Concordance of self-reported hormonal contraceptive use and presence of exogenous hormones in serum among African women

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Abstract

Objectives—Studies that rely on self-report to investigate the relationship between hormonal contraceptive use and HIV acquisition and transmission, as well as other health outcomes, could have compromised results due to misreporting. We determined the frequency of misreported hormonal contraceptive use among African women with and at risk for HIV.

Study design—We tested 1,102 archived serum samples from 664 African women who had participated in prospective HIV prevention studies. Using a novel high-performance liquid

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chromatography mass-spectrometry assay, we quantified exogenous hormones for injectables (medroxyprogesterone acetate or norethisterone), oral contraceptives (OC) (levonorgestrel or ethinyl estradiol), and implants (levonorgestrel or etonogestrel) and compared them to self-reported use.

Results—Among women reporting hormonal contraceptive use, 258/358 (72%) of samples were fully concordant with self-report, as were 642/744 (86%) of samples from women reporting no hormonal contraceptive use. However, 42/253 (17%) of samples from women reporting injectable use, 41/66 (62%) of samples from self-reported OC users, and 3/39 (8%) of samples from self-reported implant users had no quantifiable hormones. Among self-reported non-users, 102/744 (14%) had 1 hormone present. Concordance between self-reported method and exogenous hormones did not differ by HIV status.

Conclusion—Among African women with and at risk for HIV, testing of exogenous hormones revealed agreement with self-reported contraceptive use for most women. However, unexpected exogenous hormones were identified among self-reported hormonal contraceptive users and non-users, and an important fraction of women reporting hormonal contraceptive use had no hormones detected; absence of oral contraceptive hormones could be due, at least in part, to samples taken during the hormone-free interval. Misreporting of hormonal contraceptive use could lead to biased results in observational studies of the relationship between contraceptive use and health outcomes.

Implications—Research studies investigating associations between hormonal contraceptive use and HIV should consider validating self-reported use by objective measures; because both over- and under-reporting of use occurs, potential misclassification based on self-report could lead to biased results in directions that cannot be easily predicted.

Introduction

Associations between use of hormonal contraceptive methods and the transmission or acquisition of HIV[1–4], bacterial vaginosis[5,6], and other sexually transmitted infections[7–9] have been topics of substantial interest for decades. Of particular concern is a possible increase in HIV acquisition and transmission among women using the injectable contraceptive DMPA (depot medroxyprogesterone acetate) [10]. Such research in general has relied on self-reported hormonal contraceptive use, rarely validated by biomarkers[11]. However, like many sensitive topics, self-reported hormonal contraceptive use is subject to possible misclassification due to social desirability bias, a need for privacy from sexual partners, confusion about methods, recall difficulties, and potentially intentional misreporting because of desire to participate in research studies in which contraceptive use is a requirement[12–14]. Misclassification of women with respect to their hormonal contraceptive use could produce biased results in epidemiologic studies. In this analysis, we examined the degree of discordance between self-reported hormonal contraceptive use and exogenous hormones in serum from a cohort of HIV-uninfected and infected African women.

Materials & Methods

Data for this analysis came from three prospective studies (Partners in Prevention HSV/HIV Transmission Study, Couples Observation Study, and Partners PrEP Study) [15–20]. Briefly, women were members of HIV-serodiscordant heterosexual couples in seven African countries (Botswana, Kenya, Rwanda, South Africa, Tanzania, Uganda, Zambia) who were followed between 2004 and 2013.

This analysis includes a randomly selected subset of HIV-uninfected women who did not acquire HIV and HIV-infected women who did not transmit HIV to their male partner, but who had a risk profile similar to women who acquired/transmitted HIV during the study follow-up, thereby representing a high-risk population [21]. A total of 843 women were initially selected for this analysis; each woman contributed up to two samples. Of the initial 1,419 samples, we excluded: 50 samples from women with surgical contraception or IUD use (only copper IUDs without hormones were available in these settings); 188 samples from women 40 years old or older; and 79 samples from women who were pregnant at the time of sample collection. However, samples from the first visit at which the pregnancy was detected were not excluded as these women may have been particularly likely to misreport their contraceptive use.

