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Research Article

Early Changes in *Plasmodium falciparum* Asexual and Sexual Populations in Children with Acute Infections Following Treatment with Artemisinin-Based Combination Drugs

Akintunde Sowunmi,[1,2] Titilope M. Okuboyejo,[1] Grace O. Gbotosho,[1,2] and Christian T. Happi[1,2]

[1] *Department of Pharmacology and Therapeutics, University of Ibadan, Ibadan, Nigeria*
[2] *Malaria Research Laboratories, Institute for Medical Research and Training, University of Ibadan, Ibadan, Nigeria*
Address correspondence to Akintunde Sowunmi, akinsowunmi@hotmail.com

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Abstract Artemisinin-based combination therapies (ACTs) may influence malaria transmission but the early changes in parasite populations have not been frequently evaluated. The changes in *Plasmodium falciparum* asexual and sexual populations in the first 16 h following treatment with artemether-lumefantrine (AL) or artesunate-amodiaquine (AA) were evaluated in 443 children with acute infections. The effects of gametocyte density on gametocyte sex ratio (GSR) were characterized in another cohort of 52 children treated with AL and AA. Stages of asexual and sexual parasites in peripheral blood were determined morphologically. In 167 children there were significant increases in peripheral asexual parasitemia at 1 h, and in 15 of these, an insignificant increase in gametocytemia at 1 h, followed thereafter by a precipitous and significant fall in all patients. Time-course of GSR showed a female-male-female-biased cycle at 0 h, 4 h, and 8 h. Pre-treatment GSR and time-course of GSR post-treatment were independent of density in the additional cohort of 52 gametocyte carriers treated with AL or AA. Population changes were similar in AL- and AA-treated children. Treatment with AL or AA is associated with early increases in asexual and sexual parasites and is closely followed by rapid elimination of these parasites.

Keywords *P. falciparum*; gametocytes; sex ratio; transmission; ACTs; children

1 Introduction

Artemisinin-based combination therapies (ACTs) are currently recommended first-line treatments of *Plasmodium falciparum* malaria globally because they rapidly reduce asexual parasite biomass, gametocyte carriage and density, and subsequently the chances of transmission [47]. In low transmission settings, for example, in Thailand, ACTs reduced transmission considerably [23]. However, it is unclear whether ACTs can reduce transmission in endemic settings in Africa after consistent use. Although much is known of the rapid clearance of asexual parasitemia between 12 h and 24 h after administration of ACTs, little is known of their effects on the dynamics of asexual parasites in the hours preceding this period.

The uptake from a human blood meal of viable *Plasmodium falciparum* male and female gametocytes and their subsequent development into gametes in the mosquito vector are essential to malaria transmission [2, 30]. Mosquito infectivity after a human blood meal, and subsequent transmission, are dependent on gametocyte density [18, 26, 42] and may be perturbed by the gametocyte sex ratio (GSR) in the blood meal [14, 26]. In the latter context, in experimental studies, Robert et al. [26] showed that the proportion of infected mosquitoes and the mean oocyst load increased as sex ratio in the infected human blood meals increased towards 0.5. Mitri et al. [14] showed that in cultured gametocytes, at low gametocyte densities, increasing male sex ratio increases mosquito infectivity rate and consequently it reduces infectivity rate at high gametocyte densities. Although much is known of the lethal effects of artemisinin drugs on young gametocytes [12] and the reduction in mosquito-infectivity in individuals treated with ACTs [17], little is known of the effects of ACTs on the population dynamics of gametocytes and GSR in the first hours following treatment. Therefore, an understanding of the dynamics of asexual and sexual parasites in the early hours following ACTs may assist in identifying the basis of their effects on transmission.

The present study evaluated the early changes in the populations of asexual and sexual parasites in the first 16 h following treatment with ACTs in a group of 443 children with acute falciparum malaria in an endemic area. The primary aim was to use the dynamics of asexual and sexual parasitemias to characterize the basis of the effects of ACTs on malaria transmission. In addition, the study examined the effects of ACTs on the interaction between gametocyte density and sex ratio in another cohort of 52 children with

Table 1: Treatment regimens and time of study.

Drugs	Regimens[†]	No. of patients	Year
AL[*]	Artemether (20 mg) plus lumefantrine (120 mg) given according to body weight: 5–14 kg received 1 tablet, 15–24 kg received 2 tablets, 25–34 kg received 3 tablets, $>$ 34 kg received 4 tablets at presentation, 8 h later and at 24, 36, 48 and 60 h after first dose.	170	2009-10
AAcp[**]	Artesunate 4 mg/kg daily for 3 d plus amodiaquine 10 mg/kg daily for 3 d (co-packaged) or according to age: 1-5 years received 1 tablet each of AA,; 6–10 years received 2 tablets each; 11–15 years received 3 tablets each.	193	2009-10
AAcf[***]	Co-formulated amodiaquine plus artesunate given as follows: children weighing $\geq$ 9–$<$ 18 kg or aged 1–5 years received 0.5 tablet; children weighing $\geq$ 18–36 kg or aged 6–13 years received 1 tablet; children weighing $\geq$ 36 kg or aged $\geq$ 14 years received 3 tablets.	72	2009-10

