

# RISK FACTORS AND EFFECTS OF ANTIMALARIAL DRUGS ON PLASMODIUM FALCIPARUMGAMETOCYTAEMIA IN NIGERIAN CHILDREN

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BABA

BY

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#### ABSTRACT

Gametocytes are the sexual forms of Plasmodium species that are essential for the transmission of malaria. However, there is little information on the factors that influence their generation or carriage, a measure of transmission potential of Plasmodium falciparum, in Nigerian children. The objectives of the studies were to determine (1) the risk factors for gametocyte carriage and effects of seasons on carriage, in children with acute falciparum malaria, and (2) the effects of selected antimalarial drugs on gametocyte carriage, intensities of carriage, morphological stages and sex ratio changes (GSR).

Overall, 2,317 symptomatic children (M.F. 1024.1293), aged 0.5-14 years, with microscopically-confirmed P. falciparum malaria, treated with standard doses of the antimalarial drugs (chloroquine (CQ), pyrimethamine-sulfadoxine (PS). trimethoprimsulfamethoxazole (TS), chloroquine plus chlorpheniramine (CQCP), pyrimethamine-sulfadoxine plus probenecid (PSP) and amodiaquine (AQ) plus pyrimethamine-sulfadoxine, AQPS) were studied Before, during and following therapy, densities of asexual parasites and of the morphologically distinct developmental stages of gametocytes were quantified in blood over 14-28 days, using Temporal changes in density ratio of Peripheral Young standard methods Gamctocytes (PYG) Peripheral Mature Gamctocyte (PMG) was used as an index of continuing gametocyte generation after treatment. GSR, defined as the proportion of gametocytes that were morphologically males, was also quantified scrially over 14 CQ blood concentrations were determined by high performance liquid chromatography Responses of infections to therapy were classified as sensitive or resistant. Data were analyzed using student to test, analysis of variance, chi square, Kaplan Meier survival test and Multiple logistic regression models

Gametocyte carriage in the recruited patients was 15% at enrolment. Four factors were found to be independent risk factors for gametocyte carriage at presentation male gender (adjusted odd ratio, AOR=0 55, 95% Confidence Interval, CI= 0 36.0 83, P= 0.005), absence of fever (AOR=1 61, 95% CI= 1.05-2 5, P= 0.03), duration of illness >3d (AOR=1 57, 95%, CI= 1.0-2.4, P= 0.047), and asexual parasitaemia <5000/µl (AOR=0 04, 95% CI= 0.02-0.07, P< 0.05). However, 15.6% of all the children developed patent gametocytaemia following treatment. Except for AFRICAN DIGITAL HEALTH REPOSITORY PROJECT

male gender in the low transmission season, the identified risk factors for gametocyte carriage were little affected by season. A total of 220 children failed to respond to antimalarial drug treatment. Children with CQ-resistant infections (37.1%), and those treated with PS irrespective of outcome, were significantly at risk of gametocyte carriage compared with other antimalarials. CQCP-resistant infection and treatment with PS significantly increased PYG-PMG ratio (P < 0.05). In contrast, AQPS significantly reduced the ratio. PSP and TS, like PS, alone, enhanced gametocyte carriage and increased GSR.

Antimalarial drugs modulate gametocyte generation particularly when resistance has developed and may potentially facilitate disease transmission. Presence of peripheral young gametocytes (PYG) was an indicator of resistance to CQCP but not to PS or AQPS PS may enhance influx of young gametocyte into circulation.

Keynvords: Plasmodium falciparum, Gametocytaemia, Antimalarial drugs, Transmission, Children (word count=459).

### **DEDICATION**

To

Allah, The Almighty, who makes all things possible

His Rasul

His Dinnmy course of struggle, and

Liberation of the Oppressed all over the world

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#### CERTIFICATION

I certify that this work was carried out by Mr. A.A. Adedejt in the Department of
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### Original papers

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#### **GLOSSARY OF ABBREVIATIONS**

af asexual forms

ANOVA analysis of variance

AQ amodiaquine

AQPS amodiaquine plus pyrimethamine-sulphadoxine

AUC<sub>270</sub> area under the curve of gametocytaemia versus time

°C degree celcius
Ca\*\* Calcium ion

CI confidence interval

Cl Chloside ion

CLuza volume of blood completely cleared of micro- or macro-gametocytes

per unit time

cGMP cyclic guanosine monophosphate

COM combination antimalarials or pyrimethamine-sulfadoxine combined

with chloroquine or amodiaquine

CQ. chloroquine

CQCP, chloroquine plus chlorpheniramine

d day

F Semale

FCT fever clearance time

FGD fractional gametocyte density

GMGD geometrie mean gametocyte density

GMPMGD geometric mean peripheral mature gametocyte density

GMPYGD geometric mean peripheral young gametocyte density

GSR gametocyte sex ratio

h, hr hou

HCO<sub>3</sub> bicarbonate ion

HCl hydrochloric acid

lf halofantrine

HPLC high performance liquid chromatography

HTS high transmission season

IOR interquarile range K DOI CHASSING IOD kg kilogramme LTS low transmission season 81 male 34 molar HUS minute min. millmeter П unuper N normal NaOH sodium hydroxide not significant ns OR odds ratio level of significance PCR polymerase chain reaction PCT parasite clearance time PCV packed cell volume **PMG** peripheral mature gametocyte PSP. PPS pyrimethamine-sulfadoxine combined with probenecid PRR paraule reduction ratio PS pyrimethamine-sulfadoxine PYG peripheral young gametocyte QT-NASBA quantitative nucleic acid sequence-based amplification RBC red blood cells RI resistance response type I (mild resistance) RII resistance response type II (moderate resistance) RIII resistance response type III (acvere resistance) RR relative risk S sensitive response standard deviation 60 standard citor

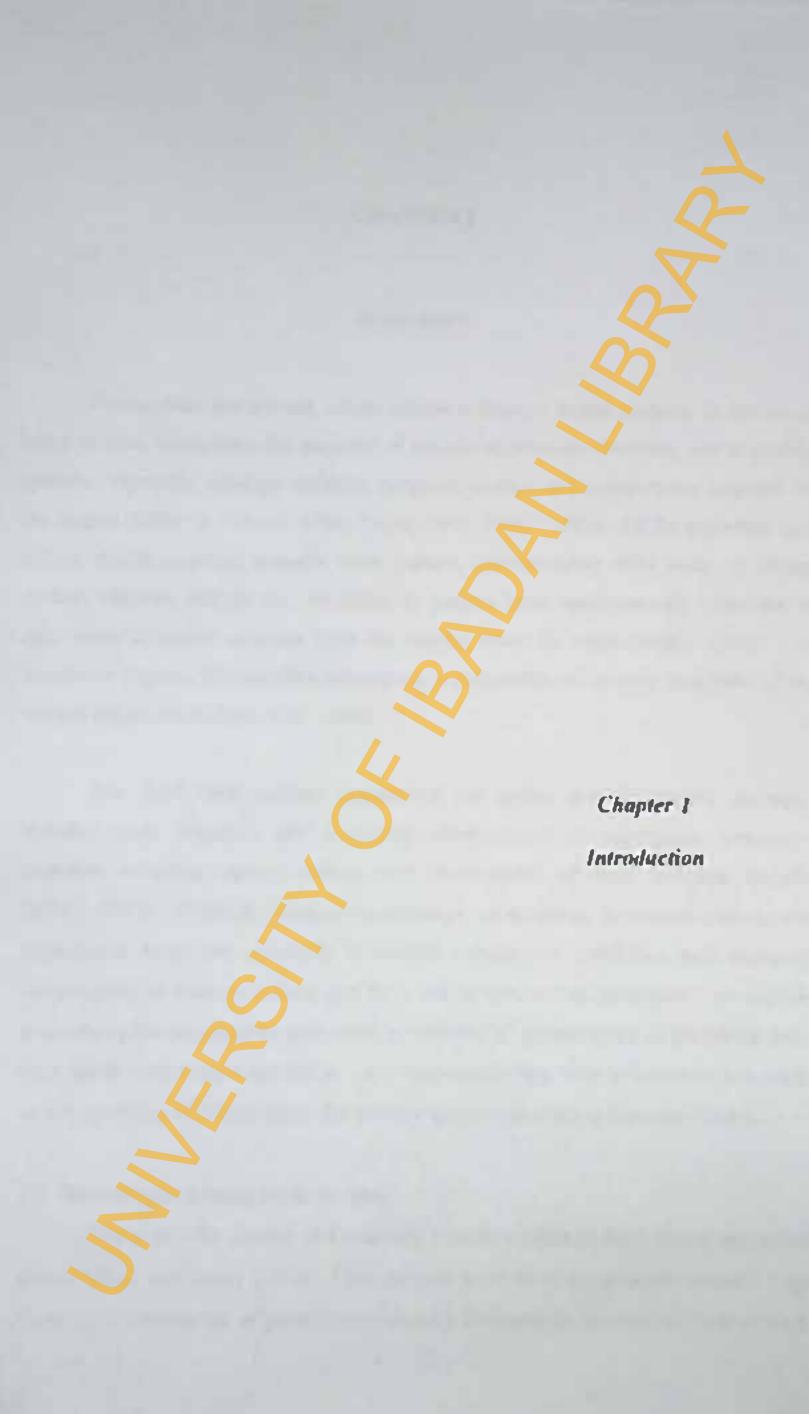
apparent half-life of gametocytacmia

apparent half life

112

11/100

Temp temperature TS trimethoprim-sulfamethoxazole μł microliter UV ultraviolet V5 VETSUS WBC white blood cells WHO World Health Organization X chi square



#### CHAPTER 1

### Introduction

Plasmodium falciparum, which causes malignant tertian malaria, is the most lethal in man, accounting for majority of malaria attributable morbidity and mortality globally, especially amongst children, pregnant women and non-immune travelers in the tropics (Gilles & Warrell, 1993, Trapc, 2001, WHO, 2003). Of the estimated one million deaths reported annually from malaria, approximately 80% occur in young African children, infants are vulnerable to malaria from approximately 3 months of age, when immunity acquired from the mother starts to wane (WHO, 2003). In southwest Nigeria, Plasmodium falciparum is responsible for greater than 95% of the malaria infections (Salako et al., 1990).

The Roll back malaria programme for global malaria control strategies includes early diagnosis and treatment, development of appropriate preventive measures including vector control, and development of local technical capacity (WHO, 2003). However, intense transmission, urbanization, increasing resistance to antimalarial drugs and unusually favourable transmission conditions have hampered the progress in malaria control and have led to unexpected epidemics. In addition, large mosquito populations and ready availability of gametocytes in peripheral blood have made transmission inevitable. It is noteworthy that little information is available on the dynamics of *Plasmodium foletparum* gametocytaemia in Nigerian children.

# 1.1 Gametocyte development in man

The over 170 species of Plasmodia parasites undergo both sexual and asexual phases (Paul and Bray, 2003). Gametocytes arise from erythrocytic asexual stages. Contrary to production of gametocytes directly from hepatic merozoites as described

in other species (Gamham, 1966), Plasmodium falciparum gametocytes (Figure 1 1) arise from mature asexual forms. The mechanism of the switch from asexual to sexual stage and its modulation are complex and incompletely understood (Carter and Miller, 1979, Mons, 1985) These mature asexual stages are absent from peripheral circulation due to cytoadherence of their carrier enythrocytes to microvascular endothelia of organs and tissues, such as heart, lung, liver, skin and brain (MacPherson et al., 1985) to avoid phagocytic clearance from spleen during maturation. The resulting young gametocytes from committed merozoites avoid peripheral blood and sequester preferentially in the bone marrow and spleen (Thomson and Robertson, 1935, Smalley et al., 1980). Bruce and colleagues (1990) showed that merozoites emerging from a single schizont developed further either into asexual stages or into gamctocytes. Smith et al. (2000) and Silvestrini et al. (2000) demonstrated that the gametocytes from one schizont are all male or all female. This observation suggests that the trophozoites of the preceding asexual generation were already committed to either sexual development or continuing asexual cycling Thus, overall, studies on gametocytogenesis consistently support the hypothesis that Plasmodium is committed to sexual development in the proceeding asexual generation rather than differentiating following invasion of the crythrocyte by uncommitted merozoites (Carter and Miller, 1979, Inselberg, 1983, Mons, 1986, Bruce et al. 1990)

Field and Shute (1956) were first to describe five different maturation stages of P. folciperum gametoeytes. The steps were further characterized, using light microscopy by Hawking and co workers (1971) in P. folciperum-infected Actus monkeys. Gametocytes growth process is in stages, I-V. (Table I.1) and maturation process has been described in rodent parasites and P. folciperum. Immature gametocytes of P. folciperum (stages I-III) are sequestered in deep tissues of the body, notably the bone narrow (Smalley et al., 1981). One of the most striking features of gametocytes is the presence of pellicular complex, which originates from a small membraneous vesicle beneath the gametocyte plasmolema in late stage I. This structure is absent from asexual stages. It consists of a subpellicular membraneous vacuole subtended by an array of longitudinally- oriented microtubules (Sinden, 1983). This strengthens the parasite and explains the lack of

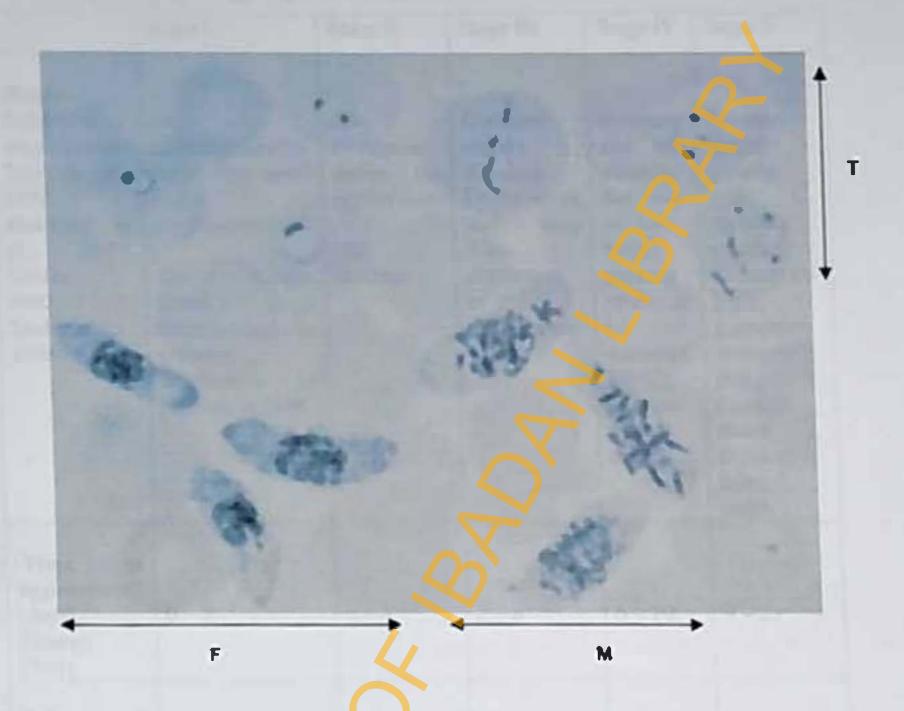


FIGURE 1.1. Mature female (F) and male (M) gametocytes, and trophozoites (T) of Plasmodium falciparum in the blood of malaria-infected patient. This picture is a composite of several pictures originating from the same Giemsa-stained thin smear. (From Talman et al., 2004b).

TABLE 11 Morphology of gametocytogenesis

	Stage I	Stage !!	Stage III	Stage IV	Stage V
	I A Indistinguishable from the small round trophozoite  IB Larger round shape distinguished by granular distribution of pigment in food	erythrocyte  IIB  D-shape	D-shaped, slightly distorted Erythrocytes with pink /blue distinction of male/female	red cell, Male pigment tends to	Sausage- shaped parasite with rounded/ angular extremities- Male: pigmented scattered pink Female: dense pigment, light, violet.
Time of appearance* (days). (Sinden, 1998)		1-4	2-8	6-10	9-23
Point in cell cycle (Sinden and Smalley, 1979)	GI	GI	GO	GO	GO

GO, a temporary resting period or more permanent, GI, cells increase in size, produce RNA and synthesize protein \* time refer to the period after sequestration

amoeboid forms in the esexual parasite (Langreth et al., 1978). The function of this structure is largely unknown.

Gametocyte development takes longer than asexual schizogony, for example. 26 hr as opposed to 22.5 hr in *P. berghei* (Mons et al., 1985) or in the more extreme case of *P. falciparum* 8-12 days as opposed to 48 hr (Sinden, 1983). The proportion of parasites that develop into gametocytes varies greatly during the course of natural infections, even at its peak, but is very low in relation to the total parasitaemia (Smalley et al. 1981) and is reversed during recrudescent infections (Sowunmi and Fateye, 2003 a)

The growth and differentiation of gametocytes of *P. folciparum* spans a period of eight days from merozoite invasion to mature gametocyte, each stage of the gametocyte has been distinguished by successive changes in the organization of the cell (Hawking et al., 1971). The frequency of conversion of asexual parasite to sexual development varies within parasite clones and differs consistently between clones (Carter and Miller, 1979, Graves et al., 1984). These findings suggest that both innate and environmental factors may predispose or trigger the parasite switch from asexual to sexual development (Carter and Miller, 1979, Graves et al., 1984, Bruce et al., 1990).

In a culturing experiment, Suhrbier et al. (1987) showed that sexual commitment is highest in the merozoites derived directly from liver schizont. Day et al. (1993) also showed that continuous asexual passaging was identified as a factor that may lead to sexual commitment. The latter has correlates with accumulated chromosomal breakage and resortment (Frontali, 1994, Birago et al., 1982) and could be particularly important if the sexual stage genes in Plasmodium are concentrated in certain chromosome, for example, chromosome 12 of Plasmodium falcipatrum.

The investment in securing a male and the risk of mixing genes with another are noteworthy costs associated with sexual reproduction but at disadvantage compared to asexual reproduction (Dyer and Day, 2000, Otto and Lenormand, 2002)

Therefore gametocytogenesis in P. falciparum and favourable conditions may

considerably add to the increasing epidemiologic burden of the infection.

Paucity of specific markers that can identify the immature gametocytes or could be used to track the development made full elucidation of the process of gametocytogenesis difficult. Although, there are genes that are expressed later in gametocytogenesis, these were of little usefulness for these studies. The genes, Pf 11-1 (Scherf et al., 1992; Feng et al., 1993) and tubulus 11 (Rawlings et al., 1992). Pfg27 (Carter et al., 1989, Pologe, 1994) and the S form of RNA (Thompson and Sinden, 1994, Waters et al., 1989) are expressed significantly after commitment to gametocytogenesis has taken place. Upregulation of the mRNAs for the early gametocyte markers Pfs16 and Pfg27 was readily detected in 3D7 parasite line (Silverstrini et al., 2005). One hundred and seventeen genes had expression profiles that correlated to those of pfs/6 and pfg27. The northern blot analysis and published proteomie data have been used to identify those proteins whose expression was gametocyte-specific Immunoflourescence analysis with antibodies against two of these gene products identified two novel parasite membrane proteins associated with sexual stage specific proteins (Silverstrini et al., 2005). One was produced from stage I gametocytes and the second showed peak production in the stage II gametocytes (Silverstrini et al., 2005). The two proteins were named Pfpeg-3 and Pfpeg-4, as P. fulciparum proteins of early gamctocytes (Silverstrini et al., 2005). The search for more molecular markers that will enable improved comprehension of the process of gametocytogenesis is on-going

## 1.2 Gametocyte carriage in man

in human, the presence of gametocyte in peripheral blood, gametocytaemia, arises 7-15 days after the initial asexual wave (Eichner et al., 2001, Day et al., 1998) compared to other human species with 1 to 3 days. The ratio of gametocytes to asexual stages in P. falciporum is less than 1 10 (Kitchen and Putnam, 1942, Sinden, 1983, Carter and Graves, 1988). Eichner et al. (2001) even reported a much lower ratio (1 156). The half-life of the mature gametocyte in blood is reported to be 2.4 days (Smalley and Sinden, 1977) but longer half life and consequent longevity in

blood stream may also occur, for example up to 4 weeks. (Smalley and Sinden, 1977) Eichner et al. (2001) and Sowunmi and Fateye (2003b) also estimated mean circulation time to be 6.4 days or more. Because the mechanism of the switch from asexual to sexual stages is incompletely understood it is necessary to study the dynamics of gametocytes in both host and mosquito vector.

## 1.3 Studies on gametocyte carriage in man

In sub Saharan Africa, antimalarial drugs used in the treatment of the infection and increasing drug resistance in P. falciparum to these drugs have been thought to contribute to gametocyte carriage in man and gametocyte infectivity to mosquitoes (Robert et al., 1996 a, 2000, Hogh et al., 1998). Children, in general, constitute a significant risk group and reservoir of the infection in sub Saharan Africa (Githeko et al., 1992, Bonnet et al., 2003) with observed pretreatment gametocyte carriage rate during the acute falciparum infections, in West Africa, ranging between 14-17% (von Seidlein et al., 2001)

In Nigeria, the development and increase in antimalarial drug resistance (Falade et al., 1997, Sowunmi et al., 1998 a, b. Sowunmi, 2002) has been associated with increases in gametocyte carriage in children (Sowunmi and Fateye, 2003 a, b). However, there is little or no information on gametocyte carriage rates and factors contributing to gametocyte carriage in Nigerian children. These factors, when identified, may provide support for control of transmission of the infection.

Several factors have been described as risk factors for gametocyte carriage in children in Africa and Asia. In Tanzania, Akim et al. (2000) reported young age, high asexual parasitaemia on presentation and gametocyte positivity on presentation as risk factors for gametocyte carriage in children with acute malaria infections. In children, in The Gambia, hyperparasitaemia, anaemia at enrollment, age, season and location of study site were identified as independent risk factors for gametocyte carriage before treatment (von Seidlein et al., 2001). The development or persistence of gametocytemia during follow up, patent gametocytemia on admission, anaemia, no coincident P. vivex malatia infection, presentation with a recrudescent infection and a history of illness longer than two days were risk factors for gametocyte

Chloroquine (CQ) and pyrimethamine-sulfadoxine (PS) are schizonticidal and both have on effects on mature gametocytes. However, CQ and PS are active against young gametocytes before their appearance in the peripheral circulation (Smalley, 1977; Butcher, 1997). Although it was previously thought that during treatment, CQ or PS does not induce gametocytogenesis, it is now well known that CQ increases gametocytogenesis in P. chabaudi in-vivo and P. falciparum in-vitro (Buckling et al., 1997, 1999). Pyrimethamine-sulfadoxine, which inhibit dihydrofolate reductase and dihydropteroate synthase, may damage the ookinete and reduce the number of oocyst (Robert et al., 2000), and may consequently affect gametogenesis. Hogh and others observed that CQ enhances P. falciparum infectivity to mosquitoes, while PS reduces it (Hogh et al., 1998).

Thus, antimalarials, amongst other factors that trigger and regulate the generation or development of gamctocyte, are important and must be continuously evaluated for their impacts on gamctocyte carriage in human and infectivity to mosquitoes (Butcher, 1997; Hogh et al., 1998, Chutmongkonkul et al., 1992; Handunnetti et al., 1996, Jones, 1997, Koella et al., 1998, Robert et al., 1996a; Robert and Trape, 1998)

In Nigeria, CQ and PS were antimalatial drugs of choice prior to change of treatment policy to artemisinin based combination therapy (ACTs) in 2004 (Federal Ministry of Health, 2004). Both CQ and PS are still readily available and readily used as monotherapy due to economic reasons and non-affordability of the ACTs, for example, artesunate-PS, by many individuals. It is essential to study the effects of CQ and PS or other antimalarial drugs used for treatment of malaria in endentic areas on the gametocyte generation and carriage during acute infections in children. This will provide useful information that may guide drug combination strategies and deployment with a view to minimizing transmissibility.

# 1.6 Gametocytes infectiousness to mosquito

Mature and functional gametocytes ingested by an appropriate species of mosquito in a bloodmeal are stimulated to transform into the stages that establish the parasites in their vector (Gardham, 1966). Under the influence of changes in the

mosquito midgut environment, the gametocytes become extracellular within 8-15 min of ingestion. Following emergence from the red blood cell (exflagellation), the male gametes fertilize the female gametes within 60 min of ingestion of blood. The fertilized macrogamete (zygote) differentiates into a single motile ookinete over the next 10-25 hr, and migrates from the bloodmeal through the midgut wall to form an oocyst underneath the basal lamina of the midgut. Each oocyst produces many thousands of invasive sporozoites over a period of 7-12 days. The sporozoites escape from the oocyst and then invade the salivary glands, where they stay for possibly very long periods until injected into another vertebrate host when the next bloodmeal is taken (Sinden, 1984; Carter and Graves, 1988).

### 1.7 Studies involving gametocytes infectiousness to mosquito

Various triggers are responsible for induction of gametocytes differentiation Microgametogenesis in vitro is, optimally, dependent upon a rise in pH (Nijhout and Carter, 1978), a tise in CO2 and bicarbonate levels (Carter and Nijhout, 1977, Nijhout and Carter, 1978), a fall in temperature of a few degrees below that of the vertebrate host (Sinden and Croll, 1975) or a very potent factor termed mosquito exflagellation factor (MEF). The latter is a small heat stable molecule from the mosquito head and gut that stimulates gametogenesis via a bicarbonate- and pHindependent mechanism (Nijhout, 1979). Kawamoto et al. (1991) showed in vitro that induction of exflagellation of P. hergher is triggered by a rise in the intracellular pH (pHi) that is mediated by Ca and cGMP regulation pHi can be modulated by alkaline media and is controlled by a complex series of interdependent ion pumps and channels controlling Na K. Cl and IICO transport between the parasite and the environment. Other influential factors described include cAMP analogues and inhibitors of phosphodiesterase (Martin et al., 1978). The duration of microgametogenesis is both temperature and species dependent, for example, at 20-22 °C, microgametogenesis may take 7-15 min for P. falciparum in vitro, although explagellation may be detected after shorter periods in the fluid excreted by feeding Amopheles (Sinden, 1983) There is no evidence that explagellation is influenced by factors released by digestion of the blood meal since digestion normally begins several hours later (Grafet al., 1986)

The microgamete formation involves three mitotic divisions with a rapid assembly of eight axonemes on the single microtubule organizing centre that divides and passes to the spindle poles. This division simultaneously segregates the genome and the axoneme so that each of the eight emergent gametes receives a single axoneme and haploid genome, both being connected to a common microtubule-organizing centre. After exflagellation the microgametes, normally bearing a single axoneme, condensed nucleus and kinetosome with its sphere and granule at the distal end, are torn from the microgametocyte surface and rapidly move away into the blood meal (Sinden and Croll, 1975).

Macrogametogenesis at the morphological level involves little more than escape from the host cell (Sinden, 1984). At the cellular level, there is de novo synthesis of the proteins that are expressed on the surface of macrogamete (Kumar and Carter, 1984). Scherf and co workers (1992, 1993) identified a gametocyte specific protein of P. falciparum called Pfi 1-1 with some evidence that this protein might be involved in the emergence of gametes from the infected crythrocyte. The contributions of these protein factors to gametogenesis are still under studies.

# 1.8 Studies on gametocyte development in Mosquitoes

Many factors influence the development, survival and infectivity of the parasite during its residence in the midgut lumen of the mosquito. Eyles (1952) has shown that the parasite development ceases at the ookinete stage unless a macromolecular (non-dialyzable) component is present in the blood meal. Studying the influence of red blood cells on the ability of P. gallinoceum zygotes fertilized in entre to infect Acides accepts. Rosenberg et al. (1984) found a linear relationship between erythrocyte density and the number of oocysts up to a 50% hematocrit. Furthermore, they deduced that there are one or more nondialyzable substances (erythrocytic factors) contained in normal crythrocytes, and released by mosquito digestion, that are essential for ookinete invasion of the gut epithelium, where they further develop. In a mosquito feeding experiment with cultured P. falciparum (Ponnadurai et al., 1989) and P. berghei (Sinden, 1989), gametocytes, dilution with fresh red cells resulted in more oocysts at initial (low) dilutions whereas further dilution reduced oocyst counts.

The involvement of blood factors and/or its digestive products in infectivity has been studied in different parasite-vector models. Using a selected line of An. stephensi. Feldmann and Ponnudurai (1989) found mature P. falciparum ookinetes in the midgut lumen of refractory mosquitoes but no further penetration of the gut epithelium was observed. The reasons for this limited development in non-compatible mosquitoes could be related to digestive function, since early initiation of hemoglobin degradation and higher aminopeptidase activity have been described in refractory strains of An. stephensi (Feldmann et al., 1990). It has also been shown that P. gallinaceum develops up to the ookinete stage in the non-compatible mosquito, An. Stephensi, at the same time period with those infecting the compatible vector, Ae. Aegypti. However, P. gallinaceum ookinetes did not escape from the midgut lumen in An. stephensi mosquitoes (Rudin et al., 1991).

A possible mechanism causing inhibition of parasite development involves damage of the parasite by digestive enzymes present in the vector. The addition of trypsin inhibitor to blood meal resulted in inhibition of midgut penetration by ookinetes (Rosenberg et al., 1984). Thus trypsin, in particular, and other aminopeptidases are the major proteolytic enzymes involved in blood digestion by female mosquitoes (Briegel and Lea, 1975; Graf and Briegel, 1982; Billingsley, 1990; Billingsley and Hecker, 1991). In animal models, P. gallmaccum ookinetes 0-10 hr old (i.e. zygote to ookingte transition) were shown to be susceptible to mosquito enzymes in double feeding experiments (Gass, 1977) and in vitro damage was observed to cultured ookinetes by proteinases from An. aegypti (Gass and Yeates, 1979) However, the finding of Shahabuddin et al. (1993) using the same parasite/vector system suggest that the parasite secretes an inactive or partially active chitinase that is activated by a mosquito- produced serine protease. In a recent study, Chego et al (1996) further examined the effect of digestive enzymes on the kinetics of P. falciparum ookinete development and oocyst infection rates in Air. albumanus, An freeborn and An gambioe. Their data indicated that proteolytic enzymes alone do not limit the early stages of sporogonic development in these vector species of Anopheles Studies involving vertebrate host factors are limited

### 1.9 Effects of drugs on gametocyte infectiousness to mosquito

Sub-therapeutic doses of antimalarial drugs have been reported to enhance insectivity of Plasmodium species to their vectors (Shute and Maryon, 1954) Additionally, numerous compounds including chloroquine (Williamson et al., 1976), trimethroprim-sulphamethoxazole (TS), (Wilkinson et al., 1973), pyrimethamine (Shute & Maryon, 1951), pyrimethamine-sulfadoxine (Carter & Graves, 1988) and berenil (Ono et al., 1993) have been suggested to induce gametocyte formation. In some studies, lack of influence of chloroquine (Jelsey et al., 1956, Smalley, 1977, Chutmongkonkul et al., 1992, Hogh et al., 1995) and PS (Hogh et al., 1995) on gametocyte infectivity was observed. It has been demonstrated that pyrimethamineand halosantrine-treated gametocytes of P. falciparim are more insective to Air stephensi mosquitoes than untreated controls (Chutmongkonkul et al., 1992). Other studies examined the effects of some schizontocidal agents on the sporogonic cycle of P. falciparum and P. bergher in anopheline mosquitoes (Coleman et al., 1988, Do Rosario et al., 1988). These studies found that chloroquine, when fed during late sporogony (10-12 days post-infection), may increase the vectorial capacity of some mosquito species.

The effects of chloroquine on the infectivity of chloroquine-sensitive and resistant populations of P. poelit ingertensis to An. stephensi mosquitoes showed an enhancement of infectivity in sensitive strains but no effect was detected in resistant clones and sublines (Ichimori et al., 1990). Chloroquine use and the subsequent development of resistance over the past years is associated with an increasing human malaria infectiousness (Lines et al., 1991) which may be indirect effects of parasitaemia on the host. Pyrimethamine but not chloroquine or halofantrine showed sporontocidal activity when evaluated by administration with infected blood meal to An. stephensi mosquitoes (Chutmongkonkul et al., 1992). Atovaquone (566C80) was noted to have inhibitory activities against ookinete, oocysts and sporozoites of Plasmodium berghet in An. stephensi (Fowler et al., 1994, 1995).

The reduction of oocyst burden in mosquito and potential to decrease the rate of transmission of resistant parasites have been reported with combination antimalarial drugs, for example, in chloroquine-artesunate (CQ-AS) treated parasite

(Hallett et al., 2004, Drakeley et al., 2004, Sutherland et al., 2003). Thus, combination therapy may prevent the transmission advantage enjoyed by drug-resistant parasites during gametocytes infectiousness and development in mosquitoes.

## 1.10 Morphological studies on gametocyte sex determination

In order to be transmitted by their mosquito vector, malaria parasites undergo sexual reproduction, which occurs between specialized male and female parasites (gametes) within the blood meal in the mosquito. Until recently, little was known about how Plasmodium determines the sex of its gametocytes (gamete precursors), which are produced in the vertebrate host. Recently, crythropoietin, the vertebrate hormone controlling crythropoiesis in response to anaemia, was implicated in Plasmodium sex determination in animal models of malaria (Paul et al., 2000). The sex ratio of malaria parasites may become progressively more male as conditions that allow motility and subsequent fertilization by the male parasites become adverse in animal models (Paul et al., 2002) and in human host following treatment (Sowurmi and Fateye, 2003 c). Natural and artificial induction of crythropoiesis in vertebrate hosts provokes a shift toward male parasite production. This change in parasite sex ratio often lead to reproductive success in the parasite, suggesting that sex determination is adaptive and could be regulated by the heamatologic state of the host (Paul et al., 2000).

High levels of gametocytacmia (Tchuinkam et al., 1993) and a male biased sex ratio (Robert et al. 1996b) may increase the infectivity of gametocytes to the mosquito feeding on humans. While the effects of some antimalarial drugs on the levels of gametocytacmia following treatment are known (Robert et al., 2000, Sowunmi and Fateye, 2003 a, b), there is little or no information on the temporal changes in sex ratio of *P. falciparum* following treatment with antimalarial drugs in children, the group most at risk for malaria in endemic areas. The effectiveness of an antimalarial drug to contribute to malaria control can be measured, in addition to rapidly clearing parasitacmia and other symptoms of infections, by production of temporal changes in sex ratios that will reduce gametocyte infectivity to mosquitoes.

### 1.11 Gametoeyte sex ratio determination

Carter and Graves (1988) and Robert et al (1996b) described the microscopic determination of gametocytes sexes. Under the microscope, the male gametes (microgametocytes) are smaller than females (macrogametocytes), the nucleus is larger in the males than the females, the ends of the cells are rounded in males and angular in females, with Giemsa the cytoplasm stains pale purple in males and deep blue in females, and the granules of malaria pigment are centrally located in females and more widely scattered in male. The sex ratio is defined as the proportion of peripheral gametocytes that are males (Pickering et al., 2000, West et al., 2001; Sowunmi and Fateyc, 2003 c).

Gametocyte sex is not determined by segregation of sex determining genes or chromosomes because malaria parasite is haploid in the vertebrate host and a single clone can produce both male and female gametocyte (Carter and Graves, 1988). Thus an adaptive significance of gametocyte sex ratio exists in parasite transmission. The malaria parasite reproductive success is constrained by the effect of immune system of the host directly on the process of fertifization (Paul et al., 2000). Therefore, parasites maintain their transmission success during course of an infection by either increasing overall gametocytacmia as parasitacmia rises or adjust the gametocyte sex ratio in response to the changing host environment (Paul et al., 2000). This adaptive transmission capacity provides insight to the mechanism of sex determination in malaria parasite.

Several concepts have been developed in the efforts to explain gametocyte sex ratios dynamics in malaria (West et al., 2001). Natural selections often favour genes that maximize their transmission to the next generation- survival of the fittest. A close examination of sex determination in Plasmodium and sex ratios changes are enucial to our understanding of the transmission success, disease epidemiology and evolution, for example, of resistant infections. The occurrence and rate of inbreeding and outcrossing contribute to sex allocation and dynamics of unbeatable sex ratio, the sex tatio that maximize successful transmission (Read et al., 1992, Nee et al., 2002, West et al., 2002). However, little is known of the direct effects or contributions of

host and antimalarial drugs factors to altering the dynamics of sex allocation and transmission success that may result due to gametocyte sex ratio changes.

## 1.12 Effects of chemotherapy on gametocyte sex ratio

There have been very few studies on the effects of malaria chemotherapy on gametocyte sex ratio in man or in vivo in artificials. Sometiment and Fateye (2003 c) showed that pyrimethamine-sulfadoxine treatment of acute fateiparum malaria in children enhanced gametocyte maleness. However, these authors (Sowunni and Fateye, 2003 d) also showed that chloroquine treatment of chloroquine sensitive infections in children produced little or no effect on gametocyte sex ratio. Talman et al., (2004 a) demonstrated that following treatment of P. fulciparum malaria in human and treatment of P. vinckei petteri in experimental animals with chloroquine and pyrimethamine-sulfadoxine, the sex ratio of the gametocytes did not become male biased. These differences in the findings by Sowunmi and Fateye (2003 c, d) and Talman et al. (2004 a), suggest that more studies are urgently needed to evaluate the effects of antimalarial drugs on gametocyte sex ratio.

# 1.13 Recent advances in estimation of gametocytacmia

For many years, presence and estimation or quantification of gametocytes in peripheral blood have been done by microscopy. Gametocyte detection by microscopy is laborious. Recently, it has been shown that submicroscopic gametocytaemia is common in children in areas of high and low transmission in Africa using molecular gametocyte detection technique, the real-time nucleic acid sequence-based amplification, (QT-NASBA) (Bousema et al., 2006; Shekalaghe et al., 2007). The technique is based on the amplification of gametocyte specific messenger ribonucleic acid (Pfs25 mRNA). The lower limit of sensitivity of this method for the quantification of P. falciparum gametocytaemia is 20- 100 per milliliter of blood. Using this method, it has been shown that following treatment of acute infections with mono and combination therapies, submicroscopic gametocytaemia is common and can be infectious to mosquitoes (Bousens et al., 2006).

### 1.14 Areas where studies are lacking and additional knowledge is required

Although gametocyte carriage has long been documented in Nigerian children with acute falcipatum infections, (Bruce-Chwatt, 1951) and contribution of drug resistance of *P. falcipatum* to increasing the prevalence and intensities of gametocytaemia have been documented (Sowunmi and Fateye, 2003 a, b, c), there is dearth of information on gametocyte carriage in Nigerian children, the host factors that support gametocyte carriage and transmission, and role of seasonality of infection on gametocyte generation and carriage. In addition, the effects, following treatment, of mono and combination drug therapies on carriage, intensity and sex ratio dynamics that contribute to continuing transmission are unknown. Certain compounds have been used to reverse chloroquine resistance in vivo in children with acute malaria infections in Nigeria (Sowunmi et al., 1997) and to chemosensitize *P. falciparum* to PS in vitro (Nzila et al., 2003), little is known of the potential effects of these compounds on gametocyte generation and contribution to transmission success of *P. falciparum*, should field and clinical evaluation of their use be favoured by health policy in the near future.

## Aims of the present study

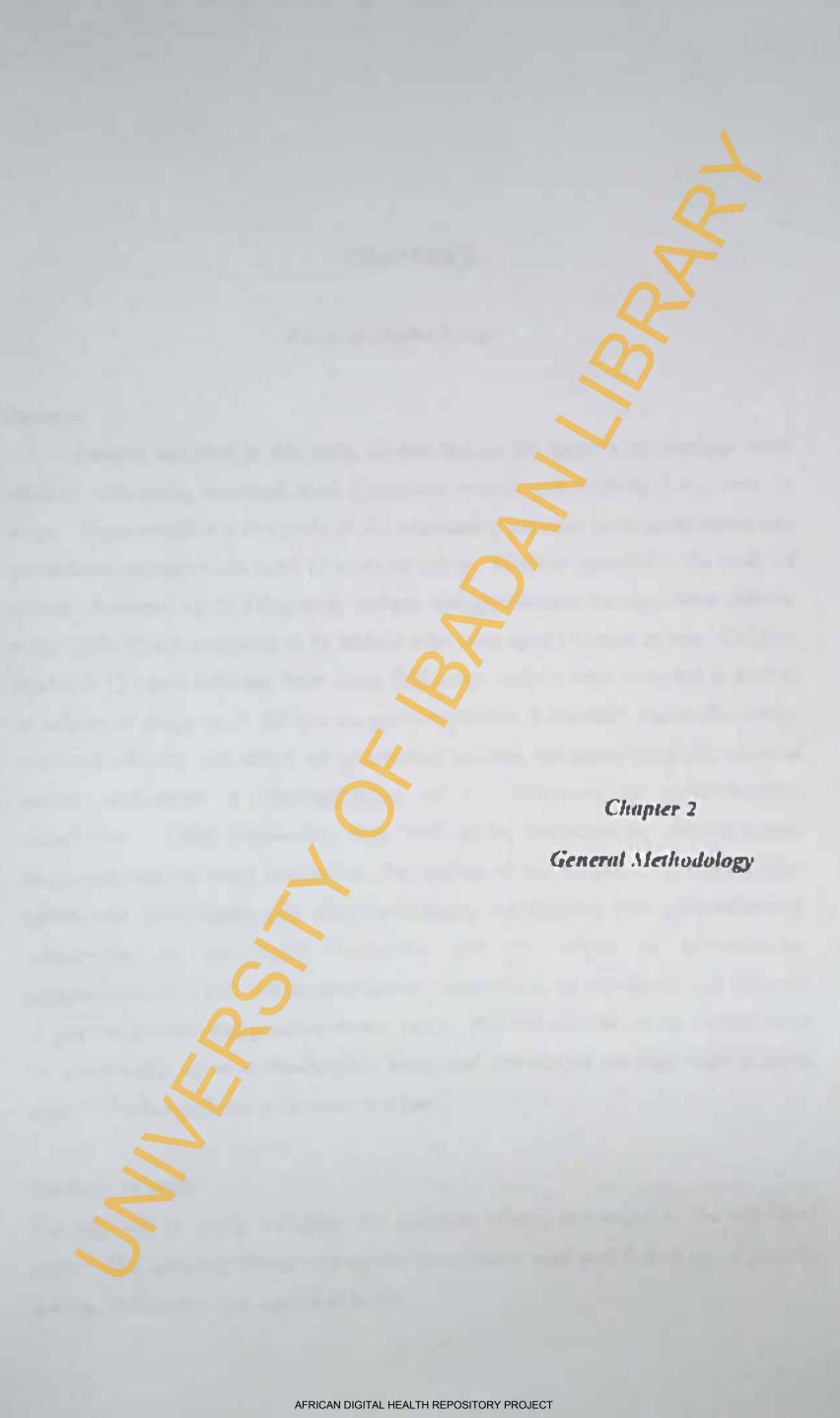
From the foregoing, it is clear that an essential component for successful control of malaria is understanding of the dynamics of gametocytacmia, a measure of transmissibility of P. faletpurum, and the influence of currently available antimalarials including potentially used combination adjuncts on malaria transmissibility. In addition, there is a need for systematic study of the epidemiology of gametocyte carriage and the influence of antimalarial drugs on gametocyte dynamics and gametocyte sex ratio.

It is for these reasons that the studies reported in this dissertation were carried out. The objectives of the studies reported in this dissertation were:

- 1. To determine the risk factors for gametocyte carnage in Nigerian children with acute uncomplicated Plasmodnim falciparum malaria
- To evaluate the effects of season on gametocyte carriage and response to therapy in children during acute l'lapnodium falciparum malaria

- To evaluate the relationship between hyperparastacmia and gametocyte carriage in children.
- 4 To compare the response to treatment with chloroquine in children who had with those who did not have gametocytes and assess the relationship between chloroquine blood levels and gametocyte carriage
- To assess the safety, treatment efficacy, and effects on gametocyte carriage of adding probenecid, a chemosensitizer of *P. falciparum* to pyrimethamine-sulphadoxine, to pyrimethamine sulfadoxine.
- 6. To compare the effects of probenecid when added to PS and PS alone on gametocyte carriage, gametocyteemia and gametocyte sex ratios in children with acute uncomplicated falciparum malaria.
- 7. To evaluate the effects of pyrimethamine-sulfadoxine, chloroquine plus chlorpheniramine and amodiaquine plus pyrimethamine-sulfadoxine on gametocyte production in children with acute, symptomatic, uncomplicated falciparum malaria
- 8. To compare the effects of trimethoprim-sulfamethoxazole and pyrimethamine-sulfadoxine on prevalence and intensity of gametocytaemia and gametocyte sex ratios in children with acute, symptomatic, uncomplicated lasciparum malaria





#### CHAPTER 2

## General Methodology

#### Patients

Patients included in the study of risk factors for gametocyte carriage were children with acute, uncomplicated, falciparum malaria and were aged less than 12 years. Those enrolled in the study of the relationship between hyperparasitacmia and gametocyte carriage were aged 12 years or below. Children included in the study of effects of season on P. falciparum malaria and gametocyte carriage were children with febrile illness suspected to be malaria who were aged 15 years or less. Children aged 0.5-12 years suffering from acute falciparum malaria were included in studies of effects of drugs on P. falciporum gametocytacmia in children; and in the safety, treatment efficacy, and effects on gametocyte carriage and gametocyte sex ratios of adding probenecid, a chemosensitizer of P. falciparum to pyrimethaminesulfadoxine. Under-twelve-year olds with acute, symptomatic, uncomplicated, falciparum malaria were included in the studies of the effects of pyrimethaminesulfadoxine, chloroquine plus chlorpheniramine, amodiaquine plus pyrimethaminesulfadoxine, on gametocyte production and the effects of trimethoptimsulfamethoxazole (TS) and pyrimethamine -sulfadoxine on prevalence and intensity of gametocytacmia and gametocyte sex ratios. Patients included in the evaluation of the relationship between chloroquine levels and gametocyte carriage were children aged 3-15 years with acute falciparum malaria

# Methods of study

The methods of study, including the selection criteria are stated in the individual study. The sampling times, appropriate biochemical tests and follow up of patients are also indicated in the individual study.

