REGIONAL DIFFERENCES IN THE RESPONSE OF PLASMODIUM VIVAX MALARIA TO PRIMAQUINE AS ANTI-RELAPSE THERAPY

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Abstract. We used logistic regression to assess effectiveness of primaquine as Plasmodium vivax anti-relapse therapy using data extracted from studies of P. vivax relapses in Brazil, India, and Thailand. The risk of relapse in Thailand was 10 times that in India and twice that in Brazil. In comparison with no primaquine treatment, the risk of relapse decreased by approximately 80% for a total adult primaquine regimen of 210 mg and by ≥95% for regimens of 315 mg and 420 mg. In addition, we used logistic regression to estimate the risk of P. vivax relapse according to weight-based primaquine dose using data from case studies. There was a three-fold increase in the likelihood of successful treatment of each additional milligram of primaquine per kilogram of body weight. Tailoring primaquine therapy to a region requires consideration of factors including body weight, natural relapse rates, and local response to primaquine.

INTRODUCTION

Plasmodium vivax malaria is an acute debilitating illness characterized by recurrent paroxysmal fevers. It causes 70–80 million cases annually, occurs largely in regions of low-to-moderate malaria endemicity, and can repeatedly infect persons of all ages. Relapses arising from hypnozoites in the liver are a feature of P. vivax malaria. These can occur weeks to years after the initial infection and place further health burdens on those affected. Relapses provide an important source of reinfection and relapse prevention requires chemotherapy that targets the latent liver stages of P. vivax.

Primaquine is currently the only commercially available drug that combats hypnozoites in the liver,4,5 and ensuring this drug is available for future generations as anti-relapse therapy is of public health importance. However, with several primaquine regimens in common use,6,7 and variance in relapse patterns geographically and across P. vivax strains2,3,8 and in responses to primaquine,9–11 the issue of determining the best regimen of primaquine for anti-relapse therapy is complex.

Current primaquine regimens and much of the knowledge about primaquine as P. vivax anti-relapse therapy is derived from experimental studies conducted in non-immune subjects up to 50 years ago. A primaquine regimen of 15 mg/day for 14 days in adults is commonly termed standard anti-relapse therapy and is based on studies that found many P. vivax strains to be almost totally susceptible to a total dose of 200 mg of primaquine base.9,10 Other studies demonstrated variability in tolerance of primaquine with the Chesson strain requiring at least 22.5 mg/day for 14 days to prevent relapse.11–13

The total dose of primaquine is more important than the schedule of delivery, with a regimen of 30 mg/day for 14 days (total dose = 420 mg) as effective as 60 mg/day for 7 days (total dose = 420 mg).11 Primaquine can cause acute hemolysis in people with glucose-6-phosphate dehydrogenase (G6PD) deficiency and the use of 45 mg as a weekly dose for 8 weeks was safer in terms of adverse hemolytic effects than standard therapy.13 Other primaquine regimens in current use include shortened regimens of 15 mg/day for 5 days (often used because of concern over compliance with 14-day regimens) and high-dose regimens of 22.5 or 30 mg/day for 14 days in areas with increased tolerance to primaquine or in non-immune persons.15

Recently, there have been reports of failure of five-day regimens6,16 and mixed success of standard therapy.6,17 A review article examined relapse rates of P. vivax after 5 and 14 days of primaquine therapy and concluded that the 5-day regimen is not effective and the 14-day regimen is often not effective.5 Failure of primaquine as anti-relapse therapy suggests the possibility of primaquine resistance in the hepatic stages of P. vivax but may be explained by other factors such as sub-therapeutic primaquine dosing,17 or misclassification bias in which recurrence of P. vivax symptoms due to reinfection or recrudescence (failure to eliminate the blood stages of P. vivax) is misclassified as relapse.5 The issue of determining effectiveness of primaquine as P. vivax anti-relapse therapy is also compounded by the heterogeneity of relevant studies, a paucity of randomized controlled trials (RCTs), and lack of a comparison group in other studies.

In this study, we assessed effectiveness of total primaquine dose as P. vivax anti-relapse therapy across different geographic regions through an analysis of the available evidence from studies of P. vivax relapses after blood stage treatment with or without primaquine therapy in any dose.

MATERIALS AND METHODS

Interventional and observational studies that assessed recurrence rates of P. vivax malaria in humans living in malaria-endemic countries after appropriate blood stage treatment with or without primaquine were considered. Studies were identified by a search of the Cochrane Library, Cochrane Central Register of Controlled Trials, Medline (1966 to November 2004), and Embase (1988 to November 2004) electronic databases using combinations of the following words: human, malaria, vivax malaria, primaquine, therapeutics, treatment outcome, recurrence, and relapse. Reference lists of selected studies were checked for potentially relevant studies. Studies were included if study data was less than 15 years old (after 1988), if follow-up was for ≥ 60 days, if P. vivax...
malaria was microscopically confirmed, and if the number of cases experiencing recurrence of malaria symptoms was provided.

