1521-009X/47/12/1415–1424$35.00
Drug Metabolism and Disposition

https://doi.org/10.1124/dmd.119.088062
Drug Metab Dispos 47:1415–1424, December 2019



# Pregnancy and *CYP3A5* Genotype Affect Day 7 Plasma Lumefantrine Concentrations

Ritah F. Mutagonda, Omary M.S. Minzi, Siriel N. Massawe, Muhammad Asghar, Anna Färnert, Appolinary A.R. Kamuhabwa, and Eleni Aklillu

*Department of Clinical Pharmacy and Pharmacology, School of Pharmacy (R.F.M., O.O.M.S.M., A.A.R.K.), and Department of Obstetrics and Gynecology, School of Medicine (S.N.M.), Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania; Division of Infectious Diseases, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden (M.A., A.F.); Department of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden (A.F.); and Division of Clinical Pharmacology, Department of Laboratory Medicine, Karolinska Institutet at Karolinska University Hospital, Huddinge, Stockholm, Sweden (E.A.)*

Received May 21, 2019; accepted September 27, 2019

## ABSTRACT

**Pregnancy and pharmacogenetics variation alter drug disposition and treatment outcome. The objective of this study was to investigate the effect of pregnancy and pharmacogenetics variation on day 7 lumefantrine (LF) plasma concentration and therapeutic responses in malaria-infected women treated with artemether-lumefantrine (ALu) in Tanzania. A total of 277 (205 pregnant and 72 nonpregnant) women with uncomplicated *Plasmodium falciparum* malaria were enrolled. Patients were treated with ALu and followed up for 28 days. *CYP3A4*, *CYP3A5*, and *ABCB1* genotyping were done. Day 7 plasma LF concentration and the polymerase chain reaction (PCR) – corrected adequate clinical and parasitological response (ACPR) at day 28 were determined. The mean day 7 plasma LF concentrations were significantly lower in pregnant women than nonpregnant women [geometric mean ratio = 1.40; 95% confidence interval (CI) of geometric mean ratio (1.119–1.1745), $P < 0.003$]. Pregnancy, low body weight, and *CYP3A5*1/*1* genotype were significantly associated with low day 7 LF plasma concentration ($P < 0.01$). PCR-corrected ACPR was 93% (95% CI = 89.4–96.6) in pregnant women and 95.7% (95% CI = 90.7–100) in nonpregnant women. Patients with lower day 7 LF concentration had a high risk of treatment failure (mean 652 vs. 232 ng/ml, $P < 0.001$). In conclusion, pregnancy, low body weight, and *CYP3A5*1* allele are significant predictors of low day 7 LF plasma exposure. In turn, lower day 7 LF concentration is associated with a higher risk of recrudescence.**

## SIGNIFICANCE STATEMENT

**This study reports a number of factors contributing to the lower day 7 lumefantrine (LF) concentration in women, which includes pregnancy, body weight, and *CYP3A5*1/*1* genotype. It also shows that day 7 LF concentration is a main predictor of malaria treatment. These findings highlight the need to look into artemether-LF dosage adjustment in pregnant women so as to be able to maintain adequate drug concentration, which is required to reduce treatment failure rates in pregnant women.**

## Introduction

Artemether-lumefantrine (ALu) has been the first-line drug for the treatment of uncomplicated malaria in Tanzania since the end of 2006. It is also the antimalarial drug of choice for the treatment of uncomplicated malaria in pregnant women in the second and third trimesters (http://www.who.int/selection_medicines/country_lists/Tanzania_STG_052013.pdf). Day 7 lumefantrine (LF) concentration has been shown to be a potent predictor of the treatment outcome in different study populations and correlates with the area under the concentration time curve (White et al., 2008; WorldWide Antimalarial Resistance Network (WWARN) Lumefantrine PK/PD Study Group, 2015). To be able to achieve adequate clinical and parasitological response (ACPR) during the course of ALu treatment, adequate LF plasma concentration after complete elimination of artemether and dihyroartemisinin needs to be achieved and maintained long enough. Sufficient LF plasma concentration is essential for the drug to be able to clear all residual malaria parasites and reduce the risk of the treatment failure and drug resistance among patients.

Lower LF plasma exposure has been described in young children (WorldWide Antimalarial Resistance Network (WWARN) Lumefantrine PK/PD Study Group, 2015; Kloprogge et al., 2018), pregnant women (Kloprogge et al., 2013, 2018; Mosha et al., 2014; Mutagonda et al., 2016), smokers (Tarning et al., 2009), or when ALu is taken unsupervised (WorldWide Antimalarial Resistance Network (WWARN) Lumefantrine PK/PD Study Group, 2015), without fat meal (Borrmann et al., 2010; Jain et al., 2017) or with concurrent use of efavirenz (Byakika-Kibwika et al., 2012; Huang et al., 2012; Maganda et al., 2015; Banda et al., 2018) and rifampicin (Lamorde et al., 2013; Olafuyi et al., 2017).

This work was supported by grants from the Swedish International Development Cooperation Agency Programme under Muhimbili University of Health and Allied Sciences Reproductive health projects.

The authors declare that they have no competing interests.

https://doi.org/10.1124/dmd.119.088062.

**ABBREVIATIONS:** ACPR, adequate clinical and parasitological response; ALu, artemether-LF; ART, Antiretroviral therapy; CI, confidence interval; ITT, intention-to-treat; LF, lumefantrine; LLOQ, lower limit of quantification; MUHAS, Muhimbili University of Health and Allied Sciences; PCR, polymerase chain reaction.

Physiologic, immunologic, and hormonal changes during pregnancy not only increase the risk of acquiring malaria but also alter the pharmacokinetic profile of drugs used for treatment of malaria (Rogerson et al., 2007). The metabolism of drugs catalyzed by *CYP3A4* and *CYP3A5* is increased during pregnancy, and these enzymes metabolize both artemether and LF (Anderson, 2005; Isoherranen and Thummel, 2013). LF is extensively metabolized into desbutyl-benflumetol in the liver mainly by *CYP3A4* enzymes (Staehli-Hodel et al., 2013) and eliminated through the bile via p-glycoprotein, a major biliary efflux pump encoded by *ABCB1* (Oga et al., 2012; Wahajuddin et al., 2014). Due to the considerable sequence similarity and overlapping substrate specificity with *CYP3A5*, it is difficult to determine the independent effect of *CYP3A4* variability on the safety and efficacy of CYP3A-mediated drug metabolism, particularly in black population where *CYP3A5* enzyme is primarily expressed (Williams et al., 2002; Klein and Zanger, 2013). *CYP3A5* expression level and enzymatic activity display significant variation between populations due to genetic polymorphisms. Most whites are non-*CYP3A5* expressors because of the high frequency of *CYP3A5*3* allele in this population. In contrast, about 70% of the black African population are *CYP3A5* expressers, as they carry one or two *CYP3A5*1* alleles. *CYP3A5* genetic variation may therefore contribute significantly to the variation in metabolism of CYP3A substrates in black Africans.

Because of polymorphic expression and wide interethnic variations in variant allele frequency distributions, *CYP3A5* genotype may be the most important contributor to interindividual and interethnic differences in CYP3A-dependent drug disposition (Mukonzo et al., 2010). Indeed, previous studies in different populations reported significant influence of *CYP3A5* genotype for variability in metabolism of quinine (Mukonzo et al., 2010), a known *CYP3A4* probe drug, and 4$\beta$-hydroxycholesterol (Diczfalusy et al., 2008; Gebeyehu et al., 2011), an endogenous marker for *CYP3A4* activity.

Recently, few studies highlighted relevance of pharmacogenetics variations in determining patient variability in plasma LF exposure (Maganda et al., 2016; Mutagonda et al., 2017; Vos et al., 2017). In a small sample size study, it was reported that pregnant women who were carriers of functional *CYP3A5* and *CYP3A4*1B* allele had lower day 7 LF concentrations and recrudescence, respectively, compared with the carriers of defective alleles (Mutagonda et al., 2017). However, a study in nonpregnant population did not yield significant associations between LF pharmacokinetic parameters with *CYP3A* and *ABCB1* single-nucleotide polymorphisms (Staehli-Hodel et al., 2013).