## Drug treatment

Children enrolled in the studies were treated with chloroquine (CQ), amodiaquine (AQ), pyrimethamine sulfadoxine (PS), chloroquine plus chloropheniramine (CQCP), chloroquine plus pyrimethamine sulfadoxine (CQPS), amodiaquine plus pyrimethamine sulfadoxine (PPS). Probenecid plus pyrimethamine sulfadoxine (PPS) The dosing regimens are indicated in the individual study as appropriate

Quantification of asexual and sexual parasitaemia and determination of gameiocyte sex and sex ratio

All blood films for examination of malaria parasite densities were stained with Giemsa. Parasites were counted against white cells in thick films under an oil immersion objective. Five hundred asexual forms of P. falciparum or the number of such parasites corresponding to 1000 white cells were counted, whichever occurred first. From this figure, parasite density was calculated from the known white blood cell count or by assuming a white cell count of 6000/µl if the actual white blood cell count was unknown

Gametocytaemia was quantified using the Giemsa stained thick blood smears. Levels of gametocytaemia (sexual forms/ µl) were estimated by counting gametocytes against 1000 leucocytes and assuming each patient had 6000 leucocytes/µl blood. If the level of gametocytaemia was at least 10 sexual forms/µl, the gametocytes were sexed on the basis that males (microgametocytes) are smaller than females (macrogametocytes), the nucleus is larger in the males than the females, the ends of the cells are rounded in males and angular in females, with Giemsa the cytoplasm stains pale 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 (Carter and Graves, 1988, Robert et al., 1996b). Gametocyte sex ratio was defined as the proportion of gametocytes in peripheral blood that were microgametocytes (Pickering et al., 2000). A minimum of 200 fields was counted before declaring any slide negative

## Disposition kinetics of gametocytaemia

Gametocyte kinetic parameters were estimated from the levels of micro- and macrogametocytaemia by a non-compartmental method, using the computer programme Turbo Ken (Clinical Pharmacology Group, University of Southampton, UK., through the courtesy of Professor A.G. Renwick), generally as previously described (Sowunmi and Fateyc, 2003 b). The time taken to attain a sex ratio of 1 (SR1) was defined as the time elapsing from drug treatment until this ratio was achieved and was calculated for each patient, from a plot of sex ratio vs time, by computer extrapolation. The data from the patients who did not have at least three estimates of gametocyte sex ratios were excluded from the estimation of SR1 and the exploration of the disposition kinetics of gametocytacmia. After determining SR1, the absolute counts of micro- and macro-gametocytaemia were log-transformed for each patient and plotted against time. The following parameters were noted or determined (1) time to attain SRI (ts<sub>R1</sub>). (2) area under the curve of the plot of micro- or macrogamctocytacmia v. time, from tsg1 to day 14 (AUCsg114), (3) the half-lives (11/2) of the micro- and macro-gametocytaemia, calculated from tset, and (4) the volume of blood completely cleared of micro- and macro-gametocytacmia from ton, defined as (the level of micro- and macro-gametocytaemia at tsal/AUCsal-t4. Since it was difficult to determine the time that gametocyte recruitment stopped in the patient, the levels of micro- and macro-gametocytaemia at tart were assumed to be the levels when recruitment stopped

# Handling of samples

Blood for haematological and biochemical tests were collected in appropriate sample bottles and were processed according to individual specifications. In general, blood was collected in heparicized bottles and immediately processed as required for the tests. Venous blood (5 ml) was obtained for drug level determination before treatment (day 0), on the eighth day following initiation of treatment (day 7) and on the day of failure or recrudescence of parasitaemia. Blood samples for drug level estimation in plasma and red blood cells were collected into heparinized bottles and

centrifuged immediately at 2000 x g. The plasma sample was separated and both samples were stored frozen at -20°C until analysis. Blood was also collected into heparinized bottles from healthy volunteers for the chloroquine calibration curves and were processed in the same ways as samples collected from patients.

## Chloroguine assay

Red blood cell and plasma chloroquine concentration levels were estimated in 12 and 10 children with resistance and sensitive response, respectively. Chloroquine was assayed in plasma and red blood cell by high performance liquid chromatography (HPLC) with ultraviolet (UV) detection, using a modified method previously used for the estimation of quinine (Babalola et al., 1993) Plasma (Iml) and the internal standard 10µ1 of papaverine (5µg/ml) were alkalinized with 1ml of 2 M NaOH and whirl mixed for 1 minute. The mixture was extracted with 2ml diethyl ether and vortexed for I minute. The organic layer was separated following centrifugation at 1200 revolution per minute (rpm) for ten minutes 100 pt of 0.1 N HCl was added to 2ml of the organic layer and the mixture vortexed for 1 minute and centrifuged at 1200 rpm. The ether upper layer was removed and 20 µl of chloroquine extract injected into the HPLC (Beckman Gold, Model 127, Switzerland with computerized recorder). The mobile phase was a buffer consisting of 0.2 M sodium dihydrogen phosphate, methanol and acetonitrile at a ratio of 65 30 5, with 1 nil perchloric acid /100 ml of solution at pH of 3.7. The mobile phase was degassed in a sonicator just before use and pumped through the column at a flow rate of Iml/min. The column contained a Bondapak C<sub>18</sub> (3.9 x 300mm). The UV detector was set at 254nm. The thawed red blood cell, already lysed by the storage condition, was processed in the same way as the plasma. A typical chromatogram is shown in Figure 2.1.

# Calibration curve of chloroquine in plasma and red blood cell by HPLC method

Calibration curves based on peak area ratios (Drug/ Internal standard) were obtained by spiking drug-free samples with standard concentrations of chloroquine. One millilitre of red blood cell or plasma each taken in extraction tubes were spiked with chloroquine to give a concentration range of 50 - 1000ng/ml and with 10µl of 5µg/ml papaverine solution as the internal standard. 1ml of 2Af NaOl1 solution was

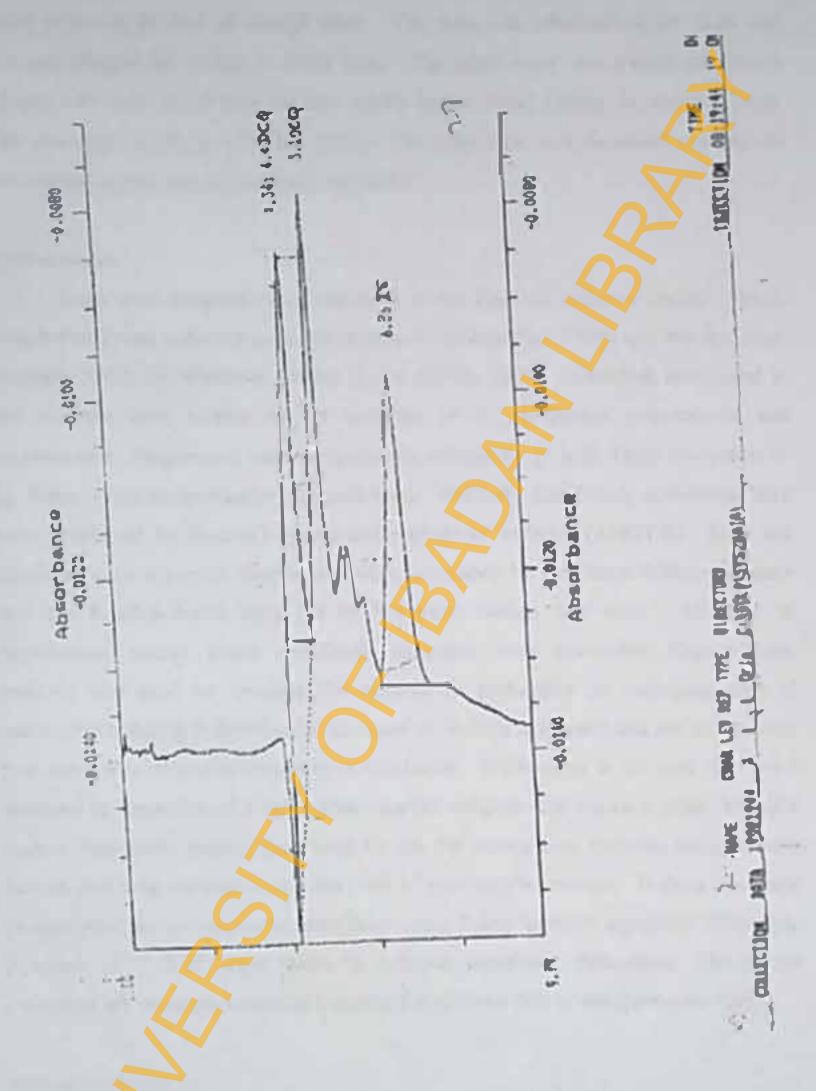


Figure 21 Chromatogram of chloroquine in red blood cell
(DCQ-desc) Ichloroquine, CQ-chloroquine, IS- interval standard)

added followed by 2ml of diethyl ether. The tube was whirl-mixed for 1min and then centrifuged for 10min at 2000 rpm. The ether layer was transferred into a sapered tube and 100µl of 0.1N HCl added before whirl mixing for another 2min. This was centrifuged for a further 10min. The ether layer was discarded and 10µl of the aqueous phase was injected into the HPLC.

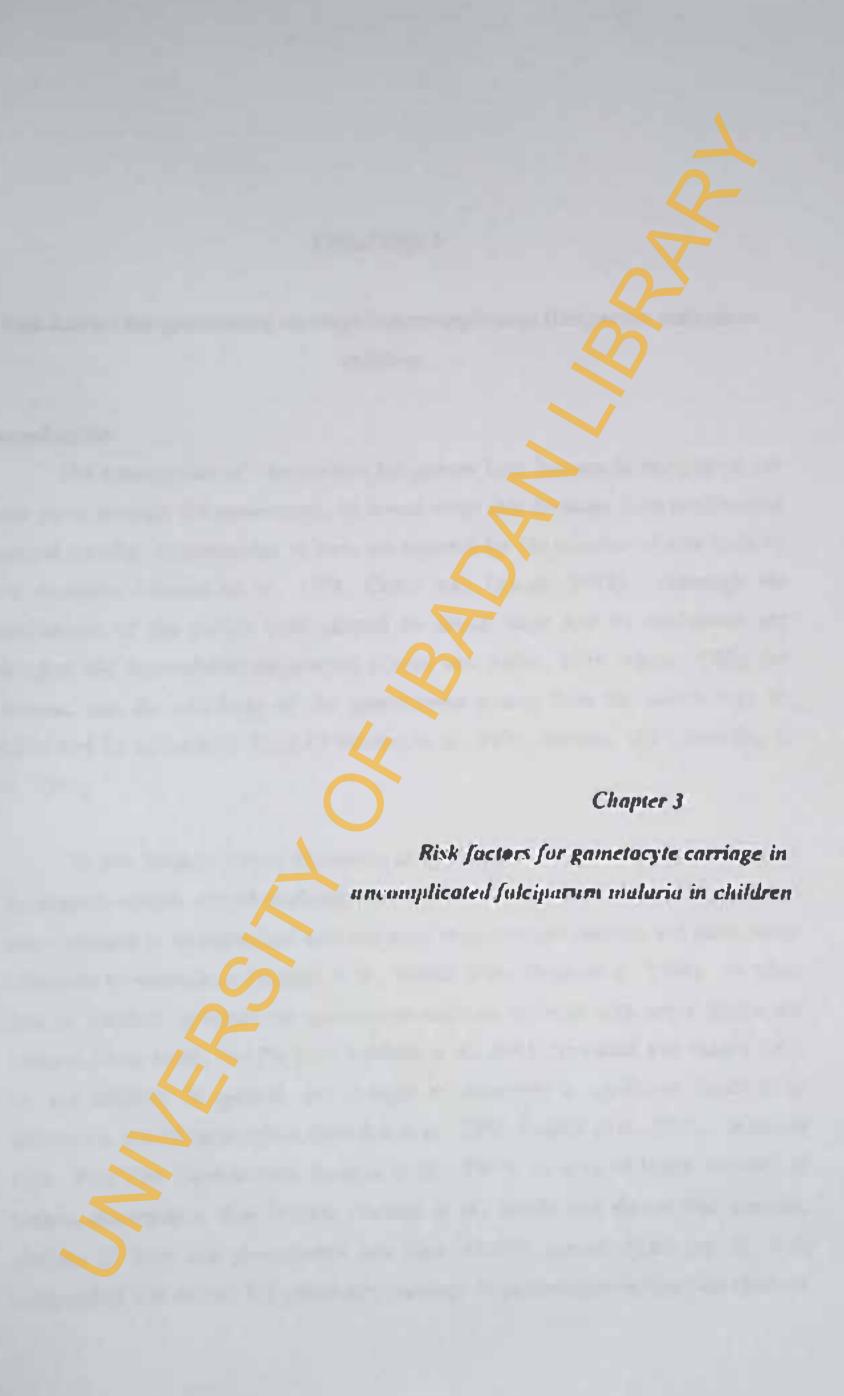
## Data analysis

Data were analysed using version 6 of the Epi-Info software (Anon., 1994). Graph Pad Prizm software package version 3.0 (GraphPad, 1999) and the statistical program SPSS for Windows version 10.01 (SPSS, 1999). Variables considered in the analysis were related to the densities of P. Jalcipanim ganietocytes and trophozoites. Proportions were compared by calculating  $\chi^2$  with Yates' correction of 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 the Mann-Whitney U-tests and the Kruskal-Wallis tests (or by Wilcoxon ranked sum test). All tests of significance, except where specifically indicated, were two tailed Kaplan-Meier analysis was used to estimate the cumulative probability of remaining free of gametocytes during follow-up for all cases of malaria combined and for those cases that were free of gametocytaemia at enrolment. Differences in survival time were assessed by inspection of Kaplan-Meier curves and pair wise log-rank tests. Multiple logistic regression models were used to test the associations between parasite, host factors and drug treatment, and the risks of gametocyte carriage. In drug treatment groups, post hoc comparisons were done using Tukey honestly significant difference P-values of  $\leq 0.05$  were taken to indicate significant differences. The values presented are generally means and standard deviations (sd) or standard error (se)

#### Ethical clearance

Ethical clearance for all the studies was obtained from the Joint University of Ibadan/ University College Hospital, Ibadan ethics review committee, and the Ethics Committee of the Ministry of Health, Ibadan





#### CHAPTER 3

# Risk factors for gametocyte carriage in uncomplicated falciparum malaria in children

#### Introduction

The transmission of Plasmodium falciparum from humans to mosquitoes can only occur through the gametocyte, its sexual stage that develops from proliferating asexual parasite. Gametocytes, in turn, are essential for the infection of new hosts by the mosquito (Sinden et al., 1978; Carter and Graves, 1988). Although the mechanisms of the switch from asexual to sexual stage and its modulation are complex and incompletely understood (Carter and Miller, 1979; Mons, 1985), the process, and the infectivity of the gametocytes arising from the switch may be influenced by antimalarial drugs (Wilkinson et al., 1976; Butcher, 1997, Buckling et al., 1999).

In sub Saharan Africa, increasing drug resistance in P. falciparum has led to increases in malaria related morbidity and mortality (Trape et al., 1998, Trape, 2001) and is thought to be associated with increases in gametocyte carriage and gametocyte infectivity to mosquitoes (Robert et al., 1996a, 2000, Hogh et al., 1998). In West African children, pretreatment gametocyte carriage in those with acute falciparum infections may reach 14-17% (von Seidlein et al., 2001, Sowunmi and Fateye 2003 b), and children, in general, are thought to constitute a significant reservoir of infection in sub-Saharan Africa (Githeko et al., 1992, Bonnet et al., 2003). A recent study from The Gambia (von Seidlein et al., 2001), an area of lesser intensity of malaria transmission than Nigeria (Salako et al., 1990), has shown that anaemia, absence of fever and parasitacmia less than 100,000 asexual forms per µL were independent risk factors for gametocyte carriage at presentation in Gambian children

In addition, treatment with pyrimethamine-sulfadoxine (PS) alone was associated with increased risk of gametocyte carriage seven days after treatment compared to chloroquine (CQ) or artemisinin-based combination therapy. It is unclear whether these factors, alone or in addition to others, are associated with gametocyte carriage in Nigerian children.

Although with increasing antimalarial drug resistance (Falade et al., 1997; Sowurm et al., 1998 a, b; Sowurm, 2002) there has been associated increases in gametocyte carriage in Nigerian children (Sowurm and Faleye, 2003 b), there is little information on the risk factors associated with gametocyte carriage pre- or post-treatment in Nigerian children. Such information is necessary as it may potentially harness the efforts aimed at the management and control of drug resistance in the community. In the present study the factors that influence the production of gametocytes were evaluated, in children presenting with acute, symptomatic, uncomplicated, P. falciparum malaria in a hyperendemic area of malaria in southwest Nigeria. The main aims were to define the host, parasite and drug factors that contribute to gametocyte production and carriage.

#### Patients and methods

Patients

The study took place between July 1996 and December 2002 in patients presenting at the University College Hospital in Ibadan, a typerendentic area for malaria in southwestern Nigeria (Salako et al. 1990). Ethical clearance was provided by the local ethics committee. During the period, a series of antimalarial drug studies were conducted to evaluate the efficacy and safety of different treatment regimens. Studies on CQ were done during the entire six years period, those of chloroquine plus chlorpheniramine (CQ-CP) in the first three years, those of PS in the first two years and the last 2 years, those of amodiaguine (AQ) alone in the last three years, and those of combination antimalarials in the last two years. However, there was considerable degree of overlap in the study periods. Details of the studies have been described before (Sowuluni et al., 1998 a, b, c; Sowuluni 2002, 2003; Sowunnii and Fatcye 2003 b). Briefly, children with symptoms compatible with acute falciparum malaria who fulfilled the following criteria were enlisted in the study age below 120 months, pure P. falciparum parasitacmia greater than 2000 asexual forms/µl blood, negative urine tests for antimalarial drugs (Dill-Glazko and lignin tests), absence of concomitant illness, no evidence of severe malaria (W110, 2000) and written informed consent given by parents or guardians. After enrolment and start of treatment (day 0), follow-up with clinical and parasitological evaluation was at days 1-7, and then on days 14, and when necessary, on days 21 and 28, for example, in patients who received PS combined with CQ (CQPS) or AQ (AQPS) Clinical evaluation consisted of a general clinical examination including measurement of weight, core temperature and physical examination.

# Assessment of parasitaemia

Thick and thin blood lilms prepared from a finger prick were Giemsa-stained and were examined by light microscopy under an oil-immersion objective, at x 1000 magnification, by two independent assessors. Parasitaemia in thick films was estimated by counting asexual parasites relative to 1000 leukocytes, or 500 asexual forms, whichever occurred first. From this figure, the parasite density was calculated assuming a leukocyte count of 6000/µL of blood. Gametocytes were also counted in thick blood films against 1000 leukocytes assuming an average leukocyte count of

6000/µL of blood (Shaper and Lewis, 1971, Ezeilo, 1971; Sowunmi et al. 1995)
Haematocrit was done at enrolment in 124 of the patients treated with CQPS or
AQPS in order to evaluate the safety of combination antimalarial therapy

## Evaluation of response to drug treatment

Response to drug treatment was assessed using World Health Organization (WHO) criteria (WHO, 1973) as follows: S = sensitive, clearance of parasitaemia without recurrence; RI (mild resistance) = parasitaemia disappears but reappears within 7 to 14 days: RII (moderate resistance) = decrease of parasitaemia but no complete clearance from peripheral blood, RIII (severe resistance) = no pronounced decrease or increase in parasitaemia at 48 hours after treatment. In those with sensitive or RI response, parasite clearance time (PCT) was defined as the time elapsing from drug administration until there was no patent parasitaemia for at least 72 h. Asexual parasite reduction ratio [PRR] (White, 1997) was defined as the ratio of day Wday 2 parasitaemia.

## Statistical analysis

Data were analysed using version 6 of the Epi-Info software (Anon., 1994), and the statistical program SPSS for Windows version 10.01 (SPSS, 1999). Proportions were compared by calculating  $\chi^2$  with Yates' correction. Normally distributed, continuous data were compared by Student's t-tests and analysis of variance (ANOVA). In the drug treatment groups posthoc comparisons were done using Tukey honestly significant difference (Tukey HSD). Data not conforming to a normal distribution were compared by the Mann-Whitney U-test and the Kruskal-Wallis test (or by Wilcoxon rank sum test). A multiple logistic regression model was used to test the association between gametocytaemia (yes or no at presentation or during follow up) and factors that were significant at univariate analysis: male gender, presence of fever, duration of illness before presentation, asexual parasitacinia at presentation, drug treatment, and rectudescence of asexual parasites within fourteen days of initiating treatment. Because the study was conducted over a

period of 6 years, time in years since the commencement of trials was included as a covariate in the model for pretreatment gametocytaemia. The values presented below are generally means and standard deviations (sd) or standard error (se) or median with interquartile range [IQR]. P-values of < 0.05 were taken to indicate significant differences.

#### Results

Patent gametocytaemia (geometric mean 26, range 6 – 1344/1tL) was present m 115 (15%) of the 767 children at enrolment

Risk factors for gametocyte carriage of enrolment.

The responses of the asexual parasitaemia to drug treatment and gametocyte carriage during and/ or after treatment are shown in Table 3.1. PRR in children treated with AQPS or CQPS was significantly higher than all other treatment groups (P < 0.001) with the exception of the AQ and PS groups, which, compared to CQPS, did not differ significantly (P = 0.099 and 0.30 respectively, Tukey HSD). PCT was significantly shorter in those treated with AQPS and CQPS compared to other treatment groups (P < 0.001) except AQ (P = 0.052 and 0.25, respectively, Tukey HSD). PCT was also significantly shorter in those treated with AQ compared to CQ (P = 0.019, Tukey HSD). Factors associated with gametocytaemia at enrolment are presented in Table 3.2. Male gender, absence of fever, duration of illness > 3 d, and asexual parasite densities less than 5000/µL were related to the presence of gametocytaemia at enrolment. Neither age nor packed cell volume at presentation was an independent risk factor for gametocyte carriage (Table 3.2).

# Risk foctors for gametocyte carriage during follow up

During follow-up, 15.6% of all patients (i.e. 120 patients) developed patent gametocytnemia, which in 85% (102 patients) had developed by day 7 following treatment. Gametocyte densities at enrolment were similar in all treatment groups, were significantly higher on day 14 in those treated with PS, and a significantly higher proportion of children treated with PS carried gametocytes throughout the duration of the study (Table 3.3). In the cohort of children in whom gametocytes were not detected at enrolment, 36 of 259 (13.9%) children treated with CQ, 9 of 82 (11.%) treated with CQCP, 3 of 93 (3.2%) treated with AQ, 1 of 64 (1.6%) treated with CQPS, 3 of 64 (4.7%) children treated with AQPS, and 50 of 90 (55.6%)

TABLE 3.1 Responses of asexual parasitaemia and gametocyte carriage following treatment with antimalarial drugs
(Standard doses of drugs were given at presentation (day 0) and asexual parasitaemia quantification was done daily for 8
d (days 0-7) and then on day 14. Gametocyte carriage was assessed on days 0, 7 and 14)

	CQ	CQCP	AQ	PS	CQPS	AQPS	P value
	(n = 315)	${n = 104}$	(n = 104)	(n = 109)	(n = 65)	(n = 70)	
%, of children with							
gametoc) tes							
at enrolment	17 8 (n = 56)	21.1 (n = 22)	10.6 (n = 11)	17.4 (n = 19)	1.5 (n = 1)	8.6 (n = 6)	0.001
on day 7	248 (n = 78)	$23 \cdot 1 \cdot (n = 24)$	106 (n = 11)	61.5 (n = 67)	3 l (n = 2)	10.0 (n = 7)	0.001
on day 14	17.1 (n=54)	106 (n = 11)	7.7 (n = 8)	48 6 (n = 53)	•	4.3 (n = 3)	0.001
PRR							
Medizo	2 27	2.30	2.75	3-18	3.94	3.70	<0.001*
Interquartile range	1 42 - 3 97	1.73 - 3.93	1.85 - 4.11	1.91 - 4.13			
Range	-0.7 - 5.77	0.10 - 5.32	-0.40 - 5.10	-0.21 - 5.6	-1 0 - 5.66	0.72 - 5.65	
PCT (days)	29+09	28+08	26 ÷ 0.8	29±11	23+08	22±08	0.001
(,-,	1-6	1-5	1-5	1-6	1-4	14	
S (no of patients)	198	97	102	78	65	70	100.0
RI	87	6	2	18	•		
RÍI	15	•		9			
RIII	15	1	. /	4			
Cure rate (%6)	62.9	93.2	98.1	71.5	100	100	100.0

PRR. paralite reduction ratio. PCT. parasite elearance time; PS, P) timethamine-sulfadorine; CQ, chloroquine. CQCP, elstoroquine plus chloriphentratume. AQ, emphasine; COM, p) translate combined with chloroptine or emphasize and complete elearance from Periphetal blood. Rill = no plunounced decrease or increase in parasitionnia at 48 hours after members. S = sensitive response. \*PRR of AQPS. and CQPS-treased children were significantly higher than in other treatment groups except those treated with AQ or PS (compared with COPS. P = 0.09 and 0.30).

\*\* PCT was significantly shorter in those trented with AQPS and CQPS compared to other treatment groups (P < 0.001) except AQ (P = 0.052 and 0.25, respectively. PCT was also significantly shorter in those trented with AQ compared to CQ (P = 0.019. Tuley HSD)

TABLE 3.2. Risk factors for P. falciparum yametocytaemia at enrolment

		No of children with gamelocytes	Crude OR (95% CI)	P. value	Adjusted OR (95% CI)	P. value
Age (y)					2	
< 5	420	69	1			-
≥5	347	46	0.78 (0.52 - 1.2)	0.26		
Gender						
male	354	66	1		1	
femule	413	49	0.6 (0.4-0.9)	001	0.55 (0.36 - 0.83)	0.005
Parasitaemia						
(/µL)						
< 5000	82	21	1		1	
> 5000	685	94	0.46 (0.26 - 0.83)	0.007	0.42 (0.24-0.73)	0.002
Fever •						
Febrile	533	73	L		1	
Afebrile	208	42	1 6 (1 06-2 13)	0 04 ª	1.61 (1.05 - 2.5)	0.03
Duration of						
illness						
≤3 d	575	76	1		1	
>3 d	162	39	17(11-27)	0.001	1.57 (1.0 - 2.4)	0.047
PCV (%)						
< 25%	24	3	1 (0)			
> 25%	100	10	0.78 (0.52 - 1.2)	0.71		

OR odds ratio CI, confidence interval

PCV packed cell volume

"Fores, acillary temperature ≥ 37.5°C

+ Time was included as a constrate in the analysis

<sup>&</sup>quot; x' with Yate's correction

TABLE 3.3. Gametocyte densities at enrolment and following treatment with various antimalarial drugs

	CQ (n = 315)	CQCP (n = 104)	AQ (n = 104)	PS (n = 109)	CQPS* (n = 65)	AQPS (n = 70)	P value
Gametocytaemia	( 513)	(10 10 0)	(11	(6, 100)			
At enrolment							
GMGD (/µL)	25 (n = 56)	24 (n = 22)	29 (n =   1)	24 (n = 19)	132 (n = 1)	40 (n = 6)	0.55
Range	6 - 1344	12 - 576	12 - 740	6 - 444	<b>/</b> 132	12 - 288	
On day 7							
GMGD (/此)	34 (n = 78)	43 (n = 24)	34 (n = 11)	75 (n = 67)	54 (n = 2)	31 (n = 7)	0.054
Range	6 – 1476	12 - 696	12 - 636	6 - 3520	24 - 120	12 - 468	
On day 14							
GMGD (/此)	21 (n = 54)	41 (n = 11)	16 (n = 8)	50 (n = 53)	-	19 (n = 3)	0.003
Range	6-144	12 – 168	12 - 36	6-480		12 - 48	
Proportion (%) of							
children with gametocytaemia on days 0. 7 & 14	6.7 (n = 29)	77 (n = 8)	3.8 (n = 4)	12.8 (n = 14)		1.4 (n = 1)	0.030

GNOD Generale mean gameton te density, PS, pyrachamine salladorine; CQ, chloroquine, CQCP, chloroquine plus chlorohomounine. AQ, amodinquine, COM, pyrimethamine salladorine combined with

chlerogeine or amoduquine
\*COPS not included in the compareson due to small number

children treated with PS developed patent gametocytaemia within 7 days of enrolment. Thus, the proportion of children who developed gametocytaemia following treatment were significantly higher in those treated with PS compared with other treatment regimens ( $\chi^2 = 136.9$ , P = < 0.001)

Presence of patent gametocytacmia at enrolment, and recrudescence of asexual parasites within 14 d were associated with presence of gametocytacmia 7 or 14 days after enrolment (Table 3.4). Delay in the time taken to clear the initial parasitacmia was associated with increased risk of subsequent gametocyte carriage, but this association was not significant following multivariate analysis (Table 3.4). Children treated with AQ, AQPS or CQPS were significantly less likely to have delayed (> 2 d) parasite clearance compared with those treated with CQ or PS alone  $(\chi^2 = 41.7, \text{ degree of freedom (df)} = 5, P < 0.001)$ 

Presence of gametocytes on day 7 or 14 was significantly associated with treatment outcome by day 14 in children treated with CQ ( $\chi^2 = 18.3$ , df = 1, P = <0.001) and CQCP ( $\chi^2 = 10.1$ , df = 1, P = 0.001), but not PS ( $\chi^2 = 0.21$ , df = 1, P = 0.64), and AQ ( $\chi^2 = 0.24$ , df = 1, P = 0.62) and AQPS or CQPS in which all children were clinically cured.

TABLE 3.4 Risk factors for P. Salciparum gametocytaemia 7 days after treatment

Starus et enrolment	Total	No of children with gametocytes on day 7	Crude OR (95% CI)	P. value	Adjusted OR (95% Cl)	P value
Gametocytes						
Present	115	86	1		1	
Absent	652	102	0.06 (0 04-0 09)	<0.001	0.04 (002 - 0.07)	< 0 001
PCT						
≤2 d	298	61	1		1	
>2 d	469	127	1.4 (1.00-2.07)	0.047	1.4 (0.9 – 2.1)	0.20
Patent asexual paras- itaemia within 14days						
Present	157	68	1		1	
Absent	610	121	0.32 (0.22 - 0.47)	<0.001	0.50 (0.3 – 0.8)	0.007
Drug treatment*						
PS	109	67		1	1	1
CQ	315	78	48 (30-79)	<0.001	8.5 (4.9 - 14.6)	<0.001
COCP	104	24	5.3 (2.8 - 10.1)	<0.001	94 (4.5 - 197)	< 0.001
AQ	104	H	13.5 (6 2 – 30.2)	< 0.001	17.4 (7.3 – 41.0)	< 0.001
AQPS	70	6	14.4 (5.7 – 38.0)	100.0>	14.9 (5.5 -10.2)	<0001
CQPS	65	1	30.2 (112 - 313.7)	< 0.001	35.6 (7.8 - 163.5)	<0.001

OR odds rates. CI Confidence interval. "X with Yake's correction. PCT, parable clearance time
PS, primethamine-sulfadoxine. CQ, chieroquine, CQCP, chieroquine plus chierpheniramine. AQ. amoduquine;
COM, pyrimethamine-sulfadoxine combined with chloroquine or amodiaquine

C) considered interval

<sup>\*</sup>Values of OR represent chances of being gametory to free

#### Discussion

Gametocytes are often detectable in peripheral blood for a variable period after acute falciparum infection, with morphologically mature gametocytes being detectable in the blood 10-14 days after originating from merozoites (Thomson, 1911, Smalley, 1976). Carriage rates may vary widely and are dependent on several factors. In the current study, gametocyte prevalence was much higher than those reported from western Thailand (2%, Price et al., 1999) and Tanzania (8%, Akim et al., 2000) but similar to that from The Gambia (17%, von Seidlein et al., 2001) in the same region of Africa. However, despite regional differences in prevalence rates, the risk factors associated with gametocyte carriage were remarkably similar.

Gametocyte prevalence in the study area before the 1990s, a period of full sensitivity to CQ, was less than 2% (L.A. Salako, unpublished observation). Presently, in the area, CQ treatment of CQ-resistant infections is associated with significant gametocyte carriage and gametocytaemia, and slower clearance of gametocytaemia (Sowunmi and Fateye, 2003 a, b). Therefore, it would appear that the present relatively high prevalence rate, in part, may be due to increasing CQ resistance. Seventy percent of all cases of acute malaria infections in our area of study occur in children aged less than ten years (Salako et al., 1990), the similar gametocyte carriage in all age groups (Table 3.2) suggests children aged below 10 years were uniformly susceptible to gametocyte carriage. In other studies involving a broader age range than we evaluated, younger age was associated with increased gametocyte prevalence, for example, in Tanzania (Akim et al., 2000).

It is unclear why male gender is a risk factor for gametocyte carriage at presentation despite similar duration of illness and other characteristics in both gender groups enrolled in the present study. So far in records, this is the flist report of the association between male gender and gametocyte carriage in African children with falciparum malaria. Could this simply be a chance finding? Plasma testosterone is often significantly mased in pre-pubertal- male than -female children (Griffin and Wilson,

gametocytogenesis in vitro (Maswoswe et al., 1985, Linguau et al., 1993). It seems possible that differences in sex hormone levels may be contributory, but hormone concentrations were not measured in the children. Gender-related differences as risk factors for gametocyte carriage require further evaluation in African children.

As was expected, duration of illness longer than 3 days was associated with increased risk of gametocyte carriage on presentation. In areas of low transmission, duration of illness longer than 2 days has also been associated with gametocyte carriage (Price et al., 1999). As longer established P. falciporum infections are more likely to produce gametocytes (Smalley et al., 1981), it is likely that longer duration of illness before presentation allowed sufficient time for the progression of committed asexual parasites to gametocytes. Since absence of fever is associated with increased risk of gametocyte catriage, afebrile children may have harboured the infection for longer periods. Alternatively, children with longer duration of illness may have had a relatively shorter duration of fever resulting in reduced noxious effects of fever on gametocyte development. Low parasitacmia (as in the present study) and anaemia are also significantly associated with gametocyte carriage (Price et al., 1999, von Seidlein et al., 2001) but haematocrit values less than 25% was not associated with gametocyte carriage in our cohort of children. There is no clear explanation for this observation. Anaemia in uncomplicated falciparum malaria may be enhanced by pre-existing helminth infections (Nacher et al., 2002), and both conditions may enhance gametocyte carriage (von Seidlein et al. 2001, Nacher et al. 2002), frequently co-exist and, are common in tropical endentic regions

Despite lower efficacy (Table 3.1), CQ treatment resulted in lower gametocyte carriage than PS. A similar observation has been made in Senegal and The Gambia (Robert et al., 2000, von Seidlein et al., 2001). The ability to release more gametocytes into the circulation following PS treatment may, in part, be independent of parasite sensitivity to PS (Sowunmi and Fateye, 2003 c) and may partly explain this observation

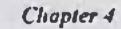
presentation were significantly more likely to be gametocytemic 7 days later than children without patent gametocyteemia. This suggests the drugs evaluated had little or no effect on mature circulating gametocytes.

As was expected, recrudescent infections were associated with higher gametocyte prevalence, as was delay in peripheral parasite clearance as parasites develop resistance to drugs. The increase in gametocyte carriage and density as resistance develops to antimalarial drugs may confer survival and propagations advantages on the parasite in the population (Handunnetti et al., 1996, Robert et al., 1996 a; Sowmuni and Fateye, 2003 a, b). In the current study, delayed clearance of peripheral parasitaemia and increased recrudescence rates were most frequently seen in those treated with CQ or PS and least frequent in those treated with AQ, AQPS or CQPS. Similar observations have been made elsewhere (Price et al., 1999, Robert et al., 2000, Akim et al., 2000; von Seidlein et al., 2001). The significantly higher gametocyte density in those treated with PS than CQ at recrudescence of asexual parasitacmia would suggest that the former may increase the propensity for the transmission of drugresistant infections than the latter since gametocyte infectivity to mosquitoes may correlate with level of gametocytecmia (Tchuinkam et al., 1993, Robert et al., 2000). Since leukocyc counts may vary widely, one of the possible sources of errors in the estimation of gametocyte density is assuming an average leukocyte count of 6000/µL of blood.

The findings of the present study may have potential implications for the management of acute infections in this endemic area prompt treatment of falciparum infections with effective drugs is often associated with low gametocyte carriage (and may invariably reduce transmission of gametocytes to mosquitoes), treatment of acute infections should, preferably, employ rapidly acting schizontocide to reduce the development of gametocytes. The artemisinin derivatives may reduce transmissibility by this mode of action (Price et al., 1996). Finally, the findings may have important

implications with respect to malarial control in sub-Saharan Africa, where combination antimalarial therapy (WHO, 2001 a, b) is presently being proposed for the treatment of malaria in the region.

Low parasitaemia, amongst other factors in this study, is associated with gametocyte carriage. However, *P. Julciparum* infections in children in an endemic area could present with hyperparasitaemia- a feature of severity of infection. This condition can be managed using oral treatment with antimalarial drugs and may provide a favourable condition for asceval parasite committed to gametocytogenesis. Little is known of the effects of drug treatment of hyperparasitaemia on gametocyte dynamics-release, carriage and intensity in children. It is important to determine the contributions of hyperparasitaemia in acute malaria and effects of its management on gametocyte carriage in children.



Plasmodium Inteinarum hyperparasitaemia in children:
risk factors, treatment outcomes, and gametocytaemia
following treatment

#### CHAPTER 4

Plasmodium falciparum hyperparasitaenia in children: risk factors, treatment outcomes, and gametocytaemia following treatment

#### Introduction

parasites and astronomical increases in circulating peripheral parasites particularly in the relatively non-immune or, less frequently, in the semi-immune. These astronomic increases may reach or surpass a threshold referred to as hyperparasitaemia. Hyperparasitaemia, defined as 5% or more parasitized crythrocytes or a parasitaemia greater than 250,000/µL blood, is considered one of the several features of severe malaria (Warrell et al., 1990)

(uncomplicated hyperparasitaemia) often poses management problems in patients resident in endemic areas. Apart from a general recommendation of parenteral antimalarials (WHO, 2000), there are no other clear-cut guidelines for the management of uncomplicated hyperparasitaemia in children resident in such areas. However, it has been suggested that uncomplicated hyperparasitaemia in children in these endemic areas be treated with oral antimalarial drugs providing the drug is rapidly absorbed and the parasites are fully sensitive to the antimalarial drug(s) chosen (Sowunmi et al., 1992, 1996, 2000 a). Such a suggestion needs review in view of the increasing resistance in P. falciparum to many antimalarials and the lack of facilities to monitor drug sensitivity of P. falciparum to many antimalarials and the lack of facilities to monitor drug sensitivity of

There is little information on, for example, the risk factors associated with uncomplicated byperparasitacinia or the time-course of gametocytacinias following oral antimalaria treatment of uncomplicated hyperparasitacinias in African children. Such information is necessary in view of the increasing resistance in *P. folciparum* to chloroquine (CQ) and other commonly available antimalarials and the increasing morbidity and mortality associated with drug resistance (Trape et al., 1998; Trape, 2001). In addition, it may improve the overall management of these cases. The present study was designed to address these issues. The main aims of the study were to evaluate the risk factors associated with hyperparasitacinia in a group of children presenting with acute, symptomatic, apparently uncomplicated, *P. folciparum* malaria in an endemic area; to assess the outcomes of oral antimalarial treatment of uncomplicated hyperparasitacinia, and to follow the course of changes in gametocytacinias in children with hyperparasitacinias who were treated with oral antimalarial drugs.

#### Patients and methods

Patients

The study took place between July 1996 and September 2003 in patients presenting at the University College Hospital in Ibadan, a hyperendemic area for malaria in southwestern Nigeria (Salako et al., 1990). Ethical clearance was provided by the local ethics committee During the period, a series of antimalarial drug studies were conducted to evaluate the efficacy and safety of different treatment regimens. All antimalarial drugs were given orally. The details of the studies have been described before (Sowiguini et al., 1998 a, b, c, 2000 a; Sowunmi, 2002, 2003, Sowunmi and Fateye, 2003 a) Briefly, children with symptoms compatible with acute falciparum malaria who fulfilled the following criteria were enlisted in the study: age below 12 years, pure P. Salciparum parasitaemia greater than 2000 asexual forms/µL blood, negative urine tests for antimalarial drugs (Dill-Glazko and lignin tests), absence of concomitant illness, no evidence of severe malaria (WHO, 2000) and written informed consent given by parents or guardians. After enrolment and start of treatment (day 0), follow-up with clinical and parasitological evaluation was at days 1-7, and then on days 14, and when necessary, on days 21 and 28, for example, in patients who received pyrimethamine-sulfadoxine (PS) combined with chloroquine or amodiaguine (COM). Clinical evaluation consisted of a general clinical examination including measurement of weight, core temperature and physical examination.

# Assessment of porasitaemia

Thick and thin blood films prepared from a finger prick were Giemsa-stained and were examined by light microscopy under an oil-immersion objective, at x 1000 magnification, by two independent assessors. Parasitacmia in thick films was estimated by counting asexual parasites relative to 1000 leukocytes, or 500 asexual forms, whichever occurred first. From this figure, the parasite density was calculated assuming an average leukocyte count of 6000/µL of blood (Shaper & Lewis, 1971, Ezeifo, 1971, Sowmani et al., 1995). Gametocytes were also counted in thick films against 1000 leukocytes assuming an average leukocyte count of 6000/µL of blood at enrolment (day 0) and on days 7 and 14. Fractional gametocyte density (FGD) at enrolment was defined

as gametocyte count divided by total asexual and sexual count (Price et al., 1999).

Haematocyte was done at envolment in 124 of the patients treated with PS or CQPS, AQPS or PPS

## Evaluation of response to drug treatment

Response to drug treatment was assessed using World Health Organization (WHO) criteria (WHO, 1973) as follows: S = sensitive, clearance of parasitaemia without recurrence, R1 (mild resistance) = parasitaemia disappears but reappears within 7 to 14 days, R11 (moderate resistance) = decrease of parasitaemia but no complete clearance from peripheral blood, R111 (severe resistance) = no pronounced decrease or increase in parasitaemia at 48 hours after treatment. In those with sensitive or R1 response, parasite clearance time (PCT) was defined as the time elapsing from drug administration until there was no patent parasitaemia for at least 72 h. Asexual parasite reduction ratio [PRR] (White, 1997) was defined as the ratio of day 0/day 2 parasitaemia.

## Statistical analysis

Data were analysed using version 6 of the Epi-Info software (Anon., 1994), and the statistical program SPSS for Windows version 10.01 (SPSS, 1999). Proportions were compared by calculating  $\chi^2$  with Yates' correction or Fisher exact test. 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 the Mann-Whitney U-test and the Kruskal-Wallis test (or by Wilcoxon rank sum test). A multiple logistic regression model was used to test the association between hyperparasitacmia (yes or no at presentation or during follow up) and factors that were significant at univariate analysis age  $\leq$  5 years, and presence of fever  $\geq$  39.5°C. Because the study was conducted over a period of 7 years, time was included as a covariate in the analysis. P-values of  $\leq$  0.05 were taken to indicate significant differences

#### Resuits

The demographic characteristics of the children enrolled in the study are summarized in Table 4.1. At enrolment, 303, 173, 104, 203, 143, 78 and 44 of the 1048 children were allotted to, and were subsequently treated with chloroquine (CQ) only; pyrimethamine-sulfadoxine (PS) only; amodiaquine (AQ) only; CQ plus chlorpheniramine (CQCP), PS combined with CQ or AQ (CQPS or AQPS), PS combined with probenecid (PPS); and halofantrine (IIF), respectively Hyperparasitaemia was found in 100 (9.5%) of the 1048 children at enrolment.

## Risk factors for hyperparasitaenna at enrolment.

Factors associated with hyperparasitaemia at enrolment are presented in Table 4.2.

An age  $\leq$  5 years, and a core temperature  $\geq$  39.5°C were independent tisk factors for uncomplicated hyperparasitaemia at enrolment

## Hyperparasitaenna during follow up

Following omit therapy, 1.2% of all patients (i.e. 13 of the 1048 patients) became hyperparasitaemic, which developed in all the 13 patients by day 1 of follow-up. The 13 patients who developed hyperparasitaemia following treatment were treated with CQ (10 patients), PS (1 patient) or COM (2 patients), and following treatment, all but two had sensitive response. The two children in the COM group who became hyperparasitaemic on day 1 specifically received PS combined with CQ. The two children with resistance response (1 R1, 1R1) were treated with CQ. Compared with other treatment groups, there was a significant difference in the proportion of children treated with CQ who become hyperparasitaemic on day 1 following treatment (P = 0.01).

# Treatment outcomes of hyperpurasitaenna

The clinical and parasitological characteristics of the 100 children who had hyperparasitacrate at enrolment and were treated with oral antimalarial drugs are

TABLE 4.1. Summary of demographic and other characteristics of the 1048 children carolled in the study

in the second	37-11
Variables	Value (mean + sd (range))
Age (years)	$5.5 \pm 2.5 (0.5 - 11.9)$
M F	493 : 555
Weight (kg)	15.1 ± 4.8 (6.6 - 27)
Presenting body temperature (°C)	$38.6 \pm 1.2 (36.4 - 40.8)$
Duration of illness (d)	$3.0 \pm 1.5 (1 - 14)$
Sexual parasite density (per pl)	
Geometric mean	30129
Range	2090-2341000
No. > 250000	100

TABLE 4.2. Risk factors for P. fulciparium hyperparasitaemia in children at enrolment

	, mode	, , , , , , , , , , , , , , , , , , , ,				•		
	Total no.	No of children with hyperparasitaemia	Ctude OR (95% CI)	P value	Adjusted OR (95% CI)	P value		
Age (years) ≤ 5 > 5	533 515	62 38	1.65 1 (1.06 – 2.6)	0.025	1 (1.05-2.47)	0.026		
Gender male female	493 555	<b>45</b> 55	1.1 1 (0 58–1.4)	0.7 8		*		
Duration of Illness (d) ≥ 3 < 3	696 352	66 34	0.98 1. (0.62 – 1.56)	0.9				
Fever * > 39 5°C < 39 5°C	214 834	31 69	1 (1.15-3.01)	0.008 <sup>d.</sup>	1 84 1 (1.17 – 2 89)	0.009		
Gametocytes								
Yes No	124 924	11 89	0.91	09°		÷		

OR, odds ratio

"I" with Yate's correction

Cl. confidence interval

summarized in Table 4.3 Despite enrolment at different periods, these characteristics were similar (primarily because the criteria for enrolment into all studies were similar).

No child with hyperparasitaemia was treated with AQ alone.

The responses of the asexual hyperparasitaemia to drug treatment are shown in Table 4.4. The cure rate following treatment with CQ was significantly lower than the other treatment groups (P = 0.001).

Comparison of outcomes of treatment of non hyperparasitaemia and hyperparasitaemia

Sixteen of 948 children without hyperparasitaemia had RIII response to treatment compared to 6 of 100 children with hyperparasitaemia. The difference between these proportions was significant ( $\chi^2 = 6.22$ , P = 0.001). Four children (3 treated with CQ and 1 with PS) aged  $\leq$  3 years who had hyperparasitaemia progressed to cerebral malaria, while 2 of the 948 children without hyperparasitaemia had the same outcome. The difference between these two proportions was significant (P = 0.001, by Fisher exact test). The 2 children without hyperparasitaemia who progressed to cerebral malaria were treated with CQ. Adverse reactions reported following drug treatment were similar in children with hyperparasitaemia and in age- and gendermatched children without hyperparasitaemia who were treated with the same drugs (data not shown). For example, in those treated with CQ, pruritus occurred in 5 (of 33) and 4 (of 33) children with and without hyperparasitaemia, respectively.

TABLE 4.3 Clinical and parasitological characteristics of 100 children with P. fulciparum hyperparasitaemia who were treated with oral antimplarial drugs

	CQ	CQCP	PS	COM	PP\$	HF•	P
	(n = 33)	(n = 25)	(n = 25)	(n = 11)	(n=5)	(n-1)	value
Age (years)							
Mean + sd	$4.3 \pm 2.3$	$5.1 \pm 2.3$	46+28	49+22	75 ± 1.5	5.0	0.6
Range	15-9	07-	0.5-10.5	3-8 1	6.3-10		
MF	12 21	10.5	12 13	6.5	3 2	1:0	
		13 12				- 10 50	
Duration of illness							
(d)							
Mean	28±12	3.2 ± 1.3	35+25	730+07	30+00	3.0	0.5
Range	1-6	1.6	1-14	2.4	3-3	5.0	0,5
114113-	1-0	1.0	1,		3-3		
Parasitaemia (/µL)							
<b>Geometric</b> теал	438649	467090	398318	387090	750304		0.4
Range	253091 -	253600 -	253091 -	250145 -	414750 -		
	1500000	2341000	1254000	716000	1388000		

PS. procedence sulfatorine, CQ, chlorogone, CQCP, chlorogone plus chlorohenimmine.

