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Hormonal Contraception and Female-to-Male HIV Transmission Risk Among Sero-discordant Couples, Zambia 1994-2012

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Epidemiology

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ABSTRACT

Hormonal Contraception and HIV Female-to-Male Transmission Risk Among Sero-discordant Couples, Zambia 1994-2012

By Preeti Ravindhran

Background: The effect of hormonal contraceptive methods (oral contraceptive pills (OCPs), implants, injectables) on HIV transmission risk from HIV-positive women to HIV-negative male partners has long been debated and is a critical public health issue, especially in areas with high HIV prevalence. Given the uncertainty of current literature, stakeholders and decision-makers require further evidence to make recommendations on contraceptive use among HIV-affected women.

Methods: HIV-discordant couples in Zambia were followed longitudinally from 1994-2012. Only couples where the woman was HIV-positive and the man was HIV-negative (M-F+) at baseline were considered in this study. Baseline and time-varying measures on demographics, behavioral, and clinical risk factors for HIV were collected at 3-month intervals. A multivariate Cox model was used to evaluate the association between hormonal contraception and time to HIV transmission from HIV+ women to HIV- male partners among couples where HIV infection was determined to be genetically linked.

Results: Out of 1,656 sero-discordant M-F+ couples that were considered in this study, 171 couples experienced genetically linked HIV transmission form the woman to man during the study period. Neither OCPs (aHR 0.92; 95% CI 0.55 – 1.55) nor implants (aHR 0.88; 95% CI 0.34 – 2.29) nor injectables (aHR 0.62; 95% CI 0.33 – 1.15) was found to be significantly associated with time to HIV infection when compared to non-hormonal methods (intrauterine device, condoms, permanent methods or no contraceptive method). These results are controlled for man's age, viral load of HIV-positive woman, male circumcision status, pregnancy status, and measures for unprotected sex (self-report, sperm present on wet prep, genital inflammation in either partner, genital ulceration among men).

Conclusion: Hormonal contraception usage among HIV-positive women does not appear to significantly increase the risk of HIV transmission to male partners among sero-discordant Zambian couples. However, the body of literature surrounding this issue remains relatively small and further research must be conducted to fully support evidence-based policy recommendations on contraceptive usage among HIV-positive women.

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CHAPTER I

BACKGROUND

Hormonal contraceptive methods—including oral contraceptive pills (OCPs), injectables, patches, rings, and implants—are highly effective and safe ways to prevent unintended pregnancies. Certain methods of hormonal contraception are also utilized for additional health benefits such as controlling irregular periods. Among HIV-positive pregnant women, hormonal contraception also prevents vertical HIV transmission (from mother to child). Such contraception methods are critical tools for family planning and play an important role in reducing maternal mortality and pregnancy-related morbidity (1). Contraceptive methods enable women to be empowered, reduce poverty and hunger, and ensure long-term environmental sustainability (1).

Despite their benefits, hormonal contraceptive methods have been suspected to increase the risk of HIV acquisition among HIV-negative women and onward transmission to men from HIV-positive women. Since hormonal contraceptives typically change the genital environment, there are a variety of biologically plausible mechanisms through which an increase in HIV transmission or acquisition could occur. One mechanism may be that hormonal contraceptive methods cause an increase in genital HIV shedding which in turn could lead to increased risk of transmission (2). Hormonal contraception may increase genital HIV shedding through a direct effect on the genital track or through indirect effects on conditions that increase shedding (e.g. cervical inflammation) (2).

Another mechanism is through co-infection with sexually transmitted infections (STIs). STIs lead to increased genital inflammation and therefore a breakdown in the protective barrier environment in a healthy woman's genital tract, also causing higher amounts of genital HIV and yielding an increased risk of HIV transmission (2). While the link between hormonal contraception and STIs is ambiguous, some studies have shown an

association between STIs like chlamydia and gonorrhea and increased viral shedding (2). In addition to HIV shedding, plasma viral load is also thought to be a predictor of HIV transmission and researchers speculate that hormonal contraception may have an effect on plasma viral load (2). However, a recent study using a pigtail macaque model found that receiving depot-medroxyprogesterone (DMPA), an injectable form of hormonal contraception, had little to no effect on plasma viral load or genital HIV shedding (3). While DMPA injectables may affect humans differently than primates, results from animal models may still provide insight into whether hormonal contraception can have an effect on mechanisms that may increase risk of HIV transmission.

Other plausible biologic mechanisms include changes in vaginal flora and in local or systemic immunity. Hormonal contraceptives have been shown to increase the risk of yeast vaginitis and reduce the number of protective H_2O_2 lactobacilli in the vaginal environment (4). Both of these effects can help propagate HIV transmission (4). Furthermore, hormonal contraception can cause genetic inflammation, which in turn recruits certain CCR5-expressing T cells (target HIV cells) to the genetic environment (4). The increased presence of these target HIV cells can potentially increase the risk of HIV acquisition in at-risk women and of transmission from HIV-infected women to their uninfected male partners (4). Out of the aforementioned mechanisms however, it is unclear if any play a relevant role in increased HIV transmission from women to uninfected male partners (5).

