



## The Prevalence of Hepatitis B and C Viruses among HIV Sero Discordant Couples in Kisumu, Kenya

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### Summary

**HIV–1 sero–discordant couples are at risk of transmitting to each other HIV, HBV and HCV. This cross–sectional study evaluated the prevalence of HBV and HCV infection and associated risk factors among 270 heterosexual HIV sero–discordant couples. 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 suppression to prevent HIV transmission among HIV sero–discordant couples site in Kisumu. The serum samples were analyzed for Hepatitis B surface antigen, antibodies to Hepatitis B core antigen and HCV IgG antibodies. 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. Regardless of whether the participants were HIV positive or not, HCV and HBV prevalence rates were 4%, 5.6% respectively, and HBV/HCV co–infection rate was 0.2%. HCV prevalence was associated with the HIV–1 infection. Since the prevalence of HBV was high, 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.**

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## Introduction

There are about 338,000 HIV-1 sero-discordant couples reported in Kenya [1]. Infection with HCV and HBV is common among HIV infected persons due to their shared modes of transmission [2, 3, 7, 8] and this represents an increasingly important public health problem [2]. 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.

HIV-negative individuals in sero-discordant relationships may be at increased risk of HIV sero-conversion following infection with other STIs including HCV and HBV [14].

HIV positive individuals with chronic HBV and HCV co-infection tend to experience more rapid liver disease progression than HIV negative people [15] and treatment of viral hepatitis due to dual HBV/HCV infection represents a challenge [16]. Hepatitis C may affect the course and management of HIV infection [17].

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, our study sought to evaluate the potential risk factors associated with coinfections and establish the prevalence of HCV and HBV among HIV sero-discordant couples in Kisumu, Siaya, Busia, Vihiga and Migori counties in Kenya.

## Materials and Methods

### Study design

The study participants were recruited from Kisumu, Siaya, Busia, Vihiga and Migori counties in Kenya [18, 19]. This was a cross-sectional measurement of antibodies to HCV, HBsAg and sexual behaviors in HIV

sero-discordant couples. This study was nested within a completed Phase III randomized placebo-controlled trial of acyclovir for HSV-2 suppression to prevent HIV transmission among HIV sero-discordant couples in Kenya [18, 19]. Serum samples and data from completed epidemiological questionnaires were obtained from the completed study. A sample size of 270 couples was expected to achieve the required sufficient precision for the estimated prevalence of HCV and HBV in HIV discordant couples. Two hundred and seventy couples whose sexual behavior information was complete in the questionnaires and serum samples were adequate were randomly selected from the pool of 530 HIV sero-discordant couples in the completed parent study.

### Enrollment

Details on recruitment criteria of HIV-1 sero-discordant heterosexual couples for the completed parent study have been described in other published papers [18, 19, 20]. Briefly, the study population consisted of heterosexual HIV sero-discordant couples in which the HIV-infected partner had a CD4 cell count of at least 250 cells/mm<sup>3</sup> at enrolment. 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. In addition, one partner (index participant) in the couple was infected with both HIV and HSV-2, while the partner participant was not infected with either HIV or HSV-2 at baseline. To focus on sex-related exposures, participants who reported blood transfusion, surgery or injection drug uses were excluded during analysis.

### Blood sample collection and processing



Venous blood (57.5ml) for use in this study was drawn from the participants by venipuncture and collected into anticoagulant vacutainers blood collection tubes. Serum was harvested following centrifugation at 1500 revolutions per 5 minutes. All samples were appropriately labeled with the participants study identification numbers (PID) and stored in  $-80^{\circ}\text{C}$  freezers in KEMRI-CUSF laboratories in Kisumu, Kenya until required for use.