COM preachague solidoxise combined with colorogaine or amodiaquine,

PPS pynnichamine-sulfadoxine combined with protonoid HF, hatofaranne

<sup>\*</sup>Excluded from multiple comparison because of reliainely small number of patients

<sup>\*\*</sup>Parisaries at carolment was 438660 per pil

TABLE 4.4 Therapeutic responses of 100 children with acute P. falciparum malaria who had hyperparasitaenna at enrolment

	CQ (n = 33)	CQCP (n = 25)	P\$ (n = 25)	COM (n = 11)	PPS* (n = 5)	HF*	P. value
						1)	
FCT (d)							
Mean ± sd	20±09	23±10	2.2 ± 1.2	16+05	22+13	1.0	0.5
Range	1 – 4	1-4	1-4	1-2	1-4	-	
PRR							
	36	1.0	37.1	13.1	30.2		14
Interquartile					112		
range (x 10 <sup>4</sup> )	0 006-72 2	0.03-44.6	27 3-77 6	0.09-45 1			
DCT (A)							
PCT (d)	20.11	22100	20.112	01101	26400	2.0	0.1
Mean	2.8 ± 1.1			2.6 ± 0.5	26±0.9	3.0	0.4
Range	2-6	2-5	2-6	2-3	2-4	•	
S (no. of patients)	18	22	22	11	5	1	
RI	8	1	3	0	0	0	
RII	2	1	0	0	0	0	
RIII	5	1	0	0	0	0	
Cure rate (%)	54.5	88	88	100	100		0.001

PRR, parame reduction ratio. FCT. fever elegance time, PCT, parasite elegance time, PS, pyrimethamine-sulfodoxine; CQ, chloroquine, CQCP chloroquine plus chlorophenisemine. COM, pyrimethamine-adiadoxine combined with chloroquine or amodiaquine; PPS pyrimethamine-adiadoxine combined with problematic. PPS pyrimethamine-adiadoxine combined with problematic. PPS pyrimethamine-adiadoxine combined with problematic. PPS pyrimethamine-adiadoxine; CQCP chloroquine, C

# Gamesocyte carriage and gamesocytaemia in children with hyperparasitaemia

In order to evaluate gametocyte carriage and gametocytaenia in those who were hyperparasitaemic at presentation, children with hyperparasitaemia were matched with those without hyperparasitaemia for time of presentation, age, gender, and drug treatment.

At enrolment gametocyte carriage was similar in children with hyperparasitaemia and in age- and gender- matched children without hyperparasitaemia who received the same drug treatment (6 of 100 v 11 of 100 children,  $\chi^2 = 1.03$ , P = 0.3). Similarly following treatment, gametocyte carriage was similar on day 7 (16 of 100 v 27 of 100 children,  $\chi^2 = 2.9$ , P = 0.08) and on day 14 (9 of 100 v 17 of 100 children,  $\chi^2 = 2.2$ , P = 0.14).

At enrolment gamctocytaemia was similar in children with hyperparasitaemia and in age- and gender- matched children without hyperparasitaemia who received the same drug treatment (geometric mean 12, range 6-24/uL  $\nu$  14 range 6-72, P = 0.5). Similarly following treatment gametocytaemia was similar on day 7 (geometric mean 71, range 6-1320/ $\mu$ L  $\nu$  66, range 6-828, P = 0.4) and on day 14 (geometric mean 57, range 12-480/ $\mu$ L  $\nu$  70 range 12-360, P = 0.7).

Fractional gametocyte density was insignificantly lower in children with hyperparasitaemia compared with those without hyperparasitaemia (median 0.003, range 0.001-0.005 v 0.048, range 0.0015-2.3%, P = 0.24)

### Discussion

Uncomplicated hyperparasitaemia is not uncommon in African children presenting with acute, symptomatic, *P. falciparum* malaria (Salako et al., 1990, Sowunmi et al., 1992, 1996, 2000 a) Prevalence rates in endemic and non endemic areas in Africa probably vary widely; in southwest Nigeria, the rate is approximately 10-12% (Sowunmi, unpublished data) The 10% prevalence recorded in the present study was similar to that previously reported from the same area in the early 1990's (Salako et al., 1990).

The risk factors associated with uncomplicated hyperparasitaemia at presentation are not frequently documented. In falciparum infections, younger age ( $\leq$  3 years) has been associated with hyperparasitaemia and increased risk of progression to cerebral malatia (Sowunmi et al., 2000 a). In the present study, age  $\leq$  5 years and a core temperature  $\geq$  39.5°C were independent risk factors associated with hyperparasitaemia at presentation. In falciparum infections in young children, the general trend is for parasitaemia to increase with time, and more specifically, to be accompanied by increases in body temperature. However, in severe infections there may be hypothermia. In practice many children with lower core temperatures than this model found may be hypothermia. This would be so because many parents or guardians bave ready access to over the counter remedies including antipyretics before presentation. This 'blunting' of presenting core temperature may mislead the attending health care provider and distract attention from the possible presence of hyperparasitaemia.

The responses of apparently uncomplicated hyperparasitacmia to oral therapy are less frequently reported, probably because of the dangers associated with oral therapy in a condition that may rapidly progress to a fatal outcome, and probably also because of increasing resistance in *P. falcipurum* to antimalarial drugs leading to reluctance to try oral therapy. Providing the parasites are fully sensitive to the oral drugs chosen, responses to drug therapy appears to be independent of parasite load. Thus in a comparative study, therapeutic responses of those with and without hyperparasitacmia were similar in children from an endemic area in West Africa (Sowunmi et al., 2000 a). In addition, in drug sensitive infections, the disposition of parasitacmia appears to follow

a first order kinetics (Sowunmi et al., 2000 a, b). In this cohort of children, CQ was the least effective drug in children with hyperparsitaemia and clearly represented a significant decline in the sensitivity of P. falciparum to this drug. Thus with prevailing degree of CQ resistance, this drug may not be ideal for the treatment of malaria irrespective of parasite load. The significantly higher proportions of children without hyperparasitaemia who subsequently developed it following treatment with CQ or PS compared with the other treatment groups suggest slow onset of antimalarial action or reduced sensitivity to these drugs and a risk for development of post-treatment hyperparasitaemia.

The similar frequencies of pruritus (and other adverse drug reactions following treatment in those with and in those without hyperparasitacmia who were treated with the same drugs [data not shown]) suggest that hyperparasitacmia does not predispose to undue adverse drug reactions following treatment (Sowunmi et al., 2000 a).

Hyperparasitaemia is a potentially life threatening condition, and with or without other features of severe malaria requires close clinical and parasitological monitoring. Its occurrence in children from this endemic area without other overt features of severe falciparum malaria suggests the presence of some degree of immunity, although these eluildren are, in general, considered relatively non-immune compared with adults from the same endemic area, and are prone to multiple infections (Happi et al., 2003). Should oral CQ or PS continued to be used for a potentially life threatening situation in view of increasing resistance of P. falciparum to these drugs in Africa? This should not be so A recent study suggests that AQ, a drug more effective than CQ in both CQ- sensitive and resistant. P Salciparium infections, rapidly clears hyperparasitaemia (Ndounga & Basco, 2003) In the small number of children treated with a combination of PS plus AQ in the study population. neither clearance nor parasite reduction ratio was significantly faster or higher respectively than those of other treatments. In view of the fact that artemisining and its derivatives clear parasitacmia more rapidly than most of the currently available antimeterials (Hien & White, 1993), these drugs combined with, for example, AQ may be tried for the management of uncomplicated hyperparasitaemia in children from Africa This suggestion is predicated on the fact that AQ is a relatively safe drug (Olliaro et al.

1996), and may be a suitable partner combination drug with the attemisinin derivatives, for example, artesuate for use in Africa (Adjuik et al., 2002). Studies to assess the efficacy of such combinations in uncomplicated and complicated hyperparasitacmias are under way in the study area

As was expected, gametocyte carriage and FGD were lower in children with than in those without hyperparasitacmia. The median FGD was 16 folds higher in those without than in those with hyperparasitacmia. Relatively low asexual parasitacmia and absence of fever are some of the risk factors associated with gametocyte carriage in falciparum infections (Price et al., 1999; Akim et al., 2000, von Seidlein et al., 2001). The lower gametocyte carriage and gametocytacmia following treatment indicate that oral therapy of this condition is not associated with undue generation and/or release of gametocytes into the peripheral circulation. However, it is not known whether gametocytes arising from patients who had hyperparasitacmia are more infectious to the mosquito than those arising from patients without hyperparasitacmia who were treated with the same drugs.



Plasmodium falciparum malaria in Nigerian children during high and low transmission seasons: gametocyte carriage and response to oral chloroquine

### CHAPTER 5

Plasmodium falciparum malaria in Nigerian children during high and low transmission seasons: gametocyte carriage and response to oral chloroquine

### Introduction

The incidence of *Plasmodium falciporum* malatia often has a seasonal pattern. Gametocyte generation, carriage and infectivity to mosquitoes are crucial to successful transmission of falciparum malatia infection, particularly in endemic areas. Carter and Miller (1979) demonstrated that the rate at which sexual differentiation occur in *Plasmodium falciparum* erythrocytic stages depends on certain environmental factors. Several other studies have reported immunological stress (Smalley and Brown, 1981; Ono et al., 1986), impact of host response to parasite (Mons 1986, Schnewcis et al., 1991, Sinden, 1998) and chemotherapy (Buckling et al., 1997; Roberts et al., 1996 a, 2000, Sutherland et al., 2002; Hogh et al., 1998; Sowunmi and Fatcye 2003 a, b) as important factors involved in the induction of gametocytogenesis.

Although some studies have reported seasonal influence on vectorial capacity, gametocyte carriage and trophozoite densities at the onset of dry or during tainy season in endemic area in Africa and Thailand (Rosenberg et al., 1990, McElroy et al., 1994, Molineaux 1980, Nacher et al., 2004), little is known about the effects of seasonal variations on gametocyte carriage and response to chloroquine treatment in endemic area of southwest Nigeria. Such information is crucial to our understanding of the potential contribution of seasonal changes to malaria transmission. Thus, in the present study, an evaluation of the effects of low and high transmission seasons on gametocyte carriage and response of children to chloroquine during *P. falciparum* malaria infection in hyperendemic southwest Nigeria has been made

### Patients and methods

Patients

The study took place between July 1996 and December 2002 in patients presenting at the University College Hospital in Ibadan, a hyperendemic area for malaria in southwestern Nigeria (Salako et al., 1990). Ethical clearance was provided by the local ethics committee. During the period, a series of antimalarial drug studies were conducted to evaluate the efficacy and safety of different treatment regimens spanning the two periods of high (April to October) and low (November to March) transmission seasons known in the area (HTS and LTS, respectively). The details of the studies have been described before (Sowunmi et al., 1998 a, b, c, Sowunmi 2002, 2003) Briefly, children with symptoms compatible with acute falciparum malaria who fulfilled the following criteria were enlisted in the study: age 13 years or below, pure P. falciparum parasitaemia greater than 2000 asexual forms/ful blood, negative usine tests for antimalarial drugs (Dill-Glazko and lignin tests), absence of concomitant illness, no evidence of severe malaria (WHO, 2000) and written informed consent given by parents or guardians. After envolment and start of treatment (day 0), follow-up with clinical and parasitological evaluation was at days 1-7, and then on days 14, and when necessary, on days 21 and 28. Clinical evaluation consisted of a general clinical examination including measurement of weight, core temperature and physical examination

# Assessment of purasiteamia and gametocytaemia

Thick and thin blood films prepared from a linger prick were Giemsa-stained and were examined by light microscopy under an oil-immersion objective, at x 1000 magnification, by two independent assessors. Parasitaemia in thick films was estimated by counting assexual parasites relative to 1000 leukocytes, or 500 asexual forms, whichever occurred first. From this figure, the parasite density was calculated assuming a leukocyte count of 6000/fil. of blood. Gametocytes were also counted in thick blood films against 1000 leukocytes assuming an average leukocyte count of 6000/fil of blood (Shaper & Lewis, 1971, Ezeilo, 1971, Sowunmi et al., 1995)

# Evaluation of response to drug treatment

In order to evaluate the response of children to chloroquine treatment during the HTS and LTS, 25mg/kg body weight of the drug over three days (10 mg/kg on day 1, 10mg/kg on day 2 and 5mg/kg on day 3) was administered to children. Response to drug treatment was assessed using World Health Organization (WHO) criteria (WHO, 1973) as follows: S = sensitive, clearance of parasitaemia without recurrence, RI (mild resistance) = parasitaemia disappears but reappears within 7 to 14 days; RII (moderate resistance) = decrease of parasitaemia but no complete clearance from peripheral blood, RIII (severe resistance) = no pronounced decrease or increase in parasitaemia at 48 hours after treatment. In those with sensitive or RI response, parasite clearance time (PCT) was defined as the time elapsing from drug administration until there was no patent parasitaemia for at least 72 h.

# Statistical analysis

Data were analysed using version 6 of the Epi-Info software (Anon., 1994), and the statistical program SPSS for Windows version 10.01 (SPSS, 1999). Proportions were compared by calculating  $\chi^2$  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 the Mann-Whitney U-test and the Kruskal-Wallis test (or by Wilcoxon rank sum test). A multiple logistic regression model was used to test the association between gametocytaemia (yes or no at presentation) and factors that were significant at univariate analysis: male gender, presence of fever, duration of illness before presentation and asexual parasitaemia at presentation. The values presented below are generally means and standard deviations (sd) or standard error (se). P-values of <0.05 were taken to indicate significant differences.

### Results

# Clinical and parasitological features at enrolment

The summaty of demographic and other characteristics of the children enrolled in the study is presented in Table 5.1. Of 1031 children enrolled into the studies, 693 and 338 children were recruited during the high and low transmission seasons, respectively between 1996-2003. Patent gametocytoemia (geometric mean 27, range 6 – 1344/ $\mu$ L) was present in 73 (10.5%) of 693 and 40 (11.8%) of 338 children at enrolment in both high and low transmission seasons respectively. These proportions were not significantly different ( $\chi^2 = 0.27$ , P=0.6). The parasite densities at enrolment in these children were 36748 (Geometric mean, range 209-150000) per  $\mu$ L and 27961 (Geometric mean, range 1116-565333) per  $\mu$ L in both high and low transmission seasons, respectively (P=0.001)

The responses of the asexual parasitacmia to drug treatments have been reported elsewhere. Factors associated with gametocytaemia at enrolment during the high transmission seasons (HTS) are presented in Table 5.2. Duration of illness > 3 d, and asexual parasite densities less than 10000/µL were related to the presence of gametocytaemia at enrolment. None of age, gender or fever at presentation was independent risk factor for gametocyte carriage (Table 5.2). However, during low transmission seasons, gender, duration of illness >4 d, and asexual parasite densities less than 5000/µL were the independent factors associated with gametocytaemia at enrolment (Table 5.3).

# Cinneal features and response to chlorogume

Of 333 children that were treated with chloroquine during the study, 168 were placed in the IfIS and 165 in the LTS. The clinical features at presentation and parasitological parameters of these children are summarized in Table 5.4. The clinical features were similar, although those enrolled in the LTS were significantly younger (P= 0.03), had significantly lower presenting temperature (P=0.03) and lower geometric mean parasite density (P= 0.001). Although, the fever clearance times were similar in the IfTS and LTS, the parasite clearance times were significantly different (P=0.003). The other therapeutic responses (Table 5.4) were similar in the two

TABLE 5.1. Summary of demographic and other characteristics of the 1031 children enrolled in the study

Variables	Value [mean + sd (range)]
Age (years)	5.6 ± 2.9 (0.5 - 12.0)
M: F	493 : 538
Weight (kg)	16.4 ± 4.8 (5.0 - 27)
Presenting body temperature (°C)	38.6 ± 1.2 (35.7 - 42.0)
Duration of illness (d)	3.2 ± 1.7 (1 - 21)
Asexual parasite density (per µL)	
Geometric mean	34063
Range	2090-2341000
No. > 250000	100

TABLE 5.2. Risk factors for P. falciparum gametocytaemia at enrolment during the high transmission seasons

	No of child with gameto	10001 OIL	P_value	Adjusted OR (95% CI)	P_value
Age (y) > 5 34 < 5 34		1 (8.1 – 6.0) (9.1	0.8	).	
Gender male 32	20 36	1 (0.7-2.0)	0.4	*	
Parasitaem in					
- 1000	51 51 32 21	1 1 89 (1 04 - 3.35	0.03	1.85 (1.11-3.33)	0.03
Fever * Febrile 4	37 46 58 27	0 99 (0.5-1 68)	0.9	•	
Duration of					
illness (day) >3 d					
≤3d 1	56 25 37 48	0.51 (0.31 – 0.9)	0.01	0.55 (0.33 – 1.0)	0.03

OR odds ratio

Force, avillar) temperature ≥ 37.5°C CL confidence interval

TABLE 5.3. Risk factors for P. fulciparum gametocytaemia at enrolment during the low transmission seasons

TABLE 3.3 R		No of children with gametocytes	Crude OR (95% CI)	P. value	Adjusted OR (95% CI)	P value
\ge (y) \$ \$ 5	159 179	13 27	0.5 (0.23 – 1.05)	0.7		~
Gender male female	159	28 12	1 0.34 (0.15-0.72)	0.003	1 0.3 (0, 1 – 0.6)	0.002
Parnsitaemia (/µL) < 5000 > 5000	23 315	8 32	1 0.21 (0.08 - 0.63)	0.001	1 0 22 (0.08-0.64)	0.005
Fever * Febtile Afebtile	98 240	9	0.68 (0.3–1.54)	0.4		3
Duration of iliness (day) < 4 d > 4 d	299 39	30	1 3.1 (1.2 - 7.3)	0.01	1 3.1 (1.2 - 7.3)	0.014

OR odds ratio
CI confidence interval

Force willow temperature > 37.5°C

TABLE 5.4: Comparison of clinical parameters of 333 children with acute falciparum malaria at prescutation and their therapeutic response following treatment with chloroquine during high and low transmission seasons

	HTS	LTS	P
Number of patients	168	165	values
Age (years)			
man ± sd	5.6 ± 2.8	$4.9 \pm 3.0$	0.03
range	0.7 - 13.0	0.6 - 120	
Weight (kg)			
mean ± sd	16.0 ± 5.6	15.1 ± 5.6	0.16
range	6.5 -33.0	6.5 - 31.0	
Duration of symptoms (d	272)		
mean ± sd	3.3 ± 1.8	3 1 ± 1.5	0.14
tauge	1.0 -14.0	1.0 - 8.0	
Body Temperature (°C)			
mean ± sd	38.5 ± 1.2	38.1 ± 1.1	0.03
lange	36.1 - 42.0	36.5 - 10.6	
Parasitaenia (/µL)	50		
GMPD (ascrual)	36748	27961	0.001
Range	2090 - 1500000	2116 - 565333	
FCT (d)		16100	0.00
mcan ± sd	5 ± 0.8	1.5± 0.8	0 99
LTVS¢	1)-4	1 – 5	
PCT (d)			
mean ± sd	2.7+0.9	30+09	0.003
Lange	1-6	1-5	
Response			
No Cured	97	92	0.7
No with RI	60	52	0.4
No with RII	6	10	0.4
No with RIII	5	11	0.1

CMPO, grametric mean parasite density; PRR, parasite reduction ratio; FCT, fover clearance time, PCT parasite clearance time, RI = parasitectoria disappeara but reappeara within 7 to 14 days, RII = decrease of parasitectoria but no complete clearance from peripheral blood; RIII = no pronounced decrease or increase in garantiasmia at 48 boors after treatment, d= day

seasons. Analysis of the treatment failures showed that of the 71 that had resistance response in the HTS, 60, 6 and 5 children had RI, RII, and RIII respectively; similarly in the LTS, 52 had RI, 10 had RII and 11 had RIII responses. RIII response occur more in the LTS than HTS but the difference was not significant (P= 0.1).

# Gametocytaemia during treatment with chloroquine and follow up

Gametocytaemia was found in twenty seven of 168 and twenty eight of 165 during the HTS and LTS, respectively at enrolment. There was no difference in the geometric mean gametocyte densities (24, range 12-1344/µl, vs. 26, range 6-150/µl; P= 0.3). Gametocytaemia increased significantly in densities by day 7 and 14 in children treated in the HTS when compared to the gametocyte densities obtained on these days in those treated during LTS following chloroquine treatment (Table 5.5). However, the cumulative gametocyte carriage by day 7 and 14 were significantly higher in the children treated with chloroquine during the LTS (P= 0.015 and P=0.03) than those treated during the HTS.

TABLE 5.5. Comparison of gametocyte intensities at presentation and following treatment in 333 children with acute falciparum malaria during high and low transmission seasons

	HTS	LTS	P
Number of patients	168	165	values
Parasitaemia (per µl) on day 0			
GMPD (gametocytes) Range	24 (n=27) 12 - 134	26 (n=28) 6 - 150	0.29
Parasitaemia (per µl) on day 7			
GMPD (gametocytes) Range	48 (n=29)° 12 - 1476	27 (n=48) 6 - 264	0.04
Parasituemia (per µI) on day 1.1			
GMPD (gametocytes) Range	29 (n=20)° 12 - 144	18 (n=35) 6 - 102	0.02

GMPD, geometric mean parasite density

<sup>\*</sup> number of cluldren carrying gametocyte, Gametocyte carriage was significantly higher in LTS by day 7 and 14 (P= 0.015 and 0.03)

### Discussion

The primary purpose of the present study was to evaluate the effect of seasons in the low and high transmission period characteristic of malaria infection in Nigerian children, on gametocyte earriage, response to oral chloroquine and gametocyte carriage following treatment. Gametocyte carriage rates may vary widely and depend on several factors. In this study, the observed prevalence of malaria infection was significantly higher in the high transmission season (1354 of 1986) than in the LTS (253 of 789), but the gametocyte carriage rate was slightly higher in the latter. Such seasonal effect has been observed earlier in the same area (Molineaux, 1980). Prompt visit to clinic and early treatment of the infection during HTS compared to slow response of infected individuals during LTS may be contributory. People in this setting appear to suspect malaria infection more in the rainy season once symptomatic or pyrexic. It is noteworthy that asexual parasitaemia at enrolment was markedly higher in the HTS than in the LTS. The reason(s) for this is not clear from the present study. A similar observation of low parasite rate during the low transmission period has been earlier reported for the area (Sowuami 1995; Salako et al. 1990). It may be that the features of asexual Plasmodium falciparum infectivity or clinical presentation vary with season or respond to changes in the environment in such a way to favour its propagation.

A critical evaluation of the risk factors for carriage of the sexual forms may provide some clues in respect of the above observation. In the present study, two and three independent factors were associated with gametocyte carriage in the HTS and LTS, respectively. Why mate gender should be a risk factor for gametocyte carriage in LTS and not in the HTS remains unclear. Testosterone and corticosteroids has been reported to stimulate *P. falciparum* gametocytogenesis in vitro (Maswoswe et al. 1985, Linguau et al. 1993). Could there be seasonal variation in the levels of sex hormones in the prepubertal mate and female? This finding would require further investigation in African children

The duration of illness longer than 4 days and reduced parasiteamia found as risk factors for garacteryte carriage in the LTS contrast sharply to the shorter duration of

presentation of symptoms or possibly low degree of virulence in the circulating asexual parasites during the LTS. Smalley et al. (1981) observed that longer established *P. falciparum* infections are likely to produce gametocytes. It is likely therefore that longer duration of illness before presentation in the LTS may allow sufficient time for the progression of committed asexual parasites to gametocytes.

The effects of antimalarial drugs on sexual differentiation in P. falciparum is still not fully understood. Certain antimalarial drugs, for example, chloroquine and pyrimethamine -sulphadoxine, have been reported to contribute to gametocytogenesis in vitro (Buckling et al., 1999) or gamctocyte generation or release in vivo (Butcher, 1997, Sowunmi and Fateye, 2003a, b). It is remarkable to note that the children in the cohorts treated with chloroquine in this study during LTS were significantly younger, had lower presenting temperature and low parasite density compared to those treated with chloroquine during the HTS. Although fever clearance times were similar, the parasite clearance times were significantly different in the two transmission seasons The children treated during the LTS had delayed clearance of their asexual forms suggesting difficing parasite behaviour and dynamics during transmission seasons. Thus the use of chloroquine in children in the study area in the IITS appeared more favourable and important to reduce circulating parasite load. Despite similar therapeutic outcome and resistance rates in the two transmission periods, early resistance of RII and RIII occur in more children during the LTS

Surprisingly, the post treatment gametocytaemia and gametocyte carriage differ significantly in the two seasons compared to pretreatment gametocytaemia and gametocyte carriage that were similar in the HTS, post treatment gametocyte intensity was high but significantly fewer children were carriers compared with low gametocyte intensity and high carriage rate in the LTS. Thus antimalarial drug chemotherapy may impose stress on the parasite, response to which could result in increased gametocyte production (Buckling et al., 1999, Smalley, 1977). The higher sexual parasite density in the HTS may in addition support increased parasite burden on mosquito and probability of

mosquito infection (Carter and Graves, 1988; Taylor and Read, 1997; Buckling et al., 1999). Thus creating heavy burden of malaria and high transmission in the area.

The increased resistance to chloroquine, which still remained the most common. readily available, cheap and first line antimalarial drug in the study area. may be contributory to differences in the post treatment gametocyte generation or release and carriage in children. Patients with slow response to treatment are likely to carry gametocytes than those that responded rapidly (Price et al., 1999). This may find relevance in our understanding of how the parasite ensures transmission despite chemotherapy of the infection. More studies would be needed to elucidate parasite response and behaviour to other antimalarial drugs during low and high transmission seasons.

Overall, a strategy that avoids the identified risk factors for gametocyte catriage in the two transmission seasons and controlled use of antimalarial drugs may reduce gametocyte prevalence and contribute to a reduction in malaria transmission



### CHAPTER 6

Response to chloroquine treatment of children who had or did not have gametocytes during uncomplicated *Plasmodium falciparum* malaria and influence of host chloroquine blood concentrations on gametocyte carriage

#### Introduction

Chemotherapy still remains the most widely used approach to combat malaria infections. Continuing use of chloroquine in the treatment of malaria infections has been associated with increasing rise in failure to clear parasites in patients. In endemic areas, as high as 40% and 80% in West African and East African patients, respectively may fail chloroquine treatment (Sowunmi and Fateye, 2003 a, Woklay et al., 1995). This has necessitated alternative antimalatial drug therapy. While awaiting the emergence of alternative to chloroquine in these areas, efforts need be geared towards minimizing the morbidity and mortality that may result from continuing use of chloroquine. One strategy, to consider to extend the period in which the antimalarial is useful in a patient is the parasite host-related characteristics, for instance gametocyte carriage, and clinical response to chloroquine

Gametocytes generation, carriage in host, and infectivity to mosquitoes are crucial to successful transmission of malaria infection and may contribute to susterance and spread of chloroquine resistance in endemic areas (Sowunmi and Fateye, 2003 a, b, Sinden, 1983). In addition, little is known of the effects of chloroquine concentration levels on gametocyte carriage and contribution to spread of chloroquine resistant infections in children. Thus the aims of the present study is to evaluate the effect of gametocytes at point time of treatment with chloroquine and during follow up period on clinical outcome and resistance pattern in children during acute, uncomplicated, falciparum malaria, and assess the effects of CQ blood levels on gametocyte dynamics.

### Patients and Methods

The study is a part of a large study on efficacy of antimalarial drugs carried out in Ibadan, Nigeria from July 1996 till March 2003 (Sowunmi et al., 1998a, b, c, Sowunmi, 2002, Sowunmi, 2003). One hundred and forty two children with acute uncomplicated P. falciparim malaria were enrolled consecutively into two groups of those who had gametocytes at enrolment or and during follow-up period and those who did not have gametocytes at encolment or any point during treatment. They were treated with chloroquine (25 mg/kg of body weight given over a 3-day period 10 mg/kg on days 0 and 1 and 5 mg/kg on day 2). The study was approved by local ethics committee. To be enrolled into the study, a child had to be aged  $\leq 13$  years, have pure P. falcipurum parasitacmia of > 1000 asexual forms/µl, give negative results in (Dill-Glazko and lignin) urine test for antimalarial drugs, have no concomitant illness or evidence of severe malaria and have the written informed consent of his or her parents or guardians. After enrolment and start of treatment (day 0), follow-up with clinical and parasitological evaluation was on days 1-7, 14, and when necessary, on days 21 and 28. Clinical evaluation consisted of a general clinical examination including measurement of weight, core temperature and physical examination

# Assessment of parasitecimia and gametocytaemia

Thick and thin blood films prepared from a finger prick were Giemsa-stained and were examined by light microscopy under an oil-immersion objective, at x 1000 magnification, by two independent assessors. Parasitacmia in thick films was estimated by counting asexual parasites relative to 1000 leukocytes, or 500 asexual forms, whichever occurred first. From this figure, the parasite density was calculated assuming a leukocyte count of 6000/µL of blood. Gametocytes were also counted in thick blood films against 1000 leukocytes assuming an average leukocyte count of 6000/µL of blood (Shaper and Levix 1971, Ezeilo, 1971, Sowunmi et al., 1995). Gametocytaemia was quantified on days 0, 3, 5, 7 and 14 using the thick blood smears prepared on those days (Sowuzmi and Fateye, 2003a, b).

# Evaluation of response to drug treatment

Response to drug treatment was assessed using World Health Organization (WHO) criteria (WHO, 1973) as follows: S = sensitive, clearance of parasitaemia without recurrence; RI (mild resistance) = parasitaemia disappears but reappears within 7 to 14 days, RII (moderate resistance) = decrease of parasitaemia but no complete clearance from peripheral blood; RIII (severe resistance) = no pronounced decrease or increase in parasitaemia at 48 hours after treatment. In those with sensitive or RI response, parasite clearance time (PCT) was defined as the time elapsing from drug administration until there was no patent parasitaemia for at least 72 h.

# Assessment of effects of CQ blood levels on gametocyte dynamics

Venous blood (5ml) was obtained from 22 children enrolled in the study at presentation and on day 3 and 7 for chloroquine concentrations in plasma and red blood cells. In addition, blood was obtained from all cases of treatment failure before retreatment with pyrimethamine sulfadoxine or amodiaquine at standard doses. Blood was immediately centrifuge at 1200 x g. plasma separated from red blood cell and both plasma and red blood cells stored at -20°C until analysis. Chloroquine was determined in plasma and red blood cells by high performance chromatography (HPLC), using a modified method previously used for the estimation of quinine (Babalola et al., 1993). Briefly, Plasma (1ml) and the internal standard 10µl of papaverine (5µg/ml) were alkalinized with 1ml of 2 M NaOH and whirl mixed for 1 minute. The mixture was extracted with 2ml diethyl ether and vortexed for 1 minute. The organic layer was separated following centrifugation at 1200 revolution per minute (1pm) for ten minutes 100µl of 0 l N HCl was added to 2ml of the organic layer and the mixture vortexed for 1 minute and centrifuged at 1200 1pm. The ether upper layer was removed and 20 µl of chloroquine extract injected into the HPLC.

The mobile phase was a buffer consisting of 0.2 M sodium dihydrogen phosphate, methanol and acetonitrile at a ratio of 65 30 5, with 1 ml perchlorie acid /100 ml of solution at p II of 3.7. The mobile phase was degassed in a sonicator just before use and pumped through the solumn at a flow rate of 1 ml/min. The column contained a Bondapak

C<sub>18</sub> (3.9 x 300mm). The fluorescence detector was set at 254nm. The compound eluted from the column in the following order chloroquine and papaverine The retention times were 4.6 and 6.8 min for chloroquine and internal standard, respectively

The thawed red blood cell, already lyscd by the storage condition, were centifuged at 1200 rpm for 10 min and the upper layer 1ml processed in the same way as the plasma. The lower limit of detection was 5ng/ml. Recoveries over the concentration range 50 -1000ng/ml were 90% in plasma and 85% in red blood cell) The intra and intersamples coefficient of variation was 4.5%. Calibration plots were linear ( $r^2 = 0.98$ ) up to 1000ng/ml. The peak area ratios were calculated and concentrations determined by extrapolation from the standard plots using a Graph Pad Prizm software package (GraphPad, 1999)

# Statistical analysis

Data were analysed using version 6 of the Epi-Info software (Anon., 1994), and the statistical program SPSS for Windows version 10.01 (SPSS, 1999). Proportions were compared by calculating  $\chi^2$  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 the Mann-Whitney U-test and the Kruskal-Wallis test (or by Wilcoxon rank sum test). The values presented below are generally means and standard deviations (sd) or standard error (se), P-values of < 0.05 were taken to indicate significant differences

### Results

Clinical characteristics and response to chlorognine treatment in children who had or did not have gametocytes

The seventy one children each in the two groups of those who had gametocytes at presentation or during treatment with CQ and those who did not have at presentation had similar clinical characteristics, parasite clearance times  $(2.9 \pm 1.1 \text{ vs} \cdot 3.0 \pm 0.8, P=0.9)$  and fever clearance times  $1.6 \pm 0.9 \text{ vs} \cdot 1.4 \pm 0.7, P=0.6)$ . 43 of 71 gametocytaemic children during the treatment of their acute malaria with chloroquine had gametocyte at presentation. These children were younger and had low presenting parasiteamia when compared with 43 children on consecutive enrolment who did not have gametocytes (Table 6.1). The therapeutic response (Table 6.2) to chloroquine treatment differs in these two groups. Children who had gametocytes at presentation had significantly shotter parasite clearance time and fever clearance time when compared with those who did not have gametocyte at presentation or during treatment of the infection  $(2.7 \pm 0.9 \text{ vs} \cdot 3.1 \pm 0.9 \text{ d}, P=0.03: 1.2 \pm 0.5 \text{ vs} \cdot 1.6 \pm 0.9 \text{ d}, P=0.01 \text{ respectively})$ 

red blood cells in 22 children

Gametocytes were carried in peripheral blood in three of the 22 children in whom chloroquine concentrations were determined, at presentation, and additional 3 children during treatment, with mean (geometric) gametocyte densities of 19 /µi blood (day 0) and 46 /µl blood (day 7), respectively. Four of the six children who had gametocyte at presentation or developed during treatment were aged less than 5 years. Table 6.3 shows the clinical parameters and therapeutic response of these children. All the six children who had gametocyte at presentation or developed during treatment had resistance response.

TABLE 6.1. Comparison of clinical parameters of 86 children with acute falciparum malaria who at presentation had or did not have gametocyte

Parameters	Gametocyta	Agametocyta	P
	emic	emic	values
Number of patients	43	43	
Age (years)			
mean + sd	$5.6 \pm 3.0$	6.9 ± 2.8	0.04
range	0.7-12.0	0.6-13.0	
Weight (kg)			
mean + sd	165±64	188+62	0.09
range	7 0-30 0	8.5-28.0	
Duration of symptoms (days)			
mean + sd	3.5 + 2.3	32±1.4	0.46
range	1.0-14.0	1.0-7.0	
Body Temperature (°C)			
mean + sd	38.4 ± 1.2	38.5 ± 1.2	0.69
range	36 5-40 6	36.5-40.6	
Parasitaemia (per ul)			0.00
GMPD (ascrual)	13588	21716	0.05
Range	209-262426	681-236866	

GMPD, geometric mean parasite density

TABLE 6.2. Therapeutic response following treatment with chloroquine of 86 children with acute falciparum malaria who at presentation had or did not have gametocyte

Parameters	Gametocyta	Agametocyta	P
	emic	emic	rajuco
Number of patients	43	43	
FCT (d)			
mean + sd	12 ÷ 05	16+09	0.01
range	1 – 3	1-4	
PCT (d)			
mcan ± sd	27+0.9	3.1+0.9	0.03
range	1 – 6	1-5	
Day 14 responses			
Cured (%)	20 (46.5)	33 (76.7)	0.001
RI	20	7	
RII	3	0	
RJII	0	3	

FCT, lever clearance time; PCT, parasite clearance time. RI = parasite cmia disappears but reappears within 7 to 14 days, RII = decrease of parasiteems but no complete clearance from peripheral blood. RIII = no pronounced decrease or increase in parasitaemia at 48 hours after treatment. S = sensitive response.

TABLE 6.3. Clinical parameters and therapeutic response of 22 children with acute falciparum malaria in whom chloroquine blood concentrations were assessed

Parameters	mean ± sd	range
Age (years)	6.2±3.1	1.5 -12.0
Age < 5 years	9	
Sex (M:F)	13:9	
Weight (kg)	17.6 ± 5.5	9.0 - 26.0
Duration of symptoms (days)	3.0 ± 0.6	2.0 - 6.0
Body Temperature (°C)	38.4 ± 1.2	35.7 - 40.2
Parasitaemia (per ul) GMPD (asexual)	26451	6240 262426
Fever Clearance time (d)	1.1±0.6	1.0 - 2.0
Parasite clearance time (d)	30±08	2.0 - 5.0
Response Sensitive (S)	9	
Resistant (R)	13	

GMPD, geometric mean parasite density

Plasma and red cell chloroquine profiles were available for all the 22 children. The gametocyte carriage and intensity, and obloroquine concentrations in plasma and red blood cells the 22 children are shown in Table 6.4. Following therapy, overall, 13 of the 22 children had resistance response and had significantly higher chloroquine concentrations in red blood cell compared to those (9) with sensitive response (P = 0.0001). The plasma chloroquine concentrations and the red blood cells- plasma ratio were similar in these two treatment outcomes.

There was a significant negative correlation between gametocytaemia on day 7 and red blood cells- plasma chloroquine concentration ratio obtained on day 3 in the children (Spearmans rho = -0.92, P = 0.008), but not with plasma (Spearmans rho = -0.70, P = 0.11) or red blood cell concentrations (Spearmans rho = -0.5, P = 0.9).

TABLE 6.4. Comparison of gametocytaemia and chloroquine concentrations in plasma and red blood cells of 2.2 children with resistant or sensitive response following treatment with chloroquine

Parameters	CQ resistant	CQ sensitive	P
	group	group	values
Number of patients	13	9	
Gametocyte density (/µl			
olood)			
Day 0 (n = 3)	19*		
Day 3 $(n = 6)$	32	-/-	
Day 5 (n = 6)	42		
Day 7 (n=6)	46		
Day 14 (n= 4)	21		
Plasma chloroquine			
concentration on day 7 or			
day of failure (ng/ml)	2020	1/22	0.00
mean + sem	303.8 ± 48.3	167.3 ± 54.3	0.08
range	82.3 - 683.2	22.4 - 538.7	
RBC chloroquine			
concentration on day 7 or			
day of failure (ng/ml)		439 4 . 91 6	0.0
mean ± sem	1096 1 ± 143 4	437.6±74.9	0.0
range	379.6 - 1875.4	158.6 - 876.8	
RBC-plasma chloroquine			
concentration ratio on day			
7 or day of failure (ng/ml)		20.00	0.5
mean ± sem	56+15	3.8 ± 0.5	0.3
range .	0.7 - 19.2	1.1 - 7.0	

<sup>\*</sup>GMPD, geometric mean (sexual) parasite density

#### Discussion

An interesting feature of the study was the significant difference in fever and parasite clearance times in children who had gametocytes compared to those who did not have at presentation following treatment with chloroquine. The reason for this finding is not clear from the present study. Children who presented with gametocytes are supposedly carrying trophozoites probably committed to gametocyte production (Bruce et al., 1990). The behaviour of the trophozoites committed to gametocyte production in the presence of antimalarial drug is little known. Also why the asexual forms of the parasites in the cohort of children with gametocytes at presentation appeared less visulent and cleared from the peripheral circulation earlier in the present study is unknown. However, the treatment of the infection with chloroquine may impose considerable stress and greatly reduce parasite number (Buckling et al., 1997).

The presence or absence of gametocytes at presentation in the children studied modulates significantly the therapeutic response of these children to chloroquine. As children without gametocytes at presentation responded to chloroquine treatment with significantly higher cure rates, there was comparatively significant resistance response to the drug in those with gametocytes at enrolment. It is clear that chloroquine therapy may favour malarious children who had no gametocytes at point of drug administration. In such children a combination of gametocidal drugs plus chloroquine or chloroquine in combination with all stage acting antimalarial, like artemether, may be of advantage. Once a child present with gametocytes in the periphetal blood, alternative antimalarial superior to chloroquine may be administered. However more studies are needed to evaluate the effect of presence or absence of gametocytes on clinical response of infected children to available antimalarial drugs in an area and potential contribution to controlling the infections.

It is interesting to note that chloroquine concentrations in red blood cells in children were significantly higher in children with resistance response. The reason(s) for this is not clear from the present study. Although, about 50% of these children carried gametocyte or asexual forms already committed to gametocyte formation, it is possible

that the handling of chloroquine by these parasites allows concentration of the drug in the red blood cell milieu. It is thus hypothesized that a 'conventional circulating exchange' of CQ may occur between the parasite cytoplasm and red blood cell environment, via a balance between concentration gradient and efflux mechanism (Krogstad et al., 1987; Ginsburg and Krugliak, 1992; Sanchez et al., 1997), with a consideration that relatively less exchange occur between the red cell and plasma compartment. The increasing density of gametocytes over time in this cohort and its correlation with low red blood cell-plasma CQ concentration ratio is not clear. A possible explanation is that switch to gametocyte formation of the committed asexual form and the increased gametocyte carriage and intensity is triggered following increased drug pressure and stress on the parasite, an observation earlier reported in some studies (Taylor and Read, 1997; Buckling et al., 1997). More studies would be required to investigate this observation. Whether or not this hypothesis explains the observations, it is clear from this finding that host handling of CQ creates adaptive support for the parasite in a way that contributes partly to spread of resistant parasites.



Open randomized study of pyrimethamine-sulfadoxine versus pyrimethamine-sulfadoxine plus probenecid for the treatment of uncomplicated Plasmodium falciparum malaria in children

### CHAPTER 7

Open randomized study of pyrimethamine-sulfadoxine versus pyrimethamine-sulfadoxine plus brobenecid for the treatment of uncomplicated *Plasmalinum*Sulciparum malaria in children

#### Introduction

Drug resistance in P. falciparum to chloroquine is a major public health problem in much of sub-Saharan Africa, accounting for recent increases in malaria-related morbidity and mortality (Trape et al., 1998, Trape, 2001), gametocyte carriage, and enhanced transmission of drug-resistant infections in Africa (Robert et al., 1996 a, 2000, Sutherland et al., 2002, Drakeley et al., 2004, Plappi et al., 2003; Sowunmi and Fateye 2003 a, b)

As an alternative to chloroquine, pyrimethamine-sulfadoxine is widely used in sub-Saharan Africa, but resistance is rapidly emerging (Sibley et al., 2001), is associated with point mutations in dihydrofolate reductase and dihydropteroate synthetase genes of the parasite (Plowe et al., 1997, Wang et al., 1997, Diourté et al., 1999), and confers survival and propagation advantages on the parasite in the population (Sowunmi and Fateye, 2003 b)

These developments have led to renewed search for effective alternatives to both chloroquine and pyrimethamine-sulfadoxine, and to the use of both drugs in combination with each other, or in combination with other antimalanals with modes of action different from those of chloroquine and pyrimethamine-sulfadoxine, with the aims of slowing the progression of resistance to these drugs and prolonging their lifespan (von Seidlein et al., 2000, Sowurm, 2002, Basco et al., 2002, Gasasira et al., 2003, Drakeley et al., 2004)

It has also led to the use of chloroquine in combination with resistance modulators, for example, chlorphenizamine (Sowunmi et al., 1997).

Experience with chloroquine plus chlorpheniramine for treating chloroquineresistant infections comes from southwest Nigeria where the prevalence of chloroquineresistant infection is 35-40% (Sowunmi et al., 1998 a. b. c. Sowunmi, 2003). A recent
study has shown that probenecid, an inhibitor of organic anion transporters and
multiresistance-associated proteins can chemosensitize *P. falciparum* to pyrimethamine,
sulfadoxine or chloroquine in vitro (Nzila et al., 2003), but the clinical significance is
unclear. To date no study has examined, clinically, the usefulness of probenecid in
combination with pyrimethamine-sulfadoxine for the treatment of malaria in African
children. Such a study is essential for a number of reasons: 1. It is possible that the
combination, given in appropriate doses, may improve treatment efficacy. 2. Malaria
transmission may be reduced if it modulates the gametocytogenesis-enhancing effect of
pyrimethamine-sulfadoxine. 3. It may potentially modify the management of paediatric
cases of malaria.

The present study reports the safety, antimalarial treatment efficacy, and effect on gametocyte carriage of pyrimethamine-sulfadoxine-probenecid and pyrimethamine-sulfadoxine alone in children aged 12 years or below with acute, symptomatic, uncomplicated, P. falciparium malaria

### Materials and methods

### Study area

The study was carried out in Ibadan, southwest Nigeria from July to September 2003. In this area of hyperendemic malaria, transmission occurs all year round but is more intense during the rainy season from April to October. In the area, it is difficult, clinically, to distinguish recrudescence from re-infection 14 days after commencing antimalarial treatment, and traditionally antimalarial efficacy tests have usually been conducted for 14 rather than the customary 28 days (Ekanem et al., 1990; Salako et al., 1990). Chloroquine resistance was reported in the area in the 1980s (Ekanem, 1985; Salako and Aderounmu, 1987) and pyrimethamine-sulfadoxine resistance in the 1990s (Sowunmi et al., 1993, 1998 a; Falade et al., 1997). Presently, chloroquine resistance reaches approximately 35-40% (Sowunmi, 2003) and, pyrimethamine-sulfadoxine resistance approximately 25% in the under-five-year-olds (Sowunmi and Fateye, unpublished).

# Patients, treatment and follow-up

Patients were eligible to join the study if they were aged 12 years or below, had symptoms compatible with acute uncomplicated malaria, with pure P. falciparum parasitacmia > 2000 asexual forms/pl., a temperature > 37.4 °C or recent pyrecial antecedents, absence of other concomitant illness, no history of antimalarial use in the 2 weeks preceding presentation, negative urine tests for antimalarial drugs (Dill-Glazko and lignin), and written informed consent given by parents or guardians. Patients with severe malaria (WHO, 2000), severe malnutrition, serious underlying diseases (renal, cardiac, or hepatic), and known allergy to study drugs were excluded from the study. The protocol was approved by the local ethics committee. The disease history was recorded by asking patients or their parents when the present symptomatic period had started, and was followed by a full physical exemination.

Enrolled patients were randomly assigned pyrimethamine-sulfadoxine 25 mg/kg of body weight of the sulfadoxine component at presentation (days 0) or pyrimethamine-sulfadoxine as above plus probenecid (Batch 2D13, Industria Farmaccutica Nova

lays (days 0, 1 and 2) All drugs were given orally; except the second daily doses of probenecid, all drugs were administered in the clinic, and all patients waited for at least 3 hafter drug administration to ensure the drug was not vomited. If it was, the patient was excluded form the study. If necessary, patients were provided with antipyretic (paracetamol tablets, 10-15 mg/kg 8 hourly for 24-48 h). Drug administration was controlled by a physician.

days (days 1-7) and then on days 14, 21 and 28. Thick and thin blood films prepared from a finger prick were Giemsa-stained and were examined by light microscopy under an oil-immersion objective, at x 1000 magnification, by two independent assessors who did not know the drug treatment of the patient. Parasitaemia (asexual or sexual) in thick films was estimated by counting asexual or sexual parasites relative to 1000 leukocytes, or 500 asexual or sexual forms, whichever occurred first. From this figure, the parasite density was calculated assuming a leukocyte count of 6000/µl of blood.

