

Pharmacogenetics of Efavirenz Exposure in Cervicovaginal Fluid during Pregnancy and Postpartum

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In this study, we investigated the combined influence of pregnancy and genetic polymorphisms on efavirenz pharmacokinetics in cervicovaginal fluid (CVF) of women receiving antiretroviral therapy. Women receiving efavirenz-containing antiretroviral therapy were recruited from two hospitals in Nigeria during 2017–2020. Sparse CVF and plasma samples were obtained during pregnancy to assess the possible association between drug concentration and *CYP2B6* polymorphisms (stage I). Participants were stratified into three *CYP2B6* 516G>T (rs3745274) genotype groups and re-enrolled for intensive pharmacokinetic sampling (stage II). Overall, 159 women (142 pregnant and 12 postpartum) contributed samples in stage I (88 CVF, 81 plasma and 73 paired). *CYP2B6* 516G>T (rs3745274) remained independently associated with log₁₀ efavirenz CVF concentration during pregnancy after adjusting for plasma concentration, with β (Log₁₀ efavirenz concentration, 95%CI) of 0.204 (0.027, 0.382), $P=0.025$). Median (IQR) efavirenz C_{min} in CVF during pregnancy ($n=12$) vs. postpartum ($n=12$) was 243 ng/mL (168–402) vs. 447 ng/mL (159–974), C_{max} was 1,031 ng/mL (595–1,771) vs. 1,618 ng/mL (675–2,695), and AUC_{0-24h} was 16,465 ng.h/mL (9,356–30,417) vs. 30,715 ng.h/mL (10,980–43,714). CVF-to-plasma AUC ratio was 0.36 during pregnancy and 0.46 postpartum. Upon stratification, efavirenz clearance during pregnancy was 57.9% higher than postpartum in patients with the *CYP2B6* 516GT genotype; the AUC_{0-24h} and C_{max} were 33.8% and 8.6% lower, respectively. Efavirenz C_{min} in CVF exceeded the protein binding-adjusted IC₉₀ (PBIC₉₀) of 126 ng/mL during pregnancy and postpartum. Efavirenz is well distributed into the CVF; both pregnancy and *CYP2B6* polymorphisms affect the extent of exposure.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Reducing cervicovaginal HIV-1 shedding using antiretroviral therapy is important for preventing heterosexual and mother-to-child transmission of HIV. Also, adequate drug exposure in the female genital tract is important for pre-exposure prophylaxis in women.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ The main objective of this study was to investigate the effect of pregnancy and polymorphisms in drug disposition genes on efavirenz exposure in CVF.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ For the first time, this study established that the overall exposure of efavirenz in the FGT (quantified in CVF) is lower

during pregnancy than postpartum. Women who are carriers of the *CYP2B6* 516G > T (rs3745274) loss of function allele T have significantly higher CVF concentration than those who did not. The minimum concentration in CVF was above the PBIC₉₀ in 83.3% of participants during pregnancy and 80% postpartum.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ Especially for drugs with a significant relative genetic contribution to observed variability, this expands our understanding of why cervicovaginal HIV-1 shedding continues in some women despite undetectable plasma viral load. The findings also highlight the need to investigate if pregnancy affects drug exposure in the FGT where this compartment is vital for clinical outcome.

Understanding antiretroviral (ARV) drug exposure in the female genital tract (FGT) is of interest in HIV because of its importance in selecting optimal therapies for preventing HIV transmission

in serodiscordant couples and from mother to child.¹ Although certain ARV drugs have been quantified in the FGT in a few studies,^{2–6} overall FGT exposure has not been well characterized

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for most drugs. While pregnancy and genetic polymorphisms have been established to alter ARV plasma exposure,^{7–10} the effect of these factors on ARV disposition in the FGT is unknown. With evidence of an ongoing risk of viral shedding in FGT, even in patients on suppressive antiretroviral therapy (ART) despite undetectable plasma viremia,^{11–13} there is a need to understand ARV dynamics in this compartment.

Mother-to-child transmission (MTCT) of HIV-1 is essentially minimized by suppressing HIV replication and reducing plasma viral load to an undetectable level, particularly at the early stages of pregnancy.¹⁴ The presence of HIV-1 in genital secretions has been shown to be associated with a greater risk of serodiscordant¹⁵ and infant HIV-1 transmission.¹⁶ In 2022, approximately 76.4% of people with HIV globally were reported to be receiving ART, including at least 8 out of every 10 pregnant women.¹⁷ Despite this high access to ART, new HIV infection among children remains substantial, mostly via MTCT. Since a substantial proportion of MTCT occurs during delivery,^{18,19} a significant percentage of these new pediatric HIV infections can be prevented by understanding factors that affect ARV disposition into the FGT.

