1 Sino-implant (II)® continuation and effect of concomitant tenofovir disoproxil
2 fumarate-emtricitabine use on plasma levonorgestrel concentrations among women
3 in Bondo, Kenya

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9 Funding: United States Agency for International Development and the Bill &
10 Melinda Gates Foundation. Gilead Sciences provided the study drugs.

11 COI Statement: The authors declare no conflicts of interest.

12 ClinicalTrials.gov registration number: Secondary analysis from NCT00625404

13 Abstract word count: 139

14 Text word count: 1960

15 Keywords: levonorgestrel implant, HIV pre-exposure prophylaxis, contraceptive
16 efficacy, contraceptive uptake, levonorgestrel concentration

17 Running title: SinoImplant-II uptake and use with pre-exposure HIV prophylaxis

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58 Abstract:

59 Objective: To assess associations between tenofovir disoproxil fumarate -
60 emtricitabine (TDF-FTC) exposure and levonorgestrel (LNG) concentrations among
61 Kenyan HIV prevention trial participants using Sino-implant (II) LNG implants for
62 contraception.

63 Methods: Women were offered implants among other contraceptive methods, were
64 randomized to daily TDF-FTC or placebo, and followed monthly up to 56 weeks.

65 Associations between TDF-FTC exposure and mean levonorgestrel values were
66 analyzed with linear mixed models.

67 Results: Of 739 women, 29 (3.9%) received implants with no incident pregnancies
68 and one discontinuation. Mean LNG concentrations over 56 weeks among 28
69 women contributing data ranged between 214.0 – 659.8 pg/mL with no significant
70 difference between TDF-FTC and placebo arms or between variable levels of TDF-
71 FTC adherence.

72 Conclusion: Concomitant TDF-FTC use was not associated with a significant change
73 in plasma LNG concentrations among women using Sino-implant (II) in the first year
74 of use.

75 Keywords: levonorgestrel implant; Sino-implant (II); levonorgestrel levels; long-
76 acting reversible contraception; pre-exposure prophylaxis; prospective study

INTRODUCTION

The Sino-implant (II) levonorgestrel (LNG) implant is a highly effective long-acting reversible contraceptive method [1]. This method, containing 150 mg of LNG (75 mg/rod), is registered for four years use in more than 25 countries across Africa, Asia, and Latin America and has been used by more than seven million women since its 1996 introduction [2,3]. The hypothesized contraceptive mechanism of action of the LNG implant is through thickening of cervical mucus and, to a lesser degree, suppression of or dysfunctional ovulation [4,5]. LNG levels in the 140-180 pg/mL range are typically considered to provide highly effective contraceptive protection [5,6]. There is little information regarding potential interactions between LNG implants and antiretroviral medications [7,8]. Data from Partners PrEP, a large placebo-controlled randomized trial to assess the efficacy of daily tenofovir disoproxil fumarate-emtricitabine (Truvada®; TDF-FTC) for pre-exposure prophylaxis (PrEP) to prevent HIV infection among negative partners in serodiscordant heterosexual couples in Kenya and Uganda, was assessed in a recent secondary analysis of pregnancy incidence by hormonal contraceptive method and TDF-FTC exposure [9]. Implant use was relatively uncommon (8% across all follow-up visits as compared to injectable hormonal contraception users at 33%) with only one incident pregnancy in the TDF-FTC arm and one in the placebo arm; thus, differences between arms could not be modeled using Cox proportional hazards regression [9]. Further, LNG concentrations were not measured [9]. Obtaining such information is important with expansion of both LNG implant availability and oral TDF or TDF-FTC use for HIV treatment and prevention [10].

FEM-PrEP was a prospective randomized placebo-controlled trial assessing the effectiveness of daily TDF-FTC as PrEP to prevent HIV infection among women aged 18-35 years at risk for HIV conducted in three countries from 2009 through 2012, including Kenya [11]. The purpose of this analysis is to describe the relationship between plasma LNG concentration and TDF-FTC exposure and to describe implant continuation among women participating in the FEM-PrEP trial and electing Sino-implant (II) use.

