Hormonal contraceptive use, herpes simplex virus infection, and risk of HIV-1 acquisition among Kenyan women

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Background: Studies of the effect of hormonal contraceptive use on the risk of HIV-1 acquisition have generated conflicting results. A recent study from Uganda and Zimbabwe found that women using hormonal contraception were at increased risk for HIV-1 if they were seronegative for herpes simplex virus type 2 (HSV-2), but not if they were HSV-2 seropositive.

Objective: To explore the effect of HSV-2 infection on the relationship between hormonal contraception and HIV-1 in a high-risk population. Hormonal contraception has previously been associated with increased HIV-1 risk in this population.

Methods: Data were from a prospective cohort study of 1206 HIV-1 seronegative sex workers from Mombasa, Kenya who were followed monthly. Multivariate Cox proportional hazards analyses were used to adjust for demographic and behavioral measures and incident sexually transmitted diseases.

Results: Two hundred and thirty-three women acquired HIV-1 (8.7/100 person-years). HSV-2 prevalence (81%) and incidence (25.4/100 person-years) were high. In multivariate analysis, including adjustment for HSV-2, HIV-1 acquisition was associated with use of oral contraceptive pills [adjusted hazard ratio (HR), 1.46; 95% confidence interval (CI), 1.00–2.13] and depot medroxyprogesterone acetate (adjusted HR, 1.73; 95% CI, 1.28–2.34). The effect of contraception on HIV-1 susceptibility did not differ significantly between HSV-2 seronegative versus seropositive women. HSV-2 infection was associated with elevated HIV-1 risk (adjusted HR, 3.58; 95% CI, 1.64–7.82).

Conclusions: In this group of high-risk African women, hormonal contraception and HSV-2 infection were both associated with increased risk for HIV-1 acquisition. HIV-1 risk associated with hormonal contraceptive use was not related to HSV-2 serostatus.

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Keywords: herpes simplex virus type 2, HIV acquisition, HIV transmission, hormonal contraception

Introduction

A number of epidemiologic studies have explored whether the use of hormonal contraception, including oral contraceptive pills and the injectable progestin depot medroxyprogesterone acetate (DMPA), is a risk factor for HIV-1 acquisition [1,2]. The question is one of considerable public health importance – hormonal forms...
of contraception are used by more than 100 million women worldwide [3] and women of reproductive age make up nearly half of new HIV-1 infections [4]. To date, studies of this issue have provided conflicting results, with increased risk found among some populations, particularly high-risk groups such as commercial sex workers, but not others.

A recent prospective study has generated additional debate. Among 4439 women recruited from family planning clinics in Uganda and Zimbabwe, neither oral contraceptive pills [adjusted hazard ratio (HR), 0.99; 95% confidence interval (CI), 0.69–1.42] nor DMPA (adjusted HR, 1.25; 95% CI, 0.89–1.78) was associated with HIV-1 acquisition [5]. Among the subgroup of women who were seronegative for herpes simplex virus type 2 (HSV-2) (48% of the study population), however, both hormonal methods increased HIV-1 risk (for oral contraceptive pills, adjusted HR, 2.85; 95% CI, 1.39–5.82; and for DMPA, adjusted HR, 3.97; 95% CI, 1.98–8.00), a finding that was robust in multiple sensitivity analyses. No association between hormonal contraceptive use and HIV-1 risk was seen among HSV-2 seropositive women, and this difference in HIV-1 risk for contraceptive users by HSV-2 status was highly statistically significant (P = 0.003). These results were surprising and the authors hypothesized they may have reflected a strong effect of HSV-2 (a known risk factor for HIV-1 [6]) overwhelming the effect of contraception among the HSV-2 seropositive participants. They called for additional investigations to explore whether this differential effect by HSV-2 status could be observed in other populations.

We previously reported the results of a 10-year prospective study among Kenyan female sex workers, among whom both oral contraceptive pill and DMPA use were associated with elevated HIV-1 risk [7]. In that analysis, HSV-2 infection was not assessed. In the present study, we have re-examined those data to explore the effect of HSV-2 infection on the relationship between hormonal contraception and HIV-1.

