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DYNAMICS OF *P. FALCIPARUM* GAMETOCYTEMIA IN SYMPTOMATIC PATIENTS IN AN AREA OF INTENSE PERENNIAL TRANSMISSION IN TANZANIA

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Abstract. We investigated the dynamics of *Plasmodium falciparum* gametocytemia in symptomatic patients attending a local dispensary in the Kilombero district, Tanzania. Consenting individuals aged one and above, with varying asexual and sexual parasitemias were treated appropriately and asked to return weekly for 28 days. Gametocyte prevalence was highest on Day 7 of follow-up in all age groups (overall 30.5%). Multifactorial analysis showed that young age ($\chi^2 = 18.4$; $P = 0.004$), high asexual parasitemia on presentation ($\chi^2 = 19.4$; $P = 0.0007$) and gametocyte positivity on presentation ($\chi^2 = 29.4$; $P = 0.001$) were all significantly associated with the presence of gametocytes on Days 7 and 14 of follow-up. High presentation of asexual parasitemia alone was positively correlated with higher gametocyte densities on both days of follow-up ($F_{4, 297} = 2.0$; $P = 0.049$). Gametocyte incidence rates decreased significantly with age ($\chi^2 = 7.6$, $P < 0.005$). In summary, in this group of chloroquine-treated individuals, gametocyte prevalence and incidence rates decreased with age, while densities remained relatively constant.

INTRODUCTION

The successful transmission of malarial parasites from humans to mosquitoes depends on the availability of mature infectious gametocytes in the peripheral blood. Gametocyte carriage can therefore be used as an estimate of transmission potential of malaria parasites from humans to mosquitoes. A reduction in transmission can be achieved through the removal of gametocytes from the circulation or by reducing the infectivity of gametocytes to mosquitoes. Such a reduction can be achieved through the use of drugs such as primaquine or a transmission-blocking vaccine.¹

Knowledge of infectiousness of humans to mosquitoes in endemic areas is necessary when assessing the effects of control measures aimed at reducing the transmission of malaria. The recent advances in the development of transmission-blocking vaccines have stimulated the study of factors affecting transmission of the parasite from humans to mosquitoes.² The density of gametocytemia is one factor which may influence a person's ability to infect the vector. A positive correlation has been shown between patent gametocytemia and infectiousness of the host to mosquitoes.³ Antimalarial drugs may also affect infectivity by enhancing rates of gametocyte production and/or the infectivity of gametocytes to the vector.^{4–6}

Although the dynamics of *Plasmodium falciparum* gametocytemia have been studied in experimentally induced infections,^{1,7,8} little is known about gametocyte production in endemic populations. Existing data are largely from community-based surveys of children^{9,10} and cross-sectional surveys in all age groups.^{11–14}

This study investigated the dynamics of gametocyte production in parasitemic individuals. Symptomatic individuals with *P. falciparum* parasites were recruited and followed-up for a period of 28 days. During this period, the presence, density, and persistence of peripheral blood gametocytemia were examined for association with factors including the patients' age, asexual parasitemia on presentation, and treatment.

MATERIALS AND METHODS

Study area and population. This study was conducted in Kibaoni dispensary, on the edge of Ifakara town in the Kil-

ombero district in Southwestern Tanzania. Most residents are subsistence farmers, the majority of whom grow corn and rice in rain-fed paddies. Houses are typically made of thatched roofs and mud walls. A more detailed description of the study area can be found in Tanner and others.¹⁵ Transmission of *P. falciparum* malaria is intense and perennial, and *Anopheles gambiae* and *Anopheles funestus* are the major vectors. There is no seasonality in prevalence of malaria infections and *P. falciparum* is present in over 90% of cases.¹⁶

The study took place between April and November 1997 and April and December 1998 as part of a larger study of the epidemiology of naturally-occurring transmission-blocking immunity. Informed consent was obtained from all adult participants and from the parents or legal guardians of minors. Scientific and ethical clearance was obtained from the appropriate committees of the Tanzania Commission for Science and Technology (COSTECH), and the Ifakara Health Research and Development Centre (IHRDC).