Routine haematological (haematocrit) and biochemical tests (concentrations of alanine aminotransferase, aspartate aminotransferase, bilirubin, and ereatinine) were done on a proportion of patients, pre-treatment and on day 14. Blood was spotted on Olter papers on days 0, 3, 7, 14, 21 and 28, and at the time of treatment failures for parasite genotyping

Classification of responses to drug treatment was according to WHO criteria (WHO, 1973) Parasite clearance time was defined as the time elapsing between drug administration and absence of detectable parasitaerms for at least 48 h. Fever clearance time was defined as the time from drug administration until the core temperature fell to or below 37 4°C and remained so for 48 h.

Cure rates were defined as the percentages of patients who remained free of parasitatives on days 14, 21 and 28 of follow-up [This step was necessary because of

intense transmission in the study area, making it difficult to distinguish, clinically, between re-infection and recrudescence after day 14 (Ekanem et al., 1990; Salako et al., 1990) and the relatively long half life of pyrimethamine-sulfadoxine.

### Retreatment of drug treatment failures

Patients who failed treatment (within 14 days) with pyrimethamine-sulfadoxine were retreated with pyrimethamine-sulfadoxine-probenecid and were followed for another 14-28 days. Those failing pyrimethamine-sulfadoxine-probenecid were retreated with oral amodiaquine 30 mg/kg over 3 d and were followed for another 14-28 days. Patients were retreated whenever they became symptomatic (usually between 14-21 days after initial enrolment). Patients with profound clinical (hyperpyrexia, oral fluid intolerance) and parasitological deterioration during follow-up were treated with artemether (9.6 mg/kg, over 5 days and were regarded as treatment failures.

### Data analysis

Sample size was calculated on the basis of recent cure rate (75% on day 14) for pyrimethamine-sulfadoxine in the under five-year olds. Data were analysed using version 6 of the Epi-Info software (Anon., 1994). Variables considered in the analysis were related to the densities of P, falciparum gametocytes and trophozoites. Proportions were compared by calculating  $\chi^2$  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 the Mann-Whitney U-tests and the Kruskal-Wallis tests (or by Wilcoxon ranked sum test). All tests of significance, except where specifically indicated, were two-tailed. P-values of < 0.05 were taken to indicate significant differences. The values presented are generally means and standard deviations (sd) or standard error (se).

### Results

### Patients' characteristics

A total of 529 children aged 12 years or below with symptoms compatible with acute, uncomplicated falciparum malaria was screened during the period. Parasitaemia was present in 313 children, 256 children were eligible for participation but only 153 children were enrolled. Of the 153 children enrolled, 79 were treated with pyrimethamine-sulfadoxine-probenecid and 74 with pyrimethamine-sulfadoxine. Two children, one from each of the treatment arms, were lost to follow up after day 7 because of parental relocation. These children were excluded from the data analysis. Figure 7.1 shows the trial profile. Overall results are for 151 children. The demographic and clinical characteristics of patients at enrolment are shown in Table 7.1. These characteristics were similar in the two treatment arms, but the duration of illness at presentation was significantly longer in those treated with pyrimethamine-sulfadoxine-probenecid.

## Fever and perasite clearance, and gametocyte carriage

One hundred and seven children were febrile at enrolment, 57 in pyrimethamine-sulfadoxine-probenecid and 50 in pyrimethamine-sulfadoxine groups. By day 2, fever cleared in 42 and 26 children, respectively. There was a significant difference in the proportion of patients in whom fever cleared by day 2 ( $\chi^2 = 4.5$ , P = 0.03). Overall, fever clearence was significantly shorter in those treated with pyrimethamine-sulfadoxine-probenecid (1.9 ± 1.1 vs 2.4 ± 1.2 d, P = 0.02) (Table 7.2).

Compared with pyrimethamine-sulfadoxine, pyrimethamine-sulfadoxine-probenecid substantially accelerated the clearance of parasitaemia. By day 2, 53 and 37 children in the pyrimethamine-sulfadoxine-probenecid and pyrimethamine-sulfadoxine treatment arms, respectively had cleared their parasitaemias. The difference in this proportion was significant ( $\chi^2 = 3.98$ , P = 0.04). Overall, parasite clearance was significantly shorter in those treated with pyrimethamine-sulfadoxine-probenecid (2.3 ± 0.9 vs 2.7 ± 1.1 d, P = 0.04) (Table 7.2). The cure rate on day 14 (96.2 vs 83.5%,  $\chi^2 = 5.3$ , P = 0.02) but not day

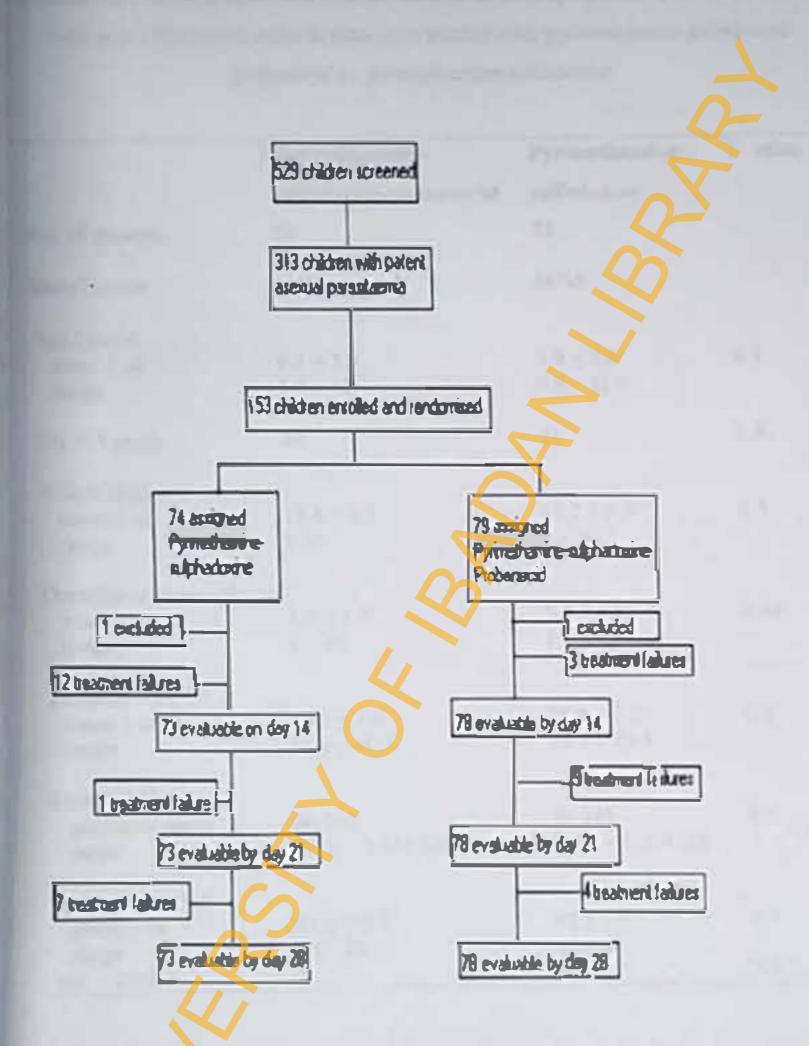


FIGURE. 7.1. Trial profile of patients enrolled in the pyrimethamine sulfadoxine vs.

TABLE 7.1. Demographic and clinical characteristics at enrolment of the 151 children with acute falciparum malaria who were treated with pyrimethamine-sulfadoxine probenecid or pyrimethamine-sulfadoxine

	Pyrimethamine-	Pyrimethamine-	P. value
	sulfadoxine-probenecid	sulfadoxine	
No. of patients	78	73	
Male/Female	41/37	38/35	
Age (years)			
mean + sd	63+29	5.9 + 2.9	0.3
range	1.5 – 12	0.8-11.5	
No < 5 years	29	25	0.8
Weight (kg)			
mean ± sd	178±61	172±56	0.5
range	7-35	5 – 30	
Duration of illness (d)			
mean ± sd	3.5 ± 1.7	$3.0 \pm 1.3$	0.04
range	1 – 10	1-9	
Temperature (°C)			
mean ± sd	38 1 ± 1.0	38.4 ± 1.2	0.2
range	359 - 40.3	36.1 – 40.5	
Parasite count (/µL)			
geometre mean	46,792	57,745	0.5
галде	2010 - 1,388,000	2020 - 1,254,000	
Haematocnt (%)			
mean ± sd	31.6 ± 5.5	33 1 ± 5 1	0.1
range	18 – 43	22 – 46	
No. < 25%	8	2	0.1

TABLE 7.2. Therapeutic responses to pyrimethamine-sulfadoxine-probenecid or pyrimethamine sulfadoxine of children with acute falciparum malaria

	Pyrimethamine-sulfadoxine-	Pyrimethamine-	P
	probenecid	sulfadoxine	value
No of patients	78	73	-
Fever clearance time (d)			
	1.9 ± 1.1	24 ± 12	0.02
mean ± sd range	1-5	1-7	
Parasite clearance time (d)			
mean ± sd	2.3 ± 0.9	27±11	0.04
range	1-5	1-6	
Day 14 responses			
No cured	75	61	
No RI	1	10	
No RII	2	1	
No. RIII	0		
Cure rate (%)	96.2	83.5	0.02
Day 21 responses			
No cured	66	60	
No RI	10	11	
No RII	2		
No. R.111	0		0.0
Cure sate (%)	84.6	82.2	8.0
Day 28 responses			
No cured	62	53	
No RI	14	18	
No RII	2		
No RIII	0		0.4
Cure rate (%)	79 4	72.6	0.4

28 (79.4 vs 72.6%,  $\chi^2 = 0.6$ , P = 0.4), was significantly higher in children treated with pyrimethamine-sulfadoxine-probenecid than in those treated with pyrimethamine-sulfadoxine Response to both treatment regimens was not related to age: one child and 2 children from the 29 and 49 < 5 and  $\geq$  5 year-olds, respectively treated with pyrimethamine-sulfadoxine-probenecid failed treatment by day 14 (P = 1.0, by Fisher exact test). Similarly, 4 and 8 children from the 25 and 48 < 5 and  $\geq$  5 year-olds, respectively treated with pyrimethamine-sulfadoxine failed treatment by day 14 (P = 1.0, by Fisher exact test).

Gametocyte carriage in those who did not have gametocytaemia at enrolment (n = 73 and 72, respectively in the pyrimethamine-sulfadoxine-probenecid and pyrimethamine-sulfadoxine) was similar on days 7 (32 of 73 (43 8%) vs 31 of 72 (43%),  $\chi^2 = 0.01$ , P = 0.9) and 14 (16 of 73 (21.9%) vs 21 of 72 (29.1%),  $\chi^2 = 0.66$ , P = 0.4) with both regimens

Response to pyrimethamine-sulfadoxine-probenecid of children with pyrimethamine-sulfadoxine-treatment failures

Seven of 12 children who failed initial treatment with pyrimethamine-sulfadoxine, were retreated with pyrimethamine-sulfadoxine-probenecid. The therapeutic responses of these children are summarized in Table 7.3. Parasitaemia and fever cleared within 2-4 days of treatment with pyrimethamine-sulfadoxine-probenecid. The child with RII response to pytimethamine-sulfadoxine during initial treatment had a RI response following retreatment with pyrimethamine-sulfadoxine-probenecid. The cure rates on days 14 and 28 were 86% and 72%, respectively. None of the three children who failed treatment with pyrimethamine-sulfadoxine-probenecid on or before day 14 (see Table 7.2) and were subsequently retreated with amodiaquine failed treatment during a 28-day follow-up period. In these children fever and parasitaemia cleared within 2-3 days of initiating amodiaquine therapy.

TABLE 7.3. Clinical and parasitological parameters of the 7 children with *Plasmoclium*folciparum malaria who had resistance response to pyrimethamine-sulphadoxine during initial treatment and subsequently treated with pyrimethamine-sulphadoxine-probenecid

4 10- 1	Pyrimethamine-	Pyrimethamine-sulfadoxine-	P.
	sulfadoxine	probeneció	suler
No of patients	7	7	
Age (years)			
mean ± sd	.8 ± 2.6		
range	3.3 – 11.5		
Weight (kg)			
mເລກ <u>+</u> sd	199±34	20 l <u>+</u> 3.8	0.5
Lauge	15 - 26	15 - 27.5	
Temperature (°C)			
mean + sd	38.8 ± 1 1	37.8 ± 1.4	0.1
range	37.0-40.3	36 0 – 39 5	
Parasite count (/µL)			
geometric mean	47,835	8,156	0.01
range	2,020 - 115,500	716 - 27,622	
Forer clearance time (d)			
mean + sd	2.1 ± 1.3	1.2 + 0.4	0.35
range	1-4	1-2	
Parasite dearance time (d)			
man + sd	36±10	28+09	0.26
range	2-5	2 – 4	
Day 14 responses			
No cured	0	6	0.00
No RI	6	l .	
No RII		0	
No RIII	0	0	
Cure rate (%)	0	86	
Day 28 responses	0	5	0.00
No cured	0	2	0.00
No RI		0	
No RII	0	0	
No RIII	0	72	

### Adverse events

Pyrimethamine-sulfadoxine-probenecid and pyrimethamine-sulfadoxine were well tolerated; no child was withdrawn because of drug intolerance. Symptoms reported within the first week and during followup were similar (Table 7.4). However, vomiting was more frequently reported by those treated with pyrimethamine-sulfadoxine. None of the 7 children who failed initial treatment with pyrimethamine-sulfadoxine and were retreated with pyrimethamine-sulfadoxine and were

### Haematological and biochemical parameters

Except for haematocrit values below 25% at enrolment in 8 and 2 children in pyrimethamine-sulfadoxine-probenecid and pyrimethamine-sulfadoxine groups, respectively, and at day 7 in 4 and 4 children, respectively, haematological, biochemical and other parameters remained normal before and after treatment in all subjects. Thrombocytopenia was present in 10 and 12 children in pyrimethamine-sulfadoxine-probenecid and pyrimethamine-sulfadoxine groups, respectively, at enrolment, but was not seen on day 14 in any child.

TABLE 7.4. Adverse drug reactions reported during the study

	Pyrimethamine	Pyrimethamine
	sulfadoxine-probenecid	sulfadoxine
No of children	78	73
investigated		
Reporting		
Pruitus	0	
Vomiting	2	2
Abdominal pain	5	2
Diarrhoea	0	2
Anorexia	0	4*
Drowsiness	0	0
Cough	4	7
Headache	3	3
Weight gain ≥ 1.0kg	38 (n = 66)	32(n=60)

<sup>•</sup> Significant statistical difference, P = 0.05

TABLE 7.4. Adverse drug reactions reported by children with acute falciparum malaria treated with pyrimethamine-sulphadoxine-probenecid or pyrimethamine-sulphadoxine

	Pyrimethamine	Pyrimethamine-
	sulfadoxine-probenecid	sulfadoxine
No of children	78	73
investigated		
Reporting		
Pruritus	0	0
Vomiting	2	/2
Abdominal pain	5	2
Diarrhoca	0	2
Anorexia	0	4.
Drowsiness	0	0
Cough	4	7
Headache	3	3
Weight gain ≥ 1.0kg	38 (n = 66)°	32 (n = 60)

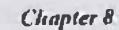
<sup>•</sup> Significant statistical difference. P = 0.05. Number with increase in weight

of pyrimethamine-sulfadoxine. Although no untoward effect was observed following retreatment, caution is required with this step, since it may increase the chances of adverse drug reactions to pyrimethamine-sulfadoxine.

The drugs used were well tolerated. The most frequently reported adverse reactions were of gastrointestinal origin, and most were indistinguishable from the symptoms of malaria. Malaria, and the drugs evaluated, can cause anorexia. It is possible the significantly reduced reporting of anorexia by those treated with pyrimethamine-sulfadoxine-probenecid was related to the accelerated clearance of fever and parasitaemia. Both probenecid and sulfadoxine can also induce haemolysis in Glucose-6 Phosphate Dehydrogenase (G6PD)-delicient subjects, but no child, following treatment, reported features suggestive of drug-induced haemolytic anaemia

It remains unclear exactly how probenecid enhanced the antimalarial effect of pyrimethamine-sulfadoxine in the cohort of children studied. Probenecid can reduce folate uptake by P. falciporum in vitro (Nzila et al., 2003), in addition to increasing plasma sulforamides concentrations by reducing renal tubular secretion of the latter. Both of these actions are independent of parasite sensitivity status to pyrimethaminesulfadoxine It is possible that following treatment with pyrimethamine-sulfadoxineprobenecid, sulfadoxine concentrations were significantly higher than in those treated with pyrimctlumine-sulfadoxine alone, but drug levels were not measured. Probenecid can also reverse resistance in cancer cells to methotrexate (Hooijberg et al., 1999) and resistance in P. falciporum to chloroquine in vitro (Nzila et al., 2003), by inhibiting the multi-drug resistance associated proteins. Inhibition of the multi-drug resistance associated proteins is an unlikely mechanism of the enhancement of the antimalarial effect of pyrimethanine sulfadoxine by probenecid since resistance to pyrimethaminesulfadozine is associated with mutations in the dihydropteroate synthetase and dibydrosolate reductase, and not mutations in the psmdrl gene of the parasite (Wang et al. 1997, Diourté et al., 1999, Duraisingh et al., 1997)

There are justifications for the dosing regimen, the relatively moderate dose was based on the dose used to retard tubular secretion of penicillin in children, a convenient starting point since the drug has not been previously co-administered with pyrimethamine-sulfadoxine for the treatment of malaria in children, the three-day dosing regimen is practicable, and compliance is more likely than if it were for longer periods. Certainly pharmacokinetic and pharmacodynamic studies are required before optimal dosing regimens can be achieved. There are potential clinical applications of these findings. If at moderate doses probenecid enhances the antimalarial efficacy of pyrimethamine-sulfadoxine, it follows that as resistance increases to pyrimethamine-sulfadoxine, higher doses of probenecid may be effective, since it is possible that enhancement may be dose related.



Comparative effects of pyrimethumine-sulfadoxine with or without probenecid on gametocytaemia and gametocyte sex ratios in children with acute, symptomatic, uncomplicated, folciparum malaria

### CHAPTER 8

Comparative effects of pyrimethamine-sulfadoxine with or without probenecid on gametocytacinia and gametocyte sex ratios in children with acute, symptomatic, uncomplicated, falciparum malaria

#### atroduction

The increasing spread of *Plasmodium folciparum* resistant to pyrimethamiciulfadoxine (PS), the first or second line treatment of malaria in most endemic
countries in Africa (Falade et al., 1997; Plowe et al., 1997; Wang et al., 1997; Dioute
et al., 1999; Omar et al., 2001 a. b; Sibley et al., 2001), has led to renewed search for
cheap, effective alternatives to PS, and to renewed efforts to prolong the clinical utility
of the drug in Africa (Nzila et al., 2003). When used as part of combination therapy,
particularly with the 4-aminoquinoline, amodiaquine, or the attemisisin derivatives,
not only is there a rapid cleatance of ascaual parasitaemia, the frequency of
gametocyte carriage and level of gametocytaemia during treatment with PS may also
be significantly reduced (Sowunni, 2002; Sowunni and Fateye, 2003 b)). However,
it is not clear whether non-antiqualarial drugs that can potentially enhance the activity
of pyrimethamine and sulfadoxine in vitro and in vivo will influence the PS-induced
increases in frequency of gametocyte carriage, level of gametocytaemia and malebiased sex ratio (Sowunni and Fateye, 2003 c).

Probenecid, an inhibitor of organic anion transporters and multiresistance-associated proteins, can champsensitiae Plasmodium fulciparum to pyrimethamine and sulfadoxine in vitro (Nzila et al., 2003), and at least, in vitro in Nigerian children treated with pyrimethamine-sulfadoxine (Sowunmi et al., unpublished data). However, its effects, if any, when added to PS for the treatment of acute, symptomatic, uncomplicated, falciparum malaria in children, on the frequency of gametocyte carriage, level of gametocytaemia, and temporal changes in gametocyte sex ratios are unknown. Such information is essential as increases in both the level of

increase the infectivity of the human population to the mosquitoes ding on it (Boyd et al., 1935, Robert et al., 1996 b). If the addition of probenecid to attenuates the potentials of PS to enhance malaria transmission, and without aducing undue toxicity, it may be the ideal chemosensitizer of P. falciporum in vivo

unetocyte carriage and the level of gametocytaemia, of the addition of probenecid to S (PSP) and (2) to follow the temporal changes in gametocyte sex ratios in children eated with PSP.

### Patients and methods

#### Patients

The study took place at the University College Hospital in Ibadan, a hyperendemic area for malaria in southwestern Nigeria (Salako et al., 1990) from July to September 2003. The subjects were 151 children presenting with acute, symptomatic, uncomplicated *Plasmodium folciparum* malaria that were randomized to the following treatment regimens: PS given orally at presentation (day 0) as 25 mg/kg of the sulfadoxine component, or PS given as above plus probenecid 20-25 mg/kg given orally in two divided doses daily for 3 d (days 0-2). The study protocol was approved by the local ethics committee.

The clinical aspects of the study were as reported in the previous chapter (Chapter 7). Briefly, to be enrolled, a child had to have acute, symptomatic, uncomplicated *P. falciparum* malaria, to be aged 12 years or below, to have a pure *P. falciparum* parasitaemia of > 2000 asexual forms/pl blood, to give negative results in (Dill-Glazko and lignin) urine tests for antimalarial drugs, to have no concomitant illness or evidence of severe malaria, and to have the written informed consent of his or her parents or guardians

After detailed clinical and parasitological assessment and drug administration at presentation, each child, as follow-up, was checked clinically and parasitologically on each of days 1-7 and 14. Fingerprick samples of blood, collected on days 0-7 and 14 were used to make thin and thick sniears so that the levels of parasitaemia could be estimated (Sowunmi and Fateye, 2003 b).

# Quantification of gomeloc larmid

Gametocytacmia was quantified on days 0, 3, 5, 7 and 14, using the thick blood smears prepared on those days (Sowunmi and Fateye, 2003 b). Levels of gametocytacmia (sexual forms/ µl) were estimated by counting gametocytes against 1000 leucocytes and assuming each patient had 6000 leucocytes/µl blood. If the level of gametocytacmia was at least 10 sexual forms/µl, the gametocytes were sexed on the basis that males (microgametocytes) are smaller than females (macrogametocytes), the nucleus is larger in the males than the females, the ends of the cells are rounded in

males and angular in females, with Giemsa the cytoplasm stains pale 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 (Carter and Graves, 1988; Robert et al., 1996 b). The time taken to attain a sex ratio of 1 (SR1) was defined as the time elapsing from drug treatment until this ratio was achieved and was calculated for each patient, from a plot of sex ratio v time, by computer extrapolation. The data from the patients who did not have at least three estimates of gametocyte sex ratios were excluded from the estimation of SR1 and the exploration of the disposition kinetics of gametocytaemia.

## Disposition kinetics of micro- and macro-gametocytaemia

Gametocyte kinetic parameters were estimated from the levels of micro- and macro-gametocytaemia by a non-compartmental method, using the computer programme Turbo Ken (Clinical Pharmacology Group, University of Southampton, U.K., through the courtesy of Professor A.G. Renwick), generally as previously described (Sowunmi & Fateye, 2003 d). After determining SRI, the absolute counts of micro- and macro-gametocytaemia were log-transformed for each patient and plotted against time. The following parameters were noted or determined: (1) time to attain SRI (tsri), (2) area under the curve of the plot of micro- or macro-gametocytaemia v. time, from tsri to day 14 (AUCsri, and (4) the half-lives (ti/2) of the micro- and macro-gametocytaemia, calculated from tsri, and (4) the volume of blood completely cleared of micro- and macro-gametocytaemia at tsri/AUCsri, Since it was difficult to determine the time that gametocytaemia at tsri/AUCsri, Since it was difficult to determine the time that gametocytaemia at tsri/AUCsri, some it has difficult to determine the time that gametocytaemia at tsri/AUCsri, some it was difficult to determine the time that gametocytaemia at tsri were assumed to be the levels when recruitment stopped.

# Statistical analysis

Data were analysed using version 6 of the Epi-Info software (Anon., 1994). Proportions were compared by calculating  $\chi^2$  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 the Mann-Whitney U-tests and the Kruskal-Wallis tests

(or by Wilcoxon rank sum test). Correlations were assessed by linear regression. All tests of significance were two-tailed. P-values of ≤ 0.05 were taken to indicate significant differences. The values presented below are generally means and standard deviations (sd) or standard error (se).

## Clinical features at enrolment and responses to therapy

Seventy eight children were treated with PSP and 73 with PS. The details of the clinical and parasitological responses to the two treatment regimens are presented in the previous chapter (Chapter 7). Briefly, the clinical and parasitological parameters at annolment were similar in the two treatment groups. Mean age at encolment was  $6.3 \pm 2.9$  and  $5.9 \pm 2.9$  years in the PSP and PS groups, respectively, (P = 0.3). Fever clearance was significantly faster in those treated with PSP ( $1.9 \pm 1.1$  d), than in those treated with PS ( $2.4 \pm 1.2$  d). The difference between these values was significant (P = 0.02). Similarly, parasite clearance was significantly faster, and the cure rate on day 14 was significantly higher in those treated with PSP than in those treated with PS. For example, the cure rate on day 14 was 96.2% in those treated with PSP and 83.5% in those treated with PS (P = 0.02).

## Frequency of gametocyte carriage and level of gametocytaemia

During the entire study period, gametocytaemia was found in 39 patients treated with PSP and in 34 treated with PS. The frequency of gametocyte carriage was significantly higher on each of days 7 and 14 (P < 0.01) than on day 0, both in the PSP- and PS-treated patients (Table 8.1). Similarly, the levels of gametocytaemia were significantly higher on each of days 3, 5, 7 and 14 than on day 0, both in the PSP- and PS-treated patients. The level of gametocytaemia was, however, significantly higher on day 5 in PS- than in PSP- treated patients (P = 0.004, Table 8.1). Two children and one child treated with PSP and PS, respectively were gametocyte carriers at all times during the study period

# Gunietocyte sex ratios

In the 39 children treated with PSP who had gametocytaemia during the study period, 42, 32,132, 460 and 138, gametocytes were counted on days 0, 3, 5, 7 and 14, respectively, and most of these gametocytes (39, 32, 130, 453, and 138 on days 0, 3, 5, 7 and 14, respectively) could be sexed. In the 34 children treated with PS who bad gametocytaemia during the study period, 12, 38, 122, 578, and 143 gametocytes were counted on days 0, 3, 5, 7 and 14, respectively, and most of these gametocytes (12, 37,

TABLE 8.1 Prevalence and intensities of *Plasmodium fulciparum* gametocytacmia at presentation and during follow-up of malarious children treated with pyrimethamine sulfadoxine-probenecid (PSP) or pyrimethamine sulfadoxine(PS)

Parameter			Day			
	0	3	5	7	14	P
PSP (n = 78)						
No with gametocytacmia	5	3	9	32	16	0.00001*
GMGD (gametocyte/µl)	17	32	33	63	44	0.002
Range	12 - 36	24 – 36	24 - 48	12 - 960	12 - 216	
PS (n≈ 73)						
No with gametogtaemia	1	3	5	31	22	0.00001 *
GMGD (earnetocyte/µl)	12	50	67	41	30	00001*
Range		36 – 72	48 - 84	12 - 687	12 - 84	

GMGD geometric mean gametocy to density

\*Kruskal-Wallis test. \* x test with Yates correction;

All comparisons were two-tail

121, 576, and 142 on days 0. 3, 5, 7 and [4, respectively) could be sexed. The data on the sex ratios for both PSP and PS were pooled because of the small number of gametocyte carriers observed pre-treatment (five among the PSP children and one among the PS children). Overall the sex ratio was malebiased, a mean (se) of 59 (12%), range 30-100% (95% confidence interval 26-92%). At presentation there was no significant correlation between the proportion of gametocytes that were male and asexual parasitaemia (r = 0.7, P = 0.17), core temperature (r = 0.2, P = 0.8) or gametocytaemia (r = 0.02, P = 0.98).

The temporal changes observed in the gametocyte sex ratios were similar for the PSP and PS treated children (Figure 8.1). There was a progressive increase in the proportion of gametocytes that were male such that by day 7, over 80% of the gametocytes were male in both treatment groups. In 3 children (one in PSP and 2 in PS) with pre-treatment female biased sex ratio, SR1 was reached by day 5. On day 7, 5 of 32 children with gametocytaemia who were treated with PSP had female biased sex ratio, while on day 14, one of 16 with gametocytaemia had a female-biased ratio. In the children treated with PS, 1 of 31 children with gametocytaemia on day 7 had female-biased ratio, while none had such ratio on day 14. In those treated with PSP, the proportions of gametocytes that were male on days 7 and 14 were significantly higher than the proportion on day 0 ( $\chi^2 = 17.1$ , P = 0.00003, and  $\chi^2 = 27.1$ , P = 0.00001, respectively). Similarly, in those treated with PS, the proportions of gametocytes that were male on days 7 and 14 were significantly higher than the proportion on day 0 ( $\chi^2 = 38.5$ . P = 0.000001 and  $\chi^2 = 51.4$ , P = 0.000001, respectively).

The levels of micro- and macro-gametocytaemia before and after treatment with PSP and PS for all the 73 children with gametocytaemia during the study period are shown in Table 8.2. In PSP treated children, the levels of micro- and macro-gametocytaemia were similar between days 0-5. However, by day 7 the level of microgametocytaemia was significantly higher than that of macrogametocytaemia. In those treated with PS, macrogametocytaemia mildly predominated between days 0-5. However, by day 7, microgametocytaemia predominated, the level of microgametocytaemia being significantly higher than those of macrogametocytamia.

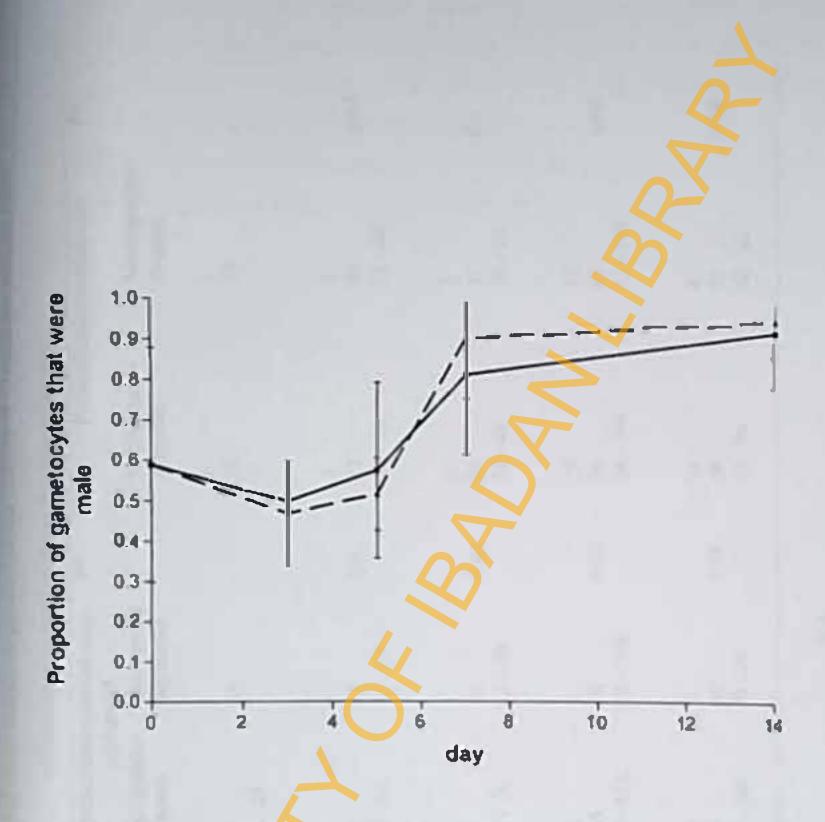


FIGURE 8.1 Changes in sex ratio of gametocytes before and after pyrimethamine-sulfadoxine-probenecid (-1-) or pyrimethamine-sulfadoxine (-1-) treatment of scute Plasmodrum fulciparum malaria in children

TABLE 8.2 Prevalence and intensities of micro- and macro-gamelocytacmia densities in 73 malarious children in ated with pyrimes minutes and intensities of micro- and macro-gamelocytacmia densities in 73 malarious children in ated with pyrimes minutes and intensities of micro- and macro-gamelocytacmia densities in 73 malarious children in ated with pyrimes minutes and intensities of micro- and macro-gamelocytacmia densities in 73 malarious children in ated with pyrimes minutes.

		e-sulfadoxine-	P*	Pyrimethan	ino-sulfadoxine	P*
	Microgameto-	Macrogand -00) tamia		Microgando-	Macrogamolo-	
Day 0						
No with constronus	3	1				
Gramatic mean level of	15	12	9	12	12	
Range (Constact tex/hi)  Day 3	12 - 24	*				
No with garactery terms	3	3		3	3	
Geometric manifestel of	17	17	1.0	17	2.4	0.65
Range (gametocytes/pl)  Day 5	12 - 24	12 - 24		12 - 24	12 - 48	
No with gardocytemma	9	8		3	3	
Geometric mean level of	21	27	0.83	30	51	0.1
Range (gamelocytes/µ ) Day 7	12-24	12 - 36		24 - 48	36 - 60	
No with gamacontomis	22	/7		29	17	
Geometric mean level of	113	28	0.02	96	43	0.01
Range (gamelocytes/µl)  Day 1.1	60 - 852	12 - 108		24 - 564	12 - 313	
No with generocitizating	20	3		16	4	
Concerne man level of	64	29	0 18	38	12	0.18
promptions (prodocytes/µ)	24 - 180	24 - 36		12 - 96	12 - 12	2

## Disposition kinetics of micro- and macro-gametocytaemia

As, at each time-point investigated, the sex ratio and levels of gametocytacmia for the PSP treated children were similar to those treated with PS (see Figure 8.1 and Table 8.2), the data for both groups were pooled for analysis of the disposition kinetics (Table 8.3). The AUC and the ha for microgametocytacmia were significantly higher than those for macrogametocytacmia, and the clearance of macrogametocytacmia was two and a half folds higher than for microgametocytacmia.

TABLE 8.3. Disposition kinetics of *P. falciparum* micro and macrogametocyte following pyrimethamine-sulfadoxine-probenecid or pyrimethamine-sulfadoxine treatment in children

Parameter*	Microgametocytaemia (N=8)	Macrogametocytacinia (N=4)	P values
AUC (sexual forms/µt.h)			
Mean and (S.E)	(7619 (3831)	4728 (691)	0.017
Range	5040 - 40017	3034 - 6250	
95% confidence interval	8561 – 26678	2530 - 6927	
t <sub>1/2</sub> (h)			
Mean and (S.E)	265.6 (59.4)	74.3 (12.8)	0.05
Rnnge	66.3 - 624.0	37.1 - 96.0	
95% confidence interval	125.1 - 406.1	33.5 – 115.1	
CL <sub>ttem</sub> (µVkg.h			
Mean and (S.E)	0.00008 (0.00003)	0.0002 (0.00008)	0.14
Range	0.00002 - 0.00015	0.00002 - 0.0004	
95% confidence interval	0.000009 - 0.00016	-0.00007 - 0.00048	

<sup>\*</sup>Calculations were from the time to attain a male; female sex ratio of 1

Wilcoxon signed rank test.

AUC. Area under the curve of gametory tacmin v. time: (1/2, apparent half-life of gametory tacmin; Class volume of blood completely cleaned of micro- or macro-gametory tes per unit time.

### Discussion

An ideal chemosensitizer of *P. fulciparum* to antimalarial drugs *in vivo* should not only accelerate the clearance of asexual parasitaemia and symptoms of infections without producing undue toxicity, but also reduce the frequency of gametocyte carriage and level of gametocytaemia. In addition, it should produce temporal changes in gametocyte sex ratios that reduce gametocyte infectivity to mosquito.

The present results show that, compared to pre-treatment, at the dose level of probenecid used, both PSP and PS, significantly increased the frequency of gametocyte carriage and levels of gametocyteemia post-treatment. With the exception of the levels of gametocytaemia on day 5 post-treatment, both these parameters were also similar in both treatment groups. It is unclear why there was a significant decrease in the level of gametocytaemia in those treated with PSP on day 5, but it may not be unrelated to the relatively rapid clearance of asexual parasites induced by the addition of probenecid. As commitment to gametocyte development occurs prior to schizont maturation (Silvestrini et al., 2000), and delay in the time taken to clear initial asexual parasitaemia (> 2 d, Sowunmi, unpublished) in children in southwestern Nigeria, is associated with increased risk of gametocyte carriage, it appears rapid clearance of asexual parasitaemia by PSP temporarity, reduced the progression of the committed populations of asexual parasites to sexual forms. This effect, however, was of short duration as levels of gametocytaemia were subsequently similar thereafter.

The gametocyte sex ratios in the small population of children at enrolment was male-biased, and at variance with earlier report (Sowunmi and Fateye. 2003 c). The relatively lower level of gametocytaemia at presentation, as a form of fertility insurance (Gardner et al., 2003), may be partly responsible. In addition, the children could have been exposed to other sex ratio modifying factors prior to presentation. These underscore an important fact gametocyte sex ratios in P, fulciparum may be variable (Paul et al., 2002, Robert et al., 2003). These, in turn, may explain why in some of the children SR1 was reached before, at or shortly after presentation.

In general, following both treatment regimens, there was significant increase in tratio. In a loogitudinal follow-up of gametocyte carriers in a village in Senegal, about et al. (2003) found a density-dependent relationship with sex ratios. Peaks of unctocytaemia were sometimes associated with minimum sex ratio. The finding of creasing sex ratio despite increasing level of gametocytaemia (and peak ametocytaemia on day 7) following treatment with PS in our small cohort of children is a greement with previous report (Sowunmi and Fateye, 2003 c), suggesting that PS, acting singly or in concert with other factors, may substantially increase gametocyte maleness. The similarity of temporal changes in sex ratios between PSP- and PS-treated children indicates that the addition of probenecid to PS had little or no effects on gametocyte sex ratios.

The findings of the present study support the notion that microgametocytes persist longer in circulation than macrogametocytes, or are longer lived (Ponnudutai et al., 1986, Reece et al., 2003, Sowunmi and Fateye, 2003 e). However, the addition of probenecid to PS did not alter micro- and macro-gametocyte disposition in the population of children. Given that levels of gametocytaemia and a male-biased sex ratio correlate with gametocyte infectivity to mosquito feeding on humans (Tehuinkam et al., 1993; Robert et al., 1996 b), it would appear that, the combination of probenecid at the present dose level with PS is unlikely to decrease the potential for transmission of malaria in the population, whether the gametocytes arise from sensitive or resistant PS infections.

# Chapter 9

Comparative effects of pyrimethamine-sulphadoxine, chlorogume plus chlorophemicumine and amodicipume plus pyrimethamine-sulphadoxine on gametocytes during and after treatment of acute, uncomplicated malaria

#### CHAPTER 9

Comparative effects of pyrimethamine-sulphadoxine, chloroquine plus chlorpheniramine and amodiaquine plus pyrimethamine-sulphadoxine on gametocytes during and after treatment of acute, uncomplicated malaria

#### Introduction

As resistance to chloroquine (CQ) increases in extent and severity, alternative regimens available to control programmes in developing endemic countries including pyrimethamine-sulphadoxine (PS), amodiaquine (AQ) (Olliaro et al., 1996, Brassour et al., 1999, Sowunmi et al., 2001) or combination of AQ with PS (AQPS) (Sowunmi 2002) or other suitable combinations have become increasingly used in the treatment of CQ-resistant falciparum infections. These alternatives have varying effects on clearance of asexual parasitaemias or sexual forms of P. fulciparum. For example, PS may (Puta and Manyando, 1997) or may not (Hogh et al., 1995) enhance gametocyte carriage during treatment of acute falciparum infections. Although the presence of gametocytes in peripheral blood after antimalarial treatment is no proof of viability, their generation is required for the transmission of the infection from the vertebrate to the anopheline host. In order to improve the management of paediatric cases of malaria and reduce transmission in our area of study, the effects of these drugs on gametocyte production needs urgent assessment. In addition, it is not clear whether the enhancement of non-enhancement of gametocyte production by PS will be influenced by its use in combination with other antimalarial drugs. It is noteworthy that antifolates are ineffective in the treatment of uncomplicated falciparum malaria in South America, for comple, in Brazil (Fontes et al., 2002)

Resistance to CQ in P. falciparum can be reversed by chlorpheniramine (CP) m vitro and m vivo (Sowanni et al., 1997, 1998 a, b, c). It has been recently shown that

the presence in peripheral blood of very young gametocytes (PYG) 72 h after commencing CQ may be used as indicator of response to CQ (Sowunmi et al., 2003). However, it is unclear whether the addition of CP to CQ will alter the use of PYG as an indicator of response to CQ or indeed as an indicator of failure of reversal of CQ resistance in vivo by CP. Although the combination of CQ with CP will not be employed by control programmes in Africa in the very near future, it is still essential to study PYG and peripheral mature gametocyte (PMG) generation during treatment with CQCP in the event that this or other similar combination become available

In other to address these issues, gametocyte generation during treatment of falciparum malaria in children with PS, CQCP and AQPS have been evaluated. The main aims of our study were: (i) to evaluate the effects of PS, CQCP and AQPS on gametocyte generation during treatment with these drugs, (ii) to determine whether or not the addition of PS to AQ will influence the generation of gametocytaemia by PS and, (iii) to evaluate PYG as an indicator of response to PS, CQCP or AQPS.

### Patients and methods

Study site

The study site, Ibadan, is a hyperendemic area for malaria in southwestern Nigeria (Salako et al., 1990). In the area, it is difficult to distinguish, clinically, reinfection from recrudescence after day 14 of treatment because of intense transmission. Antimalarial drugs have therefore generally, until recently, been evaluated on the basis of data recorded up to day 14, rather than the customary day 28 (Ekanem et al., 1990, Sowunmi and Salako, 1992).

#### Patients

The study took place at the University College Hospital in Ibadan, Nigeria Overall, 166 children who presented with acute, symptomatic, uncomplicated P. fulciparum malaria were enrolled in the study between September 1999 and September 2001.

The study was designed to elicit a 20% difference in cure rates between AQPS/CQCP on one hand and PS on the other hand with 80% power and at 95% level of confidence. The minimum number of patients required for each treatment arm is 45. In general, to be enrolled, the children had to be aged 0.5-10 years, and have symptoms compatible with acute, falciparum malaria (with fever or history of fever in the 24-48 h preceding presentation) and a pure P. falciparum parasitaemia of > 2000 asexual forms/µl blood. Those who bad taken antimalarial drugs in the 2 weeks preceding presentation, provided a urine sample found positive for 4 aminoquinolines or sulphonamides (by the Dill-Glazko and lignin tests, respectively), or who had a concomitant illness, such as sickle-cell anaemia, or severe or complicated malaria (Warrell et al., 1990, WHO, 2000) were excluded. The informed consent of a parent or guardian was obtained for each child included in the study. A child was withdrawn from the study if she/he developed concomitant illness during the follow-up period, or if his/her parent or guardian requested it. The study received ethical approval from the local ethics committee.

Before enrolment in the study, a medical history of each child was obtained from an accompanying parent/guardian and each was physically examined. Body weight and oral or rectal temperature were recorded, and thick and thin films were prepared from finger-prick blood samples. These smears were Giemsa-stained for parasite identification and quantification of any peripheral parasitaemias.

### Drug treatment

Children were randomly allotted to one of 3 treatment groups. One group received PS at presentation (day 0) at a dose 25 mg/kg of the sulphonamide component. Each tablet of PS contained 500 mg of sulphadoxine and 25 mg pyrimethamine. The other groups received chloroquine base, 30 mg/kg of body weight over 3 days (days 0-2) plus chlorpheniramine maleate, 6 mg at presentation followed by 4 mg every 8 h for 7 days (days 0-6) if the child was aged < 5 years, or 8 mg at presentation followed by 6 mg every 8 h if the child was  $\geq$  5 years, or a single dose of PS at presentation plus AQ 30 mg/kg over 3 days (days 0-2). All drugs were given by a physician orally and each child was observed for at least 3 h after each such supervised drug treatment, in order to ensure that the drug was not vomited. If it was, the child was excluded from the study. Additional management of some children included the administration of an antipyretic (e.g. 10-15 mg paracetamol/kg, every 8 h for 24 h) and fanning and tepid sponging when necessary.

# Evaluation of response

Clinical observations were recorded daily for 8 days (days 0-7) and then on day 14. Thick and thin blood films, for quantification of parasitaemia, were prepared at the same times. At each follow-up, the guardians or parents (and, when possible, the children) were actively questioned, using a standard questionnaire, and the children were examined for the presence of adverse reactions to drugs

Giernsa-stained blood films were examined by light microscopy under an oiliremersion objective, at X 1000 magnification, by two independent assessors who did
not know the drug treatment of the patients Parasitaemia in thick films was estimated

by counting asexual parasites relative to 1000 leukocytes. or 500 asexual forms, whichever occurred first. From this figure, the parasite density was calculated assuming a leukocyte count of 6000/µl blood. Young gametocytes (stage I-III) and mature gametocytes (stage IV and V) (Sinden, 1998) were also counted in thick blood films against 1000 leukocytes on days 0, 3, 4, 5, 6, 7, and 14. The responses to drug treatment were classified according to World Health Organization (1973) criteria. Treatment was considered a failure if the day-3 parasitaemia was 25% of the day 0 value, if parasitaemia did not clear by day 7, or if parasitaemia cleared before day 7 but re-appeared before day 28. The parasite clearance time (PCT) was defined as the time elapsing from drug administration until there was no patent parasitaemia. The fever clearance time (FCT) was delined as the time from drug administration until the oral or rectal temperature felt to  $\leq$  37.4°C and remained so for at least 72 h. (This definition was necessary because of the routine use of paracetamol during the first 36 h of treatment in some children). Cure rates were defined as the proportions of patients who remained free of parasitaemia on day 14 of follow-up.

### Re-treatment of drug treatment failures

All treatment failures were re-treated with AQPS on day 14 provided they were not symptomatic before this time. Patients with profound clinical (hyperpyrexia, oral fluid intolerance) and parasitological deterioration during follow-up were treated with artemether, 9 6 mg/kg of body weight over five days and were regarded as treatment failures.

## Statistical analysis

Proportions were compared by calculating  $\chi^2$  with Yates' correction or by Fisher exact 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 the Mann-Whitney U-tests and the Kruskal-Wallis tests (or by Wilcoxon rank sum test). The values presented below are generally means and standard deviations (ad). P-values of < 0.05 were taken to indicate significant differences.

