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Cervicovaginal HIV-1 Neutralizing IgA Detected among HIV-1-Exposed Seronegative Female Partners in HIV-1-Discordant Kenyan Couples

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Abstract

Objective—Cervicovaginal HIV-1-neutralizing IgA was associated with reduced HIV-1 acquisition in a cohort of commercial sex workers. We aimed to define the prevalence and correlates of HIV-1-neutralizing IgA from HIV-1-exposed seronegative (HESN) women in HIV-1-serodiscordant relationships.

Methods—HIV-1-serodiscordant couples in Nairobi were enrolled and followed quarterly up to two years, and women in concordant HIV-1-negative relationships were enrolled as controls. Cervicovaginal, seminal, and blood samples were collected at enrollment and follow-up. Cervicovaginal IgA was assessed for HIV-1-neutralizing activity by a peripheral blood mononuclear cell-based assay using an HIV-1 clade A primary isolate.

Results—HESN women in discordant relationships had significantly more HIV-1-neutralizing IgA detected in genital secretions compared to control women (36 of 155 [23%] vs. 4 of 70 [6%], respectively; odds ratio [OR] 5.0; 95% confidence interval [CI] 1.70–14.64; $P=0.003$). These responses persisted over time in all available follow-up cervicovaginal samples from women with detectable HIV-1-neutralizing IgA at baseline. Partner median HIV-1 plasma viral load was lower among women who had HIV-1-neutralizing IgA compared to women without detectable activity (4.3 vs. 4.8 log₁₀ copies/ml, respectively; OR 0.70; 95% CI 0.51–0.94; $P=0.02$). A similar trend was found with partner seminal viral load (OR 0.57; 95% CI 0.32–1.02; $P=0.06$).

Conclusion—HESN women were 5-times more likely to have neutralizing IgA in cervicovaginal secretions than low-risk control women, and these responses were inversely

associated with partner viral load. These observations support the existence of antiviral activity in the mucosal IgA fraction following sexual HIV-1 exposure.

Keywords

HIV; immunoglobulin A; discordant couple; exposed uninfected; Africa; neutralization; viral load

INTRODUCTION

Despite efforts to control the HIV-1 pandemic, 2.7 million individuals were newly diagnosed with HIV-1 in 2010, of whom 1.9 million lived in sub-Saharan Africa [1]. Although heterosexual contact is the primary mode of HIV-1 transmission worldwide, the transmission rate per contact has been estimated to be on average only 1 per 526 in male-to-female transmission and 1 per 1,000 in female-to-male transmission, based on HIV-1-discordant couples [2, 3]. This low per contact rate may be attributed to a number of viral and immunologic factors, including mucosal immunity expressed by both HIV-1-infected and -exposed, uninfected individuals.

There is limited understanding of mucosal humoral immunity and its role in decreasing HIV-1 transmission. A major component of humoral mucosal immunity is immunoglobulin A (IgA), which is secreted in response to mucosal or systemic antigenic challenges. In the case of HIV-1, repeated exposure to virus through sexual contact has been considered a mechanism for induction of humoral responses. This has prompted many to investigate mucosal IgA among HIV-1-exposed, seronegative (HESN) individuals. While some studies have reported absence of HIV-1-binding or -neutralizing genital IgA [4–7], others have demonstrated that HESN women, including commercial sex workers and uninfected women in HIV-1-discordant relationships, have HIV-1 IgA responses in cervicovaginal secretions [8–16]. Of these studies, some examined HIV-1-binding IgA [10, 12–14, 16], and others investigated functional activity by detecting IgA antibodies mediating HIV-1-neutralization and -inhibition of HIV-1 transcytosis [8, 9, 11, 14, 15].

Only one study included a prospective analysis to determine the association between cervicovaginal HIV-1-neutralizing IgA and HIV-1 acquisition. In this case-control study of sex workers, HIV-1-neutralizing activity was detected among 81% of women who remained HIV-1-uninfected and 46% of women who acquired HIV-1 over follow-up [11]. Overall, IgA with neutralizing activity against HIV-1 subtype A and C was strongly associated with reduced acquisition of HIV-1. This study did not have data on partner HIV-1 viral load (VL) to determine viral cofactors for induction of mucosal IgA.

