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Lumefantrine plasma concentrations in uncontrolled conditions among patients treated with artemether-lumefantrine for uncomplicated *plasmodium falciparum* malaria in Mwanza, Tanzania

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ABSTRACT

Background: Therapeutic efficacy of artemether-lumefantrine is highly dependent on adequate systemic exposure to the partner drug lumefantrine particularly day 7 lumefantrine plasma concentration. There has been contradicting findings on the role of the cut-off values in predicting treatment outcomes among malaria patients in malaria endemic regions. This study assesses the day 3 and 7 lumefantrine plasma concentrations including related determinant factors and their influence on treatment outcomes among treated Tanzanian children and adults in uncontrolled conditions (real life condition).

Methods: Data was nested from an efficacy study employing the WHO protocol, 2015 for monitoring antimalarial drug efficacy. Lumefantrine plasma concentration was measured by high performance liquid chromatography with ultraviolet (HPLC-UV). *Results:* Lumefantrine plasma concentrations below 175ng/ml and 200ng/ml on day 3 and 7 did not affect adequate clinical and parasitological response (ACPR) and recurrence of infection (p = 0.428 and 0.239 respectively). Age and baseline parasitemia were not associated to day 3 median lumefantrine plasma concentrations (p = 0.08 and 0.31 respectively) and day 7 lumefantrine plasma concentrations (p = 0.07 and 0.41 respectively). However, the day 3 and day 7 lumefantrine plasma concentrations were significantly higher in males compared to females (p = 0.03 and 0.042 respectively).

Conclusion: Lumefantrine plasma concentrations below cut-off points (175ng/ml and 200ng/ml) on day 3 and 7 did not influence treatment outcomes.

Introduction

Sub-Saharan countries including Tanzania are the most affected with the burden of *Plasmodium falciparum (P. falciparum)* malaria. Treatment response in *P. falciparum* malaria is influenced by a vast number of factors. Such factors can be classified as drug quality, pharmacokinetic characteristics of individual drug, parasite sensitivity and host genetics (Obua et al., 2008). Artemether-lumefantrine (ALU) is the most used artemisinin-based combination therapy (ACT) as first line drug in malaria endemic countries (Organization, 2015a). The rapid parasite clearance is associated with artemether whereas lumefantrine plays a significant role in clearing the remaining parasites after two parasite asexual cycles have been exposed to artemether (Kloprogge et al., 2015). Artemether which is a lipid soluble derivative of dihydroartemisinin is quickly absorbed and transformed to the active metabolite dihydroartemisinin (DHA). The peak concentrations of artemether and DHA are obtained within 2 hours af-

Abbreviations: ACT, Artemisinin-based combination therapy; DHP, Dihydroartemisinin-piperaquine; ALU, Artemether-lumefantrine; ACPR, Adequate Clinical and Parasitological Response; ETF, Early Treatment Failure; LCF, Late Clinical Failure; HPLC-UV, High performance liquid chromatography with ultra-violet; LPF, Late Parasitological Failure; WHO, World Health Organization.

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ter administration resulting to rapid reduction in asexual parasites biomass and quick resolution of symptoms (Djimdé and Lefèvre, 2009; White et al., 1999). Lumefantrine is highly lipophilic with 98% binding to plasma lipoproteins and fat (Chotivanich et al., 2012). The bioavailability of lumefantrine is increased by concurrent uptake of fat meals (Ashley et al., 2007a; Djimdé and Lefèvre, 2009; Organization, 2015a). The terminal elimination half-lives of artemether and lumefantrine are $\leq$ 1 hour and 3-5 days respectively (Ashley et al., 2007b; Djimdé and Lefèvre, 2009). The elimination of lumefantrine is very slow in healthy volunteers than in patients with malaria (terminal half-life 2-3 days vs 4-6 days) (Djimdé and Lefèvre, 2009; Ezzet et al., 2000). The slow elimination of lumefantrine plays a great role in the elimination of residual parasites after artemether and DHA have been cleared thus preventing recrudescence (Djimdé and Lefèvre, 2009; White et al., 1999) due to parasite's exposure to high levels of lumefantrine concentrations resulting from accumulation owing to a long half-life of the drug (White et al., 1999).