Methods

Population and procedures

Study procedures have been previously detailed [7,8]. Briefly, beginning in February 1993, HIV-1 seronegative women attending a municipal communicable disease clinic in Mombasa, Kenya were enrolled. At approximately monthly follow-up visits, sexual behavior and contraceptive use were recorded and screening for HIV-1 and sexually transmitted diseases was performed. Individualized risk reduction counseling and free condoms were provided at all visits. The study was approved by the institutional review boards of the University of Washington, the Fred Hutchinson Cancer Research Center, and the University of Nairobi. Participants provided informed consent for study procedures.

SeroLOGY and microbiology

HIV-1 serology was performed using an enzyme-linked immunosorbent assay (ELISA; Detect-HIV, Biochem Immunosystems, Montreal, Quebec, Canada), with positive results confirmed by a second ELISA (Recombigen; Cambridge Biotech, Worcester, Massachusetts, USA).

Serologic testing for HSV-2 was performed on archived frozen specimens using a type-specific, HSV-2 gG-based ELISA (HerpeSelect; Focus Diagnostics, Cypress, California, USA). A staged protocol was used. Enrollment samples for all participants were tested first. Those with index values (the ratio of the optical density of the sample to that of a standard calibrator) > 1.1 were defined as seropositive, according to the manufacturer’s instructions. Some data, however, suggest that index values between 1.1 and 3.5 may have reduced specificity for detecting HSV-2 infection, especially among individuals from East Africa [9,10]. Thus, samples with index values in this range were defined as having 'low positive' results. For women with negative or low positive serologic results at enrollment, we then tested their last follow-up sample to look for a change in HSV-2 serostatus. Finally, we tested intervening follow-up samples for women whose serostatus changed, in order to better define the timing of seroconversion. Follow-up samples with index values between 1.1 and 3.5 were defined as low positive, as at enrollment. Women whose serostatus changed from low positive to definitively positive (i.e., to an index value > 3.5) during follow-up had intervening samples tested to define the timing of this transition. Enrollment samples were serum, and follow-up samples were plasma; these sample types have been shown to provide similar results for the ELISA used [11].

Light microscopy of a vaginal wet preparation was used to diagnose vaginal Trichomonas vaginalis infection and candidiasis on the basis of identifying motile trichomons and yeast forms, respectively. Bacterial vaginosis was diagnosed by microscopy of a vaginal Gram stain [12]. Cervical secretions were cultured for Neisseria gonorrhoeae on modified Thayer–Martin media. Cervicitis was defined by an average of ≥ 30 polymorphonuclear leukocyte cells/high power field of Gram-stained cervical secretions.

Data analysis

Analyses were performed using SPSS 10.0 (SPSS, Chicago, Illinois, USA) and S-Plus 2000 (Mathsoft, Cambridge, Massachusetts, USA). All visits between February 1993 and January 2003, the period of our previous analysis [7], at which both contraceptive exposure and HSV-2 serostatus could be determined were selected. Due to the small numbers, visits at which
contraceptive methods other than oral contraceptive pills or DMPA were reported (primarily IUDs and Norplant) were excluded.

The associations between hormonal contraception, HSV-2 infection, and HIV-1 acquisition were evaluated using Cox proportional hazards analysis. We aimed to address three principal questions: (1) whether adjusting for HSV-2 serostatus changed our previously-documented association between hormonal contraception and HIV-1 acquisition [7] (i.e., whether there was confounding by HSV-2); (2) whether the relationship between hormonal contraception and HIV-1 risk differed by HSV-2 status (i.e., whether there was effect modification by HSV-2), as seen in the recent study from Uganda and Zimbabwe [5]; and (3) whether HSV-2 was associated with HIV-1 acquisition [6]. HIV-1 events were analyzed as occurring on the date HIV-1 seroconversion was detected at a clinic visit.

Hormonal contraception was analyzed in a time-dependent fashion. As we have previously done [7,8], we estimated that hormonal contraceptive methods would have a persistent effect on HIV-1 risk for 70 days after discontinuation. We assumed that HIV-1 infection would occur, on average, at the midpoint between visits, that visits occurred every 30 days, and that HIV-1 antibodies take 25 days to appear after infection [13]. Under these assumptions, we estimated that HIV-1 seroconversion would be detected 45 days after HIV-1 acquisition, that is, generally at the second clinic visit occurring after HIV-1 infection, as antibodies would not have developed at the first visit after HIV-1 was acquired. Thus, an exposure interval of 115 (70 + 45) days was used for women who stopped or changed hormonal contraceptive methods. Women who changed from one hormonal method to the other (e.g., from DMPA to oral contraceptive pills) were classified as being exposed to both methods during the time the exposure intervals overlapped. Women reporting no contraceptive method or who had had a tubal ligation served as the comparison group for contraceptive analyses. Notably, condom use was analyzed as a separate covariate since condoms were used by many in the cohort for sexually transmitted disease prevention, often in addition to another method for pregnancy prevention.

