**Impact of Pharmacogenetics and Pregnancy on Tenofovir and Emtricitabine Pharmacokinetics.**

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**Abstract**

**Aim**: Treatment and prevention of mother to child transmission of HIV in pregnancy utilises tenofovir (TFV) and emtricitabine (FTC). This study aims to evaluate the role of single nucleotide polymorphisms (snp) of TFV and FTC pharmacokinetics (PK) during pregnancy. **Method:** Sixty one (61) pregnant or postpartum women on TFV and FTC were selected from a group of pregnant and postpartum Nigerian women and both snps and drug levels evaluated. **Results:** Pregnancy decreases TFV plasma concentration by 26% (log10 β= -0.131 (-0.228, -0.034), (P= 0.009) between 4-18 h post dose. FTC concentrations in individuals with *ABCC2* rs2273897 TT genotype were 6 fold higher than heterozygous (CT) and homozygous (CC) women. All other evaluated snps were not significant. **Conclusion**: Pharmacogenetics may contribute to treatment outcome or resistance prevention.

**INTRODUCTION**

Tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) in combination are the most common components of WHO-recommended first-line combination antiretroviral therapy (cART) regimens recommended for prevention of mother-to-child transmission (PMTCT) of HIV [1-4] and first-line regimens for treatment of adults, adolescents and pre-exposure prophylaxis (PrEP) ( [4]. Knowledge of anticipated pharmacokinetic (PK) parameters has been derived in non-pregnant adult [5-7], but physiologically, pregnancy alters absorption, distribution, metabolism and elimination of drugs [8]. Pregnancy also induces changes in gastric pH, intestinal transit time, progesterone production and glomerular filtration rates, significantly changing pharmacokinetics [6, 8], inducing modifications in bioavailability, volume of distribution (Vd) and clearance of drug (CL) [8, 9].

Genetic polymorphisms of drug transporters are known to influence the pharmacokinetics of FTC and TFV active moiety (TFV) during pregnancy [1, 2]. Marked increases in 17 β estradiol significantly influence FTC and TFV excretion due to genetic modulation of ABCC4 transporters [16-19]. 17 β estradiol is a potent inhibitor of ABCC4 transporters and impact on TFV blood exposure and TFV elimination via the kidneys during pregnancy [10-12]. After an initial metabolic conversion of TDF to TFV by esterase hydroxylation and subsequent intracellular phosphorylation by nucleotide kinase to tenofovir phosphate, TFV is finally excreted unchanged by glomerular filtration and active tubular secretion via renal transporters [14, 15]. Likewise, FTC is also excreted predominantly unchanged via the kidneys, but excretion is predominantly influenced by ABCC2 in both pregnant and postpartum mothers [13, 14]. Excretion of TFV and FTC in kidneys and trans-placental transport is regulated by substrate specific efflux and influx transporters [15]. Single nucleotide polymorphisms (SNPs) of transporters regulatory genes have also been associated with maternal exposure and viral suppression during pregnancy [7-9]. These polymorphisms in ABCC class of transporters influences pharmacokinetics of FTC with consequential effect on drug distribution during pregnancy [11-13]. For instance, FTC is an ABCC1 substrate eliminated primarily unchanged via the kidneys [10]. It is also excreted in breast milk and crosses placental membrane to the fetal compartment in significant amount, and can all be modulated by pregnancy [15].

While these changes in pharmacokinetics of widely prescribed antiretroviral (ARVs), including TFV and FTC, have been reported in pregnancy, only limited data is available for combined influence of pregnancy and pharmacogenetics on TFV and FTC pharmacokinetics [8, 16-18]. This study hypothesised that combined effect of pregnancy and pharmacogenetics result in significant changes in FTC and TFV PK, and aimed at investigating the impact of pharmacogenetics on changes in FTC and TFV PK during pregnancy.

**METHODS**

**Study population and selection criteria**

HIV-positive pregnant and postpartum women were recruited from three hospitals in Benue State Nigeria: Bishop Murray Medical Centre, Makurdi; St. Monica’s Hospital, Adikpo; and St. Mary’s Hospital, Okpoga. The original study was conducted between December 2012 and October 2013 to evaluate the pharmacogenetics of pregnancy-induced changes and breastfed infants’ exposure to efavirenz and nevirapine during pregnancy and postpartum, respectively [19, 20]. For the present analysis, samples were selected to evaluate the influence of genetics and pregnancy on TFV and FTC. Pregnant and postpartum women taking ART regimens containing TDF and FTC were included. Participants were excluded if samples were collected within four hours of dosing. A total of 61 women (31 pregnant and 30 postpartum) were eligible and evaluated for both drug concentrations and selected SNPs.

