consisted of a total of 270 HIV-1 Discordant couples from the completed Phase III randomized placebo-controlled trial. The criteria used to identify the 270 couples from the 530 couples was; availability of complete data on variables to be analyzed in the current study and adequate serum sample.

A total of 154 (29%) men and 121 (23%) women were HIV-1 positive. Polygamy was not an exclusion criterion in this current study. Among these 270 couples, 265 men had only one wife or partner while five men had two wives (or partners) resulting in a total of 275 women and 270 men. The heterosexual discordant couples were classified as follows; sexual partners of the opposite gender who are married, have been living together, or otherwise consider each other a primary partner.

In a discordant couple, one partner (index participant of any gender) in the couple is infected with HIV, while the partner participant is not infected with HIV. In polygamous relationships in the study; if the man was HIV positive his wife had to be HIV negative to be recruited in this study. If the man was HIV negative, his wife had to be HIV negative to qualify for recruitment into the study. In polygamous relationships, each wife was counted as a couple.

Although for this analysis, there were 270 couples (270 men and 275 women), there was also missing data on some variables for some subjects and, therefore, frequencies indicated in the Tables are often less than the total number of couples of respondents.

4.1.2 Characteristics of couples in whom the male is HIV-1 positive

In 154 couples in whom the male was HIV+; males were on average about 7 years older than their wives (Table 4.1). In the study, equal number of men (94%) and women (94%) reported that they were married (p=0.452) (Table 4.1). There were more men (66%) to have more years of education than their wives (34%) (p<0.0001) (Table 4.1). There was no significant difference in the number of years lived together among the men (50%) and women (50%) (p=0.619) (Table 4.1). Sixty percent of the study participants had lived together for less than 5 years (Table 4.1). More men (47%) than women (16%) had a source of income (p<0.0001). Forty percent of the couples had 1-2 children (Table 4.1).

4.1.3 Characteristic of couples in whom the female is HIV-1 positive

In 121 couples in whom the female was HIV+; men were on average about 9 years older than their wives (Table 4.1). In the study, equal number of men (99%) and women (99%) reported that they were married (p=0.976) (Table 4.1). There were more men (72%) to have more years of education than their wives (28%) (p<0.0001) (Table 4.1). There was no significant difference in the number of years lived together among the men (48%) and women (51%) (p=0.918) (Table 4.1). Thirty eight percent of the study participants had lived together for less than 5 years (Table 4.1). More men (40%) than women (5%) had a source of income (p<0.0001). Thirty two percent of the couples had 1-2 children (Table 4.1).

Table 4.1: Baseline Demographic Characteristics of HIV sero-discordant couples by Gender of HIV-1 infected Partner

	Couples in Whom Male was HIV+			Couples in Whom Female was HIV+		
	HIV-(154)	**HIV+(154)**	**(p-value)df**	**HIV-(116)**	**HIV+(121*)**	**(p-value)df**
	Female	**Male**		**Male**	**Female**	
Age (mean(Standard Deviation))	28.89 (7.87)	36.14 (11.65)	ttest,(<0.0001)	41.33(11.11)	32.58 (9.41)	ttest(<0.0001
Years of education; (count (%))						
None	5 (3.3)	2 (1.3)		1 (0.9)	6 (5.0)	
1-8y	111 (72.1)	77 (50.0)	(<0.0001) Df=3	62 (53.2)	94 (77.6)	(<0.0001) Df=3
9-12y	32 (20.8)	57 (37.0)		46 (39.7)	19 (15.7)	
>12y	6 (3.9)	18 (11.7)		7 (6.0)	2(1.7)	
Years lived together						
<=5y	87 (60)	88 (60.3)		41 (36.6)	47 (39.2)	
6-10y	35 (24.1)	30 (20.6)		27 (24.1)	24 (20.0)	
11-15y	12 (8.3)	15 (10.3)	(0.619), Df=4	17 (15.2)	16 (13.3)	(0.918) Df=4
16-20y	5 (3.5)	6 (4.1)		8 (7.1)	10 (8.3)	
21-25y	6 (4.2)	7 (4.8)		19 (17.0)	23 (19.2)	
Total Number of Children						
None	22 (14.3)	19 (12.4)		4 (3.5)	5 (4.1)	
1-2.	77 (50)	61 (39.6)	<0.0001 Df=3	32 (27.6)	39 (32.2)	
3-4.	41 (26.6)	34 (22.1)		34 (29.3)	40 (33.1)	(0.540) Df=3
>=5	14 (9.1)	40 (26.0)		46 (39.7)	37 (30.6)	