Increased *CYP3A* enzyme activity during pregnancy due to induction (Jeong, 2010) may alter LF disposition in a significant manner. Previous studies reported the relevance of genetic variation on *CYP3A* enzyme induction (Habtewold et al., 2013; Ngaimisi et al., 2014). This raises the hypothesis that genetic variations in drug-metabolizing enzymes and transporters might significantly contribute to variability in day 7 LF concentrations and treatment outcome in pregnant and nonpregnant women. Therefore, the aim of this study was to examine the effects of pregnancy and role of genetic variations in *CYP3A4*, *CYP3A5*, and *ABCB1* on day 7 LF concentration and malaria therapeutic response.

## Materials and Methods

**Study Design, Population, and Procedures.** This was an observational prospective cohort study conducted at Kisarawe, Kibiti, Mkuranga, and Rufiji districts located in the Coast Region in Eastern Tanzania. The reported prevalence of malaria in pregnancy during the study period was 8.1% and *Plasmodium falciparum* was the predominant species (Mutagonda et al., 2016). The study was carried out at Kisarawe, Mkuranga, and Utete district hospitals and at Mohoro and Kibiti health centers from May 2014 to August 2017. This study was approved by the Muhimbili University of Health and Allied Sciences (MUHAS) and National Institute for Medical Research ethical committees. All women consented to the study before enrollment. Women's identification number was used throughout the data collection and analysis period so as to ensure confidentiality. All patients' information was filled in the confidential case report forms.

Pregnant and nonpregnant women diagnosed with uncomplicated *P. falciparum* malaria infection detected by microscopy were recruited and enrolled from the Reproductive and Child Health clinic and outpatient department. Inclusion criteria were women aged ≥18 years (married women <18 years were also included), resident of study areas, and with hemoglobin level of ≥8 g/dl. Exclusion criteria were pregnant women in the first trimester, allergic to ALu, unable to take oral medication, or vomited the medication within 1 hour of taking the dose, and reported intake of any antimalarial drug within the past 28 days. Also, patients with a history of renal, liver, or heart problems, or with severe malaria were excluded.

**Sample Size.** The calculation of sample size for this study used the classic statistical tool recommended by the Technical Expert Group on Malaria Chemotherapy to calculate the sample size (https://apps.who.int/iris/bitstream/handle/10665/44048/9789241597531_eng.pdf;jsessioni). The sample size was determined based on an expected proportion of treatment failures in both groups (pregnant and nonpregnant women), 95% confidence level, and 5% precision. The estimated population proportion of clinical failures in pregnant women was 18% (Mosha et al., 2014) at confidence level of 95% and precision of 5%. Therefore, a minimum sample size of 196 pregnant women would be needed for this study. For nonpregnant women, *P* was 5% (Mosha et al., 2014) at confidence level of 95% and precision of 5%, thus requiring 73 nonpregnant women as a minimum sample size for this study. Based on World Health Organization recommendations, in order for the efficacy study to be representative, a minimum sample of 50 patients is required, regardless of the rates of failure (https://apps.who.int/iris/bitstream/handle/10665/44048/9789241597531_eng.pdf;jsessioni). In this study, we recruited 205 pregnant and 72 nonpregnant women, hence sufficient to describe the efficacy of LF in the population.

**Treatment, Clinical, and Laboratory Procedures, and Patient Follow-Up.** All women received six doses of four tablets of ALu (Coartem; Novartis Pharma AG, Basel, Switzerland) (20 mg artemether and 120 mg LF) over the course of 3 days at 0, 8, 24, 36, 48, and 60 hours. For each patient, general, physical, and clinical examinations, including axillary temperature measurements and evaluation of malaria-related symptoms, were performed at enrollment and on the follow-up visits on days 2, 7, 14, 21, and 28. Full medical history, including current illnesses and medications used, was recorded. Adherence to ALu intake was assessed using a self-administered questioner and by pill counting on day 2 visit. Pregnant women gestational age was determined from the estimated first day of the last normal menstrual period and compared with clinical examination of a fundal height.

For pharmacogenetics analysis, 1 ml whole blood was taken into an EDTA-containing vacutainer tube and stored at −80°C at MUHAS laboratory. Also, approximately 50 $\mu$l blood was collected at enrollment (day 0) and on follow-up days (day 7, 14, 21, and 28) on a filter paper (Whatman grade 3) for screening and genotyping of malaria parasite using polymerase chain reaction (PCR). These filter papers were air dried and stored separately in a plastic bag and kept frozen at −80°C. For pharmacokinetics, 3 ml venous blood was collected in heparinized tubes at the enrollment day and on day 7 (corresponding to 168 hours) following initiation of ALu treatment to be able to determine plasma LF concentrations. The whole blood was centrifuged at 2000*g* for 5 minutes. Plasma obtained after centrifugation was stored in cryo tubes at −80°C until analysis.

***CYP3A4*, *CYP3A5*, and *ABCB1* Genotyping.** In the pharmacogenetics analysis, genomic DNA was isolated in whole-blood samples using QIAamp DNA Midi Kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions. Genotyping for the common functional variant alleles for *ABCB1 c.3435C>T* and *ABCB1 c.4036A>G (rs3842)*, *CYP3A4*1B*, *CYP3A5*3*, *CYP3A5*6*, and *CYP3A5*7*, which have been reported to be relevant for LF disposition (German and Aweeka, 2008; Djimdé and Lefèvre, 2009), was carried out at the Division of Clinical Pharmacology, Department of Laboratory Medicine, Karolinska Institutet, Sweden, as previously described (Mutagonda et al., 2017). The Quant Studio 12 K Flex Real-Time PCR system (Life Technologies Holding, Singapore) was used for genotyping.

**Parasite Screening and Genotyping.** Dried blood spots on filter papers were punched, and three punched-out circles 3 mm in diameter were used for DNA extraction using QIAamp DNA blood micro kit (Qiagen GmbH) following the

TABLE 1

Characteristics of study participants with *P. falciparum* malaria ($N = 277$)

| Characteristics | Pregnant Women ($n = 205$) Median (Range) | Nonpregnant Women ($n = 72$) Median (Range) | *P* Value (Mann–Whitney *U* Test) |
|---|---|---|---|
| Age (years) | 22 (15–42) | 25 (16–53) | 0.004 |
| Body weight (kg) | 56 (38–82) | 60 (39–95) | 0.05 |
| Hemoglobin (g/dl) | 10 (8–14) | 12 (8–14) | 0.001 |
| Gravida | 2 (1–8) | NA | |
| Gestation age (weeks) | 20 (14–37) | NA | |
| Pregnancy trimesters | | NA | |
| Second (%) | 176 (85%) | | |
| Third (%) | 31 (15%) | | |
| Baseline parasitemia (counts/$\mu$l) | 2000 (400–54,688) | 2600 (400–20,000) | 0.02 |

manufacturer's recommendations. Screening of *Plasmodium* parasites was performed using a species-specific PCR targeting the ssRNA gene, as previously described (Shokoples et al., 2009; Mutagonda et al., 2017). *P. falciparum* genotyping was performed by nested PCR using fluorescent primers in the nested allelic specific reaction, followed by capillary electrophoresis in a DNA sequencer for fragment sizing, as previously described (Liljander et al., 2009; Mutagonda et al., 2017).

**Quantification of LF Plasma Concentrations.** The collected plasma was analyzed using a validated high performance liquid chromatography method with UV detection (Minzi et al., 2012). Analysis was done at the Sida/MUHAS Bio-Analytical Laboratory (Dar-es-Salaam, Tanzania). The isocratic high performance liquid chromatography system consisted of a UV/VIS detector (SPD-20AV; Shimadzu, Kyoto, Japan), auto sampler (SIL-20A; Shimadzu), and a pump (LC-20AT; Shimadzu). The column RP18, 5 $\mu$m; 125 × 4 mm (LiChrospher 100) was used. The lower limit of quantification (LLOQ) was 50 ng/ml. The percentage of CV during analysis for LF was <10%.