MATERIALS and METHODS

Setting and Participants

At the Bondo, Kenya site, 739 women enrolled in the randomized placebo-controlled FEM-PrEP trial; inclusion criteria and trial procedures have been previously described [11]. Participants were required to use effective non-barrier contraception at enrollment and Sino-implant (II) was offered from 2010 onward, with 29 women receiving the LNG implant, of whom 11 received the implant at screening, six at enrollment, and 12 during study follow-up. This study was approved by the Protection of Human Subjects Committee at FHI 360 and the Kenyatta National Hospital/University of Nairobi Ethics and Research Committee in Kenya.

This secondary analysis includes all women receiving Sino-implant (II) with LNG measurements after insertion from completed follow-up visits (n=29); one woman contributed only one measure following insertion and was excluded from longitudinal analysis. Plasma specimens were obtained at routine monthly follow-

up visits between May 2009 and April 2011, stored at -70° C at the study site, and transported in liquid nitrogen to the testing facility.

Measures

The primary objectives of this analysis are describing plasma LNG concentrations among Kenyan Sino-implant (II) users in the first year of use and the association between TDF-FTC exposure and LNG concentrations. Baseline demographic and medical descriptors include age, body mass index (BMI), and prior pregnancy. For women randomized to the TDF-FTC arm, drug use was divided into three categories: “good-to-excellent adherence” (plasma concentrations of tenofovir diphosphate (TFV-DP)>100,000 femtomoles/mL and tenofovir (TFV)>10 ng/mL, reflecting probable product use four or more times each week during the preceding 28 days), “essentially no use” (TFV-DP <10,000 femtomoles/mL and TFV undetectable, reflecting an extremely low number or no doses), and “some use” (TFV-DP and/or TFV values in a range between the two prior specified indices, suggesting intermittent use) [12]. The rationale for stratifying active arm participants into exposure categories is that a relatively low proportion of women randomized to the TDF-FTC arm had plasma drug concentrations consistent with daily use, which is a key reason for lack of effect in the trial results and early trial closure [11].

Laboratory Analysis

Frozen samples were sent to PPD Development Labs (Richmond, VA, USA), where LNG and metabolites were extracted using liquid-liquid extraction and analyzed via a validated sensitive and selective high-performance liquid chromatography (HPLC)

method coupled with mass spectrometry (calibration range of 50 – 25,000 pg/mL). The linearity of this procedure was evaluated by analyzing nine calibration standards in duplicate for LNG over the calibration range using a linear weighted, 1/concentration squared, least squares regression algorithm to plot the peak area ratio of the appropriate analyte to its internal standard versus concentration. Quality controls were prepared at 50.0, 125, 1250 and 20,000 pg/mL. Intra-assay precision ranged from 11.3 to 0.766% and intra-assay accuracy ranged from 10.9 to -1.65%, while inter assay-precision ranged from 15.8 to 2.10% and inter-assay accuracy ranged from 7.40 to 3.88%. The lower limit of detection for this assay is 50 pg/mL. Plasma TFV and FTC and intracellular TFV-DP and emtricitabine triphosphate concentrations in upper layer packed cells were measured using protein precipitation and HPLC with tandem mass spectrometry at the University of North Carolina-Chapel Hill laboratories [11].

Statistical Analysis

Women included in this analysis received Sino-implant (II) at or after trial screening. Specimen collection dates were rounded to the nearest 4-week interval. Descriptive statistics were generated for implant users and differences between TDF-FTC and placebo arms assessed with two-sample t-tests and Fisher's exact tests. LNG concentration levels from post-insertion week 4 until week 36 were summarized graphically by TDF-FTC exposure. Due to limited sample size, measurements were pooled together and linear mixed models with random intercept to account for repeated measures were used to assess the association between TDF-FTC exposure and LNG concentration. TDF-FTC exposure categories were assigned to each sample

rather than to a participant as the designated category for one woman could vary across follow-up visits. Analysis was performed with SAS V9.3 (SAS Institute, Cary, NC).