Despite the uncertainty of biological mechanisms behind hormonal contraception's effect on HIV transmission, there have been several studies that have explored the epidemiological association between hormonal contraception usage and HIV transmission from HIV-positive females to male partners. However, these studies have been largely inconclusive. A recent systematic review evaluated evidence assessing the indirect relationships between hormonal contraception and proxy outcomes for HIV acquisition (e.g.

genital shedding, plasma viral load). Of 17 indirect studies discussed in this systematic review, all but one found no association between any type of hormonal contraception and proxy measures for woman-to-male HIV transmission risk (2). The one study that did find a significant association only found an increased risk of transmission among women from seven African countries using injectables as opposed to other forms of hormonal contraception (2). Again, these studies provided only indirect evidence, and HIV transmission was not directly measured (2).

The same systematic review identified one other study that did directly measure the association between hormonal contraceptive methods and HIV transmission. This study, conducted by Heffron *et al*., demonstrated a significant association between hormonal contraception and female-to-male transmission in HIV sero-discordant couples from seven African countries (6). In this study, both DMPA injectables and OCPs were found to yield elevated risks of HIV transmission but only DMPA injectables were significantly associated with HIV transmission (6). However, there were serious limitations to this study. As a confounder, condom usage was assessed based on self-report, which has been demonstrated to be inaccurate (6). Adjusting for condom usage in the analysis may not have fully eliminated bias. Also, there may be other unmeasured factors that contribute to a woman's choice of contraceptive method and sexual behavior, thus resulting in residual confounding (5). Finally, nearly all HIV-positive partners were co-infected with herpes simplex virus-2 (HSV-2), weakening the generalizability of this study (6).

Like the Heffron *et al.* study, a 2013 study conducted by Lutalo *et al.* among Ugandan couples also directly measured HIV transmission but found no significant risk increase of transmission associated with usage of OCPs or DMPA injectables in female-to-male transmission without adjusting for the viral load of infected partners (7). After adjustment, point estimates for HIV transmission increased among users of hormonal contraception but

were not statistically significant (7). Although results from the Heffron *et al.* study were not replicated, the authors concluded that the possibility of hormonal contraception affecting HIV transmission from females to males could not be ruled out (7). It is thus clear that the scientific community has not yet reached a consensus whether hormonal contraception usage increases the risk of HIV transmission and further research is required.

In 2014, World Health Organization revisited guidelines for hormonal contraception usage among women living with asymptomatic or mild HIV clinical disease (as determined by WHO stages, see reference) and determined not to place any restrictions on recommended usage of hormonal contraception (8). Women living with severe or advanced HIV clinical disease were recommended not to use levonorgestrel IUDs due to concerns regarding disease progression rather than transmission and other forms of hormonal contraception were not restricted (8). These recommendations, however, and other policies may change as the evidence base increases.

Long-acting reversible contraception (LARC), including IUDs and hormonal implants, are cost-effective methods to prevent unintended pregnancies (9). However, LARCs include several hormonal forms of contraception (e.g. implants) so the promotion of certain LARCs to HIV-affected populations may exacerbate HIV transmission if such a link is found. Consideration must be given to the competition between preventing HIV transmission and preventing unintended pregnancies, both two critical public health issues (10). A deeper understanding and evaluation of the proposed association between hormonal contraception and HIV transmission is thus absolutely critical to ensure that current recommendations and policies are accurate and advance public health.

This study aims to explore the association, if one exists, between hormonal contraceptive use (including OCPs, injectables, and implants) and increased risk of female-to-male HIV transmission while controlling for potential confounders in a cohort of HIV-

discordant couples (F+M-) in Zambia, where HIV prevalence is high and contraception uptake remains relatively low. It is estimated that about 1.1 million people in Zambia are living with HIV, of which more than half are women (11). Furthermore, an estimated 51% of women between the ages of 15 and 49 do not use any form of modern contraception (12). Because of this unmet need for contraception, efforts are underway to increase access (13). Due to the sizable HIV-affected population in Zambia however, consideration must be given to how increased contraceptive usage may adversely affect HIV transmission.

CHAPTER II | MANUSCRIPT

INTRODUCTION

Hormonal contraceptive methods—oral contraceptive pills (OCPs), implants, and injectables—are critical tools for family planning and play an important role in reducing maternal mortality and pregnancy-related morbidity (1). Despite their benefits, hormonal contraceptive methods have been suspected to increase HIV acquisition risk among HIV-negative women and onward transmission among HIV-positive women. There are a variety of plausible biological mechanisms through which increased transmission could occur but it is unclear whether they play any relevant role in HIV transmission (2-5).

