EMERGENCE AND CLEARANCE OF GAMETOCYTES IN UNCOMPLICATED PLASMODIUM FALCIPARUM MALARIA

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Abstract. We reviewed the records of 1,175 patients with uncomplicated Plasmodium falciparum malaria to determine the prevalence of gametocytemia. All patients were admitted and received artemisinin combination therapy. Blood films were checked daily until discharge. Circulating gametocytes were observed in 240 (20.2%) of patients and in most cases (222 of 240, 92.5%) gametocytemia was detected during the first 24 hours after admission. Gametocytes were first seen in 174 cases on admission, in 24 cases at 12 hours, and in 24 cases at 24 hours. The longest interval between admission and first appearance of gametocytes was 192 hours. The median gametocyte clearance time was 163 hours (range = 12–806) in the 219 patients in whom gametocytemia resolved. However, 21 patients (9.8%) still had gametocytemia on discharge. Gametocytemia generally is present within the first 24 hours after admission, and emerges in only 1.9% of patients later on during treatment with artemisinin.

INTRODUCTION

The gametocyte plays a key role in malaria transmission. When transmitted from infected humans to the mosquito, this stage of the malaria parasite completes the life cycle. The precise mechanism of gametocytegenesis is unknown, but several factors influencing gametocyte emergence have been identified, including parasite genetics, stress to the parasite, host immunologic factors, and even seasonal change. Without treatment, most patients with falciparum malaria will develop gametocytocemia within 10–40 days after the onset of parasitemia. Gametocytes have been reported to survive as long as 21 days in peripheral blood. Mature gametocytes are relatively resistant to most antimalarial compounds, and primaquine is the only drug with proven gametocidal activity. In some areas, it is recommended that primaquine be given to patients with P. falciparum malaria with gametocytocemia to prevent transmission. In Thailand, it is recommended that primaquine be given with artesunate and mefloquine to all newly diagnosed patients with P. falciparum malaria whether or not gametocytes are present. The rationale is that even if there is no gametocytocemia at the time malaria is diagnosed, it is possible that gametocytes will emerge during treatment. However, the prevalence of gametocytocemia during malaria infection is not well defined. Here, we retrospectively review data from patients with uncomplicated P. falciparum malaria enrolled in treatment trials to gather information on the emergence and clearance of gametocytes during therapy.

PATIENTS AND METHODS

We retrospectively reviewed charts from patients with uncomplicated P. falciparum malaria enrolled in nine separate trials of artemisinin combination therapy. All trials were conducted at Hospital for Tropical Diseases in Bangkok, Thailand between 1999 and 2004. Four trials are published and five trials are unpublished. Details of all trials are shown in Table 1. Most patients in our study were from Thailand’s western border with Myanmar, where malaria epidemiology has been previously described in detail. Transmission of malaria is low and seasonal. On average, each person has one P. vivax and one P. falciparum malaria infection every two years. Nearly all P. falciparum malaria infection is symptomatic.

Patients. Studies were reviewed and approved by the Ethics Committee of the Faculty of Tropical Medicine of Mahidol University and informed consent was obtained from each subject. All patients had slide-confirmed P. falciparum malaria, with no signs or symptoms of severe malaria. All patients received artemisinin combination therapy and remained in hospital for 28 days.

Laboratory methods. Thick and thin blood films were examined for asexual blood stage parasites and gametocytes every 12 hours until asexual blood stage parasites were no longer present and then daily thereafter until day 28 or until discharge. Parasite and gametocyte counts per microliter of blood were calculated per 1,000 red blood cells in a thin film or per 200 white blood cells in a thick film. Time to gametocyte detection was the number of hours from admission to the first peripheral smear positive for gametocytes. Gametocyte clearance time was the interval between the first and last positive smears for gametocytes. The parasite clearance time was the interval between starting treatment and the first peripheral blood smear with no demonstrable asexual parasites.

Statistical analysis. All statistical analyses were performed with the statistical computing package SPSS version 11 for Windows (SPSS Inc., Chicago, IL). Arithmetic means were generally used, except that initial parasite counts and maximum gametocyte counts were expressed as geometric means. Gametocyte clearance times were evaluated by Kaplan-Meier analysis.

RESULTS

Data from 1,175 patients with uncomplicated P. falciparum malaria studied between May 1999 and September 2004 and whose treatment did not include primaquine were analyzed. All patients receive artemisinin combination therapy regimen. Gametocytocemia was found in 240 of these 1,175 patients
(20.2%), and it is these individuals who are the main focus of our investigation. One hundred seventy-nine patients (74.6%) were male, the median age was 21 years (range = 2–62), and slightly more than half (57.9%) were having their first episode of malaria. The median duration of fever prior to admission was 5.0 days (range = 0–60) and the median admission parasitemia count was 5,363/μL (range = 17–156,240). The admission characteristics of the 240 patients are summarized in Table 2.