RESULTS

All women (n=29) receiving Sino-implant (II) are included in descriptive analysis (Table 1), but longitudinal analysis excludes one women with only one measure before post-insertion week 4. Women receiving TDF-FTC did not differ significantly by key characteristics, such as age, prior pregnancy, or recent unprotected sex, from women receiving placebo (Table 1). For women randomized to the TDF-FTC arm (n=17), TDF-FTC exposure for an individual visit measure was good to excellent, non-zero, and undetected at 41 (34.2%), 33 (27.5%), and 46 (38.3%) of 120 total visit measures, respectively. The 28 women included in longitudinal analyses contributed 194 plasma specimens between post-implant insertion weeks 4 to 56 prior to study closure. LNG concentration measures were missing for 42 time points; ten time points were missing due to absent or insufficient plasma volume among seven women, while the other 32 time points resulted from missed follow-up visits among a further seven women. We included all data from tested specimens in the final model, but limited graphs to 36 weeks of data due to the limited number of visits after week 24 resulting following early trial closure.

No incident pregnancies and one implant removal (3.4%, discontinuation at 7 months, with reason for discontinuation not recorded) occurred during the study period. Six LNG concentrations were >1500 pg/mL; three of these values were present at insertion in women who reported oral contraceptive use. The remaining

three post-insertion values were considered outliers and censored from analysis. LNG concentrations varied noticeably but generally decreased with time from insertion (Figure 1). For 18 women receiving implants and who had LNG concentration measures at 4 weeks post-insertion, the mean LNG concentration was 660 pg/mL (standard deviation $\pm$ 214 pg/mL; interquartile range= 539 – 830 pg/mL). Mean LNG concentrations for remaining time points until 36 weeks post-insertion ranged between 469 and 649 pg/mL. LNG concentrations were somewhat lower for women randomized to the TDF-FTC arm (Figure 1). To further distinguish possible TDF-FTC effects, the unadjusted median LNG concentrations for each of four conditions (the three separate adherence categories and the placebo arm) are displayed in Figure 2. In a model controlling for age, BMI, and time from insertion, TDF-FTC use was not significantly associated with changes in mean LNG concentration, either when TDF-FTC exposure was considered for all women randomized to this arm (estimate= -67.3 pg/mL, 95% CI: -194.7, 60.0; p=0.30) or for women with “good to excellent” (estimate= -68.9 pg/mL, 95% CI: -206.3, 68.5; p=0.32), “some use” (estimate= -79.9 pg/mL, 95% CI: -215.5, 55.7; p=0.25), or “non-use” (estimate=-58.2 pg/mL, 95% CI: -195.3, 79.6; p=0.41) TDF-FTC exposure compared to the referent of placebo.

DISCUSSION

Mean LNG levels one month after insertion and through the first year were roughly consistent with prior assessments of Sino-implant (II) and similar LNG implants; assay differences and larger sample size may account for somewhat lower LNG

concentrations in other studies [6,13,14]. Importantly, all mean concentrations exceeded previously-reported minimum levels for contraceptive efficacy [6].

There is scant evidence regarding altered contraceptive or antiretroviral efficacy with concomitant LNG implant and TDF-FTC or other nucleoside reverse transcriptase inhibitor use as part of combination antiretroviral therapy [8,9].

Although small, our study detected no significant association between TDF-FTC exposure and plasma LNG concentrations. We also found that TFV/TFV-DP adherence among implant users was similar to that for FEM-PrEP participants overall [12].

Drug concentration findings should be cautiously interpreted due to small sample size and short study duration, as small but consistent differences in PK over time may attain statistical significance in subsequent years of use. Not all women received the implant at screening, limiting LNG concentration assessment during the first four weeks, when the plasma concentration falls most markedly. Women received implants upon request, thus, these participants may not reflect the general population. TDF-FTC concentrations were assessed at each follow-up visit, reflecting adherence over the prior month [12]. However, some women receiving implants missed visits (32 time points) and others who completed follow-up visits did not have LNG concentration levels (10 visits). We attribute the latter group to insufficient plasma available at the time when this secondary analysis was conducted. While nearly 50% of women had at least one missing concentration measurement during follow-up, it is unlikely that the missing data biased the primary comparison of LNG concentrations by study arm given that the primary

outcome is a biological measure not subject to participant behavior or sample insufficiency. We presume, therefore, that any impact of missing data would be limited to a reduction in the precision of concentration estimates. Last, three of 194 (1.5%) LNG concentration results which exceeded 1500 pg/mL were censored from analysis; however, if these measurements were indeed valid, their exclusion may have biased our final results. The high levels may be attributable to concomitant oral contraceptive use for management of irregular bleeding with the implant.

