Some features of primary and recrudescent amodiaquine-resistant *Plasmodium falciparum* infections in Nigerian children

Akintunde Sowunmi/[1]/+, Sulayman T Balogun, Grace O Gbotosho, Christian T Happi

Department of Pharmacology and Therapeutics, Institute for Medical Research and Training, University of Ibadan, Ibadan, Nigeria
[1]Department of Clinical Pharmacology, University College Hospital, Ibadan, Nigeria

Characteristics of primary and recrudescent Plasmodium falciparum *infections were evaluated in 25 children who did not recover after amodiaquine (AQ) treatment. Recrudescence was detected by a thick blood smear and confirmed by polymerase chain reaction. Over half of recrudescent events occurred after 14 days of initiation of treatment and were associated with relatively low asexual parasitaemia. We examined the gametocyte sex ratio (GSR) in these children and in age and gender-matched controls that had AQ-sensitive (AQ-S) infections (n = 50). In both AQ-S and AQ-resistant (AQ-R) infections, the GSR was female-biased pre-treatment and became male-biased by the third day after treatment initiation. However, gametocyte males persisted after this period in children with AQ-R infections. AQ-recrudescent infections are relatively low (25 of 612.4%) in children from this endemic area.*

Key words: amodiaquine - recrudescence - malaria - gametocyte sex ratio - children - Nigeria

Due to increasing resistance of *Plasmodium falciparum* to antimalarial monotherapy, one of the strategies to combat the spread of drug resistance is the use of combination antimalarials, particularly artemisinin-based combination therapy (ACT) (WHO 2001). One of the most frequently used partner drugs for ACT in Africa is amodiaquine (AQ), which is more effective than chloroquine (CQ) for both CQ-sensitive and CQ-resistant infections. AQ is also used as a partner drug for non-artemisinin-based combination therapy (NACT), especially with antifolates (Schellenberg et al. 2002, Sowunmi 2002, Gasasira et al. 2003, Sowunmi et al. 2007c), and is used as monotherapy in some settings.

Despite differing mechanisms of resistance to CQ and pyrimethamine-sulfadoxine (PS) (Plowe et al. 1997, Fidock et al. 2000), resistance to CQ or PS seems to confer survival and propagation advantages to the parasite *P. falciparum* (Handunnetti et al. 1996, Robert et al. 2000, Sutherland et al. 2002, Sowunmi & Fateye 2003a, b). Recent suggestions have been made that in Africa mutant alleles of *Pfcrt* and *Pfmdr-1* associated with CQ-resistance in *P. falciparum* are also associated with resistance to AQ (Ochong et al. 2003, Happi et al. 2006, Holmgren et al. 2006). However, it is unclear if resistance to AQ also confers survival and propagation advantages to the parasite.

Despite increasing use of AQ treatment for acute falciparum infections in Africa, little information is available for the treatment's effects on gametocyte carriage and gametocyte sex ratio in West African children. A recent study indicated that carriage rates may be higher and that gametocyte sex ratio may be more male-biased after AQ than after artesunate or artesunate-AQ combination (Sowunmi et al. 2007a). In the latter context, it is unclear if the gametocyte sex ratio following AQ treatment differs between AQ-sensitive (AQ-S) and AQ-resistant (AQ-R) parasites.

The present study was designed to address these issues. The main goals were: to evaluate the features of primary and recrudescent infections in children treated with AQ and to follow the temporal changes in gametocyte sex ratios in AQ-R and AQ-S infections.

PATIENTS AND METHODS

The study was conducted in 615 children less than 13 years of age with acute, uncomplicated *P. falciparum* malaria in Ibadan, a malaria endemic area (Salako et al. 1990) in southwestern Nigeria in 2000, 2004 and 2006. Fully informed consent was obtained from the parents/guardians of each child. Briefly, children were enrolled in the study if there was fever or history of fever in the 24-48 h preceding presentation, pure *P. falciparum* parasitaemia with > 2000 asexual forms/μL, absence of other concomitant illness, no history of antimalarial use in the two weeks preceding presentation and negative urine tests for antimalarial drugs (Dill-Glazko and lignin). Children with severe malaria (WHO 2000), severe malnutrition, serious underlying diseases (renal, cardiac or hepatic), sickle cell anaemia or known allergy to AQ were excluded from the study. The study protocol was approved by the Joint University College Hospital/University of Ibadan ethics review committee (2000 study) and by the Ethics Committee of the Ministry of Health, Ibadan, Nigeria (2004 and 2006 studies).