First detection of gametocytes. Gametocytes were detected on admission in 174 patients (72.5%). Gametocytemia was observed in another 24 patients on the 12-hour blood smear and in an additional 24 patients on the smear taken 24 hours after admission (Table 3). Therefore, by 24 hours, gametocytes had been detected in 92.5% of all patients who would ultimately develop gametocytemia. Some patients occasionally developed gametocytemia at a later time, one as late as 192 hours after admission (Table 3).

Probability of gametocytemia. The overall risk of developing gametocytemia was 20.2% (240 of 1,175 patients). The probability of having gametocytemia was only 1.9% if no gametocytes were present during the first 24 hours after admission (Table 3).

Prevalence of gametocytemia. The daily prevalence of gametocytemia is shown in Figure 1. Gametocytemia prevalence peaked during the first three days after admission and decreased sharply to below 10% after completion of the seven-day course of artemisinin treatment.

Clearance of gametocytes. Only 21 patients (9.0%) were discharged with gametocytemia. Gametocytemia cleared in the other 219 individuals. The median gametocyte clearance time was 163 hours (range = 12–806). The probability of carriage of gametocytes by Kaplan-Meier survival analysis is shown in Figure 2. Gametocytes were cleared within 200 hours of detection in approximately half of the patients.

DISCUSSION

The prevalence of gametocytemia during *P. falciparum* malaria has not been well defined because most information has come from point prevalence investigations or outpatient studies. Gametocytemia on presentation has been reported to occur in 2.4–22.0% of patients.20–23 We measured an overall prevalence rate of 20.2%, and found that most (72.5%) gametocytic patients were identified on admission. However, even with artesinin treatment, 27.5% of the patients developed gametocytemia at a later time.

Primaquine plus artesunate has been shown to reduce gametocyte carriage compared with artesunate alone.24 To reduce malaria transmission, current policy in malaria-endemic areas of Thailand is to administer artesunate plus mefloquine plus primaquine to all newly diagnosed *P. falciparum* malaria patients whether or not gametocytemia is present. Implementing this policy in our study patients would have covered the 20.2% of individuals with gametocytemia. However, pri-
Primaquine would have been given unnecessarily to the other 79.7% of the patients and exposed them to the risks of glucose-6-phosphate dehydrogenase (G6PD) deficiency-related hemolysis. Testing for G6PD deficiency is not generally available at the community level, but, fortunately, G6PD deficiency in Thai individuals is generally mild.\(^{24-26}\) However, all non-hospitalized patients who receive primaquine should be advised to look for indications of hemolysis such as a change in urine color.

Follow-up blood smears should be prepared if primaquine is only given to patients with gametocytemia because gametocytes can be absent on admission and emerge during treatment. In our study, however, the risk of developing gametocytemia if none were present by 24 hours was only 1.9% (Table 3). However, patients often do not return for follow-up during community-based treatment. Artesunate treatment rapidly clears both parasites and symptoms, so most patients have no incentive to return for 12-hour and 24-hour blood film checks. In practical terms, the question is whether to give primaquine on day 0 to patients without gametocytemia, and how to answer this question is debatable.

The prevalence of gametocytemia during treatment has been shown to vary according to the antimalarial drug regimen used. Peak gametocyte prevalence rates at day 7 were 62.2% with chloroquine plus sulfadoxine and pyrimethamine and 97.2% with chloroquine and sulfadoxine alone.\(^{27}\) We measured gametocyte prevalence at 10% on day 7 and < 4% on day 14 in artemisinin-treated individuals. Our results are consistent with those of other investigations that show that artemisinin reduces gametocytemia. Since the patients reviewed in this study received different artemisinin combination therapy, as shown in Table 1, each artemisinin derivative may have some different effect on gametocytemia. However, there is no evidence to support this hypothesis.

We could not separately analyzed the prevalence of gametocyte emerging during each artemisinin treatment regimen because the number of the patients was too small. Thus, we reviewed and analyzed all patient in nine different trials as a single group, i.e., the patients with artemisinin combination therapy.

Previous studies have shown that artemisinin can reduce the transmission of malaria in the community when compared with quinine-based regimens,\(^{10}\) and primaquine has been shown to shorten the gametocyte clearance time.\(^{18}\) However, whether to recommend universal treatment of all *P. falciparum* malaria with a combination of an artemisinin derivative and primaquine remains controversial. Not all patients with uncomplicated *P. falciparum* malaria will develop gametocytemia, and a variety of factors that affect the risk benefit ratio must be considered. Information about the prevalence of gametocytemia and the effect of antimalarial treatment on this prevalence will provide information for policy makers trying to answer this question.

**Table 4**

<table>
<thead>
<tr>
<th>Risk of gametocytemia*</th>
<th>Overall risk 20.2%</th>
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</thead>
<tbody>
<tr>
<td>If gametocyte negative at D0</td>
<td>6.6%</td>
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<tr>
<td>If gametocyte negative at D0 and 12 hours</td>
<td>4.3%</td>
</tr>
<tr>
<td>If gametocyte negative at D0, 12 hours, and 24 hours</td>
<td>1.9%</td>
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</tbody>
</table>

* D0 = day 0.
REFERENCES