### Results

Clinical and parasitological characteristics at enrolment and theropeutic responses

A total of 166 children was enrolled in the study. Fifty one, 52 and 63 children were enrolled in the PS, CQCP, and AQPS groups, respectively. Of these, 49, 48, and 60 children in the PS, CQCP, and AQPS groups, respectively completed the mandatory 14-day follow-up period and were analysed. The clinical and parasitological characteristics at enrolment were similar in all groups (Table 9.1). The therapeutic responses to drug treatment are also summarized in Table 9.1. AQPS was significantly more effective than CQCP or PS in clearing fever and parasitaemia and with a significantly higher cure rate on day 14. Direct comparison of PS and CQCP showed that fever  $(2.2 \pm 1.1 \text{ vs } 1.6 \pm 0.8 \text{ day}, P = 0.008)$  but not parasite clearance in those with sensitive response  $(2.7 \pm 1.1 \text{ vs } 2.5 \pm 0.8 \text{ day}, P = 0.33)$  was significantly faster with CQCP than with PS. The cure rate on day 14 was also significantly higher with CQCP than with PS (80.8 vs 59.2%, P = 0.03)

## Gametocytoemia during follow-up

The prevalence and intensities of gametocytaemia before, during and after treatment are summarized in Table 9.2. Gametocyte castiage on days 3, 7, and 14 or days 3, 7, and 14 combined were significantly higher in the PS group than in the other treatment groups. However, the geometric mean gametocyte densities (GMGD) were similar mail the treatment groups

The median survival time for peripheral young gametocytes (PYG) (Figure 9.1) in PS, CQCP, and AQPS treatment groups were 3.5, 1.5, and 1.5 days, respectively. There was a significant difference in the overall comparison of the survival experience using Wilcoxon (Gehan) statistics ( $\chi^2 = 14.7$ , P = 0.0006). The ratios of the densities (per  $\mu$ 1 blood) of peripheral young gametocytes (PYG) to peripheral mature gametocytes are summarized in Table 9.3. The ratios were consistently below 1 in the CQCP and AQPS groups up till day 7. However, in the PS group, this ratio tose progressively to

TABLE 9.1. Clinical and parasitological parameters of the children enrolled in the study

	PS (n =49)	CQCP (n = 48)	AQPS(n = 60)	P value
Age (years)				
mean + s.d.	5.1 + 2.7	$6.0 \pm 2.3$	5.5 ± 2.5	0.52
range	0.6-10	2.0-10	12-10	0.35
Weight (kg)				
mean ± s d	154+56	16.8 + 5.2	15.5 ± 4.7	0.32
range	6.5-26	8,1-35	6.26	
Duration of illness (d)				
mean ± s d	3.1 ± 1.4	36±24	2.8 ± 1.3	0.06
range	1-7	2-14	1-8	
Presenting body tem	p			
(°C)	38.5 ± 1.2	38.6 ± 12	38.1 ± 1.0	0.05
mean ± s.d.	358-405	36.2-40.5	36.40.2	
range				
Parasitacmia (per µl)				
geometric mean	37858	29248	30482	0.56
range	3310-375476	2511-219600	878-716000	
Fever clearance time (d)				
mean ± s.d	2 2 ± 1 1	1.6 ± 0.8	12+09	0.000001
range	1-5	1-4	1-3	
Parasite clearance time (				
mean ± s.d.	27±11	2.5 ± 0.8	22±07	0.012
range	1-6	1-4	1-4	
No of children				
cured	29	38	60	0 00000
RI	10	10		
RII	7		( <del>-</del>	
RIII	3	•	-	

95% confidence interval. PS pyrimethinine-sulphadoxine, CQCP chiloroquine plus
chierphenicamoe: AQPS arrediagoine plus pyrimethinine-sulphadoxine

TABLE 9.2. Gametocytacmias before, during and after the treatment, with pyrimethamine-sulphadoxine (PS), chloroquine plus chlorpheniramine (CQCP) or amodiaquine plus pyrimethamine-sulphadoxine (AQPS), of *Plasmodium falciparum* infections in children

	PS (n = 49)	CQCP (n= 48)	AQPS (n = 60)	P value
Day 0 Garnetocytacmia				
Geometric mean (/µt)	36	22	40	0.49
Mean + S.E.	87 ± 52.1	26 ± 5.3	74 ± 43.3	0.47
Range	12-444	12-36	12-288	
Day 3 Gametocytaemia				
Geometric mean (/µl)	36	44	40/	0.92
Mean + S.E.	100 + 43.5	127 + 83.5	86 + 54.1	0.72
Range	12-876	12-612	12-408	
Day 5 Gamelocytaemia				
Geometric mean (/µl)	70	61	43	0.74
Mean ± S.E.	243 + 112.9		141 + 121.0	0.74
Range	12-468	12-518	12-504	
Day 7 Gametocytaemia				
Geometric mean (/µl)	135	39	36	0.38
Mean ± S.E.	399 ± 141.2	136 ± 73.6	98 + 74.1	
Range	12-3520	12-696	12-468	
Day 14 Gametocytaemia				
Geometric mean (/µl)	83	34	19	0.21
Mcan ± S.E.	139 ± 29	60 ± 30.1	24 ± 12	
Range	12-480	12-168	12-48	
No of patients v	vith			
gametocytaemia on *				
Day 0	9	8	6	0.42
Day 3	25	8	7	0.000004
Day 7	26	8	6	0.000002
Day 14	20	4	3	0.000001
Day 3. 7 & 14	32	- 11	3	0.000000
Day 7 & 14	27	11	3	0.000000

<sup>&</sup>quot;Wilconon (Oclust) for survival analysis ( $\chi^2 = 14.7$ , P = 0.0006)

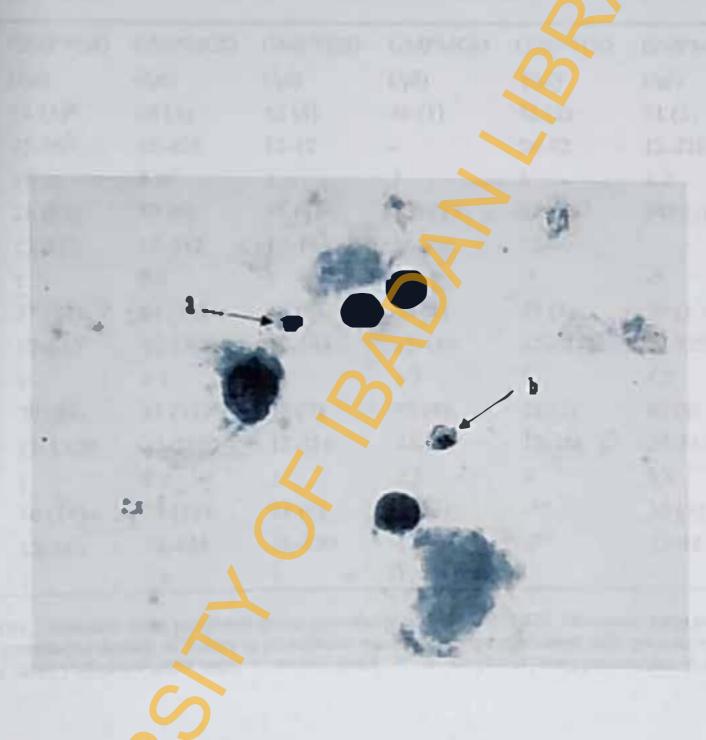


FIGURE. 9.1. Micrograph of peripheral young gametocyte (arrows a -stage II. b -stage IV) obtained in thick blood smear of a child treated with pyrimethamine sulfadoxine

TABLE 9.3 Prevalence and intensities of peripheral young gametocytes and peripheral mature gametocytes in children treated with pyrimethamine-sulphadoxine (PS), chloroquine plus chlorpheniramine (CQCP) or amodiaquine plus pyrimethaminesulphadoxine (AQPS)

		PS	cQc	CP	AQP	S
Day	GMPYGD	GMPMGD	GMPYGD	GMPMGD	GMPYGD	GMPMGD
	(/µl)	(/14)	(/µl)	(/hl)	(44)	(/µl)
0	21 (5) <sup>a</sup>	65 (4)	12 (4)	36(1)	42 (2)	51 (2)
	12-36b	12-408	12-12		24-72	12-216
	1c	3.1c	1	3	1	1.2
3	28 (20)	57 (9)	55 (4)	106 (2)	27 (2)	348 (1)
	12-372	12-552	12-144	24-468	12-60	
	1	2.0	1	1.9	.0	_*
5	37 (21)	54 (17)	46 (6)	58 (4)	23 (3)	39 (3)
	12-640	12-1840	12-348	12-180	12-84	12-420
	1	1.5	1	1.3	1	1.7
7	79 (24)	85 (21)	42 (7)	97 (4)	28 (3)	87 (2)
	12-1320	12-2210	12-216	12-600	12-156	24-312
	1	1.1	1	2.3	1	3.1
14	50 (14)	78 (12)	21 (4)	35 (3)	_40	19 (3)
	12-240	12-444	12-120	12-72		12-48
	1	1.6		17		

GMPY (ID Geometric mean perpheral found gametocyte density, GMPMGD Geometric mean penpheral martire gamerocite density, a values in parentheses represent number of children with gamelocytasmia, b range, c GMPYGD:GMPMGD ratio. \* not calculated. \*\* no penpheral young gatactocy les obsented

on day 7 indicating continuing production (or generation or mobilization) of young gametocytes PYG-PMG density ratio increased significantly from day 0-14 in those treated with PS and CQCP ( $\chi^2 = 76$ , P = 0.000001 and  $\chi^2 = 42.2$ , P = 0.000001, respectively) but decreased significantly in those treated with AQPS ( $\chi^2 = 53.2$ , P = 0.000001) (Figure 9.2)

Relationship heriveen PYG and responses to drug treatment

None of the children successfully treated with CQCP had PYG during the follow-up. In children who had sensitive response to PS treatment (n = 29), PYG was present on days 0. 3. 5. 7, and 14 in 5, 12, 13, 13, and 7 children, respectively. Similarly in those successfully treated with AQPS (n = 60), PYG was present on days 0. 3. 5. 7, and 14 in 2. 2. 3. 3, and 0 patient, respectively. The PYG rates were significantly higher in those treated with PS than in those treated with AQPS at all times during follow up ( $P \le 0.006$  in all comparisons). Post Hoc Turkey HSD test for repeated measure of the effect of PYG generation over the 14 day follow up showed significant differences in the comparisons of PS vs AQPS and PS vs CQCP (P = 0.0001 and 0.0001 respectively). There was no significant difference in the comparison of PYG generated by those treated with AQPS and CQPS (P = 0.08)

Relationship between PYG and outcomes of treatment in the children treated with PS and CQCP

The relationship between treatment outcomes and presence of PYG in children treated with PS and CQCP are shown in Tables 9.4 and 9.5. PYG rates were similar in those with sensitive or resistant responses to PS (18 of 29 1.5 13 of 20,  $\chi^2 = 0.04$ , P = 0.93) and the rates were similar from days 0.14. In contrast, PYG was seen only in those with resistant response to CQCP. In those without gametocytacmia at presentation, but who subsequently developed PYG 72 h after commeacement of CQCP, the presence of PYG was associated with treatment failure on or before day 14 (Table 9.5)

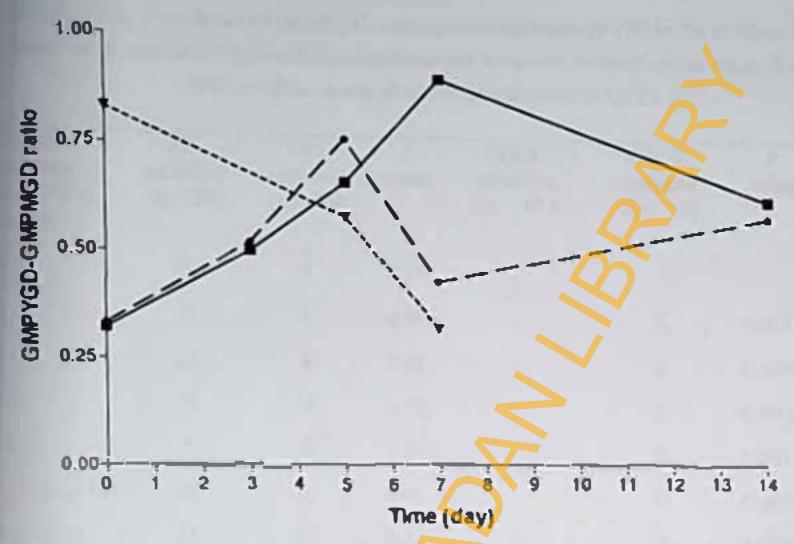


TABLE 9.4. Prevalence of peripheral young gametocytacmia (PYG) in the children with sensitive or resistant response following treatment with oral pyrimethamine-sulphadoxine (PS) or chloroquine plus chlorpheniramine (CQCP)

No. of children with PYG on day	PS- sensitive (n = 29)	PS- resistant (n = 20)	p value	CQCP- sensitive (n = 38)	CQCP- resistant (n = 10)	value
0	5	4	0.81		3	
3	12	8	0.96		4	0.001
5	13	8	0.85		6	0.00002
7	13	11	0.38		7	0.000002
14	7	7	0.34		4	0.001
3. 7 &/or 14	18	13	0.65		8	0.000002
7 & or 14	13	13	0.11		7	0.0000001

TABLE 9.5. Peripheral young gametocyte (PYG) carriage at or after day 3 in children treated with oral pyrimethamine-sulphadoxine (PS) or chloroquine plus chlorphamine (CQCP) and who did not have gametocytaemia on presentation

No. of children with PYG on day	PS- sensitive (n = 21)	PS- resistant (n = 15)	P vnlue	CQCP- sensitive (n = 38)	CQCP- resistant (n = 4)	P value
3	5	6	0.46			
4	6	6	0.72		2	0.008
5	9	7	0.91			0.08
6	10	9	0.69		The species	0.08
7	11	10	0.61	-	2	0.008
14	7	7	0.64	1	2	0.008

#### Discussion

The ideal antimalarial drugs or drug combinations for the treatment of falciparum malaria should not only promptly clear parasitaemia, sever or other symptoms of malaria, but should also prevent the generation of gametocytes from asexual forms during treatment. In the present study, PS was significantly less effective than CQCP or AQPS in clearing parasitaemia or sever in children with acute falciparum infections. This is not surprising since progressive decline in sensitivity of P. falciparum to PS has been reported from the area of study from the late 1990s (Falade et al., 1997; Solvunmi et al., 1998a). The decline in sensitivity of the parasite to PS has also occurred in many areas of Africa (Sibley et al., 2001)

In addition to their effects on the sexual forms, gametocyte carriage may be influenced to a considerable extent by the sensitivity of the asexual parasites to the drugs used for the treatment of infections. For example, as resistance of the asexual parasites to the 4-aminoquinolines, CQ, and AQ, increases, gametocyte carriage also increases (Strickland et al., 1986; Hogh et al., 1995). In these studies, gametocyte carriage rates 28 days after PS treatment were significantly less than those of CQ and AQ since PS was more effective than the 4-minoquinolines on asexual parasites in the settings of these studies. However, increased carriage may also be related to decreased sensitivity to PS in certain circumstances (Sowunmi et al., 1998a; Tjitra et al. 2002). In this cohort of children, gametocyte carriage was significantly higher at all times after treatment with PS than in the other treatment groups. In addition, PYG rates were similar in both PS-sensitive and -resistant infections supporting a known fact that PS enhanced generation or release of gametocytes during treatment of acute falciparum infections (Puta and Manyando, 1997). However, GMGD were similar in all the treatment groups.

Many antimalarial drugs appear to reduce gametocytaemia by clearing the asexual stage infections. This clearance, if exceptionally rapid, may reduce transmissibility particularly in areas of low transmission. For example, the attemisintin derivatives have reduced transmissibility in some parts of Thailand by this process (Price et al., 1996)

In order to determine the influence of treatment with antimalarial drugs on gametocyte production and densities, both young and mature gametocytes were quantified and were expressed as ratios. The ratios of PYG to PMG were consistently below 1 up to day 7 in those treated with CQCP and AQPS, but rose to 1 by day 7 in those treated with PS irrespective of the sensitivity of the asexual parasite to PS. This showed continuing and enhanced production or, preferential mobilization of gametocytes by PS irrespective of the sensitivity of the asexual parasites to PS. This process of continuing or preferential mobilization of young gametocytes by PS may explain why gametocytes persist longer in some patients treated with PS. This is plausible because the young gametocytes must grow and run the normal time-course of survival of the normal mature gametocytes.

Given that gametocyte density may correlate with mosquito infectivity and therefore transmission success (Tchuinkam et al., 1993). Drakeley et al., 1999), the effects of PS on gametocytes carriage and mobilization have implications for malaria control programmes with respect to the use of this drug. Recent WHO recommendations (WHO, 2001a, b) have focused on the use of combination antimularial therapy (CT), particularly artemisinin-based combination therapy (ACT). Although several control programmes in Africa have switched to CT, some programmes use PS-based combination, for example, AQPS (Sowunmi, 2002). The modulating effect of AQ on enhanced production of PYG by PS may provide supporting argument for the use of combination therapy. However, the reduced generation of PYG by PS despite its combination with other drugs suggests that generation of gametocyte is an inherent property of antifolate antimplarials (Hamel et al., 2005).

In a recent study, it was shown that the detection of PYG 72 h after the start of CQ therapy may be used as an indicator of response to this drug (Sowunmi et al., 2003). The results of the present study show that PYG may also be used as an indicator of response to CQCP Failure of the enhancement of the antimalarial efficacy of CQ by CP in vivo was associated with the presence, in peripheral blood, of young gamctocytes. However, PYG was not an indicator of response to PS, since both PS-sensitive and resistant infections generated PYG. In addition, the presence of PS in combination with

AQ also generated PYG and was clearly not an indicator of response to AQPS since the cure rate in this group was 100%.

The limitation of the present study is the fewer number of gametocyte carriers in the AQPS and CQCP groups following treatment. Therefore caution is required with the interpretation of the data from these two groups.



Comparative effects of antifolates- trimethoprimsulfamethoxuzole and pyrimethamine-sulfadoxine on gametocytes in children with acute, symptomatic, uncomplicated, Plasmodium falciparum malaria

#### CHAPTER 10

Comparative effects of antifolates- trimethoprim-sulfamethoxazole and pyrimethamine-sulfadoxine on gametocytes in children with acute, symptomatic, uncomplicated, *Plasmodium falciparum* malaria

#### Introduction

The antifolate antimalarial, pyrimethamine-sulfadoxine (PS), has become increasingly used as first line treatment of falciparum malaria in several African countries because of increasing resistance in *Plasmodium falciparum* to chloroquine (CQ). In spite of frequent use and of *in vivo* and *in vitro* studies (Hogh et al., 1998, Sowunmi and Fateye, 2003 b), its cliects on gametocytes in children with falciparum infections remain incompletely understood.

With increasing use, resistance in P. falciparum to PS is increasing (Sibley et al., 2001) probably as a consequence of long half lives of its components. It has recently been suggested that, trimethoprim-sulfamethoxozole (TS), an antifolate antimalarial with relatively short half-lives of its components compared to PS, may be used as alternative to the latter for the treatment of uncomplicated (alciparum infections in children because it is as efficacious as PS (Omar et al., 2001 a, Fchintola et al., 2004). It is assumed that the relatively short half-life of TS may, when compared with PS, reduce the chances of engendering resistance in P. falciparum to this drug and may provide additional advantage with transmission of drug resistance infections over PS.

However, while the effects on PS on gametocytes and gametocyte sex ratios (GSR) are known (GSR may influence infectivity to mosquitoes and transmission- see

Robert et al., 1996 b, Sowunmi and Fateye, 2003 c), the effects of TS on gametocytes are relatively unknown in African children with falciparum malaria. It is hypothesized that PS and TS have similar effects on gametocyte prevalence, density and sex ratio, and possess similar effects on gametocyte survival in children treated with these drugs. This hypothesis was tested in a group of children with acute symptomatic uncomplicated P. falciparum malaria who were tandomized to and who received PS and TS for the treatment of their infections.

#### Patients and methods

#### Patients

Between June and August 1999, a randomized trial of TS and PS for the treatment of uncomplicated falciparum malaria was conducted in 102 children at the University College Hospital in Ibadan, a hyperendemic area for malaria in southwestern Nigeria (Salako et al., 1990) Ethical clearance for the study was provided by the local ethics committee. In general, to be enrolled, the children had to be aged 0.5-12 years, and have symptoms compatible with acute, falciparum malaria (with fever or history of fever in the 24-48 h preceding presentation) and a pure Plasmodium falciparum parasitaemia of > 2000 asexual forms/µl blood. Those who had taken antimalarial drugs in the 2 weeks preceding presentation, provided a urine sample found positive for 4 aminoquinolines or sulfonamides (by the Dill-Glazko and lignin tests, respectively), or who had a concomitant illness, such as siekle-cell anaemia, or severe or complicated malaria (WHO, 2000) were excluded. The informed consent of a parent or guardian was obtained for each child included in the study. A child was withdrawn from the study if she/he developed concomitant illness during the follow-up period, or if his/her parent or guardian requested it. Thick and thin blood films from all patients who participated in the study were examined for the presence and density of asexual and sexual parasites at enrolment and start of treatment (day 0), and at follow-up at days 1-7, and then on day 14. TS was given as 20 mg/kg of the sulfamethoxazolo component twice daily for 5 days (day 0-4), PS was given as the 25 mg/kg of the sulfadoxine component at presentation (day 0). All drugs were administered orally

# Assessment of parastuemia and gametocyte sex ratio

Thick and thin blood films prepared from a finger prick were Giemsa-stained and were examined by light microscopy under an oil-immersion objective, at x 1000 magnification, by two independent assessors who did not know the drug treatment of the patients. Parasitaemia in thick films was estimated by counting asexual parasites relative to 1000 leukocytes, or 500 asexual forms, whichever occurred first. From this figure, the parasite density was calculated assuming an average leukocyte count of 6000/µL of blood (Shaper and Lewis, 1971, Ezeilo, 1971, Sowunmi et al., 1995). Gametocytes were also

counted in thick films against 1000 leukocytes assuming an average leukocyte count of 6000/µL of blood at enrolment (day 0) and on days 3, 5, 7 and 14. Gametocytes were sexed if gametocytaemia was ≥ 12 sexual forms/µl. Gametocyte sex determination was based on following criteria (Carter and Graves, 1988; Robert et al., 1996 b) males are smaller than females; the nucleus is bigger in males than females; the ends of the cells are round in males and angular in females; the cytoplasm stains pale 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. Gametocyte sex ratio was defined as the proportion of gametocytes in peripheral blood that were microgametocytes (Pickering et al., 2000; West et al., 2001).

# Statistical analysis

Data were analysed using version 6 of the Epi-Info software (Anon., 1994), and the statistical program SPSS for Windows version 10 01 (SPSS, 1999). Proportions were compared by calculating  $\chi^2$  with Yates' correction or Fisher exact test or Mantel Haenszel test. 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 the Mann-Whitney U-test and the Kruskal-Wallis test (or by Wilcoxon rank sum test). Kaplan-Meier analysis was used to estimate the cumulative probability of remaining free of gametocytes during follow-up for all cases of malaria combined and for those cases that were free of gametocytaemia at enrolment. Differences in survival time were assessed by inspection of Kaplan-Meier curves and pair wise log-rank tests. P-values of  $\leq 0.05$  were taken to indicate significant differences.

#### Results

# Demographic characteristics and therapeutic responses

A total of 104 children were enrolled into the study. Two children, one in each treatment group were excluded from the study due to parental relocation. These children were cleared of their peripheral parasitaemia at the time of exclusion. The demographic characteristics of the children enrolled in the study and the therapeutic responses to the treatment given are summarized in Table 10.1. These were similar in the two treatment groups. However, parasite clearance was significantly shorter in those treated with TS than PS.

# Prevalence of gametocytaemia

The prevalence of gametocytacmia before and after treatment with PS, and before, during and after treatment with TS is shown in Table 10.2. Gametocyte earriage was similar on days 0-7 in both treatment groups and it peaked at day 7 in both TS and PS groups. Gametocyte carriage was significantly lower on day 14 in those treated with TS than PS ( $\chi^2 = 5.7$ , P = 0.017). Eleven and 19 children treated with TS and PS, respectively were gametocyte carriers on both days 7 and 14. The difference between these proportions was significant ( $\chi^2 = 4.0$ , P = 0.046).

In general, compared to pre-treatment, both drugs significantly increased gametocyte carriage post-initiation of treatment ( $\chi^2 = 20.9$ , P = 0.003 for TS and  $\chi^2 = 28.4$ , P = 0.0001 for PS, see Table 10.2). In children without patent gametocytecmia at enrolment, there was a greater propertiely to be gametocyte-positive by day 7 with a significantly greater proportion of children treated with PS having gametocytes by day 14 of follow up compared with TS (30/47 [63.8%] vs 16/48 [43.3%],  $\chi^2 = 7.66$ , P = 0.005).

TABLE 10.1 - Summary of clinical characteristics at enrolment and therapeutic responses in patients with acute falciparum malaria treated with trimethoprim-sulfamethoxazole or pyrimethamine-sulphadoxine

Parameter	TS (n = 53)	PS (n = 49)	Pvalue
Agc (y)			
mean ± sd	6.3 ± 2.9	63 + 2.8	0.9
range	1.5 - 12.0	0.8 - 10.5	
Weight (kg)			
mean ± sd	18 2 ± 64	17.6 ± 5.2	0.6
range	7.5-34.5	7.0 - 28.0	
Temperature (°C)			
mean ± sd	381±13	384 ± 1.4	0.2
range	35.7 - 40.9	35.9 - 41.0	
Parasite density (/µl)			
Geometric mean	36543	34983	0.29
Range	2200-349636	2552-652800	
Gametocyte density (/µl)			
Geonietric mean	15 (n = 3)	17 (n = 2)	0.8
Range	12 – 24	12 – 24	
PCT (d)			
mean ± sd	$2.5 \pm 0.9 (n = 50)$	$3.2 \pm 1.2 (n = 44)$	0.002
range	1-5	1-6	
FCT (d)			
mean ± sd	20±10	2.3 ± 1.3	0.20
range	1-4	1-6	
Day 14 responses*			
No of infections			
Cured (%)	47 (88 7)	43 (87.7)	0.88
RI	6	5	
RII	0	0	
RIII	0	1	

<sup>\*</sup>Using WHO (1977) criteria
TS. trime hopetor sulfance horizone: PS, pyramethamine-sulphadounc, FCT for cr clearance time, PCT parasite chearance time, set sundard deviation. All compansons were two-tailed

TABLE 10.2. Intensity and prevalence of P. falciparum gametocytaemia following treatment of uncomplicated malaria with trimethoprim-sulfamethoxazole or pyrimethamine-sulfadoxine of 102 malarious children

	N(n=5)	<b>₹</b> 5(6=40)	E. Ane
Day 0	18 [12 - 48]* 5/ 53 (9 4%)**	17 [12 - 24] 2/ 49 (4 1%)	0 441
Day 3	27 [12 - 120] 13/ 53 (25.0%)	27 [12 –144] 12/49 (24 5%)	1.0
Day 5	33 [12 – 420]	45 [12 - 1872]	0.49
	21/51(41 2%)	25/48 (52 1%)	0.31
Day 7	42 [12 - 444]	71 [12 - 2316]	0.27
	28/49 (57.1%)	34/ 46 (73.9%)	0.13
Day 14	33 [12-120]	43 [12 -1200]	0.52
	13/37 (35 1%)	23/35 (65.7%)	0.018

<sup>•</sup> Geometric mean [cange] • Gamelocite positive/ No of patients examined, values in parentheses represents percentage of patients with gamelocitacinia • • • Mann Whiteey ics ¶ x² square test

# Gametocytaemia

Gametocytaemia before and after treatment with PS, and before, during and after treatment with TS is shown in Table 10.2. Gametocytaemia was similar throughout the duration of the study in both TS and PS-treated children with peak gametocytaemia occurring in both treatment groups on day 7. Peak gametocytaemia (on day 7) was significantly higher than day 3 gametocytaemia in both treatment groups (t = 0.066, P = 0.018 for TS; t = 0.08, P = 0.017, by Wilcoxon sign rank test for paired data). Gametocytaemias occurring on days 3-14 were not compared with pre-treatment gametocytaemia because of the small number of patients in both groups. However, multiple comparison of gametocytaemia using Friedman test showed that there was significant increase in gametocytaema with time on days 3, 5, 7 and 14 in those treated with PS (P = 0.011). In comparison, there was no significant increase in gametocytaemia with time on days 3, 5, 7 and 14 in those treated with TS (P = 0.29).

The Kaplan-Meier sativival curve of the cumulative probability of remaining gametocyte-free in children who were agametocytaemic at envolment is shown in Figure 9.1. By day 7 of follow up, children treated with PS had a significantly higher propensity to have developed gametocytes than in TS-treated children (Log-rank statistic \$35, df = 1, P = 0.02)

# Temporal changes in gametocyte sex ratios

In TS-treated children, 7, 28, 104, 134 and 44 gametocytes were counted on days

0, 3, 5, 7 and 14, respectively and approximately 77% of these gametocytes could be

sexed. In PS-treated children, 7, 34, 230, 293 and 168 gametocytes were counted on days

0, 3, 5, 7 and 14, respectively and approximately 76% of these gametocytes could be

sexed. The data on GSR at enrolment were pooled because of the small number of

gametocyte carnels absenced pre-treatment (live among TS-treated children and 2 among

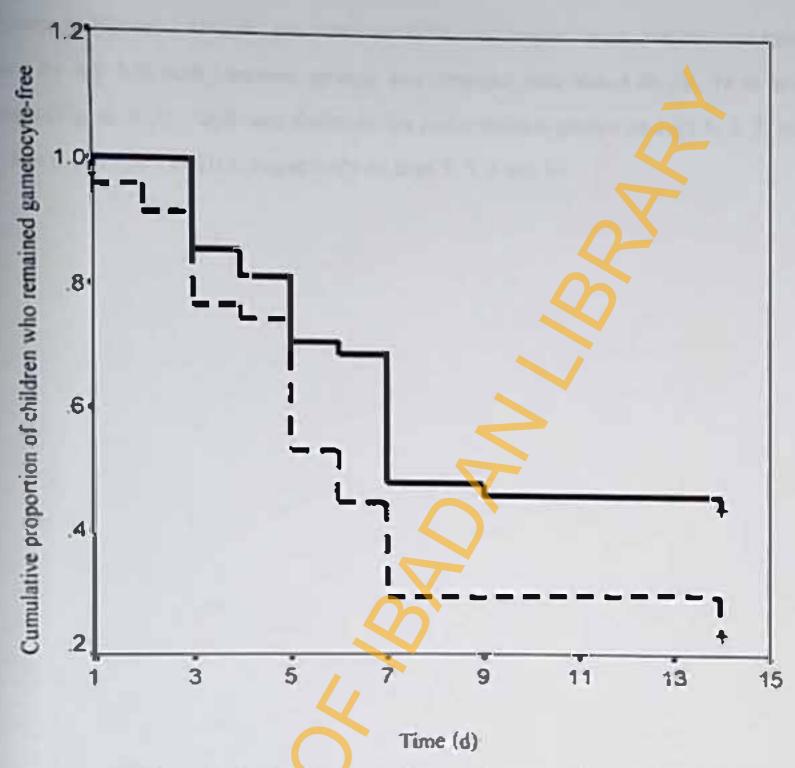


FIGURE 10.1 Figure 1 Kaplan-Meier plot (survival curve) of cumulative probability of remaining gametocyte-free in 95 children who were agametocytacmic at enrolment following treatment with trimethoprim-sulfamethoxazole (TS, broken line) or pyrimethamine-sulphadoxine (PS, solid line)

PS-treated children) Overall, pre-treatment GSR was female-biased, but became male-biased by day 3 in both treatment groups, and remained male-biased till day 14 in both groups (Figure 10.2). GSR was similar in the two treatment groups on days 3, 5, 7, and 14 (P = 0.4, 0.7, 0.7 and 0.2, respectively on days 3, 5, 7 and 14.

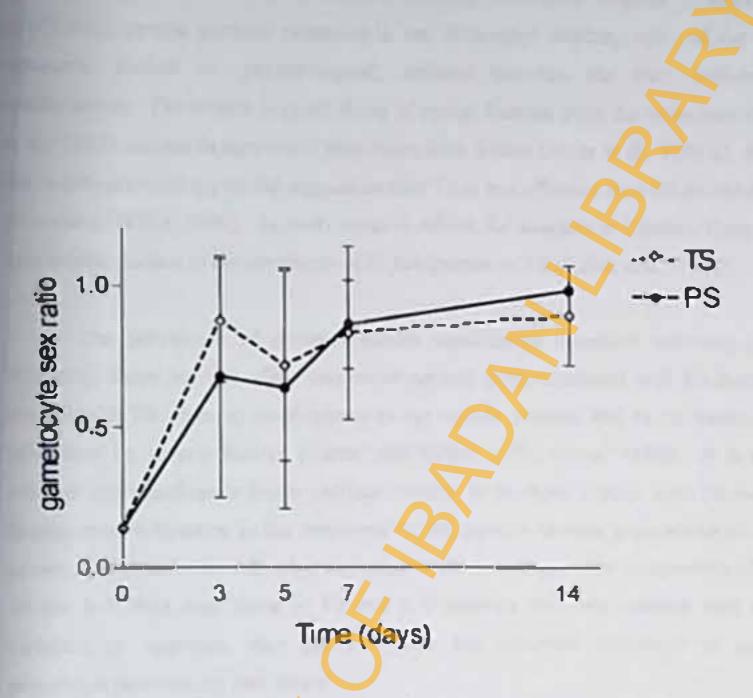


FIGURE. 10.2. Changes in sex ratio of gametocytes before and after treatment with pyrimethamine-sulfadoxine (PS), and before, during and after treatment with trincthoprim-sulfamethoxazole (TS) in children with acute, uncomplicated, falciparum malaria. The vertical lines indicate standard error

#### Discussion

malaria in children from this endemic area of southwest Nigeria. Apart from a significantly shorter parasite clearance in the TS-treated children, none of the outcome measures, clinical or parasitological, diffiered between the two antifolate drug combinations. The results support those of recent findings from the same area (Fehintola et al., 2004) and are in agreement with those from Kenya (Omar et al., 2001 a). However, the results are contrary to the suggestion that TS is less effective than PS for the treatment of malaria (WHO, 1996). In many areas in Africa, for example in Uganda, there has been appreciable decline in the sensitivity of P. falciparum to TS (Kilian et al., 1998).

The prevalence of gametocytacmia significantly increased following treatment with both drugs but this effect was more marked in those treated with PS than in those treated with TS. Sexual development in the malaria parasite and its modulation may be influenced by several factors (Carter and Miller, 1979, Mons, 1985). It is not clear whether the significantly lower carriage on day 14 in those treated with TS was due to fundamental differences in the responses of the asexual parasite populations to switch to gametocyte production following exposure to the two drugs. The components of TS have shorter half lives than those of PS and it is possible that this, coupled with individual variation in response, may partly explain the observed difference in gametocyte prevalence between the two drugs.

Although there were no significant differences in gametocyte density in the two treatment groups, the significant increases in gametocyte prevalence with time, the greater proportion of children with patent gametocytaemia on both days 7 and 14 among children treated with PS, and the significantly higher propensity to have developed gametocytes by day 7 in PS compared with TS treated children (see Figure 10.1) suggest a more marked effects of PS on gametocyte production. These findings with PS are in agreement with previous observations from the same area (Sowuluni and Fateye, 2003 b, e). Thus, the significantly teduced effects of TS on gametocyte retention may be an advantage for the use of TS over PS in endense setting.

lower gametocyte prevalence Despite insignificant and increase gametocytaemia with time in TS treated children, both TS and PS appear to have similar effects on GSR. None of the post-treatment initiation GSR data diffier between the two antifolate drug combinations; both drugs favoured gametocyte maleness. It is not clear whether the effects of the drugs on gametocytaemia are fundamentally different from their effiects on GSR. Since GSR may be influenced by several factors (West et al., 2002; Gardner et al., 2003), this may impact on the temporal changes in GSR. The male-biased sex ratio after PS treatment is in agreement with recent findings from the same area (Sowunmi and Fateye, 2003 c). The gametocyte maleness seen after initiation of treatment with both drugs suggests that antifolates, in general, may favour gametocyte maleness. Since gametocyte infectivity to mosquito is increased by gametocyte maleness (Robert et al., 1996 b) and infectivity correlates with gametocyte density (Tchuinkam et al, 1993; Robert et al, 2000), both TS and PS by enhancing gametocyte maleness, gametocyte carriage and gametocytacmia, may markedly enhance malaria transmission whether the treated patients have antifolate sensitive or resistant infections This a demerit for the use of these drugs alone for the treatment of malaria.

Conclusions and Recommendations

# Conclusions and Recommendations

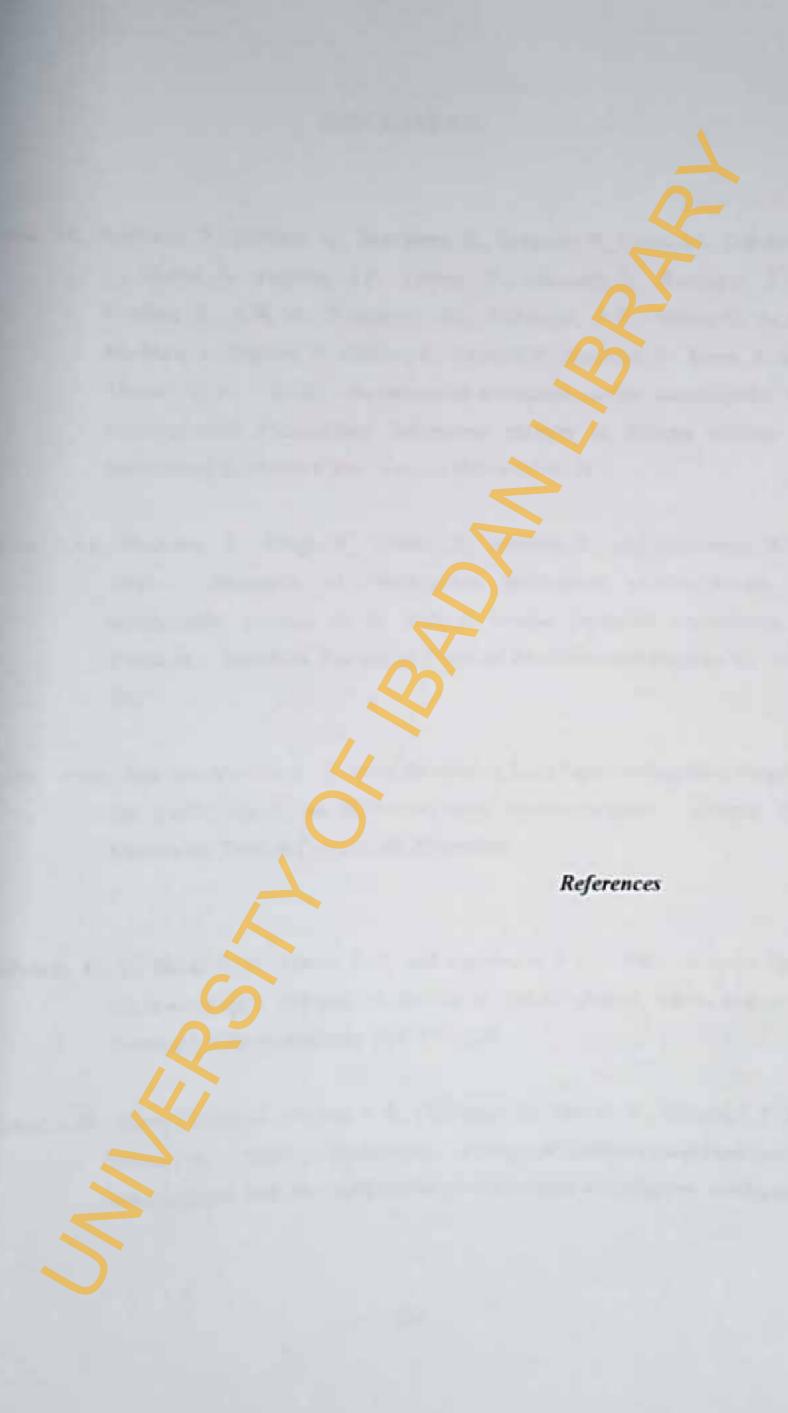
# The studies presented in this dissertation have shown that

- Children are uniformly susceptible to gametocyte carriage and that longer duration of illness, absence of fever, male gender and pamsitaemia < 5000 /µl are risk factors for gametocyte carriage.
- Apart from male gender, the risk factors associated with gametocyte carriage are little affected by season
- Children with CQ- resistant infections and those treated with PS irrespective of outcome were significantly at risk of gametocyte carriage.
- PS treatment significantly increased PYG PMG, but significant increases in this ratio were found only with CQCP resistant infections. AQPS significantly decreased the ratio-
- Presence of PYG was an indicator of response to CQCP but not to PS or AQPS
- PPS and TS, like TS alone, enhanced gametocyte carriage and gametocyte maleness, but TS has a lower propensity to cause gametocyte maleness
- Recently developed molecular assays are more sensitive than microscopic method Therefore, the estimates of the prevalence of gametocytaemia in the studies reported in this thesis are likely to be underestimates.

# Further studies are needed in the following areas;

- a. The effects of other antimalarial drugs, for example amodiaquine, a drug similar in action to chloroquine, or its combination with artesunate on gametocyte carriage and sex ratio
- b. Evaluation of the value of PYG as an indicator of resistance to amodiaquine
- c Molecular and cellular basis of the mobilization of gametocytes to peripheral blood by pyrimethamine sulfadorune
- d Infectivity to mosquitoes, of gametocytes obtained after treatment with various antimalerial drugs studied in this dissertation

- e The prevalence of submicroscopic gametocytaemia before, during and after treatment with various antimalarial drugs and combination therapies by molecular assays
- Infectivity to mosquitoes of blood obtained from children with submicroscopic gametocytaemia before, during and after treatment with various antimularial drugs and combination therapies.
- g Distinguishing between male and female gametocytes using molecular assay techniques



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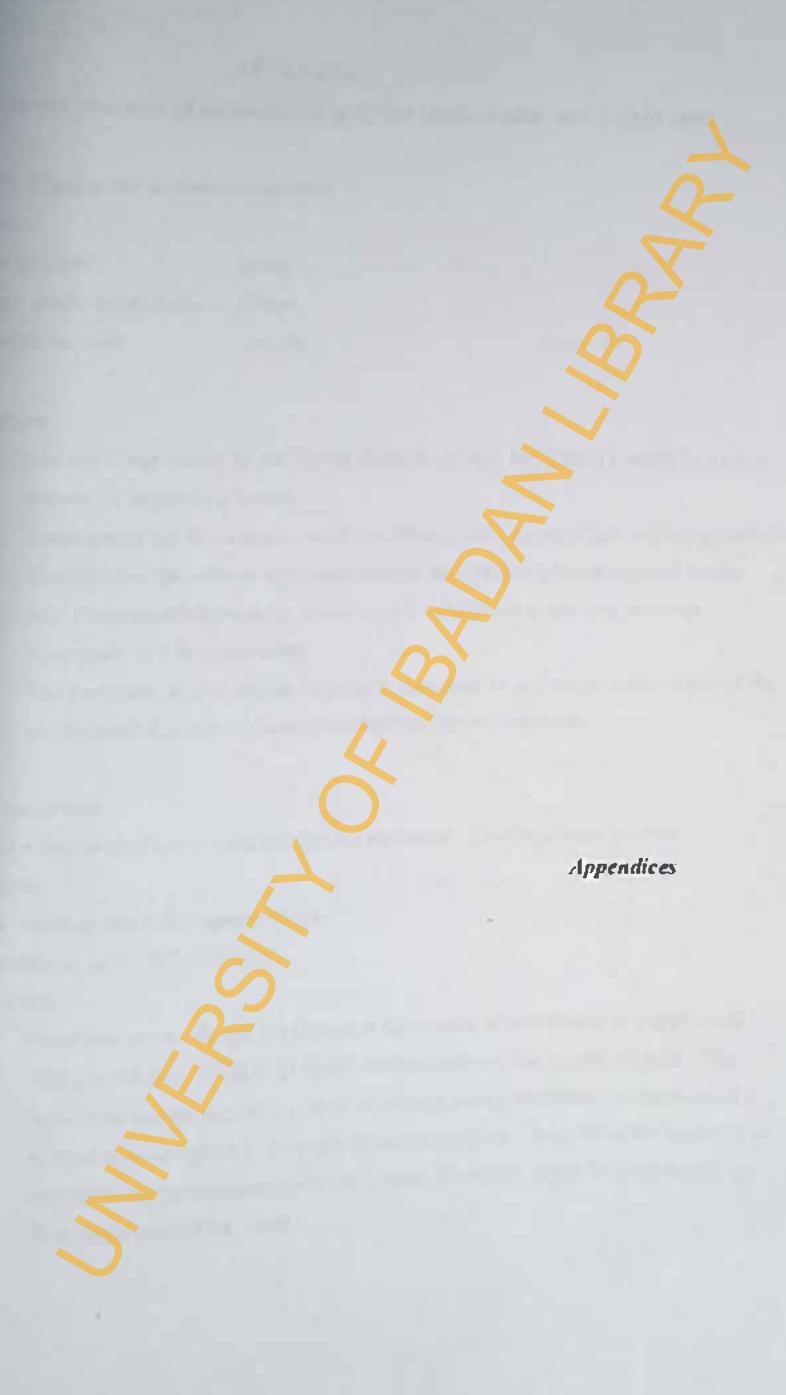
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#### APPENDIX 1

Tests for the presence of antimalarial in Urine (Dill-Glazko and Lignin test)

#### A. Dill-Glazko for 4- aminoquinoline

Reagents:

Eosine powder 50mg

Reagent grade chloroform 100ml

Hydrochloric acid Imol/L

#### Procedure

- Add the 50mg cosine to the 100ml chloroform and Im HCL (1 mol/l) in a glass stopper ed separating funnel
- 2. Shake gently for few minutes until the chloroform becomes light yellow in colour
- 3 Separate the chloroform layer and store in dark brown glass stoppered bottle
- 4. Add 10 drops of chloroform solution to 2 ml urine in a test-tube and mix vigorously or a few moments
- The presence of chloroquine in urine is indicated by a change in the colour of the precipitated chloroform layer from light yellow to violet red

# B. Lignin test

This is a simple qualitative field test for the detection of sulfonamides in urine

Reagents

Paper towel or blank newspaper strips

Hydrochloric acid, HCL (3mol/1)

# Procedure

- I Place one or two drops of urine on a blank strip of newspaper or paper towel
- 2 Add a small drop of FICL (3 moVI) to the center of the moistured area. The immediate appearance of a yellow to orange colour indicates the presence of a sulfonamide compound. The test becomes positive I hour after the ingestion of sulfonamides and stays positive for 3 days (Caution-paper of bond quality or filter paper cannot be used).



Appendix 2. Micrograph of peripheral mature make (now a) and female (arrow b) obtained in thick blood smear of a child treated with anticularial drug.

# Risk factors for gametocyte carriage in uncomplicated falciparum malaria in children

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(Recented 23 September 2003; revised 19 February 2004; accepted 24 February 2004)

SUMMARY

be risk (\*\* in a suscepted with pametory taems at presentation and after treatment with different antimalated first regions were evaluated in 767 children enrolled prospectively in 5 automalated drift in all between July 1996 and December 1921 in a hyperendemic area of southwestern Nigeria. The children were assigned to one of 6 treatment groups: chloromet ICQ) only, pyrimethamine-sulfadosace (I'S) only; anvoltaquine (AQ) only, CQ combined with chlorophentramine 1900) or I'S combined with CQ (CQIS) or AQ (AQI'S). At enrolment, 115 (ISS) of 767 children were gametocyte carein During follow-up, 15 6% of all patients (i.e. 120 patients) developed patent randomy terms, which in 85% (102 minus) had developed by day 7 following treatment. In a multiple regression model, 4 foctors were found to be independent task factors for the presence of attentiony terms at enrolment male sentler failured odds tauto (AOR) = 0.55, 95, 100 minus (AOR) = 0.000, P=0.005), absence offer or (AOR=1.01.95% C1.05-2.5, P=0.03), duration of illness of the sentless of the presence of patent gametocyteemia at enrolment (AOR=0.000) (AOR=0.42, 95% C1.02-4.1 P=0.000), and attended with the presence of saccased parasites within 14 days were associated with the presence of saccased parasites within 14 days were associated with the presence of saccased random for malarise models with increased risk of subsequent gametocyte carriage. These findings may have implications for malarise models of a subsequent gametocyte carriage. These findings may have implications for malarise models in sub-Saharan A(rice where control of the disease depends almost criticity on chemotherapy.