Among non-human studies, a recent rhesus macaque mucosal vaccine study has shown that vaccine-induced vaginal IgA showed evidence of protection from SHIV, supporting a role for cervicovaginal IgA in decreasing HIV-1 acquisition [17]. Furthermore, Xiao et al showed significant correlation between vaccine-induced mucosal sIgA responses and delayed acquisition of infection following repeated low-dose mucosal SIV challenge [18].

We conducted a study to determine whether HIV-1-neutralizing IgA was detectable in cervicovaginal secretions of HESN women in HIV-1-serodiscordant relationships, enrolling women in HIV-1-negative seroconcordant relationships as a control group. We also evaluated correlates of HIV-1-neutralizing IgA responses, including infected partner's plasma and seminal HIV-1 VL, genital tract infections, and behavioral factors in both partners.

METHODS

Study setting and participants

All study participants were recruited and screened through voluntary counseling and testing centers in Nairobi, Kenya from 2007–2009. Study participants were HIV-1-serodiscordant couples with one HIV-1-infected and one uninfected partner. In addition, concordant HIV-1-seronegative couples were recruited and enrolled as a control population. Eligible participants were >18 years of age, reported sexual intercourse with their study partner 3 times in the 3 months prior to screening, and planned to remain together for the duration of the study (up to 24 months). We excluded participants who were on antiretroviral therapy (ART), had a history of clinical AIDS (WHO stage IV), were enrolled in another HIV-1 treatment trial, had sexual intercourse with another partner two months prior to enrollment, planned to have sexual intercourse with another partner during the study, or planned to be away from Nairobi for two consecutive months during follow-up. Written informed consent was obtained from all study participants, and ethical approval granted by Institutional Review Boards at University of Washington, Karolinska Institutet, and Kenyatta National Hospital.

Clinical and laboratory procedures

Women were seen in clinic at enrollment and every 3 months for 12–24 months. During these visits, questionnaires were administered and blood specimens collected. Cervicovaginal samples (CVS) were collected at enrollment and every 6 months from HIV-1-uninfected women in HIV-1-serodiscordant couples and at enrollment from female controls in HIV-1-seroconcordant negative relationships. Two cotton-tipped swabs were used to collect secretions from the cervical os and posterior vaginal fornix. Both swabs were transferred into a vial containing 5ml of phosphate-buffered saline (PBS), transported on ice to the laboratory within 2 hours of collection, spun down to remove cellular debris, and cryopreserved at -80°C .

Enrollment IgA was purified from thawed CVS with jacalin-agarose beads (Immunkemi, Sweden). A total of 800 μL of CVS was added to 400 μL of jacalin-agarose beads and mixed for 2 hours at 4°C , followed by centrifugation and collection of the IgA-depleted fraction. The diluted supernatant with the jacalin-agarose beads was mixed for 2 hours at 4°C and then centrifuged. The jacalin-agarose beads were then thoroughly washed with PBS (pH 7.4). The bound IgA was eluted overnight at room temperature by adding 1mL 0.8M D-galactose pH 7.5. The supernatant with the purified IgA was subsequently collected and all fractions were stored at -80°C .

A previously defined HIV-1-neutralization assay [11] was performed by investigators blinded to partner's serostatus. In addition, CVS from the HESN and control women were randomly interspersed when assays were conducted. A primary isolate of HIV-1 (subtype A, UG037 isolate) was obtained from the NIH AIDS Research and Reference Reagent Program (USA) and was collected from peripheral blood mononuclear cells (PBMC) stimulated with phytohemagglutinin (PHA) and interleukin 2 (IL-2). Median tissue culture infective dose (TCID₅₀) values were determined and aliquots were stored at -80°C . As TCID₅₀ values may change depending upon the PBMC donor, three viral dilutions were used in each assay, with a target value of 10–50. The purified IgA was not further diluted or concentrated when tested for neutralizing capacity. Duplicate wells of each virus dilution and of each sample fraction were incubated for 1h at 37°C , followed by addition of a mixture of 1×10^5 PHA-stimulated PBMCs from two different donors. After 16–18h incubation at 37°C , unbound virus was washed away and fresh medium (RMPI 1640; GIBCO, USA) supplemented with fetal bovine serum and IL-2 was added. Medium was replaced on day 3, and on day 6 the

supernatant was collected and analyzed using a p24 antigen enzyme-linked immunosorbent assay (ELISA) (Murex HIV Antigen Mab kit; Abbott Diagnostics, USA). Neutralization in the PBMC assay was defined as reduction of p24 antigen (subtype A virus) in the supernatant as compared to p24 antigen content when the virus isolate was incubated in the presence of samples from Swedish low-risk HIV-1-uninfected individuals.

