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RISK FACTORS FOR GAMETOCYTE CARRIAGE IN UNCOMPLICATED FALCIPARUM MALARIA

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Abstract. The factors affecting the development of patent *Plasmodium falciparum* gametocytemia were assessed in 5,682 patients entered prospectively into a series of antimalarial drug trials conducted in an area of low and seasonal transmission on the western border of Thailand. Of the 4,565 patients with admission thick smear assessments, 110 (2.4%) had gametocytemia. During the follow-up period 170 (3%) of all patients developed patent gametocytemia, which in 89% had developed by day 14 following treatment. In a multiple logistic regression model five factors were found to be independent risk factors at presentation for the development or persistence of gametocytemia during follow up; patent gametocytemia on admission (adjusted odds ratio [AOR] = 7.8, 95% confidence interval [CI] = 3.7–16, $P < 0.001$), anemia (hematocrit <30%) (AOR = 3.9, 95% CI = 2.3–6.5, $P < 0.001$), no coincident *P. vivax* malaria (AOR = 3.5, 95% CI = 1.04–11.5, $P < 0.04$), presentation with a recrudescent infection (AOR = 2.3, 95% CI = 1.3–4.1, $P < 0.004$), and a history of illness longer than two days (AOR = 3.3, 95% CI = 1.7–6.6, $P < 0.001$). Patients whose infections responded slowly to treatment or recrudesced subsequently were also more likely to carry gametocytes than those who responded rapidly or were cured (relative risks = 1.9, 95% CI = 1.3–2.7 and 2.8, 95% CI = 2.0–4.0, respectively; $P < 0.001$). These data provide further evidence of important epidemiologic interactions between *P. falciparum* and *P. vivax,* and drug resistance and transmission potential.

The life cycle of *Plasmodium falciparum* is dependent upon the production of viable sexual stages of the parasite and their appearance in sufficient numbers in the peripheral blood of the human host. These gametocytes can then be transmitted to a feeding anopheline mosquito and on to a new human host, thus completing the life cycle of the malaria parasite. In contrast to the treatment of the three other species of human malaria, most of the antimalarial drugs used to treat the asexual stages of *P. falciparum* malaria have little or no effects on the viability of mature gametocytes. The rate of production of gametocytes is influenced considerably by host factors, notably immunity,[1] and this may have significant epidemiologic consequences. Integrated malaria control programs that aim to reduce malaria transmission are often based on identifying those individuals most likely to transmit malaria. We investigated the factors that influence the production of gametocytes following uncomplicated falciparum malaria in an area where there is a high prevalence of multidrug resistance, and a relatively low level of transmission and consequent background immunity.

METHODS

Study site. The study took place between 1990 and 1995 in patients living in a camp for displaced person of the Karen ethnic minority situated in an area of malarious hill forest on the western border of Thailand. During the five-year period, a series of 18 antimalarial drug studies were conducted to determine the optimum treatment of falciparum malaria in the face of a continuing decrease in mefloquine sensitivity. The epidemiology of malaria at this site has recently been described in detail.[2] Transmission of malaria is low (each person experiencing approximately one vivax and one falciparum malaria infection every two years) and seasonal in this area. Nearly all falciparum malaria infections are symptomatic, and the treatments of every episode have been documented.

Patients. Patients of all ages were recruited into these studies provided that they or their accompanying relatives gave fully informed consent. Each of the studies was approved by the Ethics Committee of the Faculty of Tropical Medicine of Mahidol University. All patients had slide-confirmed falciparum malaria. Pregnant women, children weighing < 5 kg, and patients with signs of severity or concomitant disease requiring hospital admission were all excluded. On admission, a questionnaire was completed recording details of symptoms and their duration, and the history of previous antimalarial medication (since health structures in the camp are the only source of antimalarial drugs in this area, the history is generally a reliable guide to pretreatment). A full clinical examination was also completed and blood was taken for routine hematology and quantitative parasite counts.