Key words: gametocyte curriage, children, risk factors, Nigeria

# INTRODUCTION

Limited to mosquitoes can only occur through the patetacyte, its accural stage that develops from problemsing ascernal parasite. Comerocytes, in turn, are estential for the infection of new hosts by the mosquito (Sinden et al. 1978, Carter & Graves, 1988). Although the mechanisms of the switch from asexual asexual asexual asexual asexual angle, and its inodulation, are complex and incompletely understood (Carter & Alifler, 1979; Mors, 1985), the process, and the infectivity of the switch may be influenced by antimalarial drugs (Wilkinson et al. 1976, butcher, 1997, Hisckling et al. 1999)

In aub Saharan Africa, increasing drug resistance in p. solin parum has led to increase in mulatlu-related multily and murtality (Trope et al. 1998. Trape at al. 199

2001. Sowumm & Pareye, 21813a), and children, an Reneral, are thought to community a aignificant reservoir of infection in sub-Saharan Africa (Catheko et al. 1992, Bonnet et al. 2003)

A recent study from The Gambia (von Seidlein et al. 2001), an area of lesser intensity of malaria transmission than Nigeria (Salaka et al. 1990), his shown that annemia, absence of lever and parasitemia less than 100 000 asexual forms per at were indefiendent risk factors for gametocyte carriage at presentation in Gambian children. In addition, treatment with Pyrimethamine sulfadoxine (115) alone was associated with increased risk of gametocyte carriage 7 days after treatment compared to chilinoquine (CO) or attentismin based combination thereby. It is unclear whether these factors alone or in addition to others, are associated with Rametocyte carriage in Nigerian children.

Although with increasing animalarial itrulicentaonce (Falade et al. 1997; Sowumit et al. 1998a, b.
Sowinimi, 2002) there have been associated increases
in gametocyte carriage in Nigerian children
(Sowumit & Fateye, 2003a), there is little inforlimitation on the risk factors associated with gametocyte
curriage pre- or president management in Nigerian children
Such information is necessary as it may parentally
harries the ellumicalists at the management and
complete of their termone in the community in the

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present study we evaluated the factors that influence the production of gametoes tea in children presenting with scute, symptomatic, uncomplicated, P folciorous malaria in a hyperendemic arcreof malaria in southwest Nigeria.

#### PATIENTS AND METHODS.

#### Pattents

The study took place between July 1996 and December 2002 in patients firesenting at the University College Hospital in Ibadan, a hyperendemic area for malaria in southwestern Gifferia (Solako et al. 1990). Ethical clearance was provided by the local ethics committee. Durink the period. a cenes of antimalanal druk studies were conducted to evaluate the efficiery and safety of different treatment regimens Studies on CQ were done during the entire 6.year period, those of chloroquine phis chlorphenicamine (COCP) in the liest I years, those of PS in the first 2 tears and the last 2 years, those of unodisquine (AQ) alime in the last 3 years, and those of combination anumularials in the last 2 years. However, there was a cansiderable descree of overlap in the study periods Dends of the atudies have been described before (Sonunmi et al. 19980, b, Somunmi, 2002, 2003, Sowunmi & Fateye, 2003 a) Briefly, children with Timptoms comfratible with acute falciparum malano who fulfilled the fullming ernerts were enlisted in the study are below 120 months, have P Sulciputum Parasitacinin greater than 2000 asexual formelit of blood, negative urine tests for antimalorial drugs IDIII. Glasko and liknin tests), absence of concomitant illness, no evidence of severe malaria (WHO, 2000) and written informed consent given by parents of Rustdiane After enrolment and start of treatment [day ol. follow-up with clinical and partialogical evaluation was ut days 1-7, and then on day 14, and when necessary, no days 21 and 28, for example, in Patienta who received PS combined with CQ (CQPS) or AQ (AQPS). Clinical cyaluation contimed of a general clinical examination includind measurement of weight, once temperature and Physical examination

# Accessment of parantacionis

Thick and thin blood films prepared from a tinter prick were Giemsantained and were examined by light microscopy under an orlumnication objective at \$1000 magnification, by 2 independent assertion by anitamism thick films was estimated by counting or anitamism thick films was estimated by counting or anitamism whichever (occurred hist firm this films, whichever (occurred hist firm this films, is parasite density was established assuming a leukacyte count of \$6000/µt of blood. Gametocytes where it is a blood films against 1000 films agains

1971, Sun unmi, Akindele & Haligun, 1993). A hormotocrat wardone at entrollment in 124 of the potients treated with CQPS or AQPS in order to evaluate the safety of combination automalarial theraps

#### Et aluation of response to drug treatment

World Health Organization (WHO) criteria (WHO, 1973) as follows: S=sensitive, clearance of parasitacinia without recutrence, RI (mild resistance) = parasitacinia dualppears but reappears within 7 to 14 days; RII (moderate resistance) = decrease of parasitacinia but no complete clearance from peripheral blood. RIII (severe resistance) = no pronounced decrease or increase in parasitacinia at 48 h ofter treatment. In those with sensitive or RI response, parasite elegrance time (PCT) was defined as the time elapsing from drug auminiatration until there was no patent parasitacinia for at least 72 h. Avexual parasite reduction mum [PRR] (White, 1997) was defined as the time reduction mum [PRR] (White, 1997) was defined as the retion of day O/day 2 parasitacinia

#### Statutical analy 14

Date were enaly ned using version 6 of the Epi-Info software (Anon 1994), and the statistical program SPSS for Windows version 10.01 (SPSS. 1999). Prohibitions were compared by calculating 21 with Coles' correction Marnally distributed, continuous data were compared by Student's 1-tests and mall as of whatee (ANOVA) In the drug teraiment groups noil-hoe companisons were done using Tukey honestly significant difference (Tukey IISD) Data not conforming to a notinal distribution were compared by the Mann-Whone's U-jest and the Kruskal-Wallis test (or by Wilcoxon tank sum test) A multiple latione retrievalor model was used to test die Respectation between Rank the Starting () es or no al presentation or during follow up) and factors that were significant of univarious analysis, male gender. presence of fever, duration of illness before presentauon, asexual fiarantaemia at presentation, drig treatment, and recrudescence of asexual harosites within 14 days of initiating treatment. Because the study was constucted over a penul of 6 years, time m years since the commencemental trials was included as a covariate in the model for pre-treatment someinchtacima The values presented below are generally means and standard deviations (5 to ) or standard error (s 12) or median with injerduariile ranke j [QR] p colues ul <0 05 were taken in indicate aignificant differences

#### PESULTA

Potent Rantetocytaemia (recometric mean 26, mare 6-1344/pl) was present in 115 (15%) of the 767 children of enrelment.

The responses of the asexual parmatments to drug matement and gametocyte carriage during and/or the treatment are snown in Table I PRR in children treated with AQPS or CQPS was significantly wher then all other treatment groups (P<0.001) with the exception of the AQ and PS groups, which, compared in COPS, did not differ siknificantly (P=0-099 and 030 respectively, Tukey HSD), PCT me in the nely harter in those treated with AQPS and COPS compared to other treatment groups (P< 0001) except AQ (P=0.052 and 0.25, respectively. Tukey HSD). PCT was also significantly shorter in those treated with AQ compared to CQ (P=0 019, Takey (15D). Factors associated with gametocytiemu at encolment are presented in Table 2 3 lale render, absence of fever, duration of illness >3 days, and asexual parasne denaities less than 5000/pl were related in the presence of gametocytacmia at molment Neither age nor packed cell volume mas an independent risk factor for imetocyte carriage (Table 2)

had factors for gametocyte carriage during follow up

During fullow-up, 15.6% of all patients (i.e. 120 patients) developed patent gametocy tacmia which, in 85% (102 patients), had developed by day 7 following freatment. Camerocyte densities at enrolment were milar in all treatment groups, were significantly theron day 14 in thuse treated with PS, and o tigmountly higher probortion of children trested with PS carried gameton te throughout the duration of the study (Table 3). In the cohort of children in blion gametucytes were not detected at encolment. 16 of 259 (13.11%) children treated with CQ 9 of 82 (11-0%) treated with CQCP, 3 of 93 (3-2%) treated 13th A(), 1 of 64 (1.6%) treated with CQPS, 3 of 64 children treated with AQIS, and 50 nf 90 13 (%) children treated with 1'S developed patent metocytaemia within 7 days of encolment. Thus, Punpartinn of children who developed gametoplaemia following treatment were significantly her in those recated with PS compared with other " | regiment (Chil = 136 9. 1 = < 0.001)

The presence of patent Kametocytaemia at enrolint, and rectudencence of arexual parasites within
days were associated with the presence of somestarnia? or 1.1 days after entolment (Table 4).
The presence of somestarnia? or 1.1 days after entolment (Table 4).
The presence taken to clear the initial harstarnia we reasociated with increased risk of subuent gametocyte corrises, but this association
must significant fullowith thulingriate analysis
finds:

(Table 4) Children treated with AQ, AQPS or
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the presence of some significantly less hard with those
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The presence of complection and day for 14 was significantly associated with treatment outcome in day 14 in children treated with CQ (Chi<sup>2</sup>=183. DF=1.P=<0.001) and CQCP(Chi<sup>2</sup>=101 in F=1.P=0.001), but not PS (Chi<sup>2</sup>=0.21, in F=1.P=0.01), and AQ (Chi<sup>2</sup>=0.24; in F=1.P=0.02) and AQPS or CQPS in which all children were clinically cured

#### DISCUSSION

Gametoc) tes are often detectable in peripheral blood for a variable period after acute fater parum infection, with morphologically mature transcriveytes being detectable in the blood 10-14 days after originating from merozoirea (Thimson, 1911, Smalley, 1976). Carrunge rates may vary widely and are dependent on several factors. In the current study, gametocyte prevalence was much higher than those reported from western Thailand (2%, Price et al. 1999) and Tanzania (8%, Akim et al. 2000) but similar to that from The Gambia (17%, von Sciulcin et al. 2001) in the same region of Africa Hinneyer, despite regional differences in prevalence rates, the risk factors associated with gametocyte carriage were remarkably similar.

Gametocy to prevalence in the study uses before the 1990s, a period of full sensitivity to C(), was less than 2% (L. A. Salako, unpublished abservation). Presently, in the area. CO treatment of CO-resistant infections is associated with significant gametocyte curringe and gamerocy themia, and stoner elemente of gametocytaemia (Souunmi & Fatere, 2003a,h) Therefore, it would appear that the present relatively high providence rate may, in part, he due to increasing CQ resultance Seventy percent of all cases of settle malaris infections in our area of study uccur in children aged less than 10 team (Salako et al 1990); the similar gametocyte carriage in all age groups auggests that children ageil helow 10 years were uniformly susceptible in gametocyte cornage in other studies involving a broader age range than we evaluated. a younger age was associated with increased generoeyie prevalence, for example. in Tenzanta (Akım et al 2000).

It is unclear why male gender is a risk factor for gametocyte carriage at presentation, despite associate duration of illness and other characteristics in both gender groups (data not shown). To our knowledge, this is the first report of the association between male gender and gametocyte carriage in African children with falciparum nuslatus. Could this simply be a chance finding? Plusma testostemme is often significantly raised in pre-pubertal-triale than feithale children (Griffin & Wilson, 1991), and testoste time and other continuous entitle may stimulate. P. falciparum gontetic) togenesis in topic (Manuscut, Peters & Warhurst, 1985, Linguage et al. 1993). It seems possible that interences in sex historium levels.

sexual parasitaemia and gametocyte carriage following treatment with antimalarial drugs Responses of able

ere given at presentation (day 0) and assexual parasitaemia quantification was done daily for 8 days (days 0-7) and then on day 14. Gametocyte carriage was 4. PRR, parable reduction ratio; PCT, parasite clearance time; PS, pyrimethamine-sulfadoxine; CQ, chloroquine; CQCP, chloroquine plus chlorphenimmine; AQ, amodiaquine, pyrimethamine-sulladoxine combined with chloroquine; AQPS, pyrimethamine-sulfadoxine combined with amodiaquine; RI = parasitaemia disappears but reappears within 7 to 14 days; RI1 = decrease of parasitaemia but no complete clearance from peripheral blood; RIII = no pronounced decrease or increase in parasitaemia at 48 h after treatment; S = sensitive response.) ancessed on days 0, 7 and Standard doses of drugs

	(4-315)	(m1-n)	AQ (4= 104)	75 (a=109)	CQPS (4=65)	AQPS (4-70)	Profine
% of children with gametocytes at enrolment on day 7 on day 14	17 & (n=56) 24.8 (n=78) 17 L (n=54)	25-1 (n=22) 25-1 (n=24) 10-6 (n=11)	10 6 (n - 11) 10 6 (n - 11) 7.7 (n - 8)	17.4 (n=19) 61.5 (n=47) 48.6 (n=53)	1.5 (n=1) 3.1 (n=2)	8-6 (n=6) 10-0 (n=7) +3 (n=3)	0.001
PRR Median Interquartile range Range	1.42-3.97	1.73-3-93	275	2 1.8	3:94	3.70	• H(U/Q >
PCT (days)	2.9±0.9	2-8±0-8	2.640.8	14700	2.3±0.8	2.2+0.8	0.101
S (no. of patients)	198 N7	26	205	78	65	2	1100
RIII RIII Cure rase (%)	15 15 62-9	93.2	118	71.5	1/02	100	101.0

 PRR of AQPS- and CQPS-treated children were significantly higher than in other treatment groups except those treated with AQ or PS (compared with AQPS and CQPS) compared to other treatment groups (P≤0.001) except AQ (P=0.052 and 0.25), respectively. PCT was significantly shorter in those treated with AQPS and CQPS compared to other treatment groups (P≤0.001) except AQ (P=0.052 and 0.25), respectively. PCT was significantly shorter in those treated with AQPS and CQPS compared to other treatment groups (P≤0.001) except AQ (P=0.052 and 0.25), respectively. those treated with AQ compared to CQ (P=0019, Tukey HSD) ugnificantly shorter in

Table 2. Risk factors for Planmodium falciparum gametocy tactnia ui enrolmente

		No of children with gameiocytes	Crude odds ratio (95% CI)	Pvalue	Adjusted OK 195% CI)	
Age (y)					1134(1)	Pralue
<5	420	69				
≥5	347	46	• 78 (O 52-1 2)	0.00		
Gender			4 10 (0 32-1 2)	0 26		
male	354	66	330			
female	413	49	06 (0+0-9)		1	
Parasitaemsa (/µ1)			V0 (V1-V3)	0116	U-55 (O-36-0-13)	0.005
<5000	82	21	77			
≥5000	685	94	0-46 (0-26-0-83)	A 200	100	
Fevert			V40 (U20-U3)	0.097	0-12 (0-24-0 73)	0-003
Febrile	533	73				
Afebrile	208	42	1 6 (1.06-2 13)	0013	1 41 (1 05 1.6)	
Duration			101106-213)	0013	1 61 (1 05-2-5)	U-03
of illness						
≤3 days	575	76	1		1	
>1 days	162	39	1.7 ().1-2.7)	D 0019	1-57 (1 1)-2-4)	0 047
PCV++	17.15					0017
€25%	24	3				
>25%	160	3	0-78 (0-52-1-2)	071		

Time was included as a covariate in the analysis

Table 3. Gametocyte densities at enfolment and following treatment with antimalanal drugs

(GMGD, Genmetrie mean gametocyte density, PS, pyrimethamine-sulfadasine, CQ, chloroduine; CQCP, chloroduine Plus chloropheniranine; AQ, amodisquine, CQM, pyrimethamine-sulfadosine cumbined with the suduing or a modis-

	CQ (n=315)	CQCP (n = 101)	AQ (n=104)	PS {n = 107}	CQ1'S* [#=65]	AQPS (n=70)	P valu
Gametocytacmia At enrolment GMGD (/μl) Range	25 (m=56) 6-13+4	24 (n = 22) 12-376	29 (u=  1)   12-740	24 (n=19) 6-44	132(e=1) 132	40 (n = 6) 12-256	n 55
On day 7 GMGD (/µ1) Range	14 (n = 78) (- 1476	11 (n = 24)	34 (n=11) 12-63h	75 (n = 67) 6-352()	54 (n = 2) 24-(20)	31 (n = 7) 12-468	0.434
On day 14 GMGD (/µl) Range Proportion (%) of children with gametocytaemia on days 0, 7 and 14	21 (n = 34) (n-)+1 (17 (d = 29)	41 (n=11) 12-168 77 (n=8)	10 (a = 5) 12-30 3-8 (m = 4)	\$0 (n=53) n=180 12.8 (n=14)	-	19 (n=3) 12-18 1 -1 (n=1)	0 (101

CQPS not included in the companion due to small number

her be contributory, but hormone concentrations are not measured in the children. Gender-selated themences as risk factors for gametoe) to carriage themes further evaluation in African children

As longer established P folciparum

infections are more likely to produce gametocytes (Sniatley, Brown & Bassett, 1981), it is likely that longer duration of illness before presentation as law ed sufficient time for the firogression of committed esexual parasites in Sametocytes. Since absence of fever is associated with increased risk of gametocyte corresse, alebrite children may have harboured the infection for a longer period. Alternatively, children with longer duration of fever sesulting in reduced lively shintler duration of fever sesulting in reduced

<sup>1</sup> Fever, axillary temperature > 37 5 C

PCV, hecked cell volume Chil with Yate's correction.

Table 4. Risk factors for Plasmodium folesporum gametocycemia 7 days after treatment

premethamme-sulfadorine, CQ, chloroduine, CQCI, chloropina plus chloropinal minus AQ, amedaninal with chloropinal or uncalculum (

Status as enrolment	l'oul	No of children with warmetich less on day 7	Ctude odda ratio	Pontue	Adjury (a) (95 - Cl)	Paulue
Cornetocytes						7 (100)
*Present Absent	652	86	0-06 (0-01-0-00)	<0.001§	1	10.001
PCTT				Cooold	0+0+ (0 02: 0 07)	< 0.001
≤2 day= >2 days	298 469	61	1 1:4 (1:00-2:07)	00175	1.4(0.0:2.4)	
Patent areaus) patentamina within 14 days			1 + (1 0 - 2 07)	0004	1-4 (0.9-3-1)	0-20
Present	157	68				
Absent	610	121	0-32 (0-22-0-47)	< 0-001	0.50 (0.3-0.8)	13-007
Daig treatment						
PS	109	67	1	-	1	1
CQ	315	78	48 (31)-79)	<0-001	x 5 14-4-14 b)	<0.001
CQCI	104	24	5-3 (2-8-10-1)	<0.001	9+(45-197)	<0 001
AQ	104	11	13-5 (6-2-30-2)	< 0.001	17 + (7.3-110)	<0.001
AQPS	70	6	14-4 (5-7-)8-0)	<0.001	147 (5 5-102)	<01001
CUPS	65		50-2 (11 2-313 7)	<0.001	35 6 17 8-163 5)	<0.001

Values of OR represent chances of being gametocyte free.

ment Low parabituemia (as in the present study) and anaemia are also aignificantly associated with sometocyte curring. (Price et al. 1999, von Seidlein et al. 2001) but hacmatocrity alues less than 25% were not associated with gameties te curringe in our cohort of children. We have no clear exclianation for this observation. Anaemia in uncomplicated falciparam majora may be enhanced by pre-existing helminth infections (Nacher et al. 2002), and both conditions and enhance gametocyte carriaged fon Seidlein et al. 2001. Nacher et al. 2002), frequently co-exist and, are common in tropical endemic regions.

Despue lower ellicacy. CO treatment resulted in lower Rametocyte carriage than PS A similar obser ation has been made in Senegal and The Cambia IRobert et al 2000, von Seidlein et al 2001) The willy to release more gainstocytes into the circulation following 15 treatment may, in part, be inde-Pendent of parasite sensitivity to 115 (Sumonmi & Faleye. 2003e) and may partly explain this obserelulds trespective of treatment regimen given shildien with patent gumetocy teen men presentation Tere significantly more likely to be gametowy themes dissoluter than children without patent Ramero-Statemia The suggests that the drugs evaluated had little or no effect on mature circulation Hamelocyles As was expected, recrudescent infections were delacedwith higher Rametocyte prevalence, as was ottay in peripheral parante clearance as parantes

develop resistance in thurs The increase in gametocyte comage and density to tensiance develops to antimalarial druge may confer survival and firopegation advantages on the paravite in the population (Handunnetti et al. 1996; Habert et al. 1996; Sutherlandet al. 2002; Sumunini & Fateye, 20030, 6). In the current study, delayed cleamnes of periphe ml parasitarmia and increased recrudescence rates were most frequently seen in these treated with CQ or PS and least frequent in those treated with AQ, AQPS ur COPS Similar obiervations have been mode else. where (Price et al. 1900; Robert et al 2000; Ahim et al 2000, von Seidlein et al 2001) The nignificantly higher gametoc) ie density in those treated with bis than CQ at recrudescence of asexual parasitaemia would suggest that the former than have a higher propensity for the transmission of drug-resultant infections than the latter since game (oc) te infectivity to intestuitors may correlate with level of gametocytacmia (Tchuinkani et al. 1993. Hobertet al. 2010). Since leukocyte counts may 1200 widely one of the possible sources til etrois in our estimation of gametucyte density is accunting in average leukocyte count of fall(X)/pl of Island

The findings of the present study may have potential implications for the management of acute infections in this endemic area. Strompt treatment of falcillatum infections with effective thrust to often associated with inw gametocyte carriage (and may invariably reduce transmission of gametocytes to

PCT, parasite clearance time.

I Chi with Yate's correction.

modifices), treatment of acute infections should, probably employ capidly acting schizantocitle to reduce the development of gametocytes The arremulan derivatives may reduce transmissibility by mode of action (Price et al 1996). Finally the indings may have important implications with resonantial control inxub-Saharan Africa, where combination antimalarial therapy (WHO, 2001 o.6) is presently being proposed for the treatment of malacial in the region.

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# PLASMODIUM FALCIPARIIM HYPERPARASTAEMIA IN CHILDREN RISK FACTORS, TREATMENT OUTCOMES, AND GAMETOCTUEMIA FOLLOWING TREATMENT

SOUTH A. ADEDEN AA. FATETE BA & BABALOUA C.P.

#### Summary

The risk factors associated with hyperparasitemia at presentation and after treatment with different antimalarial drug regimens were evaluated in 1,048 children errolled prospectively in seven artimakinal drug trials between July 1996 and September 2003 in a hyperendemic area of southwestern Nigeria. The outcomes of treatment of hyperparasitoensia, and gametocyte consage following treatment were also evaluated. The children were assigned to are all seven treatment groups : chloroquine (CQ) only; pyrimethamine sulfadoxine (PS) only; amadiaquine (AQI) only, CQ plus chlorphenizamine (CQCP), PS combined with CQ or AQ (COM); PS combined with probenecid (PPS), and halalantine (HF). Hyperparasilaemia was lound in 100 (9.5 %) of the 1,048 children at enrolment (day O). Following and therapy. 1.2 % of all patients (i.e. 13 patients) became hyperparasitaenic, which developed in all patients by day 1 of followup. In a multiple regression model, age < 5 years, and a core temperature lard or rectal) > 39.5°C were found to be independent risk factors for hyperparasitaemia at enrolment, Fallowing therapy, the cure rate on day 14 was significantly lower in those treated with CG compared to other treatment groups. Severe resistance (CII) response to treatment occurred significantly more frequently in those with hyperparasitoemia at enrolment than in those without, and was seen in five and one child with hyperparasitoenia who were treated with CQ and CQCP, respectively. Garrelocyto cantage was insignificantly lower at enrolment and at all times following treatment in children with hyperparasitaem a for in age and gender matched children without hyperparasisemia who received the same treatment. The results are discussed in the light of management of uncomplicated hyperparastaemia in children in endemic settings.

KZY WORDS : molaria, hyperparantaemia, ital factors, gamerocytaemia, children, Nigeria

Résumé : HYPEPARAUTEUR à PLAIMINNE AALCHARIN CHEZ DES ENFANTS : FACTEURS DE ENGLE ET GAMÉTICCYTÉME AVANT ET APRÈS TRAITEMENT

les facteurs de risque associés à l'hyperparasitémie à Plasmodium foldiparum à l'admission et après le traiement avec sept protocoles antipalidéens différents par voie orale ant été évalues. chez 1 048 amonts los d'une étide prospective, menée entre juillet 1996 et septembre 2003 au Sud-Ouest du Nigéria, dans une zone il perendémique. Les résultats du tratement (hyperparasiteme et durée de la persistence des gamétocytes dans le sangi art été évalués. Les groupes traités étaient chloroquire (CGI); pyriméthamine suflaxionine (PS), anaxiaquine (AGI); duraquine plus chlorpheniamine (CGCP); pyriméthamine ullador ne plus chloroquire ou anodiaquire (COV) py inellamine sulladoxine plus probenecial (PPS): halolantime (HI) Experparquitémie a élé retrouvée chez 100 enlants lors de Communion (9,5 %) Après 24 houres de traitement, 1,2 % des inlants (n = 13) sont devenus hyperparasités. Avec un programme de régression multiple, nous avons more é qu'un âge s. 3 ans et une température centrale 2 39,5°C sont des facteurs de risque indépendants pour l'hyperparasitémie à l'admission. Après deux semaires de taitement, le poucentage de guérison est significativement plus bos dans le groupe CO. Line résistance sévére à ce traitement (RIII) apparaît plus fréquenment chez coux qui sont hyperparasités à l'admission. Le nombre de gamétocytes circulants était plus bas à l'admission et pendant le traitement chez les enfants hyperparasitémiques que chez ceux du même dae et de même sover avec le même traitement, mois sons hyperparasitémie. Les résultats sont discutts dans le but d'améliane le tratement des accès palustes non compliqués avec hyperparasitémie dans les zones d'endêmie

MOTS GLES : policilane, hyperparasitimie, faceur de reque, grandocystonie, anfante, Nightia

### INTRODUCTION

rapid multiplication of asexual parasites and massive increases in circulating peripheral parasites particularly in the relatively non-immune or, less

Itequently, In the semi-immune These mainties to other team authority (WIO, 2000 ii), there are no other team and immune in children of teams of the particular in the interpretation of the interpret

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uncomplicated hyperpurationals in children in these cationic areas he trained with neal antimological drugs providing the drug is rapidly absorbed and the paraever are fully servitive to the untimabrial druggly driven (Somunmi et al., 1992, 1996, 2000 a) Such a suggesthat needs teview in view of the increasing resistance in P falchorum to many antimologials drugs and the lack of facilities to monitor drug seasitivity of P. falciporum tre titiro and lis titeo in many caklende areas

There is lattle information on, for example, the risk isethe associated with uncomplicated hyperparamemia or the time-course of gametroy versus following oral andmalayid treatment of uncomplicated hyperparate termine in African children. Such Information is notessury in view of the increasing reasonne in P fakt. Justim to chimmiulae (CQ) and other commonly available antinularists airl the lacrosing imphility and misulity associated with ding resistance (Trape of al. 1998; Franc. 2001). In addition, it may improve the meral transported of these even. The present why was designed in address these tower. The main sime of the undy were in evaluate the tisk factors acro-משובין שיוון איזוביושושאישכווון או פ פושיף הו כאושוכים जिन्द्राच्या अधि। अधिद्र क्षाताच्याद अद्यान्त्रीं पद्यान plicated, P falcipanum malare in an orderale area; in שיבים לופ ואונדישוובי הל חוש בתותשושוש ויבודים או incomplicated hyperpartitions, and to follow the tentioned changes in terministential in children with philader and was asky my was asky mile with المال لفتطيم

# PATIENTS AND METHODS

#### PATIENTS

- The study took place between July 1996 and September 2003 in patients presenting at the University College Hospital in Ihadan, a hyperendemic area for malaria in southwestern Nigeria (Salako et al., 1990). Ethical clearance was provided by the local ethics committee. During the period, a series of antimalarial drug studies were conducted to evaluate the efficacy and safety of different treatment regimens. All antimalarial drugs were given orally. The details of the studies have been described before Oceanneni et al., 1998 a, b, c, 2000 a; Sowunmi, 2002, 2003; Sowuneni & Fateye, 2003). Briefly, children with symptoms computible with acute falciparum malaria who fulfilled the following criteria were enlisted in the wady age he ow 12 ears, pure P. falciparum paraaltaemia greater than 2000 asexual forms/µL blood, and ligning tests), absence of concomitant illness, no days). Patients with RI response were re-treated with evidence of severe malaria (WHO, 2000 a) and written oral mefloquine 25 mg/kg single dose and followed

informed consent given by justines of yourdans. After entiliment and stan of treatment (thy 0) (nilina-up) with clinical and purasitological evaluation was at days. 1-7, and then on days 14, and with necessary, on days 21 and 28, for example, is fathents when totelled pyth methantine-autisationing (PS) it interest in interest in Itrichel combined with chlorestuting infractulated. May & Baker Ple, Nigerial or anicallativine (Camoquine). Parke Davis, Senerall Clinical makeation consisted of a general clinical examination including measurement of weight, cree temperature and thy ucal caundration.

#### ASSESSMENT OF PARASITALIANA

Think and thin him at the propagal from a farger fight were Gienses stained and were examined by light microscripty under an nil immeration objective, at x 1,000 numbership, by two indeposition assessment Paravacemu in thick films was estimated by counting avestal paraties teletive to 1,000 leukocytes, or 500 average fixther whichever occurred fine from this ligure, like purasite depoly was eskulated assuming an avorage lookneyre count of 6,000 pl. of blood (Shaper & Lewis 1971, Ezziki, 1971, Komsund et al., 1995) Gamero to were also crainist in thick filing against ביינים בעונים באנים או ביינים ביינים וביינים מוחוז ביינים of 6000 fil is hirror at comment (dry 6) and on dry 7 and 14 I racinnal gametocyte density (FGD) at combined was defined as guineticitie count divided by great are avail and sea util craint (Price of al., 1999) Haemajocro was done at enrolment in 121 of the national treated with PS or OQPS AQPS in PPS

#### EVALUATION OF RESPONSE TO DRING TREATMENT

performe to drug provinces was a second using world Health Organization (9710) crume (9710 1973) as follows 5 - establist, clearance of paramagnia without reconcice RI (mild restorer) - parameter discrepant but responsible 7 to 14 days, \$11 (priese relieve) - drorae of propositions has and complete cientance (mon peripheral Nood: IUII (אבייבי (בשנם חספ) - חס (אייוטנועותכים מבייבולב סר invente la paractionnic et 48 bours after treatment. In those with sensitive or II response, paraste character time (PCI) was delined as the time elapsing from these for at local 72 b. Acts and I wrong restation ratio (PRN) (When, 1997) was defined as the ratio of day Orday 2 pagragacouls

# RE-TREATMENT OF TREATMENT FAILURES

All patients with RII and RIII responses were re-treated with intramuscular artemether (9.6 mg/kg, over five

up for another 14-28 days. Patients were retreated whenever they become symptomatic or when they show personal clinical (hyperpyrexia, oral fluid into-lerance) or parasitological deterioration

#### STATISTICAL ANALYSIS

Data were analysed using version 6 of the Epi-Into software (Arkin, 1994), and the materical program SPSS for Windows version 10.01 (SPSS, 1999) Propositions were compared by calcubling x2 with Yates' contection or Fisher exact test. Normally distributed, contimunus class were compared by Student's recess and analysis of variance (ANONA) Data nex conforming to a resmal distribution were compared by the Maini-Whitney Utest and the Kruskal-Walls too (or by Wilenzon rank rum text). A multiple logistic regression mixtel was used to test the association between hyperpatasitaenala (yes or no at presentation or during failow up) and former that were Applicant at univamale analysis age \$ 5 years, and presence of fever (neal or rectal temperature) > 39 5" C. Because the שעלי בינו הבבל בינו מיני ב ושיבו כו היינו וצוח ליות was incided as a construct in the analysis. P. values of \$0.05 were taken to Indian Algoritani Illiantes

#### RESULTS

he demographic characteristics of the children enrolled in the study are summarized in Table I. At enrolment, 303, 173, 104, 203, 145, 78 and It of the 1,040 children were allocated up, and were subsequently treated with chiracopulate (CQ) only, pyrimediamine authorize (PS) unity; untextiaquine (AQ) only; CQ plus chiephenicanune (CQCP). PS combined with CQ or AQ (COM); IS combined with profunction (PPS); and talofaction (ITE) [Halfand, Giams-maintine], respectively. Hyperpolarization was found in 100 (95 %) of the 1,040 children at corolmens.

#### RUNN FACTORS FOR THE DUMRASSIALEMA AT ENBOLMENT

Pocum associated with in hellocastacings at encolnent are presented in Table II. Ago \$ 5 years, and real or recest temperature \$ 39.9°C were tradependent risk (after for uncomplicated hyperparantacing at encolment.)

Variables	Velos le [mesad (reng)]
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## HYPERPARASITAEMIA DIJRING POLLOW UP

Following oral therapy, 1.2 to of all futients (Ie 13 of the 1,048 pullents) become himportunemen which developed in all patients by day I of folkswap The 13 patients were treated with CQ (10 patients) PS (one ומולרון) מי COM (ואים ושוניתא), בואל לתלוחאיווצ עוביטובית all his two but sensitive response. The two didition in the COM group who became hyperpurationale on day I specifically received PS combined with CQ. The two children with researce response (1 RL 1810 were austral with a Compared with other action though there was a Assolitant difference in the perpending of children tresient with CQ who became hypertanal termic on they I following treatment (1 - 001)

## TREATMENT OF PRODUCES OF HYVERUAKASITAEMIA

The clinical and parastalogical characteristics of the 100 children who half hyperpartainemia ar envolment and were treated with oral andralatal drugs are summarized in Table III Despite explored at different per locks, these characteristics were similar (primaril) hecause the enterta for enrollment toto all studies were similar) No child with hyperpalastrome was treated with AQ alone.

The requested of the evental hyperpereduction in thus treatment are almost in Table IV. The cure rate fuller wing treatment with CO with algolithantly lower than the other treatment groups

Parasite: 2004, 11, 000-000.

	(m - 71) CO	(n - 3);	(A (A2))	(1) = 1)) COM	(V = 3)	(n = 11	r. raha
Apr (years)							
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Diffe	1.5-9	97-19 5	G 3-10 5	JA I	h.s.la		
M F	12 / 71	13 13	17 13	4 1	3 2	10	-
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Men	20:12	31215	35:25	30-07	3.0 ± 0.0	jo	
Carrier	1-0	1-6	1.11	344	3-3	-	
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Belle Pr. - Therefore companies of the distance with case of fifther case making who had believe presented in continued

# COMPARISON OF OUTCOMES OF TREATMENT OF NON INTERVALANTAEVILA AND INTERVALANTAEVILA

Stateon of 948 children without hyperparagraphic had Rull response in treatment compared in all of 100 childan with hyperparasitemia. The difference herween where perpendicular was significant (2? = 6.22. 19 = 0.001). Four children (three treated with CQ and one with P.D. aged 5 ) years who had hyperparadization progressed on cerebral mulanta, while own of the 1918 children without hyperparatremts had the same outerane The difference between these two proportions was significant (P - 0.001, by Picher coon rest) The two children without hyperparasitiemia who progressed in cerebral malaria were ocated with CQ Advence resethe reported following drug treatment were shally in children with hyperparabelerais and in age and matched children without hyperprisationing who were vested with the same drugs (data not shown) By example, it there treated with CQ, pro-Titue occurred in five (of 33) and four (of 33) children with and without hyperproduction to the city of

## GAMETOCYTE CARRIAGE AND GAMETOCYTAEMIA IN CHILDREN WITH HYPERPARASITAEMIA

In order to evaluate gametocyte carriage and gametocytaemia in those who were hyperparasitaemic at presentation, children with hyperparasitaemia were matched with those without hyperparasitaemia for time of presentation, age, gender, and drug treatment.

At enrolment gametocyte carriage was similar in children with hyperparasitaemia and in age, and gender-matched children without hyperparasitaemia who received the same drug treatment (6 of 100 is 11 of 100 children,  $\chi^2 = 1.03$ , P = 0.3). Similarly following treatment, gametocyte carriage was similar on day 7 (16 of 100 is 27 of 100 children;  $\chi^2 = 2.9$ , P = 0.08) and on day 14 (9 of 100 is 17 of 100 children,  $\chi^2 = 2.2$ , P = 0.14).

At enrolment gametocytaemia was similar in children with hyperparasitaemia and in age- and gender- matched children without hyperparastaemia who received the same drug treatment (generatric mean 12, range 6-24/ul. in 14 range 6-72, P = 0.5). Similarly following treatment, gametocytaemia was similar on day 7 (geometric mean 71, range 6-1320/ul. in 66, range 6-828, P = 0.4) and on day 14 (geometric mean 57, range 12-480/ul. in 70 range 12-360, P = 0.7).

Fractional generocyte density was insignificantly lower in children with hyperparasitaemia compared with in children with hyperparasitaemia (median 0.003, range those without hyperparasitaemia (median 0.003, range 0.0015-2.3 %, P = 0.24).

## RE-TREATMENT OF TREATMENT FAILURES

All treatment failures responded to restreatment with businesses attemedies of our methodoline with clearance of fever and parastreams within 72 h of commencing re-greatment and with no recommence of parastrems during additional 14-28 days of follow-up

## DISCUSSION

ncomplicated hyperparasitaemia is not uncommon in African children presenting with acute, symptomatic, P. falciparum malaria (Salako et al., 1990; Sowunmi et al., 1992, 1996, 2000 a). Prevalence rates in endemic and non endemic areas in Africa probably vary widely; in southwest Nigeria, the rate is approximately 10-12 % (Sowunmi, unpublished data). The 10 % prevalence recorded in the present study was similar to that previously reported from the same area in the early 1990's (Salako et al., 1990).

The risk factors associated with uncomplicated hyperparasitaemia at presentation are not frequently documented. In falciparum infections, younger age (< 3 years) has been associated with hyperparasimemia and increased risk of progression to cerebral malaria (Sowunmi et al., 2000 a). In the present study, age ≤ 5 years and oral or rectal temperature ≥ 39.5°C were independent risk factors associated with hyperparasitaemia at presentation. In falciparum infections in young children, the general trend is for parasitaemia to increase with time, and more specifically, to be accompanied by increases in body temperature. However, in severe infections there may be hypothermia. In practice many children with lower oral or rectal temperatures than our model found may be hyperparasitaemic. This would be so because many parents or guardians have ready access to over the counter remedies including antipyretics before presentation. This 'hlunting' of presenting oral or rectal temperature may mislead the attending health care provider and distract attention from the possible presence of hyperparasitaemia

The responses of apparently uncomplicated hyperparasitaemia to oral therapy are less frequently reported, probably because of the dangers associated with oral therapy in a condition that may rapidly progress to a fatal outcome, and probably also because of increasing resistance in *P. falciparum* to antimalarial drugs leading to reluctance to try oral therapy. Providing the parasites are fully sensitive to the oral drugs chosen, responses to drug therapy appears to be independent of parasite load. Thus in a comparative study, therapeutic responses of those with and without hyperparasitaemia were similar in children from an endemic

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area in West Africa (Sowunmi et al., 2000 a). In addition, in drug sensitive infections, the disposition of parasitaemia appears to follow a first order kinetics (Sowunmi et al., 2000 a, b). In our cohort of children, CQ was the least effective drug in children with hyperparsitaemia and clearly represented a significant decline in the sensitivity of P. falcipanim to this drug. Thus with prevailing degree of CQ resistance, this drug may not be ideal for the treatment of malaria irrespective of parasite load. The significantly higher proportions of children without hyperparasitaemia who subsequently developed it following treatment with CO or PS compared with the other treatment groups suggest slow onset of antimalarial action or reduced sensitivity to these drugs and a risk for development of post-treatment hyperparasitaemia.

The similar frequencies of pruritus (and other adverse drug reactions following treatment in those with and in those without hyperparasitaemia who were treated with the same drugs [data not shown]) suggest that hyperparasitaemia does not predispose to undue adverse drug reactions following treatment (Sowunmi et al., 2000 a).

Relatively low asexual parasitaemia and absence of fever are some of the risk factors associated with gametocyte carriage in falciparum infections (Price et al., 1999; Akim et al., 2000; von Seidlein et al., 2001). The lower gametocyte carriage and gametocytaemia following treatment of the children with hyperparasitaemia indicate that oral therapy of this condition is not associated with undue generation of gametocytes. However, it is not known whether gametocytes arising from patients who had hyperparasitaemia are more infectious to the mosquito than those arising from patients without hyperparasitaemia who were treated with the same drugs.

Hyperparasitaemia is a potentially life threatening condition, and with or without other features of severe malaria requires close clinical and parasitological monitoring. Its occurrence in children form this endemic area without other overt features of severe falciparum malaria suggests the presence of some degree of immunity, although these children are, in general, considered relatively non-immune compared with adults from the same endemic area, and are prone to multiple infections (Happi et al., 2003) Should oral CQ or PS continued to be used for a potentially life threatening situation in view of increasing resistance of P. falciparum to these drugs in Africa? We feel otherwise. A recent study suggests that AQ, a drug more effective than CQ in both CQ- sensitive and resistant- P. falciparum infections, rapidly clears hyperparasitaemia (Ndounga & Basco, 2003). In the small number of children treated with a combination of PS plus AQ in our study population, neither elearance nor parasite reduction ratio was

significantly faster or higher respectively than those of other resonance in view of the fact dust arterisions and its derivatives clear parasistents more rapidly than more of the correctly available antimalateds (then & White, 1995), these drugs combined with, for example, AQ may be trial for the management of uncomplicated hyperparationals in children from Africa. This suggestion is predicated on the fact that AQ is a relatively rafe drug (Ollians or al., 1996), and may be a suitable partner combination drug with the attendants derivatives, for example, attendants for use in Africa (Adjuik et al., 2002). Studies in about the elikacy of such combinations in uncomplicated and complicated hyperparationals are under way in our study area.

## **ACKNOWLEDGEMENTS**

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PERSONAL STREET

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# Plasmodium salciparum Malaria in Nigerian Children During High and Low Transmission Seasons: Gametocyte Carriage and Response to Oral Chloroquine

A A Adeder F A Febiatals B A Faiche T C Happi A O I Amoo G O Oboughts and A Sowunant Department of Phonocology & Therefore a was Institute for sie and Remark and Trability Chineses of thousand Mais , Vierrie

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## Summers

Plesmodiam felciperum malaria during high and him intimulation resum was challated in 1931 children treated with different antimedrial drop in a hyperendemie area of sombnestern Nikeria. Scient)-three (10.5%) of 693 and forly (11.8%) of 3.3% children were gamelocyle cautiers in the high transmission sensons (1/15) and for transmission seasons (1/15), respectively to a maillable regression model. In factors were found to be independent risk factors for the presence of Rametocytemia at combinent in the 1815: duration of illumental described in the combined in th than 10000/µ2 Similarly male gender, duration of illness > 4d and paravic density less than \$000/µ2 were found independent rick factors for presence of gameines tenia during I.T.S. The presenting Presidentia, paresite clearance times, intensity of gametocytemia and projunttion carrying gametocytes first treatment differ alguifficantly in the 333 (323%) of these children that were treated with chlorogeine in the two seasons. These findings may be important in our understanding of P. fulciperum franchistion suntenance, imposse to chieroquine therepy and contribution of chieroquine to game too te carriage as seasonal charges occur.

#### Introduction

of Phonodism Jakaparen malana often was scaled pattern Gamerico le geno ation, dermage and infocurity to mosquilos are charal to ancedul transmission of falipperum malaria ferron particulari) in endemic areas. Canier and Hiller demonstrated that the file at which sesual differentiation occur in Plannadium falciporum triphrogue stages depends on certain en vocamental General other studies have reported immu-Bolomes these impact of hour responde to mankets and chemotherapy 1 as important (ac loss savolved in the induction of Barocies resire

Although some studies have reported geatonal influence on varional superity, gametocyte carriage and imphotoite densites at the onset of dry or thatland 14-17 little is known about the effect of seasonal variations on processor carriage and response to chloroquine treatment in endemic area of southwest Nigeria Such information is crucial th our understanding of the parential contribution of executed changes to malaria transmission. Thus, in the present study, we coalusted the effect of low and high transmission wasons on gamerocyte cutture and response of children to chlorodine during ר לפון אף ממונים וחלמנותה וה h) ויסישל אוני southwest Nigeria.

## Acknowledgements

The undy received financial support from the UNDP World Bank WHO Special Programme for Research and Training in Tropical Diseases. AS was supported by a WHO TDR Career Development Award.

Correspondence: Dr. A. A. Adedeji. Department of Pharmacology, Obafemi Awolowo College of Health Sornes, Olabisi Osabanjo University, Sagamu, Nigeria. Louis <ahnoniadel syahoo.co.uk>.

#### Patients and Methods

Pas rate

The study took place between July 1996 and Describer 2002 in patients presenting at the University College Hospital in Ibaden, a hiperen. dereic area for malaria in south western Nigery Ethical elemence was provided by the level ethics committee During the period a series of antimalerial

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drug studies were conducted to evaluate the efficacy and safety of different treatment regimens spanning the two periods of high (April-October) and low (November-March) transmission seasons known in the area the details of the studies have been described before. Is 2021 Briefly, children with symlytoms compatible with scute falosparum malana who fulfilled the following column were enlisted to the study age 13 years or below, pure P Julciparium parasiemia greater than 2000 asexual forms mi blood negative urine tests for antimalanas drugs (Dill-Glarko and lignin tests), obsence of concomilant illness, no evidence of severe malaria and written informed consent given by parents or guardians. After encolment and start of inciment (day U), follow-up with clinical and parasitological evaluation was at days 1-7, and then on days 14, and when necessary, in days 21 and 28 Clinical evaluation consisted of a general elmical examination encluding measurement of weight, core temperature and physical examination

A vermient of porculternia and gametocy tenna

Thick and thin blood films prepared from a linger prick were Circums stamed and were examined by light interoscopy under an oil-immersion objective, at x1000 magnification, by two independent assessors. Parasitemia in thick films was estimated by counting assexual parasitem relance to 1000 kukocytes or 500 oscenal forms, whichever recurred first. From this figure, the parasite density was calculated assuming a leukocyte count of 6000, ml of blood. Gametocytes were also counted in thick blood blood. Gametocytes were also counted in thick blood blood against 1000 leukocytes assuming an average leukocyte count of 6000 ml of blood.