Recruitment of patients. All patients reporting to the dispensary with various symptoms had blood taken by finger prick for a thick film smear. After drying on a heating block and staining with Giemsa, 20 fields were examined for presence of parasites using a light microscope with 100 \times oil immersion lens and 10x ocular eyepieces. Fever status of patients was initially recorded, but after observing a concordance of less than 5% between reported and actual elevated temperatures on presentation (axillary temp $> 37.5^\circ\text{C}$), we decided to merely record reports of fever.

Patients with positive slides for asexual parasites were given the first-line antimalarial chloroquine (25 mg/kg over 3 days) unless they reported previous treatment with chloroquine (CQ), in which case they were given pyrimethamine-sulphadoxine (Fansidar[®], 1.25 mg/kg of pyrimethamine and 25 mg/kg of sulfadoxine) in a single dose. The study was carefully explained to patients who presented with *P. falciparum* parasites after screening. Those who consented were given a unique identification number and asked to come back to the dispensary seven days later. On their return visit, a thick film was made and examined for gametocytes. A slide was declared negative if no parasites were seen after examination of 100 fields. Positive slides were preserved and

TABLE 1
Parasite data on presentation at the dispensary*

| Age group (yr) | No. | Asexual parasites | | Gametocytes Prevalence‡ (%) |
|----------------|-------|-------------------|--------------------|-----------------------------|
| | | Prevalence (%) | Density† >4,000/μl | |
| <1 | 945 | 50.0 | 37.8 | 8.9 |
| 1–2 | 1,294 | 62.0 | 34.4 | 10.9 |
| 3–6 | 883 | 56.3 | 21.3 | 8.7 |
| 7–15 | 864 | 43.6 | 16.7 | 5.5 |
| ≥16 | 2,594 | 26.8 | 7.2 | 4.3 |
| Total | 6,580 | 43.2 | 23.7 | 7.9 |

* No. = total number of slides read.

† Proportion of positive slides with densities greater than 4,000/μl.

‡ Proportion of positive slides with gametocytes.

transferred to the IHRDC laboratory where gametocyte density was assessed by counting against 500 leukocytes. Weekly return visits continued 14, 21, and 28 days after treatment.

Pregnant women and severely anemic or incapacitated individuals were not included in the study, and when necessary, were referred to the nearby St. Francis District Hospital. Children under the age of one year were not recruited for follow-up.

Data analysis. Descriptive statistics were calculated using Epi Info (Centers for Disease Control and Prevention, Atlanta, GA) and SPSS (SPSS, Inc., Chicago, IL) statistical software. The effects of age, sex, chloroquine-treatment outcome (CQ treatment outcome) and presentation asexual parasitemia on gametocyte positivity at Days 7 and 14 were tested using logistic regression models, allowing for correlations between repeated measures on the same individuals using random effects. Similarly, the effects of these variables on gametocyte densities at Days 7 and 14 were tested using multiple regression models fitted with the SAS MIXED procedure.¹⁷ A random effect term was also included in these models to allow for the correlations within individuals.

Age was categorized as 1–2 years, 3–6 years, 7–15 years, and ≥ 16 years. Parasite density was stratified according to World Health Organization (WHO) guidelines (WHO/MAL/82.988) and designated as: 0/μl (-ve); < 800/μl; 800–1,599/μl; 1,600–3,999/μl; 4,000–19,999/μl; ≥ 20,000/μl.

RESULTS

Recruitment of study population. A total of 6,586 people reported at the dispensary with malaria-like symptoms during the study period. Forty-three percent of the patients had positive thick films for asexual parasites, and 57% of the positive patients were children aged six years and below. The prevalence of asexual parasitemia was highest in children between the ages of 1–2 years (62%). The highest proportion of higher density asexual parasitemias (≥ 4,000/μl) was found in children less than one year old, and decreased with age. Two hundred and twenty-five individuals (8%) were also positive for gametocytes on presentation (Table 1).