### **Laboratory testing**

All laboratory tests were conducted at the Kenya National Blood Transfusion Services (KNBTS). HBsAg and anti-HCV antibody testing was conducted on coded frozen serum samples from each participant using third generation HBV and HCV ELISAs. Murex HBsAg version 3 [21] and Murex Anti-HCV version 4 [22] 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 by using the ANILAB SYSTEMS HBsAg EIA PLUS kit [23] and HUMAN anti-HCV kit [24] respectively.

HBsAg reactive samples were tested further for antibody to Hepatitis B core antigen (anti-HBc-IgG/IgM) using DRG Diagnostics Anti HBC ELISA [25], 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.

### **Data collection**

Demographic, behavioral and clinical data were collected from enrolled participants through interviewer-

administered questions [19]. Data forms were scanned and entered using intelligent character recognition DataFax software (DataFax, ver 3.7-004, Clinical DataFax Systems Inc., Hamilton, Ontario, Canada) and centrally double-verified by independent data technicians [19].

### **Data analysis**

STATA statistical software was used for analyzing the data. Chi-square and logistic regression were used to test whether there was any association between HCV and HBV infection status and sexual behaviors in HIV sero-discordant couples and  $P < 0.05$  was considered as having significant association.

### **Ethics Statement**

The current study protocol was also approved by the ethical committees of JKUAT, KEMRI and the Ancillary Study Committee of University of Washington, USA as part of the requirements for using samples and data from the completed parent study. Written informed consent was obtained prior to enrollment of study participants in the completed parent study [19]. The participants were asked to consent to the completed study and other ancillary study in the future on stored specimens.

### **Results**

#### **Demographic Characteristics of Enrolled Couples by Gender of HIV-infected Partner**

The baseline demographic characteristics of the couples by gender of HIV infected partner are shown in Table 1. In 154 couples studied, males were on average about older than their wives ( $p < 0.0001$ ). There was a tendency for more men to have more years of education than their



wives ( $p < 0.0001$ ). More men than women had a source of income ( $p < 0.0001$ ).

**Table 1: Baseline Demographic Characteristics of Enrolled Couples by Gender of HIV-1 infected Partner**

	Couples in Whom Male is HIV+			Couples in Whom Female is HIV+		
	HIV- (154)	HIV+(154)	p-value	HIV-(116)	HIV+(121*)	p-value
	Female	Male		Male	Female	
<b>Age</b> (mean(SD))	28.89(7.87)	36.14(11.65)	<0.0001	41.33(11.11)	32.58(9.41)	<0.0001
<b>Married;</b> (count (%))						
yes	145(94.2)	144(93.5) *	0.452	115(99.1)	120(99.2)**	0.976
no	9(5.8)	10(6.5)		1(0.9)	1(0.8)	
<b>Years of education;</b> (count (%))						
None	5(3.3)	2(1.3)		1(0.9)	6(5.0)	
1-8y	111(72.1)	77(50.0)	<0.0001	62(53.2)	94(77.6)	<0.0001
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)	
<b>Years lived together</b>						
<=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	17(15.2)	16(13.3)	0.918
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)	
<b>Total Number of Children</b>						
None	22(14.3)	19(12.4)		4(3.5)	5(4.1)	
1-2.	77(50)	61(39.6)	<0.0001	32(27.6)	39(32.2)	
3-4.	41(26.6)	34(22.1)		34(29.3)	40(33.1)	0.540
>=5	14(9.1)	40(26.0)		46(39.7)	37(30.6)	
<b>Total number of children with study partner</b>						
None	45(29.2)	48(31.2)		14(12.1)	17(14.1)	
1-2.	66(42.9)	67(43.5)	0.622	34(29.3)	37(30.6)	
3-4.	32(20.8)	28(18.2)		36(31.0)	36(29.8)	0.955
>=5	11(7.1)	11(7.1)		32(27.6)	31(25.6)	
<b>Earns Income</b>						
yes	24(15.6)	73(47.4)	<0.0001	46(39.6)	6(5.0)	<0.0001
no	130(84.2)	81(52.6)		70(60.3)	115(95.0)	

\* There is one couple at least where one partner (man) considers he is not married while the partner (woman) believes she is married.