Contraceptive use was not a study requirement, but all study sites offered multiple contraceptive methods on site and women could choose to obtain methods from the site or other providers. Women self-reported contraceptive methods by standard interviewer-administered questionnaires at regular study visits and women in the analysis could switch between methods.

Blood samples were taken at quarterly visits and stored at −80°C. We used a validated, high-performance liquid chromatography-heated electrospray ionization-tandem triple quadrupole mass spectrometry (LC-MS/MS) assay to simultaneously test for five exogenous hormones, as well as progesterone (P4) [22]. We expected the following exogenous hormones for each self-reported method (Table 1): medroxyprogesterone acetate (MPA) or norethisterone (NET), components of two different injectable contraceptives; levonorgestrel (LNG) or ethinyl estradiol (EE2), components of oral contraceptives (OC); and LNG or etonogestrel (ENG), components of contraceptive implants. The lower limit of quantification (LLQ) for MPA, ENG, and LNG was 0.02 ng/mL; for EE2 and P4, 0.01 ng/mL; and for NET, 0.04 ng/mL. We set results below LLQ to half the LLQ value for analysis.

For each sample, we compared self-reported hormonal contraceptive use to exogenous hormones as quantified by LC-MS/MS; the quantified hormones were not used to assign contraceptive use. We categorized the quantified hormones as expected, unexpected, both expected and unexpected, or no exogenous hormones for each method, as described above (Table 1). We compared the quantification of any expected exogenous hormone (versus no expected exogenous hormone) as a binary outcome by HIV status using generalized estimating equation (GEE) models to account for repeated observations, with a Poisson distribution and exchangeable correlation matrix. We also calculated the proportion of samples with evidence of ovulation (P4 3ng/mL). Among all users and separately for all

non-users, we modeled ovulation by presence of expected exogenous hormones using the same GEE model as above; due to small samples sizes, we were unable to model each method independently. Samples from pregnant women were excluded from all analyses involving P4, as pregnancy alters P4 levels. All analyses were conducted with SAS 9.4 (SAS Institute).

Results

We analyzed a total of 1,102 samples from 664 women. Most samples (952, 86%) were from married women and 323 (29%) were from women age under 25 years old (Table 2). Roughly a third of samples (413, 37%) were from HIV-infected women. The majority of samples were taken when no hormonal contraceptive method was reported (744, 68%); injectable use was reported for 253 (23%), OC use was reported for 66 (6%), and implant use was reported for 39 (4%) of samples.

For women reporting hormonal contraceptive use, only the expected exogenous hormones were quantified in 258 (72%) of samples, including 205 (81%) of samples from injectable users, 18 (27%) of samples from OC users, and 35 (90%) of samples from implant users (Table 3). However, unexpected hormones were also quantified. Specifically, 6 (2%) of samples from self-reported injectable users, 7 (11%) OC users, and 1 (3%) of implant users had an unexpected exogenous hormone quantified. In addition, no exogenous hormones were quantified in 42 (17%) of self-reported injectable user samples, 41 (62%) of OC user samples, and 3 (8%) of implant user samples (Figure 1; Figure S1). When restricted to the first sample per woman, results were similar.

Among the 744 samples collected from women reporting no hormonal contraceptive use, 642 (86%) had no hormones quantified (Table 3). However, of the 102 samples with an exogenous hormone quantified, 68 (67%) had MPA, 16 (16%) had NET, 7 (7%) had EE2, 19 (19%) had LNG, and 3 (3%) had ENG identified, including ten samples that had multiple hormones (Figure S2).

Of the 116 samples across all methods with an unexpected exogenous hormone quantified, 89 had data on previous hormonal contraceptive use available and of these 30 (34%) had self-reported a method in the six months prior that could explain the hormones identified. Predominantly, these had quantified MPA or NET and reported prior injectable use (n=28). One sample had quantified ENG and reported prior implant use and one sample had quantified LNG and reported prior OC use.