This analysis indicates Sino-implant (II) releases LNG at concentrations adequate for contraceptive efficacy in the first year of use, with no statistically significant variation by TDF-FTC use. These results provide reassurance that provision of TDF-FTC for pre-exposure prophylaxis is not contraindicated for women using the LNG implant for contraception during the first year of use, but further study of antiretroviral drug concentrations with LNG implant use among a larger study population and over a greater duration are needed.

251 ACKNOWLEDGMENTS

252 We are grateful to the women who participated in the FEM-PrEP trial, to the study
253 staff, to the communities who partnered with us to conduct the trial as well as all
254 collaborators in Africa, Belgium and the US. FEM-PrEP was conducted under two
255 grants funded by the United States Agency for International Development (USAID):
256 the Contraceptive and Reproductive Health Technologies and Research Utilization
257 Program, and then the Preventive Technologies Agreement (No.
258 GHO- A- 00- 09- 00016-00). Early support was also provided by the Bill & Melinda
259 Gates Foundation. Gilead Sciences, Inc. donated Truvada® and placebo. The views
260 expressed in this publication do not necessarily reflect those of FHI 360, the funding
261 agencies, or Gilead Sciences, Inc.

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309

Table 1

Table 1. Baseline sociodemographic and biologic characteristics among women randomized to either placebo or tenofovir disoproxil fumarate-emtricitabine (TDF-FTC) and receiving Sino-implant (II) at the Bondo, Kenya FemPrEP trial site (n=29).

	Placebo		TDF-FTC		Total		P-value ¹
Demographics	n	(%)	n	(%)	n	(%)	
Age (in years)							
18-29	6	(50.0)	13	(76.5)	19	(65.5)	
30-35	6	(50.0)	4	(23.5)	10	(34.5)	
Total	12		17		29		0.11
Mean (SD)	29.3	(5.6)	26.1	(5.8)	27.4	(5.8)	
Median (Range)	31	(19 - 35)	26	(19 - 35)	28	(19 - 35)	
Total	12		17		29		0.15
BMI							
Mean (SD)	22.5	(2.4)	22.6	(4.2)	22.6	(3.5)	
Median (Range)	22	(19 - 28)	21	(19 - 33)	21	(19 - 33)	
Total	12		17		29		0.92
Ever been pregnant							
No	0	(0.0)	2	(11.8)	2	(6.9)	
Yes	12	(100)	15	(88.2)	27	(93.1)	
Total	12		17		29		0.34
Contraceptive method used at screening							
Oral	1	(8.3)	4	(23.5)	5	(17.2)	
Injectable	4	(33.3)	6	(35.3)	10	(34.5)	
Condom/None	7	(58.3)	7	(41.2)	14	(48.3)	
Total	12		17		29		0.07
Unprotected sex in previous four weeks							
No	2	(16.7)	2	(11.8)	4	(13.8)	
Yes	10	(83.3)	15	(88.2)	25	(86.2)	
Total	12		17		29		0.38

¹Based on two sample t-test for continuous variables, and Fisher exact test for categorical variables.

Figure 1

95% Confidence Limits denoted in dashed and solid bar-capped lines for each data point for respective mean LNG levels.

LNG=levonorgestrel

Figure 2

Figure 2. Plasma levonorgestrel concentrations over 36 weeks from Sino-Implant (II) insertion among 29 women randomized to either placebo or tenofovir disoproxil fumarate-emtricitabine (TDF-FTC) in Bondo, Kenya.