Evaluation of response to drug treatment In order to evaluate the response of children to chloroquine treatment during the HTS and LTS, 25 mg/kg body weight of the drug over three days (10 mg/kg on day 1, 10 mg/kg on day 2 and 5 mg/kg on day 3) was administered to children. Response to drug treatment was assessed using World Health Organization (WHO) criteria26 as follows: S = sensitive, clearance of parasitemia without recurrence, RI (mild resistance) paraxitemia disappears but reappears within 7-14 days; RII (moderate resistance) = decrease of parasitemia but no complete clearance from peripheral blood; RIII (severe resistance) = no pronounced decrease or increase in parasitemia at 48 h after freatment. In those with sensitive or RI response, parasite clearance time (PCT) was defined as the time elapsing from drug administration until there was no patent parasitemia for at least 72 h

Statistical analysis

Data were analysed using version 6 of the Epi-Info
software, and the statistical program SPSS

for Windows various 10.01.20 Proportions were or by Fither exact or by Mantel Haenszel 1655 Normally distributed, continuous data were compased by Student's 1-tests and analysis of variance (ANOVA). Data not conforming to a normal distribution were compared by the Mann-Whitisty U-test and the Kruskal-Walfis test (or by Wilcoxon took sum test) A multiple logistic regression model was used to test the establish between gametocytemia (Yes or No at presentation) and factors that were agnificant all univariate analysis male gender, presence of fever, duration of illness before presentation and assual parasitemia of presentation The values presented below are generally means and standard deviations (Sp) or standard error (5E) p-values of <0.05 were taken to indicate agrificant dillerences

## Results

Clinkul and paradiological features at envolvent The demographic parameters and other charocter isises of the children enrolled in the study are summarized in Table 1 Of 1031 children eurofled the studies, 693 and 338 children were recruited during the high aint low transmission seasons respectively lictured 1996-2003 Patent gameto. cylemia (reconcine ocean 27, range 6-1344 ml) was present in 73 (10 5%) of 693 and 40 (11.8%) of 338 children at corolment to both high and for 11 anomysion seasons, respectively These proportions were not significantly different (x2 = 0.27. p= 06) The lurante iscontica at carolment in these children were 36 748 (Geometric nwan range 209 150 000) and 27.96) (Geometric mean range 1116-565 333) in both high and low transmission ecasons respectively (p=0001)

the response of the assumbly perastement to drug treatments have been reported elsewhere. Factors associated with ganiciocytemes at encolonent during the high transmission seasons (1175) are presented to fable 2. Duration of illness >3 d. and asexual parastic densities has than 10000 at were related to the presence of gameton terms at encolonent. None of age, gooder or forer at presentation was independent risk factor for semesonic carriage (Table 2)

However, during low transmission seasons, gender illustration of illustration and assexual potabilic densities less than 5000 iil were the independent factors assertated with gamelocytems: at encountry (Table 3)

Clinical features and response to chievaguine
Of 335 claids that were treated with observed until
during the study, 165 were placed in the HTS and
165 in the 115. The clinical features as presentation
and furagiological parameters of these children

Journal of Tempiral Pallating

me summarized in Table 4. The clinical features were emilar although those enrolled in the LTS were appulicantly younger (1=0.03), had neuticantly lower Presenting temperature (1=0.03) and lower geometric mean parasite density (p=0.001). Though the lever clearance times were similar in the HIS and LTS, the parasite clearance times were profesionally different (p=0.003). The therapeutic responses (Table 4) were similar in the two seasons. Analysis of the treatment failures showed that of the 71 that had resistance response in the H1S 60, 6 and 5 children had RI. RII, and RIII respectively, similarly in the LTS, 52 had RI. 10 had RII and 11 had Rill responses. Rill response occur more in the LIS than IITS but the difference was not significant (n=0.1)

Summary of demographic and other characteristics of the 1031 children enrolled in the study

Variables.	Value [mean 1 sw (range)]
Age (jean)	5.6±2.9 (0.5.12.0)
N F Weight (kg)	493 555
Presentat body	16 4 ± 4.8 (5.0-27) 38 6± 1,2 (33 7-12.0)
(C) sufference	
Duration of Most (d)	32±1.7(1-21)
desmit (bet lijt	
בהשבלהל שבשון	34063
Range	2090-2 341 000
No >230 000	100

Gametocy tente during treatment with chlorogume and follow up

Gametocytemia was found in 27 out of 168 and 28 out of 165 during the HTS and LTS, respectively, at enrolment. There was no difference in the geometric mean gametocyte densities (24, range 1.21344, µ), vo. 26, range 6.150 µl, p=0.3). Gametocytemia increased agnificantly in densities by day 7 and 14 in children treated in the HTS when compared to the gametocyte densities obtained on these days in those treated during LTS following chloroquine treatment (Table 5). However, the cumulative gametocyte carriage by day 7 and 14 were significantly higher in the children treated with chloroquine during the LTS (p=0.015 and p=0.03) than those treated during the LTS (p=0.015 and p=0.03) than those treated during the LTS (p=0.015).

## Discusalon

The primary purpose of the present study was to cratuate the effect of seasons in the kiw and high transmission period characteristic of malaria infection to high an children, on gametocyte carriage following transment (fametocyte carriage rates may very undely and depend on several factors in this study, observed prevalence of malaria infection was againfrantly higher in the high transmission season (67.2%) than in the LIS (32.8%) but the famet Such seasonal effect had been observed eather in the same area. Prompt visit to chine and early treatment of the infection during HTS compared to

Prof. forces for D. falcing the liter temps of enculment theing the high trumes taken treatment

	Total No. of	No of children	Crude DR (ya+i Cl)	מלכו ק	Addition DR	p value
Age (y)			· ·	- 5	_	
>\$ <5	3.46 3.47	35 38	109(04-15)	08	<b>~</b>	1.5
Contr			1	-	-	
Male	320 373	36	12107-20)	04		
≥10(m) ≥10(m)	36l 132	51 21	1 59 (1 04-1 35)	0 03	165 (111-129)	0.03
Februar Februar Alabata	417 258	46 37	0.99 (0.5-1.68)	09		
Parating of ellipside >3d <3d	156	25 45	0 51 (0 11-0 9)	0.01	072 (072-10)	0.03

OR odds rates
From, Applant temperature >37 y'C

Cl confidence in tast

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Rick furtoes for P. falciparum gumetoe) temto of enrolment during the four transmission seconds

	Total No. of children	No of children	Crude OR (95% CI)	p take	Adjusted OR (95% CI)	A talue
AÆ (y) >5 <5	159	[] 27	0.5 (0 23-1 05)	07	0=	-
Gender Malo Female	159	28 12	0.34 (n.15-0.72)	0 003	03 (01-04)	0.002
Parasitemus (-15L) < 5000 > 5000	23 315	8 32	0.21 (0.08-0.63)	0 001	022 (0 08-064)	0.005
Fener* Februle Afebrule	98 240	<b>Q</b> 3l	0 68 102-1 541	0.1		1
Denstion of illness <4d >4d	299	30 10	34 (12-73)	0.01	) 1 (12-73)	0014

UR odds ratio.

\* Fever, axillar) (conpensione >375°C.

Cl coplidence interval

we response of infected individuals during LTS may be contributory. People in this setting appear to suspect malaria infection more in the ratoly season once symptomatic or pyrexic. It is noteworthy than ascault parasitemia at encolment was markedly higher in the HTS than in the LTS. The reason(s) for this is not clear from the present study. A similar observation of low parasite rate during the low transmission period had been earlier reported for the area. It may be that the features of assurably with season of respond to changes in the covironment in such a way to favour its parasitism and propagation.

A critical evaluation of the neighbors for carriage of the sexual forms may provide some clues in respect of the above observation. In the present study, two and three independent factors were accorated with gametocyte carriage in the HTS and LTS and LTS (carried). Why would make gooder be a nik factor for gametocyte carriage in LTS and not in the HTS remains unclear featosterone and corticosto the HTS remains unclear featosterone and corticostero the HTS remains unclear featosterone and corticosterone and corti

The duration of illness longer than 4 days and tention duration of illness and two fold parameter duration of illness and two fold parameter duration of illness and thou fold parameter duration of illness and thou fold parameter duration of symptoms or possibly law skyree of presentation of symptoms or possibly law skyree of presentation of symptoms or possibly law skyree of presentation of symptoms or possibly law skyree of

the LTS Smalley, et ut 12 had observed that longer established P fulciparum infections are tikely to produce Smetreyses. It is tikely therefore that longer duratino of Illness before presentation in the LTS may allow sufficient time for the progression of committed assessed paragraphs to gametocytes

The elect of antimalismal drugs in sexual differentiation to P. fulciporum a still not futly understood. Centain autimaianal drugs, for example chloroquine and pyrimethamine - sulphadoxine, have been reposted contribute to gametocytogesicsus in titro or gaucieote generation or release in the 121224 It is remarkable to note that the children in the cobons treated with chloroquine in this study iluting L'IS were significantly younger, had lower presenting temperature and low paramite dentity compared to those treated with elitoroquioe during the HTS Although forer dearrose times were umilier, the parasite clearance times were agnificantly different in the two transmission seasons. The children treated Juring the LTS had delated clearance of their account forms sufficient differing parasite behaviour and dynamics during transmission ecitons. Thus use of chincoquine in children to the study area in the HTS appeared more favourable and important to reduce circulating parasite load Despite umilar therapeutic outcome and resistance rules in the twil transnumon periods, early remiance of Itil and Rill occur in more children duttes the LTS

Surprisingly, the post treatment gametoeytemin and gametoeyte carriage differ algorithmently in the two generals compared to pretreatment gametoeytemia

Journal of Tropical Pediatrics

TABLE 4

Comparison of clinical parameters of 333 children with

The fall thereon and their

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muse during high and law transmission scawing

	IITS	LTS	D values
Rember of patern's	168	165	
Age (years) Moon±sp Range	5+±2.8 0.7-13.0	49±3.0 06-12.0	0 03
Wegat (kg); Meso ± 30 Ruige	16.04 5.6 6 5 -33.0	15   £ 3.6 6 5.31 <b>0</b>	0.16
Duration of symp Meso±kn Range	10-140	3.1±15 1.0-8.0	0 14
Brily temperature Mean ± so Range	38.5 ± 1.2 36.1 = 12.0	3% 1 ± 1 1 36 5-40 6	001
CMPD  [asexual)	367-5	27061	0 (10)
Range	2090-1560000	2116- 365333	
FC((J) May = 20 Real age	1.5±0\$	1.5±08 1-5	0.99
PCT (d) Mean ± 80 Range	27+04	10+09	a not
No Cwed No with RI No with RII	97 60 6	92 52 10	0.7
No with RIII	5	- 11	01

GMPD, geometric mean parasite density; PRR, parasite reduction ratio; FCT, fever clearance time; PCT, parasite clearance time; RI = parasitemia disappears but reappears within 7 to 14 days; RII = decrease of parasitemia but no complete clearance from peripheral blood; RIII = no pronounced decrease or increase in parasitemia at 48 h after treatment; d= day.

HTS, post treatment gametocyte intensity was high but significantly fewer children were carriers compared with low gametocyte intensity and high carriage rate in the LTS. This antimalarial drug chemotherapy may impose stress on the parasite response to which could result in increased gametocyte production. The higher sexual parasite density in the HTS may in addition support increased parasite burden on mosquito and probability of mosquito infection. Thus creating heavy burden of malaria and high transmission in the area.

TABLE 5

Comparison of gametocy territoristy at presentation and following transment in 323 children with ucute faktipurum medatus thering high and him transmission sections

1175	LTS	p 12 lucs
163	165	
10-27	26 (a - 28)	029
13-134	6-150	
		0.04
		003
	168  24  16 = 271  12 - 134  18 = 29)  12 - 1476  29  18 = 20)	168 165  24 26  16 28)  13-134 6-150  41 27  16 29) (n = 48)  12-1476 6-264

GMPD, pour this stop turning density

The toctease resistance to chloroquine, which still remained most common readily available. chesp and first line animalors) drug in the study area may be contributory to differences in the post trailment famiciocyte faminion or release and carriage to children Patients with slow response to licalment are likely to carry gametocytes than those that responded tapidly 16 Furthermore, high carriage rate in the LTS post treatment with chloroguine may also suggest a compensatory mechanism to ensure furtherance of transpiresion at almost same potential as in HTS relative to available transmission sids. This may lime relevance in our understanding of how the parasite ensures transmission despite charothers py of the infection. More studies would be pended to cluddate parasite response and behaviour to other anumalanal drugs during low and high transmission sessons

Orciall a strategy that avoids the identified risk factors for gametoette carriage in the two transmission may reduce gametoette prevalence and contibule to a reduce gametoette prevalence.

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## BRIEF REPORT

A. A. Adedeji . B. A. Faleye . A. O. J. Amoo . A. Sonunmi

# Response to chloroquine treatment in children with or without gametocytes during uncomplicated Plasmodlum falciparum malaria

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Characterapy still remains the most widely use dapproach to combat malera infection. However, chloroquine has ocrasingly been failing to clear parasiles in patients in endonic evens, and failure raies as high as 40 and 80% have ocen reported for West African and East African Indients, especially 11, 21. This development has necessitated the development of an alternative antimalianal drug therapy. Wale awaiting the emergence of an alternative to chlorogume, bowerer, efforts need to be greated towards minincrees the morbidity and mortality that may result from the continued use of chloroquine in codemic areas. One watery that may prove useful for extending the period diring which this anhandland agent remains effective is to consider parasite host-related characteristics, such as gacarriage, and the clinical response of patients to deroquine Gametocyte generation, bust carriage, and efectivity of mosquitoes are crucial for the successful convission of malaria infection and may contribute to the methance and spread of chloroquine resistance in cadenic 12 1. 3]. Thus, the present study was conducted to trailme the role played by Bernetocytes at the time of with chloroduine and during the follow-up period on clinical purcome and resistance patterns in children with acute nocomplicated (alciperum malana

This study is part of an extensive, long-term study on the change of minimizated drupt carried out in Ibular, Nigeria. Com July 1996 to March 2003 [4 6] A total of 142 children with scute uncomplicated P. Sole porum malaria were enrolled consecutively unto two groups; the first group included II children who had gamerocytes at enioliment endor during follow up, and the second group included 71 palients who did not have gametocytes either at envolument or at any point during treatment. All Panents war treated with chloroquine (25 m8 kg of body weight given over a 3 day period. It marks on days 0 and 1 and 5 mg/kg onday 2). For ensollment mto the study, each child had so meet the following enteria: (a) age <13 years; (b) Justiosis of P. Joloporum parusitentia with >1.000 asexual forms/HI; (c) ncBallyc results of wine tests (Dill-Glezko and lightin) for animalistial drugs; and (i) no concomitant illness or evidence of severe malaria. Writish infinitions content was obtained from the parents or guildians of each child proc to envolument. The study was approved by the local ethics committee.

After receiving a demiled climest and parasitological essented and dung administration at presentation, each child underwent clinical and parasitalogical exampation daily on days 1-7 and on day 14. At each ascenment, fingaprick blood samples were collected and used to make thick and this smeats for estimating the level of parasge. mia Gamelocyteinia was quantified on days 0. J. S. 7 and 14 ming the shick blood ancare prepared on shose days [1] Classifications of response to drug treatment were determined according to the entire outlined by the World Health One Zation [7]. Parasite eleganore was defined as the amount of time between the wart of drug administration and the absence of detectable parallemia, which was maintained for at least 48 h. Fever eleasurer was defined as the amount of time from the Mart of drug administration until the core temperature (el) to 2)7 4°C and remained to for 18 h. Data were analyzed whith version 6 of the Epi-In To software (Consers for Disease Control and Prevention. Allants, GA. USA), and differences giving p values of d) 05 were taken as significant

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The two Boups of children had similar clinical charestenution paresite cleanance times (2.9±1.1 vs. 3.0±0.8; -0.9) and lever clearance limes (1.6=0.9 vs. 1.4=0.7; 6). Of the 71 children with gametocytemia during Alamquise treatment. 43 had gametocytes at presentation These children were younger and had lower levels of parexitmia at presentation compared with 43 children from the group without gamelocytes, who were selected for compansed based on consecutive enrollment (lable 1). The therapeutic response to chloroquine treatment also differed in these two groups. Children who had gameto. one at presentation had significantly shorter times to parasite and sever clearance compared with those who did not have game to cytes at presentation or during treatment (2.7±0.9 vs. 3.1±0.9; p=0.03 and 1.2±0.5 vs. 1.6±0.9; ~0.01, respectively).

An interesting finding of the study was the significant difference in fever and parasite electrone times following treatment with chloroquine in children with gametocytes tempared to those without gametocytes. The reason for this difference could not be elucidated in the present study; however, it has previously been reported that children who present with gametocytes are probably carrying trophomies that are likely to be committed to gametocyte production [8]. Unfortunately, little is known about the behavior of trophozogies committed to gametocyte production

tion in the presence of antimalarial daugs. We are also imable to explain why the esexual forms of the parasites in our collort of children with gamelocytes at presentation appeared less vindent and were cleared from peripheral blood earlier than in the group of paperts without gametocytes. While it has been reported previously that chloroquine treatment may impose considerable stress and greatly reduce the number of parasites [9], this requires further investigation

The results of this snully indicate the presence of absence of gametocytes at presentation modulates the theraperatic response of children to chlorogume significantly. Since the children without gametocytes at presentation responded to chloroquine ocalment with significantly higher cure rates, it seems there was comparatively significant resistance to the drug in those children will pametucytes at encollment. It is clear that chloroquine thempy may be more beneficial in children with mulana who lack gametocytes at the time of treatment initiation to children with gametocytes, a combination of gurrelocidal drugs plus chloroquine or chloroguine in combination within antimalenal agent active against all parastic sages, like artemether, may be advantageous. Once a child presents with gametocytes in the peripheral blood, aherolive annualarial agents superor to chloraguing may be administered. However, more studies are needed to evaluate the effect that the presence or absence

Fible | Comparison of clinical parameters and response to chieffed in 86 chieffed with sente feloporum malariz who rither had or did too time paraetorytes at presentation

Permis	Wes gone tools	Wilton: parties remin	Prelim
Number of papents	43	43	
ABe (r)	/ Access	6.942.8	0.04
Moniso	\$6470	06-130	
Raige	0.7-120		
Meight (LE)	16.516.4	18.846.2	0.09
MonouSD	7.0-30 0	8.5-28.0	
Renge			
Survey of symptom	3,5x23	3.2±1.4	0.46
MartSD	1.0-140	1,0-7,0	
Range	. 2.351		
lody barparen (°C)	38441.2	385412	0.69
Maniso	10.1-10.6	163-40.6	
Horse	70.3 10.0		
Power (per ml)	13,588	21.716	D 05
CMPD (OCM)	209-262,426	681-236,866	
Range			
(1 (902)	1.2+0.5	16109	0.01
MesnaSD	1-1	1-4	
Response		7.1.00	0.03
Cr (days)	2740.9	3 1409	0.43
Marksp	1-6	14	
Range		11 (74 %)	100.0
Day 14 frapemies	20 (46 5)	33 (76.7)	1000
Cord (16)	20	7	
R.F	3	3	
रिहा स्रोत	0		

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of gammocytes has on the clinical response of infected children to available antimatival drugs. These fundo studies should be conducted in an endemic area and could contribute to efforts towards controlling this infection.

Acknowledgements We are grateful to our clinic nurse, Mrs. M. Amao for her assistance with the study. AS is the recipient of a WHO/ TDR Career Development Award.

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# Open randomized study of pyrimethamine-sulphadoxine vs. pyrimethamine-sulphadoxine plus probenecid for the treatment of uncomplicated *Plasmodium falciparum* malaria in children

A Sowiemi, F. A. Fehintale, A. A. Adedel, G. O. Ghotosho, C. O. Falede, E. Tambo, S. A. Fereye, T. C. Happiend A. H. J. Oduole

Deputinger of Pharmacology & The expension and Insulate for Medical Research and Training University of Handam Nature

## Summary

OACECROUND Increasing drug resistance in Planmodrum Jaloparum has reconstructed reserved season for cheap, effective alternatives to commendy available antimalateds, eliverguine and pyrimethamice-tulphadeenes, for the treatment of malaria in Africa. Probaggid, an inhibitor of organic anion issumpurees and multiparameter assembled Protein, can chomocomiuse P. Jaloparum in pyrimethamice and sulphaduseum wison, but the clinical eigenbrance is unclear. We numbed the safety, treatment efficacy, and effects on gametocite carrage of adding probanecid to pyrimethamine sulphadustice.

METHODS We evaluated 151 children aged 12 years or younger who had uncomplicated & falciporum malaria. Patients were randomly assigned pyrimethemine sulphadoxine [25 mg/kg of the sulphadoxine complication) or pyrimethamine sulphadoxine as above plus probased 20-25 mg/kg of bodyweight in two divided dones daily for 3 days. The primary endpoints were parasitulugical core rates fin days 141 and 28.

RELUCTS Both regiment were well intersted; no child was withdrawn because of days insplement. Ferest (1.9 ± 1.1 in 2.4 ± 1.2 days, P = 0.02) and paramite clearants (2.3 ± 0.9 in 2.7 ± 1.1 days, P = 0.04) were significantly shorten and the paramitological cute rate on that 14 (96.2% in 83.5%, P = 0.02) but not day 28 (79.4% in 72.6%, P = 0.4), was eignificantly higher in children present with Pyranchamine with phadoxine Camenocy is curings was similar with both treatment regiment.

CONCLUSIONS The combination of professional and profession, and profession, at a relatively moderate dose, improved treatment efficient but had no effect on gamenetic camage. The pyrimeth amore sulphadusine professional combination metric further evaluation as a posential treatment for use in Nigeria.

keywords prohesered, pymosti, mios sulphadoxide, milans, childre, North

## Introduction

Drug resistance in Plasmodium falciparum to chloroquine is a major public health problem in much of sub-Saharan Africa, accounting for recent increases in malaria-related morbidity and mortality (Trape et al. 1998; Trape 2001), gametocyte carriage, and enhanced transmission of drug-resistant malaria in Africa (Robert et al. 1996, 2000; Sutherland et al. 2002; Drakeley et al. 2004; Happi et al. 2003; Sowrammi & Fatere 2003a,b).

As an alternative to chloroquine, pyrimethaminesulphadexine is widely used in sub-Saharan Africa, but especiated with point mutations in distributed and 2001). It is associated with point mutations in distributed and reductant and distributed and instruction of the parameter (Planet et al. 1997) Wang et al. 1997; Duant et al. 1999), and conten survival and propugation radiantages on the parameter survival and propugation radiantages on the parameter in the population (Semigram) & Faceye 1003h),

These developments have ted to the control work for effective afternatives on both chlorophine and principle of enthularity with mides of action different from shore of chlorophine and process of action different from shore of chlorophine and process of action different from shore of chlorophine and process of action different from shore of chlorophine and process of action different from shore of chlorophine and process of action different from these chlorophines.

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## A Someon of al Produced plus pyrindentember of the uncomplaint melet

aims of slowing the progression of resistance to these drugs and prolonging their lifespan (von Seidlam et al. 2000; Basen et al. 2002; Sowammi 2002; Drakeley et al. 2004; Gasaura et al. 2003), It has also led to the use of chloroquine in combination with resistance modulators, e.g. chlorophenicamine (Sowunmi et al. 1997).

Experience with chienquine plus chlorphenizaine for actions chlaroquine resistant infactions course from Kanbwest Nigeria where the prevalence of chloroquine registant infection is 35-40% (Sowunga et al. 1998s bes Sommand 2003). A recent study has shown that probable ecid, an inhibitor of organic amon transporters and multiperistance associated projects can chanosensitize P. falcomm to p) meethamine, suiphedoone or chintechains in thirm (Neula et al. 2003), but the disues) significance is uncless. To date no study has examined, clinically, the unclularity of probestical in combination with pytimethemine-sulphedusine for the tresiment of malana in Almen children. Such a study is essential for a number of responsit is possible that the combination, given in appropriate doses, may improve treatment efficier. Maland transmission may be reduced if probensed modulates the same noting terreleasing effect of partineshamine sulphadazine, it can help elter the management of paech. atric cases of malana

Here we report the safety, antimalanal treatment electy, and elices on gamerocyte carnage of pyrimethatines sulphadoxios alone in children aged 12 years or younger with acute, symptomatic, uncomplicated P folioporum malina.

#### Materials and methods

#### Study area

The study was carried out in Ibadan, southwest Nigeria from July to September 2003. In this area of hyperendemic malaria, transmission occurs all year round but is more intense during the rainy season, April to October. In the area, it is difficult, clinically to distinguish recrudescence from re-infection 14 days after commencing antimalarial treatment, and usually animalarial efficacy tests have been conducted for 14 rather than the customary 28 days (Ekanem et al. 1987; Salako et al. 1990). Chloroquine resistance was reported in the area in the 1980s (Ekanem 1985; Salako & Arkemunnu 1987) and pyrimethaminesulphadoxine resistance in the 1990s (Sowunmi et al. 1993, 1998a; Falade et al. 1997). Presently, chloroquine resistance reaches approximately 35-40% (Sowunmi 2003) and, pyrimethamine-sulphadoxine resistance approximately 25% in the under 5-year olds (A. Sowunmi & B. A. Fateye, unpublished data).

Potinita, teatment and follow-up

l'accors were eligible to toin the anidy il they were ased 12 years or younger, had syroproms companible with acute uncomplicated malaria, with pure P. falopurum partistacraia >2000 are rual formatily a temporature >37.4 °C ne rount provid anterchant shows ul other concumitant iliness or dintory of animalerial use at the 2 weeks preceding presentation, negative utire tests for actimalarial drags (Dill-Glaske and Little at and written informed consent from parents or mathians. Patients with screek malana (WHO 2000), severe malnutnonni per out underlying duction from the cardisc, or benealed, and known allerry to muly dress were excluded frum the mody. Educal clearance fire the stisly was provided by the Educa-Committee of Oya State Ministry of Health, Ibadan, Nigetta. The disease history was recorded by asking patients or their parents when the present symptomatic period had reacted, and was fullowed by a full physical examination.

Entalled patients were randomly assigned pyrimethemine sulphadosine 25 metes of hinterwestly of the שולף הביות מומהף והמול זו נאפיבובגאישות (מבצים) ווני pynnoethamine-sulphandunine as above plus probenered Barch 2011, Industria Fernactica Norm Argentina. Milano, Italy); 20-25 mg/kg of bodyweight in two divided doses daily for 3 days (days 0, 1 and 2) The readomization wa, computer generated and treatment code were scaled in individual envelopes. Once envalled the study daugs were administered by a physician. Patient evaluation and follow-up siter drug edenials ration was performed by enosites physician blinded to the drug treatment All drugs were pren only, except the toward daily dones of probehedd, all drogs were administered to the clinic, and all persons waited for at least & hafter drug administration to enture that the drift was net a posited if it was, the pourist was excluded from the study. If recessary, patients were presided with empyretice (paracramal tables, 10-15 marks every 8 h for 24-46 h). The mudy nume obtained thick and thin blood films from each shild as soon as they came to the chair. The thates were carefully labelled mit the promis' codes and were air direct hefore being stations.

Follow up with clinical and paraditulogical crobustion was performed every day for 7 days (days 1-7) and then us days 14, 21 and 28. Thack and this blood films prepared from a finger Prick were Greenes stained and were examined by light encourage minder an oil emmersion less, at 1000x angentication, by two undertendent assessors who were blinked to the treatment of the patient. Paraditaemia formula in the patient. Paraditaemia promotes account or securit forms teletime to 1000 learnings. It should be compared to the security of the patient of the patient of the patient of the patients of the patient

# A. Sowunmi et al. Probenecid plus pyrimethamine-sulphadoxine for uncomplicated malaria

first. From this figure, the parasite density was calculated transpare a leasuryte count of 6000/yl of blood.

Rintine harmatilogical (basematicitit) and biochemical tests (consentrations of alamine aminotratisferase, apparate aminotransferase, hilifilitis, and organizes) were performed in 54 randomly selected children, pic-resument and on day 14 after treatment. Blund was aported on filter papers on days 0, 3, 7, 14, 21 and 28, in all patients, and at the time of treatment failures for parastic genotyping. Parastic genotyping will be reported chembers.

Response to drug textinent was assessed using WHO (1973) eniccia, as follows: S. sensitive, deacance of parathemia without recurrence RI (mild tensure). parantaemia disappean but reappeon within 7-14 dats: RI3 (musicrate resistance), decrease of parautaemia but no complete charance from peripheral blood; RIII (verere resigance), an promounced decrease or increase in 1986. utaemia at 48 h after exertoreist. In those with sentitive tie RI response, parasite clearance time was defined as the time o sands has authorisinable and above of detectable parantaemia for at least 48 b. Fever elegrance time was refined as the trove from deue administration until the core semperature fell to or below 37.4 °C and remained so lot 48 b. Cure rates were defined as the perconages of Patients whose asexual parasitsenia cleared from jestiphi eral blund and who were live of patent ascault parasitae on days 14, 21 and 28 of follow-up.

#### Reseatment of drug treatment failures

In periods who failed treatment (within 14 days), the codes were bruken, and if the prizers was instituted with primerhamine sulphadoxine, the or he was treated with primerhamine sulphadoxine probability and followed up for another 14-28 days. Those is link social treatment with Primerhamine sulphadoxine. Those is link social treatment with Primerhamine sulphadoxine. Those is link social days and followed up for accuber 14-28 days.

Patential over the treated when the they became shopker cather they became shopker cather for they became shopker cather for they became shopker with probability 14-21 days after minus emplificant. Patentia with probability 14-21 days after minus emplificant. Patentia with probability deternors for they became 5 days and other marked with artemether 19 6 pay to over 5 days and other marked of treatments follows.

## Sendy size and statistical analysis

Sample size was calculated so that the study would be able to detect a difference of 22% in the parasitological failure rate between pyrimethamine-sulphadoxine-probenecid and pyrimethamine-sulphadoxine groups, with 95%

power at a 5% significant level (it was assumed that 75% of these given pyrispethamine sulphadurine, based on the current cure rate in the under 5-year olds, and 97% of those given pyrunethamuse-sulphadoxine-prubenecid would be cured on first treatment At least 71 children were accord to each treatment orm. Data were analysed using version 6 of the Ept-Info whomes (Anonymous 1994) Variables considered in the analysis were related to the dentities of P. folaps non generocytes and trophozoites. Proportions were compared by calculating the squited value with Yater' cortection or by fisher exact or by Mantel-Harnsach tests. Normally distributed, continuous data were compared by Studeni's steets and availant of VERSON (ANOVA) Data not confirming to a normal distribution were compared by the Mano-Whitney U tests and the Krushal Walles term for by Wilcoxum ranked man 101). All leves of uprubiance, except where appealingly indicated, were two unled. It values of <0.05 were interest significant differences. Data were extend sorully using the paper to codes and were traly analysed at the cod of the study.

#### Results

#### Patiente characteristics

One buildred and filty-three children were enrolled,

79 were treated with pyrinethamine-pulphadoxine,
probancial and 74 with pyrinethamine-pulphadoxine,
Two children, one from each of the treatment arms,
were lost to follow-up after day 7 because of potential
te-location. These children were excluded from date
an attaly figure I shows the unit profile. Overall results
are for 151 children. The descriptions and clinical
characteristics of patients at carolineas are thomass
Table 1. These characteristics were similar in the two
treatment orms, but the detainer of littless at presentation
was arguificantly longer to those treated with pyrimethaccine-tulphaduator-probancial (P = 0.04).

## Fever and parasite clearance, and gametocyte carriage

One hundred and seven children were febrile at enrollment, 57 in the pyrimethamine-sulphadoxine-probenecid and 50 in the pyrimethamine-sulphadoxine group. By day 2, fever cleared in 42 and 26 children, respectively. There was a significant difference in the proportion of patients whose fever cleared by day 2 [ $\chi^2 = 4.5$ , P = 0.03, odd's ratio (OR) = 0.47, 95% confidence interval (CI) = 0.23–0.96)]. Overall, fever clearance was significantly quicker in those treated with pyrimethamine-sulphadoxine-probenecid (1.9 \* 1.1 \*\*\*5. 2.4 \* 1.2 days, P = 0.02) (Table 2).

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## A. Swunge of oil. Probenecid plus pyrimethamine-sulphadoxine for uncomplicated malaria

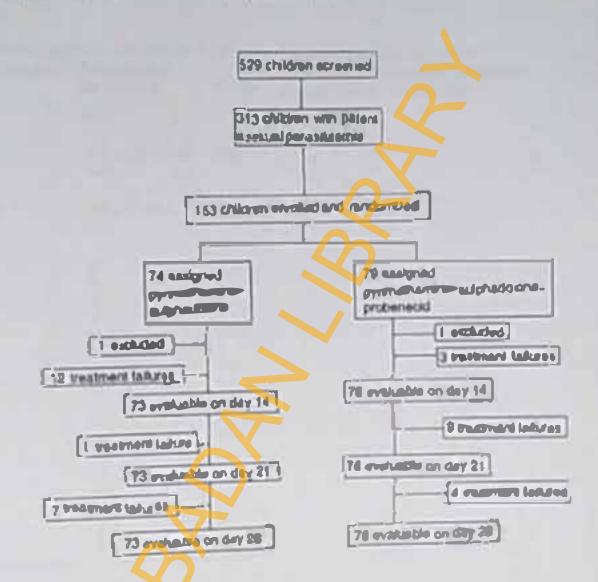


Figure 1 Trial profit

table 1 Demotrachic and climical characters of passes at

	Primarihaman galphadasan	miphadouet
No. of patrons	73 41/37	73 16/31
Age (plan) Meso a SD Respe	6.3 × 2.9 1.5-12 29	1.5 . 2.9 0.3-11.1 25
Mone SD Ramp	17.3 - 6-1 2-15	172 - 5 A 5-30
Menn of discer (d. Menn o SD Ronge	3.5 1.7	10 . 13
Mean + 10 Lamp	38.1 x 1.8 31 x 40.1	16 4 a 1.2 36 1 40 1
Commit and Pall	44 772 2010 1 331 000	17 745 mm 1 151 000
Money (%) Money  Money  () (2) (%)	31.6 + 5.5 18-45	33.1 + 3.4 12.46

Compared with promethanine sulphidouine, pyrimeth. anire al traderice protection abstentially extended the clearance of Cornectornia, By clay 2.53 and 37 children in the pyrus burine sulphadiarine Probencie and pyrisethans might down tremme eme, respectively, had their parenterns cleared. The differtece in this properties was egifcial (p. . 3.91, P-0.04, OR - 2.06, 95% CI - 1.01-422]. Overall bring general Mat selegable drigger to spore trained with gyrineshamics outphate sine probenaid [23 + 0.9 or 27 + 1.1 days ? = 0.04) (Table 1). The cure race on day 14 (96.2% m. 23.5%, g1 = 3.3, P = 0.02, OR = 4.92, 95% CI = 1.24 -28 0) but mot day 28 (77 4% m. 72.6%, 2 - 0.6, F - 04, OR = 1.46, 95% (1 = 0.64-1.15), was equificantly better in children then in short crossed with great hand an alphadorine Perspectual to perly treatment telliment and that to age, one child and row children from the 29 and 49 & and 25-year olds, respectively, treated with pyrimethericat estiphadenine probuncid fuled presences by day 14 17 = 1.0. by Fider erest true, OR = 0.34, 95% Cl = 0.01-16.5]. Seedler 17, loss and cips dallers (cm) क्रि 15 बार्च 49 र र बार्च 25 एका क्षेत्र, त्यक्वाचारांत्र, प्रत्याच with pyramethan over sulphath sine faire forestern by

## A. Sowunmi et al. Probenecid plus pyrimethamine-sulphadoxine for uncomplicated malaria

	ty more to a sure to a photo control to the sure of th	Cyrinethianace-	R-value
Nice all Patricia	74	73	
ליים לצום מיד מווא ולנ	yal		
Mean a SD	1.9 a 1.1 ln = 671	2.4 a 3.3 (m = 60)	0.02
Rappe	1-5	1-7	
Parame clearance time	(days)		
Nas . SD	2.3 + 0.9 (4 = 76)	2.7 a 1.1 (n = 71)	Q.D4
Range	1-5	1-6	
Day 14 Improved			
No cord	73	61	
Na III	3	10	
No. Ril	2		
No. RIII	9	ı	
Cur nte (%)	96.2	413	001
Day 25 responses			
No. currd	66	60	
No Ri (Combare)	10	11	
No RII	2	1	
Na NI	0	1	
Care tale [%]	846	\$2.2	01
DIL TI WHOSE			
No. cared	62	1)	
dia RI (complante)	14	18	
No IUI	2		
Na RID	0	1	44
Core PARE (76)	79.4	71.6	0.4

Table 2 Therapeutic propulates to primerhamine sulfit estimate printenerial per doubles

der 14 (P = 1.0, by Fisher exact tent, OH = 0.98. 95% O = 0.19.4.18)

Cancel only the captures in those who did not have the strength of a transfer of the strength of the strength

# Response to pyrimethamine-sulphadoxine-probenecid of children with pyrimethamine-sulphadoxine-treatment failures

Some of 12 chaires who had respectance or no distance of purviousness within 14 days of individual treatment with primarile managements of the primarile management of the distance of the dis

response to promethance sulphalinent during until
treatment had an iti response (allowing re-treatment with
primethanke sulphaline probanció. The cure rates
on days 14 and 28 were 36% and 72%, responsely.
None of the three children who failed treatment with
pyrimphanism sulphalisms probanció en er before
tay 14 (Table 2) and who were subsequently re-treated
with amodiquese failed treatment during a 28 day
follow op priod in these children fever and parantament
cleared within 2-1 days of initiation of amodiagume
thereby.

## Advance create

Pyrimether that a contract of the state on child was withdrawn because of done intulerance frequency frequency frequency reported by their present with the first a contract of the state o

O PER BASIN PARTY LIN

## A Sombara et al. Probanacid plus profeschamica autohadazina for uncomplicated metarle

Table 1 Clinical and parasemingical parameters of the seven children with Plannations (alciperum maleria who had reappearance us no clearance of parasities must hibrary initial recomment with pyramethemine sulphadnune and were unfrequently treated with pyramethemine telephadnune probenical.

	Paramethamine-	Pyrimethamine- sulphadoxine-probenecid	P-value
Nia ul Pătienis	7	1	
Age (FEATS)			
Mean = SU	6.8 - 26		
Honge	13-115		
Weight (LE)			
Mesa + 50	199 - 34	10.1 + 1 4	0.5
Reage	13-26	13-27.5	
Temperature (C)			
Meine SD	10.4 a 1.4	17.8 0 1 4	0.1
PLINE	J7 0-49.3	16.0-19.1	
Paraute cours (/ki)			
CACAMORE MAND	47 835	6116	0.01
	2020-111 500	714-37 621	
Mange Even desperant does			
Mess - SD	1.1 . 1.3	1,3 = 0.4	0,35
	14	1-3	
<b>Lags</b>			
Paraute clearance din	16.10	2.8 . 0 9	0.26
Mesa · 5D	2-5	3-4	
Reagt	5-3		
Duy 14 comono			0.001
Mor creen	0		
No RI		q	
No Ril		0	
Na RIU	0	16	
Con tast 12	0		

Table 4 Adverse drug reactions reported during the eroly

	Pyrum hamme - miphidis use-paritis accid	Pylandonia
No in dillips appearant	78	73
Vo marchine	1	1
Andones Paris	0	41
Drow mell I	0	7
Forth pos (2).0 (s)	30 (0 - 04)	32 (a = 60)

Significant statistical difference, P = 0.05.

## Handendopied and birthe mical parasiters

Employed to be manufactory to be before 25% at coordinate in the and constitution in pyr standardines extractions.

Produced and pyrim other manufactory than the group and the day 7 in from and fore children.

The manufactory and an analogoidal, birth stress of after treatment in the first and after treatment in the first and after treatment in the product.

All religions. The manufactory appears was present in 10 and 12.

children in pyrimethamine-sulphadoxine-probenecid and pyrimethamine-sulphadoxine groups, respectively, at enrollment, but was not seen on day 14 in any child.

## Discussion

Given the increasing prevalence and intensities of resistant infections to pyrimethamine-sulphadoxine in much of sub-Saharan Africa (Falade et al. 1997; Sewummi et al.

5 100 Carrier 100 100 100

## A. Someon of all Probenical plus pystmethamine-sulphadoxine for uncomplicated malaria

19982; van Dillen et al. 19991 Othat et al. 2001; Sibley et al. 2003) and the tendency for the drug to mercase processe carriage after its use for the treatment of malana (Robert et al. 2000; van Seidleln et al. 2001; Sommon & Fatere 2003a,b), there is a need to review chanotherapeutic strategies haved on the use of this doing afone. The results of the present study indicate that probancied, an inhibitor of organic anion transporters and multiresistance associated proteins (Borst et al. 2000), and a chanosensiozer of P. folcapeum in units to antifolde appear (Nella et al. 2003), enhances the antipolariot effect of pyronethemine sulphadoxine in units in children with tenompherical falciparum infections. This is the first report of the enhancement of the antipolarial activity til an antifolder appear by probanced in humans.

The relatively accelerated elegrance of lever and parawascon a produced a cure rate of 96% by day 14 after bearing with pyracthemic-julph wanter probessed. lancestingly, only one of the three children who falled bonnent was as years and Evaluation of the analytetion stann of the failures would ture been helpful. Langer demand of illness (>2.) days) is associated with instruction tak of gamejust to carriage in uncomplayied fakipaneo Thin Price of al. 1999). However, despite a langer duration of illians lectore percentation in the pyrimeth an oc-sulphadurine probanció grand children, the third cather prior and fellowing treatment was winder to above of pyramethamine allphasio kine treated children. The worder games on the cast rage full owing west was web both reporter adicates that the use of Frimethamine authorizative probetaxid may not overly demonstrate or transmission of generocitics origing from בוווים ורצובוו וחבונים וחבונים

Vicinity all of the pyricardiscase sulphalarone treatcone balance were cared of their informations when they were
coned with pyrimetimism.

Overall, the Indicates a branchial effect of probagation.

Mosque, the branchial effect may also be due to

the server, the branchial effect of probagation repeated

Minimum of pyrimethylagam sulphalaria as, Alabangh

contain as required with this way, as at realy increase that

the server of pyrimethylagam and phalaria as, Alabangh

contains as required with this way, as at realy increase that

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contains as required with this way, as at realy increase that

the server of pyrimethylagam and phalaria as, Alabangh

contains as required with this way, as at realy increase that

The drupt and were well calculated. The man inequality reported adverse reactions were of generalizations of the state of properties of the state of

probeneed and sulphadistine can also induce luctualism in glucuse 6 phosphate debydrogenase IGSPU) decient subjects but no child, full units treatment, reported features suggestive of drug-induced harmolytic anzemia

It turnsian uncleut exactly how prubenecid enhanced the andmalarial effect of pytimethamine sulphadousine in the cohort of children studed. Probenced can reduce folare uptake by P. falcipirum in 11tra (Nitto et al. 2003), in addition in increasing platers and thunsmildes encountre thans by reducing renal subular recretion of the latter, Brah of these octions are ladgression of parente sentinies ty status to pyrimethamine sulphadosine. It is possible that fullnwing treatment with princette mine-sulphalinzine probenecial, sulphadouing concentrations were significantly higher than in these treated with pynicethaminesulphadurine alore, but drig levels were rut measured Probenecial can alin reverse restitaine in cancer celle to methotres are [Hoosberg et al. 1999] and remarke in P. falcipanin to chiomquine in tirm, thinha et al. 20031. by ushibiting the multi-dang res stance areasted pristerns. lupipition of the multi-close registance and taled protecut is an walikely combanion of the construction of the and malatest effect of pyrimentumine with and other by processed as resistance to pyriare hataine-sulphadraine ( processed with outrarious in the il hydrapterhate synche inse and dilig deololate reductate, and not murations in the plande | Kene of the parame (() ura singh et al 1997, Wang 10 d. 1997; Diouste et al. 1999).

That are luntifications for our doing segumen the relatively makerage done was based on the dine wied to retaid subulat secretion of penjalim in children - 1 convented starting point as the dat bas ant book previously co-edministered with Pyrimethaminesubpludence for the treatment of malers is duldren; the stuly due of reposen is promoble, and compliance to more likely than if it were for longer points. Constally characteristic and pharmaculyment-makes are required before optimal down reputers can be exhaust There are potential clinical applications of our body of at menterale done producerid cohunces the antiquation efficiety of pyrical busing mighaboure, it follows that as CONTRACTOR IN PERSONAL STATES AND I downer, happer down of probationid may be effective, as it is possible that cohorament any he down related

## Acknowledgements

The study was supported by the UNDP/World Bank/ WHO Special Programme for Research and Training in Tropical Diseases. We are grateful to our clinic nurse, Mrs Moji Aman. AS is a recipient of a WHO/TDR Career Development Award.

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## A. Someoni et al. Probenecid plus gyrimechanismustiphedosine for uncomplicated multita

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## SHORT COMMUNICATION

Comparative effects of pyrimethamine-sulfadoxine, with and without probenecid, on Plasmodium falciparum gametocytes in children with acute, uncomplicated malaria

The increasing apread of Marmodium falaparum resistent to pyrimethanilaesulfadowne (PS), the lirst- or second-line treatment of malaria in many endemic countries in Alica (Sibley et al., 2001), bas led to a renewed search for cheap, effective alternatives to PS, and to renewed efforts to prolong the clinical utility of this drug combination in Africa (Nzila et al., 2003). The treatment of scute, P. falaparum malaria with PS is often associated with day-induced increases in the frequency of gametocyte carriage and the level of gametocytacrala (Somunni and Fairye, 2003a, b). When PS is used in combinetion with other annmalerial drugs, particularly with amodisquine or the artemisinin des ivantes, however, these unwelcome chapper may be considerably reduced (Sowunmi, 2002; Sownard and Fateye, 20034).

Probenecid, an inhibitor of organic anion transporters and multi-resistance associated proteins, can chammaning P. Salapo rom to Primarhanine and sulfadown c, both in our (Ninks er al., 2003) and at least in Nigerian children cressed with PS, or care (Southern) a d. 2004) The effects of probenesid on the frequency of sometocyte carrage, level of graces, and temporal changes in when added to PS for the treatment of maleris in children, are, house when The sign of the present the open (1) to determine the effects, on the frequency of garnerocyte carriage and the positioned to P3 (PSP) and (2) to follow would formulate the gamelocytes were would and of participants of the addition of temporal changes in game to grants as described by Carter and Graves (1986)

and gametocyte sex ratios in children wested with PSP.

The study took place in Ibadan, a hyperendemic area for malaria in south-westem Nigeria, in July-September 2003. Overali, 151 children presenung with acute, uncomplicated, P. Jakoponon majarla were randomized to receive a single treatment with PS alone the PS was given orally at presentation (day 0) as 25 mg of the sulfadozine componentikg - or the same dose of PS plus probenecid (20-25 mg/kg.day given orally, in two divided deses, on each of days 0-2). The study protocol was approved by the local ethics committee. To be entolied on the study, a child had to be aged \$12 years, have a pure P. foloporum parasitaemia of > 2000 esexual forms/il blood, give a e ga tive results in (Dill-Gierko and lignes) wrote tests for antimialarial drugs, have on concomtrant illoess or evidence of severe malaria, and have the written informed consent of his or ber Parents of guardians.

After detailed clinical and parasnological assessment and drug administration at presentation, each child was checked clinically and paraitologically on each of days 1-7 and 14 Fingerprick samples of blood, collected on days 0-7 and 14, were used to make thin and thick smears so that the levels of parasis. semis could be estimated. Gametocysemia wes quantified on days 0, 3, 5, 7 and 14, using the thick bloodsmean prepared on those days (Sourment and Fatere, 2003b), if the level of pamerocytacouls was at least 10

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and Robert et al. (1996). The time clapsing from first drug treatment until a sex ratio of I was achieved (SRI) was calculated for each patient, from a plot of sex ratio v. time, by computer extrapolation. The data from a patient were excluded, from the exploration of the disposition kinetics of gametocyteemia, if gametocyte sex ratios had not been estimated for that patient at least three times after SRI.

Gametocyte kinetic parameters were estimated, from the levels of the micro- and macro-gametocytacmias, by a nou-compartmental method, generally as previously described (Sowunml and Fateye, 2003b). Data were analysed using version 6 of the Epi-Info software (Anon., 1994), and differences giving P-values of \( \leq 0.05 \) were taken as significant.

