The effect of hormonal contraception on genital tract shedding of HIV-1

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Objective: A previous cross-sectional study reported that hormonal contraception may be associated with increased infectivity in HIV-1 infected women. We conducted a prospective study to determine if cervical shedding of HIV-1 increased after initiating hormonal contraception.

Design: Shedding of HIV-1 DNA (a marker of HIV-1 infected cells) and HIV-1 RNA were measured before and after initiating hormonal contraception.

Methods: HIV-1 seropositive women were recruited from a Kenyan family planning clinic. At baseline, cervical secretions were collected for HIV-1 DNA and RNA assays in women initiating hormonal contraception; follow-up samples were collected a median of 64 days later.

Results: One-hundred and one women chose depot medroxyprogesterone (Depo), 53 chose low-dose oral contraceptives (OC), seven high-dose OC, and 52 progesterone-only OC. At follow-up, there was a significant increase in the prevalence of cervical HIV-1 DNA detection (from 42% to 52%, odds ratio (OR), 1.62; 95% confidence interval (CI), 1.03–2.63) for all hormonal contraception combined, and a trend for an increase for each individual type. Although the prevalence of cervical HIV-1 RNA increased slightly (from 82% to 86%; OR, 1.56; 95% CI, 0.83–3.03), the concentration of cervical HIV-1 RNA did not change significantly overall (from 2.81 to 2.84 log10 copies/swab; \( P = 0.77 \)) or for individual contraception types.

Conclusions: A modest but significant increase in shedding of HIV-1 DNA but not of HIV-1 RNA was detected after starting hormonal contraception. Our results may have important implications regarding the infectivity of women using hormonal contraception, and highlight the need for epidemiologic studies of transmission rates from women using and not using hormonal contraception. © 2004 Lippincott Williams & Wilkins
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Introduction

In sub-Saharan Africa, 55% of the estimated 28.5 million HIV-1 infected individuals are women, and approximately 90% of these are between the ages of 15 and 49 years [1]. Thus, counseling about family planning methods is an essential part of health care for most HIV-1 infected women. In a recent reproductive health survey of women in sub-Saharan Africa and Southeast Asia, 30–40% of those using family planning had chosen oral contraceptives (OC). In some countries, depot medroxyprogesterone acetate (Depo) is more commonly used than OC. For example, in a survey in Kenya, 22% of women using family planning used OC and 30% used Depo [2].

More than 20 studies have investigated whether use of OC or Depo is associated with increased susceptibility to HIV-1. A meta-analysis of published studies addressing the relationship between OC and HIV-1 susceptibility reported an increased risk, with an odds ratio (OR) of 1.19 [95% confidence interval (CI), 0.99–1.42] for 28 studies [3]. This OR increased when the analysis was limited to the eight most rigorous studies (OR, 1.60; 95% CI, 1.05–2.44).

Other authors have focused on the effect of hormonal contraception on the infectivity of HIV-1 infected women. In a cross-sectional study of 318 women, the detection of HIV-1 DNA in cervical fluids was significantly associated with use of Depo, low-dose OC, and high-dose OC [4]. In another cross-sectional study, hormonal contraception was not found to increase genital tract shedding of HIV-1 RNA [5]. To provide more definitive data on the effect of hormonal contraception on the infectivity of HIV-1 seropositive women, we conducted a prospective study, with cervical secretions collected before and after initiation of high- and low-dose combination OC, progesterone-only pills, and Depo injections. In this study, a qualitative assay was used to detect the presence of HIV-1 proviral DNA, a marker for infected cells. A quantitative assay was used to measure the concentration of HIV-1 RNA (both cell-associated and cell-free virus) in cervical secretions.

Methods

Study population and procedures

From August 1996 to September 1998, women attending outpatient clinics at Coast Provincial General Hospital in Mombasa, Kenya were recruited if they planned to initiate hormonal contraception. First, informed consent for HIV serologic testing was obtained. Women testing positive for HIV-1 antibodies using a rapid detection assay underwent a second informed consent process for the prospective study.

After completing an enrollment questionnaire regarding demographic, sexual, and obstetric history, a physical examination was performed. Cervical secretions for HIV-1 DNA and RNA assays were collected by placing successive Dacron swabs gently into the cervical os and turning three times. The presence of 5% or more of the cervical surface displaying endocervical mucosa on speculum examination was defined as cervical ectopy.