Previous literature on female-to-male HIV transmission has been inconclusive in assessing the association between hormonal contraception and increased risk. One prospective cohort study in which HIV transmission was directly measured in men from seven African countries found that the only type of hormonal contraception significantly associated with increased female-to-male HIV transmission was depot-medroxyprogesterone (DMPA) injectables, as opposed to other forms of hormonal contraception (OCPs, implants) or non-hormonal methods (6). Subsequent studies have since failed to replicate these results, instead finding that no hormonal contraceptive method is significantly associated with increased HIV transmission from HIV-positive women to HIV-negative men (2, 7). However, the scientific community has not yet reached a consensus and stakeholders are calling for more stringent studies on the issue in order to support policy and programmatic recommendations.

Currently, official guidelines from World Health Organization place no restriction on recommended hormonal contraception use (OCPs, injectables, implants) among women at-risk or living with HIV (1, 8). If however such contraceptive methods are shown to increase HIV transmission risk, balance must be struck between two conflicting public health

interests: reducing HIV spread and promoting hormonal contraception, especially long-acting reversible contraception (LARC), which are cost-effective methods to prevent unintended pregnancy (9, 10). Such guidelines are especially important in HIV-affected areas like Zambia, where 1.1 million people are currently living with HIV and half of all women use contraception (11-13).

METHODS

Study Design and Population

From 1994-2012, HIV-discordant couples (married or cohabitating) identified through couples' voluntary HIV counseling and testing (CVCT) services in Lusaka, Zambia were enrolled and followed longitudinally by the Rwanda Zambia HIV Research Group (RZHRG). CVCT includes various services such as rapid HIV testing, group counseling, and combined couple counseling after HIV testing. Previous literature has described RZHRG promotions and recruitment (14-16), enrollment, retention (17), testing, counseling (18, 19), and cohort demographics (20). Couples' counseling and testing was briefly promoted by local influential leaders and community members to couples 16 years of age or above. Baseline and time-varying exposures were collected, including self-reported contraceptive use, condom use, male circumcision status, and measures of unprotected sex, such as self-report sex history and sperm on a wet prep. Couples were only included in this study if they met the following parameters: woman was HIV-positive and her male partner was HIV-negative at baseline (F+M- couples), the woman was not on antiretroviral treatment (ART), and the couple had at least one follow-up visit after enrolling.. All participants provided written informed consent and this study was approved by the Office for Humane Research Protections-registered Institutional Review Boards at Emory University and in Zambia.

Longitudinal Data Collection

Over the 17 years of follow-up (1994-2012), the type and frequency of data collection did not remain consistent. During the entire follow-up period, both partners were seen quarterly, received physical and genital examinations, and laboratory testing for sexually transmitted infections. Physical exams and STI laboratory testing was performed for each couple at baseline and afterward based on visibly present signs and symptoms. Starting in 1999, plasma banking for VL testing became available. In 2003, p24 ELISA screening of HIV-negative partners began. Fertility goals were only recorded from 2002-2011. Couples demonstrating at least one route of exposure for HIV infection (e.g. unprotected sex, sperm on a wet prep, incident pregnancy, or incident STI) received quarterly HIV testing until the next 3-month follow-up visit, during which time risk assessment for HIV transmission was repeated. From 2008-2011, HIV-negative partners were tested monthly while their HIV-positive partners were only seen quarterly.

Exposure of Interest

Type of contraception used by HIV-positive women was assessed at baseline and at three-monthly follow-up visits. Contraceptive method was categorized as follows: combined oral contraceptive pills (OCP), DMPA injectables (dosage 150mg IM), copper intrauterine device (IUD), implants (Norplant, Jadelle), or permanent methods (hysterectomy, vasectomy, tubal ligation). For this analysis, contraceptive type was re-categorized as implant, injectable, or OCP versus non-hormonal contraceptive methods (none/condoms, copper IUD, permanent methods).

Baseline Covariates

Various demographic data was collected at enrollment including age, number of years cohabitating, monthly family income, and Nyanja literacy. Other covariates assessed at baseline include behavioral and clinical risk factors for unprotected sex and HIV

transmission, such as number of previous pregnancies, pregnancy status, fertility intentions of both partners, yearly and lifetime sex partners of both partners, male circumcision status, and viral load of HIV-positive women (in copies/mL, log-transformed for the analysis).