**Data Analysis.** $\chi^2$ test was used to compare the observed and expected genotype frequencies, according to the Hardy–Weinberg equilibrium. Patients with LF concentrations > LLOQ before treatment were excluded from this analysis, and the predose concentration was set to 0 if < LLOQ. LF plasma concentration data were log 10 transformed, and data were expressed as mean with 95% CI. Independent *t* tests were done to compare log day 7 LF between the pregnant and nonpregnant women. Univariate linear regression analyses were used to identify the individual effect of independent covariates on log-transformed day 7 LF concentration, followed by a stepwise multivariable regression analysis. The final model consisted of variables with *P* values <0.05. Comparison of mean log day 7 plasma LF concentration based on the number of functional *CYP3A5*1* variant alleles in all study participants and stratified by pregnancy status was analyzed using ANOVA.

Factors influencing malaria treatment outcome were evaluated using Cox regression analysis. Comparisons of treatment outcomes both PCR uncorrected and corrected ACPR and reinfection were performed using two analysis methods,

TABLE 2

Genotype and variant allele frequency distribution among pregnant and nonpregnant women with uncomplicated *P. falciparum* infection

| Genotype | Pregnant Women Frequency *N* (%) | Nonpregnant Women Frequency *N* (%) | *P* Value |
|---|---|---|---|
| *CYP3A4*1B (- 392A>G)* | | | 0.809 |
| **1/*1* | 19 (9.4%) | 5 (6.9%) | |
| **1/*1B* | 80 (39.6%) | 30 (41.7%) | |
| **1B/*1B* | 103 (51.0%) | 37 (51.4%) | |
| *CYP3A5*3 c.6986A>G* | | | 0.881 |
| **1/*1* | 118 (59.3%) | 43 (62.3%) | |
| **1/*3* | 75 (37.7%) | 24 (34.8%) | |
| **3/*3* | 6 (3.0%) | 2 (2.9%) | |
| *CYP3A5*6 c.14690G>A* | | | 0.480 |
| **1/*1* | 145 (71.4%) | 51 (71.8%) | |
| **1/*6* | 49 (23.9%) | 19 (26.8%) | |
| **6/*6* | 9 (4.4%) | 1 (1.4%) | |
| *CYP3A5*7* | | | 0.781 |
| *27131_27132insT* | | | |
| **1/*1* | 156 (77.6%) | 53 (73.6%) | |
| **1/*7* | 43 (21.4%) | 18 (25.0%) | |
| **7/*7* | 2 (1.0%) | 1 (1.4%) | |
| *ABCB c.3435* | | | 0.672 |
| *CC* | 139 (72.0%) | 53 (74.6%) | |
| *CT* | 51 (26.4%) | 16 (22.5%) | |
| *TT* | 3 (1.6%) | 2 (2.8%) | |
| *ABCB rs3842* | | | 0.269 |
| *AA* | 116 (57.1%) | 39 (54.2%) | |
| *AG* | 70 (34.5%) | 31 (43.1%) | |
| *GG* | 17 (8.4%) | 2 (2.8%) | |
| Allele | Minor Allele | Pregnant Women | Nonpregnant Women |
| *CYP3A4*1B (- 392A>G)* | *1B | 70.8 | 72.2 |
| *CYP3A5*3 c.6986A>G* | *3 | 21.9 | 20.3 |
| *CYP3A5*6 c.14690G>A* | *6 | 16.4 | 14.8 |
| *CYP3A5*7 27131_27132insT* | *7 | 13 | 13.9 |
| *ABCB c. 3435* | T | 14.8 | 14.1 |
| *ABCB rs3842* | G | 25.7 | 24.4 |

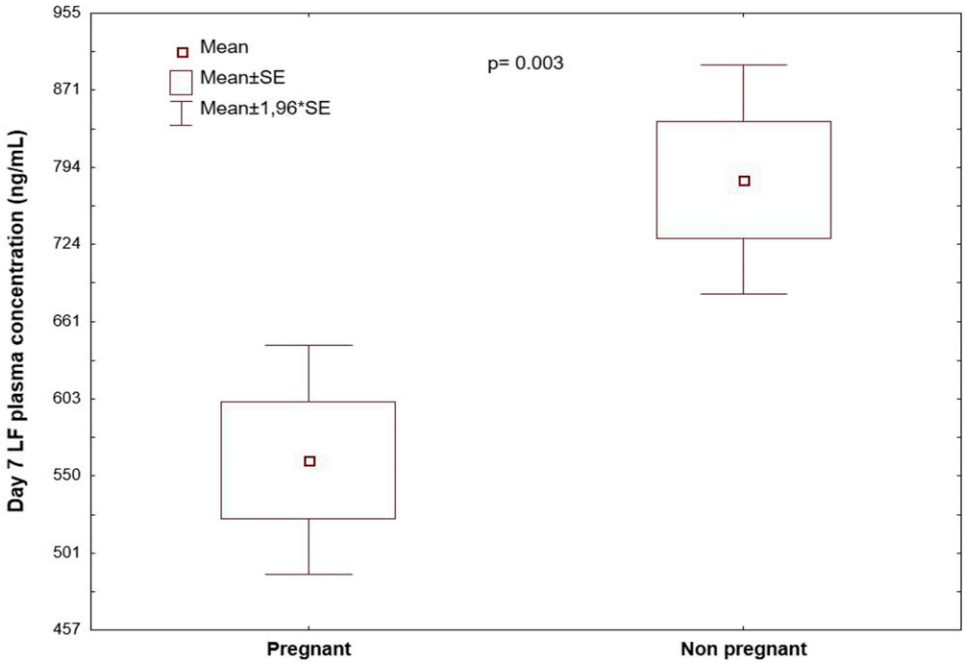


**Fig. 1.** Comparison of day 7 LF plasma concentration between pregnant and nonpregnant women with uncomplicated *Plasmodium falciparum* infection.

as follows: Kaplan–Meier analysis whereby the log–rank test for equality of survivor functions was used for comparison of survival curves and simple comparison of proportions whereby $\chi 2$ or Fisher exact test was used for categorical variables (such as pregnancy status and treatment outcome). Cox hazards regression was used to determine the relationship between treatment outcome and day 7 LF concentrations, pharmacogenetics, and other study variables.

Statistical analyses were performed using SPSS software, version 22.0 (IBM, Somers, NY). *P* values $<0.05$ were considered to be statistically significant.

## Results

**Baseline Characteristics of Study Participants.** A total of 277 women was enrolled in the study, including 205 pregnant and 72 nonpregnant women. Majority of pregnant women (85%) were in the second trimester, with the remainder being in the third trimester. Baseline data showed significant difference between the two groups. The median age, Hb levels, and baseline parasitemia were higher among nonpregnant compared with pregnant

TABLE 3

Factors affecting log day 7 LF plasma concentration

| Variable | *n* | Univariate Analysis | | Multivariable Analysis | |
|---|---|---|---|---|---|
| | | Beta Coefficient (95% CI) | *P* Value | Beta Coefficient (95% CI) | *P* Value |
| Pregnancy | 209 | −14.5 (4.9–24.2) | 0.003 | −27.1 (5.0–49.3) | 0.009 |
| Age (years) | 209 | 0.3 (−0.2 to 0.9) | 0.237 | | |
| Weight (kg) | 208 | 0.8 (0.3–1.2) | 0.002 | 1.8 (0.7–2.8) | 0.002 |
| Hemoglobin (g/dl) | 208 | 3.2 (0.2–6.2) | 0.036 | NS | |
| Trimester | 142 | 19.1 (2.8–35.5) | 0.022 | NS | |
| Gravida | 142 | 7.2 (−19.6 to 5.2) | 0.253 | | |
| Parasitemia (counts/$\mu$l) | | | | | |
| <1000 | 40 | 4.0 (−7.4 to 15.4) | 0.484 | | |
| 1000–10,000 | 158 | 3.9 (−17 to 24.8) | 0.710 | | |
| >10,000 | 11 | Reference | | | |
| *CYP3A4*1B* | | | 0.046 | NS | |
| *CYP3A4*1/*1* | 21 | −9.2 (1.4–17) | 0.022 | | |
| *CYP3A4*1/*1B* | 83 | 1.5 (−8.1 to 11.1) | 0.759 | | |
| *CYP3A4*1B/*1B* | 104 | Reference | | | |
| Number of *CYP3A5*1* allele | | | | | 0.002 |
| Zero | 50 | 9.0 (2.5–15.5) | 0.007 | 15.5 (6.3–24.8) | 0.002 |
| One | 95 | 10.1 (−0.4 to 20.5) | 0.059 | 6.6 (−18.1 to 31.2) | 0.591 |
| Two | 63 | Reference | | Reference | |
| *ABCB rs3842* | | | 0.70 | NS | |
| A/A | 118 | −2.1 (−13.0 to 8.7) | 0.695 | | |
| A/G | 80 | −0.4 (−21.3 to 20.5) | 0.969 | | |
| G/G | 11 | Reference | | | |
| *ABCB c. 3435 C>T* | | | 0.14 | NS | |
| C/C | 143 | −16.5 (−33.4 to 0.4) | 0.056 | | |
| C/T | 54 | −29.8 (−63.1 to 3.4) | 0.078 | | |
| T/T | 4 | Reference | | | |

NS, Nonsignificant variables.