Cervical and vaginal secretions were also collected to assess the presence of genital infections. Examinations and sample collections were performed by one of two investigators (C.C.W. or R.S.M.).

Women were eligible for the study if they did not have genital ulcers on examination or microbiologic evidence of any genital infections with the exception of bacterial vaginosis. Women with genital infections were given appropriate treatment and asked to return in 2 weeks. If at the second visit their infections had resolved, they were eligible for the study.

Women were given oral contraceptive pills or Depo injections, depending on their choice. Depo or progesterone-only pills were dispensed to women who were breastfeeding. High-dose estrogen combination pills were dispensed for the first three months of the study and low-dose estrogen combination pills thereafter once they became available.

Follow-up visits were scheduled at 1 and 2 months after initiation of contraception. Follow-up visits were included in the analysis if they occurred 14 to 120 days after the initial visit. If more than one follow-up visit occurred, the latter visit was used (154 cases). Comparison of paired visits was performed separately for cervical RNA concentration, prevalence of cervical DNA detection, prevalence of cervical RNA detection, and plasma RNA concentration. Because of missing samples, 10 women, two women, and 17 women were excluded from the analyses of cervical RNA, cervical DNA, and plasma RNA assay results, respectively.

Laboratory tests

Diagnoses and evaluation of HIV-1, cervical infections,
and vaginal conditions were performed as described previously [6,7]. CD4 lymphocytes were quantified in EDTA-anticoagulated whole blood by a manual method (Cytosphere; Coulter, Hialeah, Florida, USA).

Plasma and cervical swabs for HIV-1 DNA and RNA detection were stored and shipped as described previously, with care to maintain the cold chain throughout [7].

Samples were assayed in batches of swab pairs. A qualitative PCR was used to measure the presence of HIV-1 DNA (a marker of HIV-1 infected cells), as described previously [8]. Quantitative levels of HIV-1 RNA (both cell-associated and cell-free) were measured with the Gen-Probe HIV-1 viral load assay (Gen-Probe Inc., San Diego, California, USA) with a sensitivity of 92% (95% CI, 85 - 98%) and a specificity of 98% (95% CI, 93 - 100%) [9]. For the study presented here, the lower limit of quantification used reflects the adjustment for the appropriate dilution factors used; 15 copies/cervical swab and 7 copies/ml plasma. In our swab analysis, any value less than 15 was assigned the value of 14 copies/cervical swab and was considered undetectable.

Data analysis
Data analysis was performed using Stata 7.0 (Stata, College Station, Texas, USA) and SPSS 10.1.3 (SPSS Inc., Chicago, Illinois, USA) software. Paired results before and after initiation of hormonal contraception were compared using McNemar’s test for dichotomous variables and paired t-tests for continuous variables. For the analysis of cervical RNA shedding and plasma RNA levels, we used the ratio of the levels of RNA before and after initiation of contraception, on a log scale, as this measure was normally distributed for cervical shedding and approximately normal for plasma levels. Power calculations were performed using Excel 2000 (Microsoft Corporation, Redmond, Washington, USA).

Results
The demographic characteristics of the 213 women included in the study analysis are presented in Table 1. Over two-thirds of women (68%) were breastfeeding at the time of the initial visit, necessitating the choice of a progesterone-only hormonal contraceptive. Forty-eight percent of women chose to use Depo, 25% low-dose combination OC, 24% progesterone-only OC, and 3% high-dose combination OC before low-dose OC were available. Twenty percent of women had severe immunosuppression, with CD4 cell counts < 10^6 cells/l.

Median follow-up was 64 days (range, 25—120 days). At baseline, 42% of women had detectable cervical HIV-1 DNA (Table 2). When data from women using all forms of hormonal contraception were combined, the prevalence of cervical HIV-1 DNA detection increased significantly at the follow-up visit (from 42% to 52%; P = 0.03). When women were divided into groups based on the individual forms of contraception used, an increase in the prevalence of HIV-1 DNA detection was noted in all groups, but this was not statistically significant. However, the statistical power to detect a 1.5-fold increase in shedding was only 34% for Depo, and 23% for low-dose and progesterone-only OC.