The need to improve current knowledge of ARV disposition into the FGT within and outside the context of pregnancy and pharmacogenetics cannot be overemphasized. However, obtaining pharmacokinetic data to achieve this is limited by the invasiveness and increased cost of sampling genital tissues,²⁰ and accurate standardization of bioanalytic methods used to quantify ARVs in the FGT.²¹ To circumvent these challenges, cervicovaginal fluids (CVF) are used as an acceptable surrogate for vaginal tissue concentrations of ARV.²⁰ In our laboratory, we recently validated a minimally invasive LC–MS/MS method to quantify the HIV drug efavirenz in CVF collected on flock swabs,⁶ which improved our ability to conduct intensive clinical pharmacokinetic studies with CVF. In this component of the viral and antiretroviral dynamics in HIV MTCT Transmission Fluids (VADICT) study, whose protocol was previously published,²² we aimed to characterize efavirenz disposition in the CVF and investigate the combined effect of pregnancy and genetic polymorphism on ARV disposition into this compartment.

METHODS

Study sites, participants, and ethics approval

Participants in the VADICT study were recruited from four sites in Benue State, Nigeria (Federal Medical Centre, Makurdi; Bishop Murray Medical Centre, Makurdi; St. Monica's Hospital, Adikpo; and St. Thomas' Hospital, Ihugh) between December 2017 and September 2020. Pregnant or recently postpartum women living with HIV who were at least 18 years old, receiving efavirenz-based ART regimen, not taking other medications with known or suspected interaction with ART components, and who had no severe illness were eligible. Participants who contributed to the aspect of the study reported here were recruited only from the two sites in Makurdi where ultra-low temperature storage facilities were available. The study was approved by the National Health Research Ethics Committee, Abuja, Nigeria (approval number NHREC/01/01/2007). The study is registered on ClinicalTrials.gov (NCT03284645), and the full protocol has been published elsewhere.²²

Study design

The VADICT study was an observational cohort study with a pharmacokinetic component conducted in two stages. In the first stage, we assessed the association between functional single-nucleotide polymorphisms (SNPs) in efavirenz disposition gene *CYP2B6*, 516G>T (rs3745274) and 983T>C (rs28399499), and mid-dose efavirenz concentration in both CVF and plasma during pregnancy. For stage two, participants were stratified into three metabolizer groups (slow, intermediate, and extensive) using the independently associated SNP based on the number of variant alleles present. Participants from each group who still satisfied the eligibility criteria when the pharmacogenetic data became available were invited for re-enrollment into the intensive pharmacokinetic stage.

Sample collection and handling

Sparse-paired plasma and CVF samples were collected from each participant simultaneously after the last dose. About 1–2 mL of plasma was obtained from each participant by centrifugation (2300 g) of venous blood samples and transferred into cryovials. CVF samples were collected with flocked swabs by a female nurse, as described in our paper on this method.⁶ In summary, participants laid down on their backs for 5 minutes before each sample collection to allow fluid pooling in the back of the vagina. The swab was gently inserted approximately 3 inches into the vagina at first and inserted further until it touched the back of the posterior fornix. The swab was gently rubbed against the mid-vaginal walls for at least 30 seconds, withdrawn, and immediately transferred into a cryovial.

Participants who were re-enrolled in stage II after stratification into slow, intermediate, and fast metabolizers underwent intensive pharmacokinetic sampling. CVF and dried blood spot (DBS) samples were collected over a 24 hours dosing period after an observed oral dose of ART regimen containing 600 mg efavirenz. Within a few minutes of CVF collection, DBS samples were prepared by spotting drops of whole blood collected on Whatman® protein saver cards by finger prick using a 2 mm safety lancet (BD, Oxford, Oxfordshire, UK). DBS cards were dried at ambient temperature (18–25°C) for 2 hours and packed in ziplock bags with desiccants. All samples were stored at –80°C at the Federal Medical Centre, Makurdi, until transferred to the Translational Pharmacokinetics Research Laboratory, Obafemi Awolowo University, Ile-Ife, using Arctic Express® Dry Shipper (Thermo Scientific, Waltham, MA, USA) where they were stored at –80°C until analysis.

Viral load assay, pharmacogenetic analysis, and drug quantification

An in vitro nucleic acid amplification test for the determination of CVF viral load was performed using the Roche COBAS AmpliPrep/TaqMan HIV-1 test version 2.0 according to the manufacturer's instruction. The quantification range and lower limit of detection of this assay were 20–10,000,000 RNA copies/mL and 20 RNA copies/mL, respectively. The viral load assay was conducted at the Groote Schuur Laboratory, University of Cape Town, South Africa. The pharmacogenetic analysis included two *CYP2B6* SNPs: *CYP2B6* 516G>T (rs3745274) and *CYP2B6* 983T>C (rs28399499) known to be associated with efavirenz plasma exposure. Genomic DNA was extracted from DBS using the QIAamp DNA Blood Mini Kit (Qiagen, Germantown, USA) and quantified using Nanodrop® spectrophotometer (Thermo Fisher Scientific, Wilmington, USA) and stored at –70°C. Genotyping was performed by real-time PCR-based allelic discrimination using TaqMan® SNP genotyping assays for *CYP2B6* 516G>T (rs3745274) with product ID C_7817765_60 and *CYP2B6* 983T>C (rs28399499) with product ID C_60732328_20 on a DNA Engine Chromo4 system (Bio-Rad Laboratories, Hercules, CA, USA). The three-step PCR protocol included a 15-minute denaturation step at 95°C, 50 amplification cycles

at 95°C for 15 seconds each, and a 1-minute annealing step at 60°C. Opticon Monitor® version 3.1 (Bio-Rad Laboratories) enabled genotype calls based on allelic discrimination plots.

