

# Effects of Chloroquine and Sulfadoxine/Pyrimethamine on Gametocytes in Patients with Uncomplicated *Plasmodium falciparum* Malaria in Colombia

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*The effect of antimalarials on gametocytes can influence transmission and the spread of drug resistance. In order to further understand this relationship, we determined the proportion of gametocyte carriers over time post-treatment in patients with uncomplicated Plasmodium falciparum malaria who were treated with either chloroquine (CQ) or sulfadoxine/pyrimethamine (SP). The overall proportion of gametocyte carriers was high (85%) and not statistically significantly different between the CQ and SP treatment groups. However, an increased risk of carrying gametocytes on day 14 of follow up (1.26 95% CI 1.10-1.45) was found among patients having therapeutic failure to CQ compared with patients having an adequate therapeutic response. This finding confirms and extends reports of increased risk of gametocytaemia among CQ resistant P. falciparum.*

Key words: *Plasmodium falciparum* - gametocytes - antimalarial drugs - malaria - Colombia

In spite of several in vivo and in vitro studies (Hogh et al. 1998, Buckling et al. 1999, Robert et al. 2000), the effect of chloroquine (CQ) and sulfadoxine/pyrimethamine (SP) on gametocytes in patients with *Plasmodium falciparum* malaria remains unclear. If after treatment a considerable proportion of patients carry gametocytes this would increase transmission. Likewise, if resistant parasites are more likely to develop gametocytes after treatment, the spread of drug resistance would be favoured. The present study examines the effect of these two antimalarials on gametocytaemia and explores the influence of treatment failure on gametocyte carriage.

## MATERIALS AND METHODS

In 1998, a 14-day in vivo randomized trial of the efficacy of CQ and SP to treat uncomplicated *P. falciparum* malaria in Quibdó, Colombia was conducted (Osorio et al. 1999). Thick blood film slides from 98 out of 141 patients who had participated in this study were examined for the presence and density of asexual and sexual parasites. Asexual parasitaemia was measured by dividing the number of asexual parasites found in 300 leukocytes by 300 and multiplying by 8,000 (the estimated number of leukocytes per microliter of blood). The presence of gameto-

cytes was evaluated in 1,000 leukocytes (approximately 200 fields), and gametocyte density was estimated by counting the number of sexual forms in 1,000 leukocytes and multiplying by 8. Two laboratory technicians with expertise in malaria microscopy read all slides blinded. Discordant results were evaluated by a qualified third reader, who did not know the results of the previous readers. EPINFO 6.04b (CDC 1997) was used for data analysis. Data was compared using 2 x 2 tables. Continuous variables were compared by Kruskal-Wallis test, and categorical data by Chi-squared or Fisher's exact test when required.

## RESULTS

Forty-two patients received CQ and 56 SP. As in the original study, in this subset CQ- and SP-treated groups were similar in demographic and malaria characteristics at enrollment but differed in the presence of CQ in urine and treatment failure (Table). The overall proportion of patients with gametocytes at enrollment was 25.5% (25/98). Most patients in the study developed gametocytes over 14 days of follow up, and the proportion of patients carrying gametocytes in blood peaked on day 7 (85.7% in CQ-treated and 86% in SP-treated patients) (Figure). Factors such as age, parasite density at enrollment, duration of symptoms (fever) and presence of CQ in urine were not associated with gametocyte carriage at enrollment, nor at follow up.

The geometric mean gametocyte density was comparable between groups at enrollment (61 gametocytes/ $\mu$ l in CQ-treated vs 73 gametocytes/ $\mu$ l in SP-treated patients) and on all subsequent control days. Gametocyte density also peaked on day 7 for both treatment groups, being 214.3 gametocytes/ $\mu$ l in CQ-treated and 276 gametocytes/ $\mu$ l in SP-treated patients.