HIV-1 rapid tests were conducted at the study clinic using the Determine® HIV-1/2 Rapid Test (Abbott Laboratories, USA) and the Bioline™ Recombigen HIV Test (Standard Diagnostic Inc, S. Korea) every 3 months for HESN women. If either commercial rapid kit was positive, results were confirmed with HIV-1 ELISA, which was performed using the Vironostika® HIV Uni-Form II Ag/Ab ELISA kit (Biomerieux Laboratories, Netherlands).

For the HIV-1-infected male partners, CD4+ T cell counts were measured at enrollment and every 6 months using a Becton Dickinson (BD) FACSCalibur flow cytometer (BD Bioscience, USA), and HIV-1 RNA levels were measured from blood and seminal samples collected at enrollment using the Gen-Probe HIV-1 VL assay (Gen-Probe Incorporated, USA). The lower limit of detection for the VL assay was 150 copies/mL for plasma samples and 500 copies/mL for semen samples. At enrollment, HSV-2 serology using the HerpeSelect IgG ELISA kit (Focus Diagnostics, USA) was performed for all participants, and bacterial vaginosis (BV) was defined as a Nugent score of 7–10 among female participants.

Prostate-specific antigen (PSA) was measured from all CVS for this study by an external accredited laboratory (Aleris Medilab, Sweden) using the ARCHITECT Total PSA assay (Abbott Ireland Diagnostics Division, Ireland), a chemoluminescent microparticle immunoassay. PSA levels of the CVS are reported as measured in the original 5ml dilution, and positivity was defined as $\geq 1\mu\text{g/mL}$, as described previously [19]. PSA, a protein secreted during ejaculation, can be detected in CVS with high sensitivity, and clears from CVS within 48 hours with a mean clearance time of approximately 20–27 hours [19–21].

Statistical analysis

HIV-1-neutralization activity of IgA fraction in CVS was evaluated both as a continuous and a dichotomous variable. A predefined neutralization cutoff of $\geq 67\%$ was used to determine a positive response [11]. Mann-Whitney test was used to compare percentages of HIV-1-neutralizing activity between HESN and control women and Pearson chi-squared to compare the proportion of HESN and control women with positive responses using the 67% cut-off. Correlates for women with and without responses were determined using independent t-tests for continuous variables and Pearson's chi-squared tests for dichotomous variables. Logistic regression was used to assess associations in a multivariate model. All analyses were done with and without the exclusion of PSA positive samples. Plasma and seminal VL below the lower limit of detection were set to half the lower limit (i.e. 75 copies/mL for plasma and 250 copies/mL for semen).

RESULTS

Cohort characteristics of HIV-1-serodiscordant couples

We enrolled 168 HESN women in HIV-1-serodiscordant relationships, of whom 155 had CVS to determine presence of neutralizing IgA. At enrollment, median age of HESN women was 30 years (interquartile range [IQR]: 25, 35), lifetime partners was 3 (IQR: 2, 3), and years in relationship was 6 (IQR: 2, 11) (Table 1). CVS from 15 (10%) women had PSA $\geq 1\mu\text{g/mL}$, indicating recent unprotected sexual activity. Among the HESN women, 32 (22%) reported a lifetime history of sexually transmitted infections (STI), 103 (67%) were HSV-2 seropositive, and 19 (13%) had BV. Fifty-five (36%) earned an income, and 48 (31%) had

less than a primary education. HIV-1-infected male partners had a median CD4 cell count of 356 cells/ μ L (IQR: 238, 521) and a $\log_{10}$ VL of 4.7 copies/ml (IQR: 4.1, 5.3). Among the HIV-1-infected male partners, 112 (72%) were circumcised. Couples reported a median of 5 sex acts (IQR: 2, 9) in the month before enrollment and 123 (79%) reported no unprotected sex with their study partner in the last month.