The suggested pharmacokinetics determinants of treatment outcomes in *P. falciparum* uncomplicated malaria are area under the curve (AUC) and day 7 plasma concentrations of partner drugs in ACT. However, some studies report day 3 plasma lumefantrine concentrations as a strong predictor of treatment outcome in infants and young children (Tchaparian et al., 2016). A single plasma lumefantrine concentration on day 7 is a proven best correlate of the plasma AUC (Ezzet et al., 1998; White et al., 1999). Day 7 lumefantrine concentration has been suggested to be a better determinant of therapeutic response than AUC when the two are compared (White et al., 2008) although some studies report the opposite (Parikh et al., 2016). The documented therapeutic day 7 lumefantrine concentrations range between 175ng/ml and 500ng/ml. Price et al specified even a lower day 7 concentration (175ng/mL) is a predictor for treatment response (Price et al., 2006). However, the big question is whether these commonly used cutoff values are applicable to all regions.

Metabolism of drugs determines the plasma concentrations hence treatment response. Lumefantrine is metabolized mainly by cytochrome P450 (CYP) enzyme system, the CYP3A4 and CYP3A5 isoenzymes. The CYP3A4 gene is located on chromosome 7q21.3-q22.1 consisting of 13 exons (Keshava et al., 2004). The most important single nucleotide polymorphism (SNP) within the CYP3A4 family is CYP3A4*1B (rs2740574) (Alessandrini et al., 2013), an A to G transition at nucleotide 392 in the promoter sequence of the gene (El-Shair et al., 2019). This SNP is associated with poor metabolism of artemether and lumefantrine (Staehli Hodel et al., 2013; Piedade and Gil, 2011). CYP3A5*3 (rs776746) is the most important SNP in the CYP3A5 gene involving a replacement of a nucleotide adenine by nucleotide guanine at locus 6986 within intron 3 creating a mRNA splice defect thus a premature stop codon (Eng et al., 2006; Tang et al., 2010). The CYP3A5*3 is involved in the metabolism of artemether, lumefantrine, mefloquine, primaquine and chloroquine (Dandara et al., 2014).

Interindividual variability in the extent and rate of absorption, metabolism, distribution, plasma protein binding and elimination has been shown to influence the plasma concentration of drugs hence affecting treatment outcomes in turn (Pang, 2003). Interindividual variability is common in Africa since African populations are genetically diverse and heterogenous (Bolaji et al., 2019; Campbell and Tishkoff, 2008; Kampira et al., 2012) due to complex patterns of populations expansion, contraction, migration and admixture during evolutionary history (Dandara et al., 2014). Indeed, Africa is regarded as a birth place for genetic diversity (Pillai et al., 2013). There is a need to assess ACTs plasma concentrations in these populations since the plasma concentrations determine the extent of parasite exposure to the drug and treatment outcomes.

Despite the wide spread use of ALU in the country there is scanty information on the drug's plasma levels and its influence on the treatment outcomes in the population. This study focuses on the day 3 and 7 lumefantrine plasma concentrations including the related determinant factors and their influence on the treatment outcomes among children and adults treated with ALU in Tanzania.

Methods

Study area, patient enrollment and drug administration

The study was conducted in Igombe, Mwanza, Tanzania, the sentinel sites for conducting therapeutic efficacy studies on antimalarial drugs. The area is semi-urban and malaria meso-endemic. Patients who were *P. falciparum* positive after microscopy and malaria rapid diagnostic test and met the inclusion criteria as per the World Health Organization (WHO) protocol for assessment of antimalarial efficacy were enrolled after a written informed consent. Full clinical examination was performed and blood samples were taken for parasite count, hematocrit and random blood glucose determination. Malaria patients with symptoms of severe malaria according to the WHO case definition, comorbid infection(s), malnutrition, chronic diseases, history of drug allergy, history of traditional herbs use in the past 4 weeks, any antimalarial drug use in the past 4 weeks, known liver dysfunction or disease and severe anaemia were excluded from this study to avoid interference with pharmacokinetics parameters and treatment outcomes.

