RISK FACTORS FOR PLASMODIUM VIVAX GAMETOCYTE CARRIAGE IN THAILAND

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Abstract. To study the risk factors for Plasmodium vivax gametocyte carriage, the presence or absence of gametocytes was determined in 2,125 patients with P. vivax malaria participating in clinical trials at the Hospital for Tropical Diseases in Bangkok, Thailand. Stepwise logistic regression models were used to determine which variables were significantly related to gametocyte carriage. On admission, 615 patients (29%) had detectable gametocytes (before treatment). After treatment had started, an additional 245 patients (11%) developed patent gametocytemia. The variables retained by multivariate analysis were highest observed temperature (adjusted odds ratio [AOR] per °C increase = 0.82, 95% confidence interval [CI] = 0.71–0.94, P = 0.006), artesunate 0.25 mg of base/kg/day (adult dose = 15 mg of base/day) for 14 days. Patients received oral paracetamol if they had a temperature > 38°C. The effect of treatment on gametocytes will be reported elsewhere (Nacher M and others, unpublished data). This paper focuses on other factors influencing gametocyte carriage.

INTRODUCTION

The perpetuation of the plasmodial cycle between humans and mosquitoes requires that gametocytes be absorbed by an anopheline vector. Gametocytes are not always detectable in the peripheral blood of malaria patients. Identifying factors that affect the magnitude and duration of gametocyte carriage is of interest because it could lead to interventions targeting transmission. There have been a number of studies identifying risk factors for Plasmodium falciparum gametocyte carriage, but to our knowledge, there is no equivalent for P. vivax, which is still a major cause of morbidity. Therefore, the objective of this study was to study risk factors for P. vivax gametocyte carriage in Thailand.

PATIENTS AND METHODS

Study site. Between 1993 and 2002, 2,125 patients 4–83 years old with P. vivax malaria were admitted to the Hospital for Tropical Diseases in Bangkok, Thailand for clinical studies on antimalarials. Gametocytes per 200 leukocytes were counted every 12 hours by experienced microscopists on Giemsa-stained thick blood smears until parasitemias became negative. A complete blood count was performed to transform emsa-stained thick blood smears until parasitemias became negative. A complete blood count was performed to transform

maquine alone (Thai Government Pharmaceutical Organization), 0.25 mg of base/kg/day (adult dose = 15 mg of base/day) for 14 days. Patients received oral paracetamol if they had a temperature > 38°C. The effect of treatment on gametocytes will be reported elsewhere (Nacher M and others, unpublished data). This paper focuses on other factors influencing gametocyte carriage.

Ethical considerations. All patients provided informed consent before admission into the study. The study was reviewed and approved by the Ethical Committee of Mahidol University (Bangkok, Thailand).

Statistical analysis. The proportion of gametocyte carriers on admission, the peak gametocyte count, and the gametocyte carriage duration were outcome variables studied in relation to a number of clinical and paraclinical covariates. Since most variables had a non-normal distribution, the Mann-Whitney test was used to compare medians. Spearman’s correlations between variables were also used. To reduce the number of relevant covariates and to control for potential confounding between covariates, backward stepwise logistic or linear regression was used. The full model included the variables that were related to gametocyte carriage in simple analysis and variables that were biologically relevant. Given the number of observations and variables used, some of the variables on admission were missing. Therefore, the total number of observations used was lower than the initial number of case records used. The variables were assumed to be missing completely at random (i.e., when height had not been measured it was impossible to calculate the body mass index, and some laboratory results were lost). Since levels of serum bicarbonates were only measured systematically in the last five years of patient care, the number of available observations was much lower than for other variables. The stepwise regression results were verified with robust regression because of the non-normal distribution of a number of variables.

RESULTS

Of 2,125 patients, 615 (29%) had gametocytes on admission (before treatment), and 860 patients (40%) had detectable gametocytes at any time during the follow-up. Having a his-
tory of malaria was not associated with any significant increase in gametocyte carriage on admission (crude odds ratio = 1, 95% confidence interval [CI] = 0.8–1.2, \( P = 0.9 \)).

**Factors influencing the presence of gametocytes.** Table 1 shows that the comparisons of variables between patients with and without \( P. vivax \) gametocytes yielded a number of significant differences. Bivariate analysis showed that there were no significant differences between gametocyte carriers and gametocyte-free patients regarding age (22 years, interquartile range [IQR] = 19–28 versus 22 years [18–29]; \( P = 0.27 \)), duration of symptoms before admission (4 hours [3–6] versus 4 hours [3–5]; \( P = 0.22 \)), leukocytes (6,200 × 10^6/L [4,900–7,900] versus 6,100 × 10^6/L [4,900–7,600]; \( P = 0.28 \)), lymphocytes (25% [16–36] versus 25% [18–36]; \( P = 0.5 \)), neutrophils (64% [52–72] versus 63% [52–72]; \( P = 0.7 \)), aspartate aminotransferase (29 IU [22–40] versus 28 IU [21–40]; \( P = 0.24 \)), alkaline phosphatase (48.2 units/L [30–84] versus 52.1 units/L [30.6–87]; \( P = 0.13 \)), sodium (138 mmol/L [136–140] versus 138 mmol/L [136–140]; \( P = 0.16 \)), and the anion gap (11 mmol/L [9–13] versus 11 mmol/L [9–13]; \( P = 0.05 \)).