Total number of children with study partner						
None	45 (29.2)	48 (31.2)		14 (12.1)	17 (14.1)	
1-2.	66 (42.9)	67 (43.5)	(0.622) Df=3	34 (29.3)	37 (30.6)	
3-4.	32 (20.8)	28 (18.2)		36 (31.0)	36 (29.8)	(0.955) Df=3
>=5	11 (7.1)	11 (7.1)		32 (27.6)	31 (25.6)	
Earns Income						
yes	24 (15.6)	73 (47.4)	(<0.0001) Df=1	46 (39.6)	6 (5.0)	(<0.0001) Df=1
no	130 (84.2)	81 (52.6)		70 (60.3)	115 (95.0)	

Note; for mean test was used

Df; Degrees of freedom

4.1.4 Couples HIV status within different age groups

In this study, the distribution of HIV among the couples did not vary within age groups (Table 4.2). There was no significant difference in HIV status distribution within all the age groups P=0.783.

Table 4.2: Couples HIV status distribution within Age groups (P=0.783)

		HIV status		
		HIV+ve n (%)	HIV-ve n (%)	Total n (%)
Age groups	18-29	120(44.4)	113(41.2)	233(42.8)
	30-39	80(29.6)	80(29.2)	160(29.4)
	40-49	45(16.7)	54(19.7)	99(18.2)
	50 and above	25(9.3)	27(9.9)	52(9.6)
Total		270	274	544

4.2 Sero-prevalence of HBV and HCV by Couple HIV status

4.2.1 Couples in whom the male was HIV-1 positive

Among couples in whom the man is infected with HIV, there were no significant differences in prevalence's of Hepatitis B Virus surface antigen (HBsAg) among the men n=10 (6.5%) compared to women n=17 (11%) (p=0.158) (Table 4.3). As for Hepatitis B core antibody (anti-HBc-IgG/IgM Antibodies) a significant difference was observed, n=13 (8.4%) of the men compared to n=4 (2.6%) of the women tested positive

for anti-HBc (p=0.03) (Table 4.3). There was significant difference between men and women, with more women n=36 (23.5%) than men n=19 (12.4%) testing positive for anti-HCV antibodies (p=0.011) (Table 4.3).

Table 4.3: Prevalence of HBV, HCV by Couple HIV Status (Unit of analysis is couple)

	0.158	0.018
	0.03	0.001
	0.011	0.878

4.2.2 Couples in whom the female was HIV-1 positive

Among couples in whom the female was HIV+, there were significant differences with more men, n=29 (16.4%) than women n=8 (6.11%) testing positive for HBsAg (p=0.018) and more men n=13 (11.2%) than women n=1 (0.8%) testing positive for anti-HBc-IgG/IgM Antibodies, (p=0.001) (Table 4.3). There were no significant differences in the prevalence of anti-HCV antibodies between men n=21 (18.4%) and women n=21 (17.7%) in this group (p=0.878) (Table 4.3).

4.3. HBV and HCV Co-infections

4.3.1 HBV and HCV Co-infections in HIV-1 discordant couples

Mono-infection as used in this study means infection with only one virus either HBV or HCV regardless of whether the study participant is HIV positive or not, and co-infection means infection with both HBV and HCV regardless of whether the study participant is HIV positive or not.

Among HIV-1 discordant couples, 397 (74.9%) of the couples did not have HBV or HCV infection, 88 (16.6%) had HCV mono-infection, 45 (8.5%) had HBV mono-infection and 9 (1.7%) had HCV and HBV co-infection (Figure 4.1).