A standard 6-dose of artemether 20mg -lumefantrine 120mg (Coartem®, Novartis, Switzerland) was administered as per manufacturer's dosing schedule based on body weight. Participants were not restricted on their routine diet.

Sample collection and follow up

Samples were collected from efficacy study which involved 35 days follow up as per the WHO protocol, 2015 for monitoring antimalarial drug efficacy (Organization, 2016). Blood from finger pricks was collected on filter paper (FTA®Whatman paper) then dried at room temperature and stored on plastic bags on day 0,1,2,3,7,14,21,28,35 for PCR genotyping of Merozoite Surface Protein 1 (MSP1) and Merozoite Surface Protein 2 (MSP2) to distinguish between recrudescence and reinfection. Venous blood (2mls) was also collected, centrifuged at 300xg for 10 minutes and stored in cryotubes at -20°C at the clinic for few hours during the visits before final storage at -80°C at the National Institute for Medical Research (NIMR). Samples were then shipped on dry ice to the Makerere University analytical laboratory for bioanalytical measurements after storage at -80°C. Thick and thin blood smears were stained by Giemsa (on each day of the visit) according to the WHO standard protocol (Organization, 2010). Parasite identification and counting were done by two independent experienced microscopists.

Genotyping and plasma lumefantrine assay

DNA was extracted from dried blood spots (DBS) using the Invitrogen Genomic DNA extraction kit (Thermo Scientific) according to the manufacturer's instructions. Nested PCR was done to identify MSP1 and MSP2 allele variants using a method described previously (Somé et al., 2018). The results were classified as recrudescence or reinfection according to the WHO guideline (Organization, 2008). Lumefantrine in plasma was measured by high performance liquid chromatography with ultraviolet (HPLC-UV). Chromatographic conditions were adapted from a previously

Table 1
Participant characteristics at enrollment.

Characteristic	Category	values
Age (years), median (IOR)	12(4,17)	
Gender (female) n (%)	Males	40/93 (43.1%)
	Females	53/93 (56.9%)
Weight (Kg), mean (SD)	<10 years	15.38 (7.73)
	≥10 years	49.82 (17.38)
Hemoglobin (g/dL), mean (SD)	<10 years	9.56 (1.24)
	≥10 years	11.10 (1.44)
Random blood glucose	<10 years	5.1(0.72)
	≥10 years	5.02(0.89)
Hepatitis B		0/93(0%)
Parasitemia (parasite/µl), geometric mean		5044.2
Residual lumefantrine plasma concentration		18/93(19.4%)

published method (Khuda et al., 2014). Quality control samples to assess precision and accuracy were set to 170, 265 and 500 ng/ml. Measurements of plasma samples in each batch of run were compared to the quality control samples. The lower limit of quantification (LOQ) and lower limit of detection(LLOD) were 18 and 12 ng/ml respectively. Intra- and inter-day coefficient of variation values were < 5%.

Treatment outcomes

Patients were assessed on day 0,1,2,3,7,14,21 and 28 for efficacy. The WHO 2015 protocol (WHO, 2015b) was used to classify treatment outcomes as early treatment failure (ETF), late clinical failure (LCF), late parasitological failure and adequate clinical and parasitological response (ACPR). Treatment failures were classified as recrudescence or reinfection after PCR correction.

Statistical analysis

Ms-Excel was used for data entry and cleaning. All statistical analyses were performed using STATA version 13.1 (Statistical Corporation, College Station, TX, US). Descriptive statistics were used accordingly. Numeric variables were summarized using mean (SD) or median (IQR) Categorical data were compared using chi square tests or fisher exact tests where appropriate. Student t-test was used to compare continuous data for two groups where necessary. Per-protocol analysis was carried, patients who withdrew from the study or were lost to follow up or had reinfection were not included in the denominator. The difference between the median values were assessed using the Mann–Whitney U-test or Kruskal–Wallis test whereas student t-test was used to assess the mean difference. Exact logistic regression was used to estimate odds ratio and 95% confidence intervals for association between day 3 lumefantrine plasma concentration, day 7 lumefantrine plasma concentration with age group (children vs adults), sex (female vs male), and baseline parasitaemia. Tests of significance were performed using the 0.05 level to infer significance.