All 615 patients were treated with AQ 30mg/kg over three days (10mg/kg daily from day 0-2). The drugs were given orally and all patients were observed for at least three hours in order to ensure that the drug was not vomited. If the drug was vomited, the patient was excluded from the study. Oral paracetamol (acetaminophen) at 10-15 mg/kg six hourly was given for 12-24 h if body temperature was > 38°C. Follow up was performed

+ Corresponding author: akinsowunmi@hotmail.com
Received 11 February 2008
Accepted 14 November 2008

for 28 days (2000) and 42 days (2004 and 2006 studies). At enrolment and during follow up, patients underwent physical examinations and parasitological assessments as previously described (Sowunmi et al. 2001, Sowunmi 2002). Asexual parasite and gametocyte counts were measured daily for the first four days (days 0-3) and thereafter on days 7, 14, 21, 28, 35 and 42. Quantification in Giemsa-stained thick blood films was done against 500 leukocytes in the case of asexual parasitaemia and against 1000 leukocytes in the case of gametocytes. From these quantified figures, parasite density was calculated assuming a leukocyte count of 6000/μL of blood. Parasite clearance time was the time interval from the start of antimalarial treatment until the asexual parasite count fell below detectable levels in a peripheral blood smear. Blood was spotted on filter paper on days 0, 1, 3, 7, 14, 21, 28, 35 and 42 and at the time of re-appearance of peripheral parasitaemia after its initial clearance for parasite genotyping.

An infection was considered recrudescent if it occurred after an initial complete clearance and parasites reappeared in blood within 28 (2000 study) or 42 days (2004 and 2006 studies), which was detected by a thick blood smear and confirmed by polymerase chain reaction (PCR), as previously described (Happi et al. 2006). Briefly, genotypes of the parasite population in each sample collected from patients with microscopically confirmed *P. falciparum* infections at enrolment and during follow-up were determined using the nested PCR methodology. Paired pre and post-treatment parasites were analysed using parasite loci that exhibit repeat numbers of polymorphisms in order to distinguish true treatment failures from new infections. Block 2 of *Msp-1* (merozoite surface proteins-1), Block 3 of *Msp-2* (merozoite surface protein-2) and region II of *Glurp* were amplified by two rounds of PCR using family specific primers and amplification conditions described previously (Happi et al. 2006). Primer sequences and PCR conditions for the nested PCR strategy are described in Table I. PCR products (10 μL) were resolved by electrophoresis on a 2% agarose gel and sized against a 100 bp molecular weight marker (New England Biolabs, Beverly, MA). The banding pattern of the post-treatment parasites was compared to matched primary parasites. Post-treatment and primary infection parasites showing identical bands were considered as true treatment failure, while non-identity indicated a new infection. *P. falciparum* clones K1, 3D7 and FC27 were used as positive controls for each reaction.

In patients with recrudescence, clinical and parasitological parameters of the primary infection were compared to those of the recrudescent infection. In addition, patients with recrudescent infections were matched for age and gender with patients who had a sensitive response to AQ. Gametocyte sex was determined as described by Carter and Graves (1988) and Robert et al. (1996). Gametocyte sex ratio was defined as the proportion of gametocytes that were male (Pickering et al. 2000).