Determination of efavirenz concentration in CVF, plasma, and DBS samples was conducted on a TSQ Quantum Access LC–MS/MS system (Thermo Fisher Scientific, Hemel Hempstead, Hertfordshire, UK) at the Translational Pharmacokinetics Research Laboratory Obafemi Awolowo University, Nigeria using previously reported methods.^{6,23} Plasma efavirenz concentration was calculated from the DBS concentration using the equation initially reported by Eyles et al.,²⁴ which accounts for the effect of plasma protein binding and hematocrit.

Statistical analysis

Compliance with Hardy–Weinberg Equilibrium (HWE) was tested as previously described.²⁵ Before statistical analysis, efavirenz plasma and CVF concentration datasets were subjected to the Kolmogorov–Smirnov normality test. Pearson correlation was performed to test the relationship between continuous variables. The univariate linear regression analysis was used to determine variables associated with efavirenz concentration in plasma and CVF. Variables that showed significant association ($P < 0.05$) with efavirenz plasma or CVF concentration in the univariate linear regression analysis were included in a multivariate stepwise linear regression analysis. Differences in efavirenz plasma and CVF concentrations and pharmacokinetic parameters between patient groups were investigated using one-way analysis of variance and the Mann–Whitney U test. All these analyses were performed using IBM SPSS Statistics version 21.0.0.0 (IBM, Armonk, New York) and GraphPad Prism 10 (GraphPad Software, La Jolla, CA).

RESULTS

Study participants

Sparse pharmacokinetic samples available for analysis in the preliminary stage I were obtained from 96 pregnant women at a mean (SD) gestational age of 34.5 weeks (3.12), age 28.9 years (4.9), and body weight 64.3 kg (11.9). The duration of therapy prior to initial enrollment was 46.5 months (36.8). Twelve pregnant women aged 28.9 years (4.9) old with a body weight of 65.7 kg (7.00) participated in stage II intensive pharmacokinetic sampling at a gestational age of 26.0 weeks (6.70). Postpartum intensive pharmacokinetic sampling was conducted in 12 women aged 32.9 years (5.1), body weight of 59.5 kg (8.8), at 37.1 weeks (4.1) after delivery. Total duration on ART prior to enrollment for stage II was 41.2 weeks (36.7) during pregnancy and 55.8 weeks (27.3) for postpartum women. All study participants received a combination ART containing 600 mg efavirenz. A total of 169 sparse samples (88 CVF, 81 plasma, and 73 paired samples in the third trimester), 168 intensive CVF samples (84 samples from 12 pregnant women and 84 samples from 12 postpartum women), and 98 DBS (48 samples from 7 pregnant and 48 samples from 7 postpartum women) were available for analysis.

Allele and genotype frequencies of CYP2B6 SNPs in pregnant women

The allele and genotype frequencies of *CYP2B6* 516G>T(rs3745274) and *CYP2B6* 983T>C during pregnancy are presented in **Table 1**. Comparison of expected genotype frequencies with the observed frequencies in this cohort indicated compliance with HWE, with P -value of 0.947 for *CYP2B6* 516G>T(rs3745274) and 0.147 for 983T>C(rs28399499) SNPs (**Table S1**).

This suggests that our study population is representative of the general population.

Are CYP2B6 516G>T (rs3745274) and 983T>C (rs28399499) associated with efavirenz CVF concentration?

In stage I, 88 sparse CVF and 81 plasma samples were obtained from a total of 96 participants during pregnancy (73 paired samples) to assess the possible association between efavirenz concentration and the two *CYP2B6* polymorphisms. CVF and plasma samples were collected at 14.45 hours (1.30–17.02) after the last dose. The median (IQR) CVF and plasma efavirenz concentrations were 788 ng/mL (502–1,542) and 1,487 ng/mL (1,041–2,363), respectively. There was a significant correlation between efavirenz CVF and plasma concentration (Pearson's $r = 0.76$, $P = 1.32 \times 10^{-6}$). The average CVF-to-plasma ratio computed from the 73 paired sparse samples was 0.55.

Only *CYP2B6* 516G>T (rs3745274) was independently associated with efavirenz concentration in CVF and plasma during pregnancy. The unstandardized regression β coefficient (which represents incremental change in $\log_{10}$ EFV concentration per unit change) for this association is 0.268 (95% CI = 0.130, 0.406, $P = 2.2 \times 10^{-4}$) and 0.193 (0.109, 0.277, $P = 1.7 \times 10^{-5}$), respectively. Notably, *CYP2B6* 516G>T (rs3745274) alone accounted for 15.9% (adjusted $R^2 = 0.159$) and 21.7% (adjusted $R^2 = 0.217$) of the observed interindividual variability in efavirenz concentration in CVF and plasma during pregnancy, respectively. The association of *CYP2B6* 516G>T (rs3745274) with CVF concentration remained significant ($P = 0.025$) after adjusting for plasma concentration with β values of 0.204 (0.027, 0.382). The association between *CYP2B6* 516G>T (rs3745274) and efavirenz CVF as well as plasma concentration is presented in **Figure 1**. No significant association was observed between efavirenz CVF concentration and *CYP2B6* 983T>C (rs28399499) ($P = 0.898$), age ($P = 0.340$), body weight ($P = 0.646$), body mass index ($P = 0.656$), time after dose ($P = 0.055$), and duration of therapy ($P = 0.379$) (**Table 2**). Due to evidence of *CYP2B6* 983T>C (rs28399499) association with plasma efavirenz concentration,²⁶ we explored potential association of composite *CYP2B6* 516G>T (rs3745274) and 983T>C (rs28399499) with efavirenz CVF exposure. We also included *CYP2B6* 983T>C (rs28399499) in the multivariate analysis. Percentage variability in efavirenz CVF concentration explained by *CYP2B6* polymorphisms increased with the composite genotype (adjusted $R^2 = 0.172$ compared with 0.160 for *CYP2B6* 516G>T (rs3745274) alone) and only *CYP2B6* 516G>T (rs3745274) was retained in the final model. Additionally, age, body weight, body mass index, time after dose, and duration of therapy were not associated with efavirenz plasma concentration.