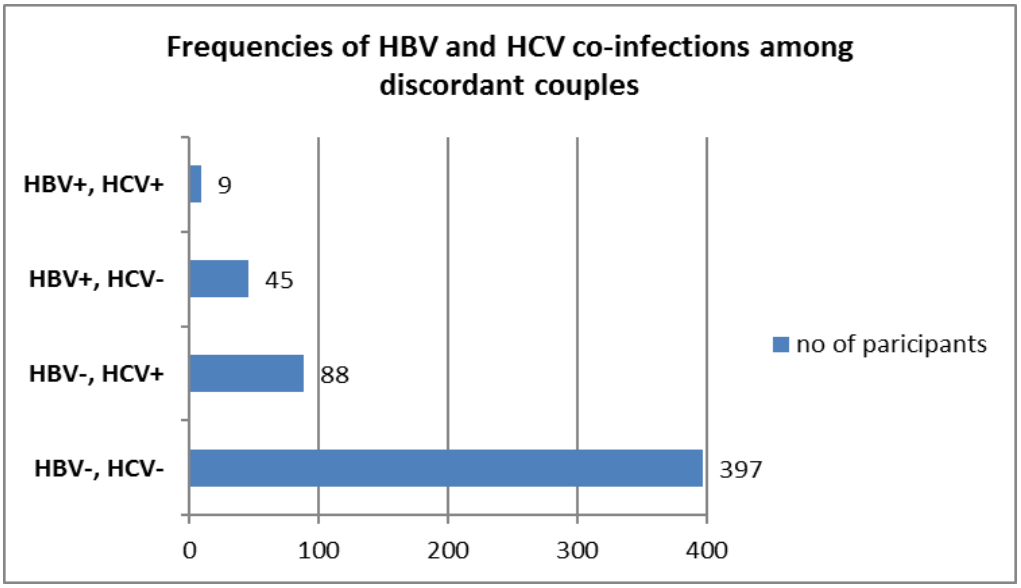

Figure 4.1 Frequency of HBV and HCV Co-infections in HIV-1 Discordant couples

4.3.3 HBV and HCV Co-infections by Couple HIV Status

Among couples in which the man was infected with HIV, more women n=31 (20.3%) than men n=18 (11.8%) had HCV mono-infection (p=0.027) while more men n=16

(10.5%) than women n=5 (3.3%) had HBV mono-infection (p=0.039), and HBV and HCV co-infection among women and men was n=5 (3.3%) and n=1 (0.7%) respectively (Table 4.4).

Among couples in which the woman was infected with HIV, equal numbers of men n=19 (16.7%) and women n=20 (16.8%) had HCV mono-infection (p=0.519), while more men n=17 (14.9%) than women n=7 (5.9%) had HBV mono-infection (p=0.015), and HBV and HCV co-infection (p=0.538) was similar in both men n=2(1.8%) and women n=1(0.8%) (Table 4.4).

Table 4.4: HBV, HCV Co-infections by Couple HIV-1 Status

HBV, HCV by Couple HIV Status

	Couple where Man is HIV+		**Couple where Woman is HIV+**	
HBV and HCV Status	**HIV-**	**HIV+**	**HIV-**	**HIV+**
HBV-, HCV-	112(73.2)	118(77.1)	76(66.7)	91(76.5)
HBV-, HCV+	31(20.3)	18(11.8)	19(16.7)	20(16.8)
HBV+, HCV-	5(3.3)	16(10.5)	17(14.9)	7(5.9)
HBV+, HCV+	5(3.3)	1(.7)	2(1.8)	1(.8)
Total	153	153	114	119

4.4. Analysis of Sexual risk factors for HBV and HCV infection among HIV-1 Discordant couples

4.4.1 Sexual behaviors - birth control methods in women with HCV or HBV

Among women in this study, there were no significant differences in the sexual behaviors - birth control methods (oral pills, intrauterine devices (IUDs), Injectables, implants and condoms) between women with HCV or HBV. All sexual behaviors analyzed in this study were not associated with infection with HBV or HCV (Table 4.5).

