Pregnancy and CYP3A5 Genotype Affect Day 7 Plasma Lumefantrine Concentrations

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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 (1.119–1.745), \( P < 0.003 \)]. 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.
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-benfluometol 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 expressers 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 blacks.

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 4β-hydroxycholesterol (Diczfalusy et al., 2008; Gebechyu 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. Prior studies reported the relevance of genetic variation on CYP3A enzyme induction (Habtewold et al., 2013; Ngaïmisi 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/10665/40448/9789241597531_eng.pdf?jesionii). 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/10665/40448/9789241597531_eng.pdf?jesionii). 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 vacuum tube and stored at −80°C at MUHAS laboratory. Also, approximately 50 μl 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 2000g 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 Aweke, 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
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 mm; 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 1**

| 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 | 176 (85%)                    | NA                                          |                                 |
| Baseline parasitemia (counts/µl) | 2000 (400–54,688) | 2600 (400–20,000) | 0.02                           |

**TABLE 2**

| Genotype            | Pregnant Women Frequency N (%) | Nonpregnant Women Frequency N (%) | $P$ Value |
|---------------------|-------------------------------|----------------------------------|-----------|
| CYP3A4*1B (-392A>G) |                               |                                  |           |
| *1/*1               | 19 (9.4%)                     | 5 (6.9%)                         | 0.809     |
| *1/*1B              | 80 (39.6%)                    | 30 (41.7%)                       |           |
| *1B/*1B             | 103 (51.0%)                   | 37 (51.4%)                       |           |
| CYP3A5*3 c.6986A>G  |                               |                                  |           |
| *1/*1               | 118 (59.3%)                   | 43 (62.3%)                       | 0.881     |
| *1/*3               | 75 (37.7%)                    | 24 (34.8%)                       |           |
| *3/*3               | 6 (3.0%)                      | 2 (2.9%)                         |           |
| CYP3A5*6 c.14690G>A |                               |                                  |           |
| *1/*1               | 145 (71.4%)                   | 51 (71.8%)                       | 0.480     |
| *1/*6               | 49 (23.9%)                    | 19 (26.8%)                       |           |
| *6/*6               | 9 (4.4%)                      | 1 (1.4%)                         |           |
| CYP3A5*7 27131_27132insT |                           |                                  |           |
| *1/*1               | 156 (77.6%)                   | 53 (73.6%)                       | 0.781     |
| *1/*7               | 43 (21.4%)                    | 18 (25.0%)                       |           |
| *7/*7               | 2 (1.0%)                      | 1 (1.4%)                         |           |
| ABCB c.3435         |                               |                                  |           |
| CC                  | 139 (72.0%)                   | 53 (74.6%)                       | 0.672     |
| CT                  | 51 (26.4%)                    | 16 (22.5%)                       |           |
| TT                  | 3 (1.6%)                      | 2 (2.8%)                         |           |
| ABCB rs3842         |                               |                                  |           |
| AA                  | 116 (57.1%)                   | 39 (54.2%)                       | 0.269     |
| 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) |                               | 70.8                              | 72.2       |
| CYP3A5*3 c.6986A>G  |                               | 21.9                              | 20.3       |
| CYP3A5*6 c.14690G>A |                               | 16.4                              | 14.8       |
| CYP3A5*7 27131_27132insT |                           | 13                                | 13.9       |
| ABCB c. 3435        | T                              | 14.8                              | 14.1       |
| ABCB rs3842         | G                              | 25.7                              | 24.4       |
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 χ² 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  | Beta Coefficient (95% CI) | P Value | Beta Coefficient (95% CI) | P Value |
|-----------------------------------|----|--------------------------|---------|--------------------------|---------|
|                                   |    | Univariate Analysis      |         | Multivariable Analysis   |         |
| 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   | 1.8 (0.7–2.8)            | 0.002   |
| Weight (kg)                      | 208| 0.8 (0.3–1.2)            | 0.002   | NS                       | NS      |
| Hemoglobin (g/dl)                | 208| 3.2 (0.2–6.2)            | 0.036   | NS                       | NS      |
| Trimester                        | 142| 19.1 (2.8–35.5)          | 0.022   | NS                       | NS      |
| Gravida                          | 142| 7.2 (−19.6 to 5.2)       | 0.253   | NS                       | NS      |
| Parasitema (counts/μl)           |     |                          |         |                          |         |
| <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                        | 21 | −9.2 (1.4–17)            | 0.046   | NS                       | NS      |
| CYP3A4*1/*1                      | 83 | 1.5 (−8.1 to 11.1)       | 0.759   | NS                       | NS      |
| CYP3A4*1/*1B                     | 104| Reference                |         |                          |         |
| Number of CYP3A5*1 allele        |     |                          |         |                          |         |
| 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)      | 0.391   |
| Two                              | 63 | Reference                |         | Reference                |         |
| ABCB rs3842                      |     |                          |         |                          |         |
| A/A                              | 118| −2.1 (−13.0 to 8.7)      | 0.695   | NS                       | NS      |
| A/G                              | 80 | −0.4 (−21.3 to 20.5)     | 0.969   | NS                       | NS      |
| G/G                              | 11 | Reference                |         | Reference                |         |
| ABCB c. 3435 C>T                |     |                          |         |                          |         |
| C/C                              | 143| −16.5 (−33.4 to 0.4)     | 0.056   | NS                       | NS      |
| C/T                              | 54 | −29.8 (−63.1 to 3.4)     | 0.078   | NS                       | NS      |
| T/T                              | 4  | Reference                |         | Reference                |         |

