Depot-medroxyprogesterone acetate use and the concentration of *Gardnerella vaginalis* in vaginal secretions: A cross-sectional study

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Abstract

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Studies have observed an association between depot-medroxyprogesterone acetate (DMPA) use and bacterial vaginosis (BV). This well-powered cross-sectional study found no association between DMPA use and vaginal fluid concentrations of *Gardnerella vaginalis* or measures of bacterial community diversity. Further research is needed to identify microbial mechanisms linking DMPA and BV.

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INTRODUCTION

Bacterial vaginosis (BV) is a common vaginal infection characterized by replacement of *Lactobacillus* species with diverse anaerobic and facultative bacteria.[1] *Gardnerella vaginalis* has long been associated with BV, and may contribute to its pathogenesis through formation of biofilms.[2] The use of depot-medroxyprogesterone acetate (DMPA) has been associated with a lower incidence of BV.[3–5] A recent prospective study of fifteen women found that DMPA initiation was associated with a significant 0.20 $\log_{10}$/month decrease in the concentration of *G. vaginalis*, providing one possible mechanism to explain lower BV incidence with this injectable contraceptive.[6] The cross-sectional analysis presented here tested the hypotheses that women using DMPA have lower vaginal concentrations of *G. vaginalis* compared to women not using hormonal contraception.

METHODS

Data for this analysis originated from a nested case-control study that assessed the relationship between the vaginal microbiota and HIV-1 acquisition in five cohorts of women from Eastern and Southern Africa. Detailed methods from this parent study have been published.[7] Enrollment procedures common to all cohorts included collection of demographic, medical, and sexual history, a urine pregnancy test, an HIV-1 test, and collection of vaginal swabs for laboratory analysis. All protocols were approved by country-specific IRBs and participants provided written informed consent.[7]

For this cross-sectional analysis, female participants were included if, at the time of vaginal swab collection, they were HIV-PCR negative, 15-45 years old, not pregnant, and either using no hormonal contraception, a non-hormonal intrauterine device (IUD), or DMPA. Pregnant women, women using contraceptive pills or implants, and women older than 45 were excluded. Women using DMPA were compared to women not using hormonal contraception plus eight women using IUDs.

Vaginal swabs were stored at -80C and shipped on dry ice for analysis at the Fred Hutchinson

Cancer Research Center in Seattle, WA. *Gardnerella vaginalis*-specific real time quantitative polymerase chain reaction (qPCR) and deep sequencing of broad-range 16S rRNA gene PCR products were conducted on vaginal fluid samples.[7]

Analyses were performed using R (Version 1.0.153, © 2009-2016). In the primary analysis, *Gardnerella vaginalis* concentrations were compared between women using DMPA versus no hormonal contraception using linear regression in a base model controlling for cohort as a group level confounder. Multivariate analysis adjusted for cohort plus additional variables identified *a priori* as potential confounding factors including age, number of recent sex partners, frequency of sex in the past month, and recent unprotected sex.

Vaginal bacterial community diversity (Shannon Diversity Index) and richness (Chao1 Richness Estimator) were examined in the subset of 67 vaginal samples analyzed using deep sequencing of broad range 16S rDNA gene PCR products. Linear regression was used to compare these indices in a base model adjusting for cohort as a group level confounder. Given the small sample size, a forward stepwise model building approach was used to adjust for potential confounding factors. Standard errors remained acceptably similar in a multivariate model including age, number of recent partners, frequency of sex, and recent unprotected sex. It was not possible to adjust for cohort in this multivariate model, as the model would not converge.

In visits at which Nugent scores were performed (N=187), additional analyses compared BV prevalence (Nugent score ≥7 versus <7) in women using DMPA versus women not using hormonal contraception.[8] A base model using logistic regression adjusted for cohort as a group level confounder, and Wald 95% confidence intervals (CIs) were used for fixed effects. A second model adjusted for age, number of recent partners, frequency of sex, and recent unprotected sex. It was not possible to adjust for cohort in this multivariate model as the model would not converge.

RESULTS

Baseline characteristics of the 215 women included in this analysis are presented in Table 1. Participants' median age was 30 years (interquartile range [IQR] 23-35). Recent unprotected sex was reported by 62 (29%) women.