Upon stratification based on *CYP2B6* 516G>T (rs3745274), we observed significant differences ($P < 0.0001$) in efavirenz median (IQR) CVF concentrations: GG ($n = 28$), 559 ng/mL (342–874); GT ($n = 45$), 791 ng/mL (630–1,475); TT ($n = 15$), 3,310 ng/mL (1,332–4,718). Similarly, plasma concentrations varied based on *CYP2B6* 516G>T (rs3745274) ($P < 0.0001$): GG ($n = 21$), 1,085 ng/mL (888–1,305); GT ($n = 48$), 1,495 ng/mL (1,095–1,983); TT ($n = 13$), 4,164 ng/mL (2,698–5,903). Only

Table 1 Demographic, clinical and genetic characteristics of study participants

Characteristics	Pregnancy (Sparse, <i>n</i> = 96)	Pregnancy (Intensive, <i>n</i> = 12)	Postpartum (Intensive, <i>n</i> = 12)
Age (years) ^a	28.9 (4.9)	28.9 (4.9)	32.9 (5.1)
Body weight (kg)	64.3 (11.9)	65.7 (7.0)	59.5 (8.8)
Gestational age (weeks)	34.5 (3.12)	26.0 (6.7)	NA
Postnatal age (weeks)	NA	NA	37.1 (4.1)
Time after last dose (h)	13.75 (3.05)	23.8 (1.02)	24.1 (1.14)
Duration on regimen (months)	46.5 (36.8)	41.2 (36.7)	50.8 (27.3)
Time since HIV diagnosis (months)	50.4 (32.7)	49.2 (39.0)	53.1 (25.9)
Viral load (copies/mL) ^b			
Undetectable	74/96 (77.0%)	12/12 (100%)	10/12 (83.3%)
<1,000	11/96 (11.5%)		1/12 (8.3%)
>1,000	11/96 (11.5%)		1/12 (8.3%)
Genotype frequencies ^b			
<i>CYP2B6</i> 516G>T (rs3745274)			
GG	34/86 (39.5)	6/12 (50%)	4/12 (33.3%)
GT	39/86 (45.3)	6/12 (50%)	5/12 (41.7%)
TT	13/86 (15.1)		3/12 (25%)
T	53.5/86 (62.2)		
C	32.5 (37.8)		
HWE <i>P</i> -value	0.947		
<i>CYP2B6</i> 983T>C (rs28399499)			
TT	79/86 (91.9%)		
TC	6/86 (6.9%)		
CC	1/86 (1.2%)		
T	82/86 (95.3%)		
C	4/86 (4.7%)		
HWE <i>P</i> -value	0.147		
Composite <i>CYP2B6</i> 516G>T and 983T>C			
0 (GG; extensive metabolizers)	28/86 (32.6%)		
1 (GT; intermediate metabolizers)	43/86 (50.0%)		
2 (TT; slow metabolizers)	15/86 (17.4%)		
G	49.5/86 (57.6)		
T	36.5/86 (42.4)		
HWE <i>P</i> -value	0.976		

Abbreviation: NA, not applicable. ^aData presented as Mean (SD). ^bData presented in frequencies (%).

CYP2B6 516G>T (rs3745274) was used to stratify participants invited for the stage II intensive pharmacokinetic sampling.

What is the combined influence of pregnancy and genetics on efavirenz CVF exposure?

In stage II, 12 pregnant women (GG, *n* = 6 and GT, *n* = 6) and 12 postpartum women (GG, *n* = 4; GT, *n* = 5 and TT, *n* = 3) were successfully re-enrolled for intensive pharmacokinetic sampling. The concentration–time profiles of efavirenz in CVF and plasma during pregnancy and postpartum in the combined cohort and the stratified groups are presented in **Figure 2**. The corresponding pharmacokinetic parameters are presented in **Table 3**. In pooled analysis, efavirenz clearance in CVF was 87.7% higher during

pregnancy compared with postpartum, while the AUC_{0-24h}, *C*_{max}, and *C*_{min} were 46.4%, 36.3%, and 45.6% lower, respectively. When stratification based on *CYP2B6* 516G>T (rs3745274), efavirenz AUC_{0-24h}, *C*_{max}, and *C*_{min} in CVF during pregnancy were 33.8%, 8.54%, and 59.9% lower than postpartum in patients with *CYP2B6* 516GT genotype. On the contrary, in patients with the *CYP2B6* 516GG genotype, the overall CVF exposure was similar during pregnancy and postpartum (**Table 3**). However, these changes were not statistically significant probably due to the limited sample size included in stage II of this study, particularly for the genotype-based comparisons. There were no patients with the *CYP2B6* 516TT genotype among re-enrolled pregnant participants for a similar comparison. Efavirenz CVF concentration