Table 4.5: Sexual behaviors among women with HCV or HBV

	Women		
	HBV- and HCV-	**HBV+/HCV+**	***p-value**
Contraceptive use			
No	93(37.5)	23(27.71)	0.106
Yes	155(62.5)	60(72.29)	
Oral			
No	246(99.19)	83(100)	NA
Yes	2(0.81)	0(0)	
IUD			
No	248(100)	83(100)	NA
Yes	0(0)	0(0)	
Injectable			0.826
No	222(89.52)	75(90.36)	
Yes	26(10.48)	8(9.64)	
Implant			0.314
No	245(98.79)	83(100)	
Yes	3(1.21)	0(0)	
Condom			
No	199(80.24)	72(86.75)	0.183
Yes	49(19.76)	11(13.25)	

* chi-square tests

IUD; Intrauterine devices

4.4.2 Other specific sexual behaviors and STIs in HIV-1 discordant couples with HCV or HBV

The sexual behaviors such as number of sex acts with partner, number of times condom used, number of other partners, number of new partners, number of sex acts with other partner, times condom used with other partner, presence of STIs were not considered in further analysis of their effect on infection with HBV or HCV because only 51 study participants reported such behaviors. Data for the other study participants was missing.

4.5. Analysis of Predictors for HBV infection among HIV-1 Discordant Couples

4.5.1 Univariate Analysis of Predictors of HBV infection among HIV-1 Discordant Couples

In a HIV-1 discordant couple, the age gap between the two partners, a partner not earning income, an educated partner and living with partner, the number of children with study partner and the HIV-1 status of partner being positive were not significantly associated with HBV infection. Only being male was significantly associated with HBV infection at p=0.013 (Table 4.6).

4.5.2 Multivariable Analysis of Predictors of HBV infection among HIV-1 Discordant Couples

In a HIV-1 discordant couple, the age gap between the two partners, being male, a partner not earning income, an educated partner and living with partner and the HIV-1 status of partner being positive were not significantly associated with HBV infection (Table 4.6).

Although being male in a HIV-1 discordant couple was significantly associated with increased odds of not detecting HBV infection in univariate analysis AOR 1.99, CI (1.159-3.417) p=0.013, multivariable logistic regression however showed no significant

association AOR 2.679, CI (0.919-7.812) p=0.071 (Table 4.6). Therefore, in a HIV-1 discordant couple, the prevalence of HBV is not associated with the age gap between the two partners, the gender of the partners, a partner not earning income, the number of years of education of partners, the number of years lived together as a couple, the number of children had with partner and the HIV-1 status of partner (Table 4.6).

Table 4.6: Univariate and Multivariable Analysis of Predictors of HBV infection among HIV-1 discordant Couples

	Univariate Analysis			Multivariate Analysis		
Covariate	**OD**	**p**	**95% CI**	**AOR**	**p**	**95%CI**
Age	1.012	0.542	0.973 - 1.052	0.985	0.659	(0.921-1.053)
Gender						
female*	1[Reference]			1[Reference]		
Male	1.99	0.013	(1.159-3.417)	2.679	0.071	(0.919-7.812)
Earns Income						
yes*	1[Reference]			1[Reference]		
no	0.837	0.554	(0.463-1.510)	2.027	0.292	(0.545-7.539)
Education Years						
None*	1[Reference]			1[Reference]		
1-8y	0.756	0.721	(0.162-3.518	0.732	0.786	(0.078-6.915)
9-12y	1.074	0.928	(0.229 -	1.424	0.773	(0.129-15.725)
>12y	1.000	1	(0169 - 5.904			
Years lived						
1y*	1[Reference]			1[Reference]		
2-5y	2.303	0.076	(0.916-5.788)	0.999	0.789	0.456 - 3.674
>5y	1.723	0.233	(0.704-4.216)	3.637	0.131	(0.682-19.396)
No. Children						
None*	1[Reference]					
1-2.	0.527	0.15	(0.220-1.260)			
3-4.	0.81	0.639	(0.337-1.950)			
>=5	0.496	0.149	(0.192-1.284)			
HIV Status						
HIV-*	1[Reference]			1[Reference]		
HIV+	0.787	0.382	(0.460-1.346)	1.32	0.451	(0.641-2.721)

* Baseline category for logistic regression

AOR; Adjusted Odd Ratio

CI; Confidence Interval

OD; Odds Ratio

4.6. Analysis of Predictors for HCV infection among HIV-1 Discordant Couples

4.6.1 Univariate Analysis of Predictors of HCV infection among HIV-1 Discordant Couples

In a HIV-1 discordant couple, the age gap between the two partners, a partner not earning an income, an educated partner, living with partner and the number of children with partner were not significantly associated with HCV infection (Table 4.7).