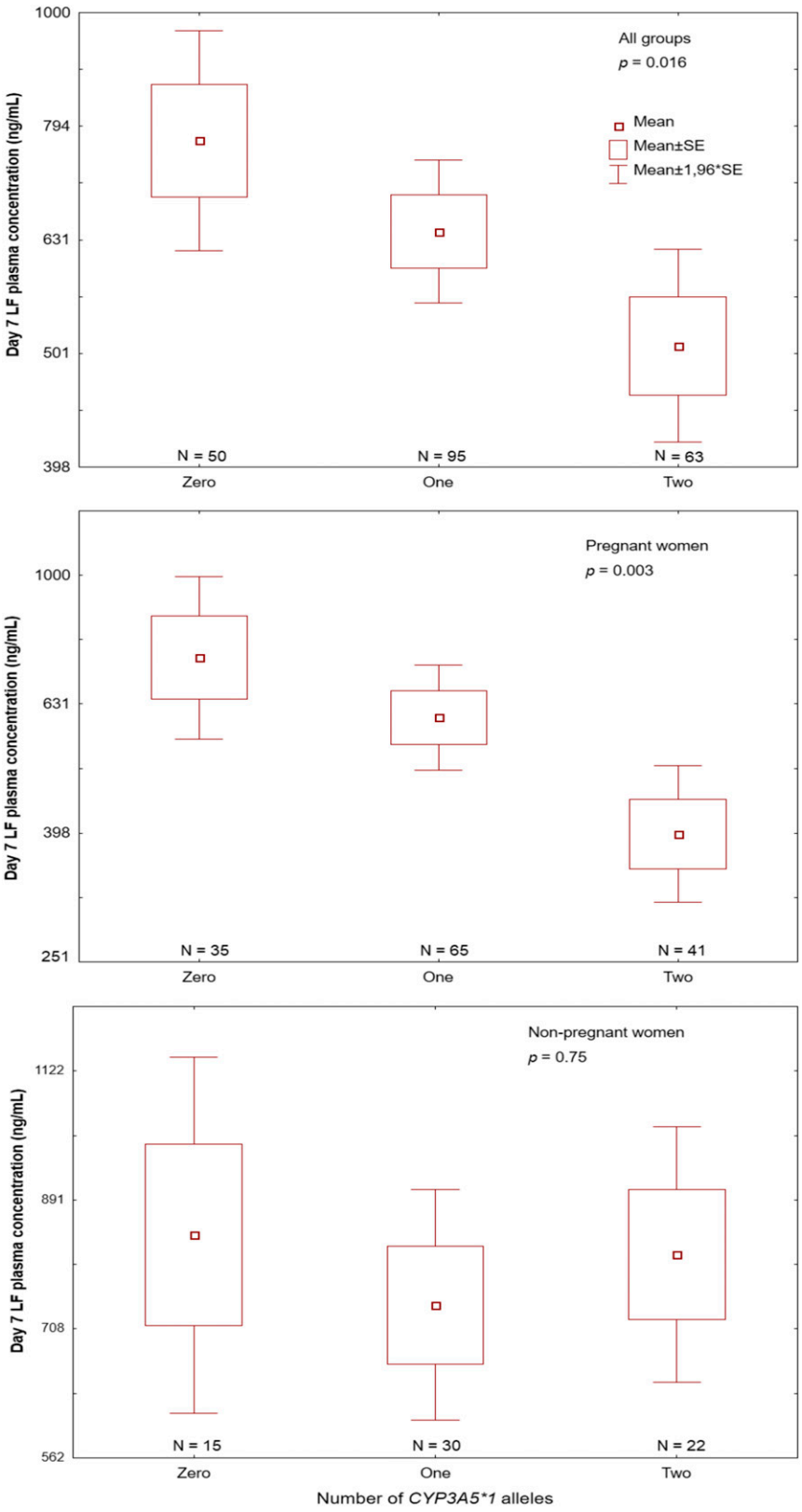


**Fig. 2.** Comparison of mean day 7 LF plasma concentration based on the number of functional *CYP3A5*1* alleles in all study participants and stratified by pregnancy status. Zero = homozygous for **3*, **6*, or **7*; one = heterozygous for **3*, **6*, or **7*; two = homozygous *CYP3A5*1/*1* genotype. Vertical bars denote 95% CIs of the mean.

women. Baseline characteristics of study participants are shown in Table 1.

Genotype distribution for *CYP3A4*, *CYP3A5*, and *ABCB1* was similar among pregnant and nonpregnant women, as presented in Table 2. There were no significant differences in the distribution of genotype or allele frequencies between the two groups. There was no significant deviation between the observed and expected genotype frequencies from Hardy–Weinberg equilibrium.

TABLE 4

Kaplan–Meier estimates of treatment outcome by pregnancy status (*P* values were calculated with log–rank test)

| Treatment Outcome | Pregnant | | Nonpregnant | | *P* Value |
|---|---|---|---|---|---|
| | *n*/*N* | Kaplan–Meier Estimate (95% CI) | *n*/*N* | Kaplan–Meier Estimate (95% CI) | |
| PCR-corrected ACPR | | | | | |
| ITT | 185/199 | 93.0% (89.4–96.6) | 66/69 | 95.7% (90.7–100) | $P = 0.443$ |
| PP | 126/140 | 90.0% (85–95) | 45/48 | 93.8% (86.7–100) | $P = 0.442$ |
| PCR-uncorrected ACPR | | | | | |
| ITT | 181/204 | 88.7% (88.4–93.1) | 64/69 | 92.8% (85.1–98.2) | $P = 0.363$ |
| PP | 128/151 | 84.8% (79–90.6) | 46/51 | 90.2% (79.9–97.5) | $P = 0.434$ |
| New infection | 15/199 | 7.6% (3.9–11.4) | 5/69 | 7.2% (0.9–13.5) | $P = 0.934$ |

PP, per protocol.

**Determinants of Day 7 LF Concentration.** Eighty-six (46.5%) pregnant women and 20 (29.9%) nonpregnant women had LF day 7 concentration below 600 ng/ml ($P = 0.023$). The geometric mean of day 7 plasma LF concentrations were 560.1 [95% confidence interval (CI) (487.8–643.3)] ng/ml in pregnant women and 782.7 [95% CI (680.9–899.9)] ng/ml in nonpregnant women ($P = 0.003$). The geometric mean ratio = 1.40; 95% CI of geometric mean ratio (1.119–1.1745). A comparison of the mean ± S.E. of log day 7 LF plasma concentrations between pregnant and nonpregnant women using independent *t* test is presented in Fig. 1.

In a univariate analysis, several factors, including pregnancy, body weight, hemoglobin concentration, trimester, and *CYP3A4*1B* and *CYP3A5* genotype (number of *CYP3A5*1* functional allele), were independent significant predictors of day 7 LF plasma concentration (Table 3). *CYP3A4*1/*1* and *CYP3A5*1/*1* genotype had significantly lower log day 7 plasma LF concentration than *CYP3A4*1B/*1B* and homozygous mutant *CYP3A5* genotype, respectively.

Carriers of both homozygous and heterozygous functional *ABCB1 c.3435C>T* alleles had lower LF concentration compared with the homozygous mutant carriers, but the results were not statistically significant ($P > 0.05$). Comparison of mean log day 7 plasma LF concentration based on the number of functional *CYP3A5*1* variant alleles in all study participants and stratified by pregnancy status is presented in Fig. 2 using ANOVA. In a multivariable regression analysis and using backward stepwise elimination, only pregnancy, body weight, and *CYP3A5* genotype were retained in the model as a significant predictor of day 7 LF plasma concentration (Table 3).