Twenty studies from 18 references met the inclusion criteria\textsuperscript{6,7,14–30} and the relevant study arms (using appropriate blood stage treatment with or without primaquine as anti-relapse therapy) were identified. Five study arms from three studies using primaquine as blood stage treatment\textsuperscript{29} and tafenoquine\textsuperscript{28} or buloquine\textsuperscript{22} as anti-relapse therapy were excluded from analysis. Information from relevant study arms was extracted including study design, country, blood stage treatment, primaquine regimen, participant numbers, duration and completeness of follow-up, the number of cases with recurrence, and the timing of recurrences.

The primary outcome measure was a \textit{P. vivax} relapse rate > one month and ≤ six months. This was calculated by dividing the number of subjects experiencing one or more \textit{P. vivax} recurrences after one month and up to six months after blood stage treatment of the primary \textit{P. vivax} episode by the number of subjects remaining in the study after six months or at the end of the follow-up period for studies of less than six months duration. Recurrences occurring before one month of follow-up were classified as blood stage treatment failure (re-crudecense) and excluded from calculated \textit{P. vivax} relapse rates. Subjects reported in the published results as lost to follow-up or excluded from analysis were excluded from the denominator. Recurrences occurring after six months were excluded to decrease the chance of counting reinfections as relapses. Only two studies used polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) to exclude reinfection,\textsuperscript{18,19} and for consistency with other studies in this analysis, our extracted data reflects recurrences that have not been confirmed by PCR.

Total primaquine dose in milligrams was the main predictor variable in which a total primaquine dose of 75 mg is equivalent to a five-day regimen of 15 mg/day, 210 mg is equivalent to standard anti-relapse therapy of 15 mg/day for 14 days, 315 mg is equivalent to 22.5 mg/day for 14 days, and 420 mg is equivalent to 30 mg/day for 14 days. For studies that administered primaquine to children at a daily dose of 0.25 mg/kg of body weight for 5 or 14 days, the total primaquine dose was recoded to be equivalent to adult doses of 75 mg or 210 mg that assume a body weight of 60 kg.

The above search strategy also identified a number of case studies and case series that described treatment of primary episodes and relapses of \textit{P. vivax} malaria and contained details of primaquine dose and body weight.\textsuperscript{31–36} Since these studies had no denominator, relapse rates could not be calculated, but information regarding primaquine dose, body weight, and success or failure of primaquine treatment was extracted and analyzed separately. Weight-based primaquine dose was calculated by dividing the total primaquine dose in milligrams by body weight in kilograms. An outcome of failed or successful treatment was allocated to each \textit{P. vivax} episode.

Statistical analysis. Results were analyzed using Stata version 8\textsuperscript{37} and are presented as odds ratios (ORs) with 95% confidence intervals (CIs) using a 5% significance level. The \textit{P. vivax} relapse rate was treated as a binary outcome measure and a total primaquine dose of 0, 75, 210, 315, and 420 mg was treated as a categorical predictor variable. Models weighted by sample size of the included studies were fitted using grouped logistic regression to estimate the risk of \textit{P. vivax} relapse according to total primaquine dose. Country of study was then added to the models, followed by publication year and study design (observational study or RCT).

Data from studies from which we calculated weight-based primaquine dose was analyzed as individual (rather than grouped) relapse risk data. We modeled each person’s relapse as a binary (0 or 1) outcome variable and included patient as a random-effects cluster variable. We used logistic mixed model regression analysis to estimate the risk of failed or successful treatment of a \textit{P. vivax} episode according to weight-based primaquine dose.

RESULTS

Twenty studies from Brazil, India, Pakistan, and Thailand met the inclusion criteria.\textsuperscript{6,7,14–30} Study designs included RCTs (n = 11) and observational studies (n = 9) that comprised comparative studies with parallel control groups but non-randomization of subjects (n = 3), interrupted time series without a parallel control (n = 1), and case series with prospective follow-up (n = 5). The subjects in all studies except one were recruited to their respective studies based on being a confirmed primary \textit{P. vivax} malaria case. The 113 subjects in study 27b (Table 1)\textsuperscript{27} were recruited after the first relapse of \textit{P. vivax} malaria after chloroquine treatment of their primary \textit{P. vivax} episode.