The mean concentration of *G. vaginalis* was 6.76 $\log_{10}$ copies/swab (standard deviation [SD] 2.75) in the 54 (25%) women using DMPA versus 6.54 $\log_{10}$ copies/swab (SD 2.65) in the 161 (75%) women not using hormonal contraception; in the base model, these means were not statistically significantly different (ß=-0.17 $\log_{10}$ copies/swab, 95% CI -0.97-0.63, p=0.677). The multivariate model adjusting for cohort, age, number of recent partners, frequency of sex, and recent unprotected sex, resulted in a larger negative association between DMPA use and *G. vaginalis* concentrations, but this association remained non-significant (ß =-0.42 $\log_{10}$ copies/swab, 95% CI -1.24-0.39, p=0.307). A post-hoc power calculation showed that this analysis had 94.8% power to detect a 1 log difference in the concentration of *G. vaginalis.*

In the subset of 67 women with deep sequencing data, bacterial community diversity was not significantly different in 20 (30%) women using DMPA (mean 0.94, SD 0.90) compared to 47 (70%) women not using hormonal contraception (mean 1.08, SD 1.06) in a base model adjusted for cohort (ß =-0.09 95%CI 0.64-0.46, p=0.750) and in the multivariate model (ß =-0.06, 95%CI 0.63-0.51, p=0.842). Similarly, bacterial community richness was not significantly different in women using DMPA (mean 23.24, SD 19.39) compared to women not using hormonal contraception (mean 34.69, SD 27.23) in a base model adjusted only for cohort (ß =-5.33, 95% CI -18.34-7.68, p=0.416) or in the multivariate model (ß =-8.44, 95% CI -22.10- 5.22, p=0.222).

In the subset of 187 women with vaginal Gram stain scores, the prevalence of BV was slightly lower in women using DMPA (15/50, 30%) compared to women using no hormonal contraception (47/137, 34%), although the difference was not statistically significant in either the base model adjusted

for cohort (OR 0.82, 95% CI 0.40- 1.65, p=0.580) or in the multivariate model (aOR 0.76, 95% CI 0.36-1.54, p=0.456).

DISCUSSION

In this large cross sectional analysis, DMPA use was not significantly associated with differences in vaginal fluid concentrations of *G. vaginalis* or with differences in vaginal bacterial community diversity, richness, or BV prevalence. These findings were somewhat unexpected, given the consistent association between DMPA use and lower BV incicence.[3–5] In addition, a recent prospective study of 15 women evaluated before and for up to twelve months after DMPA initiation demonstrated a significant 0.20 $\log_{10}$ copies/swab decrease in the concentration of *G. vaginalis* for each additional month of DMPA exposure (nearly 2 $\log_{10}$ copies/swab decrease at one year).[6] The present analysis had more than sufficient statistical power to detect differences of this magnitude.

There are several plausible explanations for why these study results contradicted with the prospective study mentioned above. First, the prospective study could have observed a significant decrease in *G. vaginalis* concentration with DMPA use by chance (Type I error), or this cross-sectional analysis may have failed to detect a true difference by chance (Type II error). Second, the prospective design, in which each woman serves as her own control, may have been better suited to detect changes in the vaginal microbiota that are not captured in a cross-sectional study. Third, this cross-sectional analysis did not measure the duration of DMPA use; if most participants had recently initiated DMPA, this could have led to the observation of small, nonsignificant differences in *G. vaginalis* concentrations. Finally, unique and possibly unmeasured characteristics of either population could explain a true difference in the effect of DMPA on the vaginal microbiota.

In this population, women using DMPA had about 20% lower odds of having BV by Nugent score in both unadjusted and adjusted analyses, but this association was not statistically significant. Previous studies have reported somewhat larger, and statistically significant, effects of DMPA on BV prevalence

and incidence.[3–5] Given the smaller sample of women with Nugent scores recorded, this analysis may have been under-powered to see a modest association between DMPA and BV. The selection of cases and controls in the parent study may also have introduced design-related bias.

One strength of this study was the large sample size, which provided >90% power to detect a 1 $\log_{10}$ copies/swab difference in the concentration of *G. vaginalis* between women using DMPA versus women not using hormonal contraception. In addition, the analyses included adjustment for important potential confounding factors. There were several limitations of the present study. Concentrations of *G. vaginalis* vary over the course of the menstrual cycle,[9] but the timing of menses was not recorded in this study. This limitation was mitigated by avoiding sample collection during menses; however, this approach does not entirely eliminate the effect of menstrual cycle variation. The smaller sample size (n=67) of the deep sequencing subset may have limited the power of these analyses to detect associations between DMPA and bacterial community characteristics. Finally, while adjustment was made for potential confounding factors using multivariable models, residual confounding is always possible.

In conclusion, this study did not find a significant association between DMPA use and *G. vaginalis* concentrations. Future studies aimed at understanding the microbial mechanisms through which DMPA may reduce BV incidence should incorporate larger sample sizes, use a prospective study design, and explore other key bacteria associated with BV.[1] Analysis of the vaginal microbiota in the context of a randomized controlled trial, where women are randomized to DMPA and to non-hormonal contraceptive methods,[10] could provide the strongest evidence for DMPA-induced changes in the vaginal microbiota.