Cohort characteristics of control women

We enrolled 75 HIV-1-uninfected women in concordant HIV-1-negative relationships, of whom 70 had cervicovaginal HIV-1-neutralizing IgA data. Control women were younger compared to HESN women (25 vs. 30 years, respectively), reported shorter duration of living together (2 vs. 6 years), and reported more sex acts with study partners in the last month (7 vs. 5 acts). In addition, a smaller proportion of control compared to HESN women reported no unprotected sex in the last month (27% vs. 79%), were HSV-2 seropositive (32% vs. 67%), and had less than a primary level education (16% vs. 31%)(Table 1). Additionally, a larger proportion of control women had detectable PSA in CVS compared to HESN women (33% vs. 10%) (all p values<0.05).

Prevalence and durability of HIV-1 neutralizing cervicovaginal IgA in HESN and control women

HIV-1-neutralizing activity in the IgA fraction ranged from 0–98% in 225 HESN and control CVS. One hundred fifty-nine samples (71%) demonstrated 0–33% neutralization, 25 (11%) samples 33–67% neutralization, and 41 (18%) samples had 67% neutralization, consistent with a positive response. A statistically significant difference was seen when comparing the level of antiviral activity as a continuous variable between HESN and control women ($p=0.02$). However, because HIV-1-inhibitory activity as a continuous percentage was not normally distributed, all subsequent analyses were performed using the 67% cut-off as a positive response. Thirty-six (23%) HESN women and 4 (6%) control women had neutralizing activity using this cut-off. HESN women were 5-fold more likely to have positive responses than controls (odds ratio [OR] 5.0; 95% confidence interval [CI] 1.70–14.64; $P=0.003$) (Figure 1).

After excluding CVS with PSA $\geq 1\mu\text{g/ml}$, HIV-1-neutralizing IgA was detected in 34 (24%) of 140 HESN women and 3 (6%) of 47 control women, and this difference was also statistically significant (OR 4.7; 95% CI 1.37–16.12; $P=0.01$). In addition, 5 HESN women acquired HIV-1 during the 2-year follow-up study period, and none had cervicovaginal HIV-1-neutralizing IgA at baseline. Furthermore, 44 HESN women had follow-up CVS at 6 months ($n=24$) and 12 months ($n=20$) after enrollment, and these samples were used to determine the durability of IgA responses. Follow-up samples were available for 30 of the 36 women who had neutralizing IgA at baseline, and all had detectable HIV-1-neutralizing IgA. Percent neutralization ranged from 67–94%. We did not perform neutralizing IgA assays for follow-up CVS from women who did not have neutralizing IgA at baseline.

Partner's plasma and seminal viral load and other correlates of IgA

Women with cervicovaginal HIV-1-neutralizing IgA had a male partner with lower plasma VL than women without neutralizing IgA (median 4.3 vs. 4.8 $\log_{10}$ copies/ml, respectively; OR 0.70; 95% CI 0.51–0.94; $P=0.02$)(Table 2 and Figure 2). This association remained after adjusting for partner's CD4 cell count, no unprotected sex, frequency of sex, duration of relationship, and partner's income (data not shown). Excluding samples with PSA $\geq 1\mu\text{g/ml}$, this association remained statistically significant (OR 0.68; 95% CI 0.49–0.93; $P=0.02$). Similarly, a trend was seen for lower median HIV-1 seminal RNA VL to be associated with HIV-1-neutralizing IgA among HESN women (2.4 vs. 3.2 $\log_{10}$ copies/ml, respectively; OR 0.57; 95% CI 0.32–1.02; $P=0.06$). This association also remained statistically significant

after excluding CVS with PSA $\geq 1\mu\text{g/ml}$ (OR 0.51; 95% CI 0.27–0.97; $P=0.04$; data not shown).