HSV-2 serostatus was also analyzed as a time-dependent variable, with HSV-2 seronegative women serving as the comparison group. For most analyses, all HSV-2 seropositive results were considered as a group. To explore the effect of low positive HSV-2 serologic results (those with index values between 1.1 and 3.5), additional analyses were performed excluding those visits at which low positive results were measured. Finally, an analysis was performed examining HIV-1 risk associated with recent incident HSV-2 infection (within the previous 6 months), detected during follow-up [14,15].

To investigate whether HSV-2 modified the effect of hormonal contraception on HIV-1 risk, interaction terms between HSV-2 serostatus and contraceptive method were added to the multivariate model. Evidence of effect modification was considered to be present if the P-value for these interaction terms was \( \leq 0.05 \). Stratified analyses by HSV-2 status were also performed.

Multivariate models included contraceptive method and HSV-2 serostatus. In addition, models controlled for several demographic and behavioral variables, and incident sexually transmitted diseases. These included data collected at study enrollment: educational level (≤ 8 versus > 8 years, the median for the cohort), parity (0, 1–2, > 2), workplace (bar versus nightclub [8]), and vaginal washing practices (none, with water, with soap/other substances [16]). Time-dependent adjustment for several variables was also performed: age (< 25, 25–29, ≥ 30 years), duration of sex work (< 1 versus > 1 year, the median for the cohort at enrollment), number of sexual partners per week (< 1 versus > 1), condom use (< 100% versus 100%), and incident genital tract infections (genital ulcer disease, bacterial vaginosis, vaginal candidiasis, trichomoniasis, cervicitis, and gonorrhea — each analyzed as a separate variable). For the sexual behavior variables (number of sex partners and condom use), an average was calculated for each year of follow-up, in order to capture average behavior over time. As we have done previously [8], we assumed an effect window of 60 days to capture the influence of genital tract infections on HIV-1 susceptibility. Excluding incident genital ulcer disease from the analyses had negligible effects on the results of the multivariate models (data not shown).

**Results**

Between February 1993 and January 2003, 1498 women were enrolled in the cohort, of whom 1272 (84.9%) returned for follow-up. Of these, 1206 (94.8%) met the criteria for inclusion in this analysis.

The median age at enrollment was 26 years [interquartile range (IQR), 22–31 years]. Sexual activity was relatively low, with a median of 1 (IQR, 1–2) sexual partner per week, as most participants (74%) had primary employment as barmaids and supplemented their income with sex work. None reported injection drug use and only three (<1%) practiced anal sex, making heterosexual vaginal intercourse the principal HIV-1 risk factor for study participants. Those included in this analysis were slightly older (26 versus 25 years, \( P = 0.03 \)) and were less likely to be bar workers (74 versus 81%, \( P = 0.02 \)) compared with those who were not included or did not return for follow-up.

At enrollment, 171 women (14.2%) used oral contraceptive pills and 244 (20.2%) used DMPA. In total, 269
(22.3%) and 369 (30.6%) women used oral contraceptive pills and DMPA, respectively, during follow-up.

At enrollment, 234 women (19.4%) were HSV-2 seronegative and 972 (80.6%) were HSV-2 seropositive, of whom 148 (15.2%) had low positive HSV-2 serologic results. Among those who were HSV-2 seronegative at enrollment, 84 seroconverted to HSV-2 during follow-up (incidence 25.4 cases/100 person-years). Of these 84 seroconverters, 39 (46.4%) had only low positive HSV-2 results.

The median duration of follow-up was 456 days (IQR, 148–1254 days), the median number of follow-up visits was 6 (IQR, 2–15), and visits were separated by a median of 35 days (IQR, 28–54). A total of 14 448 follow-up visits were accumulated, reflecting 2682 person-years of follow-up. Two hundred and thirty-three women seroconverted to HIV-1 (incidence 8.7 cases/100 person-years).