TABLE I

Primers sequences and thermocycling conditions for the amplification reactions

Locus and reactions	Primers names and sequences	Cycling conditions
Msp-1 (block2)		
Primary amplification	CHM1-OF: 5' CTAGAAGCTTTAGAAGATGCAGTATTG-3' CHM1-OR: 5' CTTAAATAGTATTCTAATTCAAGTGGATCA-3'	95°C/5 min; 45cycles 94°C/1 min; 58°C/1 min; 72°C/1 min; 72°C/10 min.
Secondary amplification	CHM1-KF: 5' AAATGAAGAAGAAATTACTACAAAAGGTGC-3' CHM1-KR: 5' GCTTGCATCAGCTGGAGGGCTTGCACCAGA-3' CHM1-MF: 5' AAATGAAGGAACAAGTGGAACAGCTGTTAC-3' CHM1-MR: 5' ATCTGAAGGATTTGTACGTCTTGAATTACC-3' CHM1-RF: 5' TAAAGGATGGAGCAAATACTCAAGTTGTTG-3' CHM1-RR: 5' CATTTGAAGGATTTGCAGCACCTGGAGATC-3'	95°C/5 min; 35cycles 94°C/1 min; 61°C/2 min; 72°C/2 min; 72°C/10 min.
Msp-2 (block3)		
Primary amplification	M2-OF: 5' ATGAAGGTAATTAAAACATTGTCTATTATA-3' M2-OR: 5' CTTTGTTACCATCGGTACATTCTT'3'	95°C/5 min; 45cycles 94°C/1 min; 55°C/2 min; 72°C/2 min; 72°C/10 min.
Secondary amplification	M2-FCF: 5' AATATTAAGAGTGTAGGTGCARATGCTCCA-3' M2-FCR: 5' TTTTATTTGGTGCATTGCCAGAACTTGAAC-3' M2-ICF: 5' AGAAGTATGGCAGAAAGTAAKCCTYCTACT-3' M2-ICR: 5' GATTGTAATTCGGGGGATTCAGTTTGTTCG-3'	95°C/5 min; 35cycles 94°C/1 min; 62°C/2 min; 72°C/2 min; 72°C/10 min.
Glurp (region II)		
Primary amplification	CHG-OF: 5' TGAATTTGAAGATGTTCACACTGAAC-3' CHG-OR: 5' GTGGAATTGCTTTTTCTTCAACACTAA-3'	95°C/5 min; 45cycles 94°C/30 s; 45°C/1 min; 68°C/2 min; 72°C/15 min.
Secondary amplification	CHG-OR: 5' GTGGAATTGCTTTTTCTTCAACACTAA-3' CHG-NF: 5' TGTTCACACTGAACAATTAGATTTAGATCA-3'	95°C/5 min; 35 cycles 94°C/30 s; 45°C/1 min; 68°C/2 min; 72°C/15 min.

Capillary blood was collected before and during follow-up and was used to measure packed cell volume (PCV). PCVs were measured using a microhaematocrit tube and microcentrifuge (Hawksley, Lancing, UK). Routine PCV was done on days 0, 3, 7, 14, 21, 28, 35 and 42.

Recrudescent infection was considered to be asymptomatic at the moment of the parasitological examination if the patient had parasitaemia but no symptoms or clinical signs upon physical examination.

Re-treatment after treatment failures - Children with treatment failure were re-treated immediately after parasitaemia was detected with thick blood smear. Five children were retreated with a combination of AQ plus chlorpheniramine as previously described (Sowunmi et al. 2007b) in order to demonstrate the reversing effect of AQ resistance by chlorpheniramine in vivo. All other children with recrudescent infections were re-treated with AQ-artesunate as previously described (Sowunmi et al. 2007a).

Data analysis - Data were analysed using version 6 of 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 test. Normally distributed, continuous data were compared by Student's *t* tests and analysis of variance (ANOVA). Data that did not conform to a normal distribution were compared by Mann-Whitney U-tests and Kruskal-Wallis tests (or by Wilcoxon ranked sum test). Correlations were assessed by linear regression. All tests of significance were two-tailed. P values of < 0.05 were considered to indicate significant differences. Data were (double) entered serially using the patients' codes and were only analysed at the end of the study.

RESULTS

Study population - During the three study periods, a total of 612 children were treated with AQ; 105 in 2000, 290 in 2004 and the remainder in 2006. There were six recrudescent infections in 2000, eight in 2004 and 11 in 2006. Recrudescence occurred on days 14, 21, 28, 35 and 42 in 12, 4, 1, 7 and 1 child, respectively.

Clinical features of primary and recrudescent infections - The characteristics of primary and recrudescent infections are summarised in Table II. The frequency of symptoms upon presentation of the primary infections and during the recrudescent infections that emerged af-

TABLE II

Clinical parameters of the primary and recrudescent infections in the 25 malarious children