\*\*Five of the 116 HIV negative men had two wives resulting in 121 women. In five couples where the man had two wives, these were considered as two couples for each wife.



### Sero-prevalence rates of HBV and HCV by Couple HIV status

Results of testing for HBV and HCV viral markers by Couple HIV status are shown in Table 2 and Figures 1 and 2.

**Table 2: Prevalence of HBV, HCV by Couple HIV Status (Unit of analysis is couple)**

Status	Couples with HIV+ Male			Couples with HIV+ women		
	HIV-	HIV+ Male	p-	HIV- Male	HIV+	p-
	(No. of couples (%))			(No. of couples (%))		
<b>HBsAg</b>						
Negative	144(93.5)	137(89.0)	0.158	97(83.6)	113(93.4)	0.018
Positive	10(6.5)	17(11.0)		29(16.4)	8(6.11)	
<b>Anti-HBc</b>						
Negative	150(97.4)	141(91.6)	0.03	103(88.8)	120(99.2)	0.001
Positive	4(2.6)	13(8.4)		13(11.2)	1(0.8)	
<b>Anti-HCV</b>						
Negative	117(76.5)	134(87.97)	0.011	93(81.6)	98(83.4)	0.878
Positive	36(23.5)	19(12.4)		21(18.4)	21(17.7)	

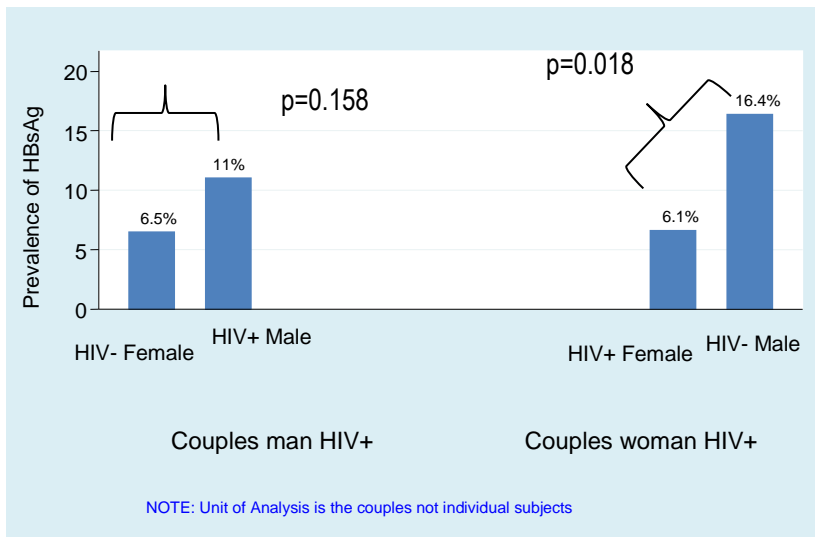
#### Couples in whom the male is HIV-1 positive

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 10(6.5%) compared to women 17(11%) ( $p=0.158$ ). As for Hepatitis B core antibody (anti-HBc-IgG/IgM Antibodies), about 13(8.4%) of the men compared to 4(2.6%) of the women tested positive for anti-HBc ( $p=0.03$ ). More women 36(23.5%) than men 19(12.4%) tested positive for anti-HCV antibodies ( $p=0.011$ ).