[†]All drugs were administered orally.
[*]AL, artemether-lumefantrine; AAcp, amodiaquine plus artesunate co-packaged; AAcf, amodiaquine plus artesunate co-formulated.
[**]Each tablet of amodiaquine contains 153 mg base and each tablet of artesunate contains 50 mg.
[***]Each co-formulated tablet contains artesunate 100 mg and amodiaquine base 270 mg.

gametocytemia before and following treatment with ACTs. The secondary aim was to examine if ACTs modify the density-dependent impact of sex ratio that may contribute to mosquito infectivity recently reported in vitro [14].

2 Methods

2.1 Patients

Patients were recruited from April 2009 to March 2010 at the malaria clinic of the University College Hospital in Ibadan, southwest Nigeria, an endemic area of malaria [28], into antimalarial efficacy studies of artemether-lumefantrine (AL) or artesunate-amodiaquine (AA). Patients were enrolled if their attending relatives gave informed consent; they were aged 0.5–15 years, had single species asexual *P. falciparum* parasitemia $\geq$ 2,000/μL blood, did not have a history of significant antimalarial drug intake in the 2 weeks preceding presentation, and had a good likelihood of being able to complete 4–6 weeks of follow-up. Patients with severe malaria [46] or serious underlying diseases (renal, cardiac or hepatic) or severe malnutrition were excluded from the study. Additionally, all children who were gametocyte carriers before and after treatment with AL or AA between 2006 and 2009 were included in the study. These latter children were used to examine the influence of ACTs on gametocyte density-dependent impact of sex ratio. The study received approval from the local ethics committee.

2.2 Drug management

At enrolment (day 0) and at follow-up on days 1–7, 14, 21, 28, 35, and 42, patients underwent full physical examination. Patients were randomized to receive AL or AA (co-packaged) between April and September 2009 ($n = 210$), and to AL or AA (co-packaged) or AA (co-formulated) between October 2009 and March 2010 ($n = 225$). Drug treatment was according to standard schedules (Table 1) [37].

2.3 Laboratory investigations

Thin and thick blood films were obtained from a finger prick for species identification and quantification of asexual and sexual parasitemia before treatment (0 h), and at 1, 2, 4, 8, 16, 24, 36, 48, and 72 h, and on days 4–7, 14, 21, 28, 35, and 42 after treatment began.

Quantification of asexual and sexual parasites in thick films was done against 500 and 1000 leukocytes, respectively, assuming a leukocyte count of 6000/μL blood. Stages of asexual parasite development in peripheral blood were estimated as follows: R1: width of cytoplasm/diameter of nucleus $\leq$ 0.5, that is, ring form aged 0–$<$ 6 h. R2: width of cytoplasm/diameter of nucleus $>$ 0.5–$<$ 1, that is, ring form aged 6–$<$ 18 h. R3: width of cytoplasm/diameter of nucleus $>$ 1, that is, ring form aged 18–24 h. Late trophozoite, that is, aged $>$ 24–40 h and containing 2 nuclei. Schizonts, that is, aged $>$ 40 h and containing at least 3 nuclei [13].

All gametocytes were sexed if gametocytemia was $>$ 10/μL blood and according to the following criteria [2, 26]: males (microgametocytes) are smaller than females (macrogametocytes), the nucleus is larger in males than females, the ends of the cells are rounder in males and angular in females, with Giemsa the cytoplasm stains purple in males and deep blue in females, and the granules of malaria pigment are centrally located in females and more widely scattered in males. Gametocytes were classified morphologically as male or female if at least three of the five criteria stated above were present. The sex ratio was defined as the proportion of gametocytes in peripheral blood that were male [21, 44, 45]. GSR was considered male-biased if sex ratio was $>$ 0.5. Gametocytes were considered immature or young when they are Stage I–III, or mature when they are Stage IV–V [31]. Gametocytes were classified as Stage II if they were enlongated in the erythrocytes or had a D-shape and distinguished from late trophozoites with 2 nuclei. Blood obtained from a finger prick into heparinized capillary tubes was used to estimate hematocrit.

Since there were rapid increases followed by rapid decreases in asexual and sexual parasite populations, the areas inscribed by the increases and decreases roughly represented triangles. The area was calculated as half the

Table 2: Characteristics of 435 children enrolled in the study.