The \textit{P. vivax} relapse rate was calculated from the relevant arms of 17 studies from Brazil, India, and Thailand (Table 1).\textsuperscript{6,7,14–29} Since three studies from Pakistan\textsuperscript{16,30} did not differentiate between recurrences occurring before or after one month of follow-up, primary treatment failure could not be excluded and the \textit{P. vivax} relapse rate could not be calculated. These three studies were not included in the grouped logistic regression and the recurrence rates according to the length of follow up are provided in Table 2.

The risk of \textit{P. vivax} relapse according to primaquine dose, country, year of study, and study design is shown in Table 3 and \textit{P. vivax} relapse rates according to primaquine dose and country are shown in Figure 1. There is a dose-response relationship between primaquine dose and \textit{P. vivax} relapse rates, with primaquine response and \textit{P. vivax} natural relapse rates varying between regions. After adjustment for country, study year, and study design and in comparison to no primaquine treatment, the risk of relapse decreased by approximately 60% for total adult primaquine doses of 75 mg (OR = 0.42, 95% CI = 0.34–0.52), by approximately 80% for primaquine doses of 210 mg (OR = 0.22, 95% CI = 0.16–0.30), and by ≥95% for primaquine doses of 315 mg (OR = 0.01, 95% CI = 0.00–0.08) and 420 mg (OR = 0.05, 95% CI = 0.01–0.20). The risk of relapse in Thailand was 10 times the risk of relapse in India (OR = 10.07, 95% CI = 7.76–13.08) and double the risk of relapse in Brazil (OR = 4.59, 95% CI = 2.64–7.97), which indicated that \textit{P. vivax} relapse was more likely in Thailand without primaquine treatment and that higher doses of primaquine were required to prevent \textit{P. vivax} relapse in Thailand than in Brazil and India. Although primaquine effectiveness did not differ according to publication year, the risk of relapse according to primaquine dose was higher in RCTs than in observational studies.

Weight-based primaquine dose (mg/kg) was calculated from six studies\textsuperscript{31–36} of returned travelers (n = 9), military
personnel (n = 1), and residents of Bangkok (n = 10) with \textit{P. vivax} malaria acquired in East Timor, Borneo, Guatemala, Thailand, and Ethiopia. Of the 52 \textit{P. vivax} episodes included in the analysis, treatment failure leading to relapse was recorded for all \textit{P. vivax} primary episodes (n = 20) and for 13 of the 32 \textit{P. vivax} relapses. The mean total weight-based primaquine dose received according to failed treatment (n = 33) was 3.2 mg/kg (range = 2.1–4.8 mg/kg) and successful treatment (n = 19) was 4.6 mg/kg (range = 2.4–7.1 mg/kg). Logistic regression models estimated an OR of 3.0 (95% CI = 1.58–5.73) for successful treatment of \textit{P. vivax} relapses, which indicated a three-fold increase in the likelihood of successful treatment for each additional milligram of primaquine according to body weight.

**DISCUSSION**

We analyzed data from 1989 to 2004 extracted from relevant literature published since 1994 and confirmed that increasing primaquine dosage results in a greater impact on the reduction of \textit{P. vivax} relapses. Our analysis showed that primaquine effectiveness varies widely between and within countries and demonstrated the geographic variability of \textit{P. vivax} relapse rates when primaquine is not used. These findings are consistent with previous studies in which tropical strains hold a higher risk of relapse than temperate strains and some strains of \textit{P. vivax} require higher doses of primaquine to prevent relapses. The results highlight the need for countries to undertake periodic local evaluation of \textit{P. vivax} relapse prevention regimens to determine an appropriate dose for their situation.

The studies included in this analysis vary in design, rigor, and sample size, and presented a number of methodologic challenges. First, large losses of subjects occurred in some studies and it was not known whether these non-evaluable subjects experienced a recurrence of \textit{P. vivax} symptoms. To avoid overestimation of primaquine effectiveness, we excluded non-evaluable subjects from analysis. Second, the virulence of \textit{P. vivax} and its response to primaquine varied between countries. To overcome this problem, we used grouped logistic regression to assess the relative effectiveness of primaquine in India, Brazil, and Thailand and to adjust for sample size. Third, there is potential for misclassification of reinfection as a relapse in malaria-endemic areas and the only way to ensure \textit{P. vivax} parasitemia is a relapse is by comparison of the parasite from the primary and recurrent episode using PCR-SSCP genotyping analysis. This technique is expensive and requires a level of skill difficult to achieve in resource-poor settings or in large studies. Another way to exclude this possibility is to assess efficacy of primaquine as anti-relapse therapy in non-endemic areas.