NS, Nonsignificant variables.
women. Baseline characteristics of study participants are shown in Table 1.

Genotype distribution for \textit{CYP3A4}, \textit{CYP3A5}, and \textit{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.

**Fig. 2.** Comparison of mean day 7 LF plasma concentration based on the number of functional \textit{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 \textit{CYP3A5*1/*1} genotype. Vertical bars denote 95% CIs of the mean.
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 was 1.40; 95% CI of geometric mean ratio (1.119–1.745). 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*I functional allele), were independent significant predictors of day 7 LF plasma concentration (Table 3). CYP3A4*1/I and CYP3A5*1/I genotype had significantly lower log day 7 plasma LF concentration than CYP3A4*1B/I*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*I variant alleles in all study participants and stratified by pregnancy status is presented in Fig. 2 using ANOVA. 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;jsessionid) 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 = 89.3–96.6) in pregnant women versus 95.7% (95% CI = 91.5–100) were non 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 univariate 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 (1.83–19.066) 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*I/I 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

| 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) | 0.443 |
| PP                         | 126/140  | 90.0% (85–95) | 45/48    | 93.8% (86.7–100) | 0.442 |
| PCR-uncorrected ACPR       |          |             |           |
| ITT                        | 181/204  | 88.7% (88.4–93.1) | 64/69    | 92.8% (85.1–98.2) | 0.363 |
| PP                         | 126/151  | 84.8% (79.9–90.6) | 46/61    | 90.2% (79.9–97.5) | 0.434 |
| New infection              | 15/199   | 7.6% (3.9–11.4) | 5/69     | 7.2% (0.9–13.5) | 0.934 |

PP, per protocol.
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 antiretroviral 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 $\geq 35$ kg
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 recrudescence in malaria-endemic areas has 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. 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 13 (65%) 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

### 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           |                     |                     |         |
| <600 ng/ml                | 11/85 (12.9%)       | 5.32 (1.48–19.07)   | 0.010   |
| >600 ng/ml                | 3/118 (2.5%)        | 1 (Reference)       | 0.45    |
| Pregnancy                 |                     |                     |         |
| Pregnant                  | 14/199 (7.0%)       | 1.61 (0.46–5.61)    | 0.37    |
| Nonpregnant               | 3/69 (4.3%)         | 1 (Reference)       | 0.59    |
| Age                       | 17/268 (6.3%)       | 0.97 (0.91–1.04)    | 0.29    |
| Hb                        | 17/268 (6.3%)       | 0.91 (0.65–1.26)    | 0.29    |
| Trimester                 |                     |                     |         |
| Second                    | 15/170 (8.8%)       | 0.04 (0.00–16.54)   | 0.46    |
| Third                     | 0/50 (0%)           | —                   |         |
| Gravida                   |                     |                     |         |
| Primigravida              | 7/82 (8.5%)         | 1.49 (0.52–4.24)    | 0.79    |
| Multigravida              | 7/117 (6.0%)        | 1 (Reference)       | 0.79    |
| 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)       | 0.65    |
| CYP3A4*1B                 |                     |                     |         |
| CYP3A4*1/*1               | 4/24 (16.7%)        | 3.25 (0.95–11.09)   | 0.06    |
| CYP3A4*1/*B               | 6/106 (5.7%)        | 1.08 (0.36–3.20)    | 0.89    |
| CYP3A5*1                  | 7/135 (5.2%)        | 1 (Reference)       |         |
| Number of CYP3A5*1        |                     |                     |         |
| Zero                      | 2/262 (3.2%)        | 0.50 (0.92–2.59)    | 0.94    |
| One                       | 10/122 (8.2%)       | 1.28 (0.44–3.74)    | 0.65    |
| Two                       | 5/80 (6.2%)         | 1 (Reference)       | 0.65    |
| 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%)           | —                   |         |
| ABCB1 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%)            | —                   |         |
findings were also reported in a meta-analysis examining the pharmacokinetics 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 observed in pregnant women than nonpregnant. The determinants of day 7 LF concentrations in pregnant women compared with nonpregnant women.

Outcome in pregnant women and nonpregnant women.

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 demographic changes? How does drug disposition change during pregnancy and what are the mechanisms that cause such changes? A systematic review of antimalarial pharmacology in pregnancy.

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