Permission was obtained from the hospitals management and consent forms signed by participant before recruitment. Protocol and materials transfer agreements were approved by the National Health Research Ethics Committee (NHREC) Abuja Nigeria.

**Study Design**

This was an observational study of HIV-positive women conducted to evaluate the relationship between selected single nucleotide polymorphisms (snps) and drug

147

Concentrations in pregnant and postpartum women. Drug concentrations were quantified at single time points (4-18 h post observed dose) for each patient, and allele and genotype frequencies evaluated to determine association between TFV and FTC concentrations in pregnant and postpartum women. Five (5) ABCC2 and ABCC4 transporter SNPs were evaluated for impact of polymorphism on drug exposure in pregnancy and postpartum. SNPs with minor allele frequency of ≥25% were investigated for polymorphisms in renal tubular transporters that significantly affect drug pharmacokinetics in pregnancy. Drug concentrations in pregnant and postpartum women were also measured and relationship between genetic polymorphisms and TFV and FTC blood concentrations were assessed. The reference minor allele frequency of the Yoruba ethnic group, a subset of Nigerian population, was used to determine the polymorphic genes to be evaluated. Genes with minor allele frequency ≥25% include: ABCC2 rs2273897, 61% and 39%, ABCC2 rs3749966, 61% and 39%, ABCC4 rs1059751, 75% and 25%, ABCC4 rs3742106, 75% and 25%, ABCC4 rs1751034 73% and 27% [8].

**Tenofovir and Emtricitabine Quantification**

TFV and FTC concentrations in blood was measured from dried blood spots (DBS) using validated LC-MS method [21]. Assay calibration range 16- 4000 ng/mL and internal standards 2CA and TFV-d6 were used for assay proficiency. Measured drug concentrations were used to evaluate the relationship between drug concentrations and genetic polymorphism of SNPs using a regression model.

**Genotyping**

Genotyping for ABCC2 (rs2273897), ABCC2 (rs3749966), ABCC4 (rs1059751), ABCC4 (rs1751034) and ABCC4 (rs1751034) was performed by real time polymerase chain reaction (PCR) allelic discrimination using standard Taqman assays. Genotypes assignment and allelic discrimination plots were performed on a chromo4 system (Bio-Rad Laboratories, Hercules, CA) and Opticon Monitor version 3.1 software (Bio-Rad Laboratories). The PCR protocol involved denaturation of DNA at 95oC for 10 minutes, 40 cycles of amplification at 95oC for 15 seconds and annealing at 60 oC for 1 min.

**Statistical analyses**

Allelic and genotype frequencies were evaluated to ensure Hardy-Weinberg equilibrium was maintained. SNPs were in Hardy-Weinberg equilibrium (P value >0.05) except ABCC2 rs3749966 (X2 = 4.8, P<0.01) and ABCC4 rs1751034 (X2 = 5.05, P<0.01) respectively which compromised their interpretation. Normality was checked for continuous variables using Shapiro Wilk test, which was statistically significant (p<0.001), and variables were log transformed. Linear regression models were used to determine the relationship between drug concentrations and other variables (age, regimen, time post dose) in SPSS version 23.0. Covariates with P-value ≤ 0.2 were further entered into a multivariate model in a stepwise fashion and analysed for relationship between the groups. Missing covariate >10% were managed by excluding the sample from the regression analysis. Within groups relationships were analysed by first creating dummy variables and subsequently entered into a model

to determine specific relationships with genotypes. Output of this analysis enabled determination of the relationship between drug concentrations at different time points with pregnancy, breast feeding and the SNPs. All charts were plotted using Graph pad prism 5.0 (GraphPad Software Inc)

**RESULTS**

Pregnant and postpartum women on once daily FTC and TDF were evaluated at single time points between 4 and 18 h post dose for drug levels and associations with polymorphisms in genes using regression models. Of the 61 (31 pregnant and 30 postpartum women) evaluated, the median (range) age and weight of pregnant women was 29 (17-42) years and 57 (48-79) kg respectively, and postpartum women were 30 (18-40) years and 59 (45-73) kg respectively (Table 1). Women on nevirapine regimen were 9 (14.8%) and 52 (85.2%) were on efavirenz regimens. Linear and multiple regression (for co-founders) models, were used to adjusted for associations with time post dose, pregnancy, postpartum and drug concentrations. Genotype frequencies of both pregnant and postpartum women were similar to previously reported genotype and allele frequencies in the region (Table 2).