**Malaria Treatment Outcome.** Malaria treatment outcomes were defined following the World Health Organization protocol (https://apps.who.int/iris/bitstream/handle/10665/44048/9789241597531_eng.pdf;jsessioni) as ACPR, new infection, and treatment failure, designated as early treatment failure, late clinical failure, or late parasitological failure. Analyses of treatment response were performed using both intention-to-treat (ITT) population that included all enrolled patients and per protocol population, including all patients who were part of the ITT and did not deviate from the protocol for other reasons than failure.

PCR-corrected ACPR at day 28 was 93.0% (95% CI = 89–96.6) in pregnant women versus 95.7% (95% CI = 91.5–100) were not statistically different between the two groups ($X^2$ test, $P = 0.572$). The day-28 new infection rate was 7.6% (95% CI, 0.4–11.4%) in pregnant women versus 6.7% (95% CI, 0.9–12.4) in nonpregnant women ($X^2$ test, $P = 0.781$). These results were consistent with those obtained by Kaplan–Meier analysis (Table 4).

**Factors Associated with Treatment Failure.** Day 7 LF concentration was significantly lower among women with therapeutic failure than those with ACPR. The mean LF concentration among women with ACPR as per ITT was 651.6 [95% CI (589.1–720.6)] ng/ml, whereas, for women with late parasitological failure, it was 231.6 [95% CI (136.0–394.5)] ng/ml ($P < 0.001$). The mean day 7 LF concentration among pregnant women with ACPR was 590.3 [95% CI (516.8–647.2)] ng/ml and 231.5 [95% CI (114.3–468.7)] ng/ml for those with treatment failure ($P < 0.0002$). For nonpregnant women, it was 801.5 [95% CI (704.2–912.2)] ng/ml and 232 [95% CI (174.2–309.4)] ng/ml for those with ACPR and treatment failure, respectively ($P < 0.0001$). Comparison of mean log day 7 plasma concentration between patients with PCR-corrected ACPR (success) versus treatment failure stratified by pregnancy status is presented in Fig. 3.

In univariable analysis (Table 5), there was a significant association between risk of recurrent infection and day 7 LF concentration ($P < 0.001$), whereby, using the previous cutoff point of 600 ng/dl (Mutagonda et al., 2016), the risk of recurrence was 5.3 (95% CI) (1.483–19.066) times higher among patients with concentration <600 ng/dl ($P = 0.010$). The risk was also higher in pregnant women (1.6 times than nonpregnant), primigravid (1.5 times than multigravida), and higher among carriers of functional *CYP3A4*1B* (3.2 times) and *ABCB1 c.3435* (1.1 times) alleles than mutants, but the association was not statistically significant ($P > 0.05$). Multivariable analysis could not be conducted due to lack of association of most of the study variable.

## Discussion

In the present study, investigation on the effect of pregnancy, pharmacogenetic variations, and other clinical and sociodemographic factors on day 7 plasma LF concentration and malaria treatment outcome was conducted. The main findings include the following: 1) significantly lower day 7 LF plasma concentration in pregnant compared with nonpregnant women, 2) significant association of *CYP3A5*1/*1* genotype and low body weight with low day 7 LF plasma concentration, and 3) low day 7 LF plasma concentration as a significant predictor of treatment failure. To date, there are limited studies investigating the effect of *CYP3A* and *ABCB1* genotype on LF concentration and malaria treatment outcome (Maganda et al., 2016; Vos et al., 2017), and only one small sample size study reported effect of genotype on LF plasma concentration in pregnant women (Mutagonda et al., 2017). Therefore, the present study described the effect of pregnancy and *CYP3A* genotype on day 7 LF plasma concentration and malaria treatment outcome. To the best of our knowledge, this is the first study to investigate the effect of pregnancy and genotyping on day 7 LF concentration and malaria treatment outcome among pregnant and nonpregnant women living in the same area.

In this study, ALu was effective in both pregnant and nonpregnant populations with the cure rate (PCR-corrected) of >90% (lower in pregnant women than nonpregnant women). The most important determinant of malaria treatment outcome in both pregnant and nonpregnant women was day 7 LF plasma concentration, indicating

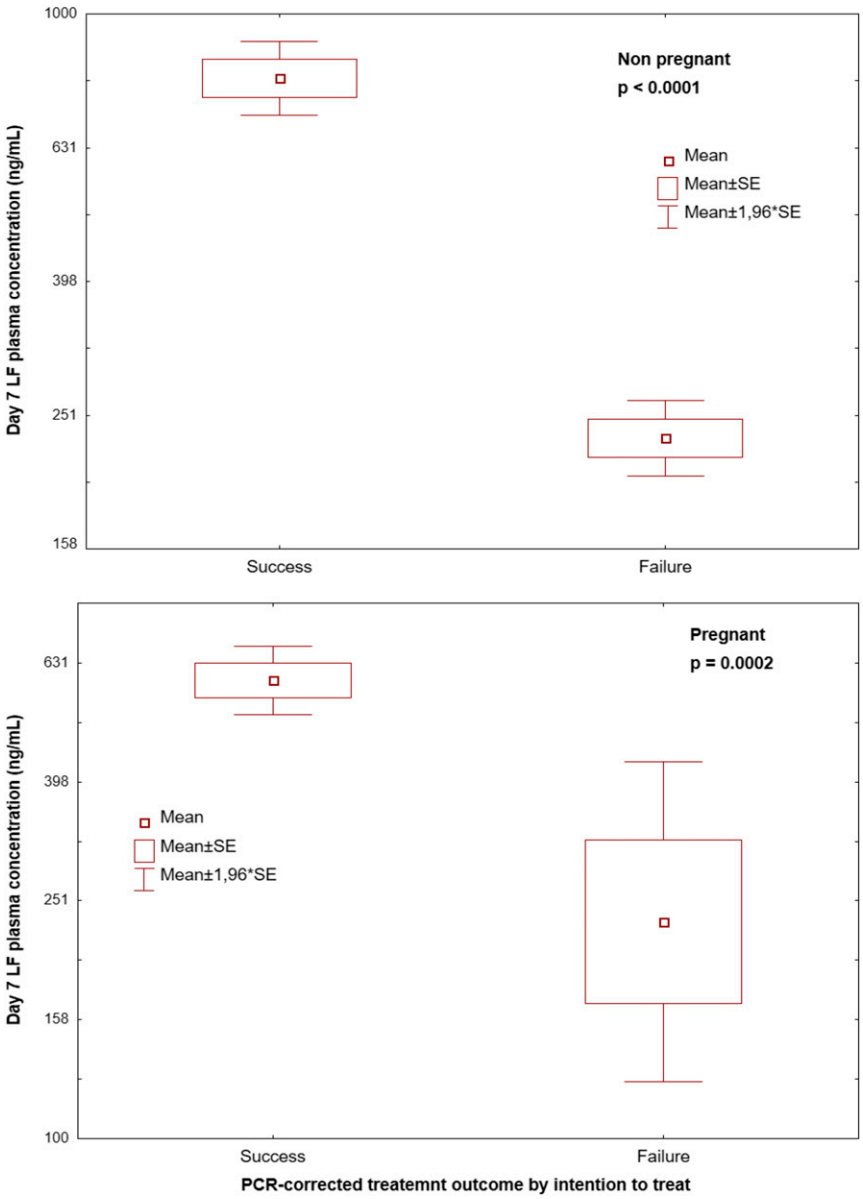


**Fig. 3.** Comparison of day 7 LF concentrations among nonpregnant women (top) and pregnant (below) and with ACPR and women with treatment failure (ITT PCR-corrected) using independent *t* test.

that the concentration of <600 ng/ml is associated with treatment failure. Day 7 LF concentration was lower by 27.1% in pregnant women compared with nonpregnant ones. This is similar to what has been reported previously from Africa (Tanzania, Uganda, and Rwanda) and Thailand (McGready et al., 2006; Tarning et al., 2009, 2013; Kloprogge et al., 2013; Mosha et al., 2014; Lohy Das et al., 2018). Lower drug concentrations in pregnant women contribute to lower cure rates and selection of parasite resistance. To achieve similar LF exposure to nonpregnant women, dosage adjustment has been suggested (Kloprogge et al., 2018).