Variable	AA (273)	AL (170)	ALL (443)	P value
Male:female	136:137	89:81	225:210	.67
Age (year)	7.1 ± 0.2 [1–15]*	6.7 ± 0.2 [1–13]	7.0 ± 0.1 [1–15]	.18
< 5 years	59	51	110	.06
Duration of illness (d)	2.6 ± 0.1 [1–7]	2.8 ± 0.1 [1–10]	2.7 ± 0.1 [1–10]	.18
Weight (kg)	19.6 ± 0.4 [6–46]	18.8 ± 0.5 [6–36]	19.3 ± 0.32 [6–46]	.23
Temperature °C	38.4 ± 0.1 [36–41]	38.5 ± 0.1 [36–41.1]	38.4 ± 0.1 [36–41.1]	.56
> 40 °C	22	15	37	.91
Hematocrit (%)	32.3 ± 0.3 [17–45]	32.1 ± 0.4 [17–40]	32.2 ± 0.2 [17–45]	.61
$< 30\%$	51	34	83	.82
Number with hepatomegaly	62	61	123	.003
Number with splenomegaly	36	22	58	.94
GMPD (/μL of blood)	64546	78397	70129	.053
Range	2791–1125000	2837–1105263	2837–1125000	
GMGD (/μL of blood)	48	88	55	.06
Range	6–168	72–108	6–168	

*Values are given as mean $\pm$ sem [range], GMPD geometric mean parasite density, GMGD geometric mean gametocyte density.
AA, amodiaquine-artesunate; AL, artemether-lumefantrine.

time interval between the increases and decreases, that is, half the base of the triangle multiplied by the height, that is, the numerical increase in asexual or sexual parasites from baseline. These areas were assumed, theoretically, to represent the biomass of the parasites mobilized and eliminated from the body.

2.4 Data analysis

Data were analyzed using version 6 of the *Epi-Info* software [4] and the statistical programme *SPSS for Windows* version 10.01 [41]. Variables considered in the analysis were related to the densities of *P. falciparum* gametocytes and trophozoites. Proportions were compared by calculating χ^2 with Yates' correction or by Fisher exact test. Normally distributed, continuous data were compared by Student's t-test, and analysis of variance (ANOVA). Data not conforming to a normal distribution were compared by the Mann-Whitney U-tests or the Kruskal-Wallis tests or by Wilcoxon ranked sum test. All tests of significance were two-tailed. P-values of < 0.05 were taken to indicate significant differences. Data were (double)-entered serially using the patients' codes and were only analyzed at the end of the study.

3 Results

3.1 Characteristics of patients enrolled in the study

The characteristics of patients enrolled in the study are summarized in Table 2. One hundred and ten children were aged < 5 years. Geometric mean asexual parasitemia was 70,129/μL, 95% CI 60,072–79,231.

3.2 Changes in asexual parasitemia during first hours following treatment

Overall, there was an increase in asexual parasitemia in 167 of 443 children (62 of 170 treated with AL and 105 of 273 treated with AA, $P = .74$) during the first 2 h after treatment commenced. Figure 1(a) shows the changes in asexual parasite density in the first 8 h following treatment with ACTs. In the 167 children, there were significant increases in asexual parasitemia at 1 h after commencing treatment compared with pre-treatment (74,291 v 60,101/μL, $P < .001$) and was immediately followed by a precipitous and significant fall in parasitemia at 8 h onward. Enrolment characteristics of those with increase or no increase in asexual parasitemia at 1 h were similar: age 7.1 ± 0.2 v 6.7 ± 0.2 years, $P = .12$; body temperature 38.4 ± 0.1 v 38.5 ± 0.1 °C, $P = .48$; hematocrit 32.6 ± 0.3 v $32.6 \pm 0.5\%$, $P = .91$. However, enrolment asexual parasitemia was significantly lower in those with increase compared with those without increase 60,101 (range 2,657–1,125,000) v 81,429 (range 2,837–1,105,263)/μL, $P = .003$. In addition, patients with no increase in asexual parasitemia had no gametocytemia at enrolment.

Figure 1(a_1) shows that increases and decreases in those treated with AL and AA between 1 and 2 h roughly represent a triangle. The areas inscribed by the triangles were similar for the two drugs (47,663 v 50,853 af (asexual forms)/μL.h, $P = .28$).

3.3 Changes in gametocyte density during first hours following treatment

Gametocytemia was present at enrolment in 17 of 443 children and in 2 children by the end of one week after treatment began. The difference in the proportions was significant ($\chi^2 = 10.5$, $P = .001$). Overall, gametocytemia increased in 14 of 17 gametocyte carriers at 1 h and peaked at 4 h, but there was no significant increase in density ($P = .18$) (see Figure 1(b)). Fifteen of 17 gametocyte carriers also had associated increase in asexual parasitemia