Gametocyte dynamics. A total of 1,205 individuals were recruited into the study, with 64%, 43%, 31%, and 22% of these patients returning on Days 7, 14, 21, and 28, respectively. As not all patients were able to return on the specified day, for the purpose of analysis we have included all individuals who returned within two days of their appointment date. Although the number of individuals returning on Days 21 and 28 was significantly lower, age distributions among patients sampled on Day 7 and on Day 14 did not differ from those at baseline (see denominators in Table 2). Of those who had at least one return visit, 44% (382 of 865) became gametocyte positive. The point prevalence figures by day are shown in Figure 1. There was a more than two-fold increase in prevalence of gametocyte carriers between Day zero and Day seven of follow-up for all age groups. Prevalence of gametocytemia was found to be highest in the 1–2 years age group and decreased significantly with age (Day 7: χ^2 for trend = 20.6, $P = 0.0001$; Day 14: χ^2 for trend = 14.2, $P = 0.00017$). Geometric mean gametocyte densities in positive individuals, however, did not vary significantly with age (Day 7, ANOVA $F = 0.694$, $df = 3$; $P = 0.557$; Day 14, ANOVA $F = 1.234$, $df = 3$; $P = 0.297$). This was true for all age categories.

Estimates of incidence rates of gametocyte-positive cases by age are shown in Figure 2. Incidence of new cases was highest on Day 7 for all age groups. On all days of follow-

TABLE 2
Multifactorial analysis of the relationship between selected parameters and gametocyte positivity on Days 7 and 14 of follow-up*

| Exposure variable | | Day 7 | | Day 14 | | Likelihood ratio test† |
|-------------------------------|--------------|--------|---------------------|--------|---------------------|------------------------|
| | | n/N | Odds ratio (95% CI) | n/N | Odds ratio (95% CI) | |
| Age (yr) | 1–2 | 96/241 | 2.3 (1.4, 3.8) | 55/186 | 2.3 (1.2, 4.6) | $P < 0.0004$ |
| | 3–6 | 52/185 | 1.6 (0.9, 2.6) | 31/113 | 2.5 (1.2, 5.0) | |
| | 7–15 | 36/121 | 1.9 (1.1, 3.3) | 31/122 | 1.2 (0.5, 2.9) | |
| | ≥16 | 33/183 | 1.0‡ | 14/113 | 1.0‡ | |
| Presentation parasitemia/μl | <800 | 12/75 | 1.0‡ | 9/53 | 1.0‡ | $P < 0.0007$ |
| | 800–1,599 | 47/176 | 2.6 (1.2, 5.5) | 23/112 | 1.5 (0.6, 3.9) | |
| | 1,600–3,999 | 86/279 | 3.0 (1.4, 6.2) | 46/190 | 2.0 (0.8, 4.9) | |
| | 4,000–19,999 | 60/155 | 3.9 (1.8, 8.3) | 27/97 | 2.2 (0.8, 5.7) | |
| | ≥20,000 | 12/45 | 2.1 (0.8, 5.5) | 5/33 | 0.9 (0.3, 3.3) | |
| Chloroquine treatment outcome | Sensitive | 43/162 | 1.0‡ | 29/162 | 1.0‡ | $P = 0.4$ |
| | RI | 84/278 | 2.2 (1.1, 4.3) | 16/77 | 0.8 (0.3, 1.9) | |
| | RII | 47/124 | 2.0 (1.0, 3.8) | 22/79 | 0.6 (0.3, 1.5) | |
| | RIII | 17/78 | 1.6 (0.8, 3.3) | 17/54 | 0.6 (0.3, 1.3) | |

* N = total number of slides read; CI = confidence interval.

† Likelihood ratio tests from a logistic model testing effects on gametocyte positivity of the variables listed, allowing for the other variables.

‡ Reference group.