#### Couples in whom the female is HIV-1 positive

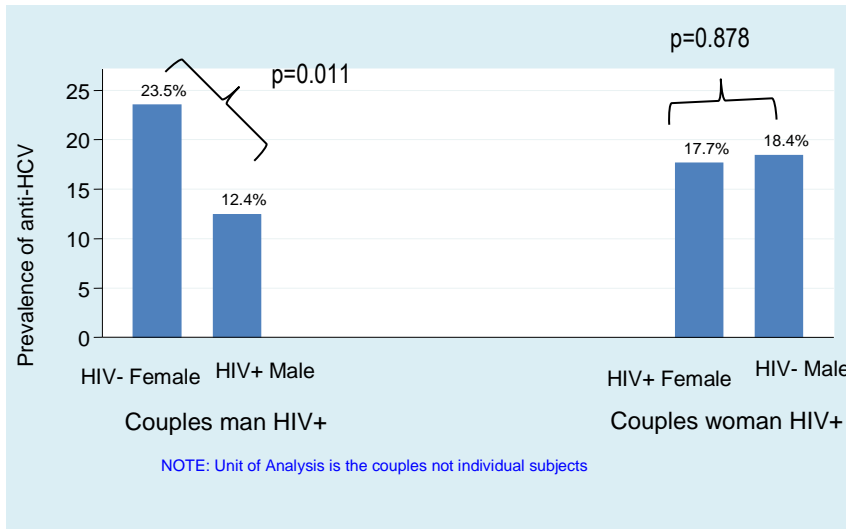
Among couples in whom the female was HIV+, there were significant differences with more men, 29(16.4%) than women 8(6.11%) testing positive for HBsAg ( $p=0.018$ ) and more men 13(11.2%) than women 1 (0.8%) testing positive for anti-HBc-IgG/IgM Antibodies, ( $p=0.001$ ). There were no significant differences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

**Figure 1: Prevalence of HBsAg by Couples HIV Status.**





**Figure 2: Prevalence of anti-HCV by Couple HIV Status**



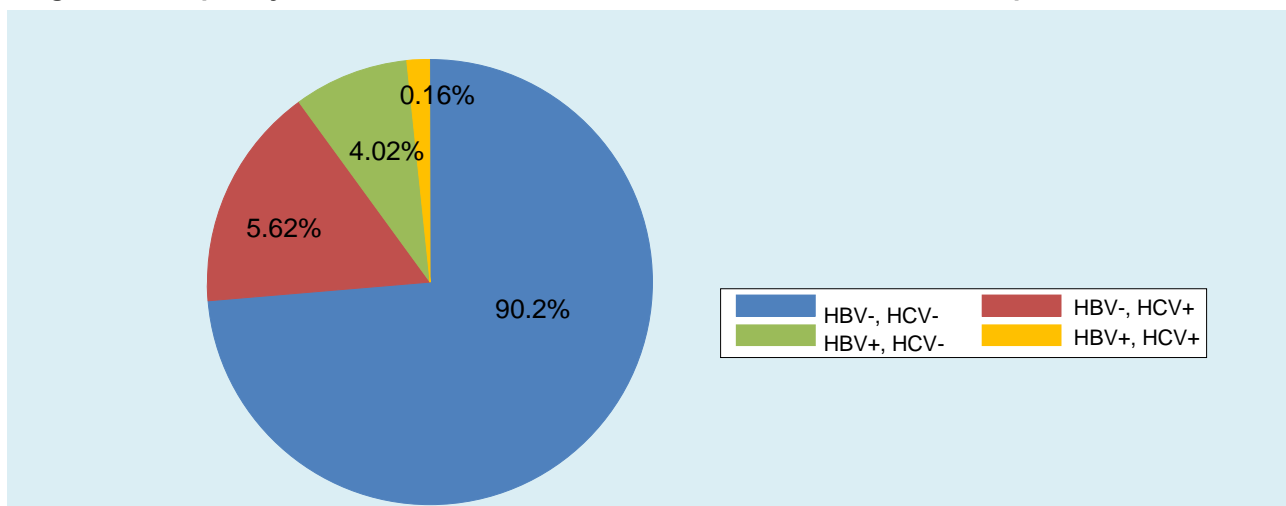
**HBV and HCV Co-infections**

**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, 90.2% of the couples did not have HBV or HCV infection, 4.02% had HCV mono-infection, 5.62% had HBV mono-infection and 0.16% had HCV and HBV co-infection (Figure 3).

