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SHORT REPORT: GAMETOCYTES, CHLOROQUINE PRESSURE, AND THE RELATIVE PARASITE SURVIVAL ADVANTAGE OF RESISTANT STRAINS OF FALCIPARUM MALARIA IN WEST AFRICA

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Abstract. Patients with *Plasmodium falciparum* infections were selected with an in vivo chloroquine sensitivity assay. Fourteen days after treatment, the gametocytes were studied in relation to asexual parasite responses classified as drug-sensitive or showing RI or RII resistance. Gametocyte prevalence and density appeared significantly higher in RII than RI strains and higher in RI than in sensitive strains. This finding on gametocyte variation in vivo may explain why the RII type of chloroquine resistance has became more prevalent than RI everywhere in tropical Africa in the short time since its emergence. The biological and epidemiologic advantage of chloroquine-resistant malaria mediated through gametocytes is discussed in the context of the present drug pressure in Africa.

In the city of Dakar, Senegal, a hypoendemic area for malaria where chloroquine-resistant strains of *Plasmodium falciparum* have been detected since 1988[1,2] and have now reached a prevalence of 45%, we studied gametocyte numbers among sensitive (S) and RI and RII resistant parasites. In vivo chloroquine sensitivity assays, at a standard dose of 25 mg of chloroquine/kg of body weight, distributed over three days, were performed in 68 individuals (ages 2–70 years, mean 19.4, median 17) and blood slides were taken on days 0, 2, 4, 7, and 14. Thick blood films were Giemsa-stained and 200 microscopic oil-immersion fields were examined (corresponding to about 0.5 µl of blood),[3] from which the gametocyte density was estimated. The parasitologic incidence was low (about 0.6/person/year).[4]

Results were 42 chloroquine-sensitive responses (62%), 11 RI (16%), and 15 RII (22%). The prevalence rates of *P. falciparum* gametocytes in the three groups were similar on days 0 and 2; however, on days 4 and 7, resistant strains had double the rate of sensitive ones and on day 14, it was three times higher (Figure 1) ($P = 0.038$ on day 4, $P = 0.001$ on day 7, and $P < 10^{-4}$ on day 14, by Fisher's exact test). Combined data from days 7 and 14 showed that 30% of the patients with sensitive strains presented with gametocytes, as compared with 79% in patients with resistant strains ($P < 10^{-4}$, by Fisher's exact test); the relative proportions in RI and RII strains were 59% and 93%, respectively ($P = 0.005$, by Fisher's exact test).

Gametocyte densities on days 4, 7 and 14 were lower in sensitive as compared with both RI and RII strains (Figure 2). Combined data from days 7 and 14 showed that the geometric mean numbers of gametocytes from patients with sensitive, RI, and RII strains were, respectively, 0.7, 2.2, and 12.6/µl of blood (for S-RI, S-RII, and RI-RII comparisons; $P = 0.026$, $P < 10^{-4}$, and $P = 0.0006$, respectively, by the Mann-Whitney U test).

Although therapeutic responses were linked with age (age S > RI > RII; 21.8, 16.1, and 15.0 years old, respectively), a stratification in relation to age ruled out any influence of age on gametocyte prevalences and densities as measured by in vivo response. For children less than 15 years of age (n = 25), combined data from days 7 and 14 showed that gametocyte prevalences and densities of sensitive and resistant strains were significantly different ($P < 10^{-4}$, by Fisher's exact test for prevalences and $P < 10^{-4}$, by the Mann-Whitney U test for densities). A similar result was obtained for those 15 years of age and older (n = 43) ($P = 0.002$ by Fisher's exact test and $P = 0.002$ by the Mann-Whitney U test).