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TABLE 1

	Combined (N=215) Median (IQR) or n (%)		DMPA users (N=54) Median (IQR) or n (%)		No Hormonal Contraception (N=161) Median (IQR) or n (%)	
Age (years)	30	(23.0, 35.0)	29	(24.0, 33.8)	30	(23.0, 36.0)
Nationality						
Kenya	113	(52.5)	25	(46.2)	88	(54.6)
Uganda	80	(37.2)	26	(48.1)	54	(33.5)
South Africa	9	(4.1)	2	(3.7)	7	(4.3)
Tanzania	5	(2.3)	0		5	(3.1)
Botswana	5	(2.3)	1	(1.8)	4	(2.4)
Zambia	3	(1.4)	0		3	(1.8)
Education (years)	8	(5.5, 10.0)	8	(5.0, 9.0)	8	(6.0, 10.0)
Married	169	(78.6)	43	(79.6)	126	(78.2)
Enrollment cohort						
Mombasa Cohort	24	(11.1)	8	(14.8)	16	(9.9)
Mama Salama Study	38	(17.6)	7	(12.9)	31	(19.2)
Partners in Prevention HSV/HIV Transmission Study	42	(19.5)	5	(9.2)	37	(22.9)
Couples Observational Study	21	(9.7)	3	(5.5)	18	(11.1)
Partners PrEP Study	90	(41.8)	31	(57.4)	59	(36.6)
Number of recent sex partners*						
0	34	(16.0)	6	(11.0)	28	(17.0)
1	173	(80.0)	46	(85.0)	127	(78.0)
>1	8	(3.7)	2	(3.7)	6	(3.7)
Frequency of sex in the past month†	3	(2.0, 6.0)	3	(2.0, 5.0)	3	(1.0, 7.0)
Any recent unprotected sex‡	62	(28.8)	17	(31.4)	45	(27.9)
On examination§‡‡						
Abnormal vaginal discharge	18/189	(9.5)	6/50	(12.0)	12/139	(8.6)
Genital ulceration	6/188	(3.1)	0/50		6/138	(4.3)
Cervical mucopus	1/188	(0.5)	1/50	(2.0)	0/138	
Vaginal gram stain Nugent score‖ ‡‡						
Normal (0-3)	98/187	(52.4)	28/50	(56.0)	70/139	(51.0)
Intermediate (4-6)	27/187	(14.4)	7/50	(14.0)	20/139	(14.5)
BV (7-10)	62/187	(33.1)	15/50	(30.0)	47/139	(34.3)
Laboratory confirmed STIs‡‡						
Neisseria gonorrhoeae¶	5/167	(2.9)	4/45	(8.8)	1/122	(0.8)
*Chlamydia trachomatis***	3/153	(1.9)	1/41	(2.4)	2/112	(1.7)
Trichomonas vaginalis††	15/207	(7.2)	3/53	(5.6)	12/154	(7.7)

Table 1: Baseline characteristics of 215 women in five African cohorts included in the primary analysis. Of 349 women in the parent study,[7] 134 women were excluded from this analysis. These included 64 who were pregnant, 18 using oral

contraceptives, 18 using contraceptive implants, 19 over age 45, and 15 with early or acute HIV infection. Variables used in the analyses were: age (continuous), number of recent sex partners (categorical), frequency of sex in the past month (continuous), and recent unprotected sex (binary).

BV, bacterial vaginosis; DMPA, depo medroxyprogesterone acetate; HIV, human immunodeficiency virus; HSV, herpes simplex virus; IQR, interquartile range; IUD, intrauterine contraceptive device; PrEP, pre-exposure prophylaxis; STI, sexually transmitted infection
Cohorts: MSS, Mama Salama Study; MC, Mombasa Cohort; COS, Couples Observational Study; HSV2, Partners in Prevention HSV/HIV Transmission Study, PrEP, Partners Pre-Exposure Prophylaxis Study.

* Past week for Mombasa Cohort of female sex workers, past month for other cohorts of general population women.
† Imputed for Mombasa Cohort women as 4 times the past week frequency.
‡ Past week for Mombasa Cohort, past month otherwise.
§ Abnormal vaginal discharge data were not available for 26 women from the MSS. Genital ulceration and cervical mucopus data were not available for 26 women from the MSS and 1 woman from the HSV2 study.
‖ Data on Nugent score were not available for 28 women (1 from MSS, 1 from HSV2, 21 from COS, 5 from PrEP).
¶ *Neisseria gonorrhoeae* data were missing in a total of 48 women (38 from MSS, 4 from HSV2, 2 from COS, 4 from PrEP).
** *Chlamydia trachomatis* data was missing from a total of 62 women (14 from MC, 38 from MSS, 4 from HSV2, 2 from COS, 4 from PrEP).
†† *Trichomonas vaginalis* data was missing from a total of 8 women (4 from HSV2, 2 from COS, 2 from PrEP).
‡‡ Missing values varied by variable so denominator shown is total n for that category.