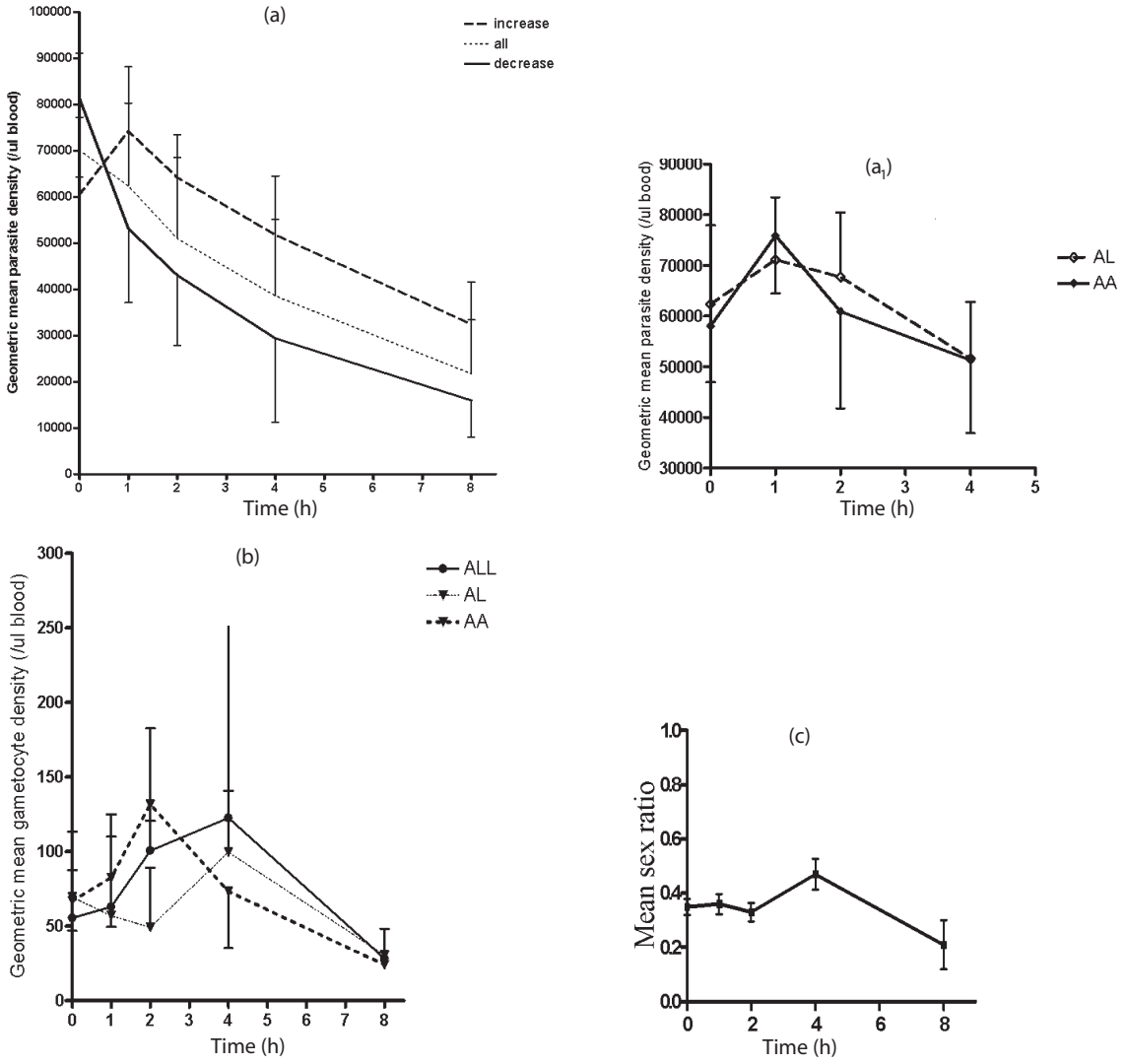

Figure 1: Variations in asexual parasite density (a), gametocyte density (b) and gametocyte sex ratio in the first 16 h following treatment with artemether-lumefantrine (AL) or artesunate amodiaquine (AA). Error bars indicate standard error of mean.

at 1 h. Peak increase in gametocyte density occurred at 2 h and 4 h in patients treated with AA and AL, respectively, and represented two triangles with similar areas: (62.3 v 112.7 sf (sexual forms)/μL.h, $P = .57$ (see Figure 1(b)). In the two children who had gametocytemia at the end of one week, gametocytes were no longer detected in peripheral blood at the end of two weeks.

3.4 Changes in gametocyte sex ratio during the first hours following treatment

Figure 1(c) shows the variation in gametocyte sex ratio in these children. Overall, there was a female-male-female-biased cycle at 0 h, 4 h, and 8 h, ($n = 17$, 10, 3, resp.), respectively. The sex ratio at 4 h was not significantly higher than at 0 h (weighted mean 0.39 v 0.33, $P = .44$) but the sex ratio at 8 h was significantly lower than at 0 h (weighted mean 0.21 v 0.39, $P = .047$).