Chloroquine is not thought to induce *P. falciparum* gametocytogenesis[5] or to interfere with transmission once gametocytes have appeared in the bloodstream[6–8] despite the higher infectivity of chloroquine-resistant strains in the rodent parasites *P. berghei* and *P. yoelii nigeriensis*.[9,10]

Most studies of the response of *P. falciparum* to chloroquine have focused on asexual stages, with gametocyte responses rarely mentioned and not considered separately by the in vivo response. In areas of chloroquine resistance,

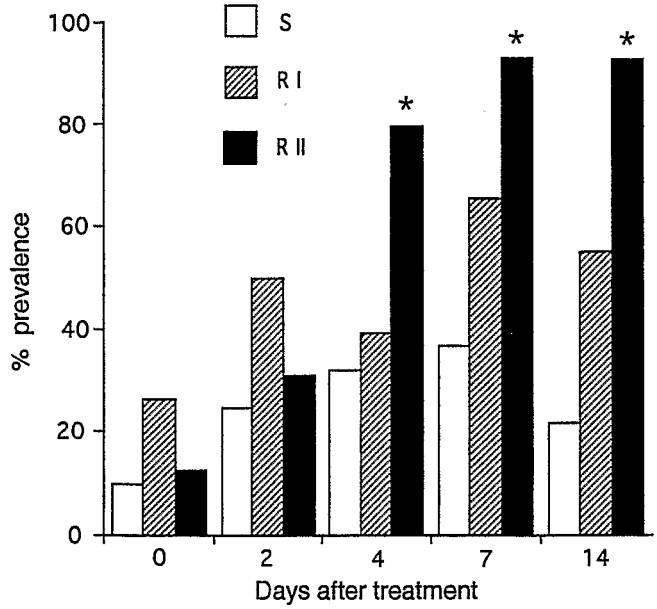

FIGURE 1. Evolution after treatment with chloroquine of gametocyte prevalence in the blood of patients with falciparum malaria attacks. Asterisks indicate significant differences ($P < 0.05$, by Fisher's exact test) from sensitive (S) patients (n varied on the different days of testing between 40 and 42 for S patients, 10 and 11 for RI patients, and 13 and 15 for RII patients). R = resistant.

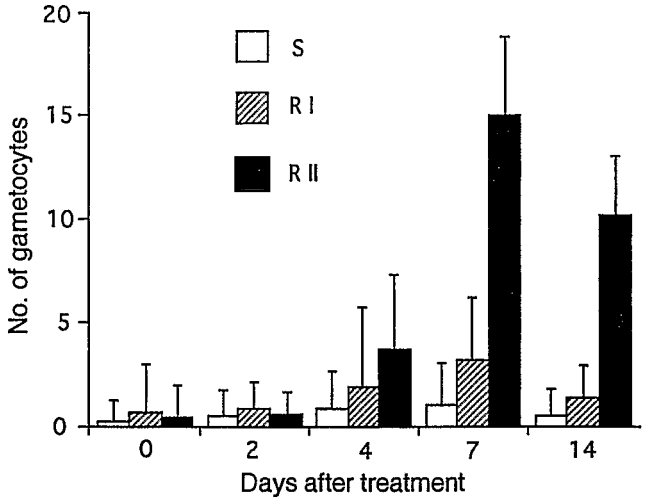

FIGURE 2. Evolution after treatment with chloroquine of the geometric mean gametocytemia per microliter of blood on patients with falciparum malaria attacks (upper bars indicate the standard deviation). S = sensitive; R = resistant.

data from Mozambique showed an increase in gametocytes on day 7 post-treatment, but this was not seen in Punjab.[11,12]

Thorough observations on gametocytes are often difficult to extrapolate in terms of transmission, it is clearly established that gametocyte density is a critical factor in their infectivity to anophelines.[7] Our observations suggest that when sensitive and RI or RII resistant strains coexist in a human population, the careful use of treatment with chloroquine leads to preferential transmission of the most resistant parasites. This may give a clue as to why the RII type of chloroquine resistance has became more prevalent than that of RI everywhere in tropical Africa in the short time since its emergence.