Patients with a palpable spleen were less likely to have detectable gametocytes on admission (odds ratio = 0.7, 95% CI = 0.5–0.98, \( P = 0.03 \). To control for the interactions between these variables and reduce the number of explanatory variables, the variables in Table 1 (excluding bicarbonates) and the presence or absence of splenomegaly were included in a stepwise logistic regression model retaining variables with a \( P \) value < 0.2. The only variables that remained significant after this procedure were the highest observed temperature (adjusted odds ratio [AOR] per °C increase = 0.82, 95% CI = 0.71–0.94, \( P = 0.006 \)), asexual parasitemia > 9,200/μL (AOR = 2.8, 95% CI = 1.9–4.2, \( P < 0.0001 \)); erythrocyte counts (AOR = 0.8/million/μL increase, 95% CI = 0.67–0.95, \( P = 0.01 \)); monocyte percentage (AOR = 0.93 per % increase, 95% CI = 0.89–0.96, \( P < 0.0001 \)); lymphocyte percentage (AOR = 0.98 per % increase, 95% CI = 0.97–0.99, \( P = 0.006 \)); and albumin (AOR = 0.67 per 10 g/mL increase, 95% CI = 0.5–0.9, \( P = 0.007 \)).

A separate model including the same variables plus bicarbonates (only the patients admitted in the last five years benefitted from this measurement) and the anion gap (Na^+, Cl^−, HCO_3^−) confirmed our results, and showed that the anion gap was positively associated with gametocyte carriage (AOR = 1.1 per unit increase, 95% CI = 1.02–1.14, \( P = 0.009 \)).

**Factors influencing magnitude and duration of carriage.** The median gametocyte count was 510/mm^3 (IQR = 330–910) and the median duration of gametocyte carriage was 24 hours (IQR = 12–48). Among patient with gametocytes, the peak number of gametocytes was positively correlated with the initial parasitemia (Spearman’s \( r = 0.26, P < 0.0001 \)) and negatively correlated with the highest observed temperature (Spearman’s \( r = -0.07, P = 0.045 \). No other variables other than treatment were significantly associated with the magnitude of gametocyte carriage. Because of the obvious influence of treatment, these associations were tested using multivariate methods, and remained significant after controlling for the same variables reported earlier and treatment using stepwise regression, and controlling the final models with robust regression models (\( P < 0.0001 \) and \( P = 0.01 \), respectively).

**The duration of gametocyte carriage was proportional to gametocyte count (Spearman’s \( r = 0.36, P < 0.0001 \)) and asexual parasites count (Spearman’s \( r = 0.10, P = 0.001 \). Conversely, there was a negative correlation between albuminemia and the duration of gametocyte carriage (Spearman’s \( r = -0.09, P = 0.01 \)). There was a negative correlation

<table>
<thead>
<tr>
<th>Variables measured on admission (available observations)</th>
<th>Presence of gametocytes*</th>
<th>No gametocytes*</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index (kg/m^2) (n = 2,035) (n = 2,035)</td>
<td>20 (18.6–21.7)</td>
<td>19.7 (18.3–21.5)</td>
<td>0.09</td>
</tr>
<tr>
<td>Highest fever (°C) (n = 2,123)</td>
<td>37.7 (37.1–38.3)</td>
<td>37.7 (37.1–38.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>Parasitemia (μL) (n = 2,120)</td>
<td>13,900 (8,300–23,105)</td>
<td>9,215 (3,710–16,040)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hemoglobin (g/dL) (n = 2,118)</td>
<td>11.9 (10.3–13.3)</td>
<td>12.1 (10.6–13.4)</td>
<td>0.08</td>
</tr>
<tr>
<td>Erythrocytes (10^12/mm^3) (n = 2,063)</td>
<td>4.45 (3.92–4.91)</td>
<td>4.55 (4–5.01)</td>
<td>0.002</td>
</tr>
<tr>
<td>Monocytes (%) (n = 2,117)</td>
<td>4 (2–8)</td>
<td>5 (3–8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Platelets (10^12/L) (n = 1,784)</td>
<td>79 (53–124)</td>
<td>94 (53–124)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Albumin (g/mL) (n = 2,116)</td>
<td>40 (36–42.5)</td>
<td>40 (37–43)</td>
<td>0.002</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL) (n = 2,117)</td>
<td>1.36 (0.9–2.01)</td>
<td>1.24 (0.8–1.9)</td>
<td>0.006</td>
</tr>
<tr>
<td>Potassium (mmol/L) (n = 1,703)</td>
<td>3.5 (3.3–3.9)</td>
<td>3.6 (3.3–3.9)</td>
<td>0.03</td>
</tr>
<tr>
<td>Chloride (mmol/L) (n = 1,700)</td>
<td>104 (102–106)</td>
<td>104 (102–106)</td>
<td>0.06</td>
</tr>
<tr>
<td>Bicarbonates (mmol/L) (n = 1,035)</td>
<td>23 (21–25)</td>
<td>23 (22–25)</td>
<td>0.01</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dL) (n = 2,113)</td>
<td>14.8 (12–18.1)</td>
<td>14.4 (11.3–18)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* Values are the median (interquartile range). A rank sum test was used for comparisons.
between the highest observed temperature and gametocyte carriage duration (Spearman’s ρ = −0.05, P = 0.2). After controlling for their respective effects and the influence of treatment, the observed correlations between gametocyte carriage duration, gametocyte counts, and albuminemia were still significant (P < 0.0001, and P = 0.008, respectively), but no longer with the highest observed temperature (P = 0.27) or asexual parasite counts (P = 0.24).

