A Prospective Study of Hormonal Contraceptive Use and Cervical Shedding of Herpes Simplex Virus in Human Immunodeficiency Virus Type 1–Seropositive Women

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Cross-sectional analyses have demonstrated an association between use of hormonal contraceptives and shedding of herpes simplex virus (HSV). This prospective study evaluated the effect of initiating use of hormonal contraception on cervical HSV detection. Two hundred women who were seropositive for HSV-2 and human immunodeficiency virus (HIV) type 1 were examined for cervical mucosal HSV by use of quantitative DNA polymerase chain reaction before and after beginning the use of hormonal contraceptives. Cervical HSV was detected in 32 women (16.0%) before initiating and in 25 women (12.5%) after initiating use of hormonal contraception (P = .4).

There were no significant differences in HSV shedding among the subgroups of women starting combination oral contraceptives containing both estrogen and progesterone or progesterone-only contraceptives. Among the 54 women who shed HSV at least once, the median change in cervical HSV after initiation of hormonal contraception was −313 copies/swab. In this prospective study, use of hormonal contraceptives did not increase detection of cervical HSV.

High rates of coinfection with herpes simplex virus (HSV) type 2 and human immunodeficiency virus (HIV) type 1 have been demonstrated in African populations [1, 2]. Infection with HSV is an important cause of genital ulcer disease (GUD), which increases HIV-1 susceptibility and transmissibility [3–6]. Understanding the factors that influence HSV reactivation among individuals with HIV-1 may help to reduce transmission of both viruses.

Several risk factors for genital tract shedding of HSV have been demonstrated. Viral shedding is detected more frequently in women seropositive for HSV-2 than in women seropositive for HSV-1 [7]. Recent infection, frequent symptomatic recurrences, young age, and immunosuppression have also been associated with increased genital shedding of HSV [7–11]. A recent cross-sectional study among HIV-1–seropositive women found that cervical shedding of HSV was associated with the use of oral contraceptive pills (OCPs) (odds ratio [OR], 5.3; 95% confidence interval [CI], 1.8–15.2; P = .002) and injectable progestrone (OR, 3.6; 95% CI, 1.5–9.0; P = .006) [1]. To further explore the relationship between HSV reactivation and hormonal contraception, a prospective study of HSV shedding was conducted among women initiating use of hormonal contraception.

**Methods**

**Study population and procedures.** Between September 1996 and May 1999, women presenting to the family planning clinic at Coast Provincial General Hospital, Mombasa, Kenya, for initiating use of hormonal contraception were offered confidential counseling and testing for HIV-1 infection. After giving informed consent, women were screened for HIV-1 infection by use of a rapid serological test (Capillus HIV-1/HIV-2; Cambridge Biotech). Samples found to be HIV-1 positive on the screening test were confirmed by EIA (Detect; BioChem Immunosystems). Women with positive test results were invited to participate in the study.

Patients were interviewed about demographic, sexual, obstetrical, and medical history by use of a standardized questionnaire. A physical examination was performed, and genital tract specimens were collected for diagnosis of sexually transmitted diseases (STDs)
and detection of HSV and HIV-1. Ten milliliters of anticoagulated blood was collected in tubes containing EDTA for HSV serologic testing (MRL Diagnostics), confirmation of HIV-1 serostatus, and lymphocyte subset analysis. All examinations and sample collection were performed by 1 of 2 investigators (R.S.M. or C.C.W.).

Endocervical secretions were collected by inserting separately 3 dacron swabs 1 cm into the cervical os and rotating each swab 3 times. The first swab was placed in a dry cryovial for detection of HIV-1 DNA and quantitation of HSV DNA. The second and third swabs were placed in 1 mL of freezing medium (70% RPMI, 20% fetal calf serum, and 10% dimethyl sulfoxide with added penicillin, streptomycin, and amphotericin B) for quantitation of HIV-1 RNA. Swabs were stored on ice for up to 4 h and then transferred to a −70°C freezer (dry swabs) or liquid nitrogen (swabs in freezing medium). Next, cervical and vaginal secretions were collected for STD diagnosis. Women found to have STDs or vaginitis were treated according to the Kenyan National Treatment Guidelines (Kenya Ministry of Health). STD counseling and latex condoms were provided by trained study nurses.

Women who desired hormonal contraception could choose either combination OCPs (30 mg ethinyl estradiol plus 0.1 mg levonorgestrel daily for 21 days, followed by an inert pill for 7 days), progestosterone-only OCPs (0.35 mg norethindrone daily), or injectable progesterone (150 mg depot-medroxyprogesterone acetate) every 3 months. Early in the study, 7 women started at a higher-dose combination OCP (50 mg ethinyl estradiol plus 0.5 mg norgestrel). Hormonal contraception was started following the cessation of menstrual bleeding. Women with irregular menses and those who were breastfeeding had a urine pregnancy test to rule out pregnancy.