Next, we examined the associations between behavioral factors and HIV-1-neutralizing cervicovaginal IgA response (Table 2). For every 5-year increase in relationship duration, there was a 32% (OR 0.68; 95% CI 0.49–0.94; $P=0.02$) decreased likelihood of a response, and those with male partners who earned an income were 68% (OR 0.32; 95% CI 0.13–0.81; $P=0.02$) less likely to have an IgA response than those whose male partners did not earn an income. When adjusted for partner's VL, the associations between relationship duration and partner's earned income and IgA response remained significant. In addition, for every 5-year increase in age in women, there was a 30% (OR 0.70; 95% CI 0.53–0.94; $P=0.20$) decreased likelihood of a response, but this association was no longer significant after excluding samples with PSA $\geq 1\mu\text{g/ml}$.

DISCUSSION

We found that HESN women in HIV-1-discordant relationships were 5-fold more likely to have HIV-1-neutralizing cervicovaginal IgA compared to women in HIV-1-negative concordant relationships. Similar results were found when HIV-1-inhibition by the IgA fraction was examined as a continuous variable. These results support the hypothesis that inhibitory activity in the IgA fraction in CVS is produced by women who are exposed to HIV-1 but remain uninfected. In addition, cervicovaginal HIV-1-neutralizing IgA was persistently detected during 12 months of follow-up among those who had neutralizing IgA at baseline. This finding suggests that this is a durable humoral response in the cervicovaginal mucosa of HESN women.

Our study is unique in that it draws from a large cohort of HESN women in a HIV-1-serodiscordant relationship. Previous studies among HESN women in commercial sex work and HIV-1-serodiscordant relationships have yielded conflicting results regarding mucosal HIV-1-specific neutralizing antibodies in HESNs – some have detected neutralizing antibodies [8–16] while others have not [4–7]. One reason for these conflicting results may be the lack of standardized assays to measure HIV-1-binding mucosal IgA antibodies. Another may be varying methods for collecting CVS. For example, some studies used cervicovaginal lavage and others used cervicovaginal swabs with varying dilution factors, and the more concentrated cervicovaginal swab may have a better IgA yield than a cervicovaginal lavage. In our study, we collected secretions from both the cervical os and the posterior vaginal fornix to increase the yield of relevant immune molecules. Depending on the assay, mode of collection, and specimen dilution, IgA yield may vary significantly. In addition, functional antibodies that are specific for the native trimer of the virus envelope glycoprotein may not bind to the monomeric form, which is typically detected in an ELISA format. The HIV-1-inhibitory activity may not be specific for HIV-1 itself, but rather to host factors that are critical to HIV-1 entry (e.g. HIV-1 receptor/coreceptors or carbohydrates) and this also would not be detected by a HIV-specific binding assay. In the present study, a PBMC-based virus neutralization assay was used to assess IgA functionality, and this assay uses a relevant cell target for HIV-1 infection. Using this approach, we and others have been able to detect HIV-1-neutralizing activity in the purified mucosal IgA fraction from HESN individuals [9, 11, 14, 15]. This approach also allows detection of antibodies directed at both conformational HIV-1-specific epitopes, as well as of host antigens that play a role in HIV replication.

Another strength of this study is that we have included control women from HIV-1-seroconcordant negative relationships from the same geographical region as the HESN group. These controls were at some risk of HIV-1 exposure despite being “low risk,”

because HIV-1 is a generalized epidemic in Nairobi. This may explain the 4 control women who had a positive IgA response in our study and sets our controls apart from controls identified in other parts of the world with low HIV prevalence in the general population. Our study also measured PSA and presented data both with and without PSA-positive CVS. The latter analysis decreases the possibility of neutralization through seminal fluid-derived HIV-1-specific IgG and IgA.

Contrary to our original hypothesis that exposure to higher VL in the infected partner would elicit more neutralizing IgA responses from HESN women, there was an inverse association between the partner's VL and the likelihood of detecting an HIV-1-neutralizing cervicovaginal IgA response. We further analyzed the infected partner's seminal HIV-1 VL and found a similar trend. A potential mechanism for this association is that different HIV-1 strains have varying abilities to elicit neutralizing IgA responses, and IgA responses may be different than IgG responses. Studies have shown that VL is positively correlated with plasma neutralizing antibody, which predominantly measure IgG responses [22–24]. It is possible that the relationship is different between mucosal HIV-neutralizing IgA and HIV-1 VL. In order to interpret the relationship between VL and induction of HIV-1-neutralizing IgA, further studies should examine the activity against several different virus isolates (including the partner's own isolate), quantify the antibody titers, and complement with additional functional assays. Furthermore, estimating HIV-1 exposure should not only take VL into account, but also the hormonal and inflammatory status of the genital mucosa.