Hormonal contraception and HIV-1 acquisition, adjusted for herpes simplex virus-2 status

In our previously-reported analysis [7], oral contraceptive pill and DMPA use were each associated with increased HIV-1 infection risk (adjusted HR, 1.5; 95% CI, 1.0–2.1 and adjusted HR, 1.8; 95% CI, 1.4–2.4, respectively). Further adjustment for HSV-2 infection had little effect on these findings, with statistically significant increases in HIV-1 susceptibility maintained for both contraceptive methods (Table 1), demonstrating that HSV-2 serostatus did not confound the relationship between contraceptive use and HIV-1 acquisition.

Effect of herpes simplex virus-2 status on the relationship between hormonal contraceptive use and HIV-1 risk

There was no statistically significant evidence of effect modification by HSV-2 status on the relationship between oral contraceptive pill or DMPA use (interaction terms $P = 0.7$ and $P = 0.2$, respectively) and HIV-1 acquisition. Among HSV-2 seropositive women, use of hormonal contraception was associated with statistically significantly elevated HIV-1 risk (Table 2). Among HSV-2 seronegative women, the risk estimate for the relationship between DMPA and HIV-1 was markedly elevated, although the confidence interval was wide, reflecting that the association was based on a small number of cases.

Herpes simplex virus-2 infection and HIV-1 risk

Most HIV-1 infections occurred among HSV-2-seropositive women (Table 2). In univariate analysis, HSV-2 seropositivity was associated with a >3-fold increased risk of HIV-1 acquisition, a finding that was maintained in the multivariate model (Table 1). There was little change in the risk estimate for the effect of HSV-2 on HIV-1 when low positive HSV-2 serologic results were dropped from the analysis (adjusted HR, 3.50; 95% CI, 1.58–7.73;
HSV-2 infection who seroconverted to HIV-1, eight (72.7%) had HIV-1 and HSV-2 seroconversions detected at the same study visit.

**Discussion**

Among this group of high-risk Kenyan women, use of hormonal contraception was associated with increased risk for HIV-1 acquisition. HSV-2 infection did not confound this relationship, nor was there statistical evidence that the effect of hormonal contraception on HIV-1 risk differed between women who were HSV-2 seropositive and those who were seronegative. HSV-2 infection was independently associated with significantly increased risk of HIV-1.

Several possible mechanisms by which hormonal contraception might influence HIV-1 susceptibility have been suggested, including changes in vaginal epithelial structure and promotion of cervical ectopy [17,18], increased susceptibility to sexually transmitted diseases and altered genital tract flora [19,20], and upregulation of HIV-1 co-receptors in the genital tract [21]. Prospective epidemiologic studies to investigate whether use of hormonal contraceptive increases HIV-1 risk have, however, generated conflicting findings [5,7,8,22–33]. Issues relating to study quality, including poor measurement of hormonal contraception exposures and timing of HIV-1 seroconversions, high loss to follow-up, and failure to adjust for potential confounding factors, probably explain some of this disagreement [2,5,34]. Studies that have shown elevated HIV-1 risk associated with hormonal contraceptive use have tended to be those among high-risk populations, such as commercial sex workers [7,8,22,32], suggesting that the effect may vary for different at-risk groups.

The impetus for the present analysis was a recent report from a community-based study of women from Uganda and Zimbabwe, among whom hormonal contraceptive use had no relationship with incident HIV-1 overall, although strong associations (HR, approximately 3–4) between oral contraceptive pill and DMPA use and HIV-1 were present for women who were HSV-2 seronegative [5]. Our results do not replicate these findings. We found statistically significantly increased HIV-1 risk overall for women who used oral contraceptive pills and DMPA, as well as among those in the subgroup who were HSV-2 seropositive. Among those who were seronegative for HSV-2, small numbers limited our ability to detect an association between oral contraceptive pill use and HIV-1, but DMPA showed a strong relationship. Thus, our results argue against the hypothesis that HIV-1 risk is associated with hormonal contraceptive use only among HSV-2 seronegative women, but support previous findings that high-risk women using hormonal contraception are at elevated risk for HIV-1.

A potentially central role for HSV-2 in the expansion of the HIV-1 epidemic has come to be recognized in the last few years [35]. A recent meta-analysis concluded that HSV-2 is associated with an approximately three-fold increased risk for HIV-1 among men and women in the general population [6], an effect that is likely mediated through mucosal epithelial disruption. The authors did not, however, demonstrate a significant relationship between HSV-2 and incident HIV-1 in studies conducted among high-risk women (summary relative risk, 1.0). Our results argue that HSV-2 is, in fact, a strong HIV-1 risk factor for women at high-risk for HIV-1.