Drug treatment significantly affects the subsequent development of gametocytemia[3] at this study site, and for this reason patients in this study were categorized into four antimalarial treatment groups for the purposes of this analysis: mefloquine (single or split dose without artemisinin derivatives), halofantrine (high or low dose), artemisinin derivatives (artesunate or artemether with and without mefloquine), and quinine (Table 1). Drug administration was observed in all cases. All patients were examined daily until they became asymptomatic and aparasitemic, and were then seen weekly during the follow-up period. Before 1993, this was for was four weeks, and since then for nine weeks. A blood smear was taken at each weekly visit or if symptoms returned between follow-up appointments. Recrudescent infections were treated with a seven-day regimen of quinine sulfate (30 mg of salt/kg/day) in combination with tetracycline (16 mg/kg/day) if more than eight years old, or more recently with a seven-day regimen of artesunate or artemether (12 mg/kg given over a seven-day period).

TABLE 1
Antimalarial treatments used

Drug and regimen	Total	Dose*
Mefloquine		
M_{25}	n = 1,302	25 mg/kg
Halofantrine		
H_{24}	n = 106	24 mg/kg in 1 day
H_{72}	n = 357	72 mg/kg over 3 days
Artesunate + Mefloquine		
MA	n = 323	As 10 mg/kg (3 doses in 1 day) + M15
MAS1	n = 152	As 4 mg/kg (1 dose) + M25
MAS3	n = 1,968	As 4 mg/kg daily for 3 days + M25
MAS5	n = 57	As 12 mg/kg (over 5 days) + M25
MAS7	n = 139	As 12 mg/kg (over 7 days) + M25
Artesunate		
AS5	n = 159	12 mg/kg over 5 days
AS7	n = 477	12 mg/kg over 7 days
Artemether		
MAM3	n = 199	Am 4 mg/kg/day for 3 days + M25
AM7	n = 206	12 mg/kg over 7 days
Quinine ± tetracycline		
Q7	n = 38	Q 30 mg/kg/day for 7 days
Q7T7	n = 199	Q 30 mg/kg/day for 7 days + T16 mg/kg/day

* As = artesunate; M15 = mefloquine (15 mg/kg); M25 = mefloquine (25 mg/kg); Am = artemether; Q 30 = quinine, 30 mg/kg; T16 = tetracycline, 16 mg/kg.

Parasite counting. Parasite counts were made on Giemsa-stained thick and thin blood films and parasitemia was expressed as the number of parasitized erythrocytes per 1,000 red blood cells or the number of parasites seen on the thick film per 500 white blood cells. Thin films were counted if the thick film exceeded 1,000 parasites per 500 white blood cells. Parasite counts on thick films were calculated assuming a total white blood count of 8,000/μl. Gametocyte counts were made only on thick blood films. Those instances where parasite counts were recorded per 1,000 red blood cells (i.e., parasitemias >0.5%) were therefore regarded as missing data when assessing gametocytemia in these series.

Data analysis. Data were analyzed using the statistical programs SPSS for Windows (SPSS Institute, Chicago, IL) and Epi-Info 6 (Centers for Disease Control and Prevention [Atlanta, GA] Public Domain Software). Normally distributed continuous data were compared by Student's *t*-test and analysis of variance. Data not conforming to a normal distribution were compared by the Mann-Whitney U test and Kruskal-Wallis analysis of variance. The association between two continuous variables was assessed using Spearman's rank correlation coefficient.

Following antimalarial treatment, gametocyte carriage was assessed on days 7 and 14, and expressed as the proportion of patients with patent gametocytemia (gametocyte positivity rate [GPR]). A multiple logistic regression model was used to determine adjusted odds ratios (AORs) for risk factors for gametocyte carriage on admission and the variables associated with the GPR. Any variables found to be associated significantly with the dependent variable in a univariate analysis were entered into the equation and the model was constructed using a forward stepwise analysis using the Wald statistic. In this area, there are significant differences in gametocyte carriage following different treatment regimens.[3] For this reason, independent risk factors were assessed after stratifying by the four treatment categories to account for potential drug effects.

RESULTS

Between November 1990 and December 1995, 5,682 patients (3,200 males and 2,482 females) were enrolled into 18 different antimalarial drug studies and completed their course of treatment. Of these, 896 (16%) were less than five years old, 2,671 (47%) were 5–14 years old, and 2,115 (37%) were more than 14 years old. Overall, 4,158 (73%) were primary infections and 1,524 were recrudescences of earlier falciparum malaria. The geometric mean asexual parasitemia on presentation was 5,769/μl (95% CI = 5,453–6,104). Mixed *P. falciparum* and *P. vivax* infections occurred in 880 (16%) cases. Follow-up was achieved in 4,642 (82%) patients to day 28 and in 2,853 (50%) to day 42.