Results

In this study, venous blood samples were collected from 93 patients with uncomplicated *P. falciparum* malaria among 365 who were followed up to 35 days during the ALU efficacy study (published elsewhere). The median age of participants was 12 years and more than half (56.9%) were female. Details of the participants are provided on the Table 1 below.

Lumefantrine plasma concentration

Participants had at least two pharmacokinetic samples on day 0, 1, 3, 7 or 14. Residual lumefantrine plasma concentration was recorded in 19.4% of the patients where by the median concentration was 357ng/ml. The median day 0 concentration for all patients was 67ng/ml. The median day 1, 2, 3, 7 and 14 lumefantrine concentrations were 817ng/ml, 1,065ng/ml, 859ng/ml, 238ng/ml and 95ng/ml respectively.

Sex was significantly associated with both day 3 and 7 lumefantrine plasma concentrations below the minimum cutoff values ($p = 0.03$ &0.042 respectively) (Figure 1). Age was not significantly associated with day 3 and day 7 lumefantrine concentrations below the minimum cut-off values ($p = 0.084$ and 0.071) (Figure 1). The day 3 and 7 lumefantrine plasma concentrations were not significantly influenced by the day 0 base line parasitemia ($p = 0.313$ and 0.413 respectively) (Figure 1). Sex of a patient showed a significant association with day 7 plasma concentration. That is, male patients had more than 4 times odds of lumefantrine plasma concentration about 200ng/ml compare to female patients. However, this association was not found with day 3 plasma concentration (Table 2).

Treatment outcomes

We assessed the association of day 3 and 7 lowest cut-off lumefantrine plasma concentration (175ng/ml) and the most commonly used cut-off lumefantrine plasma concentration (200ng/ml) with day 28-day outcomes. Day 3 lumefantrine plasma concentration below the minimum cut-off values predicting treatment response (175ng/ml) was not associated with low ACPR ($p = 0.433$). Day 7 lumefantrine plasma concentration below the minimum cut-off values (175ng/ml) was also not associated with low ACPR compared to concentration above the 175ng/ml values ($p = 0.313$) (Figure 2). Both lumefantrine plasma concentrations below 200ng/ml and above 200ng/ml on day 3 and 7 did not affect ACPR ($p = 0.428$ and 0.239 respectively) Figure 2.

Discussion

Therapeutic efficacy of ALU is highly dependent on adequate systemic bioavailability to the partner drug lumefantrine (Fogg et al., 2004; Parikh et al., 2016). The present study documents lumefantrine plasma concentrations in routine conditions/uncontrolled diet intake in the population. A substantial proportion of the patients had day 7 lumefantrine plasma concentration below the cut-off values predicting for treatment failure. Hodel et al performed simulations in a similar population and suggested a substantial proportion of patients would have day 7 lumefantrine concentrations below the cut-off values proposed (Hodel et al., 2013). We understand the low levels of lumefantrine concentrations could be due to uncontrolled dietary pattern during the study period unlike in other PK studies, the study focus was on determining plasma concentrations in uncontrolled conditions (real life situation) to reflect what is really happening in the population. An intake of 16g of milk has been shown to increase lumefantrine concentration 6 folds compared to a fasted state (White et al., 1999). However a small intake of fat (1.2g) has been shown to be associated with adequate lumefantrine exposure (Djimdé and Lefèvre, 2009). Recent studies have suggested the typical african diet is sufficient to archieve adequate lumefantrine exposure (Borrmann et al., 2010). This study is one of the few studies which have assesed lumefantrine concentrations under real life situation in African population considering a previous study in another area of the country had indicated only 0.4% of malaria patients do take ALU with food despite the empasize given by health care providers that ALU works better if taken with food (Kabanywanyi et al., 2010). It is easy to record high concentrations in controlled PK studies thus showing adequate lumefantrine con-