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**TABLE 1**

<table>
<thead>
<tr>
<th>Country</th>
<th>Reference</th>
<th>Patients enrolled*</th>
<th>Patients completing follow-up</th>
<th>\textit{P. vivax} relapse rate (%) following a total primaquine dose (milligrams)</th>
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<td></td>
<td></td>
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<td>Brazil</td>
<td>17</td>
<td>56</td>
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<tr>
<td></td>
<td>18†</td>
<td>79</td>
<td>61</td>
<td>26.7</td>
</tr>
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<td>India</td>
<td>19†</td>
<td>273</td>
<td>204</td>
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<td></td>
<td>29†</td>
<td>55</td>
<td>39</td>
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* Number in relevant study arms.
† Randomized control trial.
‡ Polymerase chain reaction–adjusted relapse rate = 3.5%.
§ Reference 27 provides results from two studies.

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**TABLE 2**

Summary of reports from Pakistan of \textit{Plasmodium vivax} malaria recurrence rates according to total primaquine dose for studies from which a \textit{P. vivax} relapse rate could not be calculated.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study period (months)</th>
<th>Patients completing follow-up</th>
<th>Recurrence rate (%) following a total primaquine dose (milligrams)</th>
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<tr>
<td>16†</td>
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<tr>
<td>16†</td>
<td>12</td>
<td>200</td>
<td>49</td>
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* Randomized controlled trial.
† Reference 16 provides results from two studies.
In India, a five-day course of primaquine (total adult dose = 75 mg) is commonly used as *P. vivax* anti-relapse therapy. Low relapse rates in some studies\(^1,21,23\) suggest this regimen has some benefit. However, the 75-mg regimen failed to show an effect in RCTs,\(^7,20,22\) which suggested that results from observational studies\(^14,21,23,24\) may not be indicative of the true relationship between primaquine and primaquine. In one observational study, different primaquine doses were compared in different geographic areas\(^21\) and different *P. vivax* transmission rates in each area may have biased results. Low relapse rates were calculated from the two RCTs using the 14-day regimen,\(^7,19\) which indicated that standard therapy is still effective in some regions in India, although low relapse rates were also calculated in comparison groups that did not receive primaquine. In contrast, *P. vivax* relapse rates from studies in Thailand were as high as 80% without primaquine,\(^28\) and 7–25%\(^6,27–29\) after standard anti-relapse therapy. Primaquine doses as high as 315 mg and 420 mg were necessary to reduce the risk of relapse substantially.

In the South American region, *P. vivax* malaria represents most malaria cases\(^1\) and the 210-mg regimen is commonly used as anti-relapse therapy. The two Brazilian studies in this analysis were conducted in the Amazon region. Our analysis demonstrated a higher risk of relapse in Brazil than in India and greater success with the 210-mg regimen than with the 75-mg regimen in preventing relapses. These Brazilian studies comprised small numbers of participants, and with few reports of natural relapse rates for comparison, it is difficult to further assess the effectiveness of *P. vivax* anti-relapse therapy in this region.

Although we were unable to include the studies from Pakistan in the logistic regression, the results from these studies add to the evidence regarding the response of *P. vivax* to primaquine. *Plasmodium vivax* is the predominant malaria parasite in Pakistan and with G6PD deficiency common in refugee communities in this region,\(^38\) a five-day regimen is commonly used as anti-relapse therapy, but showed no effect in the one study presented here.\(^16\) Standard therapy showed better results, but up to one-third of the cases still experienced a recurrence,\(^16,30\) which suggested that higher doses are required.

Failure of primaquine as anti-relapse therapy suggests primaquine resistance in *P. vivax*, although treatment failure may also be caused by sub therapeutic dosing resulting from failure to complete a course of treatment,\(^7\) substandard manufacturing of primaquine,\(^39\) or an inadequate weight-based dose.\(^17\) We estimated from our sub-analysis of weight-based primaquine dose a three-fold increase in the likelihood of successful treatment of each additional milligram of primaquine given per kilogram of body weight. This is consistent with other findings in which higher relapse rates have been found in patients with higher body weights.\(^17\)

Although sub-therapeutic primaquine dosing is a concern, primaquine therapy is extensively prescribed without consideration of body weight. A recent review recommends 0.5 mg/kg of primaquine/day for 14 days,\(^3\) which translates to a total dose of 420 mg for a 60-kg adult. There is a need for greater consideration of body weight in any context but it is important that the optimum dose per kilogram is tailored to the local context. Factors such as natural relapse rates, the local response to primaquine, treatment compliance, the ability to weigh patients, and the risk of adverse hemolytic effects also need to be considered. Future studies to evaluate primaquine effectiveness will need to be well designed and uniform to allow monitoring for emerging drug resistance over time and in different geographic areas.

![Image](image_url)

**Figure 1. Plasmodium vivax** relapse rate according to total primaquine dose and country.
REFERENCES


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