When we stratified results by HIV status (Table 3), no significant differences were noted in quantification of any expected hormones (versus no expected hormone) between HIV-infected and HIV-uninfected women (p=0.92 among non-users, p=0.19 among injectable users, p=0.58 among OC users, and p=0.08 among implant users).

Among all non-pregnant hormonal contraceptive method users with the expected exogenous hormones quantified, only 12/266 (5%) of the samples showed evidence of ovulation (Table 4). When there were no expected hormones, the proportion with ovulatory P4 levels was significantly higher, 25/88 (28%, p<0.0001). Furthermore, among all non-pregnant OC

users, 11/65 (17%) had no exogenous hormones quantified and had evidence of ovulation, possibly distinguishing them from women on the placebo week. Among self-reported non-users, 17/102 (17%) of samples with any quantifiable exogenous hormones had evidence of ovulation, compared to 193/618 (31%) of samples with no quantifiable hormones (p=0.005).

Finally, three women reported injectable use when a pregnancy was detected, only one of whom had quantifiable MPA, at 0.025 ng/mL. In addition, one woman had a pregnancy detected while reporting OC use, with no quantifiable exogenous hormones.

Discussion

In this large study of African women with and at risk for HIV, we tested serum samples for multiple hormones to objectively assess self-reported hormonal contraceptive use. We identified self-reported use that could not be verified by serum levels as well as unreported use that was identified by serum results. Overall, 82% of samples were perfectly concordant with self-reported hormonal contraceptive use. However, 17% of samples from women reporting injectable use, 8% of samples from reported implant users, and 62% of samples from reported OC users had no exogenous hormones detected, and unexpected exogenous hormones were also detected in 2–11% of samples. Among self-reported non-users, 14% had at least one exogenous hormone present. These results illustrate the potential for both under- and over-reporting of hormone use and emphasize the limitations of self-report for epidemiologic analyses of the relationship between hormone exposure and health outcomes, such as HIV acquisition.

A recent study, limited to self-reported contraceptive non-users from Zimbabwe, found 27% had detectable exogenous hormones, mostly related to OC or injectable use [14]. In a qualitative component, they also found that women misrepresented their contraceptive use in order to be eligible to participate in research studies and access services[14]. Similarly, in a U.S.-based study of oral-contraceptive users, non-use was associated with poverty and the authors posited that participants may have joined the study for its benefits[23]. For the present analysis, hormonal contraceptive use was not a criterion for participation in the studies, which may explain the lower rate of misreporting among non-users that we found compared to the Zimbabwe study. However, our results broadly agree that hormonal contraceptive use may go underreported.

In addition, some women may report hormonal contraceptives when they are not using them, and we found such over-reporting of use for women who reported oral, injectable, and implantable hormonal methods. Even among users of longer-acting methods, where adherence should have limited impact, we found evidence of overreporting. In addition, 67% of samples from self-reported OC users had no LNG nor EE2. While one explanation for this lack of hormone detection is the hormone free interval of OC use, 17% of samples from reported OC users had no expected hormones while also having ovulatory P4 levels. For comparison, a study of consistent OC users in the U.S. found ovulation (by ultrasound) in 2.7% of participants[24]. Although poor compliance may still be a factor, the true proportion of misreporting among OC users is likely between 17% and 67%. One study in the U.S. found 9–13% of OC users had undetectable LNG and EE at some point during follow-up

[25], while another found that 17% of OC users were inconsistent or non-users, by LNG [24].

We found evidence that quantification of unexpected hormones in some cases may have been due to prior hormonal contraceptive use, particularly of injectable methods, which are known to have a long half-life [26,27]; additional work understanding the pharmacokinetics of these drugs in African populations is still needed. However, only one-third of samples had a prior method reported that could possibly explain the discordant results; LNG and ENG have shorter half-lives and prior use is less likely to explain unexpected presence of these hormones[28,29]. Women also may have taken exogenous hormones for non-contraceptive purposes, such as treating menorrhagia, which could explain some results, and we did not specifically collect data on hormone use for other reasons. Finally, a total of four women were pregnant yet reported hormonal contraceptive use; this may be due to either misreporting (3 of the 4 had no quantifiable exogenous hormones) or contraceptive failure.