Time-Varying Covariates

Further data was collected at regularly scheduled three-monthly follow-up visits or visits initiated by the client. Data reflecting the last three months was collected on pregnancy status, number of sex acts with and without condoms, protected and unprotected sex with study partner, and whether or not sperm was present on a wet prep of a vaginal swab. Summary variables were created for genital inflammation and genital ulceration for men and women using time-varying measures collected by physical examinations or laboratory tests, whether diagnosed or treated at the research clinic or reported by the study participant. Genital ulceration included erosion or friability of the vagina or cervix in women and positive RPR serology for syphilis (21).Physical examination measures for genital inflammation include cervical or vaginal inflammation in women, genital discharge (urethral discharge in men and vaginal discharge in women), and laboratory diagnosis and treatment for sexually transmitted infections (trichomoniasis, gonorrhea, chlamydia, candida, or bacterial vaginosis) (22).

Outcome of Interest

The outcome of interest is time to HIV transmission from HIV-positive women to their HIV-negative male partners. Any HIV infection in male partners were genetically linked to HIV-positive women (i.e. infection acquired from study partner rather from outside the study couple). Negative male partners were tested for HIV infection every three months during follow-up visits using a screening and confirmatory rapid HIV serologic test, as described in previous literature (18). Time of infection was determined, when possible, through testing of plasma obtained from the last antibody negative sample with p24 ELISA

and RNA polymerase chain reaction (PCR). Infections were determined to be genetically linked through PCR-amplified comparisons of nucleotide sequences from each partner (23). Eleven couples with missing linkage information were reclassified as being linked (23, 24).

Data Analysis

All analyses were conducted using Statistical Analysis Software (SAS version 9.4). Descriptive analyses for baseline covariates (demographics, family planning and sexual history, clinical indicators, and HIV risk factors) were stratified by couples' HIV transmission status (all couples, non-seroconverters, and linked infections). Continuous baseline covariates were described by means and standard deviations while counts and percentages were used for categorical covariates. Significant differences between non-seroconverting couples and couples with linked infections were determined using unequal variance 2-sample t-tests (for continuous variables) and chi-squared tests of association (for categorical variables).

Bivariate associations between time to HIV infection and baseline or time-varying covariates were evaluated using crude hazard ratios (HRs) and 95% confidence intervals (CIs). P-values for hazard ratios were computed using Wald tests. Time to outcome was determined as time taken for the HIV-negative male partner to seroconvert. Couples were censored if seroconversion did not occur during the study period, if either partner died, the couple separated, the HIV-positive woman began antiretroviral treatment (ART), or if either partner was lost to follow-up.

Variables with significant bivariate associations with the outcome of interest (using a Bonferroni adjusted p-value of 0.002 as the significance level), along with method of contraceptive use, were included in an initial multivariate Cox model. All time-independent variables satisfied the proportional hazards assumption, which was checked using Schoenfeld residuals and log [-log(survival probability)] versus log(time) plots. Multi-

collinearity was assessed using condition indices of 30 and variance decomposition proportions of 0.50 as cutoff criteria. Effect-measure modification was evaluated between the contraceptive method and male circumcision status, male/woman genital inflammation and male genital ulceration. Variables with significant bivariate associations with both the exposure and outcome were considered as confounders. Using a final multivariate Cox model, adjusted hazard ratios and 95% CIs were reported for all levels of contraceptive method.

RESULTS

Baseline Demographics (Table 1)

Of the 1,656 sero-discordant couples (HIV- men, HIV+ women) considered in this study, 86% of them were non-seroconverters (n=1,430), 10% (n=171) experienced seroconversion genetically linked to the HIV-positive woman in the study, and 3% (n=55) experienced incident HIV infection obtained from a partner not in the study. No couples in this study were found to have unknown linkage. At α=0.05, mean age among non-seroconverting couples was significantly higher (35.4 men and 29.0 women) than for couples with linked HIV infections (33.1 men and 27.0 women). Non-seroconverting couples had significantly greater number of years cohabitating (6.0 years) than linked couples (4.7 years). Couples experiencing linked HIV infections lived in households with lower monthly incomes ($65.15/month) than non-seroconverting couples ($95.59/month). Fertility intentions and circumcision status of the male partner differed significantly between non-seroconverting couples and linked couples. HIV-positive women in linked infection couples had higher viral load than HIV+ women in non-seroconverting couples.

At baseline, 77% of women (n=1261) were using condoms or using no contraceptive method at all. In non-seroconverting couples, 76% (n=1086) of HIV+ women used no method or condoms while 79% (n=134) of women in couples experiencing

seroconversion used no method or condoms. Differences in contraceptive use between women in non-seroconverting couples and in seroconverting couples were not significant. No significant differences was found in pregnancies, yearly and lifetime sex partners (male and woman), and women's fertility intentions between non-seroconverting couples and linked couples.

Bivariate analyses (Tables 2 and 3)

Bivariate associations between some baseline risk factors and time to HIV infection were significant at a significance level of 0.05: man's age, woman's age, years cohabitating, number of previous pregnancies, fertility intentions of the male, viral load of the woman (continuous categorization and categorical), and male circumcision status (Table 2).