Recent HSV-2 acquisition may have particularly strong effects on HIV-1 susceptibility, although not all studies

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**Table 2. HIV-1 incidence and multivariate association with hormonal contraceptive method, stratified by herpes simplex virus (HSV)-2 serostatus.**

<table>
<thead>
<tr>
<th>HSV-2 seropositive</th>
<th>Number of HIV-1 cases/person-years of follow-up</th>
<th>HIV-1 incidence per 100 person-years</th>
<th>Multivariate HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>None/tubal ligation</td>
<td>116/1647</td>
<td>7.0</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral contraceptive pills</td>
<td>37/255</td>
<td>1.5</td>
<td>1.51</td>
<td>1.03–2.21</td>
<td>0.04</td>
</tr>
<tr>
<td>DMPA</td>
<td>73/511</td>
<td>1.4</td>
<td>1.68</td>
<td>1.23–2.29</td>
<td>0.001</td>
</tr>
<tr>
<td>HSV-2 seronegative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None/tubal ligation</td>
<td>2/171</td>
<td>1.2</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral contraceptive pills</td>
<td>1/65</td>
<td>1.3</td>
<td>0.77</td>
<td>0.05–11.40</td>
<td>0.9</td>
</tr>
<tr>
<td>DMPA</td>
<td>6/48</td>
<td>12.5</td>
<td>32.50</td>
<td>1.19–885</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Cl, confidence interval; DMPA, depot medroxyprogesterone acetate; HR, hazard ratio.

$P = 0.002$. Recent incident HSV-2 infection (within 6 months of a study visit) was associated with high risk for HIV-1 (adjusted HR, 4.94; 95% CI, 1.91–12.80; $P = 0.001$). Among the 11 women with recent incident HSV-2 infection who seroconverted to HIV-1, eight (72.7%) had HIV-1 and HSV-2 seroconversions detected at the same study visit.
have found this effect and few controlled for potential confounding factors [6]. There is biological plausibility to suggest this association, as HSV-2 reactivations occur with greater frequency and severity early after HSV-2 acquisition [36]. We found that recent HSV-2 infection, defined as within the prior 6 months, was associated with a nearly 5-fold increased risk for HIV-1, after adjusting for demographic and behavioral characteristics and incident sexually transmitted diseases. Most women with recent HSV-2 who seroconverted to HIV-1 had both infections detected at the same visit, however, making it impossible to exclude co-transmission of these two viruses.

Several features support the validity of this study’s findings. Prospective, monthly data collection allowed precise measurement of the timing of hormonal contraceptive exposures and HIV-1 outcomes. Multivariate analyses adjusted for strong predictors for HIV-1, including incident sexually transmitted diseases and sexual behavior, which were also measured at each study visit. Importantly, behavioral confounding may be less of an issue for high-risk women, who may use condoms for HIV-1 and sexually transmitted disease protection independent of choices for pregnancy prevention, compared with women from the general population, whose use of (or inability to use) condoms with stable partners may be related to HIV-1 risk within that partnership. Finally, our large sample size and high HIV-1 incidence allowed us to detect associations between hormonal contraception and HIV-1 with hazard ratios of approximately 1.5–1.7, risk estimates that few other prospective studies would have had the statistical power to measure.

Safe and effective contraceptive choices are essential for women at-risk for HIV-1. Given the available data, both high-risk women such as commercial sex workers and, paradoxically, women from the general population without HSV-2 (nearly half of the sample in the recent study [5]) face elevated HIV-1 risk as a result of hormonal contraceptive use. Debate regarding the true risk that use of hormonal contraception poses for women at-risk for HIV-1 will continue. Nonetheless, it is clear that hormonal contraceptives are not protective against HIV-1 acquisition, and the public health message must be that dual-protection with condoms should be the goal for women using hormonal contraception. Our findings also confirm that HSV-2 infection itself is a strong risk factor for HIV-1. On-going clinical trials of HSV-2 therapy to prevent HIV-1 acquisition have the potential to demonstrate a powerful prevention intervention for women, both for those at high and lower risk for HIV-1.

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