Of the 159 children entalled, 73 (39) treated with PSP and 34 with PS) were found gametoey memic at least once during the study. Although the entolment characterizes for the PSP and PS groups were similar, mean (s.p.) fever-clearance times [19 (08) days, with a range of 1-4, p. 2.5 (10) days, with a range of 1-7; P=0.009] and parable clearance times [22 (0.8) days, with a range of 1-4, p. 2.7 (1.0), with a range of 1-5; P=0.001) were significantly faster, and the frequency of parablelogical cure on day 14 (37 of 39 v 26 of 34 children; P=0.02) was significantly higher in those treated with PSP then in those even PS.

The frequency of game to cyte carriage was a specificantly higher on each of days 7 and the three children (one given PSP and two given PS) who had a female-biased sex ratio given PS) who had a female-biased with PSP, five of the Of the children treated with PSP, five of the Of the children treated with PSP, five of the Of the children treated with PSP, one of Among the children treated with PS, one of the 31 gametocytechnic on day 7 but note that the children treated with PSP, one of the 31 gametocytechnic on day 14 had of the children treated with PSP, one of the 31 gametocytechnic on day 14 had of the 22 gametocytechnic on day 14 had of the children treated with PSP, one of the 31 gametocytechnic on day 7 and one of the 22 gametocytechnic on day 14 had of the children treated with

(Barnetocyterful) in the PSP-treated garnetocytaemics, for example, were 17 (range = 12-365; N=5), 32 (range = 24-36; N=3), 33 (range = 24-18; N=9), 63 (range = 12-960; N=32) and 44 (range = 12-216; N = 16), respectively. The corresponding values for the PS-treated subjects were 12 (N=1), 50 (range=36-72; N=3), 67 (range = 48-84; N=5),41 (range = 12-687; N=31) and 30 (range=12-81; N=22). The mean level of gametocyteemla was, however, significantly bigher on day 5 in the PS-treated subjects than in the PSP-treated (P=0.004). Two of the children treated with PSP and one of those given PS were found to be gametocyte carriers every time they were checked

Sex could be determined for > 90% of all gametocyles. At presentation the overall sex ratio was male-based - with a mean of 59% leange = 30%-100%; 95% confidence interval (CI) = 26%-92%) of all gamerocytes sexed then being identified as male - and there was no significant contlation between the proponion of gametocytes that were male and the level of asexual parasitacmia (r=0.7; P=0.17), core temperature (r=0.2; P=0.8), or level of gametocycemia (r=0.02; P=0.98). The temporal changes observed in the gametocyte tex ratios were sinular for the PSP- and PS-trasted children (Fig.), In both trestment groups there was a progressive increase in the proportions of gametocytes that were male such that > 80% of the gametocytes were make by day 7 lo the three children (one given PSP and two given PS) who had a female-biased sex ratio on presentation, SR1 was reached by day 5 Of the children trested with PSP, five of the 32 gametocytecmie on day 7 and one of the 16 garnetocytamic on day 14 hod femalebiased games ocytecenias at those thmes Among the children treated with PS, one of the 31 gametocymernie on day 7 but note of the 22 parotoxymerate on day 14 had o female-biased ratio at those times. In the 14 were significantly higher than the proportion on day 0 (with Pavalues of 0.00003 and 0.00001, respectively). Similarly, in those treated with PS, the proportions of gametocytes that were male on days 7 and 14 were significantly higher than the proportion on day 0 (with a Pavalue of 0.000001 for each companison; see Figure).

in the PSP-treated children, the level of microgametocyteemia in the peripheral blood was similar to that of macrogametocyteemia between days 0.5 (see Table) but male gametocytes became significantly more numerous than the female by day 7. In those treated with PS, the level of microgametocyteemia alightly exceeded that of macrogametocyteemia between days 0.5. By day 7, however, the mean intensity of the microgametocytaemias was significantly higher than that of the macrogametocytaemias.

As, at each time-point investigated, the text ratios and levels of gametocytaemia for the PSP-treated children were similar to those treated with PS (with the exception of the intensities of the day-5 gametocytaemias; see Figure and Table), the data from both

treatment groups were pooled for the analynis of the disposition kineties. For the male gametocytes, the mean (5.E.) 'area under the cure' of the plot of gametoste density o. time (AUC) - calculated from SR! and expressed in units of account formefulb was significantly higher [17,619 (3831), with a range of 5040-40,017 and a CL of 8561-26,678; N=8] than that for the female gametocytes [4728 (69)], with a range 3034.6250 and a C1 of 2530 4927; N=4; P=0.017). The mean (se) half-life (th) of the mair gametocytes [265.6 (59.4) b, with a range of 66.3-624.0 and a CI of 125.1-406.1 h] was nice significantly longer than that of the semale gametoeytes [74.3 (12.8) h, with a range of 37.1-96.0 and a Cl of 33.5-115.1 b; P=0.05]. Although the mean (5.E.) desparce of the levele gamerocytes, expressed in units of sulkg.h [0.0002 (0.00008), with a range of 0.00002-0.0004 and a C1 of -0.00007. 0.00048} was 2.5-fold bigher than that of the male game locytes 10.00008 (0.00003), with a range of 0.00002-0.00015 and a Cl of 0.000009 0.00016), this difference was not omtistically significant (P=0.14).

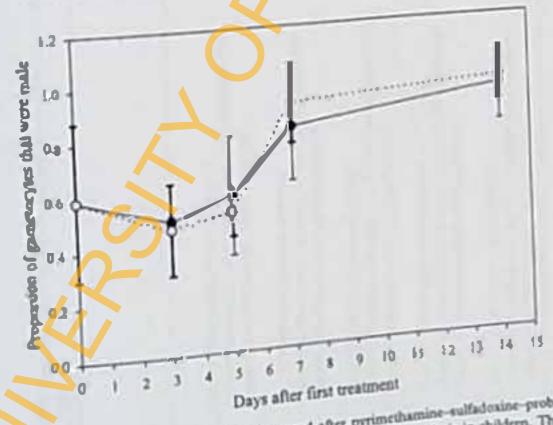


FIG. Changes in the sex ratio of gametocytes before and after pyrimethamine-sulfadoxine-probenecid (●) or pyrimethamine-sulfadoxine (○) treatment of acute, Plasmodium falcipanum malaria in children. The vertical lines indicate s.e.

			PSP			5	
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	No. 4 demetocytacmia (gametocytacmia (gametocytev)d) Range el gametocytacmias (gametocytes/pl)	20 64 24-180	3 29 24-36	0.18	12-46	12 12	98.0

\*Calculated by the Witness or one ore.

Overall, PSP appeared to be therapeutically superior to PS, in clearing asexual parasitaemia and fever. Treatment with PSP or PS was, however, generally associated with increases in the frequency of gameto-cyte carriage and the levels of gameto-cytacmia. The between-treatment difference in the level of gameto-cytacmia on day 5 is perhaps attributable to the relatively rapid elearance of asexual parasites induced by the addition of probenecid to PS.

The observation of a predominance of male gametocytes over the semple, in both treatment groups at most times duting follow-up (Fig.), is in contrast to the general, carly predominance of female gametocytes observed, in the same atudy area, by Sowunmi and Fateye (2003b). It may be related to the relatively low level of gametocytaemia seco at presentation in the present study - B male bias when there are few gametocytes in the peripheral blood being a form of fertility cosurance (Gardner et al., 2003), In addition, the children in yesogated in the present study could have been exposed to other sex-ratio-modifying factors prior to presencation (Paul et al., 2002; Robert et al., 2003). Such factors may explain wby, in the present study, SRI was reached in some of the children before, at or shoully after presenta-D03.

Despite the increasing intensing of the generocythemias, there were significant more in the personnes of pametoches that were male following reaument with enter PS or PSP (Fig.), Although, in a long. indical follow-up of gametocyte curriers in a of Senegal, Robert et al. (2003) detected a density dependent relationship with wer ratios, they reported that peaks of Charles Wert turnstienes theoristics with very low male female set ration it that PS, scoop singly or to comment with other factors, substantially because the promptings of garotpeyers that the male when added to PS, probeneral opposite to have no effect on gametocyte sex

The present observations support the notion that male gametocytes pendit longer in the peripheral circulation than female gametocytes, or are longer-lived (Ponnudural et al. 1986; Reece et al., 2003; Sowwaml and Fateye, 2003b, .). The addition of probenecid to PS had no significant impact on the disposition of male of female gametocytes in the peripheral blood. Given that intensities of gametocytacmia and the maie bias in gametocyte sex ratios correlate positively with gametocyte infectivity to mosquitoes feeding on humans (Tchuinkain et al., 1993; Robert et al., 1996), it would appear that the addition of probenecid to treatment with i'S is unlikely to decrease (or increase) the transmission of malaria in the population, whether the gametocytes involved arise from PS-sensitive or -resistant infections.

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# Effects of pyrimethamine-sulphadoxine, chloroquine plus chlorpheniramine, and amodiaquine plus pyrimethaminesulphadoxine on gametocytes during and after treatment of acute, uncomplicated malaria in children

A Sowunmi/+, AA Adedeji, GO Gbotosho, BA Fateye, TC Happi

Department of Phormaculogy & Thereprovies and Pongraduate Institute for Medical Research and Stability University of Italia, Hada, Nigoli

The effects of pyrimethamine-sulphuibstino (PS) chlamiquine plus chlorphentramine, a 111 receptor anengunist that neverses chlandaum neststance to Plasmodium fakipanum in vitra and in the (COCP) and anno-Doyuble plus pyrimethamitic-sulphalosism (AQPS) on gametors to prophation were evaluated in 137 chiliben with acute symptomatic uncomplicated falcipionin malarly with were received with these drugs. PS was significoult less effective than CQCP or 1QPS or clearing oversal parallamin or other symptoms of malaris Generocyte corrioge on days 1. 7 and 14 nero significantly higher in these tentral with P.S. The ratio of the doubt ther hi placing of bertheson come house (61.2) that is a riose ill to be him in wante and the Ote [PMG] that is stage II' and V. on index of cuntimiling Reneration of sametaceto a reve to 1 by clos 7 of transacrit be those treatest with PS, but remulated consistently below I in the other treatment Bruspy PrG.P.M. down rollo incredent significantly from the 0-14 in those would with PS and CQCP (x2 - 76 P - 9 00000) and x2 = 42 ? P = 0.00001, respectively) but the second significantly in these treated with AQPS (x2 = 55 2 1. - accomply Both PS-senditive and resistant infections someted BYG (18 of 29 to 13 of 20. 22 - 0 nd P -493) his PEG was present unly in those with recisions response to CDCP Combountain of PS with amortioquine (10) that is, (10PS) resulted in less production of PTG but to this sering PTG and not ballerine of response to IQPS These data linkers that PS estimated production or releast of soung gamelocites when med alone but greatened less young grantoes as when well in combination with AQ. PYG own be used as on budious of respons to COCP has not PS or PS-tweet combination dries.

Key words: malaria - garnetocytaernia - pyrimethamine - salphadexine - chloroquine - chloropheniramine - amediaquine -

As resistance to chloroquine (CQ) increases in extent and severity, alternative regimens available to control programmes in developing endemic countries including pyrimethamine sulphadoxine (PS), amodiaquine (AQ) (Olliaro et al. 1996, Brasseur et al. 1999, Sowunmi et al. 2001) or combination of AQ with PS (AQPS) (Sowunmi 2002) or other suitable combinations have become increasingly used in the treatment of CQ-resistant falciparum infections. These alternatives have varying effects on clearance of asexual parasitaemias or sexual forms of Plasmodium falciparum. For example, PS may (Puta & Manyando 1997) or may not (Hogh et al. 1995) enhance gametocyte carriage during treatment of acute falciparum infections. Although the presence of gametocytes in peripheral blood after antimalarial treatment is no proof of viability, their generation is required for the transmission of the infection from the vertebrate to the anopheline host. In order to improve the management of paediatric cases of malaria and reduce transmission in our area of study, the effects of these drugs on gametocyte production needs urgent assessment. In addition, it is not clear whether the enhancement or non-enhancement of gametocyte production by PS will be influenced by its use in combination with other antimalarial drugs. It is noteworthy that antifolates are ineffective in the treatment of uncomplicated falciparum malaria in South America, for example, in Brazil (Fontes et al. 2002).

Resistance to CQ in P. falciparum can be reversed by chlorpheniramine (CP) in vitro and in vivo (Sowunmi et al. 1997, 1998a, b, c). We have recently shown that, the presence in peripheral blood of very young gametocytes (PYG) 72 h after commencing CQ may be used as indicator of response to CQ (Sowunmi et al. 2003). However, it is unclear whether the addition of CP to CQ will alter the use of PYG as an indicator of response to CQ or indeed as an indicator of failure of reversal of CQ resistance in vivo by CP. Although the combination of CQ with CP will not be employed by control programmes in Africa in the very near future, it is still essential to study PYG and peripheral mature gametocyte (PMG) ger eration during treatment with CQCP in the event that this or other similar combination become available

the UNDP/World Bank/ WHO Special

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Received 16 January 2006 Attented 21 November 2006 In order to address these issues, we have evaluated gametocyte generation during treatment of falciparum malaria in children with PS, CQCP and AQPS. The main aims of our study were (i) to evaluate the effects of PS, CQCP and AQPS on gametocyte generation during treatment with these drugs, (ii) to determine whether or not the addition of PS to AQ will influence the generation of gametocytaemia by PS, and (iii) to evaluate PYG as an indicator of response to PS, CQCP or AQPS.

## PATIENTS AND METHODS

Study site - The study site, Ibadan, is a hyperendemic area for malaria in Southwestern Nigeria (Salako et al. 1990). In the area, it is difficult to distinguish, clinically, re-infection from recrudescence after day-14 of treatment because of intense transmission. Antimalarial drugs have therefore generally, until recently, been evaluated on the basis of data recorded up to day 14, rather than the customary day 28 (Ekanem et al. 1990, Sowunmi & Salako 1992).

Patients - The study took place at the University College Hospital in Ibadan, Nigeria. Overall, 166 children who presented with acute, symptomatic, uncomplicated P falciparum malaria were enrolled in the study between September 1999 and September 2001.

The study was designed to elicit a 20% difference in cure rates between AQPS/CQCP on one hand and PS on the other hand with 80% power and at 95% level of confidence. The minimum number of patients required for each treatment arm is 45. In general, to be enrolled, the children had to be aged 0.5-10 years, and have symptoms compatible with acute, falciparum malaria (with lever or history of fever in the 24-48 h preceding presentation) and a pure P falciparum parasitaemia of 2000 asexual forms'µl blood. Those who had taken animalarial drugs in the 2 weeks preceding presentation, provided a urine sample found positive for 4 aminoquinolines or sulphonamides (by the Dill-Glazko and again tests, respectively), or who had a concomitant illness, such as sickle-cell anaemia, or severe or complicated malaria (Warrell et al. 1990/WHO 2000) were excluded. The informed consent of a parent or guardian was obtained for each child included in the study. A child was withdrawn from the study if she he developed concomitant illness during the follow-up period, or if his her purent or guardian requested it. The study received thical approval from the local ethics committee.

Before enrolment to the study, a medical history of much child was obtained from an accompanying parent made and each was physically examined. Body weight and oral or rectal temperature were recorded, and thick and thin films were prepared from finger-prick blood tamples. These insears were Gierma-stained for paratire identification and quantification of any peripheral parameter.

Drug Processor - Children were randomly allotted to one of 3 treatment groups. One group received PS or present on (day 0) at a dose 25 mg/kg of the sulphosamide component. Each tablet of PS contained 500 mg of sulphadoxine and 25 mg pyrimethamine. The other

weight over 3 days (days 0-2) plus chlorpheniramine maleate, 6 mg at presentation followed by 4 mg every 8 h for 7 days (days 0-6) if the child was aged < 3 years, or 8 mg at presentation followed by 6 mg every 8 h if the child was ≥ 5 years, or a single dose of PS at presentation plus AQ 30 mg/kg over 3 days (days 0-2). All drugs were given by a physician orally and each child was observed for at least 3 h after each such supervised drug treatment, in order to ensure that the drug was not vomitted. If it was, the child was excluded from the study. Additional management of some children included the administration of an antipyretic (e.g. 10-15 mg paracetamol/kg, every 8 h for 24 h) and fanning and tepid sponging when necessary.

Evaluation of response - Clinical observations were recorded daily for 8 days (days 0-7) and then on day 14. Thick and thin blood films, for quantification of parasitaemia, were prepared at the same times. At each follow-up, the guardians or parents (and, when possible, the children) were actively questioned, using a standard questionnaire, and the children were examined for the presence of adverse reactions to drugs.

Giemsa-stained blood films were examined by light microscopy under an oil-immersion objective, at × 1000 magnification, by two independent assessors who did not know the drug treatment of the patients. Parasitsemia in thick films was estimated by counting asexual parasites relative to 1000 leukocytes, or 500 asexual forms, whichever occurred first. From this figure, the parasite density was calculated assuming a leukocyte count of 6000/ ul blood. Young gametocytes (stage 1-III) and mature gametocytes (stage IV and V) (Sinden 1998) were also counted in thick blood films against 1000 leukocytes on days 0, 3, 4, 5, 6, 7, and 14. The responses to drug treatment were classified according to World Health Organization (1973) criteria. Treatment was considered a failure if the day-3 parasitaemia was > 25% of the day 0 value, if parasitaemia did not clear by day 7, or if parasitaemia cleared before day 7 but re-appeared before day 28. The parasite clearance time (PCT) was defined as the time clapsing from drug administration until there was no patent parasitaemia. The fever clearance time (FCT) was defined as the time from drug administration until the oral or rectal temperature fell to ≤ 37.4°C and remained so for at least 72 h. (This definition was necessary because of the routine use of paracetamol during the first 36 h of treatment in some

children).

Cure rates were defined as the proportions of patients
who remained free of parasitaemia on day 14 of fol-

Re-treatment of drug treatment failures - All treatment failures were re-treated with AQPS on day 14 provided they were not symptomatic before this time. Pavided they with profound clinical (hyperpyrexia, oral fluid intolerance) and parasitological deterioration during intolerance) and parasitological deterioration during follow-up were treated with artemether, 9.6 mg/kg of follow-up were five days and were regarded as treat-body weight over five days and were regarded as treatment failures.

Satisfical analysis. Data were analysed using version 6 of the Epi Info sultware (Anon 1994). Proportions were compared by calculating  $\chi^2$  with Yotes' connection or by Fisher exact tests. Normally distributed, compared by Student's I tests and analysis of rannace (ANOVA). Data not conforming to a normal distribution were compared by the Alana-Whitney U-tests and the Kruskal-Waltis tests (or by Wilcoxon rank sum test). The vidues presented below are generally means and mandatal deviations (ad). Paralysis of < 0.05 were taken to indicate significant differences.

#### RESULTS

Clinical and parasitological characteristics at endiant and therapeutic responses. A total of 166 children was entolled in the 195, CQCI, and AQIS groups, respectively. Of these, 49, 48, and 60 children in the 195, CQCP, and AQI'S groups, respectively completed the manually 14-day follow up period and were analyzed. The clinical and parasitological characteristics at citological water suntiar in all groups (Table 1). The therapeutic responses to drug treatment are also summarized in Table 1. AQPS was sign the analyzed effective than

COCP or I'S in clearing fewer and parasitaents and with a significantly higher cure rate on day 14. Direct comparison of I'S and CQCP showed that fever (2.2. 1.1 to 1.6. 0.8 day, 1' 0.008) but not parasite eletance in those with sensitive tesponse {2,7. 1.1 vi 2.5. 0.8 day. I' 0.33) was at goilicantly faster with CQCI than with I'S. The cure rate on day 14 was also significantly higher with CQCI than with I'S 180.8 vi 59.2% I' 0.03).

tions for them to the ting follow-up. The prevalence and intensit end garnetory terms before, than 18 and after treatment are knowned to Table II. Garciocyto carriage up they 3. 7. and 14 or they 3. 7. and 14 combined were against with higher in the I'S group than in the other treatment groups. However, the geometric mean game for itematics IGMGD) were similar in all the treatment groups.

The molian survival time for peripheral young gamesurytes (PA'G) in PS. COCI; and AOPS meanment groups were 15. 1.5, and 1.5, respectively. There was a significant difference in the overall comparison of the significant difference using Wilcoxon (Gehan) statistics turnival expensions using Wilcoxon (Gehan) statistics (X) = 1.17, P = 0.00061. The ratios of the densities (per planet) of pempheral young gametocytes (I'VO) to pempheral limiture gametocytes are sammarized in Table

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	المراسع المزوندودم المراس احي	24 of the Capping of August	AOFS(n = 60)	Produc
Oi		COCP (n = 48)	VALUE	
(years) Geom + s.d.	FS (n →19) 5 1 ± 24	0022J 2010	3 5 a 2.5 12.10	0.52
Congration (leg)	15.4±3.6	168±52 8.135	15 5 ± 4 7 6-26	032
Destroy of These (d)	6.5-30	36±24 2.14	28=13 14	0.09
Personal and resp. (*C)	1.7	38 6 ± 1 = 362-405	18 1 0 1.0	0.00
	358-40.5	31/31/400 34384	THE THEFT	<b>a</b> 56
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The ratus were consistently below I in the CQCI and AQPS groups up till day 7. However, in the PS group, that ratio rose projection for generation or mobilization) of young gametocytes. PYG-PMG density ratio intersect significantly from day 0-14 in those treated with PS and CQCP ( $\chi^2$  - 76. P = 0.000001 and  $\chi^2$  - 42.2, P = 0.00001, respectively) but decreased significantly in those treated with AQPS ( $\chi^2$  = 53.2, P = 0.000001)

Relationship between PYG and responses to drug treatment. None of the children successfully iteated with CQCP had PYG during the follow-up. In children who had sensitive response to PS treatment (n = 29), PYG was present on days 0, 3, 5, 7, and 14 in 5, 12, 13, 13, and 7 children, respectively. Similarly in those are restully treated with AQPS in = 601, PYG was present on days 0, 3, 5, 7, and 14 in 2, 2, 3, 3, and 0 senious, respectively. The PYO sales were significantly higher in those treated with PS than in those treated with AQPS in all times during follow up (F' 5 0,006 in all companions).

Post line Turkey IISD test for repeated measure of the effect of PYG generation over the 14 day follow up showed significant differences in the compatisons of I'S vs AQI'S and I'S vs CQCP (P = 0.000) and 0.0001 respectively). There was no significant difference in the compatison of I'YO generated by those treated with AQPS and CQPS (I' = 0.08)

Relationship between PYG and outcomes of treos.

ment in the children treated white PS and CQCP. The relationship between usalment outcomes and presence of PYG in children treated with I'S and CQCP ato shown in Tables IV and V. PYG rates were similar in those with sensitive of resistant responses to I'S (18 of 29 cs 13 of 20. 2 0.04, I' 0.93) and the lates were similar from days 0.14. In contrast, PYG was seen only in those with resistant response to CQCP in those without genetocytecnia is presentation, but who subsequently developed I'YO 72 h after commencement of CQCP, the Presence of PYO was associated with restruction failure on or before day 14 (Table V)

Gamesocytaemias before, during and after the treatment, with pyrimethamine-sulphadoxine (PS), chloroquine plus chloroheniramine

(COCT), or a second and after the treatment, with pyrimethamine-sulphadoxine (ADPS), of Plasmodium fulciparum infections in children

(COCS.) or attroctradmine bin	в ругинения	COCP	AQPS	P value
	PS (n = 49)	(n-48)	(n = 60)	
Man + 2.2 Man + 2.2 - Man ( ) july - Man + 2.2 - Man ( ) july - Man + 2.2 - Man ( ) july - Man (	36 17=52.1 12-041	22 26 ± 5 J 12 × 16	40 74 ± 43.3 12-288	0.49
(mark mark (pl))  Mare S.E.	36 100±13-5 13-478	137±135 13412	40 86 ± 54.1 12-408	0.74
Day 5 (2) == tox 3 taxenum  Con-mate-in-thirt  Mican + \$.E.	243 ± 113 9	61 118±643 12-518	41 141 x 521 0 12-506	038
Day 7 plantage year max Gen hit comment (pil)— —Mann w S E.	300 ± 141.2	136 ± 73 6 12 690	36 98 ± 74.1 12-468	021
Continuence terms ( p.l)  Most = 3.1	(3) (3) (2) (2)	34 40 = 30   12   168	19 24 ± 12 12-48	
Charles and Charle				041 0(1119) 0 (2 p) 1 0 (3 f) 1
Duy I	25 20 21 21	0 0 11 11		0 (01110) 0 (1127)

TABLUUM

Prevalence and intensities of peripheral young guardich tesoning photosimuse games are in children arealed with PARTIE CONTROL CONTROL

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0	21 (5)*	6514) 12-409	12(4) 12-12	36(11	42 (2) 34-72	36 (2) 13-216 13
3	28(20)	57(9) 12 353 10	55141) 12-144	106 (3) 24-460 1.0	12 40	MI(1) 4 39(51)
3	37 (21) 12 640	54((7) (7.1840 1.3	46 (6)	50 (4) 12 180 1 3	15 W	12-4(%)
7	79 (24) 13-1320	51(21) 12-2210	47(7)	97(4) 12 msi 2.3	12-15A 1	87(Z) 24.1(3 1.1
14	50 (14) 12-2-40	78 (12) 12 444 1 8	21 (4) 12 120 1	11(1) 12-11 1 7		19(3) 13-40 <sub>180</sub>   180 <sub>21</sub> yin de 1007

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the (TYG) as the children with remain was restricted the principle presented as the

Providence of purple	and prime others	and the state of the last	a delate	COCP and the	COCP-nulstand	yalar
No. of children with PVG on day	(2 = 29)	(10° 20)	051	(4 = 28)	3	ting
0	5		0.49			o mate
3	E	3	0.85	-	7	♦ (MINIS)
5	D		0.35		4	Atrit:
7	U		0.14			OR(mont)
14	7	13	0.65		7	0.000000
A Constitution	25	13	0.11			
Testo II	13	11				

TABLE V

	4/1-100	and the state of	) je children (FC	and each each price		P
		13-10-10-1	7	CIPT - MARINE	CULT-SECTION	- California
No. of colders with PVG on Ser	(n = 29)	- 30	(de		1	0.000
	18		6.52		1	605
	*	7	649		1	6.000
	17	10	664			

#### DISCUSSION

The ideal antimalarial drugs or drug combinations for the treatment of falciparum malaria should not only promptly clear parasitaemia, fever or other symptoms of malaria, but should also prevent the generation of gametocytes from asexual forms during treatment. In the present study, PS was significantly less effective than CQCP or AQPS in clearing parasitaemia or fever in children with acute falciparum infections. This is not surprising since progressive decline in sensitivity of P. falciparum to PS has been reported from the area of study from the late 1990s (Falade et al. 1997, Sowunmi et al. 1998a). The decline in sensitivity of the parasite to PS has also occurred in many areas of Africa (Sibley et al. 2001).

In addition to their effects on the sexual forms, gametocyte carriage may be influenced to a considerable extent by the sensitivity of the asexual parasites to the drugs used for the treatment of infections. For example, as resistance of the asexual parasites to the 4-aminoquinolines, CQ, and AQ, increases, gametocyte carriage also increases (Strickland et al. 1986, Hogh et al. 1995). In these studies, gametocyte carriage rates 28 days after PS treatment were significantly less than those of CQ and AQ since PS was more effective than the 4minoquinolines on asexual parasites in the settings of these studies. However, increased carriage may also be related to decreased sensitivity to PS in certain circum-Mances (Sowunmi et al. 1998a, Tjitra et al. 2002). In our cohort of children, gametocyte carriage was significantly higher at all times after treatment with PS than in the other treatment groups. In addition, PYG rates were timilar in both PS- sensitive and -resistant infections supporting a known fact that PS enhanced generation or release of gametocytes during treatment of acute falciparum infections (Puta & Mayando 1997). However, GMGD were similar in all the treatment groups.

Many antimalarial drugs appear to reduce gametocytaemia by clearing the asexual stage infections. This clearance, if exceptionally rapid, may reduce transmissibility particularly in areas of low transmission. For example, the artemisinin derivatives have reduced transmissibility in some parts of Thuiland by this process (Price et al. 1996)

In order to determine the influence of treatment with antimalarial drugs on gametocyte production and densilies, we have quantified both young and mature gameto-Cyles and expressed these as ratios. The ratios of PYG to PMG were consistently below I up to day 7 in those treated with CQCP and AQPS, but rose to 1 by day 7 in those treated with PS irrespective of the sensitivity of the asexual parasite to PS. This showed continuing and enhanced production or, preferential mobilization of Exmetocytes by PS irrespective of the sensitivity of the preferential and PS. This process of continuing or preferential mobilization of young gametocytes by PS may explain why gametocytes persist longer in some patients treated with PS. This is plausible because the young gameto yes must grow and run the normal timecourse of survival of the normal mature gametocytes.

Given that gametocyte density may correlate with mosquito infectivity and therefore transmission success (Tchuinkam et al. 1993, Drakeley et al. 1999), the effects of PS on gametocytes carriage and mobilization have implications for malaria control programmes with respect to the use of this drug. Recent WHO recommendations (WHO 2001a, b) have focused on the use of combination antimalarial therapy (CT), particularly artemisinin-based combination therapy (ACT). Although several control programmes in Africa have switched to CT, some programmes use P5-based combination, for example, AQPS (Sowumi 2002). The modulating effect of AQ on enhanced production of PYG by PS may provide supporting argument for the use of combination therapy. However, the reduced generation of PYG by PS despite its combination with other drugs suggests that generation of gametocyte is an inherent property of antifolate antimalarials (Sowunmi et al. 2005, Hamel et al. 2005).

In a recent study, we have shown that the detection of PYG 72 hafter the start of CQ therapy may be used as an indicator of response to this drug (Sowunmi & Fateye 2002). Our results show that PYG may also be used as an indicator of response to CQCP. Failure of the enhancement of the antimalarial efficacy of CQ by CP in vito was associated with the presence in peripheral blood of young gametocytes. However, PYG was not an indicator of response to PS, since both PS-sensitive and resultant infections generated PYG. In addition, the presence of PS in combination with AQ also generated PYG and was clearly not an indicator of response to AQPS since the cure rate in this group was 100%.

The limitation of the present study is the fewer number of gametocyte carriers in the AQPS and CQCP groups following treatment. Therefore caution is required with the interpretation of the data from these two groups.

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## Effects of antifolates - co-trimoxazole and pyrimethaminesulfadoxine - on gametocytes in children with acute, symptomatic, uncomplicated, Plasmodium falciparum malaria

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Animolarial drugs including the antifolate, pyrimethamine-sulfadazine (PS), can modulate the providence and months of game ioch poemia following treatment of poute malaria infrasions They may also directly influence the boundston and spread of drug inscruitivity. Little is known of the effects of co-rimaticale (Co. 7), and antifolise and on gametos) les in children with acute malana infections. He compared the effects of Co-T and PS on the prevalence and untensities of game (OC) taemta and game (OC) te sex ratios in 102 children and 05.12 years proving with acres and uncomplicated falciparum molaria. Compared to pre-vecument, both drugs significantly removed gametocyte carriage post-initiation of incoment flowever greater te carriage was strifteenth longer meday 14 in those treated with Co-T than PS. Significant increase in gameiochiaemia with time occurred in PS\_but and Co.T. was as children Kaplan-Meier sormal ourse of the cumulance probability of remarking goods of the incheldren who were organicios) taemic at enrolments howed that by day 7 of follow up, children treated with 15 had a significantly higher propensity to have developed Bameiochter than in Co-Tibrated children (Log rank statistic 3 1). If = 1. P = 0.02) Gametocy ie sex ratio changes were similar following treatment with both the PS and Co I brown of ocule molar la infections in children from this endenic area is associated with significant increases in privalence and intensities of gamelocy toemla but these effects are more marked in those around with PS than Co-T.

Key words: co-trimocarois - pyrocybamus substours - makes - procesylamus - ses mon - children - Nigeria

The authorise antimaterial, pyronethumine sulfadorine (PS), has become increasingly used as first line treatment placipalum malana ni cerela Alnean countres because of tacreating resistance in Plasmodium Jalesparum to the spite of frequent use and of in vivo and in Hoghet al 1998, Sowania & Falere 2003a). the lices on same tocytes in children with falciparum inreceipt incompletely understood

With tources in By Jole purum to PS to tog (Sibley et al 2001) probably as a consequence of long half lives of its components. It has recently been elected that, co-inmoxazole (Co.7), an antifolate anti-Balanal with relatively short half-lives of its components to PS, may be used as alternative to the latter the trespont of ancomplicated fair parum infections because it is as efficacious as PS (Omar et al 2001. Fediratole et al. 2004) It is assuroed that the relahardy short half-life of Co-T may, when compared with the chance of engendering resistance in P. les thousand the his des and the his blonge additional ad-

vantage with transmission of drug resistant infections over PS. It is noteworthy that antifolate antimalarials are not effective in the treatment of uncomplicated falciparum malaria in South America, for example, Brazil (Fontes et al. 2002).

However, while the effects on PS on gametocytes and gametocyte sex ratios (GSR) are known (GSR may influence infectivity to mosquitoes and transmission - see Robert et al. 1996, Sowunmi et al. 2003 a,b), the effects of Co-T on gametocytes are relatively unknown in African children with falciparum malaria. We hypothesized that PS and Co-T have similar effects on gametocyte prevalence, density, and sex ratio, and possess similar effects on gametocyte survival in children treated with these drugs. We tested this hypothesis in a group of children with acute symptomatic uncomplicated P. falciparum malaria who were randomized to and who received PS and Co-T for the treatment of their infections.

## PATIENTS AND METHODS

Patients - Between June and August 1999, a randomized trial of Co-T and PS for the treatment of uncomplicated falciparum malaria was conducted in 102 children at the University College Hospital in Ibadan, a hyperendemic area for malaria in Southwestern Nigeria (Salako et al. 1990). Ethical clearance for the study was provided by the local ethics committee. In general, to be enrolled, the children had to be aged 0.5-12 years, and have symptoms compatible with acute, falciparum malaria (with fever or history of fever in the 24-48 h preceding presentation) and a pure P. falciparum parasitaemia of > 2000 asexual forms/µl blood.

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These who had taken antimalarial drugs in the two weeks presentation, provided a unne sample found songve for four ammoquinolines or sulphonamides (by the Dill-Glazko and lignin tests, respectively), or who had a constitutant illness, such as sickle-cell anaemia, or seresear complicated malaria (WHO 2000) were excluded. The informed consent of a parent or guardian was obbesed for each child meluded in the study. A child was wildrawn from the study if she/he developed concomithe follow-up period, or if his/her paron or grandum requested it. Thick and thin blood films from all patients who participated in the study were exammed for the presence and density of asexual and sexual paralities at engolment and start of treatment (day 0), and at follow-up at days 1-7, and then on day 14. Co-T was gree as 20 mg/kg of the sulfamethoxazole component The daily for five days (day 0.4); PS was given as the 25 marky of the sulfadoxine component of presentation (day 1) All drigs were odministered orall y.

Assessment of parasitoemio and gametoc) te sex ra-6 - Thick and thin blood films prepared from a finger Mick were Greense-chained and were examined by light micolcopy under an oil-immersion objective, at x 1000 magailication, by two independent assessors who did not two the drug treatment of the patients Parasitaemia in the films was esummed by counting ascaunt parasites Miline to 1000 leukocytes, or 500 asexual forms, which In occurred first. From this figure, the parasite density real culated assuming an average leukocyte count of 6090/11 of blood (Shaper & Lewis 1971, Ezeilo 1971, Somumi et al. 1995). Gametoeyles were also counicdio films against 1000 leukocytes assuming on average Count of 6000/µl of blood at enrolment (day 0) on days 3, 5, 7, and 14. Gametocyles were sexed, to bicod lilm if gametocytactio was 2 12 sexual forms Genetocyte sex determination was based on the fol-Graves 1988, Robert etal 1996) are smaller than females, the nucleus is bigger in than females: the ends of the cells are round in and angular in females, the cytopiasm stains pale people is males and deep blue in females; and the giarwe of malaria plyment are centently located in females and more widely scattered in males. GSR was defined as proportion of gametocytes in peripheral blood that TO DE CONTRA (Pickenos et al. 2000, West et al.

Statistical analyze - Data were analyzed using verthe 6 of the Epi-lafo software (Anon 1994), and the sta-Popular SPSS for Windows version 10 01 (SPSS for Windows version 10 01 (SPSS with the popular distributions) with the popular distributions were compared by calculating a with contection of Fisher exact lest Normally distrib-"Collavous data were compared by Student's 1-1831 tod tod) sis of variance (ANOVA) Data not conforming to a compared by the Mann-Wallery U. test and the Kruskal-Wallis test (or by William rank rum test), Kaplan Meier analysis was used the cumulative probability of remaining free of by during follow-up for all cases of malana comand for gotocares that were free of garretochtreture Dillacoct 10 tanning nue were estented

by inspection of Kaplan-Merics curves and pairwise lograpk tests. P-values of \$0.05 were taken to indicate allm ficant differences.

#### RESULTS

Demographic characteristics and therapeutic responses - A total of 104 children were capolled into the study. Two children cae in each treatment group, were excluded from the study due to parenul sclocation. These children were cleared of their peripheral parasitaemia at the time of exclusion. The demographic characteristic of the children enrolled in the study and the their partie responses to the treatment given are summarized in Table 1 These were similar in the two creamont groups. However, parasite dentance was significantly shorter in those treated with Co.T than PS

Providuce of general terms . The previlence of gametocy Werness before and after treatment with PS, and before, dunns and after trestment with Co-T is shown in Table II Gametoc) te carriage was similar on days 0-7 ip both treatment groups and it peaked at day? In both the PS and Co-T poups. Gametos) te campge was significapily lower on day 14 in those treated with Co-T than PS (22-5.6, P=0.018) Eleven and 19 children vented with Co-T and PS, respectively were gametocyte camers on both days 7 and 14. The difference between these proportrous was significant (x2 - 4.0, P = 0.046)

In general, compared to pre- uestment, both drup agmilicantly ignessed gamelogge carriage post-initiation of treatment (x2 = 20.9, P = 0.003 for Co-Tand x1 = 28.4, P - 0.0001 for PS, see Table 11) In children without patent gametorytaemis at emplement, there was a greater propensity to be gamelocyte-positive by day 7 with a significantly greater proportion of children treated with PS have ing gardencytes by day 14 of follow up compared with Co-T (63.6% of 34.3%, 12=5 9.1-0.016) (Table 11)

Gomeros Juenia - Gametocytemia before and after treatment with PS. and before, Juring and after treatment with Co. T Is shown in Table II Gameiocy terrils was aimi. has throughout the duration of the study in both Co-Tand PS-treoled children with peak gamelocy themis occurring in both treatment groups on day 7. Peak gamesocytaeras (on day 7) was significantly higher than day 3 gametocytecinia in both treatment groups (1=0.066, P=0.018 for Co.T; 1=008, P=0017, by Wilconon sign lank less for paired state). Gamelocy tacinias occurring on days 3.14 were not compared with pre-irealment gametocyleania because of the small number of patients in both groups However multiple comparison of gametoc) taenus using Friedman test showed that there was significant locresse in gazzelocy weems with time on days 3, 5, 7, and 14 in those treated with PS (P = 0.011) In comparison, there was no significant increase in gamelocy to conja with time on days 3, 5. 7, and 14 in those treated with Co-T (P =

The Kapian, Nicker survival curve of the cumulauve probability of remaining gunelocy te-free inchildren who were agametocytaemle at enrolment is shown in Fig. 1. By day 7 of follow up, children treated with PS had a alguificantly higher Propensity to have developed game

TABLE I Summary of clinical characteristics at enrolment and therapeutic responses in patients with acute falciparum malaria treated with co-trimoxazole (Co-T) or pyrimethamine-sulphadoxine (PS)

	co-trimoxazole (Co-T) or pyrim	euramine-suipnaouxine (rS)	
Parameter	Co-T (n = 53)	PS (n = 49)	P value
Age (y)			
mean ± sd	63 ± 2.9	6.3 ± 2.8	0.9
range	1.5 - 12.0	08-105	
Weight (kg)			
mean ± sd	18.2 ± 6.4	17.6 ± 5.2	0.6
nege	7.5 - 3.1.5	70-280	
Temperature (°C)			
EE + N	38.1 ± 1.3	38.4 ± 1.4	0.2
<b>COR</b>	35.7 - 40.9	35.9 + 41.0	
المعدد طحمت (الما)			0.29
provide and the second	36543	34983	0.27
pake	2200-349636	2557-052800	
Controve density (1911)			0.8
Fredrit men	15 (a = 3)	17 (a = 2)	424
Gire	12 - 24	12 - 24	
KT (d)		101	0.002
man ± ed	$2.5 \pm 0.9 (n - 50)$	12±1,2(a-44)	
<b>O</b> g	1-5	1.6	
fct (s)			0.20
च्या ± स्र	2.0 ± 1.0	2.3 ± 1.3	
COLO.	1 - 4	1.6	
Det 14 response o			
to of subcolors		43 (977)	0.88
(%)	47 (88.7)	43 (877)	
	6	Ó	
	0		
	0	time at warder der sale	All pumple

will (1973) criteria FCT fever clessance line, PCT: paralle clearace time, al carded devalues All carpeters were 100 biled

TABLE II

Intensity and prevalence of Plasmodium for ciparum gametocytaemia following treatment of uncomplicated malaria with co-

many and	prevalence of Plasmodium falciparum	gametocytaethia for (PS) of 102 malarious chi amine-sulphadoxine (PS) of 102 malarious chi PS (n = 49)	Padu
	trimoxazole (Co-1) as pyrime	PS (n = 49)	1.0 €
by O e	Co-T (n - 53)	17 [12 . 24]	0.44
-70-	18 (12 48)	2/ 49 (41%)	10
73	51 53 (9.4%)	27 [12 - 144]	1.0
13	27 (12 - 120)	12/49 (24.5%)	0.49
9 5	13/ 53 (25%)	45 [12 - 1872]	0.11
·	33 [12 - 420]	25.48 (52.1%)	027
y 7	21/51(41.295)	71 [12-2316]	0.13
2.(4)	42 [12 - 444]	34/46 (73 9%)	0.52
714	28 49 (57 1%)	47 ]12 -1200]	ouls
711	33 [12 - 120] 13/37 (33: 1%)	23/35 (65 7%)	devent become of palice

Language of Description of Descripti Day 10000 [ 1300] 6 general positive of patients cumined when in perceives represent Co-T prespond (22 = 20 9, 7 with both drap.

than in Co.T-treated children (Log-rank statistic 535, d(=1, P=002).

Temporal changes in gametos ne sex ratios - In Co-Tmared children, 7, 28, 104, 134, and 44 paroctocytes were counsed on days 0, 3, 5, 7, and 14, respectively and approximately TT% of these gardery to could be sexed. In 15-treated-hildren, 7, 34, 230, 293, and 168 gametocytes ser counted on days 0, 3, 5, 7, and 14, respectively and oppositionally 76% of these gametocytes could be sexed The data on GSR at envolment were pooled because of the mail number of gametocyte carriers observed pre-westnext (three among Co-T-treated children and two among 15-valed children) Overall, pre-treatment GSR was febalobiased, but became male-biased by day 3 in both tratachi groups, and remained male-biased till day 14 in toberoups (Fig. 2). GSR was similar in the two treatment groups on days 3, 5, 7, and 14 (P = 0.4, 0.7, 0.7, and 0.2, respectively on days 3, 5, 7, and 14.

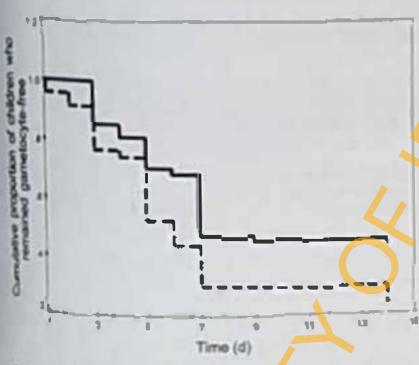


Fig. 1: Kaplan-Meier plot (survival curve) of cumulative probabila) of remaining gametocyte-free in 95 children who were aganetocytaemic at enrolment following treatment with cotrimoxarole (broken line) or pyrimethamine-sulphadoxine (solid line).

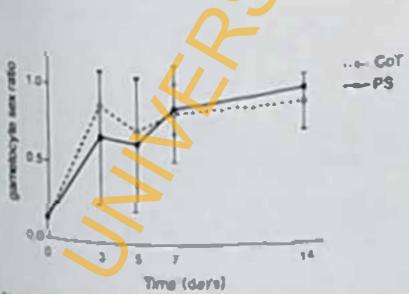


Fig. 2: changes in sex ratio of gametocytes before and after tresther bear pyrimethamine-sulfadoxine (PS), and before, during and ther treatment with co-trimoxazole (Co-T) in children with acute, the design that the vertical lines indicate stan-

#### DISCUSSION

Co-T and PS were both effective in the treatment of uncomplicated falciparum malaria in children from this endemic area of Southwest Nigeria. Apart from a significantly shorter parasite clearance in the Co-T-treated children, none of the outcome measures, clinical or parasitological, differed between the two antifolate drug combinations. The results support those of recent findings from the same area (Fehintola et al. 2004) and are in agreement with those from Kenya (Omar et al. 2001). However, the results are contrary to the suggestion that Co-T is less effective than PS for the treatment of malaria (WHO 1996). In many areas in Africa, for example in Uganda, there has been appreciable decline in the sensitivity of P. falciparum to Co-T (Kilian et al. 1998).

The prevalence of gametocytaemia significantly increased following treatment with both drugs but this effect was more marked in those treated with PS than in those treated with Co-T. Sexual development in the malaria parasite and its modulation may be influenced by several factors (Carter & Miller 1979, Mons 1988). It is not clear whether the significantly lower carriage on day 14 in those treated with Co-T was due to fundamental differences in the responses of the asexual parasite populations to switch to gametocyte production following exposure to the two drugs. The components of Co-T have shorter half lives than those of PS and it is possible that this, coupled with individual variation in response, may partly explain the observed difference in gametocyte prevalence between the two drugs.

Although there were no significant differences in gametocyte density in the two treatment groups, the significant increases in gametocyte prevalence with time, the greater proportion of children with patent gametocytaemia on both days 7 and 14 among children treated with PS, and the significantly higher propensity to have developed gametocytes by day 7 in PS compared with Co-T treated children (see Fig. 1) suggest a more marked effects of PS on gametocyte production. These findings with PS is in agreement with our previous observations (Sowunmi & Fateye 2003 a,b). Thus, the significantly reduced effects of Co-T on gametocyte retention may be an advantage for the use of Co-T over PS in endemic

setting.

Despite lower gametocyte prevalence and insignificant increase in gametocytaemia with time in Co-T treated children, both Co-T and PS appear to have similar effects on GSR. None of the post-treatment initiation GSR data differed between the two antifolate drug combinations; both drugs favoured gametocyte maleness. It is not clear whether the effects of the drugs on gametocytaemia is fundamentally different from their effects on GSR. Since GSR may be influenced by several factors (West et al. 2002, Gardner et al. 2003), this may impact on the temporal changes in GSR. The male-biased sex ratio after PS treatment is in agreement with our recent findings from the same area (Sowunmi & Fateye 2003b). The gametocyte maleness seen after initiation of treatment with both drugs suggests that antifolates, in general, may favour gametocyte maleness. Since gametocyte infectivity to mosquito nd infectivity correlates with gametocyte density (Ithmhametal 1993, Robert et al. 2000), both Co-T and is synthmong gametocyte maleness, gametocyte carries and gametocyte carries and gametocyte carries and gametocyte carries may markedly enhance malaria may markedly enhance malaria mayor or resistant infections. This a dement for the use of these drugs alone for the treatment of malaria.

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