All women were asked to return at 4 and 8 weeks after starting hormonal contraception. At each follow-up visit, a brief history was obtained, and a physical examination and collection of specimens were repeated. If STDs were diagnosed or yeast infections were observed, women were treated and asked to return in 2 weeks to repeat the examination, to confirm that the infection was cured and to collect specimens for the HSV shedding study in the absence of other genital tract infections.

Baseline visits (prior to initiating the use of hormonal contraceptives), when women were free of Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis, and vaginal yeast infection, were identified. Women were excluded if they had received treatment for these infections within 2 weeks of initiating use of hormonal contraceptives. The baseline visit was compared with the final follow-up visit at which women were free of genital tract infections. To be included in the analysis, the follow-up visit had to be completed within 6 months of starting hormonal contraceptives.

Microbiology The number of polymorphonuclear leukocytes in 3 nonadjacent high-power fields on microscopy of cervical Gram stains was quantified. Culture for N. gonorrhoeae was performed on modified Thayer-Martin media. Presence of C. trachomatis was determined by EIA (MicroTrak II; Behring Diagnostics). Gram-stained vaginal secretions were scored for bacterial vaginosis [12], and a wet mount was examined for the presence of yeast and T. vaginalis.

Lymphocyte subsets were counted using a manual method (Cytosphere; Coulter) until September 1998, after which a semiautomated system (Zymmune; Bartels) was used.

Detection and quantitation of HSV DNA. Cervical dry swab samples were assayed for HSV DNA by use of a high-throughput, semiautomated, quantitative, fluorescence-based PCR technique, as described elsewhere [13]. The assay uses primers to the common region of HSV glycoprotein B and detects both HSV-1 and HSV-2. The linear limit of quantitation for this assay includes values from 10 to $10^7$ copies/reaction (2.5 x 10$^2$ to 2.5 x 10$^7$ copies/swab).

Data analysis. Infection with HSV-2 is associated with a significantly greater frequency of detection in genital secretions in comparison with infection with HSV-1 [7]. The analysis presented here was restricted to HSV-2–seropositive women (including dually seropositive women). McNemar’s test was used for comparison of paired observations for binary data, and the Wilcoxon signed rank test was used for comparison of paired continuous data. Statistical tests were performed using SPSS software, version 10.0 (SPSS).

Results

Patient characteristics. Among 209 women who initiated use of hormonal contraceptives, 200 (95.7%) were seropositive for HSV-2, and 198 (94.7%) were seropositive for HSV-1. Baseline characteristics of the 200 HSV–2–seropositive women are shown in table 1. Thirty-five (17.9%) of these women had CD4 cell counts <200 cells/µL. Plasma HIV-1 loads of >30,000 copies/mL were observed in women with no. (%) of women

<table>
<thead>
<tr>
<th>Variable (n = 200)$^a$</th>
<th>Median (IQR) or no. (%) of women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>25 (22–29)</td>
</tr>
<tr>
<td>Years of education</td>
<td>8 (5–10)</td>
</tr>
<tr>
<td>Married</td>
<td>159 (81)</td>
</tr>
<tr>
<td>Monogamous or abstinent in past year</td>
<td>176 (89)</td>
</tr>
<tr>
<td>Days since last intercourse</td>
<td>14 (5–90)</td>
</tr>
<tr>
<td>No. of pregnancies</td>
<td>2 (1–3)</td>
</tr>
<tr>
<td>Currently breastfeeding</td>
<td>132 (66)</td>
</tr>
<tr>
<td>CD4 cell count, cells/µL</td>
<td></td>
</tr>
<tr>
<td>&lt; 200</td>
<td>35 (18)</td>
</tr>
<tr>
<td>200–499</td>
<td>94 (48)</td>
</tr>
<tr>
<td>≥500</td>
<td>66 (34)</td>
</tr>
<tr>
<td>Plasma HIV-1 load &gt; 30,000 copies/mL</td>
<td>133 (71)</td>
</tr>
<tr>
<td>HSV-1 seropositive</td>
<td>187 (94)</td>
</tr>
<tr>
<td>Genital ulcer on exam</td>
<td>11 (6)</td>
</tr>
<tr>
<td>Hormonal contraceptive type</td>
<td></td>
</tr>
<tr>
<td>Combination OCP</td>
<td>59 (30)</td>
</tr>
<tr>
<td>Progestrone-only OCP</td>
<td>49 (25)</td>
</tr>
<tr>
<td>DMPA</td>
<td>92 (46)</td>
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</tbody>
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NOTE. Combination OCP contained 30 mg ethinyl estradiol plus 0.1 mg levonorgestrel (n = 52) or 50 mg ethinyl estradiol plus 0.5 mg norgestrel (n = 7), and progestrone-only OCP contained 35 mg norethindrone. DMPA, 150 mg depot-medroxyprogesterone acetate intramuscular injection; IQR, interquartile range; OCP, oral contraceptive pill.