In a HIV-1 discordant couple, the HIV-1 status of partner being positive was significantly associated with reduced odds of not detecting HCV infection as compared to being HIV negative, AOR 0.787, CI (0.460-1.346) p=0.037 . Also being a male had increased odds of not detecting HCV infection as compared to female; AOR 1.99, CI (1.159-3.417) p=0.013 showed a significant association (Table 4.7).

4.6.2 Multivariable Analysis of Predictors of HCV infection among HIV-1 Discordant Couples

In a HIV-1 discordant couple, the age gap between the two partners, being male, a partner not earning an income, number of years of education of partner, living with partner and number of children had with partner were not significantly associated with HCV infection (Table 4.7).

In a HIV-1 discordant couple, the HIV-1 status of partner being positive was significantly associated with reduced odds of not detecting HCV infection, AOR 0.028, CI (0.191-0.909) p=0.028 (Table 4.7).

This association did not however apply in the age gap between the two partners, gender of the partners, a partner not earning an income, the number of years of education of partners, the number of years lived together as a couple and the number of children had

with partner. To ensure validity of the results, the study did not recruit any participant who had history of Injection drug use thus proof that the study results are valid.

Table 4.7: Univariate and Multivariable Analysis of Predictors of HCV infection among HIV-1 Discordant Couples

Covariate	Univariate Analysis			Multivariate Analysis		
	OR	**p**	**95%CI**	**AOR**	**p**	**95%CI**
Age	0.969	0.07	(0.935-	0.984	0.61	(0.927-
Gender						
female*	1[Reference			1[Reference		
male	0.665	0.06	(0.434-	0.795	0.59	(0.340-
Earns Income						
yes*	1[Reference			1[Reference		
no	1.344	0.26	(0.800-	1.093	0.86	(0.393-
Education						
None*	1[Reference			1[Reference		
1-8y	1.396	0.66	(0.309-	2.282	0.47	(0.235-
9-12y	1.286	0.75	(0.270-	1.246	0.85	(0.123-
>12y	0.828	0.83	(0.134-	4.668	0.23	(0.361-
Years lived						
1y*	1[Reference			1[Reference		
2-5y	1.619	0.16	(0.815-	3.101	0.07	(0.905-
>5y	1.214	0.57	(0.618-	1.937	0.35	(0.476-
No. Children						
None*	1[Referen			1[Referen		
1-2.	1.204	0.68	(0.489-	0.267	0.06	(0.068-1.058)
3-4.	1.815	0.19	(0.732-	0.482	0.39	(0.091-2.545)
>=5	0.555	0.26	(0.197-	0.277	0.19	(0.040-1.941)
HIV Status						
HIV-*	1[Referen			1[Referen		
HIV+	0.635	0.03	(0.415-	0.416	0.02	(0.191-0.909)

* Baseline category for logistic regression

AOR; Adjusted Odd Ratio

CI; Confidence Interval

OD; Odds Ratio

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. DISCUSSIONS

The prevalence and risk factors for HBV and HCV in HIV sero-discordant couples have not been well characterized in Kenya. This study illustrates that the prevalence of HBV and HCV was high among HIV-1 sero-discordant couples. The prevalence of HBV in HIV-1 sero-discordant couples is not associated with the HIV-1 status of partners and the prevalence of HCV in HIV-1 sero-discordant couples is associated with the HIV-1 status of partners. This cross-sectional study was conducted based on the need to determine the prevalence and potential risk factors of HCV and HBV among HIV-1 sero-discordant couples since these are not well characterized in Kenya.

In this study, regardless of the HIV status of the partners, the prevalence of HBV among HIV-1 discordant couples was 8.5%. The HBV prevalence was within the expected range in Kenya, where other studies among different study populations the prevalence of HBV was 8% - 30% (Bagshawe and Nganda, 1973; Bowry *et al*, 1985; Kerubo *et al*, 2015; Kiire, 1996; Margolis *et al*, 1991; Muriuki *et al*, 2013; Mutuma, 2011). The HBV prevalence obtained in our study was high compared to that expected in the WHO Africa region, where approximately 6.1% of the adult population is infected with HBV (WHO, 2017a). In this study, where the male was HIV+ve and where female was HIV+ve, the prevalence of HBV was 11% and 6.11%. The rates of HIV-HBV co-infection are reported as high as 10–20% in countries where HBV infection is either endemic or intermediate to high HBV cases (Thio, 2009). Previous studies results in Kenya have indicated HIV/HBV coinfection rate of between 12 to 40% which is within this study findings (http://apps.who.int/medicinedocs, 2017). A recent study to determine Prevalence of hepatitis B and C viral co-infections among HIV-1 infected individuals in Nairobi indicated 6% of study participants had HIV-1 and HBV infections (Muriuki *et*