**Figure 3: Frequency of HBV and HCV Co-infections in HIV-1 Discordant couples**





**Analysis of Sexual risk factors for HBV and HCV infection among HIV– Discordant couples**

**Sexual behaviors – birth control methods in women with HCV or HBV**

Regardless of their HIV status, 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 3). Other sexual behaviors were not analyzed due to few study participants reporting such behaviors in our study.

**Table 3: Sexual behaviors among women, Birth Control Methods**

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

**Analysis of Predictors for HBV infection among HIV–1 Discordant Couples**

Results of univariate and multivariate analysis of predictors of HBV infection among HIV–1 discordant couples are shown in Table 4.



**Table 4: Univariate and Multivariate Analysis of Predictors of HBV infection among HIV-1 discordant Couples**

Covariate	Univariate Analysis			Multivariate Analysis		
	OR	p	95%CI	OR	p	95%CI
<b>Age</b>	1.012	0.542	0.973 – 1.052	0.985	0.659	(0.921–1.053)
<b>Gender</b>						
female*	1[Reference]			1[Reference]		
male	1.99	0.013	(1.159–3.417)	2.679	0.071	(0.919–7.812)
<b>Earns Income</b>						
yes*	1[Reference]			1[Reference]		
no	0.837	0.554	(0.463–1.510)	2.027	0.292	(0.545–7.539)
<b>Education Years</b>						
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	(0.169 – 5.904)			
<b>Years lived</b>						
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)
<b>No. Children</b>						
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)			
<b>HIV Status</b>						
HIV-*	1[Reference]			1[Reference]		
HIV+	0.787	0.382	(0.460–1.346)	1.32	0.451	(0.641–2.721)

\* Baseline category

#### **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  $p=0.013$ , regardless of HIV status of self or partner.





### Multivariate 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.

Although being male (regardless of HIV status of self or partner), was significantly associated with HBV infection in univariate analysis ( $p=0.013$ ), multivariate analysis showed no significant association ( $p=0.071$ ). 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.

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

Results of univariate and multivariate analysis of predictors of HCV infection among HIV-1 discordant couples are shown in Table 5.

**Table 5: Univariate and Multivariate Analysis of Predictors of HCV infection among HIV-1 Discordant Couples**

Covariate	Univariate Analysis			Multivariate Analysis		
	OR	p	95%CI	OR	p	95%CI
<b>Age</b>	0.969	0.072	(0.935-1.003)	0.984	0.611	(0.927-1.046)
<b>Gender</b>						
female*	1[Reference]			1[Reference]		
male	0.665	0.06	(0.434-1.017)	0.795	0.597	(0.340-1.858)
<b>Earns Income</b>						
yes*	1[Reference]			1[Reference]		
no	1.344	0.264	(0.800-2.259)	1.093	0.865	(0.393-3.038)
<b>Education Years</b>						
None*	1[Reference]			1[Reference]		
1-8y	1.396	0.664	(0.309-6.301)	2.282	0.477	(0.235-22.109)
9-12y	1.286	0.752	(0.270-6.118)	1.246	0.852	(0.123-12.674)
>12y	0.828	0.839	(0.134-5.113)	4.668	0.238	(0.361-60.416)
<b>Years lived together</b>						
1y*	1[Reference]			1[Reference]		
2-5y	1.619	0.169	(0.815-3.214)	3.101	0.072	(0.905-10.627)
>5y	1.214	0.573	(0.618-2.385)	1.937	0.356	(0.476-7.881)
<b>No. Children</b>						
None*	1[Reference]			1[Reference]		
1-2.	1.204	0.686	(0.489-2.965)	0.267	0.06	(0.068-1.058)
3-4.	1.815	0.198	(0.732-4.504)	0.482	0.39	(0.091-2.545)
>=5	0.555	0.266	(0.197-1.566)	0.277	0.196	(0.040-1.941)
<b>HIV Status</b>						
HIV-*	1[Reference]			1[Reference]		
HIV+	0.635	0.037	(0.415-0.972)	0.416	0.028	(0.191-0.909)



\* Baseline category

### **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, being male, 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.