Pregnancy-related hormones such as estrogen and cortisol induce CYP3A activity, which can result in subtherapeutic plasma concentrations of drugs (Jeong, 2010). One of the determinants of day 7 LF concentration in this study was *CYP3A5* genotype in gene dose-dependent manner (Fig. 2). In a univariate analysis, *CYP3A4*1B* genotype was significantly associated with higher LF plasma concentration, but not in the multivariable analysis. The variant allele frequency of *CYP3A4*1B* is more than 70% in the study population, which is unlikely to account for the day 7 LF concentration observed between different *CYP3A4*1B* genotypes. *CYP3A5* may contribute to the complexity of these unexplained differences, as almost all *CYP3A4* substrates, with a few exceptions, are also metabolized by *CYP3A5* (Desta and Flockhart, 2017). In this study, carriers of homozygous wild-type *CYP3A5*1* allele had 15.5% times higher day 7 LF concentration compared with the homozygous wild type, and the difference was significant. Further analysis showed that pregnant women with at least one mutant *CYP3A5*1* allele had a higher day 7 LF concentration compared with the carriers of homozygous wild-type alleles (Fig. 2). In HIV patients' cohort study, it was observed that patients receiving Efavirenz-based antiretoviral therapy (ART) (CYP3A inducer) had lower LF concentration compared with those receiving nevirapine-based ART and ART naive control group (Maganda et al., 2016). Previous studies showed absence of *CYP3A5*1* influence on LF concentration in nonpregnant population (Maganda et al., 2016; Vos et al., 2017), which could not be ascertained in this study due to a small sample size of nonpregnant women.

Weight of patients is among the important factors in the determination of the doses of ALu to be prescribed. For instance, patients with ≥35 kg

TABLE 5

Cox modeling of factors associated with risk of treatment failure by day 28 in the intent-to-treat population ($N$ = 268, 17 treatment failure)

| Variable | Events/Patients (%) | Hazard Ratio 95% CI | $P$ Value |
|---|---|---|---|
| Day 7 concentration | 14/203 (6.9%) | 0.02 (0.01–0.11) | <0.001 |
| Day 7 LF cutoff | | | 0.010 |
| <600 ng/ml | 11/85 (12.9%) | 5.32 (1.48–19.07) | |
| >600 ng/ml | 3/118 (2.5%) | 1 (Reference) | |
| Pregnancy | | | 0.45 |
| Pregnant | 14/199 (7.0%) | 1.61 (0.46–5.61) | |
| Nonpregnant | 3/69 (4.3%) | 1 (Reference) | |
| Age | 17/268 (6.3%) | 0.97 (0.91–1.04) | 0.37 |
| Hb | 17/268 (6.3%) | 0.91 (0.65–1.26) | 0.59 |
| Trimester | | | |
| Second | 15/170 (8.8%) | 0.04 (0.00–16.54) | 0.29 |
| Third | 0/30 (0%) | — | |
| Gravida | | | 0.46 |
| Primigravida | 7/82 (8.5%) | 1.49 (0.52–4.24) | |
| Multigravida | 7/117 (6.0%) | 1 (Reference) | |
| Baseline parasitaemia | | | |
| <1000 | 3/48 (6.2%) | 0.75 (0.08–7.17) | 0.79 |
| 1000–10,000 | 13/208 (6.2%) | 0.77 (0.10–5.87) | 0.79 |
| >10,000 | 1/11 (8.3%) | 1 (Reference) | |
| *CYP3A4*1B* | | | |
| *CYP3A4*1/*1* | 4/24 (16.7%) | 3.25 (0.95–11.09) | 0.06 |
| *CYP3A4*1/*1B* | 6/106 (5.7%) | 1.08 (0.36–3.20) | 0.89 |
| *CYP3A4*1B/*1B* | 7/135 (5.2%) | 1 (Reference) | |
| Number of *CYP3A5*1* | | | |
| Zero | 2/62 (3.2%) | 0.50 (0.09–2.59) | 0.41 |
| One | 10/122 (8.2%) | 1.28 (0.44–3.74) | 0.65 |
| Two | 5/80 (6.2%) | 1 (Reference) | |
| *ABCB1 rs3842* | | | |
| *A/A* | 8/147 (5.4%) | 0.61 (0.24–1.58) | 0.31 |
| *A/G* | 9/100 (9.0%) | 1 (Reference) | |
| *G/G* | 0/19 (0%) | — | |
| *ABCB c. 3435* | | | |
| *C/C* | 13/188 (6.9) | 1.12 (0.37–3.43) | 0.84 |
| *C/T* | 4/63 (6.3) | 1 (Reference) | |
| *T/T* | 0/4 (0%) | — | |

body weight are given four tablets of ALu (each containing 20 mg artemether and 120 mg LF) at predefined interval (http://www.who.int/selection_medicines/country_lists/Tanzania_STG_052013.pdf). In this study, it was observed that day 7 LF concentration was increased by 1.8% with increase per kg body weight. The effect of patients' weight on day 7 LF plasma concentration and subsequent malaria treatment outcome was reported in a systematic review and meta-analysis using individual patient data showing a substantially higher risk of recrudescent malaria and lower LF concentrations at day 7 with decreasing weight-for-age $z$-scores (WorldWide Antimalarial Resistance Network (WWARN) Lumefantrine PK/PD Study Group, 2015).

LF is a substrate of P-glycoprotein, and the effect of different transporters on LF concentration has been reported. The role of *ABCB1c.3435* (Multi-Drug Resistance - MDR1) in HIV patients receiving Nevirapine-based ART and that of *ABCC2* ( MDR2) in children under <5 years has been described (Maganda et al., 2016; Vos et al., 2017). It has been reported that *ABCB1* is highly expressed during pregnancy as a defense mechanism to protect the fetus from toxicity starting from the first trimester to term (Sun et al., 2006). In the current study, *ABCB1 c.3435* did not show the significant association with day 7 LF concentrations, but lower LF concentration was observed among patients with the functional allele compared with homozygous mutants. The effect of *ABCB1c.3435* would have been described well if the proportion of patients with homozygous mutation would have been higher. Because levels of protein expression decrease with advancing gestational age, it is also likely that inclusion of pregnant women in the first trimester would have given better association of the effect of *ABCB1c.3435* on day 7 LF plasma concentrations (Mathias et al., 2005; Sun et al., 2006). However, for the purpose of this study, we could only recruit pregnant women in the second and third trimesters because ALu was contraindicated to be used during the first trimester of pregnancy at the time of study. There is still limited information on the effect of ABC transporter proteins on plasma LF concentration and malaria treatment outcome. Therefore, we recommend further studies to determine the association between LF concentration and malaria treatment outcome with other ABC drug transporters such as *ABCB11* (bile salt export pump), *ABCG2* (breast cancer resistance protein), and *ABCC* (multidrug resistance protein).

The efficacy of ALu in pregnant compared with nonpregnant women showed the ACPR of greater than 90%, indicating adequate therapeutic efficacy. The efficacy of ALu for treatment of uncomplicated malaria was not significantly different between pregnant and nonpregnant women. In this study, 86 pregnant women had day 7 LF concentration below 600 ng/ml, but only 14 (16.3%) had treatment failure, whereas in nonpregnant women 20 women had concentration below 600 ng/ml, but only three (15%) had treatment failure. Despite the significant differences in day 7 LF concentration between the two study populations, the majority of women with lower day 7 LF concentrations in both groups were still cured. Higher cure rates in malaria-endemic areas have been associated with the immunity that acts in synergy with antimalarial chemotherapy (White, 1997; Rogerson et al., 2010). Also, more than half of enrolled pregnant women were multigravida, which can also explain a nonsignificant difference in treatment outcome between pregnant and nonpregnant women, as shown in Table 4. However, the mean day 7 LF concentration between those who were cured and those with treatment failure was significant, as observed in Fig. 3. Similar

findings were also reported in a meta-analysis examining the pharmacokinetic and pharmacodynamics of LF, in which the main determinant of treatment failure was lower LF exposure (Kloprogge et al., 2018).