For the pregnant women, the highest TFV concentration was 553.259 ng/mL, and lowest was 35.520ng/mL at 12 and 14.5 h post dose respectively. Pregnancy was found to decrease plasma concentration of TFV by a factor of log10 0.13 (log10 β= -0.131 (-0.228, -0.034), (P= 0.009) between 4-18 h post dose. Over 50% had TFV concentration greater than IC50 (2.3 μM or 10ng/ml) at 18 h post dose and there was no significant association of drug concentration with any SNP. The homozygous genotype ABCC2 rs2273897 TT, a variant allele in the population was associated with a significant increase concentration of FTC by a factor of log10 0.766 in both pregnant and postpartum women irrespective of time post dose (β = log 10 0.766 (0.084, 1.448) (P= 0.028). There was no association of FTC concentrations with other SNPs.

**Table 1: Demographic and genetic characteristics of study population**

|  |  |  |
| --- | --- | --- |
| Characteristic | Pregnancy | Postpartum |
| Number (%) of women | 31 (50.8%) | 30 (49.2%) |
| Median (Range)* Age (years)
* Weight (Kg)
 | 29 (17-42)57 (48-79) | 30 (18-40)59 (45-73) |
| Median (Range) CD4 Count ( cells/mm3)* Baseline CD4 count
* Last CD4 Count
 | 234 (9-533)443 (108-1206) | 336 (73-898)574 (96-1290) |
| **Median (Range) Time Post Dose (Hrs)** | 12 (7-18.5) | 14 (4-16) |
| Drug Regimen * TDF/FTC/EFV
* TDF/FTC/NEV
 | 24 (77.4%)7 (22.6%) | 28 (93.3%)2 (6.7%) |
| Median (range) Duration on regimen (months) | 27 (1-48) | 13 (2-36) |
| Genotype Frequencies |
| *ABCC2* 12:g.154962860T>C (rs2273897)CCCTTTMAF*ABCC4* 11:g.95021537A>C (rs3742106)AAACCCMAF*ABCC4* 11:g.95020696A>G (rs1059751)AAAGGGMAF*ABCC2* 12:g.32293730T>C (rs3749966)CCCTTTMAF*ABCC4* 13: 95062722T>C (rs1751034)CCCTTTMAF | 0.330.540.130.350.500.380.130.250.540.460.000.290.080.580.330.380.040.500.460.27 | 0.320.450.230.350.550.360.090.250.590.320.090.290.140.590.270.380.000.590.410.27 |

**Figure 2: Tenofovir and Emtricitabine Mean (Standard Deviation) concentrations compared between genotypes in pregnant and postpartum women**