3.5 Stages of gametocytes found in peripheral blood during the first hours following treatment

Mature male and female gametocytes were mainly found in peripheral blood in those with gametocytemia (Figure 2). However in 7 children, who had no young gametocytes in their pre-treatment blood slides, young gametocytes were also present in a very small number within 8 h of initiating treatment (Figure 2). In these children, young gametocytes were not found after 16 h.

3.6 Relationship between gametocyte density and gametocyte sex ratio during the first hours

Figure 3 shows that there was no significant relationship between parasite density and sex ratio at enrolment ($r = 0.08$, $P = .9$, $n = 17$, 13, 14, 10, and 3 at 0, 1, 2, 4, and 8 h, resp.).

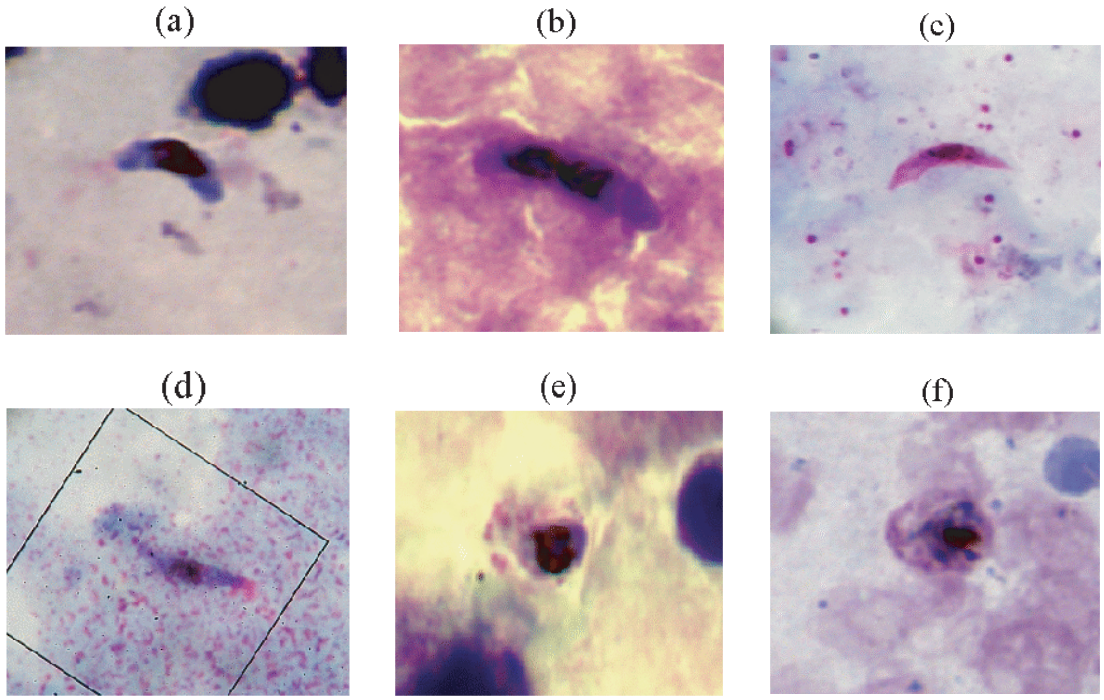

Figure 2: Light micrograph of *P. falciparum*; (a)-(b) mature male gametocytes, (c)-(d) mature female gametocytes, (e)-(f) young gametocytes following treatment with artemether-lumefantrine (AL) or artesunate amodiaquine (AA). Note the D-shaped appearance suggesting stage IIb and oat grain appearance suggesting stage IIa.

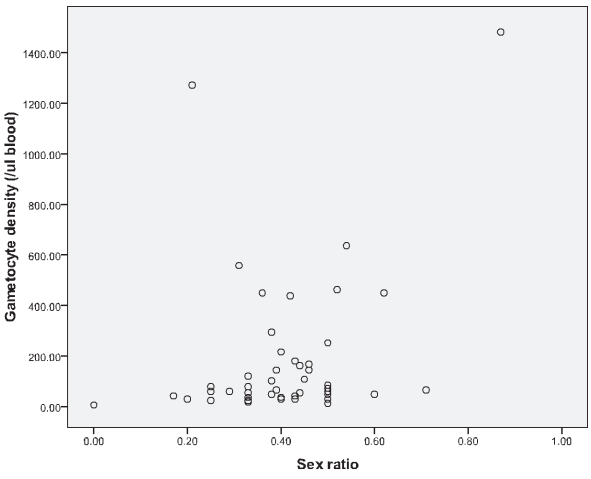

Figure 3: Relationship between gametocyte density and gametocyte sex ratio in the first 8 h following treatment with artemether-lumefantrine and artesunate-amodiaquine. Data for both drug combinations have been combined.