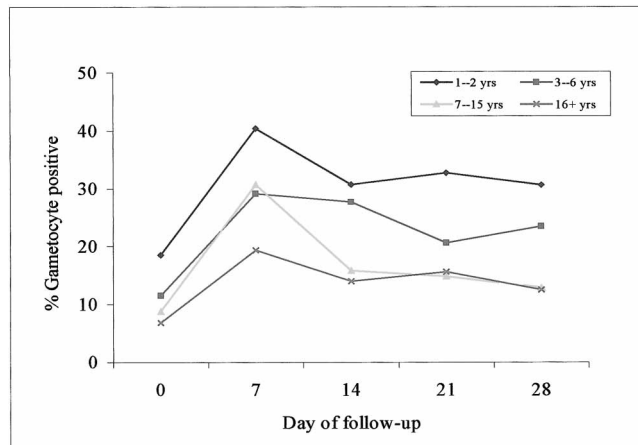


FIGURE 1. Prevalence of gametocytemia among those patients who returned at least once for a weekly blood slide. Gametocyte-positive cases for each follow-up day are expressed as a percentage of all recruits returning for each visit. Of the 1,205 individuals recruited, 770 returned on Day 7, 514 on Day 14, 370 on Day 21, and 270 on Day 28.

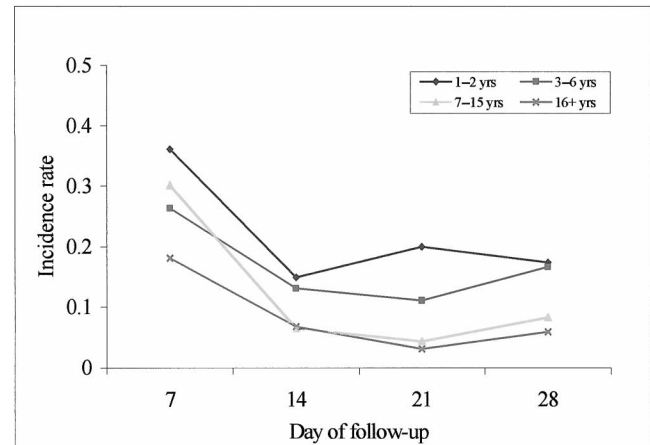


FIGURE 2. Incidence rate of gametocytemia, the occurrence of new gametocyte-positive cases during follow-up. This was estimated using the number of previously gametocyte-negative individuals who became positive, divided by the total number of gametocyte-negative individuals for each weekly time point.

up, incidence was highest in the youngest age group and showed an age-dependent decrease. Mean incidence rates for the follow-up period decreased significantly with age (χ^2 for trend = 20.37, $P = 0.00001$). The majority of new gametocyte positive cases (70%) were seen on Day seven.

Factors influencing gametocytemia. Given the low rate of compliance on Days 21 and 28, further investigations were restricted to the data obtained on Days 7 and 14.

Age (years), sex, presentation parasitemia, CQ treatment outcome, and gametocyte positivity on the previous visit were analyzed for association with the presence of gametocytes on Days 7 and 14 (Table 2). Chloroquine treatment outcome was categorized according to parasitological response using the WHO's S, RI, RII, RIII, grading system.¹⁸ There was a statistically significant inverse association between a person's age and the presence of gametocytes on both Days 7 and 14. There was also an increase of gametocytemia with increasing presentation parasitemia density

on both days of follow-up. Presence of gametocytes on presentation and on the previous visit were also associated with increased rates of gametocyte carriage on both Days 7 and 14. There was no association between a person's sex and the presence of gametocytes (data not shown).

Similarly, gametocyte densities were tested for association with the variables, age (years), sex, presentation parasitemia, and CQ treatment outcome, on Days 7 and 14 (Table 3). Predictably, there was no significant association between age and gametocyte densities on both days. Presentation parasitemia, however, showed a significant positive correlation with gametocyte densities on both days. Males had a slight but significantly higher mean density than females on both days (data not shown).