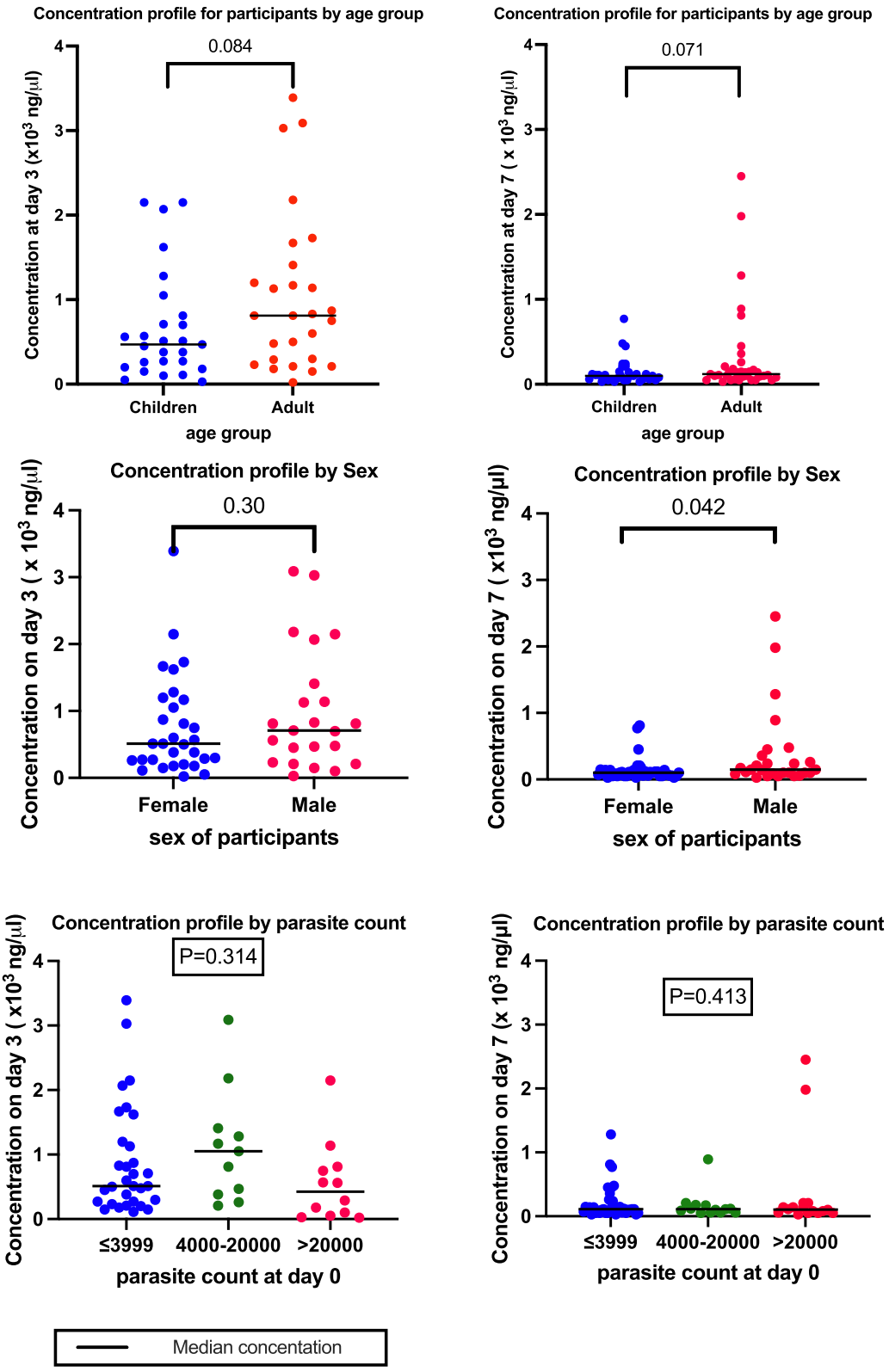

Figure 1. Day 3 and Day 7 lumefantrine plasma concentrations in relation to age, sex and baseline parasitemia.

centrations but this may be unrealistic because in clinical practice patients receive drugs without control of dietary intake.