The effect of this misreporting on the results of an epidemiological study is not immediately clear. To fully understand the direction of bias in studies of hormonal contraception and HIV risk, for example, the sensitivity of self-report within each contraceptive method stratified by HIV-acquisition status and the proportion of women in each strata would be needed. If misreporting of hormonal contraceptives is the same among women who acquire HIV and those who do not, then the reported increases in risk among DMPA users would be underestimated.[1] On the other hand, if misreporting differs between women who acquire HIV and those who do not (known as differential misclassification), results may be overestimated or underestimated [30,31]. This supports the need for well-done clinical trials to answer the question of whether hormonal contraception increases the risk of HIV acquisition.[32]

This study has several limitations. We did not have data on the exact composition and date of initiation for each contraceptive method, nor on last menses. In this region, we believe that OC was limited to LNG/EE2 combinations; while it is possible that some women could have had access to NET- or ENG-based OC, there were only two OC users with detectable NET in our sample and none with ENG. Hormonal contraceptive use was not a requirement for these studies; rates of misreporting may differ (and potentially be much higher) in studies with such requirements. Likewise, the rates of misreporting may depend on the prevalence of each method and may not be generalizable to settings with a different contraceptive mix. Additionally, serum progesterone is a marker of presumed ovulation [33], but sampling without regard to the date of last menstrual period may have missed some ovulations; however, ultrasound for ovulation confirmation was not a part of this study.

The strengths of this study include the ability to test for a range of hormonal contraceptive methods and endogenous hormones simultaneously using highly sensitive laboratory techniques. The P4 results demonstrate that hormones were identified at biologically relevant concentrations, as evidence of ovulation was more common when exogenous hormones were not quantified. These data underscore the value of the LC-MS/MS assay, which provides quantitative data on a range of exogenous and endogenous hormones, providing objective data to supplement self-reported use [22]. In addition, we had a large

sample size, including women representing many African countries and throughout the reproductive age range.