Except for injectables, no other hormonal contraceptive method (with non-hormonal methods grouped and ungrouped) was found to have a significant bivariate association with time to HIV infection (Table 3). Couples in which the HIV+ woman was using injectables as a contraceptive method had a significant association with time to HIV infection compared to couples where the HIV+ woman did not use any contraceptive method or used condoms [crude HR 0.55 (95% CI 0.30 – 0.98), P=0.0412]. Time-varying covariates that did have significant associations with time to HIV infection included pregnancy during interval and pregnancy status during follow-up visits, whether woman had sex with study partner in the last 3 months and number of times (self-report by the woman), sperm present on wet prep, genital inflammation in either partner, and genital ulceration in the man (Table 3).

Multivariate analyses (Table 4)

Hormonal contraception was not associated with any incident HIV infection among couples where HIV infection was linked to the study partner in multivariate analyses. No effect-measure modification was found between hormonal contraception and male

circumcision status, genital inflammation in men or women, or genital ulceration in men. Only variables with significant associations (using α=0.002 as cutoff criteria) with the outcome of interest were evaluated for multi-collinearity and none were found to be collinear. During confounding assessment, seven variables (viral load of HIV+ woman, unprotected sex in past 3 months, sperm on wet prep, genital inflammation of either partner, genital ulceration of male, and male circumcision status) were found to be non-confounders because they were not significantly associated with contraceptive method. However, they were retained in the final model because these risk factors were known *a priori* to be strongly associated with time to HIV infection.

Among couples experiencing linked HIV infection, use of OCPs (aHR 0.92; 95% CI 0.55-1.55), implants (aHR 0.88; 95% CI 0.34-2.29), or injectables (aHR 0.62; 95% CI 0.33-1.15) was not found to be significantly associated with HIV transmission from a HIV+ woman to her male partner relative to using non-hormonal contraceptive methods (IUD, condoms, permanent methods, or no method). Results are controlled for male's age (per year increase), woman's baseline log viral load (per $\log_{10}$ copies/mL increase), male circumcision status, pregnancy status during interval, pregnant at follow-up visit, unprotected sex with study partner in last 3 months (reported by woman), sperm present on wet prep, genital inflammation in either partner, and genital ulceration in males.

DISCUSSION

The use of hormonal contraception (whether OCP, implant, or DMPA injectable) was not associated with an increased risk of HIV transmission from HIV+ Zambian women to their HIV- male partners after adjusting for demographic, behavioral, and clinical risk factors. In other words, hormonal contraceptive use among HIV+ women was not found to lead to faster HIV transmission from women to men in sero-discordant Zambian couples. Although still non-significant, injectable use resulted in the lowest adjusted hazard ratio for

seroconversion among men (aHR 0.62; 95% CI 0.33-1.15), suggesting that women who use injectables actually take longer to transmit HIV to their male partners than women who use non-hormonal methods. These results are, for the most part, in accordance with previous studies (e.g. the Lutalo *et al.* study) that have similarly found no significant association between hormonal contraception and increased HIV transmission risk. This does however conflict with the Heffron *et al.* study, which found injectables to be significantly associated with an increased risk of HIV transmission (aHR 1.97; 95% CI 1.06-3.58) (6). Due to the conflicting evidence, further research is required to truly assess which forms of hormonal contraception, if any, increase risk of HIV transmission from HIV+ women to HIV- men in sero-discordant couples.

The design and analysis of this investigation overcomes several challenges faced by previous studies (25). Measures of unprotected sex were taken by self-report and compared to clinical indicators of unprotected sex over 17 years of prospective follow-up. Contraceptive use was measured frequently (every three months) to accurately capture rates of usage, stopping, and switching methods. Unlike many other studies, this study makes a distinction between all HIV infections and HIV infections in men that are genetically linked to the HIV+ women enrolled in the study, which ensures that modeling covariates for the woman (including contraceptive use) is appropriate to describe HIV infection in men. Previous literature describing RZHRG data has described the accuracy of self-reported contraceptive methods among the study population of interest (26). Finally, the Zambian sero-discordant couples captured in this population are inherently an analysis group with similar within-sample risk of HIV exposure.

CVCT clients are not necessarily representative of the general population in Zambia. Due to having received counseling and contraceptive education, CVCT clients are more

likely to use condoms and have full access to a variety of contraceptive methods (including LARC methods) at the research clinic (27-29).