Sex has been suggested to affect pharmacokinetic and pharmacodynamic parameters between men and women for various drugs (Soldin et al., 2011; Whitley and Lindsey, 2009). The influence of sex on lumefantrine exposure is not well established in humans. In this study, sex influenced both day 3 and 7 lumefantrine concentrations where by males had higher concentrations than females similar to findings reported in malawi (TEKETE, 2020). Evidence from animal (rats) study showed higher AUC and bioavailability in

Table 2
Day 3 and Day 7 Lumefantrine plasma concentrations in relation to age sex and baseline parasitemia.

Characteristics	Day 3 Lumefantrine plasma concentration			Day 7 Lumefantrine plasma concentration		
	OR	95%CI	P value	OR	95%CI	P value
Age group						
<=10 years	Ref			Ref		
>10 years	2.251	0.418-15.651	0.467	1.232	0.346-4.550	0.937
Sex						
Female	Ref			Ref		
Male	1.587	0.293-11.035	0.816	4.689	1.251-20.272	0.018
Baseline Parasitemia						
<=3999	Ref			Ref		
4000-20000	2.016	0.231-+inf	0.562	0.515	0.047-3.045	0.696
>20000	0.217	0.031-1.298	0.104	0.935	0.178-4.127	1.000

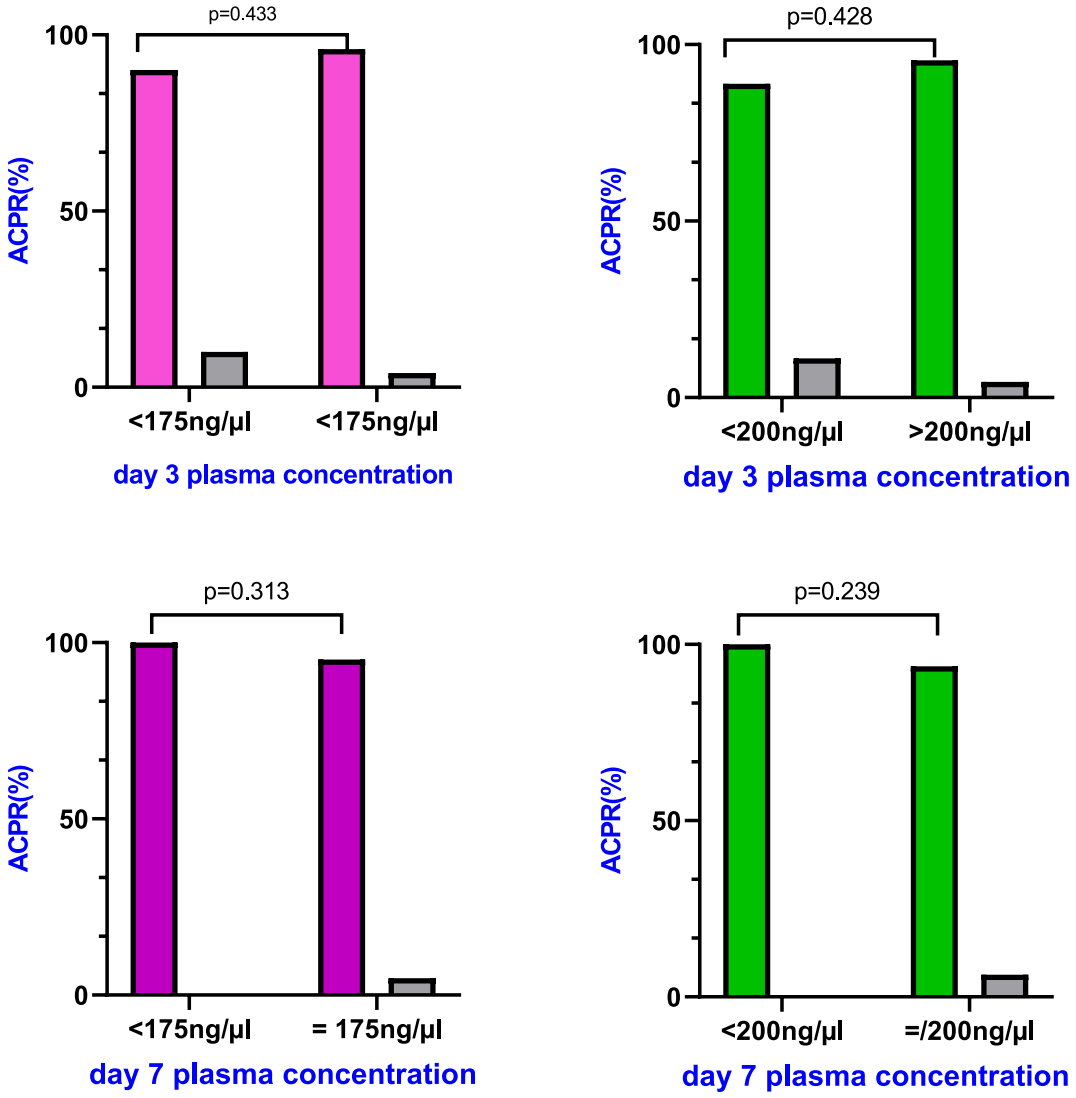

Figure 2. Association between lumefantrine plasma concentrations and treatment outcomes.

males than females (1.66 times higher) possibly due reduced absorption of lumefantrine in female rats (Wahajuddin et al., 2012).

The present study has also reports a lack of association between age and day 3 and 7 lumefantrine plasma concentrations. Our findings are similar to those reported previously in Thailand (Ezzet et al., 2000). However, these findings are in contrast with those from other areas (Tchaparian et al., 2016; TEKETE, 2020). The discrepancy observed may be due to most of children who participated in our study being not very young (above 3 years) since most studies have reported lower day 7 lumefantrine concentrations among very young children than older children and adults (Kloprogge et al., 2018; org, 2015). Similarily, day 3 lumefantrine concentration was not affected by age contrarily to findings in Uganda (Tchaparian et al., 2016), although our findings are in match with another previous study in Uganda (Checchi et al., 2006). The explanation above on age differences of the study participants may accentuate for the contradiction observed.