Gametocytes on admission. Of the 4,565 patients who had thick smears taken on admission, 110 (2.4%) had detectable gametocytemia at this time (Table 2). The geometric mean gametocyte density was 175/μl (95% CI = 133–230). The median fractional gametocyte density in these patients (gametocyte count divided by total asexual and sexual parasite count) was 8.2% (interquartile range [IQR] = 2.4–35). The fractional gametocyte density was correlated positively with age (r_s = 0.23, P = 0.02) and hematocrit (r_s = 0.26, P = 0.01). Patients presenting with pure *P. falciparum* infections had higher fractional gametocyte counts than those with mixed infections (median [IQR%] = 8.9 [2.6–37.1] versus 1.6 [0.6–5.7], P = 0.02) as did patients presenting without a fever compared with febrile patients (median [IQR]% = 9.7 [3.0–41.5] versus 4.8 [1.5–16.1], P = 0.03).

There were five independent risk factors for a patent gametocytemia on admission: presenting without fever (AOR =1.9 [95% CI = 1.2–3.1], P = 0.006), low asexual parasit-

TABLE 2
Gametocyte carriage on admission and after treatment

Drug and regimen	Total	Gametocyte carriage on admission*	Gametocyte positivity rate
Mefloquine	1,302	1.9% (23/1,221)	8.7% (78/899)
Halofantrine	463	1.5% (7/454)	3.3% (12/365)
Artemisinin derivatives	3,680	2.8% (77/2,712)	1.5% (36/2,329)
Quinine ± tetracycline	237	1.7% (3/178)	22.1% (25/113)
Total	5,682	2.4% (110/4,565)	4.1% (151/3,706)

* Only patients with thick smears on admission.

emia (< 1,000/μl) (AOR = 1.8 [95% CI = 1.1–2.8], P = 0.02) (Figure 1), anemia (hematocrit < 30%) (AOR = 5.5 [95% CI = 3.5–8.7], $P < 0.001$), pure *P. falciparum* infections rather than mixed *P. falciparum* and *P. vivax* infections (AOR = 4.4 [95% CI = 1.4–14.3], $P = 0.01$), and a palpable spleen (AOR = 1.7 [95% CI = 1.1–2.8], $P = 0.02$). Age and a prolonged history of illness were not significant risk factors in this analysis.

Gametocytemia following treatment. Overall 170 patients had gametocytes noted during follow-up: 6.6% (86 of 1,302) in those receiving mefloquine, 2.6% (12 of 463) in those receiving halofantrine, 11.3% (27 of 237) in those receiving quinine, and 1.2% (45 of 3,680) in those patients receiving an artemisinin derivative. Data from some of these cases have been previously reported.[3] Although in 20 (12%) cases gametocytes were present on more than one visit, by day 14, 88% (151 of 170) of the patients who were to develop gametocytemia during the follow-up period had already done so (Table 2), and by day 28 this proportion had increased to 95% (162 of 170). Because of the transient nature of gametocytemia, 35% (1,976 of 5,682) of the patients who missed their follow-up appointments on either day 7 or day 14 had to be excluded from the calculation of the cumulative GPR. Compliance to early follow-up was slightly worse in males compared with females (63% versus 68%, respectively; $P < 0.001$), in recrudescent infections compared with primary infections (63% versus 66%; $P = 0.04$), and in pure *P. falciparum* infections compared with mixed infections (64% versus 70%; $P = 0.001$), but for none of the other risk factors examined. The geometric mean maximum gametocytemia in patients with a patent gametocytemia during follow-up was 228/μl (95% CI = 183–285). The following were independent risk factors for the presence of gametocytes during the post-treatment follow-up period: patent gametocytemia or anemia on admission, recrudescent infections, pure *P. falciparum* infections, and prolonged preadmission history of fever (Table 3). Patients were therefore categorized as potential gametocyte producers if they had a pure *P. falciparum* infection with a prolonged history of fever, and one of the following: anemia on admission, gametocytes on admission, and presentation with or subsequent development of a recrudescent infection. This categorization predicted subsequent patent gametocytemia with a sensitivity of 53% and a specificity of 82%.