In conclusion, for studies evaluating the biological relationship between contraceptive use and acquisition and transmission of HIV and other sexually transmitted infections, reliance on self-reported hormonal contraceptive use will be limited by some degree of misreporting and could result in misclassification. Importantly, use of hormones may be either under- or over- reported, and even women reporting use of a particular hormonal contraceptive may have been exposed to other exogenous hormones. Studies using hormonal contraception as an inclusion or exclusion criteria may benefit from objective measures to screen women, or at least retrospective testing to identify a subgroup fully meeting the entry criteria. Studies examining associations with hormonal contraceptive methods should use objective methods to verify self-reported use and, in particular, determine if misclassification is differential, thus quantifying the impact of exposure misclassification.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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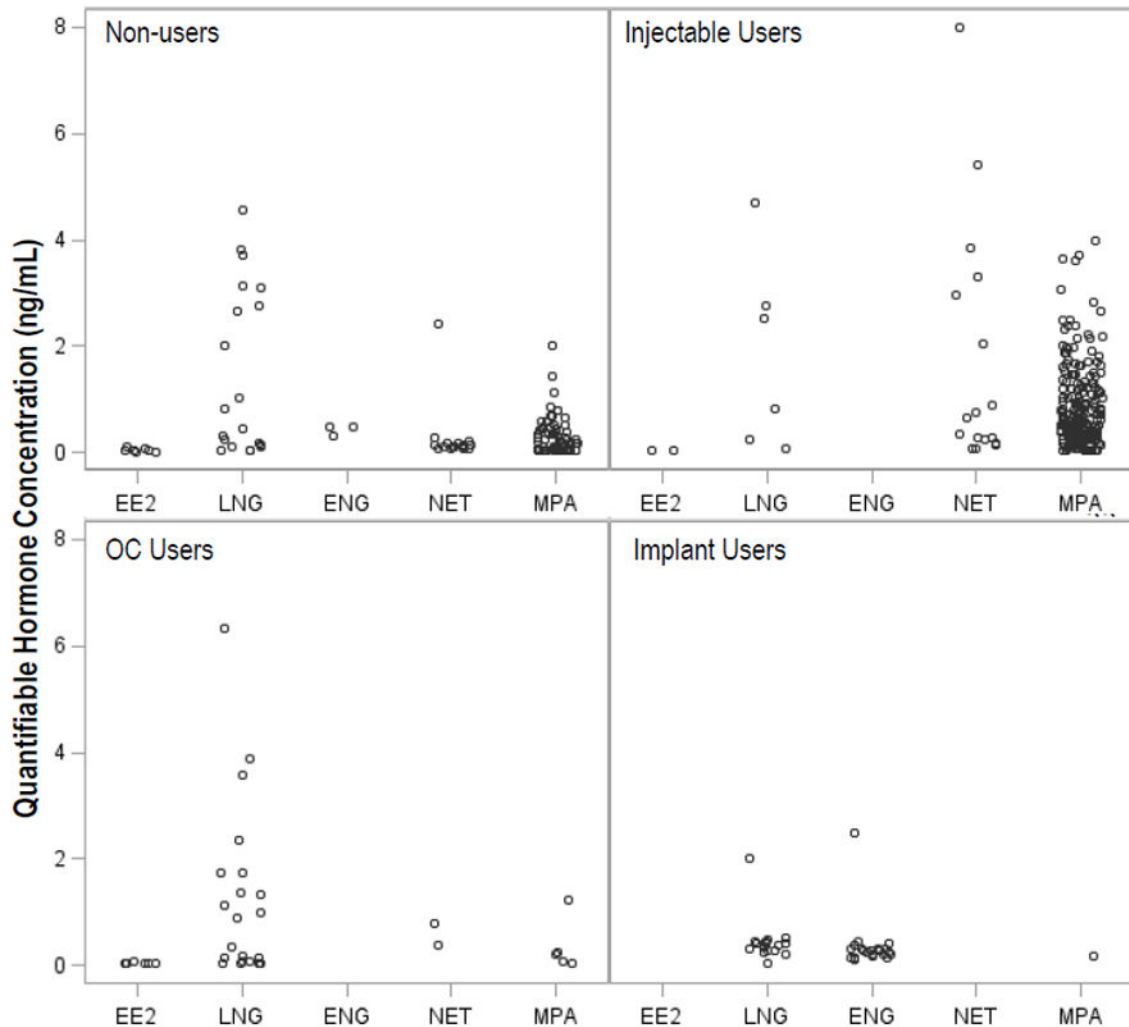
Figure 1. Quantities of Exogenous Hormones by Self-Reported Hormonal Contraceptive Use
The concentration of quantifiable hormones are presented for each women, according to their self-reported hormonal contraceptive use at the time of the sample, for those with quantifiable hormones; women may be represented by more than one symbol if more than one hormone was detected in the sample. The expected hormones were as follows: for injectables users, medroxyprogesterone acetate (MPA) and norethisterone (NET); for oral contraceptive (OC) users, levonorgestrel (LNG) and ethinyl estradiol (EE2); and for implant users, LNG and etonogestrel (ENG). The lower limit of quantification (LLQ) for MPA, ENG, and LNG was 0.02 ng/mL; for EE2 and P4, 0.01 ng/mL; and for NET, 0.04 ng/mL. Results below LLQ were set to half the LLQ value for analysis.