Day 3 lumefantrine plasma concentration is associated with absorption and distribution taking into account the peak lumefantrine concentration after treatment occurs at 70 hours since adminstration, whereas day 7 lumefantrine concentration is suggested to be a result of metabolism and elimination (Checchi et al., 2006). Day 7 lumefantrine concentrations below cut-off values (175ng/ml & 200ng/ml) were not associated with treatment failure. Our findings are comparable with similar studies in other African populations (Bell et al., 2009; Checchi et al., 2006; Hodel et al., 2013; Kilonzi et al., 2020). Studies done in other areas have reported that patients with day 7 lumefantrine levels below 175ng/ml are likely to experience treatment failure than their counterpart contrarily to our findings (Price et al., 2006). The lack of correlation between therapeutic day 7 lumefantrine concentrations (175ng/mL and 200ng/mL) and treatment outcomes suggest that these cut-off values may not be applicable to all regions/populations as documented in Malawi and Northern part of Tanzania (Bell et al., 2009; Kilonzi et al., 2020). The lack of predictive effect of the lumefantrine plasma concentrations cut-off values observed in malaria endemic areas may be due to early acquisition of natural immunity against malaria infections unlike to the current concept that children below 5 are naïve. This may be attributed to an increase in frequency of mosquito bites during early childhood. Background immunity acts in synergy with antimalarial chemotherapy in malaria endemic areas (Ezzet et al., 2000; Kloprogge et al., 2013). The high parasite sensitivity in the studied countries may also account for the lack of correlation between the day 7 plasma concentrations and treatment outcomes observed.