Shorter duration of relationship and lack of income for the male partner were also associated with having a cervicovaginal HIV-1-neutralizing IgA response among HESN women. Both associations were independent of partner's VL. A plausible explanation for the association may be due to more risky behavior in the women with shorter relationships, leading to greater frequency and magnitude of viral exposure. Additionally, lack of income may be a marker for lower socioeconomic status, and previous studies have shown that lower condom use was seen in people reporting lower wealth or difficult financial situations [25, 26]. Having a male partner without an income may have an indirect association with greater unprotected sexual exposures which may lead to more neutralizing IgA responses.

Finally, our study was initially designed to determine if the presence of cervicovaginal HIV-1-neutralizing IgA would decrease the acquisition of HIV-1. Despite our large numbers of HESN women in this cohort, only 5 women acquired HIV-1 when followed for two years. Of these 5 women, none had HIV-1-neutralizing cervicovaginal IgA responses. However, with such a small number of HIV-1 acquisitions, our study was not powered to determine if the presence of HIV-1-neutralizing IgA decreases the risk of acquiring HIV-1.

Another limitation of this study is that we only used one type of assay and one virus isolate to determine neutralization of HIV-1, and thus, may have underestimated detection of HIV-1-neutralizing IgA. In addition, our study did not normalize for total IgA content despite measuring for HIV-1-neutralization from purified IgA, and we did not define whether the majority of the cervicovaginal antibodies were derived from the systemic or mucosal (secretory IgA) compartment. Finally, we lacked detailed information about the HIV-1-serostatus of former sexual partners of the women in the control group. The study site in Nairobi represents an HIV-1-endemic region and presence of HIV-1-neutralizing IgA in cervicovaginal secretions of the control women could possibly reflect previous sexual HIV-1 exposure.

In summary, our study found that HIV-1-neutralizing IgA is present in cervicovaginal mucosa of HESN women, and there is a 5-fold higher odds of detecting HIV-1-neutralizing IgA in HESN women compared to control women. While the association of lower partner's

plasma VL and presence of cervicovaginal neutralizing IgA differs from our hypothesized mechanism that greater exposure to HIV-1 virus would better elicit neutralizing IgA, this could be explained by a preferential induction of IgA by certain viral strains. Understanding the role of mucosal HIV-1-inhibiting IgA, how it differs in function and specificity from systemic monomeric IgA as studied in vaccinated individuals [27], as well as in already infected individuals [28], and the mechanisms contributing to its induction could make a significant contribution in developing a vaccine or a microbicide with activity against HIV-1 at mucosal surfaces.

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Figure 1. Presence of cervicovaginal HIV-1 neutralizing IgA in HIV-1 exposed seronegative (HESN) and control women

Cervicovaginal samples were collected from HESN and control women. Thirty-six (23%) of 155 HESN women and 4 (6%) of 70 control women had neutralizing activity ($P=0.003$). After excluding samples with detectable prostate specific antigen ($1\mu\text{g/ml}$), this association remained significant ($P=0.01$)

Figure 2. Plasma and seminal HIV-1 viral load in male partners of HIV-1 exposed seronegative (HESN) women with and without detectable cervicovaginal HIV-1-neutralizing IgA

A comparison was made for (a) plasma and (b) seminal HIV-1 viral loads in male partners among HESN women with and without detection of cervicovaginal HIV-1-neutralizing IgA. Plasma and seminal viral loads below the lower limit of detection were set to half the lower limit (75 copies/mL for plasma and 250 copies/mL for semen), as indicated by the broken line. Solid lines indicate median concentration, whiskers indicate 5–95 percentile. P-values for plasma and seminal viral loads comparisons were 0.02 and 0.06, respectively.