Effects of the therapeutic response. Delay in the time taken to clear the initial parasitemia increased the risk of subsequent gametocyte carriage significantly ($\chi^2 = 95$, $P < 0.001$). After stratifying by treatment group, patients still parasitemic 48 hr after starting treatment were 1.9 (95% CI = 1.3–2.7]) times more likely to become gametocytemic (assessed at day 14) compared with those who cleared their parasitemias more rapidly ($P = 0.001$). Patients whose infections recrudesced subsequently were also more likely to become gametocytemic during follow up (relative risk after stratifying by treatment group = 2.8 [95% CI = 2.0–4.0], $P < 0.001$).

DISCUSSION

The gametocytes of *P. falciparum* develop over a period of approximately 10 days.[4,5] Most of this development occurs in the microvasculature of the deep tissues. Gametocytogenesis is thought not to begin at the onset of the asexual infection, but requires sufficient exposure of the asexual parasite population to a critical parasite density for a developmental switch to occur.[6] Longer established infections with falciparum malaria are therefore more likely to produce gametocytes both *in vivo*[7] and *in vitro*.[1] Several factors have been shown to trigger the production of gametocytes, including the clinical manifestations of malaria,[8] drug treatment,[9] and hemolysis of infected erythrocytes.[10]

In the natural course of falciparum malaria, the peak of gametocytemia follows 7–10 days after the peak of asexual parasitemia. As the asexual parasite count plateaus with control of the infection, the gametocyte count increases. This leads to an increase in the proportion of parasites in the

TABLE 3
Population attributable risk (PAR) for gametocyte carriage during follow-up, after stratifying by treatment group

Risk factor	Frequency	AOR* (95% CI)	PAR%	*p*
Gametocytes on admission	2.4%	7.8 (3.7–16)	14.0	<0.001
Anemia on admission	18%	3.9 (2.3–6.5)	34.3	<0.001
Pure *Plasmodium falciparum* infections	84%	3.5 (1.04–11.5)	67.7	0.04
Recrudescent infections	27%	2.3 (1.3–4.1)	26.0	0.004
Prolonged preadmission history	64%	3.3 (1.7–6.6)	59.5	<0.001

* Adjusted odds ratio (AOR) and 95% confidence interval (95% CI) of the risk associated with the presence of the factor when compared with its absence after adjustment for the other factors in the table. The AORs were calculated by logistic regression analysis.

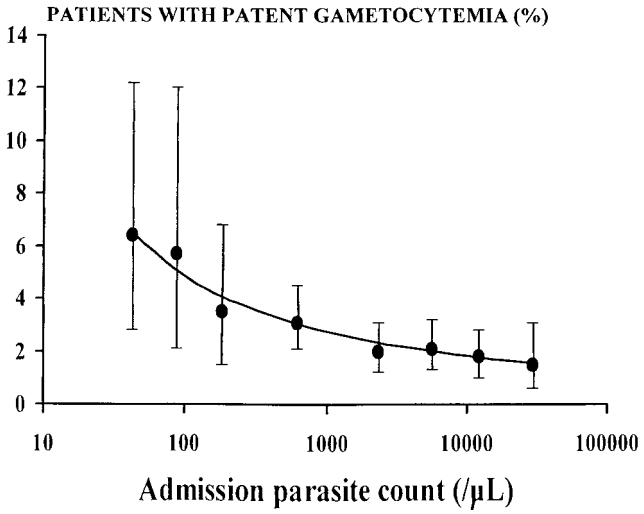

FIGURE 1. Relationship of the risk of patent gametocytemia on admission with the presenting asexual parasitemia (means and 95% confidence intervals).

