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Plasmodium falciparum gametocyte sex ratios in symptomatic children treated with antimalarial drugs

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ABSTRACT

The sex ratios of *Plasmodium falciparum* gametocytes, defined as the proportion of gametocytes in peripheral blood that were male, were evaluated in 1609 children with acute, symptomatic, uncomplicated malaria, pre- and post-treatment with various antimalarial drugs, over an 8-year period (1999–2006) in an endemic area of southwest Nigeria. Gametocyte carriage on presentation was 10% (162 children). In 162 children in whom 5797 gametocytes were sexed on presentation, the weighted mean sex ratio was 0.18 (95% confidence interval 0.13–0.25). Following therapy, in 446 children in whom 38,519 gametocytes were sexed, the weighted mean sex ratio was 0.38 (95% CI 0.33–0.43) on day 3 and 0.70 (95% CI 0.63–0.75) ($P < 0.000001$) by day 7 after therapy commenced. Non-artemisinin monotherapy significantly increased sex ratio producing a male-biased ratio, but artemisinin combination therapy significantly reduced the sex ratio producing a female biased ratio. Pre-treatment sex ratio correlated negatively with haematocrit ($r = -0.229$, $P = 0.003$) or gametocytaemia ($r = -0.435$, $P < 0.0001$) but not with other clinical or parasitological parameters. The ratio of the sex-specific half lives male:female, the 'gametocyte maleness index' (GMI), was >1 with non-artemisinin monotherapy but <1 with artesunate and artemisinin-based combinations. In a multiple regression model, anaemia, low gametocytaemia, and enrolment before 2004 were independent predictors of a male-biased sex ratio pre-treatment. A pre-treatment haematocrit <25%, enrolment before 2004 and treatment with non-artemisinin monotherapy were independent predictors of a male-biased sex ratio 7 days postinitiation of therapy. These findings may have implications for malaria management efforts in sub-Saharan Africa.

1. Introduction

Gametocytes, the sexual forms that arise when a small proportion of asexual forms of *Plasmodium spp*. undergo developmental switch, are essential for the transmission of malaria after a female *Anopheline* mosquito obtains a human blood meal. In endemic areas, virtually all age groups contribute to the reservoir of infection, but children older than 1 year and adults constitute the major reservoir (Githeko et al., 1992; Drakeley et al., 2000; Bonnet et al., 2003; Ross et al., 2006). In African children with acute *Plasmodium falciparum* infections, gametocyte carriage may vary from 8 to 17% (Akim et al., 2000; von Seidlein et al., 2001; Sowunmi et al., 2004a,b). These rates may be considerably modified following treatment with antimalarial drugs (Sowunmi and Fateye, 2003a; Sowunmi et al., 2004a,b; Bousema et al., 2006).

Many variables, for example, high gametocyte density, a less female-biased sex ratio, and antimalarial drugs have been shown to enhance gametocyte infectivity to mosquitoes (Tchuinkam et al., 1993; Robert et al., 1996; Hogh et al., 1998; Bousema et al., 2006). In a recent study in a Senegalese village, Robert et al. (2003) found a female-biased sex ratio in adults and children who were largely asymptomatic for *P. falciparum* infections. In addition, these authors showed that a male-biased sex ratio was associated with anaemia and a wave of gametocytes with female-biased sex ratio. It is not clear whether these findings are applicable to the individuals resident in other endemic areas in West Africa or indeed in children with acute, symptomatic malaria, or if there are other factors that may be associated with a male-biased sex ratio in children from other endemic areas of West Africa.

Community management of malaria transmission, in addition to other measures, requires evaluation of factors that may influence gametocyte sex ratio [defined as the proportion of gametocytes in peripheral blood that were male (Pickering et al., 2000)] to produce ratios that discourage transmission. In addition, it requires monitoring the effects of currently available antimalarial drugs on gametocyte generation and sex ratios. There is little information

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Table 1
Treatment regimens of the children enrolled in the study[a].

Drugs[b]	Regimens[c]
CQ	30 mg/kg of chloroquine base over 3 days, that is, 10 mg/kg daily
AQ	30 mg/kg of amodiaquine base over 3 days, that is, 10 mg/kg daily
PS	Pyrimethamine–sulfadoxine given as 25 mg/kg of the sulfadoxine component at presentation
COT	Cotrimoxazole given as 25 mg/kg of the sulphamethoxazole component twice daily for 5 days
AS	Artesunate given as 28 mg/kg over 7 days, that is, 4 mg/kg daily
PSP	Pyrimethamine–sulfadoxine given as in PS above plus probenecid at 20–25 mg/kg in two divided doses daily for 3 days
AL	Artemether (20 mg) plus lumefantrine (120 mg) given as follows: 5–14 kg received 1 tab; 15–24 kg received 2 tab; 25–34 kg received 3 tab; >34 kg received 4 tab at presentation, then 8 h later and at 24, 36, 48 and 60 h after first dose
ASAQ	Artesunate given as in AS above plus amodiaquine given as in AQ above
ASP	Amodiaquine given as in AQ above plus sulfalene–pyrimethamine given as 25 mg/kg of the sulfalene component

CQ, chloroquine; AQ, amodiaquine; PS, pyrimethamine–sulphadoxine; COT, cotrimoxazole; AS, artesunate; PSP, pyrimethamine–sulphadoxine plus probenecid; AL, artemether plus lumefantrine; ASAQ, artesunate plus amodiaquine; ASP, amodiaquine plus sulfalene–pyrimethamine.

[a] See Sowunmi and Fateye (2003a) and Sowunmi et al. (2007a).