TABLE 1

Demographic data for HIV-1 discordant couples with female HIV-1 exposed seronegative (HESN) partners and control women in HIV-1 seroconcordant negative relationships

Characteristics	N	Median (IQR) or n (%)	N	Median (IQR) or n (%)
		<i>HESN Women</i>		<i>Control Women</i>
Age	155	30 (25–35)	70	25 (22–30)
Lifetime partners	154	3 (2–3)	70	3 (2–3)
Years in relationship	152	6 (2–11)	69	2 (0.8–7)
Presence of cervicovaginal PSA ($1\mu\text{g/ml}$)	155	15 (10%)	70	23 (33%)
History of sexually transmitted infections	148	32 (22%)	68	17 (25%)
HSV-2 seropositivity	155	103 (67%)	66	21 (32%)
Bacterial vaginosis	152	19 (13%)	56	14 (25%)
Earn income	155	55 (36%)	70	28 (40%)
Less than primary school education	155	48 (31%)	70	11 (16%)
		<i>HIV-1 Infected Male Partner</i>		<i>HIV-1 Uninfected Male Partner</i>
Age	155	36 (32–43)	69	30 (25–34)
Lifetime partners	154	6 (4–9)	69	5 (3–8)
CD4 cell count (cells/ μl)	151	356 (238–521)		N/A
Plasma viral load ¹	154	4.7 (4.1–5.3)		N/A
Seminal viral load ¹	93	2.9 (2.4–4.2)		N/A
History of sexually transmitted infections	147	70 (48%)	67	26 (39%)
HSV-2 seropositivity	155	102 (66%)	67	18 (27%)
Circumcision	155	112 (72%)	69	62 (90%)
Earn income	155	132 (85%)	69	56 (81%)
Less than primary school education	155	23 (15%)	69	7 (10%)
		<i>Couple</i>		<i>Couple</i>
Sex acts ²	155	5 (2–9)	70	7 (3–12)
No unprotected sex ²	155	123 (79%)	70	19 (27%)
Combined income (KSh/month) ³	155	9000 (4000–16,000)	69	10,000 (5000–17,000)

Abbreviations: IQR, Interquartile range

¹Log₁₀ copies/ml

²With study partner in the past month

³Approximately 1 US dollar = 85 Kenyan Shillings

TABLE 2

Correlates of cervicovaginal HIV-1-neutralizing IgA, among HIV-1 exposed seronegative (HESN) women

	IgA positive n=36	IgA negative n=119	OR (95% CI)	p-value
	Median (IQR) or n (%)			
<i>HESN Female Participant Factors</i>				
Age ¹	28 (24–31)	31 (26–36)	0.70 (0.88–0.99)	0.02
Sex acts ²	3.3 (2–7)	5 (3–9)	0.94 (0.86–1.03)	0.17
Years in relationship ^{1,3}	4 (2–8)	7 (2–13)	0.68 (0.49–0.94)	0.02
Presence of cervicovaginal PSA (1µg/ml)	2 (6%)	13 (11%)	0.48 (0.10–2.23)	0.35
Lifetime STI	7 (21%)	25 (22%)	0.92 (0.36–2.37)	0.87
HSV-2 seropositivity	22 (61%)	81 (69%)	0.72 (0.33–1.56)	0.40
Bacterial Vaginosis	6 (17%)	13 (11%)	1.58 (0.55–4.53)	0.39
<i>HIV-1 Infected Male Partner Factors</i>				
CD4 cell count (cells/µl)	398 (196–498)	354 (253–533)	1.00 (0.84–1.20)	0.96
Plasma viral load ⁴	4.3 (3.1–4.9)	4.8 (4.1–5.4)	0.70 (0.51–0.94)	0.02
Seminal viral load ⁴	2.4 (2.4–3.5)	3.2 (2.4–4.3)	0.57 (0.32–1.02)	0.06
Lifetime STI	13 (36%)	60 (52%)	0.53 (0.24–1.14)	0.10
HSV-2 seropositivity	26 (72%)	76 (64%)	1.47 (0.65–3.34)	0.36
Circumcision	23 (64%)	89 (75%)	0.60 (0.27–1.32)	0.20
Earn income ³	26 (72%)	106 (89%)	0.32 (0.13–0.81)	0.02
<i>Couple Factors</i>				
No unprotected sex ²	29 (81%)	94 (79%)	1.10 (0.43–2.81)	0.84

Abbreviations: OR, Odds ratio; CI, Confidence interval; IQR, Interquartile range

¹Per 5 years²With study partner in the past month³Statistically significant when excluding PSA 1µg/ml⁴Log₁₀ copies/ml