There was no early treatment failure or late clinical failure throughout the follow-up period of 28 days, an indication that ALu is still effective for treatment of uncomplicated malaria in pregnant women. There was no significant effect of the study variables such as *CYP3A* enzymes, *ABCB1* transporters, obstetrics, and other patients' demographic characteristics on malaria treatment outcome.

The limitation for this study was a relatively small sample size of nonpregnant women compared with pregnant women. Due to availability of predetermined schedules and sensitization of pregnant women to attend antenatal clinics, it was much easier to recruit and retain them for the entire period of follow-up after treatment. In contrast, nonpregnant women with uncomplicated malaria are not admitted, and they only attend at the outpatient department when feeling unwell, so it was difficult to recruit and retain them for the entire period of follow-up after treatment. Despite differences in sample size in the two groups of women, the findings of this study show the importance of genetic variation in the *CYP3A5* locus for LF pharmacokinetics and treatment outcome in pregnant women and nonpregnant women.

In conclusion, this study assessed the effect of *CYP3A* enzymes and *ABCB1* transporter on day 7 ALu concentrations and treatment outcome in pregnant and nonpregnant women. Lower day 7 LF concentration was observed in pregnant women than nonpregnant. The determinants of day 7 LF concentration were pregnancy status, the number of *CYP3A5*1*, and weight of patients. Slightly lower therapeutic efficacy of ALu was observed in pregnant women compared with nonpregnant women.

**Acknowledgments**

We are grateful to our fellow malaria and reproductive and child heath team members at MUHAS, Dr. Billy Ngasala, Dr. Rose Mpembeni, and Dr. Dinah Gasarasi, for providing research inputs throughout the study period. We thank Dorisia Nanage for dedicated technical assistance. We also thank the health care providers and the study participants from Mkuranga, Kisarawe, and Utete district hospitals and from Kibiti and Mohoro Health Centers for all the cooperation given to us throughout the study period.

**Authorship Contributions**

*Participated in research design:* Massawe, Kamuhabwa, Aklillu, Mutagonda.

*Conducted experiments:* Mutagonda.

*Contributed new reagents or analytic tools:* Minzi, Färnert, Aklillu.

*Performed data analysis:* Mutagonda, Aklillu, Asghar.

*Wrote or contributed to the writing of the manuscript:* Mutagonda, Aklillu, Kamuhabwa, Minzi, Färnert, Massawe, Asghar.

**References**

Anderson GD (2005) Pregnancy-induced changes in pharmacokinetics: a mechanistic-based approach. *Clin Pharmacokinet* **44**:989–1008.

Banda CG, Dzinjalamala F, Mukaka M, Mallewa J, Maiden V, Terlouw DJ, Lalloo DG, Khoo SH, and Mwapasa V (2018) Impact of efavirenz-, ritonavir-boosted lopinavir-, and nevirapine-based antiretroviral regimens on the pharmacokinetics of lumefantrine and safety of artemether-lumefantrine in *Plasmodium falciparum*-negative HIV-infected Malawian adults stabilized on antiretroviral therapy. *Antimicrob Agents Chemother* **62**:e01162-18.

Borrmann S, Sallas WM, Machevo S, González R, Björkman A, Mårtensson A, Hamel M, Juma E, Peshu J, Ogutu B, et al. (2010) The effect of food consumption on lumefantrine bioavailability in African children receiving artemether-lumefantrine crushed or dispersible tablets (Coartem) for acute uncomplicated *Plasmodium falciparum* malaria. *Trop Med Int Health* **15**:434–441.

Byakika-Kibwika P, Lamorde M, Mayito J, Nabukeera L, Namakula R, Mayanja-Kizza H, Katabira E, Ntale M, Pakker N, Ryan M, et al. (2012) Significant pharmacokinetic interactions between artemether/lumefantrine and efavirenz or nevirapine in HIV-infected Ugandan adults. *J Antimicrob Chemother* **67**:2213–2221.

Desta Z and Flockhart DA (2017) Pharmacogenetics of drug metabolism, in *Clinical and Translational Science*, 2nd ed, Elsevier, London EC2Y 5AS, United Kingdom In press.

Diczfalusy U, Miura J, Roh HK, Mirghani RA, Sayi J, Larsson H, Bodin KG, Allqvist A, Jande M, Kim JW, et al. (2008) 4Beta-hydroxycholesterol is a new endogenous CYP3A marker: relationship to *CYP3A5* genotype, quinine 3-hydroxylation and sex in Koreans, Swedes and Tanzanians. *Pharmacogenet Genomics* **18**:201–208.

Djimdé A and Lefèvre G (2009) Understanding the pharmacokinetics of coartem. *Malar J* **8** (Suppl 1):S4.

Gebeyehu E, Engidawork E, Bijnsdorp A, Aminy A, Diczfalusy U, and Aklillu E (2011) Sex and *CYP3A5* genotype influence total CYP3A activity: high CYP3A activity and a unique distribution of *CYP3A5* variant alleles in Ethiopians. *Pharmacogenomics J* **11**:130–137.

German PI and Aweeka FT (2008) Clinical pharmacology of artemisinin-based combination therapies. *Clin Pharmacokinet* **47**:91–102.

Habtewold A, Amogne W, Makonnen E, Yimer G, Nylén H, Riedel KD, Aderaye G, Bertilsson L, Burhenne J, Diczfalusy U, et al. (2013) Pharmacogenetic and pharmacokinetic aspects of CYP3A induction by efavirenz in HIV patients. *Pharmacogenomics J* **13**: 484–489.

Huang L, Parikh S, Rosenthal PJ, Lizak P, Marzan F, Dorsey G, Havlir D, and Aweeka FT (2012) Concomitant efavirenz reduces pharmacokinetic exposure to the antimalarial drug artemether-lumefantrine in healthy volunteers. *J Acquir Immune Defic Syndr* **61**:310–316.

Isoherranen N and Thummel KE (2013) Drug metabolism and transport during pregnancy: how does drug disposition change during pregnancy and what are the mechanisms that cause such changes? *Drug Metab Dispos* **41**:256–262.

Jain JP, Leong FJ, Chen L, Kalluri S, Koradia V, Stein DS, Wolf MC, Sunkara G, and Kota J (2017) Bioavailability of lumefantrine is significantly enhanced with a novel formulation approach, an outcome from a randomized, open-label pharmacokinetic study in healthy volunteers. *Antimicrob Agents Chemother* **61** (9):e00868-17.

Jeong H (2010) Altered drug metabolism during pregnancy: hormonal regulation of drug-metabolizing enzymes. *Expert Opin Drug Metab Toxicol* **6**:689–699.

Klein K and Zanger UM (2013) Pharmacogenomics of cytochrome P450 3A4: recent progress toward the "missing heritability" problem. *Front Genet* **4**:12.

Kloprogge F, Piola P, Dhorda M, Muwanga S, Turyakira E, Apinan S, Lindegårdh N, Nosten F, Day NP, White NJ, et al. (2013) Population pharmacokinetics of lumefantrine in pregnant and nonpregnant women with uncomplicated *Plasmodium falciparum* malaria in Uganda. *CPT Pharmacometrics Syst Pharmacol* **2**:e83.

Kloprogge F, Workman L, Borrmann S, Tékété M, Lefèvre G, Hamed K, Piola P, Ursing J, Kofoed PE, Mårtensson A, et al. (2018) Artemether-lumefantrine dosing for malaria treatment in young children and pregnant women: a pharmacokinetic-pharmacodynamic meta-analysis. *PLoS Med* **15**:e1002579.

Lamorde M, Byakika-Kibwika P, Mayito J, Nabukeera L, Ryan M, Hanpithakpong W, Lefèvre G, Back DJ, Khoo SH, and Merry C (2013) Lower artemether, dihydroartemisinin and lumefantrine concentrations during rifampicin-based tuberculosis treatment. *AIDS* **27**:961–965.

Liljander A, Wiklund L, Falk N, Kweku M, Mårtensson A, Felger I, and Färnert A (2009) Optimization and validation of multi-coloured capillary electrophoresis for genotyping of *Plasmodium falciparum* merozoite surface proteins (msp1 and 2). *Malar J* **8**:78.