3.7 Stages of asexual parasites in peripheral blood during the first hours following treatment

Table 3 summarizes the stages of parasites in peripheral blood in 40 randomly selected children, who had increase (20 children) or no increase (20 children) in asexual parasitemia in the first 4 h of treatment. In each group, the proportion of each stage is similar at 0 h and at 2–4 h. However, in patients with increase in asexual parasitemia in the first 2 h, the proportion of ring-shaped asexual parasites 18–24 h old increased significantly at 2–4 h after treatment ($P = .02$). Schizonts were found in peripheral blood of 1 child at 0 h and in 4 children at 2–4 h (Figure 4).

3.8 Effects of ACTs on relationship between density and sex ratio in children before and after treatment

In order to examine more carefully the relationship between gametocyte density and sex ratio, an evaluation of all patients treated with AL or AA between 2006 and 2009 was carried out. There were 52 children who carried gametocytes in the first 8 days.

Figure 5(a) shows the distribution of gametocyte densities and sex ratios at enrolment in 52 gametocyte carriers. Sex ratio did not alter significantly with gametocyte density: the proportion of patients with a male-biased sex ratio was 10/22 (45%) and 12/30 (40%) when gametocyte densities were $< 20/\mu$L and $\geq 20\,\mu$L, respectively ($\chi^2 = 0.012$, $P = .91$). The weighted mean sex ratios were similar at enrolment in those with low and high gametocyte densities (0.43 v 0.35, $P = .28$). Prior to treatment with AL or AA, there was no significant correlation between gametocyte density and sex ratio ($r = -0.14$, $P = .29$) (Figure 5(b)).

Figure 6 shows the temporal changes in GSR in children with low or high gametocytemia. Irrespective of density,

Table 3: Stages of asexual parasites in the periphery during the first hours following treatment.

	Increase in asexual parasitemia ($n = 20$)			No increase in asexual parasitemia ($n = 20$)		
Stages of parasite	Before	After[†]	P value	Before	After[†]	P value
Very young rings (0–< 6 h) [R1]	0[‡]	0[‡]	—	0[‡]	0[‡]	—
Young rings (6–< 18 h) [R2]	9	1	0.8	2	3	1.0
Mature rings (18–24 h) [R3]	10	18	0.02	15	14	1.00
Late trophozoites (24–40 h)[*]	0	1	0.5	3	3	0.7
Schizonts (> 40 h)[**]	1	4	0.3	0	0	—

[†]2–4 h after oral ACTs.
[‡]Indicate number of patients.
[*]Containing 2 nuclei.
[**]Containing at least 3 nuclei.

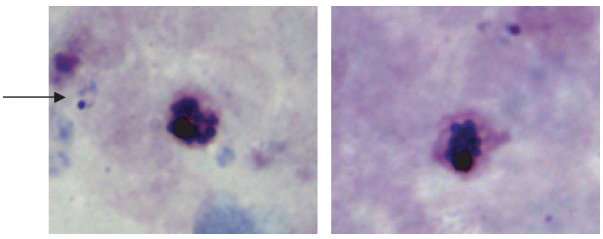

Figure 4: Schizonts obtained from two patients, note the ring form in the background.

GSR was female-biased during follow-up. There was no difference in sex ratio values at all times during follow-up in children with low or high gametocyte density. For example, on day 3, mean sex ratio values were 0.40 ($n = 6$) and 0.13 ($n = 12$) in patients with low and high gametocyte densities, respectively.

4 Discussion

The study showed increases in asexual and sexual parasitemia in approximately 40% of patients in the first few hours of initiating treatment with AL or AA. Virtually all of gametocyte carriers who had increases in their gametocytemias also had increases in their asexual parasitemias suggesting a common mode(s) for increases in asexual and sexual parasitemias. Increase was significant for mature rings 18–24 h old. The increases were followed by a rapid decline in parasitemias. This process and sequence, taken together, may be beneficial in a number of ways: they may reduce the subsequent sequestered mass and limit the deleterious consequences of sequestration and/or facilitate removal, by pitting, of parasites from the red cells by the spleen. Thus, increases (the hypothetical mobilization) may be effects of ACTs during early hours of treatment and may contribute to their parasitological actions. The latter is supported by ex vivo parasite viability studies that showed that the viability of circulating asexual parasites is significantly reduced by artemisinin drugs about 6–12 h after start of treatment compared to quinine or sulfadoxine-pyrimethamine [15,40]. An alternative explanation for the