Parameters	Primary infection	Recrudescent infection	p value
Age (year)			
mean age (sd)	6.5 (3.1)	-	-
range	1.5-12.0	-	-
Weight (kg)			
mean (sd)	16.6 (6.3)	17.4 (6.2)	< 0.0001
range	7.5-28.0	8.5-29.0	-
Axillary temperature (°C)			
mean (sd)	38.4 (1.3)	36.7 (0.9)	< 0.0001
range	36.0-40.0	36.1-39.5	
n with axillary temp > 37.4 (°C)	15	5	0.002
Packed cell volume (PCV) (%)			
mean (sd)	30.9 (3.6)	33.3 (2.3)	0.001
range	25.0-37.0	29.0-37.0	-
n with PCV < 30%	8	1	0.02
Parasite density (/µL)			
geometric mean	28,571	760	< 0.0001
range	3368-1,140,000	120-54,769	-
Gametocyte density (/µL)			
geometric mean	15	12	-
range	12-24 (n = 3)	12 (n = 1)	
Gametocytaemia: parasitaemia ratio	0.0025	-	-
N of children with:			
Hepatomegaly only	4	4	1.00
Splenomegaly only	1	0	1.00
Hepato-splenomegaly	4	2	0.67

sd: standard deviation.

ter AQ treatment of the primary infections is shown in Fig. 1. The prevalence of gametocytaemia in primary and recrudescent infections were similar (3/25 = 12% and 1/25 = 4%, respectively, p = 0.6). There was no correlation between gametocytaemia and asexual parasitaemia in the primary infection (r = -0.8, p = 0.39). The clinical features of children with recrudescent infections and of age and gender-matched children with sensitive infections were similar (data not shown).

Temporal changes in gametocyte sex ratio in children with recrudescent infection and age and gender-matched children with a sensitive response - Three children that had recrudescent infections and three age and gender-matched children with a sensitive response to AQ were gametocyte carriers at enrolment. In addition, two and three children from the recrudescent and drug sensitive groups, respectively, became gametocyte carriers within two weeks of treatment initiation (Fig. 2). Out of the children who were gametocytaemic, a total of 168, 112, 204, 114, 54 and 36 gametocytes were counted on days 0, 1, 2, 3, 7 and 14, respectively. Of these, 156, 106, 192, 102, 54 and 36 gametocytes could be sexed on days 0, 1, 2, 3, 7 and 14, respectively. The corresponding number of patients in whom the gametocytes were counted was 6, 5, 2, 3, 3 and 3, respectively.

In children with recrudescent infections and in age and gender-matched children with sensitive infections, the gametocyte sex ratio, which was initially female-biased at enrolment, became male-biased by day three after initiation of treatment but gametocyte maleness persisted beyond this period in those with recrudescent infections (Fig. 2).

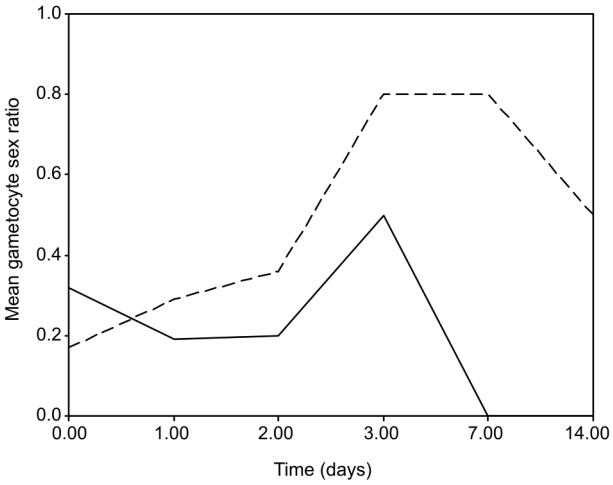

Fig. 2: changes in gametocyte sex ratio in age and gender matched children with amodiaquine-sensitive (solid line) and amodiaquine-resistant (broken line) uncomplicated falciparum malaria.