Hemoglobinopathies and gametocytes. Among a subgroup of patients for which hemoglobin electrophoresis was available (n = 1,014), there were no significant differences between patients with normal hemoglobins and patients with the hemoglobin E trait for median gametocyte counts (495/ mm³ [IQR = 330–830] versus 445/mm³ [305–795]; P = 0.5) and for median durations of gametocyte carriage (24 hours [12–48] versus 24 hours [12–48]; P = 0.31).

DISCUSSION

Patients with detectable *P. vivax* gametocytes on admission had higher asexual parasitemias, but lower erythrocyte, monocyte and lymphocyte counts, lower albumin concentrations, and lower body temperatures than patients with no detectable gametocytes. To our knowledge, the factors that influence *P. vivax* gametocyte carriage have not been described. In this large series, *P. vivax* gametocyte carriage has some similarities and some differences with that of *P. falciparum*. The negative association between fever and gametocyte carriage has also been described in *P. falciparum* malaria. Anemia, but not the hemoglobin E trait (thalassemia), was also predictive of gametocyte carriage. In *P. falciparum* malaria, high asexual parasitemia (> 5%) has been associated with increased gametocyte carriage in one study. Conversely, increased parasitemia was associated with absence of gametocyte carriage in another study. In Thailand, patients with hyperparasitemia had a lower ratio of gametocytes to asexual forms, but they were more likely to have *P. falciparum* gametocytes, perhaps indicating that excessive multiplication compensated for reduced gametocytogenesis. Another study on the Thailand-Burma border showed that *P. vivax* gametocyte densities were positively correlated with trophozoite densities. The duration of symptoms was not associated with *P. vivax* gametocyte carriage. This is in contrast with the observations in malaria therapy patients in which the proportion of patients with observable *P. vivax* gametocytes increased with time. Since most patients at the Hospital for Tropical Diseases had a short symptoms evolution, the relationship between time and gametocytes may have been affected by early treatment. Thus, the role of parasitemia and anemia may be a reflection of the duration of the malaria episode, with each cycle committing a few gametocytes while asexual parasites progressively multiply and destroy erythrocytes until the patient becomes anemic and concomitantly presents patent gametocytemia. However, in our study, there was no obvious effect of evolution duration. An alternative causal explanation to the strong link between anemia and gametocytes would be that the destruction of the oxygen transporter and the tissue hypoxia concomitantly to erythrocyte destruction might be conducive to gametocytogenesis, and thus might represent the finality of asexual parasite multiplication. The lower bicarbonate concentrations in gametocyte carriers might have reflected slight acidosis due to increased anaerobic metabolism leading to increased lactates. This might explain the observed association between the importance of the anion gap and gametocyte carriage.

Finally, the increase in gametocyte carriage in patients with low albuminemia suggests that the host’s nutritional status has an important impact on plasmodial physiology, possibly interference with parasite multiplication, the attenuation of malaria symptoms, or hematologic consequences of malnutrition favor gametocytogenesis.

Although the results of this study show a variety of factors influencing patent gametocyte carriage, most of them could be grouped into two compatible unifying perspectives, one that does not seem to be well supported by the present data where most findings would be consequences of the duration of the infection, and another in which parasite multiplication and erythrocyte destruction would increase gametocytogenesis through the events surrounding tissue hypoxia.

Although the infectivity of *P. vivax* gametocytes is only weakly correlated with gametocyte densities, factors influencing the magnitude and duration of gametocyte carriage may translate into differences in mosquito infection. This would require prospective studies on the factors influencing transmission to the mosquito.

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REFERENCES