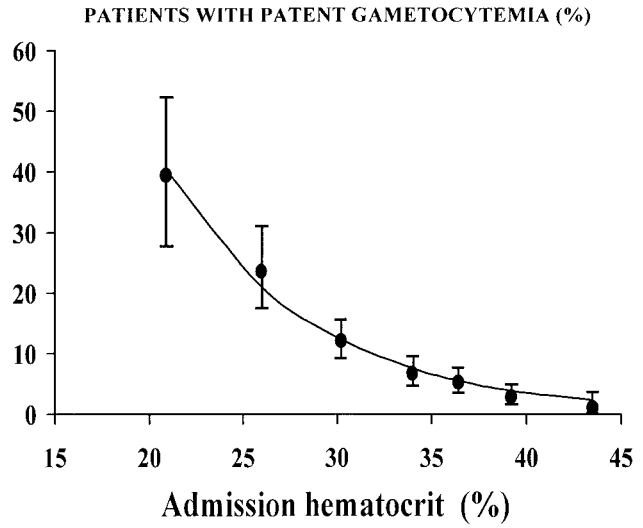

FIGURE 2. Relationship of the risk of patent gametocytemia at any time with the presenting hematocrit (means and 95% confidence intervals).

blood smear that are gametocytes.[11] The sexual stages are not pyrogenic, and are commonly seen in subjects without fever, in whom the asexual count has decreased below the pyrogenic density. In this study, 2.4% of the patients had gametocytes present on admission. Patients who were afebrile and presented with a low asexual parasitemia were 2.7 (95% CI = 1.8–4.0) times more likely to present with a patent gametocytemia compared to those without both of these factors ($P < 0.001$) (Figure 1). This subgroup of patients may have been harboring the blood stage infection for longer. This is consistent with the finding that anemia and splenomegaly were also associated with an increased risk of gametocyte carriage (Figure 2). Five factors were associated with the appearance of gametocytes during follow up (Table 2). Three of these factors (anemia on presentation, recrudescent infections, and a prolonged preadmission history of fever), could all be construed as markers of a prolonged infection. Once *P. falciparum* gametocytogenesis has been triggered, then a fraction of each parasite generation undergoes sexual differentiation with each successive cycle.[12] This presumably accounts for the observation that the presence of gametocytes on admission was associated with a nearly eight-fold increase in subsequent gametocyte carriage during follow-up.

Patients with a mixed *P. falciparum* and *P. vivax* infections were four times less likely to present with a patent gametocytemia when compared with pure *P. falciparum* infections. This effect persisted after correcting for differences in age and baseline parasitemia. Furthermore, if gametocytemia was present then the density of these sexual stages was lower in mixed infections. The pyrogenic density of *P. vivax* is significantly lower than that of *P. falciparum*[2] and this may bring patients with coexistent vivax malaria to medical attention earlier. Indeed, in this study patients with mixed infections were 1.3 (95% CI = 1.2–1.4) times more likely to present with a shorter history of fever (less than 2 days) compared with patients with pure *P. falciparum* infections ($P < 0.001$). Mixed infections also decreased subsequent post-treatment gametocyte carriage by more than three-fold, independent of the length of pre-admission history of fever and presenting parasitemia. This further supports the supposition that coinfection with *P. vivax* may attenuate infections with *P. falciparum*.[13] Whether this is because of earlier presentation to medical attention, and thus treatment (with drugs that affect immature gametocytes), or a more specific inhibition of *P. falciparum* gametocytogenesis, is not clear.

As drug resistance takes hold within a parasite population, there is a delay in the time taken to clear the peripheral parasitemia and an increase rate of recrudescence. Both these parameters of response to antimalarial treatment were associated significantly with subsequent gametocyte production. The increase in gametocyte carriage with resistant infections must be an important factor driving resistance of *P. falciparum*. [3,14]

Once formed, the sexual stages often persist for up to three weeks and show a persistent ability to infect mosquitoes.[15,16] Although asexual and immature gametocytes of *P. falciparum* show similar sensitivity to most antimalarials, after about the sixth day of maturation the gametocytes become less susceptible to drug action.[16] Artemisinin and its derivatives may have a broader stage specificity, but the only drugs that kill mature gametocytes of *P. falciparum* reliably are the 8-aminoquinolines (primaquine, pamaquine, and tafenaquine)[17] The principal drawback of primaquine is that is causes hemolysis in patients with glucose-6-phosphate dehydrogenase deficiency and requires an extended treatment course (14 days). In this study, potential gametocyte producers could be predicted with a sensitivity of 53% and a specificity of 82%. Eliminating gametocytemia in these high risk patients could help rationalize health care initiatives aimed at reducing malaria transmission. Alternatively the widespread use of artemisinin derivatives could be advocated, reducing gametocyte carriage, reducing the development of resistance, and in areas of low endemicity, reducing the incidence of malaria.[3,14,18]

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