$^a$n = 197 for “Age,” “Married,” “Monogamous or abstinent in past year,” and “No. of pregnancies”; n = 196 for “Years of education”; and n = 188 for “Plasma HIV-1 load >30,000 copies/mL.”
Hormonal contraception and HSV shedding. Cervical swabs from the 200 HSV-2–seropositive women were tested for the presence of HSV before and after starting use of hormonal contraceptives. There was no association between hormonal contraceptive use and cervical shedding of HSV (table 2). The virus was detected in 32 samples (16.0%) at baseline and in 25 samples (12.5%) at follow-up (P = .4). Among 59 women who started use of combination OCs, cervical HSV was detected in 12 (20.3%) at baseline and in 8 (13.6%) at follow-up (P = .5). Among 141 women who started use of progesterone-only contraceptives, HSV was detected in 20 (14.2%) at baseline and in 17 (12.1%) at follow-up (P = .7).

Overall, there was no significant difference in the quantity of HSV in cervical secretions before and after initiating use of hormonal contraceptives (median 0 vs. 0 copies/reaction; P = .4). Less than half the women had any cervical HSV at either visit. Among the 54 women who shed HSV, the median cervical mucosal HSV level at visits when shedding occurred was 4.0 x 10^4 copies/swab (range, 2.5 x 10^4 to 2.5 x 10^6 copies/swab). Among these women, the median levels before and after initiating use of hormonal contraceptives were similar, with either low copy numbers or no detection of HSV (413 vs. 0 copies/swab; P = .4). The median change in HSV load among the 54 patients with HSV shedding was -313 copies/swab (IQR, -1.6 x 10^4 to 6.6 x 10^3 copies/swab). Only 3 women had detectable HSV at both the baseline and follow-up visit.

There was no association between initiating use of hormonal contraceptives and the presence of a genital ulcer on physical examination. Overall, there were 11 women (5.5%) with GUD at baseline and 10 (5.0%) with GUD at follow-up (P = .8). Only 3 women had GUD present at both examinations.

### Discussion

This prospective study found no evidence that initiating use of hormonal contraceptives increases cervical shedding of HSV. Sixteen percent of women had detectable cervical HSV at baseline, and there was no significant change after initiation of hormonal contraception. Furthermore, there was no significant change in the quantity of cervical mucosal HSV among women initiating hormonal contraception. These data also demonstrated that HSV-2 shedding was a very frequent complication of HIV-1 infection among Kenyan women.

In a previous cross-sectional study, mucosal HSV was detected in 26% of women using hormonal contraceptives, compared with 11% of women not using hormonal contraceptives [1]. Comparison of the findings from this prospective investigation with those of the cross-sectional analysis illustrates several reasons why it is difficult to infer causality from cross-sectional studies. Although adjustment for CD4 lymphocyte count was performed in the cross-sectional analysis, residual confounding due to the immune status of patients cannot be ruled out. In addition, factors associated with HSV shedding may not be distributed evenly between women who use hormonal contraceptives and those who do not. For example, younger women and those more recently infected with HSV have more frequent HSV shedding [7, 8]. Uneven distribution of these factors between women who use hormonal contraceptives and women who do not use hormonal contraceptives would result in confounding in cross-sectional analyses.

There are limitations to this study. The intermittent nature of HSV reactivation means that collection of a single sample on 1 day could miss some episodes of HSV shedding [14]. In addition, vulvar and perianal HSV shedding were not tested and may occur independently of cervical shedding [15]. Both of these factors could have decreased the ability to detect changes in HSV reactivation. However, the previous cross-sectional study used the same sample-collection method and demonstrated a significantly higher prevalence of cervical HSV among women who use hormonal contraceptives [1]. The current analysis had 97% power to detect a 2-fold difference in detection of cervical HSV after starting use of hormonal contraceptives. Thus, changes in HSV shedding of the magnitude suggested by earlier cross-sectional analyses did not occur in this cohort during the first 2 months after initiation of hormonal contraception. This analysis cannot rule out the possibility of changes occurring after a longer period.

In conclusion, it is reassuring that, in contrast to previous cross-sectional data, this prospective study found that initiating use of hormonal contraceptives does not increase cervical shedding of HSV. This study also illustrates the value of prospective investigations as a means of further exploring the relationships discovered in cross-sectional analyses. Additional prospective studies are needed to gain greater understanding of the factors associated with symptomatic and asymptomatic HSV reactivation, because both may serve to amplify HIV-1 transmission.
Acknowledgments

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References