The influence of therapeutic failure on the presence of gametocytes was assessed among CQ-treated patients. It was found that the risk of having gametocytes on day

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TABLE  
Demographic and malaria characteristics at enrollment, and therapeutic response in patients treated with chloroquine (CQ) or sulfadoxine/pyrimethamine (SP)

Characteristic	CQ n = 42 (%)	SP n = 56 (%)
Males	23 (55)	33 (59)
Mean of age (years)	23.3	19.7
1-14	15 (36)	23 (41)
15-44	24 (57)	28 (50)
45+	3 (7)	5 (9)
Mean (SD) of days of symptoms (fever)	6 (4.2)	5.8(5.2)
Presence of CQ in urine	11/28 (69)	5/32 (31)
Geometric mean of asexual parasitaemia/ $\mu$ l (range)	4,275 (1,000 - 35,399)	4,897 (1,000 - 26,668)
Presence of gametocytes at enrollment	12 (29)	13 (23)
Therapeutic failure	18 (43)	3 (5)

14 was higher for those patients who presented therapeutic failure than for those who respond to treatment RR = 1.26 (95% CI 1.10-1.45) (Figure). There were no differences in gametocyte density between CQ therapeutic failure and therapeutic response groups during the follow up (data not shown).

### DISCUSSION

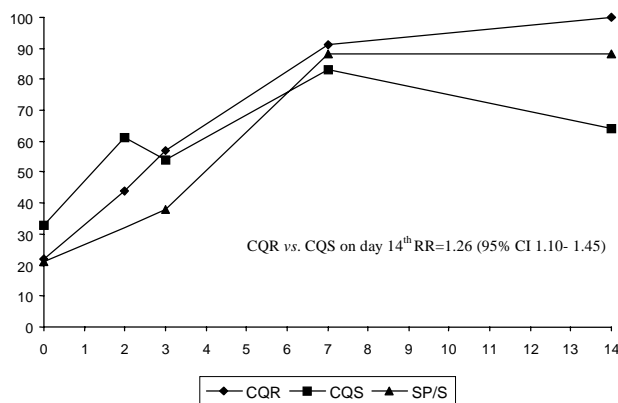
In the present study a high proportion of patients (overall 85%) carried gametocytes after treatment with either CQ or SP. Hence, the increased prevalence of gametocyte carriers seen in SP-treated vs CQ-treated patients reported by others was not detected (Robert et al. 2000, von Seidlein et al. 2001). However, the higher risk of carrying gametocytes in patients harboring CQ-resistant parasites was confirmed (Robert et al. 1996).

The proportion of CQ-treated patients who developed gametocytes was higher in our study than those reported both in Senegal (hypoendemic area) (Robert et al. 2000) and Gambia (hyperendemic area) (von Seidlein et al. 2001). The study conducted in Senegal and ours were comparable with respect to the level of CQ resistance, age of participants, duration of symptoms prior to diagnosis, and method for detecting gametocytes. Therefore, other factors such as differences in the propensity of strains to produce gametocytes (Buckling et al. 1999) or prevalence of anaemia, which has been identified as an independent risk factor for gametocyte carriage (Price et al. 1999), are more likely to explain the difference in prevalence of gametocyte carriers between studies. Haematocrit levels were not measured in the present study.

In contrast to the 62.2% gametocyte carriers on day 7 of follow up found in Senegal and 85.7% in our study, in Gambia only 18% of CQ-treated patients carried gametocytes on day 7. The lower prevalence of gametocyte carriers could be explained in part by the lower level of CQ resistance (30.2% vs 58% in Senegal and 43% in Quibdó, Colombia) and the smaller number of thick-blood fields examined in the Gambian study (100 fields instead of 200 fields). Measuring the effect of antimalarials on gameto-

cytes, in addition to their therapeutic efficacy, is becoming more important due to the potential impact of gametocyte development on the spread of drug resistance. Therefore, methods to detect the presence of live gametocytes as well as gametocyte density require standardization.