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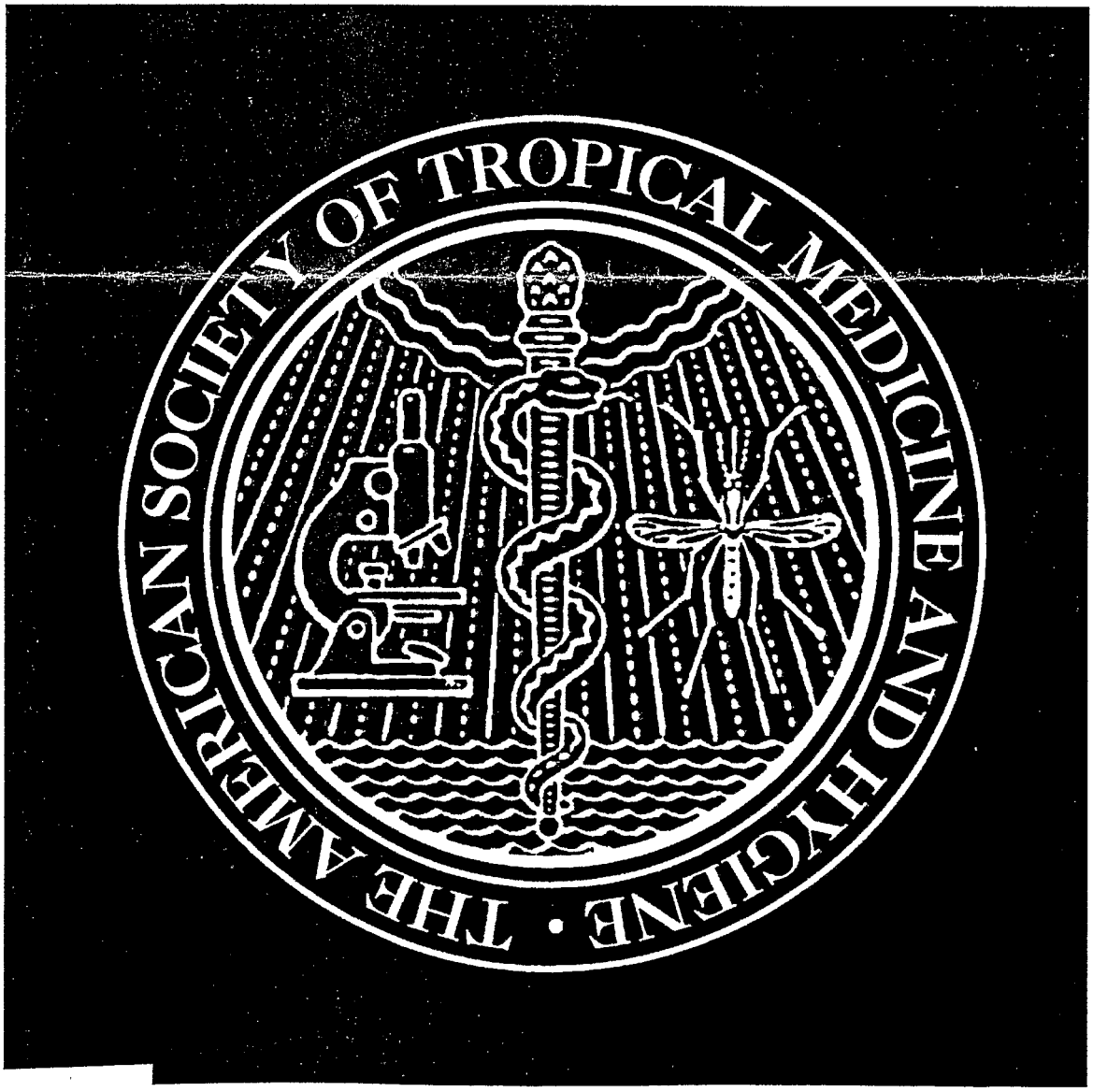

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