Of 635 cases that were treated with CQ and returned within 14 days, 475 (74.8%) had recrudescences. Of these, 276 (43.5%) were classified as RI, 123 (19.4%) as RII, and 123 (11.9%) as RIII. Outcome after CQ treatment had no significant association with presence of gametocytes. However,

TABLE 3
Multifactorial analysis of the relationship between selected parameters and densities of gametocytes on Days 7 and 14 of follow-up

| Exposure variable | | Geometric mean density/ μl^* (25, 75 th percentiles) | | Significance test [†] |
|---|---------------|---|---------------------|--------------------------------|
| | | Day 7 | Day 14 | |
| Age (yr) | 1-2 | 73.9 (60.3, 90.6) | 57.5 (48.5, 68.3) | $F_{3,297} = 0.7, P = 0.6$ |
| | 3-6 | 61.7 (47.1, 80.7) | 54.9 (43.3, 69.7) | |
| | 7-15 | 72.0 (49.5, 104.8) | 56.8 (41.0, 78.8) | |
| | ≥ 16 | 46.6 (36.3, 59.7) | 45.2 (36.3, 56.3) | |
| Presentation parasitemia/ μl | <800 | 44.9 (33.1, 61.0) | 41.8 (30.3, 57.7) | $F_{4,297} = 2.0, P = 0.049$ |
| | 800-1,599 | 56.1 (44.2, 71.2) | 41.8 (30.0, 60.1) | |
| | 1,600-3,999 | 72.2 (58.3, 89.5) | 55.4 (46.4, 66.1) | |
| | 4,000-19,999 | 66.6 (50.0, 88.7) | 59.7 (46.7, 76.28) | |
| | $\geq 20,000$ | 170.4 (56.9, 510.4) | 103.6 (48.8, 268.0) | |
| Chloroquine treatment outcome | Sensitive | 57.9 (43.7, 76.7) | 45.0 (35.9, 56.5) | $F_{3,227} = 2.4, P = 0.4$ |
| | RI | 55.8 (44.6, 69.8) | 52.4 (42.2, 64.9) | |
| | RII | 86.7 (61.1, 123.1) | 64.0 (47.9, 85.4) | |
| | RIII | 57.7 (41.0, 81.1) | 45.0 (33.3, 60.7) | |

* Geometric mean density of gametocyte-positive individuals.

† Sequential F-tests from a mixed model testing effects on gametocyte densities of the variables listed. Analysis included only gametocyte-positive samples.

odds ratios values were generally higher in carriers of resistant parasites, on Day 7, where there was a trend of decreasing risk of gametocyte carriage with increasing levels of resistance (Table 2). Chloroquine treatment outcome also showed no significant relationship with gametocyte densities on either day. Data for Fansidar® treatment outcome were not included in the analysis because of insufficient numbers.

DISCUSSION

The overall prevalence of gametocyte carriers in our follow-up group was much higher than that seen in those screened on presentation (44.0% versus 8.0%), and prevalence that was highest on Day 7 did not decline to pre-screening levels. Of the gametocyte-positive cases, the majority (70%), were first patent on the first follow-up visit, i.e. Day 7. Haji and others also reported low prevalences of gametocytemia in the same area in a community survey which involved repeated random sampling of asymptomatic individuals.¹⁹ One possible explanation for the higher rate of gametocytemia in the follow-up group could be the examination of more fields per slide during follow-up. On admission we read 20 fields for screening purposes, while during follow-up 100 fields were examined for gametocytes. In West Africa, Muirhead-Thomson¹¹ reported increases in gametocyte prevalences after increasing the number of fields screened by 50%. To test this possibility, a proportion of presentation slides were re-read against 100 fields. We were able to detect 5% more gametocyte-positive slides in this way, which does not adequately explain the difference in our findings.