In a HIV-1 discordant couple, the HIV-1 status of partner being positive was significantly associated with HCV infection at  $p=0.037$ .

### **Multivariate 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.

In a HIV-1 discordant couple, the HIV-1 status of partner being positive was significantly associated with HCV infection at  $p=0.028$ .

Therefore, in a HIV-1 discordant couple, the prevalence of HCV is associated with HIV-1 status of partner being positive, but not the age gap between the two partners, the 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.

### **Discussion**

Our study results observed 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 establish 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 rate of HBV and HCV mono-infection, and HBV and HCV co-infection among HIV-1 discordant couples was 5.6%, 4% and 0.2% respectively. Our results are consistent with other studies where prevalence of HBV is expected to be high since HBV is endemic in sub Saharan Africa and the prevalence of HCV is expected to be less than 5% due to low presence of high risk factors for HCV such as unsafe blood transfusions, intravenous drugs and homosexuality [9, 10, 11, 12, 13]. In a study conducted in Kisumu Kenya, 53% of the patients presenting with jaundice had HBV and HIV co-infection [26]. In Kenya, the prevalence rate of HCV and other risk factors for transmission of HCV and HIV among HIV/AIDS medical in-patients was low at 3.7% [27]. In other studies the prevalence of HBV and HCV co-infection among HIV-1 sero-discordant couples was between 0.3% to 1.5% [28, 29, 30].

The prevalence of HBV infection in our study participants was high but not associated with HIV status of partners, suggesting that partners in HIV-1 sero-discordant couples are likely to be infected with HBV since its endemic in the region [5, 6, 31, 32], but not as a result



of HIV infection. This was also consistent with the assumption that in Sub-Saharan Africa, association between HBV with HIV is not expected to be strong since HBV is endemic in this region, and most sexually active adolescents and adults in this region have already been exposed to HBV before being exposed to HIV [5, 6, 31, 32].

The rate of HBV and HCV co-infection is generally low as confirmed by the results of our study and as well as other studies where the prevalence of HBV and HCV co-infection among HIV-1 sero-discordant couples was between 0.3% to 1.5% [28, 29, 30]. It has also been observed in other studies, that prevalence of triple infection with HIV, HBV and HCV among HIV positive inpatients, outpatients and injecting drug users is usually low [29, 37]. This proves that since the three viruses share the same modes of transmission, co-infection with the three viruses is not uncommon, especially in HBV and HCV endemic areas and among people at high risk for parenteral infection [5, 6, 16]. 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 [15, 16]. Although, our studies results reported low HBV and HCV coinfection, 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.

Our study confirmed the presence of persistent chronic HBV infection among HIV-1 patients especially men (84%) and this has also been evident in other studies in Kenya [26]. Therefore, 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. Consequently, men in HIV-1 discordant couples are more prone to be chronic carriers of HBV infection compared to women.

Our study was able to prove 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. Only the HIV-1 status of a partner being positive was associated with the presence of HCV infection in the study group.

### **Conclusion**

The prevalence of HBV and HCV was high among HIV-1 sero-discordant couples regardless of HIV status of partners.

The prevalence of HBV and HCV in HIV-1 sero-discordant couples was not associated with any of the following factors: age, gender, income, education, number of children or years lived together and use of barrier and non-barrier contraceptives.

The prevalence of HBV in HIV-1 sero-discordant couples was not associated with the HIV-1 status of a partner being positive and the prevalence of HCV in HIV-1 sero-discordant couples was associated with the HIV-1 status of a partner being positive.

Results of this study have clearly demonstrated that HBV prevention measures should be encouraged among HIV-1 sero-discordant couples to reduce transmission of HBV and other STIs including HIV. 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.



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