Table 1

Classification of Quantifiable Hormone Results

Self-reported Method	Only Expected Hormones	Expected & Unexpected Hormones	Only Unexpected Hormones	No Hormones
Injectable	Only MPA or NET	MPA or NET, plus LNG, ENG, or EE2	Only LNG, ENG, or EE2	None
Oral	Only LNG or EE2	LNG or EE2, plus MPA, NET, or ENG	Only MPA, NET, or ENG	None
Implant	Only LNG or ENG	LNG or ENG, plus MPA, NET, or EE2	Only MPA, NET, or EE2	None
None	None	--	Any detected	--

Table 2

Characteristics of 664 Women Contributing Samples

| | Self-Reported Method | | | |
	Injectable, % (n)	OC, % (n)	Implant, % (n)	None, % (n)
N Samples	23.0% (253)	6.0% (66)	3.5% (39)	67.5% (744)
Age				
<25	22.5% (57)	30.3% (20)	15.4% (6)	32.3% (240)
25–29	25.3% (64)	28.8% (19)	25.6% (10)	28.5% (212)
30–34	29.2% (74)	30.3% (20)	28.2% (11)	26.6% (198)
35–39	22.9% (58)	10.6% (7)	30.8% (12)	12.6% (94)
Married	87.7% (222)	90.9% (60)	100% (39)	84.8% (631)
>8 years education	32.4% (82)	21.2% (14)	23.1% (9)	37.0% (275)
Recent Condomless Sex	19.4% (49)	31.8% (21)	28.2% (11)	15.1% (112)
Sexual Partners Outside of Study Partner	2.4% (6)	1.5% (1)	0% (0)	3.9% (29)
Pregnant	1.2% (3)	1.5% (1)	0% (0)	3.2% (24)
HIV-infected	35.6% (90)	36.4% (24)	10.3% (4)	40.0% (295)
Study				
Couples Observational Study	2.0% (5)	1.5% (1)	0% (0)	6.7% (50)
Partners in Prevention HSV/HIV Transmission Study	42.7% (108)	36.4% (24)	2.6% (1)	66.7% (496)
Partners PrEP Study	55.3% (140)	62.1% (41)	97.4% (38)	26.6% (198)

Table 3

Quantifiable Exogenous Hormones by Self-Reported Hormonal Contraceptive Method

Self-Reported Method	Only Expected Hormones(s), % (n)	Expected & Unexpected Hormones, % (n)	Only Unexpected Hormone(s), % (n)	No Hormones, % (n)
Injectable	81.0% (205)	1.6% (4)	0.8% (2)	16.6% (42)
HIV-infected	87.8% (79)	0% (0)	0% (0)	12.2% (11)
HIV-uninfected	77.3% (126)	2.5% (4)	1.2% (2)	19.0% (31)
OC	27.3% (18)	6.1% (4)	4.5% (3)	62.1% (41)
HIV-infected	29.2% (7)	8.3% (2)	12.5% (3)	50.0% (12)
HIV-uninfected	26.2% (11)	4.8% (2)	0% (0)	69.0% (29)
Implant	89.7% (35)	2.6% (1)	0% (0)	7.7% (3)
HIV-infected	75.0% (3)	25.0% (1)	0% (0)	0% (0)
HIV-uninfected	91.4% (32)	0% (0)	0% (0)	8.6% (3)
None [*]	86.3% (642)	N/A	13.7% (102)	N/A
HIV-infected	85.4% (252)	N/A	14.6% (43)	N/A
HIV-uninfected	86.9% (390)	N/A	13.1% (59)	N/A
Total	**81.7% (900)**	**0.8% (9)**	**9.7% (107)**	**7.8% (86)**

[*] For non-users, expected hormones=none.

Table 4

Proportion of Samples[*] with Evidence of Ovulation[**]

Quantifiable Hormones	Self-Reported Method			
	Injectable, % (n)	OC, % (n)	Implant, % (n)	None[***], % (n)
Only Expected Hormones	3.9% (8)	11.1% (2)	5.7% (2)	31.2% (193)
Expected & Unexpected Hormones	0% (0)	0% (0)	0% (0)	--
Only Unexpected Hormones	0% (0)	0% (0)	--	16.8% (17)
No Hormones	35.0% (14)	27.5% (11)	0% (0)	--
Total	8.8% (22)	20.0% (13)	5.1% (2)	29.2% (210)

[*]
Excludes pregnant women

[**]
P4 3ng/mL

[***]
For non-users, expected hormones=none.