Lohy Das J, Rulisa S, de Vries PJ, Mens PF, Kaligirwa N, Agaba S, Taming J, Karlsson MO, and Dorlo TPC (2018) Population pharmacokinetics of artemether, dihydroartemisinin, and lumefantrine in rwandese pregnant women treated for uncomplicated *Plasmodium falciparum* malaria. *Antimicrob Agents Chemother* **62** (10):e00518-18.

Maganda BA, Minzi OM, Ngaimisi E, Kamuhabwa AA, and Aklillu E (2016) CYP2B6*6 genotype and high efavirenz plasma concentration but not nevirapine are associated with low lumefantrine plasma exposure and poor treatment response in HIV-malaria-coinfected patients. *Pharmacogenomics J* **16**:88–95.

Maganda BA, Ngaimisi E, Kamuhabwa AA, Aklillu E, and Minzi OM (2015) The influence of nevirapine and efavirenz-based anti-retroviral therapy on the pharmacokinetics of lumefantrine and anti-malarial dose recommendation in HIV-malaria co-treatment. *Malar J* **14**:179.

Mathias AA, Hitti J, and Unadkat JD (2005) P-glycoprotein and breast cancer resistance protein expression in human placentae of various gestational ages. *Am J Physiol Regul Integr Comp Physiol* **289**:R963–R969.

McGready R, Stepniewska K, Lindegardh N, Ashley EA, La Y, Singhasivanon P, White NJ, and Nosten F (2006) The pharmacokinetics of artemether and lumefantrine in pregnant women with uncomplicated falciparum malaria [published correction appears in Eur *J Clin Pharmacol* (2009) 65:84]. *Eur J Clin Pharmacol* **62**:1021–1031.

Minzi O, Ngaimisi E, Shewiyo DH, Sasi P, and Ignace AM (2012) Interlaboratory development and cross validation of a chromatographic method for determination of lumefantrine in human plasma - a proficient capacity assessment of bioanalytical laboratories in East Africa. *J Anal Bioanal Tech* **3**:131.

Mosha D, Guidi M, Mwingira F, Abdulla S, Mercier T, Decosterd LA, Csajka C, and Genton B (2014) Population pharmacokinetics and clinical response for artemether-lumefantrine in pregnant and nonpregnant women with uncomplicated *Plasmodium falciparum* malaria in Tanzania. *Antimicrob Agents Chemother* **58**:4583–4592.

Mukonzo JK, Waako P, Ogwal-Okeng J, Gustafsson LL, and Aklillu E (2010) Genetic variations in *ABCB1* and *CYP3A5* as well as sex influence quinine disposition among Ugandans. *Ther Drug Monit* **32**:346–352.

Mutagonda RF, Kamuhabwa AA, Minzi OM, Massawe SN, Maganda BA, and Aklillu E (2016) Malaria prevalence, severity and treatment outcome in relation to day 7 lumefantrine plasma concentration in pregnant women. *Malar J* **15**:278.

Mutagonda RF, Kamuhabwa AAR, Minzi OMS, Massawe SN, Asghar M, Homann MV, Färnert A, and Aklillu E (2017) Effect of pharmacogenetics on plasma lumefantrine pharmacokinetics and malaria treatment outcome in pregnant women. *Malar J* **16**:267.

Ngaimisi E, Minzi O, Mugusi S, Sasi P, Riedel KD, Suda A, Ueda N, Bakari M, Janabi M, Mugusi F, et al. (2014) Pharmacokinetic and pharmacogenomic modelling of the CYP3A activity marker 4β-hydroxycholesterol during efavirenz treatment and efavirenz/rifampicin co-treatment. *J Antimicrob Chemother* **69**:3311–3319.

Oga EF, Sekine S, Shitara Y, and Horie T (2012) Potential P-glycoprotein-mediated drug-drug interactions of antimalarial agents in Caco-2 cells. *Am J Trop Med Hyg* **87**:64–69.

Olafuyi O, Coleman M, and Badhan RKS (2017) Development of a paediatric physiologically based pharmacokinetic model to assess the impact of drug-drug interactions in tuberculosis co-infected malaria subjects: a case study with artemether-lumefantrine and the CYP3A4-inducer rifampicin. *Eur J Pharm Sci* **106**:20–33.

Rogerson SJ, Mwapasa V, and Meshnick SR (2007) Malaria in pregnancy: linking immunity and pathogenesis to prevention. *Am J Trop Med Hyg* **77**(6 Suppl):14–22.

Rogerson SJ, Wijesinghe RS, and Meshnick SR (2010) Host immunity as a determinant of treatment outcome in *Plasmodium falciparum* malaria. *Lancet Infect Dis* **10**:51–59.

Shokoples SE, Ndao M, Kowalewska-Grochowska K, and Yanow SK (2009) Multiplexed real-time PCR assay for discrimination of *Plasmodium* species with improved sensitivity for mixed infections. *J Clin Microbiol* **47**:975–980.

Staehli Hodel EM, Csajka C, Ariey F, Guidi M, Kabanywanyi AM, Duong S, Decosterd LA, Olliaro P, Beck HP, and Genton B (2013) Effect of single nucleotide polymorphisms in cytochrome P450 isoenzyme and N-acetyltransferase 2 genes on the metabolism of artemisinin-based combination therapies in malaria patients from Cambodia and Tanzania. *Antimicrob Agents Chemother* **57**:950–958.

Sun M, Kingdom J, Baczyk D, Lye SJ, Matthews SG, and Gibb W (2006) Expression of the multidrug resistance P-glycoprotein, (ABCB1 glycoprotein) in the human placenta decreases with advancing gestation. *Placenta* **27**:602–609.

Tarning J, Kloprogge F, Dhorda M, Jullien V, Nosten F, White NJ, Guerin PJ, and Piola P (2013) Pharmacokinetic properties of artemether, dihydroartemisinin, lumefantrine, and quinine in pregnant women with uncomplicated plasmodium falciparum malaria in Uganda. *Antimicrob Agents Chemother* **57**:5096–5103.

Tarning J, McGready R, Lindegardh N, Ashley EA, Pimanpanarak M, Kamanikom B, Annerberg A, Day NPJ, Stepniewska K, Singhasivanon P, et al. (2009) Population pharmacokinetics of lumefantrine in pregnant women treated with artemether-lumefantrine for uncomplicated *Plasmodium falciparum* malaria. *Antimicrob Agents Chemother* **53**:3837–3846.

Vos K, Sciuto CL, Piedade R, Ashton M, Björkman A, Ngasala B, Mårtensson A, and Gil JP (2017) MRP2/ABCC2 C1515Y polymorphism modulates exposure to lumefantrine during artemether-lumefantrine antimalarial therapy. *Pharmacogenomics* **18**:981–985.

Wahajuddin, Raju KS, Singh SP, and Taneja I (2014) Investigation of the functional role of P-glycoprotein in limiting the oral bioavailability of lumefantrine. *Antimicrob Agents Chemother* **58**:489–494.

White NJ (1997) Assessment of the pharmacodynamic properties of antimalarial drugs in vivo. *Antimicrob Agents Chemother* **41**:1413–1422.

White NJ, Stepniewska K, Barnes K, Price RN, and Simpson J (2008) Simplified antimalarial therapeutic monitoring: using the day-7 drug level? *Trends Parasitol* **24**:159–163.

Williams JA, Ring BJ, Cantrell VE, Jones DR, Eckstein J, Ruterbories K, Hamman MA, Hall SD, and Wrighton SA (2002) Comparative metabolic capabilities of CYP3A4, CYP3A5, and CYP3A7. *Drug Metab Dispos* **30**:883–891.

WorldWide Antimalarial Resistance Network (WWARN) Lumefantrine PK/PD Study Group (2015) Artemether-lumefantrine treatment of uncomplicated *Plasmodium falciparum* malaria: a systematic review and meta-analysis of day 7 lumefantrine concentrations and therapeutic response using individual patient data [published correction appears in *BMC Med* (2016) 14:214]. *BMC Med* **13**:227.

**Address correspondence to:** Dr. Ritah F. Mutagonda, Department of Clinical Pharmacy and Pharmacology, School of Pharmacy, Muhimbili University of Health and Allied Sciences, P.O. Box 65013, Dar es Salaam, Tanzania. E-mail: rittdavisrida@yahoo.com