There are some limitations to this study. One, there is a potential for unmeasured confounders (e.g. genetic factors specific to the population) that may bias estimated hazard ratios in an unknown direction. Furthermore, data collection over time was not consistent (e.g. fertility intentions were only measured from 2002-2011 rather than for the entire study period) due to changes in funding. Selection bias at enrollment and number of couples lost to follow-up may also introduce bias into this study. Previous work with this population has found that F+M- discordant couples are less likely to be eligible and enroll more likely to be lost to follow-up than F-M+ couples (17). Factors that were predictive of attrition include residence far from the clinic, younger age, and women's age at first intercourse as 17 years or less (17). F+M- couples are slightly younger with shorter durations of union than M-F+ couples in this study so this study's findings may be more generalizable to younger, less experienced couples (17). Time since enrollment was also not controlled for in this study. This may potentially bias results since those who just joined the study may be more likely to engage in unprotected sex and therefore have a higher risk of HIV transmission. This investigation also estimated total effects (exposure or covariate-mediated pathways in addition to direct effects) controlling for confounding. Although this does not clarify on questions regarding the biological plausibility of hormonal contraception on HIV transmission risk, estimates of total effects may be more relevant when using estimates to inform public policy.

The importance of assessing whether hormonal contraception increases HIV transmission risk from HIV+ women to HIV- men must be considered when developing and implementing public policy surrounding HC. This current evidence must be weighed against other factors (e.g. effectiveness of contraceptive method, especially LARC methods, rate of

unintended pregnancies, maternal and child mortality, and vertical HIV transmission) before making a public health recommendation on whether HC should or should not be used in certain scenarios. In addition, context-specific HIV prevalence, maternal mortality rates, access and utilization of HC, and cost-effectiveness of HC promotion and uptake must also be taken into consideration when framing public policies and public health interventions.

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TABLES

Table 1. Descriptive analyses of baseline covariates by seroconversion outcomes

	Total couples		Non-seroconverting couples		Linked infections		P-value**
	N / Mean	% / SD	**N / Mean**	% / SD	**N / Mean**	% / SD	
Total	**1,656**	100%	**1,430**	86%	**171**	10%	
Demographics							
Man age (mean, SD)	35.10	8.42	35.43	8.48	33.08	8.03	0.0004
Woman age (mean, SD)	28.71	6.69	28.96	6.76	27.02	6.25	0.0002
Years cohabiting (mean, SD)	5.88	5.87	6.04	5.98	4.69	5.02	0.0015
Monthly family income (US dollar equivalent)	91.54	114.78	95.59	117.47	65.16	101.71	0.0003
Woman reads Nyanja (N, %)							
Yes, easily	428	26%	379	27%	36	22%	0.1478
With difficulty/not at all	1208	74%	1037	73%	131	78%	
Family planning and sexual history							
Number of previous pregnancies (mean, SD)	3.17	2.22	3.20	2.22	2.96	2.20	0.1795
Pregnant (N, %)							
Yes	241	15%	199	14%	31	18%	0.1377
No	1,415	85%	1231	86%	140	82%	
Contraceptive method (N, %)							
None/condoms alone	1,261	77%	1,086	76%	134	79%	0.4540
OCPs	153	9%	127	9%	19	11%	
Injectables	149	9%	133	9%	11	6%	
Implant	50	3%	46	3%	3	2%	
IUD	24	1%	21	1%	3	2%	
Permanent method	11	1%	10	1%	0	0%	
Fertility intentions of man (N, %)*							
Yes, next year	105	18%	92	19%	10	16%	0.0149
Yes, but not next year	216	38%	169	35%	33	53%	
Don't know/No	254	44%	226	46%	19	31%	
Fertility intentions of woman (N, %)*							
Yes, next year	207	28%	183	28%	16	24%	0.3522
Yes, but not next year	169	23%	144	22%	20	30%	
Don't know/No	367	49%	321	50%	31	46%	
Man lifetime sex partners (mean, SD)	10.93	16.87	11.15	17.77	9.36	8.80	0.0318
Man last year sex partners (mean, SD)	1.65	1.74	1.63	1.80	1.69	1.10	0.5507
Woman lifetime sex partners (mean, SD)	3.88	6.24	3.87	6.34	3.77	5.27	0.8214

Woman last year sex partners (mean, SD)	1.13	1.10	1.14	1.17	1.13	0.45	0.7655
Clinical (HIV+ woman)							
Log viral load, log10 copies/ml (mean, SD)	4.39	0.93	4.29	0.95	4.76	0.69	<0.0001
Viral load categories, copies/ml (N, %)							
<10,000	182	31%	137	35%	24	16%	
≥10,000 to <100,000	244	41%	157	40%	66	44%	<0.0001
≥100,000	169	28%	102	26%	60	40%	
Male circumcision status (N, %)							
Yes	284	17%	267	19%	12	7%	<0.0001
No	1369	83%	1160	81%	159	93%	

Fertility intentions collected from 2002-2011
OCP: oral contraceptive pill; IUD: copper intrauterine device
*unpooled p-value comes from 2-sample t-test for differences between seroconverting and non-seroconverting couples