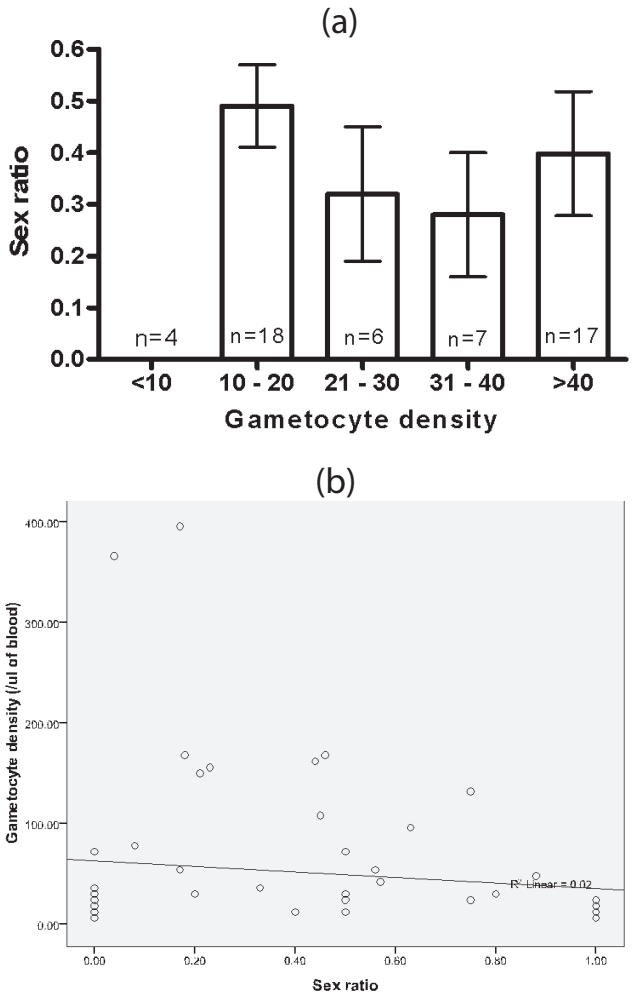

Figure 5: (a) Distribution of gametocyte densities and sex ratios at enrolment in a cohort of 52 children before treatment with artemether-lumefantrine or artesunate-amodiaquine. Data for both drug combinations have been combined. (b) Relationship between gametocyte density and sex ratio at enrolment in a cohort of 52 children before treatment with artemether-lumefantrine or artesunate amodiaquine. Data for both drug combinations have been combined.

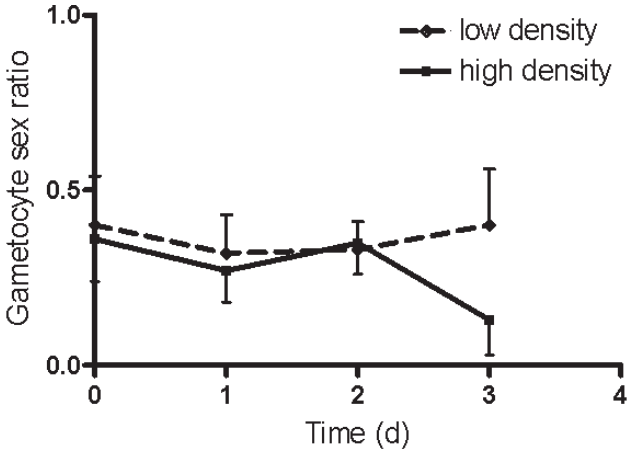

Figure 6: Time-course of sex ratio in 52 gametocyte carriers with high or low gametocytemia following treatment with artemether-lumefantrine or artesunate-amodiaquine.

increases in the number of late stage asexual parasites found in peripheral blood is the asynchrony of the infections and the differences in time interval between release of merozoites and the time of presentation.

Gametocyte carriage in the children, on presentation, was very low (3.8%) and, compared with carriage in the years preceding 2009, showed a significant decline: gametocyte carriage on presentation was 21.6% (21/97) in 2002, 22.7% (41/181) in 2004, 13% (51/391) in 2005, 12% (42/350) in 2007–2008, and 2% (9/435) in 2009 [34,35,36,39]. However, these rates are likely to be underestimates because of significant submicroscopical gametocytemia detectable by polymerase chain reaction (PCR) in children from this and other endemic areas [10, 18,29] that may substantially contribute to the already large gametocyte reservoir and mosquito infectivity even after ACTs [18] or primaquine. Additionally, an efficient vector, *Anopheles gambiae*, and environmental conditions permit easy transmission. Thus, in this endemic area, despite significant decreases in gametocyte carriage in children with acute malaria after 5 years of adoption of ACTs as first-line treatments, asexual parasite rate of 37%, a measure of transmission intensity, is little affected [6].

Late trophozoites, schizonts, and young gametocytes of *P. falciparum* are scarcely found in peripheral blood of African children [32]; young gametocytes are sequestered in bone marrow and spleen [5,32,43]. In the present cohort of children, schizonts and stage IIb gametocytes were found in peripheral blood within 8 h of initiating treatment and they disappeared within 16 h in 11 (2.5%) of the children. If the presence, in peripheral blood, of young gametocytes, can be confirmed during the early hours of initiating ACTs and their disappearance after 24 h of treatment, by immunoassay or reverse transcription-PCR, this would suggest mobilization from sequestered sites by ACTs. Following ACTs use, 60–90% of all gametocytes would have emerged by 24 h [22, 39]. Therefore, as insensitivity develops to ACTs in this endemic area, and as has been shown in areas of Southeast Asia where insensitivity has now developed to ACTs, gametocyte carriage would increase [1].