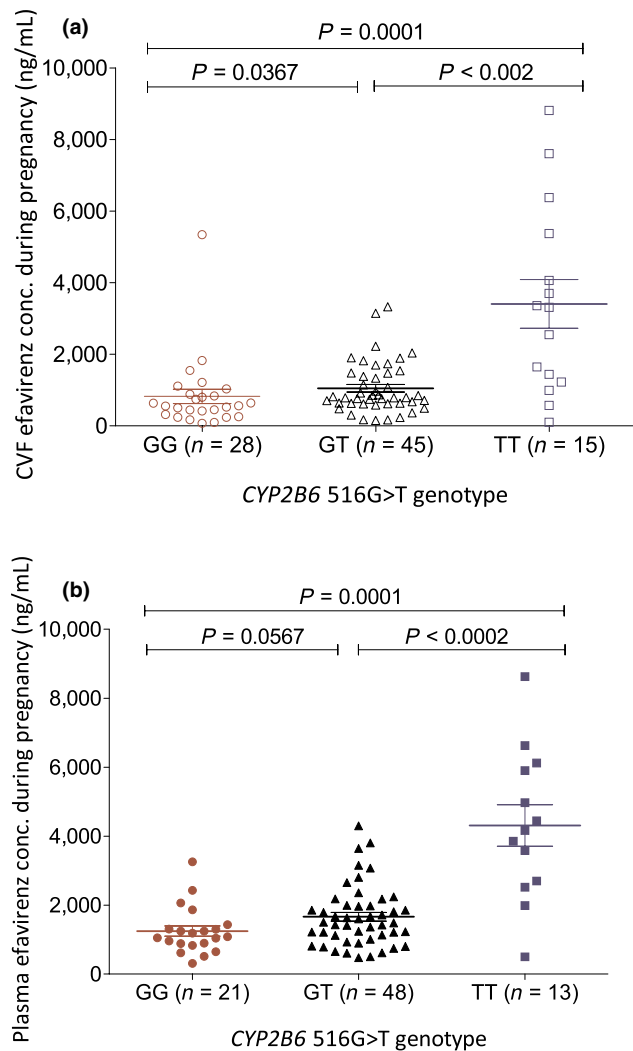


Figure 1 Differences in efavirenz concentration in maternal plasma during pregnancy based on maternal *CYP2B6* 516G>T genotypes (GG, *n* = 21; GT, *n* = 48; TT, *n* = 13) and CVP (GG, *n* = 28; GT, *n* = 45; TT, *n* = 15).

exceeded the protein binding adjusted IC_{95} (PBIC₉₀) of 126 ng/mL for wild-type HIV-1²⁷ in 100% of pregnant women and 75% of postpartum women with the *CYP2B6* 516GG (Table 3).

Paired efavirenz CVP and plasma pharmacokinetics during pregnancy and postpartum

The pharmacokinetic parameters of efavirenz in CVP and plasma during pregnancy and postpartum derived from paired samples (*n* = 7) are presented in Table 4. During pregnancy, median (IQR) efavirenz AUC_{0-24h} in CVP was 17,576 ng.h/mL (13,624–27,239) compared with 48,983 ng.h/mL (41,414–54,311) in plasma (DBS-derived), giving a CVP:plasma AUC₀₋₂₄ ratio of 0.36. This ratio increased to 0.46 during the postpartum period. The time to reach C_{max} in CVP was 8 hours both during pregnancy and postpartum, ~6 hours after plasma C_{max} . An increase in CVP:plasma C_{max} ratio during the postpartum period was also observed (0.39 vs. 0.27). Efavirenz C_{min} in CVP was above the PBIC₉₀ in 91.6% in both the pregnant and postpartum cohorts (Table 3).

Table 2 Univariate and multivariate linear regression analyses of variables associated with efavirenz concentration in cervicovaginal fluid (CVF) during pregnancy

Patient characteristics	β (Log ₁₀ efavirenz concentration) (95%CI)	P-values
Univariate linear regression		
Age (years)	0.010 (−0.011, 0.032)	0.340
Body weight (kg)	−0.002 (−0.009, 0.006)	0.646
Time after dose (h)	0.030 (−0.001, 0.062)	0.055
BMI (kg/m ²)	−0.005 (−0.025, 0.016)	0.656
Duration of therapy (months)	0.001 (−0.001, 0.004)	0.379
<i>CYP2B6</i> 516G>T (rs3745274)	0.268 (0.130, 0.406)	2.2×10^{-4}
<i>CYP2B6</i> 983T>C (rs28399499)	−0.021 (−0.341, 0.300)	0.898
<i>CYP2B6</i> 516/983G>T	0.278 (0.143, 0.413)	9.6×10^{-5}
Multivariate linear regression		
<i>CYP2B6</i> 516G>T (rs3745274) ^a	0.268 (0.130, 0.406)	2.2×10^{-4}
<i>CYP2B6</i> 516G>T (rs3745274) ^b	0.204 (0.027, 0.383)	0.025
<i>CYP2B6</i> 983T>C (rs28399499)	Excluded	