al, 2013). This study results are consistent with other studies conducted in Kenya and other sub-Saharan countries, although in some countries there are some differences, this could be largely be due to diversity of patients from different population groups, sample size, test kit sensitivity and specificity (Muriuki *et al*, 2013; Taiwo *et al*, 2012).

In this study, regardless of the HIV status of the partners, the prevalence of HCV among HIV-1 discordant couples was 16.6%. In this study, where the male was HIV+ve and where female was HIV+ve, the prevalence of HCV was 12.4% and 17.7% respectively. This study results were high even after excluding participants who had high risk factors such history of being been IDUs, had surgery, tattoos, unsafe blood transfusion, homo sexuality among other risk factors. This study results are consistent with other study results that obtained high prevalence of HIV/HCV co-infection in other countries namely; 10.3% in Kenya (Muriuki *et al*, 2013), 13.8% and 18.1% in Tanzania (Nagu *et al*, 2008; Telatela *et al*, 2007). The study findings were also contrarily to those of other studies which indicated lower HIV/HCV coinfection rates 5.8% in Nigeria (Lesi *et al*, 2007), 4.9% in Rwanda and Uganda (Pirillo *et al*, 2007). In addition, a global systematic review and meta-analysis of the Prevalence and burden of HCV co-infection in people living with HIV indicated that HIV–HCV co-infection was 2·4% within general population samples, 4·0% within pregnant or heterosexually exposed samples, 6·4% in men who have sex with men), and 82·4% in PWID (Platt *et al*, 2016). Consequently, the probable reason for the high prevalence in this study could be due to the shared modes of transmission of both viruses among HIV sero-discordant couples and changes in prevalence of HCV over time or the study participants did not provide accurate information about other risk factors associated with high transmission of HCV such as unsafe blood transfusions, IDUs, homosexuality or the study site was a hot spot for HCV transmission. In addition, with increased access to antiretroviral drugs for HIV patients, migrating populations and social networking by intravenous drug use, cases of HBV and HCV co infections have been on the rise (Lacombe *et al*, 2010), coupled with the dramatic rise in survival rates of these individuals (Sulkowski *et al*, 2000).

The low prevalence of 1.7 % HBV, HCV, HIV triple infection in this study was consistent with other studies where prevalence of triple infection with HIV, HBV and HCV among HIV positive inpatients, outpatients and injecting drug users was usually low (Harania *et al,* 2008; Borkakoty, 2007). In other studies, the prevalence of HBV and HCV co-infection among HIV-1 sero-discordant couples was between 0.3% to 1.5% (Kelley *et al,* 2008; Harania *et al,* 2008; Olanisun *et al,* 2009). Despite the low HBV, HCV, HIV triple infection, prevention measures to reduce the risk factors that increase the chances of transmission of HCV/HBV among partners in HIV-1 sero-discordant couples should still be advocated. Related studies indicate that when HCV-infected patients also are infected with HIV, their risk of chronic liver disease is increased up to two fold compared with the risk for HCV-infected patients without HIV (Kramer *et al*, 2006). Therefore, due to dual HBV/HCV infection, partners in HIV-1 sero-discordant couples are at risk of more severe liver disease, and are at an increased risk for progression to hepatocellular carcinoma and treatment of viral hepatitis will represent a challenge (Liu and Hou, 2006; Highleyman, 2009). Consequently, due to shared modes of transmission among HBV, HCV and HIV, it is paramount to advocate for integrated public health measures to prevent and control transmission of the viruses among HIV sero-discordant couples thus reduce morbidity and mortality related to infection with the HBV, HCV and HIV.