Although infectivity to mosquitoes seems to be lower in SP-treated rather than CQ-treated patients, whether CQ or SP enhances infectivity to mosquitoes is still controversial (Hogh et al. 1998, Robert et al. 2000, Targett et al. 2001). There is more agreement on the selective advantage of CQ-resistant over CQ-sensitive parasites in terms of transmission, based on both prevalence of gametocyte carriers and infectivity to mosquitoes (Hogh et al. 1998, Robert et al. 2000). As in the present report, most studies have found that the proportion of CQ-treated patients harboring gametocytes is higher among those presenting therapeutic failures than patients with adequate therapeutic response (Robert et al. 1996, 2000, von Seidlein et



CQR= Patients with therapeutic failure to CQ  
CQS= Patients with adequate therapeutic response to CQ  
SP/S= Patients with adequate therapeutic response to SP

Proportion of gametocyte carriers at enrollment and follow up per treatment and therapeutic response. CQ: chloroquine; SP: sulfadoxine/pyrimethamine

al. 2001). Nevertheless, the relative risk found in our study (1.26 95% CI 1.10-1.45) was lower than that of other reports (3.5 95% CI 1.67-7.34) (Robert et al. 2000). Although further studies are needed, these findings suggest that the transmission advantage of CQ resistant parasites, and its impact on the spread of drug resistance, could vary geographically due to differences in the proportion of gametocyte carriage in patients harboring CQ-susceptible parasites. If this is the case, it would in part explain the lower speed with which CQ resistance has increased in some regions.

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#### REFERENCES

- Buckling A, Ranford-Cartwright L, Miles A, Read AF 1999. Chloroquine increases *Plasmodium falciparum* gametocytogenesis *in vitro*. *Parasitology* 118: 339-346.
- Hogh B, Gamage-Mendis A, Butcher G, Thompson R, Begtrup K, Mendis C, Enosse S, Dgedge M, Barreto J, Eling W, Sinden R 1998. The different impact of chloroquine and pyrimethamine/sulfadoxine upon the infectivity of malaria species to the mosquito vector. *Am J Trop Med Hyg* 58: 176-182.
- Osorio LE, Giraldo LE, Grajales LF, Arriaga AL, Andrade AL, Ruebush II T, Barat L 1999. Assessment of therapeutic response of *Plasmodium falciparum* to chloroquine and sulfadoxine/pyrimethamine in a low malaria transmission area in Colombia. *Am J Trop Med Hyg* 61: 968-972.
- Price R, Nosten F, Simpson J, Luxemburger C, Phaipun L, ter Kuile F, van Vugt M, Chongsuphajaisiddhi T, White N 1999. Risk factors for gametocyte carriage in uncomplicated falciparum malaria. *Am J Trop Med Hyg* 60: 1019-1023.
- Robert V, Awono-Ambene H, Le Hesran J, Trape J 2000. Gametocytemia and infectivity to mosquitoes of patients with uncomplicated *Plasmodium falciparum* malaria attacks treated with chloroquine or sulfadoxine plus pyrimethamine. *Am J Trop Med Hyg* 62: 210-216.
- Robert V, Molez J, Trape J 1996. Short report: gametocytes, chloroquine pressure, and the relative parasite survival advantage of resistant strains of falciparum malaria in West Africa. *Am J Trop Med Hyg* 55: 350-351.
- Targett G, Drakeley C, Jawara M, von Seidlein L, Coleman R, Deen J, Pinder M, Doherty T, Sutherland C, Walraven G, Milligan P 2001. Artesunate reduces but does not prevent posttreatment transmission of *Plasmodium falciparum* to *Anopheles gambiae*. *J Inf Dis* 183: 1254-1259.
- von Seidlein L, Jawara M, Coleman R, Doherty T, Walraven G, Targett G 2001. Parasitaemia and gametocytaemia after treatment with chloroquine, pyrimethamine/sulfadoxine combined with artesunate in young Gambians with uncomplicated malaria. *Trop Med Int Health* 6: 92-98.