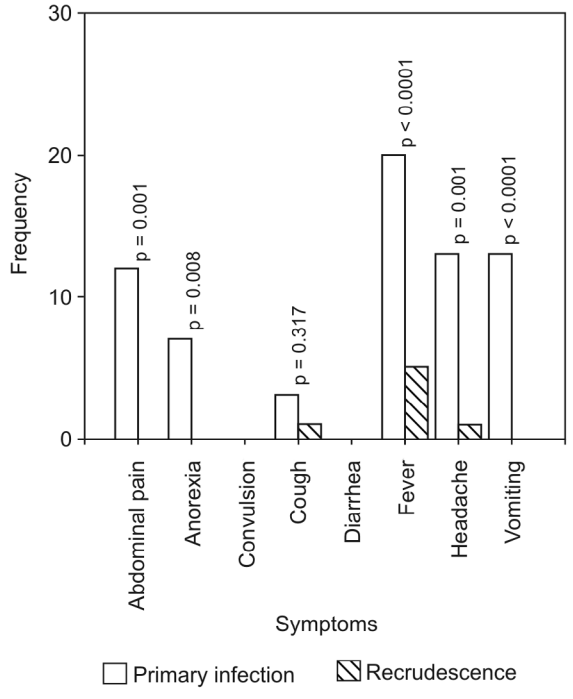

Fig. 1: frequencies of symptoms at enrolment (during the primary infection) and recrudescence among the 25 malarious children (p values indicate the differences in the frequency of symptoms during primary and recrudescent infections).

Response of recrudescent infections to AQ plus chlorpheniramine or AQ-artesunate - All AQ-treatment failures responded to AQ plus chlorpheniramine or AQ-artesunate with fever and parasitaemia that cleared within two and three days, respectively, and had no recurrence of symptoms or parasitaemia after 28-42 days of follow-up.

DISCUSSION

Malaria is hyperendemic in all of southwestern Nigeria. Although response to AQ monotherapy was positive in early 2000 (Sowunmi et al. 2001), recent reports suggest resistance to AQ monotherapy is increasing (Happi et al. 2006) and is conferred by the same mutations in the parasite that confer resistance to CQ (Ochong et al. 2003, Happi et al. 2006, Holmgren et al. 2006). Our results after evaluating recrudescent infections in 615 children enrolled in AQ efficacy studies over a seven-year period in an endemic area of malaria showed that AQ resistance is at 4%, a figure that is likely to rise as the use of the drug as monotherapy continues. Although combination antimalarials are officially recommended for the management of malaria in Nigeria (Anon 2004), AQ monotherapy is still used considerably by many people primarily because combination antimalarials are unaffordable (Sowunmi, unpublished observation). In this context, the results of the present study are important for the control of malaria in Nigeria. Furthermore, in the event of artemisinin-AQ treatment failure, it is likely that AQ, not artemisinin, will be the most likely cause of failure since in vivo parasites resistant to artemisinin and artemisinin derivatives have not been encountered in the area.

Given the results of the molecular analyses, it seems likely that all of the patients examined had true recrudescence and not re-infections. In this context, over half

of the patients that had recrudescence after 14 days of initial treatment, especially those occurring at days 35 and 42, would have been considered to be new infections by clinical criteria in the absence of confirmation by molecular analysis. It is particularly noteworthy that in a third of children with recrudescent infections, recrudescence occurred 35 or 42 days after initiation of therapy.

Although resistance to AQ monotherapy in the present study is relatively low, the extent to which this may modify the local reservoirs of infection is unknown. The present results showed that a recrudescent infection (that emerges after AQ treatment of a primary infection has failed) is clinically different from a primary infection. The significant modifications observed include a propensity to produce few symptoms and signs of infection and a lower asexual parasitaemia. These characteristics are generally similar to those documented for CQ (Handunnetti et al. 1996, Sowunmi & Fateye 2003a) and pyrimethamine-sulfadoxine (Sowunmi & Fateye 2003b). Additionally, recrudescence was not associated with anaemia. These differences may significantly affect the ability of a healthcare provider to make a prompt diagnosis of recrudescence and can therefore affect the appropriate treatment of the recrudescent infections.

Although fewer patients were gametocytaemic at recrudescence (and recrudescence occurred late and as promptly treated), it is likely that if patients were followed for a longer time, then more notable gametocytaemia may have developed and favoured the transmission of drug resistant parasites since low asexual parasitaemia and absence of fever are risk factors for gametocyte carriage (Price et al. 1999, von Seidlen et al. 2001, Sowunmi et al. 2004). In addition, in the absence of symptoms, it is unlikely that patients would seek treatment, thus increasing the number of days with gametocytaemia that would follow recrudescence and increasing the chances of transmission.

The proportion of male gametocytes present in a blood meal obtained by the mosquito from a human host, in addition to other factors, may be crucial to the infectivity of the gametocytes to the mosquito (Robert et al. 1996). Although the children who carried gametocytes were small, overall the gametocyte sex ratio was initially female-biased and became male-biased within four days of the initiation of treatment. This finding is an agreement with that of a larger cohort of children from the same study area (Sowunmi et al. 2007a) and suggests that, in patients who became gametocytaemic, the drug may have likely encouraged transmission, particularly in recrudescent patients where male-biased gametocytaemia was still present for a week after treatment initiation for the primary infection.