In this study, there were couples who were discordant for HBV and HCV. For example, among couples in whom the male is HIV-1 positive, more women 36(23.5%) than men 19(12.4%) tested positive for anti-HCV antibodies and among couples in whom the female was HIV+, more men, 29(16.4%) than women 8(6.11%) tested positive for HBsAg and more men 13(11.2%) than women 1 (0.8%) tested positive for anti-HBc-IgG/IgM Antibodies. Although this study focused on HIV sero-discordant couples, it is evident that there is high prevalence of the HBV and HCV among HIV negative population, thus the need to for constant surveillance (Muriuki *et al,* 2013) of these infections among HIV sero-discordant couple and the general population.

Since positive HBsAg and anti-HBc IgG are indicative of persistent chronic HBV infection (Lee, 1997), this implies that people who test positive for HBsAg and anti-HBc in a HIV-1 sero-discordant couple pose as a source of infection to their partner who is not positive for HBV. The presence of persistent chronic HBV infection among HIV sero-discordant couples in this study was consistent with results of the study conducted in Kisumu to determine the prevalence of HBV, HIV co-infection in patients who presented with jaundice (Otedo, 2004). In this Kisumu study, IgG anti-HBc and IgM anti-HBc were detected in 17 (5%) and 317(95%) patients respectively (Otedo, 2004). The presence of discordancy of HCV and HBV in the current study group poses a great public health risk in couples since there is potential for couple transmission of viruses due to shared modes of transmission.

Consequently, this study also indicated that the following risk factors were not associated with the presence of HCV or HBV infection among HIV sero-discordant couples; age, gender, income, education, number of children or years lived together and use of barrier and non-barrier contraceptives by women. This could be because they do not play a key role in transmission of the virus. Only the HIV-1 status of a partner being positive was associated with the presence of HCV infection in the study group; indicating that presence of HIV contributes to infection/transmission with HCV among HIV sero-discordant couples.

For many pathogens, infection by multiple strains or other pathogens may have little or no epidemiological impact – the key distinction is simply whether a host is infected, or not However, there are pathogens for which coinfection alters pathogen dynamics in two ways namely; coinfection can lead to genetic exchange between co-infecting pathogens that may be essential to immune escape, or host jumps and coinfection may be associated with within-host competition (Metcalf *et al*, 2015). The evolution and prognosis of patients co-infected with HIV and HBV or HCV is not well known not to mention the immunological, virological and biochemical including the elastographic responses (Duri *et al*, 2015).

Inadequate immune responses towards the hepatitis viruses in co-infected individuals may lead to the development of cirrhosis and end stage liver disease (Ionescu *et al*, 2011). Co-infection with HIV poses a greater risk of mortality than either HCV or HBV infection alone and is frequently associated with hepatitis flares following ART initiation (Andrade *et al*, 2013). Based on the current study results, there is still need to follow up the group after several years to determine if there was transmission of the viruses to the partners who were initially negative and the risk factors that promoted the transmission.

5.2. LIMITATIONS OF THE STUDY

1) There was insufficient data on sexual behaviors for analysis of their effect on infection with HBV or HCV. The sexual behaviors data that was not available or was inadequate was number of sex acts with partner, number of times condom used, number of other partners, number of new partners, number of sex acts with other partner, times condom used with other partner and presence of STIs.

2) The study was identifying prevalent cases, and the prevalent cases may not be representative of all cases that have developed in the population.

3) The presence or absence of both exposures and the infections were determined at the same time in each study participant. Therefore, it was not possible to determine a temporal relationship between the exposures and the onset of disease (s).

5.3. CONCLUSIONS

1) The prevalence of HBV, HCV, and HBV HCV HIV co-infection was 8.5%, 16.6% and 1.7% respectively.

2) The prevalence of HCV in HIV-1 sero-discordant couples studied was only associated with the HIV-1 status of a partner being positive.

5.4. RECOMMENDATIONS

1) Although the prevalence of HBV and antibody to Hepatitis B core antigen was low among the study group, HBV prevention measures such as vaccination, knowing your sexual partner HBV status, use of condoms, being cautious about body piercing and tattooing and not practicing IDUs should be encouraged among HIV-1 sero-discordant couples to reduce transmission of HBV and other STIs including HIV.
2) All HIV-1 discordant couples should be tested for both HBV and HCV, since they are likely to have HBV or HCV infection. Generally, early testing of HBV and HCV among the HIV-1 discordant couples will encourage the start of integrated public health management measures for the prevention and control of HIV, HBV and HCV among HIV discordant couples.