Association between *CYP2B6* 516G>T and efavirenz CVP remained significant after adjusting for plasma concentration. Abbreviations: β , is the unstandardized regression coefficient which represents incremental change in log₁₀ EFV concentration per unit change in a patient characteristic. BMI, body mass index; CI, confidence interval; P-value, probability value. ^aThe result of multivariate analysis before adjusting for plasma concentration. ^bThe result of multivariate analysis after adjusting for plasma concentration.

HIV-1 viral load in CVF during pregnancy and postpartum

HIV RNA was detectable in 22 (23%) out of the 96 pregnant women who contributed to the sparse CVP samples. The median (IQR) HIV RNA in these 22 participants was 770 copies/mL (144–3,986). Efavirenz CVP concentration in participants with detectable (*n* = 22) and undetectable (*n* = 74) HIV RNA were 743 (364–850) ng/mL and 789 (478–1,431), respectively. Median efavirenz CVP concentrations in participants with HIV RNA <1,000 copies/mL and >1,000 copies/mL were 617 ng/mL (393–797) and 610 ng/mL (276–871), respectively. HIV RNA was undetectable in all the 12 pregnant women enrolled in the intensive pharmacokinetic study. Two of the postpartum women who contributed to the intensive pharmacokinetic sample had a median detectable HIV RNA of 2,690 copies/mL (Table 1).

DISCUSSION

The FGT is a sanctuary site for HIV-1 and contributes to MTCT during delivery in the absence of ART. The use of ART reduces cervicovaginal HIV-1 RNA,²⁸ contributing to the prevention of MTCT and heterosexual transmission of HIV. Reasons for ongoing cervicovaginal HIV-1 shedding in women taking ART are not well understood. However, available evidence indicates differences in the penetration of different ARVs into the FGT.²⁹ In this study, we confirmed that efavirenz distributes into the FGT of pregnant and postpartum women following oral dosing. There was a strong

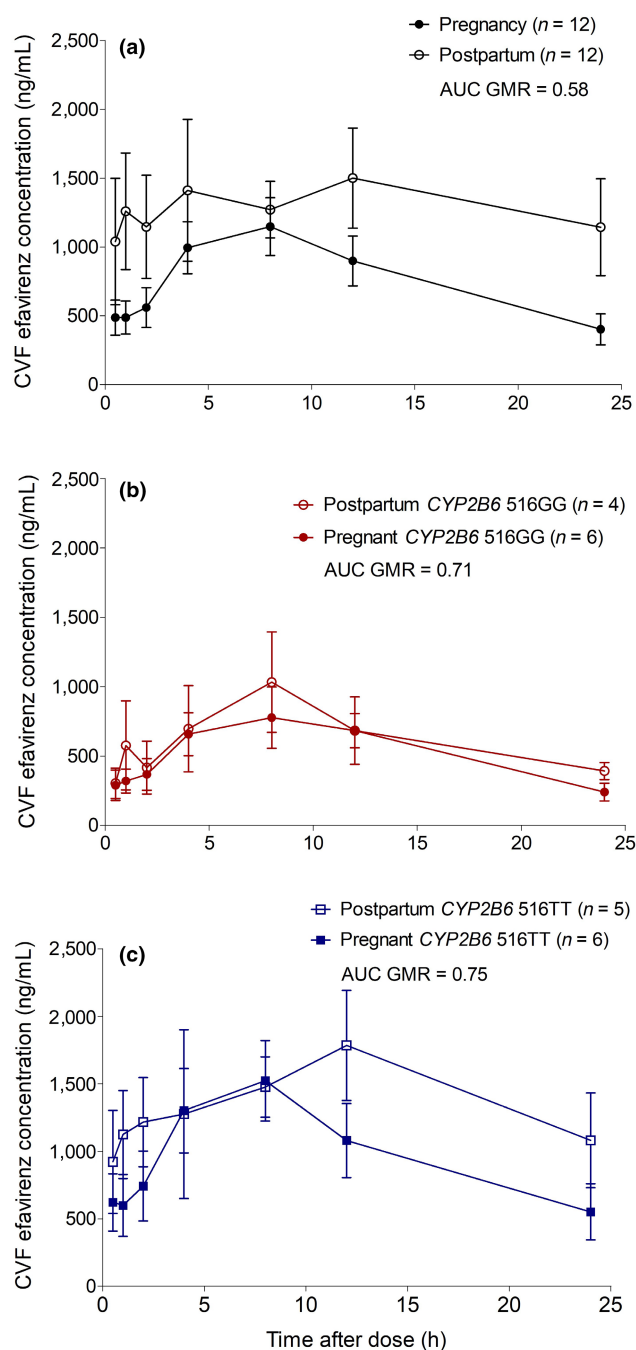


Figure 2 Efavirenz concentration–time profiles in cervicovaginal fluid (CVF) during pregnancy and postpartum in the combined cohort and the groups stratified by *CYP2B6* genotype. AUC GMR indicates the ratio of the geometric mean of the AUC during pregnancy/AUC during postpartum periods.

correlation between CVF and plasma exposure and CVF:plasma AUC_{0–24h} ratios of 0.36 during pregnancy and 0.46 postpartum. Beyond its well-established impact on plasma efavirenz concentration, we confirmed for the first time the influence of *CYP2B6* 516G>T (rs3745274) on CVF efavirenz exposure. Although efavirenz minimum effective concentration in the FGT has not been established, CVF concentrations exceeding the PBIC₉₅ (126 ng/mL) for wild-type HIV-1 were achieved within 30 minutes

after drug administration in 91.6% of both pregnant and postpartum women studied.