ACKNOWLEDGEMENTS

To our clinic staff, especially Moji Amoo and Adeola Alabi, for assistance with running the study.

REFERENCES

Anon 1994. Epi Info Version 6. A word processing data base and statistics program for public health on IBM-compatible microcomputers. Centers for Disease Control and Prevention, Atlanta, GA.

Anon 1999. SPSS for Windows Release 10.01 (standard version). SPSS Inc, Chicago, IL.

Anon 2004. *National antimalarial treatment policy*. Federal Ministry of Health, Abuja.

Carter R, Graves PM 1988. Gametocytes. In WH Wernsdorfer, I McGregor (eds.), *Malaria. Principles and Practice of Malariology*, Vol. I, Churchill Livingstone, Edingburgh, p. 253-303.

Fidock DA, Nomura T, Talley AK, Cooper RA, Dzekunov SM, Ferdig MT, Ursos LN, Sidhu AB, Naude B, Deitsch KW, Su X-Z, Wootton JC, Roepe PD, Wellems TE 2000. Mutations in *P. falciparum* digestive vacuole transmembrane protein *PfCRT* and evidence for their role in chloroquine resistance. *Mol Cell 6*: 861-871.

Gasasira AF, Dorsey G, Nzarubara B, Staedke SG, Nassali A, Rosenthal PJ, Kamya MR 2003. Comparative efficacy of aminoquinoline-antifolate combinations for the treatment of uncomplicated falciparum malaria in Kampala, Uganda. *Am J Trop Med Hyg 68*: 127-132.

Handunnetti SM, Gunewardena DM, Pathirana PPSL, Ekanayake K, Weerasinghe S, Mendis KN 1996. Features of recrudescent chloroquine-resistant *Plasmodium falciparum* infections confer a survival advantage on parasite and have implications for disease control. *Trans R Soc Trop Med Hyg 90*: 563-567.

Happi CT, Gbotosho GO, Folarin OA, Bolaji OM, Sowunmi A, Kyle DE, Milhous W, Wirth DF Oduola AMJ 2006. Association between mutations in *Plasmodium falciparum* chloroquine resistance transporter and *P. falciparum* multidrug resistance 1 genes and *in vivo* amodiaquine resistance in *P. falciparum* malaria-infected children in Nigeria. *Am J Trop Med Hyg 75*: 155-161.

Holmgren G, Gil J, Ferreira PM, Veiga MI, Obonyo CO, Bjorkman A 2006. Amodiaquine resistant *Plasmodium falciparum* malaria *in vivo* is associated with selection of *pfcrt* 76T and *pfmdr 1* 86Y. *Inf Gen Evol 6*: 309-314.

Ochong EO, Van Den Broek IVF, Keus K, Nzila A 2003. Association between chloroquine and amodiaquine resistance and allelic variation in the *Plasmodium falciparum* multiple drug resistance 1 gene and the chloroquine resistance transporter gene in isolates from the Upper Nile in southern Sudan. *Am J Trop Med Hyg 69*: 184-187.

Pickering J, Read AF, Guerrero S, West SA 2000. Sex ratio and virulence in two species of lizard malaria parasites. *Evol Ecol Res 2*: 171-184.

Plowe CV, Cortese JF, Djimde A, Nwanyanwu OC, Watkins WM, Winstanley PA, Estrada-Franco JG, Mollinedo RE, Avila JC, Cespedes JL, Carter D, Dumbo O 1997. Mutations in *Plasmodium falciparum* dihydrofolate reductase and dihydropteroate synthetase and the epidemiologic patterns of pyrimethamine-sulphadoxine use and resistance. *J Inf Dis 176*: 1590-1596.

Price R, Nosten F, Simpson JA, Luxemburger C, Paiphun L, ter Kuile F, van Vugt M, Chongsuphajaisiddhi T, White NJ 1999. Risk factors for gametocyte carriage in uncomplicated falciparum malaria. *Am J Trop Med Hyg 60*: 1019-1023.

Robert V, Awono-Ambene HP, Hesran J-YL, Trape J-F 2000. Gametocytaemia and infectivity to mosquitoes of patients with uncomplicated *Plasmodium falciparum* malaria attacks treated with chloroquine or sulfadoxine plus pyrimethamine. *Am J Trop Med Hyg 62*: 210-216.