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APPENDICES

Appendix I: Map of Kenya, Kisumu County

Figure 3.1: Map of Kenya, Kisumu County

Appendix II: Questionnaire derived from the ongoing study

1) In the past month, how many times did you have sexual intercourse with your partner?

    a. -Of those times, how often was a condom used?

2) When having sex, do you and your study partner ever use anything to reduce the secretions or make the vagina dry?

3) Besides the study partner, how many partners have you had sex with in the past month?

    a. -How many of these are new partners?

    b. -In the past month, how many times did you have sexual intercourse with someone other than your study partner?

    c. -Of those times, how often was a condom used?

4) In the past three months, have you had sores on your genital area?

5) In the last three months, has a health care provider diagnosed you with or treated you for any of the following sexually transmitted or genital infections? Urethritis/Urethral discharge, Genital ulcer disease, Lymphogranuloma venereum, Vaginitis/vaginal discharge, Cervicitis/Cervical discharge/Pelvic inflammatory disease, Gonorrhea, Chlamydia, Chancroid, Trichomonas, Syphilis, Bacterial vaginosis and Candidiasis.

6) In the last three months, have you had any of the following: blood transfusion, surgery, injection?

Appendix III: Ethical Approval

KENYA MEDICAL RESEARCH INSTITUTE

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KEMRI/RES/7/3/1 **30 SEPTEMBER 2008**

FROM: **SECRETARY, KEMRI/NATIONAL ETHICAL REVIEW COMMITTEE**

THROUGH: **DR. N WAMAE,**
THE DIRECTOR, CMR,
NAIROBI

TO: **MR. HILARY KIGUME (PRINCIPAL INVESTIGATOR)**

RE: **SSC PROTOCOL No. 1350 (2ND *REVISION*): DESCRIPTIVE EPIDEMIOLOGY OF HEPATITIS C VIRUS AND HEPATITIS B VIRUS IN HIV SERO-DISCORDANT COUPLES IN KISUMU, KENYA**

Dear Sir,

I make reference to your letter dated 29 September 2008. We acknowledge receipt of the following documents:

a. The revised study protocol;
b. Appendix I: Questionnaire derived from SSC 861: Phase III Randimized Placebo-Controlled Trial of HSV-2 Suppression to Prevent HIV Transmission Among HIV-Discordant Partners, version 4.1.1 dated 15 December 2005;
c. Appendix II: Specimen storage and possible future research testing plan for the study SSC 861;
d. Appendix III: Sample Informed Consent Forms- Screening only for the study SSC 861;
e. Appendix IV: Sample Informed Consent Forms- Enrollment for Index Participants- for the study SSC 861;
f. Appendix V: Enzyme Immunosorbent Assay Procedures;
g. Appendix VI: Investigator and site-specific activities.

The Committee notes that:

1) The proposed cross-sectional study seeks to provide evidence of risk factors associated with co-infections and establish the prevalence of HCV and HBV among HIV sero-discordant couples in Kisumu;
2) This study will obtain serum samples and completed epidemiological questionnaires from SSC Protocol No. 861 and thus there will be no contact with research participants. The sample size for the HBV arm of the study is 724 persons or 362 couples and for the HCV arm 209 couples or 418 persons;
3) All samples will be stored will be stored at the National Blood Transfusion Services in Nairobi during the course of the study;
4) Any emerging IP issue arising from the study will addressed in accordance with existing policies in KEMRI, Jomo Kenyatta University of Agriculture and Technology and University of California in San Francisco, USA.

In Search of Better Health

The Committee is satisfied that the issues raised at the 154th ERC meeting of 8 May 2008 have been adequately addressed. The study is hereby granted approval for implementation effective today, the **30th day of September 2008** to **29th September 2009.**

Please note that you are responsible for reporting any changes to the research study to the Scientific Steering Committee and to the Ethical Review Committee prior to implementation. This includes changes to research design, personnel, equipment, funding and procedures that could introduce new or more than minimum risk to research participants. You may embark on the study.

Sincerely,

C. WASUNNA,
FOR: SECRETARY,
KEMRI/NATIONAL ETHICAL REVIEW COMMITTEE