These findings are clinically relevant for multiple reasons. First, confirming the influence of polymorphisms in drug disposition genes on the extent of drug exposure in the FGT could help in understanding why cervicovaginal HIV-1 shedding continues in some women despite undetectable plasma viral load. Though the present study focused on efavirenz, a similar scenario could potentially be at play for other drugs where there is significant genetic contribution to observed interindividual variability. For instance, we previously reported a relative genetic contribution of 81% for efavirenz and 65% for tenofovir³⁰; the latter continues to be a major component of first-line ART regimens. This may help in optimizing treatment and preventative therapies for women. Second, instead of focusing on plasma alone, this study highlights the importance of assessing the potential influence of pregnancy on drug exposure in the FGT where it plays a key role in clinical outcome. This could reveal opportunities to optimize therapy for pregnant women.

Other studies have reported the extent of penetration of some other ARV drugs into the FGT, with CVF:plasma concentration ratio of 2.3 (1.4–4.1) for raltegravir,³¹ 3.2 (1.2–8.5) for lamivudine² and 5.2 (1.2–22.6) for tenofovir.² Several factors are expected to affect the extent of drug distribution in the FGT, including molecular weight and protein binding within the FGT. The changes in CVF:plasma concentration ratio observed for efavirenz in the present study show the need to interpret single timepoint data with caution. AUC ratio is a more reliable estimate. Sufficiently permeable low molecular weight drugs are expected to distribute into the FGT to some extent, and poor protein binding is expected to further facilitate this.³² Efavirenz protein binding in the CVF was not determined in this study. However, based on previous reports, the concentrations of drug-binding proteins have been reported to be ~1% of what is obtained in blood plasma.³³ CVF protein binding of the antiretroviral drug maraviroc was reported to be 10% of plasma protein binding.³⁴ Hence, efavirenz CVF protein binding is likely to be limited, contributing to the observed level of exposure.

Our results indicate a 46% reduction in efavirenz CVF AUC_{0–24h} during pregnancy relative to postpartum values. Both efavirenz CVF C_{min} and C_{max} during pregnancy were substantially lower than postpartum by 45.6% and 36.3%, respectively. Efavirenz clearance from the CVF was markedly higher (87.6% increment) during pregnancy compared with postpartum. The resulting lower drug exposure was above the PBIC₉₀ and did not appear to contribute to detectable HIV-1 RNA in the CVF. The situation may be different in women initiating ART late pregnancy and who are extensive metabolizers, the lower exposure potentially leading to sub-optimal suppression of viral replication in the FGT and increased risk of MTCT of HIV. The onset of pregnancy is well known to be associated with maternal physiological changes.³⁵ These changes could alter drug disposition and may explain the reduction in the CVF exposure of efavirenz observed in this study.

Efavirenz is predominantly metabolized by *CYP2B6*.³⁶ The *CYP2B6* 516G>T (rs3745274) is a missense polymorphism within the *CYP2B6* gene characterized by loss of *CYP2B6* functional allele and altered enzymatic activity. The loss of functional *CYP2B6* alleles is known to alter the pharmacokinetics of drugs

Table 3 Efavirenz CVF pharmacokinetics in genetically stratified pregnant and postpartum women

	C_{min} ($\mu\text{g/mL}$)	Participants with CVF $C_{min} > \text{PBIC}_{90}$ (%)	C_{max} ($\mu\text{g/mL}$)	AUC_{0-24h} ($\mu\text{g.h/mL}$)	CL/F (L/h)	T_{max} (h)
Combined						
Pregnancy (n=12)	243 (168–402)	91.6	1,031 (595–1,771)	16,465 (9,356–30,417)	36.6 (19.7–65.1)	8.0 (7.0–9.0)
Postpartum (n=12)	447 (159–974)	91.6	1,618 (675–2,695)	30,715 (10,980–43,714)	19.5 (13.8–54.7)	8.0 (8.0–8.0)
Preg/PP GMR (90% CI)			0.69 (0.50–1.67)	0.58 (0.36–1.59)	1.72 (1.44–2.99)	
CYP2B6 516GG						
Pregnancy (n=6)	169 (164–242)	100	664 (596–1,069)	11,577 (7,507–13,284)	52.0 (42.0–79.9)	8.0 (4.0–12.0)
Postpartum (n=4)	151 (116–225)	75.0	636 (583–1,107)	10,630 (9,848–15,875)	57.0 (46.0–61.1)	8.0 (7.0–8.0)
Preg/PP GMR (90% CI)			0.79 (0.22–1.73)	0.71 (0.24–1.37)	1.40 (0.62–2.51)	
CYP2B6 516GT						
Pregnancy (n=6)	330 (250–440)	83.3	1,550 (1,090–2,090)	20,671 (15,993–28,712)	30.0 (21.0–37.6)	8.0 (8.0–8.0)
Postpartum (n=5)	814 (496–981)	80.0	1,695 (1,540–3,003)	31,229 (27,660–41,873)	19.0 (14.0–22.0)	8.0 (8.0–8.0)
Preg/PP GMR (90% CI)			0.79 (0.02–2.39)	0.75 (0.05–2.21)	1.33 (0.47–3.29)	
CYP2B6 516TT						
Postpartum (n=3)	972 (587–2,383)	100	2,587 (1,633–4,365)	39,673 (25,147–64,938)	15.0 (10.9–35.8)	8.0 (8.0–8.0)