Table 2. Associations between baseline covariates and time to linked HIV infection (n=1,656 M-F+ couples)				
	HR*	**95% CI**		**P-value**
Demographics				
Man age (per year increase)	0.97	0.95	0.99	0.0013
Woman age (per year increase)	0.97	0.94	0.99	0.0069
Years cohabiting at baseline (per year increase)	0.95	0.92	0.98	0.0021
Monthly family income (per US dollar increase)	1.00	1.00	1.00	0.2610
Woman reads Nyanja				
Yes, easily (ref)	n/a	n/a	n/a	n/a
With difficulty/not at all	1.19	0.83	1.73	0.3475
Family planning and sexual history				
Number of previous pregnancies (per pregnancy increase)	0.90	0.83	0.98	0.0162
Pregnant				
Yes	1.28	0.86	1.88	0.2220
No (ref)	n/a	n/a	n/a	n/a
Fertility intentions of man				
Yes, next year	1.26	0.59	2.72	0.5542
Yes, but not next year	2.08	1.18	3.67	0.0115
Don't know/No	n/a	n/a	n/a	n/a
Fertility intentions of woman				
Yes, next year	1.26	0.68	2.33	0.4700
Yes, but not next year	1.35	0.77	2.36	0.3023
Don't know/No (ref)	n/a	n/a	n/a	n/a
Man lifetime sex partners (per partner increase)	0.99	0.98	1.01	0.2441
Man last year sex partners (per partner increase)	1.02	0.94	1.09	0.6769
Woman lifetime sex partners (per partner increase)	1.00	0.98	1.02	0.9846
Woman last year sex partners (per partner increase)	0.96	0.72	1.26	0.7495
Clinical (HIV+ woman)				
Log viral load (per log10 copies/mL increase)	1.90	1.54	2.34	<.0001
Viral load categories, copies/mL				
<10,000 (ref)	n/a	n/a	n/a	n/a
≥10,000 to <100,000	2.30	1.45	3.65	0.0004
≥100,000	3.55	2.20	5.72	<.0001
Male circumcision status				
Yes (ref)	n/a	n/a	n/a	n/a
No	2.69	1.49	4.85	0.001

*Non-seroconverting couples are the reference group

Table 3. Associations between time-varying covariates and time to linked HIV infection (n=1,656 M-F+ couples)						
	Linked infections (N = 171)					
	N intervals	%	HR*		95%CI	p-value
Current contraceptive method at follow-up visit						
Non-hormonal (IUD, nothing/condoms, tubal ligation) (ref)	130	77%	n/a	n/a	n/a	n/a
Implant	5	3%	0.59	0.24	1.46	0.2548
Injection	13	8%	0.54	0.30	0.97	0.0399
OCPs	21	12%	0.79	0.49	1.26	0.3153
Current contraceptive method at follow-up visit						
IUD	3	2%	1.21	0.38	3.88	0.7434
Nothing/condoms (ref)	125	74%	n/a	n/a	n/a	n/a
Permanent method	2	1%	0.86	0.21	3.47	0.8261
Implant	5	3%	0.59	0.24	1.47	0.2605
Injection	13	8%	0.55	0.30	0.98	0.0412
OCPs	21	12%	0.79	0.49	1.26	0.3206
Pregnant during interval						
Yes	26	16%	2.32	1.52	3.54	<.0001
No (ref)	132	84%	n/a	n/a	n/a	n/a
Pregnancy status						
Pregnant	26	16%	2.24	1.47	3.42	0.0002
Not pregnant	132	82%	n/a	n/a	n/a	n/a
Post-partum (up to 6 months post delivery)	3	2%	0.70	0.22	2.22	0.5496
Sexual history						
Number of times sex with partner in project with a condom in the last 3 months reported by woman	21.37	26%	1.00	0.99	1.01	0.9609
Number of times sex with partner in project without a condom in the last 3 months reported by woman	7.70	20%	1.01	1.01	1.02	0.0002
Sex with study partner with a condom reported in past 3 months reported by woman						
Yes (ref)	144	84%	n/a	n/a	n/a	n/a
No	27	16%	0.94	0.62	1.43	0.7774

Sex with study partner without a condom reported in past 3 months reported by woman						
Yes	100	58%	2.37	1.74	3.23	<.0001
No (ref)	71	42%	n/a	n/a	n/a	n/a
Sperm present on wet prep						
Yes	25	17%	2.40	1.50	3.84	0.0003
No (ref)	124	83%	n/a	n/a	n/a	n/a
Clinical history						
Genital inflammation in man in the past 3 months						
Yes	64	37%	7.03	4.46	11.08	<.0001
No (ref)	107	63%	n/a	n/a	n/a	n/a
Genital inflammation in woman in the past 3 months						
Yes	75	44%	2.73	1.84	4.06	<.0001
No (ref)	95	56%	n/a	n/a	n/a	n/a
Genital ulceration in man in the past 3 months						
Yes	39	23%	4.00	2.76	5.80	<.0001
No (ref)	132	77%	n/a	n/a	n/a	n/a
Genital ulceration in woman in the past 3 months						
Yes	31	18%	1.53	1.02	2.30	0.0401
No (ref)	139	82%	n/a	n/a	n/a	n/a