For all women combined, 82% and 86% had quantifiable cervical HIV-1 RNA concentrations before and after initiation of hormonal contraception, respectively.

| Table 1. Enrollment characteristics of 213 human immunodeficiency virus type 1 seropositive women in Mombasa, Kenya. |
|-----------------|-----------------|
| Characteristic | Value |
| Age, years [median (range)] | 25 (17–41) |
| More than primary school education [n (%)] | 56 (27%) |
| Sexual/obstetric/gynecologic history | |
| Number of pregnancies [median (range)] | 2 (0–10) |
| Described herself as monogamous in past year [n (%)] | 182 (88%) |
| Currently breastfeeding [n (%)] | 144 (68%) |
| Lifetime number of sex partners [median (range)] | 3 (1–20) |
| Money for sex in past year [n (%)] | 0 (0%) |
| Physical and laboratory evaluation [n (%)] | |
| Bacterial vaginosis | 66 (31%) |
| Cervical ectopy | 94 (44%) |
| CD4 count < 200 x 10^3/l (n = 184) | 36 (20%) |
| Contraceptive type [n (%)] | |
| Depot medroxyprogesterone | 101 (48%) |
| Low-dose combination pills | 53 (25%) |
| High-dose combination pills | 7 (3%) |
| Progesterone oral contraceptive pills | 52 (24%) |
Overall, no change in mean cervical or plasma HIV-1 RNA concentration was detected (2.81 to 2.84 log_{10} copies/swab, \( P = 0.77 \) and 4.84 to 4.88 log_{10} copies/ml, \( P = 0.47 \), respectively). Although no significant changes in prevalence or concentration of cervical HIV-1 RNA was noted for individual hormonal contraceptive types during follow-up, there was a slight increase in both the prevalence and the concentration of HIV-1 RNA shedding for all groups except the high-dose OC. Finally, no significant changes in plasma HIV-1 RNA concentration were noted for individual hormonal contraceptive methods.

Discussion

In this prospective study, women initiating hormonal contraception demonstrated a modest but significant increase in the prevalence of HIV-1 infected cells (from 42% to 52%; \( P = 0.03 \)) and a slight increase in the prevalence of HIV-1 RNA detection in cervical secretions, although this was not statistically significant (from 81% to 86%; \( P = 0.18 \)). No changes were detected in the concentration of cervical HIV-1 RNA, but our power to detect small changes was limited. In addition, plasma HIV-1 RNA concentration did not change.

The findings from this prospective study support previous findings of a cross-sectional study showing that cervical shedding of HIV-1 infected cells was significantly higher among 55 women using Depo (OR, 2.9; 95% CI, 1.5–5.7), 25 women using low-dose OC (OR, 3.8; 95% CI, 1.4–9.9), and 11 women using high-dose OC (OR, 12.3; 95% CI, 1.5–101) for a minimum of 6 months compared to women not using hormonal contraception [4]. Levels of viral RNA were not examined, but it was assumed that HIV-1 RNA would also increase because infected cells are thought to be the primary source of cell-free virus [10].

In our study, the increase in cervical HIV-1 proviral DNA without a commensurate increase in the concentration of cervical HIV-1 RNA was surprising, although not inconsistent with the findings of two other cross-sectional studies [4,10]. Our data suggest that the increase in infected cells in cervical secretions may reflect an influx of infected cells from systemic compartments rather than changes in local virus replication. In such a model, hormonal changes would not influence the levels of virus replication in the genital mucosa, but would alter the number and types of infected cells in the mucosa. Over time, the concentration of replicating virus may increase slowly in response to increases in the number of infected cells. However, our follow-up period may have been too short to detect these changes.

These findings demonstrate that short-term use of hormonal contraception by HIV-1 infected women is associated with a modest increase in shedding of HIV-1 infected cells, but not of the concentration of HIV-1 RNA, although we had low power to detect the latter. The relative impact of HIV-1 proviral DNA versus HIV-1 RNA on infectivity is uncertain, but the presence of HIV-1 provirus and HIV-1 RNA in
maternal cervical secretions [11,12] and breastmilk [13] have been found to increase vertical transmission risk.

Hormonal contraception is an effective, affordable contraceptive method [14]. However, these results raise the possibility that use of hormonal contraception may increase the infectivity of HIV-1 infected women to uninfected partners, thus providing another reason for barrier contraception for partner protection. This study may provide guidance for future studies of hormonal contraception and shedding in HIV-1 infected women, incorporating longer follow-up periods, as well as the enumeration of mucosal lymphocyte populations. In addition, epidemiologic studies to determine if HIV-1 transmission rates by women using hormonal contraception differ from women not using hormonal contraception would provide valuable information about the effect of hormonal contraception on infectivity of HIV-1 infected women.

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