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**Table 2: Univariate and multivariate analysis of pregnant and breastfeeding women combined**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| TDF | Univariate analysis | Multivariate analysis |  |  |
| Log10 | **P-value** | **β(95%CI)** | **P-value** | **β(95%CI)** | **% Effect** |  |  |
| Age (years) | 0.420 | 0.004 (-0.007, 0.015) |   |   |  |  |  |
| Drug regimen | 0.215 | 0.159 (-0.096, 0.414) |   |   |  |  |  |
| Time Post dose | 0.047 | 0.028 (0.001, 0.055) | 0.027 | 0.017 (0.002, 0.032) | 104.0 |  |  |
| Pregnant | 0.005 | -0.154(-0.259, -0.049) | 0.009 |  -0.131 (-0.228, -0.034) | 26.0 |  |  |
| Postpartum | 0.005 | 0.154 (0.049, 0.259) | 0.009 | 0.131 (0.034, 0.228) |  |  |  |
| Genotype  |   |   |   |   |  |  |  |
| *ABCC2* rs2273897 TT | 0.106 | 0.141(-0.031, 0.312) |   |   |  |  |  |
| *ABCC2* rs2273897 CC | 0.703 | 0.021 (-0.089, 0.131) |   |   |  |  |  |
| *ABCC2* rs3749966 TT | 0.431 | 0.046 (-0.071, 0.163) |   |   |  |  |  |
| *ABCC2* rs3749966 CC | 0.854 | -0.018 (-0.210, 0.175) |   |   |  |  |  |
| *ABCC4* rs3742106 AC | 0.166 | 0.082(-0.035,0.199) |   |   |  |  |  |
| *ABCC4* rs3742106 CC | 0.863 | -0.018 (-0.224, 0.188) |   |   |  |  |  |
| *ABCC4* rs1059751 | 0.119 | 0.083(-0.022, 0.189) |   |   |  |  |  |
| *ABCC4* rs1751034 | 0.678 | -0.021(-0.123,0.081) |   |   |  |  |  |
|   |   |   |   |   |  |  |  |
| FTC | **Univariate analysis** | **Multivariate analysis** |  |  |  |
| Log10 | **P-value** | **β(95%CI)** | **P-value** | **β(95%CI)** |  |  |  |
| Age (years) | 0.215 | 0.035 (-0.021,0.091) |   |   |  |  |  |
| Drug regimen | 0.974 | 0.022 (-1.287,1.330) |   |   |  |  |  |
| Time Post dose | 0.941 | -0.066(-1.845,1.712) |   |   |  |  |  |
| Pregnant | 0.820 | 0.061(-0.479, 0.602) |   |   |  |  |  |
| postpartum | 0.820 | -0.061(-0.602, 0.479) |   |   |  |  |  |
| Genotype  |   |   |   |   |  |  |  |
| *ABCC2* rs2273897 TT | 0.112 | 0.724 (-0.175,1.622) | 0.028 | 0.766(0.084,1.448) | 583.4 |  |  |
| *ABCC2* rs2273897 CT | 0.527 | 0.179 (-0.387, 0.746) |   |   |  |  |  |
| *ABCC2* rs3749966 TT | 0.665 | -0.130 (-0.728, 0.469) |   |   |  |  |  |
| *ABCC2* rs3749966 CC | 0.570 | 0.280 (-0.708, 1.268) |   |   |  |  |  |
| *ABCC4* rs3742106 AC | 0.520 | 0.194 (-0.407,0.746) |   |   |  |  |  |
| *ABCC4* rs3742106 CC | 0.919 | 0.054 (-1.006, 1.113) |  |  |  |  |  |
| *ABCC4* rs1059751 | 0.791 | -0.072 (-0.615,0.471) |   |   |  |  |  |
| *ABCC4* rs1751034 | 0.561 | 0.153 (-0.372,0.677) |   |   |  |  |  |

**Discussion**

This study report for the first time the link between increase FTC blood concentration and ABCC2 154962860T>C (rs2273897) TT. An estimated 6-fold increase in FTC blood concentration was observed in pregnant and postpartum women with ABCC2 154962860T>C (rs2273897) TT allele compared to women with CT and CC alleles. Other transporters evaluated in this study for their impact on TFV and FTC concentrations during pregnancy or postpartum were ABCC2 rs3749966, ABCC4 rs3749966, ABCC4 rs1059751, ABCC4 rs3742106, and ABCC4 rs1751034. All allele frequencies, genotype frequencies and common allele were consistent with previously reported genes in the region [24]. TFV concentration was 26% lower in pregnant women, consistent with previous reports of PANNA and IMPAACT P1026 studies [22, 23]. This is an important finding in understanding ART exposure in pregnancy, in this patient population since previous report of the pharmacogenetics of pregnancy induced changes of EFV pharmacokinetics and EFV in this cohort, demonstrated significantly lower EFV exposure during pregnancy in CYP2B6 516GG genotype women [13]. Our findings now suggest a potential decrease in both EFV and TFV in pregnant women with CYP2B6 516GG irrespective of the transporter involve in TFV elimination. The significance of this findings are not clear, but optimal drug exposure without adverse side effect is required to ensure viral suppression and adherence to drugs. Good adherence is an important factor of ensuring treatment efficiency and prevention of increasing clades of resistant mutants virus against a wide class of antiretroviral agents This study was performed in a small sample size and this is recognised as a limitation. It aimed at elaborating the research need of further investigating the impact of pharmacogenetics and pregnancy on ARVs (EFV, TDF and FTC or DTG, TDF and FTC combinations) pharmacokinetics in larger population across different ethnic background and regions of the world.

**Conclusion**

In conclusion, pregnancy and the puerperium are periods of considerable physiological changes in women, and the interplay between pregnancy and pharmacogenetics have significant impact on FTC and TFV exposure. To attain the global aims of elimination of mother to child transmission, effort should be made to achieve optimal treatment throughout pregnancy and breastfeeding whilst ensuring safety to both mother and infant.

**Future Perspective**

In view of these findings and limitations of this study, further pharmacogenetic investigation of the ABCC2 rs2273897 and other transporters is required in larger population and multiple regions. Proposed intensified ART in sub-Saharan Africa and other low and middle income countries with TDF/FTC NRTI backbone will increase frequency PLWH on formulation containing TDF and FTC in pregnancy, and long term efficacy of these two important NRTI will need to be protected.

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