Robert V, Read AF, Essong J, Chuinkam T, Mulder B, Verhave JP, Carnevale P 1996. Effects of gametocyte sex ratio on infectivity of *Plasmodium falciparum* to *Anopheles gambiae*. *Trans R Soc Trop Med Hyg 90*: 621-624.

Salako LA, Ajayi FO, Sowunmi A, Walker O 1990. Malaria in Nigeria: a revisit. *Ann Trop Med Parasitol 84*: 435-445.

Schellenberg D, Kahigwa E, Drakeley C, Malende A, Wigayi J, Msokame C, Aponte JJ, Tanner M, Mshinda H, Menendez C, Alonso PL 2002. The safety and efficacy of sulfadoxine-pyrimethamine, amodiaquine and their combination in the treatment of uncomplicated falciparum malaria. *Am J Trop Med Hyg 67*: 17-23.

Sowunmi A 2002. A randomized comparison of chloroquine, amodiaquine and their combination with pyrimethamine-sulfadoxine in the treatment of acute, uncomplicated, *Plasmodium falciparum* malaria in children. *Ann Trop Med Parasitol 96*: 227-238.

Sowunmi A, Ayede AI, Falade AG, Ndikum VN, Falade CO, Happi TC, Oduola AMJ 2001. Randomized comparison of chloroquine and amodiaquine in the treatment of acute, uncomplicated, *Plasmodium falciparum* malaria in children. *Ann Trop Med Parasitol 95*: 549-558.

Sowunmi A, Balogun T, Gbotosho GO, Happi CT, Adedeji AA, Fehintola FA 2007a. Activities of amodiaquine, artesunate and artesunate-amodiaquine against asexual and sexual-stage parasites in falciparum malaria in children. *Antimicrob Agent Chemother* 51: 1694-1699.

Sowunmi A, Fateye BA 2003a. Asymptomatic, recrudescent, chloroquine-resistant, *Plasmodium falciparum* infections in Nigerian children: clinical and parasitological characteristics and implications for the transmission of drug-resistant parasites. *Ann Trop Med Parasitol 97*: 469-479.

Sowunmi A, Fateye BA 2003b. Gametocyte sex ratios in children with asymptomatic, recrudescent, pyrimethamine-sulfadoxine-resistant, *Plasmodium falciparum* malaria. *Ann Trop Med Parasitol 97*: 671-682.

Sowunmi A, Fateye BA, Adedeji AA, Fehintola FA, Happi TC 2004. Risk factors for gametocyte carriage in uncomplicated falciparum malaria in children. *Parasitol 129*: 255-262.

Sowunmi A, Gbotosho GO, Happi CT, Adedeji AA, Bolaji OM, Fehintola FA, Fateye BA, Oduola AMJ 2007b. Enhancement of the antimalarial efficacy of amodiaquine by chlorpheniramine in vivo. *Mem Inst Oswaldo Cruz 102*: 417-419.

Sowunmi A, Gbotosho GO, Happi CT, Adedeji AA, Fehintola FA, Folarin OA, Tambo E, Fateye BA 2007c. Therapeutic efficacy and effects of artemether-lumefantrine and amodiaquine-sulfalene-pyrimethamine on gametocyte carriage in children with uncomplicated *Plasmodium falciparum* malaria southwestern Nigeria. *Am J Trop Med Hyg 77*: 235-241.

Sutherland CJ, Alloueche A, Curtis J, Drakeley CJ, Ord R, Duraisingh M, Greenwood BM, Pinder M, Warhurst DC, Targett GAT 2002. Gambian children successfully treated with chloroquine can harbor and transmit *Plasmodium falciparum* gametocytes carrying resistant genes. *Am J Trop Med Hyg 67*: 575-585.

von Seidlein L, Drakeley C, Greenwood B, Walraven G, Targett G 2001. Risk factors for gametocyte carriage in Gambian children. *Am J Trop Med Hyg 65*: 523-526.

WHO - World Health Organization 2000. Severe falciparum malaria. *Trans R Soc Trop Med Hyg 94* (Suppl. 1): 1-90.

WHO - World Health Organization 2001. *Antimalarial drug combination therapy. Report of a WHO technical consultation. WHO/CDS/RBM/2001.35,* WHO, Geneva.