*Non-seroconverting couples are the reference group

Table 4. Multivariate models of hormonal contraception use and time to HIV infection (M-F+ couples)

	Linked infections			
Current contraceptive method at follow-up visit	**aHR***	**95%CI**		**p-value**
Non-hormonal (IUD, nothing/condoms, permanent method)	ref	ref	ref	ref
OCP	0.92	0.55	1.55	0.7580
Implant	0.88	0.34	2.29	0.7930
Injectables	0.62	0.33	1.15	0.1305
Number of events in model = 129				

**Controlling for…man's age, log viral load, male circumcision status, pregnancy status, number of times woman had sex with partner without a condom in the last 3 months, sperm present on wet prep, genital inflammation in man or woman in the past 3 months, genital ulceration of man in past 3 months*

CHAPTER III

SUMMARY

From 1994-2012, 1,656 sero-discordant couples (HIV+ women and HIV- men) were followed longitudinally through a research clinic in Lusaka, Zambia, where HIV serostatus and demographic, behavioral, and clinical risk factors were measured every three months. A multivariate Cox model analysis found that hormonal contraceptive methods (OCPs, implants, injectables) are not significantly associated with increased time to HIV infection compared to non-hormonal methods (copper IUD, permanent methods, condoms, or no method) among sero-discordant Zambian couples after adjusting for demographic, behavioral, and clinical risk factors. These findings are in alignment with some previous studies that have also found no association between hormonal contraceptive methods and HIV transmission risk. However, conflicting evidence from other literature indicates that further research with more rigorous standards of evaluation must be conducted before a consensus can be reached in the scientific community.

The design and analysis of this investigation add certain strengths and minimize certain sources of error and bias present in other studies. The cohort of Zambian sero-discordant couples in this study all had relatively similar risk for HIV exposure, minimizing bias that may result if this was not the case. Proxy measures for unprotected sex (e.g. clinical indicators such as sperm from a vaginal swab on a wet prep) were gathered over 17 years of follow up and contraceptive method was assessed every three months to accurately capture usage, method switching, and stoppage. Furthermore, this study only looks at infections where HIV transmission was genetically linked to both partners in the study, reducing confounding from This study therefore overcomes several challenges faced by previous studies in that couples were followed over 17 years and risk factors were measured frequently.

However, certain limitations to this study reduce generalizability. RZHRG CVCT clients (especially F+M- couples) are less likely to enroll and more likely to be lost to follow-up, potentially resulting in selection bias (17). Furthermore, CVCT clients are more likely than the general population to use contraceptive methods and practice safe sex due to the counseling, education, and contraceptive methods they receive at the research clinic (27-29). Unmeasured confounders such as genetic factors and missing data from inconsistent data collection may further bias hazard ratio estimates in an unknown direction.

PUBLIC HEALTH IMPLICATIONS

There are about half a million women in Zambia currently living with HIV (11). Furthermore, there is a growing need for increased access and uptake of contraception in Zambia; 51% of women aged 15-49 do not use any form of contraception (12, 13). With the government, NGOs, and other stakeholders working to increase access to contraception – including certain highly efficient and cost-effective hormonal contraceptive methods (e.g. LARC) – consideration must be given to the potential impact of such programs on the sizable HIV-affected population in Zambia. If there truly is an association between hormonal contraception and female-to-male HIV transmission, efforts to increase hormonal contraception uptake in areas with high HIV prevalence may inadvertently lead to increased HIV incidence among HIV-negative males. This study adds to a growing body of literature that has found no association between hormonal contraception and female-to-male HIV transmission and can be used to inform WHO guidelines and recommendations on hormonal contraception. However, further research must be conducted to absolutely ensure that policies surrounding hormonal contraception are not in conflict with HIV-related public health priorities.

FUTURE POSSIBLE DIRECTIONS

While this study's findings of no significant association between hormonal contraception and HIV transmission are in accordance with other studies that have had similar results, these results must be replicated in a multitude of settings and populations before consensus can be reached and public health policy can be well-substantiated by evidence. The epidemiologic link between hormonal contraception and female-to-male HIV transmission, as well as the biological mechanism, need to be determined. Future studies that look into this topic should adequately control for confounding from other factors (history of unprotected sex, pregnancy status, demographics, co-infections with other STIs) measured from both partners while ensuring that HIV infection in the seroconverter has been genetically linked to the woman taking hormonal contraception. Contraceptive usage should also be accurately measured to account for switching methods and stopping. Furthermore, research is also required regarding plausible biological mechanisms by which hormonal contraception can increase risk of transmitting HIV among HIV+ women and acquiring HIV among HIV- men.