An intriguing finding is the peak in gametocyte-positive cases on Day 7. It takes 10–12 days for *P. falciparum* gametocytes to mature and appear in the peripheral circulation,²⁰ although morphologically mature gametocytes may be seen on the seventh day of infection *in vitro* (Lensen A, unpublished data). This relatively long maturation period is probably a mechanism for evading the deleterious effects of clinical paroxysms related to asexual infection. These have been shown to abrogate gametocyte infectivity of other malarial *in vivo*,²¹ and in *P. falciparum in vitro*.^{22,23} Thus, gametocytes resulting from sequestration of parasites seen on Day 0 should appear in circulation after Day 7. Since this was not the case, it is reasonable to conclude that induction of the gametocytes seen in the majority of our patients had occurred before they had presented at the dispensary. Factors that modulate gametocytogenesis *in vivo* are poorly understood,^{1,24} though conditions adverse to the survival of the asexual parasite such as chemotherapy,^{7,25,26} clinical manifestations of malaria²⁷ and hematological disruptions²⁸ are thought to influence the production of gametocytes. Such symptom-related stress conditions detailed above may have been the reason for the observed increase in gametocyte rates. Nevertheless, it is unlikely that the majority of individuals presenting at the dispensary at different stages of infection would all have patent gametocytes at the same time; other external factors may have influenced gametocytogenesis. The factor common to all the study patients was that they received chloroquine treatment. Evidence of self-medication with CQ was tested in a subgroup of patients and seen in 20% (9 of 45) individuals. Treatment with CQ or

self-treatment with locally available antimalarials prior to attendance at the dispensary may well have contributed to the observed peak in gametocyte prevalence on Day 7.

Several parameters were analyzed for associations with the presence and density of gametocytes during follow-up. In agreement with other studies, gametocyte prevalences decreased with age.^{11–14,19} Interestingly, while age and presentation parasitemia were significantly related to gametocyte prevalence when examined independently, only presentation parasitemia was significantly associated with gametocyte prevalence and density in multi-factorial analysis. Thus, the higher a person's parasitemia on presentation, regardless of age, the greater the chance of being gametocyte positive and the higher the density of gametocytemia. The most important determinant for the prevalence and density of gametocytes, therefore, is the density of asexual parasitemia infection. While prevalence declined with age, mirroring the decline in high density asexual parasitemias, gametocytes densities remained low and did not vary with age, a finding reported before in Ifakara¹⁹ and in Kisumu, Kenya,¹⁴ an area of similar endemicity. Other studies, however, have reported age-dependent decreases in gametocyte densities.^{3,12,13} The observed differences may be due to differing endemicities of the study areas; however, the varying methodologies used do not allow for a detailed comparison.

In this study incidence rates were highest in the 1–2 year olds and decreased with age as expected, implying that rates of gametocyte production are higher in children. As children have higher prevalences and densities of asexual parasitemia, intuitively we expect them to produce more gametocytes. This would mean higher densities of gametocytes in younger ages, which is not corroborated by our findings. MacDonald²⁹ proposes that the ability to restrain gametocyte production comes much earlier in life than that of limiting schizont growth. In areas of high endemicity, high-density gametocyte infections are limited to the first year of life while in areas of low transmission the decrease in gametocyte loads is more gradual. Our data seem to support this hypothesis. Despite higher prevalences and incidence rates, children do not have higher densities of gametocytes. Examining the converse, although fewer adults produce gametocytes, those that do produce densities similar to younger age groups. This implies that the kinetics of sexual stage immunity are different to the kinetics of asexual stage immunity. A study by Baird and others³⁰ found evidence for the specific suppression of *P. falciparum* gametocytemia in semi-immune natives of Irian Jaya. This suppression was independent of immune control of asexual parasitemia. Furthermore, non-immune Javanese transmigrants developed immunity against gametocytes at a much faster rate than to asexual parasites.

In summary, we have followed-up a group of symptomatic parasitemic individuals to investigate the dynamics of gametocyte production in an area of intense and perennial transmission. Gametocyte prevalence and incidence rates decreased with age, while densities remained relatively constant. The explanation for this finding will be related to production and clearance of gametocytes, which may differ with age. Given these observations, further studies are needed on the effects of age-specific clearance of gametocytes and the capacity of gametocytes to infect mosquitoes. This may have

epidemiologically important implications for the development of sexual stage immunity and the contribution of adults to the infectious reservoir of malaria.

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