Overall, as expected, GSR was female-biased on enrolment and remained so following treatment until gametocytemia was no longer detectable after 3 days of initiating treatment. However, there was no significant correlation between gametocyte density and sex ratio before and after four hours following initiation of treatment and even in the additional cohort of 52 children. A correlation between density and gametocyte sex ratio has been reported in children treated with non-artemisinin drugs from this endemic area [34]. If low gametocytemia is associated with increasing sex ratio, a negative correlation would have been expected. Thus, it is probable that AL or AA may influence the parasite's mechanism of facultatively adjusting their sex allocation in response to low gametocytemia. Alternatively, they may have little or no effects and the observations in the present study may reflect a natural phenomenon.

In this endemic area, falciparum infections in children are multi-clonal [9,8,11,38] and in accordance with evolutionary theory that predicts that sex ratio becomes less female-biased as clone number increases due to competition among clones for mating success—local mate competition (LMC) [7,16,25], increasing clone number is associated with less female-biased sex ratio in naturally infected children [38]. However, rapid elimination of asexual parasites by AL or AA may, presumably, significantly reduce the number of circulating clones and produce a less male-biased sex ratio (MBSR) when the remaining viable committed asexual parasites eventually develop to gametocytes. This effect should become apparent after one week of ACTs, since development from asexual to sexual parasites takes approximately 10 days [30]. This is consistent with a recent finding from this endemic area showing that by the end of 7–14 days, GSR in children treated with artesunate or artesunate-amodiaquine became female-biased irrespective of pre-treatment GSR [35].

Parasites are also thought to alter their sex allocation to ensure successful fertilization by producing enough males to fertilize its females particularly when gametocyte density is low and gametocyte viability compromised, for example, from host immune response—the so-called fertilization insurance [45] and as the infection progresses and anaemia develops [19,20,27]. ACTs may not worsen the anaemia of malaria infection [3,33,37] and therefore less likely to promote the male-biased sex ratio associated with anaemia.

In order to quantify the increases in parasites in peripheral blood over time, the concept of area of triangle resulting from the sharp increase and rapid decline of

parasitemias was used. These triangles showed that increases in asexual and sexual parasites overlapped, but the sex ratio triangle occurred later. The reasons for the lag are unclear. The time-course and areas of the triangles for asexual and sexual parasites for AL- and AA-treated children were not similar suggesting that artesunate in AA may cause relatively early increases compared with artemether in AL. These differences, however, did not translate to a significant difference in asexual and gametocyte clearance in patients treated with AA and AL.

Based on the findings in the first few hours following administration of AL or AA to individuals with acute falciparum malaria, and on the time-course of GSR in the one week following initiation of treatment, it would appear that a rapid elimination of large parasite biomass involving both committed and non-committed parasites prevented these parasites from progressing to development into gametocytes and averting changes in GSR that may favor increased mosquito infectivity.

There are limitations of the present study. Although gametocyte densities and sex ratio changes were evaluated and used to surmise the effects of ACTs on transmission, there is a need to consider gametocyte viability in order to reveal the full impact of ACTs on transmission. Additionally, gametocytes sex ratio data were derived from few patients necessitating caution with extrapolation. The assumption that the hypothetically mobilized parasites were actually all eliminated cannot be proven completely. It is possible that some of the parasites may escape killing effects of ACTs. Overall, the sequestered parasite biomass is unknown in any of the patients making it difficult to estimate the fraction of the sequestered biomass that were hypothetically mobilized. The contribution of immunity to the observed changes could not be evaluated. Finally AL or AA concentrations were not measured making it difficult to examine the relationship between the drug effects and drug concentrations during the critical early stage of drug administration.

Currently, primaquine, the gametocytocidal 8-amino-quinoline, when combined with artemisinin drugs is thought to accelerate gametocyte clearance in infected patients [24]. However, the effects of these combinations or of primaquine alone on the early changes in sexual populations during the first few hours of initiating treatment are unknown. Additionally, the effects of primaquine alone or in combination with artemisinin drugs on gametocyte sex ratios in the first few hours after treatment are also unknown. It is essential that these change if any be evaluated.

In conclusion, treatment with AL or AA is associated with early increases in peripheral asexual or sexual parasites during the first 8 h of initiating of therapy in 40% of children, and is followed by early, rapid elimination of large parasite biomass that may contribute to reduction in transmission.

Nomenclature

ACT: Artemisinin combination therapy
GSR: Gametocyte sex ratio
AA: Artesunate-amodiaquine
AL: Artemether-lumefantrine
ANOVA: Analysis of variance

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