[b] 162, 648, 184, 53, 120, 78, 90, 183 and 91 children were enrolled in CQ, AQ, PS, COT, AS, PSP, AL, ASAQ and ASP groups, respectively.

[c] All drugs were administered orally.

on the effects of antimalarial drugs on gametocyte sex ratios. Such information is necessary to optimize control of malaria transmission.

In order to address these issues in an area of intense transmission, we prospectively evaluated, over an 8-year period, gametocyte sex ratios in cohorts of children participating in antimalarial drug efficacy studies involving chloroquine, amodiaquine, pyrimethamine–sulphadoxine, cotrimoxazole, artesunate, pyrimethamine–sulphadoxine plus probenecid, artemether–lumefantrine, artesunate–amodiaquine and amodiaquine–sulphalene–pyrimethamine in southwestern Nigeria. Our aims were to determine, over time, the spectrum of sex ratios in children presenting with acute, symptomatic, apparently uncomplicated falciparum malaria; evaluate the temporal changes in sex ratios post-treatment with antimalarials; determine the factors influencing a male-biased sex ratio, pre- and post-treatment with antimalarial drugs.

2. Patients and methods

Patients were recruited from 1999 to 2006 at the malaria clinic of the University College Hospital in Ibadan, southwest Nigeria, an endemic area of malaria (Salako et al., 1990) into various antimalarial efficacy studies and were enrolled if the following criteria were met: an age 0.5–14 years, fever or history of fever in the 24–48 h preceding presentation, pure *P. falciparum* parasitaemia $\geq$2000 μl^{-1} blood, absence of concomitant illness, negative urine tests for 4-aminoquinoline (Dill-Glazko) and sulfonamides (lignin), and written informed consent of a parent or guardian. Patients with severe malaria (WHO, 2000) or serious underlying diseases (renal, cardiac or hepatic) or severe malnutrition were excluded from the study. The studies received approval from the local ethics committee.

Drug treatment was according to standard schedules (Sowunmi and Fateye, 2003a; Sowunmi et al., 2007b; see Table 1 for details). At enrolment (day 0) and at follow-up on days 1–7, 14, 21, and 28 (up to 2003) and on 1–3, 7, 14, 21, 28, 35 and 42 (after 2003), patients underwent full physical examination and thin and thick blood films examination for quantification of asexual and sexual parasitaemia. Quantification of asexual and sexual parasites in thick films was done against 500 and 1000 leukocytes, respectively assuming a leukocyte count of 6000 μl^{-1} blood. All gametocytes were sexed if gametocytaemia $\geq$10 μl^{-1} blood and according to the following criteria (Carter and Graves, 1988): males (microgametocytes) are smaller than females (macrogametocytes), the nucleus is larger in males than females, the ends of the cells are round 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. The sex ratio was defined as the proportion of gametocytes in peripheral blood that were male (Pickering et al., 2000). A gametocyte sex ratio was considered male-biased if it was $\geq$0.5.

Blood obtained from a finger prick into heparinized capillary tubes was used to estimate the haematocrit.

2.1. Kinetics of sex-specific gametocytaemia

Gametocyte kinetic parameters were estimated from gametocyte and sex-specific densities by a non-compartmental model using the computer programme *Turbo Ken* (Clinical Pharmacology Group, University of Southampton, UK, through the courtesy of Professor A.G. Renwick) as previously described (Sowunmi and Fateye, 2003a,b; Sowunmi et al., 2007a). The following parameters were calculated from the curve of sex-specific gametocytaemia by using the real times of sampling from each patient: areas under the curves of gametocytaemia *versus* time until the last detectable gametocyte concentration (C_{tgm}), (AUC_{gmlast}), were calculated using the trapezoidal method. Area under the sex-specific gametocytaemia–time from zero to infinity (AUC_{gm0-00}) was calculated by adding to AUC_{gmlast} the extrapolated AUC_{gm} calculated as C_{tgm}/k_{el}, the elimination rate constant derived from the semilogarithmic plot of sex-specific gametocytaemia *versus* time (visual inspection of the final part of the gametocytaemia–time curve was used to identify the elimination phase). Terminal elimination half life, $t_{1/2\beta}$, was calculated as $0.693/k_{el}$. The final sex-specific gametocytaemia at the apparent time of clearance was taken to be 0.001 sexual forms/μl blood (a level assumed to be below microscopical detection). Sex-specific areas under the curve and half lives were determined only in patients who had gametocytaemia at enrolment and for at least three times during the first 7–14 days after enrolment. Gametocyte maleness index (GMI) was defined as the ratio of sex-specific half lives male:female. This ratio was determined for each drug treatment group. AUC-related data will be reported elsewhere.

3. Data analysis

Data were analyzed using version 6 of the *Epi-Info* software (Anon., 1994), and the statistical programme *SPSS for Windows* version 10.01 (Anon., 1999). 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 or by Mantel Haenszel tests. Normally distributed, continuous data were compared by Student's *t*-tests and analysis of variance (ANOVA). Data not conforming to a normal distribution were compared by Kruskal–Wallis and the Mann–Whitney *U*-tests (or by Wilcoxon ranked sum test). Association between sex ratio and any of the clinical or parasitological parameters was assessed by Spearman's rank correlation coefficient. A multiple logistic regression model was used to test the association between a male-biased sex ratio, that is, sex ratio $\geq$0.5 (yes or no at presentation or during follow-up) and factors that were significant at univariate analysis: presence of fever, haematocrit <25%, asexual parasitaemia >20,000 μl^{-1}, and gametocytaemia <18 μl^{-1}. A multiple logistic regression model was also used to