Data are presented in median (IQR). Note: Because of the parallel nature of the study design, the pregnancy and postpartum samples were not paired. Abbreviations: 90% CI, 90% confidence interval; AUC_{0-24h} , area under the concentration–time curve over a 24 h dosing interval; CL/F, apparent clearance; C_{max} , maximum CVF or plasma concentration; C_{min} , minimum CVF or plasma concentration; GMR, geometric mean ratio; PBIC_{90} , protein-bound IC_{90} ; T_{max} , time to reach maximum drug concentration.

Table 4 Comparison between efavirenz CVF and DBS-derived plasma pharmacokinetics (paired) in pregnant and postpartum women

	C_{min} ($\mu\text{g/mL}$)	C_{max} ($\mu\text{g/mL}$)	AUC_{0-24h} ($\mu\text{g.h/mL}$)	CL/F (L/h)	T_{max} (h)
Pregnancy (n=7)					
Plasma	844 (534–1,109)	5,080 (4,535–5,364)	48,983 (41,414–54,311)	12.2 (11.0–14.4)	2.00 (2.00–4.00)
CVF	271 (243–422)	1,368 (829–1,808)	17,576 (13,624–27,239)	34.1 (22.3–44.9)	8.00 (4.00–8.00)
CVS:Plasma ratio	0.32	0.27	0.36	2.80	4.00
Postpartum (n=7)					
Plasma	2,192 (1,637–3,495)	5,174 (4,643–6,988)	67,339 (57,492–106,868)	8.91 (6.21–10.5)	2.00 (2.00–2.00)
CVF	600 (423–905)	2,021 (1,579–2,415)	30,927 (28,463–36,256)	19.4 (16.7–21.1)	10.0 (8.00–12.0)
CVS:Plasma ratio	0.27	0.39	0.46	2.18	5.00

Data are presented in median (IQR). Abbreviations: AUC_{0-24h} , area under the concentration–time curve over a 24 h dosing interval; CL/F, apparent clearance; C_{max} , maximum CVF or plasma concentration; C_{min} , minimum CVF or plasma concentration; T_{max} , time to reach maximum drug concentration.

that are metabolized by this enzyme, including efavirenz.^{7,37} Efavirenz concentration in the CVF varied depending on *CYP2B6* 516G>T (rs3745274) genotypes during pregnancy and postpartum, with the lowest CVF exposure observed in women with *CYP2B6* 516GG genotype. For pregnant women with *CYP2B6* 516GG genotype, the overall efavirenz exposure in CVF was 43.9% lower, compared with those with *CYP2B6* 516GT genotype. On

the other hand, the overall efavirenz CVF exposure in the limited number ($n=3$) of postpartum women with *CYP2B6* 516TT genotype in this cohort was 273% higher than those with *CYP2B6* 516GG and 27.0% higher than those with *CYP2B6* 516GT. These findings suggest that *CYP2B6* may play a role in efavirenz CVF disposition. Efavirenz CVF C_{min} was above 126 ng/mL (PBIC_{90} for the wild-type HIV-1) in 75% of postpartum women with the

CYP2B6 516GG genotype, compared with 100% in pregnant women with the same genotype.

The expression of CYP2B6 isoenzyme, its activity in the FGT, or the potential contribution of other SNPs was not assessed in this study. This is considered one of the limitations of this study, and further studies focused on these areas are recommended. Additionally, the dynamic nature of pregnancy means many of the participants in the initial genotyping stage were no longer eligible when stage II started. This resulted in limited sample size for the genotype-based comparisons of efavirenz pharmacokinetics in CVF during pregnancy and postpartum. However, sufficient sparse samples were available to establish the role of CYP2B6 polymorphism on efavirenz exposure in CVF during pregnancy.

In conclusion, the overall exposure of efavirenz in the FGT (quantified in CVF) is lower during pregnancy than postpartum. In addition, women who are carriers of the CYP2B6 516G>T loss of function allele T have significantly higher CVF concentration than those who did not.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICTS OF INTEREST

The authors declared no competing interests in this work.

AUTHOR CONTRIBUTIONS

O.E., O.B., and A.O. wrote the manuscript. A.O. and E.A. designed the research. O.E., U.A., J.O., and A.O. performed the research. O.E. and A.O. analyzed the data. A.O. and O.B. contributed analytical tools.

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