Few studies have reported day 3 lumefantrine concentration as a strong predictor of treatment failure in young children (Tchaparian et al., 2016) and is regarded as a close surrogate predictor of treatment outcomes (Checchi et al., 2006) than day 7 plasma concentration (Tchaparian et al., 2016). The peak lumefantrine concentration is attained approximately 70 hours after the first dose (Ezzet et al., 2000; White et al., 1999) thus measuring lumefantrine concentration approximately at 72 hours (day 3) is substantial. Since the day 3 lumefantrine concentration cut-off values predicting treatment failure are inadequately defined, we decided to employ similar cut-off values to day 7 lumefantrine concentration(s) which are widely used. Day 3 lumefantrine concentrations below cut-off values (175ng/ml & 200ng/ml) were not associated with treatment failure. The reasons given for day 7 lumefantrine concentrations may also explain the observed findings above.

Studies have suggested plasma lumefantrine concentrations are lower in younger children than older children and adults (Barnes et al., 2008; Tchaparian et al., 2016). Difference in bioavailability of oral administered drugs (which in turn affects plasma concentration) between adults or older children and young children has been attributed to the differences in gastric pH, immaturity of secretion and activity of bile and pancreatic fluid, intestinal transit time and gastric emptying time. Our study has not established a significant difference in lumefantrine plasma levels between adults and children similar to other previous studies. The lack of the difference in lumefantrine exposure between the two age groups could be due to a small number of young children as most of the children in our study were older children. Older children have greater food intake and low vomiting tendency than young children (Borrmann et al., 2010) which could explain a high absorption compared to young children.

Although inadequate lumefantrine concentrations (<175ng/ml & <200ng/ml) in real life did not affect treatment outcomes in terms of ACPR and recurrence of parasites at individual level, its contribution to the risk for development of parasite resistance at population level due to the parasite exposure to sub-optimal concentrations cannot be ruled out thus posing a major public health issue.

Our study has recorded a high proportion of patients with residual lumefantrine concentrations despite patients declaring they had not taken ALU tablets for the past 28 days. Hodel et al recorded similar findings (Hodel et al., 2009). The presence of low residual lumefantrine concentrations is alarming since exposure of parasites to sub-optimal concentrations may select for resistant parasites. The high proportion of patients with residual lumefantrine concentrations indicates self-medication is common to most patients before coming to hospitals and there is a high drug selection pressure to parasites in the population. Residual drug levels may also expose patients to toxicity upon initiating the treatment. A large proportion of patients with drug concentration prior treatment also suggests that, the patient's history may be not reliable thus there is a need for measuring plasma concentrations at enrollment prior initiation of treatment in antimalaria efficacy studies in malaria endemic regions since the impact of residual plasma concentrations to treatment outcomes is unknown. There may be a need for a modification in the WHO guidelines for antimalarial drugs efficacy surveillance specifically in malaria endemic countries. A similar suggestion was made by Hodel et al. (2009).

Limitations

We collected samples on 24 hours basis thus samples between time 0 hours and 24 hours were not collected thus limiting the predictions of absorption related kinetics. Another shortcoming of the present study is unavailability of CYP3A4*1B and CYP3A5*3 data in order to have a pharmacokinetic (PK)/pharmacogenetic (PG) picture. However, our recent review (to be published else where) has established a broader PG/PK picture on antimalarial drugs used for uncomplicated *P. falciparum* malaria patients in terms of drug exposure, efficacy and safety in Sub-Saharan Africa.

Conclusion

Lumefantrine plasma concentrations below cut-off points (175ng/ml and 200ng/ml) on day 3 and 7 did not influence treatment outcomes among uncomplicated malaria patients with uncontrolled dietary intake. Age, sex and level of parasitemia at enrollment did not predict for both day 3 and 7 lumefantrine plasma concentrations.

Authors' contributions

KJM participated in proposal development, sample collection, genotyping of MSP-1 and 2, data analysis and manuscript drafting. ETK, SM and AL carried out data analysis and manuscript reviewing. EK and GS participated in proposal development, supervision of the research group, revising and approving the manuscript for publication.

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Ethical approval and consent to participate

Ethical and study approval was granted by the joint Catholic University of Health and Allied Sciences (CUHAS) /Bugando Medical Centre (BMC) Institutional Review Board. All patients or parent/guardian signed a written informed consent.

Consent for publication

Not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. All authors declare no competing interests

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