

Evaluation of Efficacy of Chloroquine for *Plasmodium Vivax* Infection Using Parasite Clearance Times: A 10-Year Study and Systematic Review

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Abstract

Introduction: Chloroquine, in combination with primaquine, is used as the firstline treatment for uncomplicated *P. vivax* malaria in Thailand. In view of the declining efficacy of chloroquine in many *P. vivax* endemic areas, the possibility of emergence of chloroquine-resistant *P. vivax* in Thailand is a concern. The aim of this study was to assess the trends in therapeutic efficacy of chloroquine and primaquine for the treatment of uncomplicated *P. vivax* malaria and to assess the utility of parasite clearance times as a measure of efficacy. **Materials and Methods:** This study consisted of: 1) review of medical records of patients who were hospitalised for a period during their treatment for uncomplicated *P. vivax* malaria at the Hospital for Tropical Diseases, Bangkok, Thailand between 2004 and 2013. Treatment consisted of chloroquine (1500 mg base administered over 3 days) or chloroquine (as before) plus primaquine (15 to 30 mg base/daily for 14 days from day 2); and 2) systematic review of the literature in English to assess current standards in the reporting of parasite clearance times. **Results:** The 28-day cure rate was 99.1%. The range of median parasite clearance time over the 10-year period was 46 to 59 hours, and there was statistical evidence for an increasing trend in parasite clearance times between 2009 and 2013. Heterogeneity was noted among previous chloroquine efficacy studies in the measurement and reporting of parasite clearance. **Conclusion:** The treatment of *P. vivax* infection with a combination of chloroquine and primaquine has remained efficacious in Thailand. Increasing rates of parasite clearance in a population over time may be a useful early warning mechanism for the emergence of chloroquine resistance. The utility of monitoring time-trends in parasite clearance to detect resistance may be enhanced if parasite clearance measurements are standardised.

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Key words: *Vivax* malaria, Parasite clearance rates, Chloroquine resistance, Time-trends

Introduction

Malaria is a major public health problem, causing an estimated 655,000 deaths worldwide in 2010.¹ It is a protozoan disease which occurs throughout the tropical regions of the world and is caused by the genus *Plasmodium* and is transmitted by *Anopheles* mosquitoes. Five species of the genus *Plasmodium* cause all malarial infections in human beings: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*.¹ *P. vivax* is currently endemic in about 50 countries. It is estimated that 2.6 billion people worldwide live at risk of infection by *P. vivax*, of whom between 72 and 390 million are estimated to become infected each year.² For many centuries, vivax malaria has been referred to as “benign tertian fever” because of

its fever periodicity and because it was considered to be rarely severe or fatal.³ However, case reports have shown that *P. vivax* infection may cause severe complications and may occasionally be associated with fatal outcomes. Complications of vivax malaria reported in the literature include severe anaemia and thrombocytopaenia, acute respiratory distress syndrome, acute renal failure, and splenic rupture.^{4,5} *P. vivax* infection during pregnancy is associated with maternal anaemia, low birth weight, and increased risk of miscarriage.⁶ Infections in young children may be associated with malnutrition and may have adverse consequences for education and development.⁷ Thus, the disease may cause substantial morbidity and socioeconomic disruption in endemic regions.

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Since *P. vivax* may exist in a dormant form in the liver as hypnozoites which may reactivate to cause relapses of infection weeks to months after the initial attack, treatment of *P. vivax* ideally involves the administration of a schizonticidal drug such as chloroquine in combination with a hypnozoitocidal agent. The only licensed hypnozoitocidal agent currently available is primaquine.^{2,8} The goal of treatment of malaria is to cure the infection rapidly and reliably, and early diagnosis and effective treatment significantly lowers the risk of progression to severe disease and the morbidity and costs associated with treatment failure.⁷ The combination of chloroquine and primaquine is highly effective against both the acute illness and in prevention of relapses in the treatment of chloroquine-sensitive *P. vivax* infections.⁷ However, the efficacy of chloroquine against *P. vivax* varies in different countries. In Papua New Guinea and parts of Indonesia, treatment failure rates greater than 20% have been recorded, and there have been reports of lower level resistance to *P. vivax* in various countries, including Cambodia, Ethiopia, Madagascar and in South America.^{7,9}

Resistance to antimalarial drugs is a major impediment to the effective treatment and control of malaria. It is therefore important to implement effective surveillance systems to detect emerging resistance of *P. vivax* against chloroquine. In view of the declining efficacy of chloroquine in many *P. vivax* endemic areas, the possibility of the emergence of chloroquine-resistant *P. vivax* in Thailand is a concern. Previous studies have generally shown that chloroquine is effective for the treatment of *P. vivax* malaria in Thailand,⁹ but there have been no studies demonstrating the trends in treatment efficacy over time. Variation in reporting of parasite clearance was also noted in a preliminary review of the literature.

This study has 2 components: a clinical study and a systematic review. The primary objectives of the clinical component were to investigate the trends in parasite clearance time (PCT) in patients treated for uncomplicated *P. vivax* malaria with chloroquine and primaquine at the Hospital for Tropical Diseases, Bangkok, Thailand between 2004 and 2013, and to determine the proportion of treatment failures in each year of the study period. The secondary objective was to assess trends in fever clearance time (FCT) over the 10-year period. The objective of the systematic review was to assess standards in the scientific literature for the measurement, reporting and interpretation of PCTs in the treatment of uncomplicated *P. vivax* malaria with chloroquine.

Materials and Methods

Clinical Study

The study population consisted of patients diagnosed with uncomplicated *P. vivax* malaria from 2004 to 2013 and who were treated with chloroquine only or chloroquine in combination with primaquine. Data were obtained from medical records of these patients. The inclusion criteria were: i) age ≥ 15 years at time of diagnosis, ii) mono-infection with *P. vivax* diagnosed by microscopy, and iii) completion of 28-day follow-up period. The exclusion criteria were: i) mixed infection diagnosed by microscopy (i.e. *P. vivax* and another *Plasmodium* species), ii) severe malaria (based on the World Health Organisation (WHO) criteria for severe malaria¹⁰), and iii) *P. vivax* in pregnancy.

Patients diagnosed with *P. vivax* malaria were admitted to hospital and treated with chloroquine 1500 mg base in divided doses over 3 days, either as monotherapy or in combination with primaquine which was started on day 2. Most patients were administered a primaquine regimen of 15 to 30 mg base/day for 14 days; a few patients with G6PD deficiency received an alternative regimen of 45 mg base/week for 8 weeks. Treatment administered in the hospital was supervised. Patients were monitored for clinical and parasitological responses, and were discharged if they were clinically well and blood smears were negative. Temperature was obtained from the oral site and was measured 4 hourly. At the time of discharge from hospital, patients were advised to complete the course of primaquine treatment, and were scheduled for follow-up visits on days 7, 14 and 28. At each visit, patients were examined clinically and thin and thick blood smears were obtained to check for parasitaemia.

Thick and thin blood films were prepared from a capillary blood sample using Giemsa's stain, and examined under a light microscope at 1000X magnification. Microscopic examination was performed every 12 hours until blood smears were negative for parasites. The number of parasites were counted for every 200 white blood cells and for every 1000 red blood cells in the thick and thin blood film respectively.

Treatment failure was defined according to WHO guidelines.¹⁰ The categories of treatment failure include i) early treatment failure (ETF), ii) late clinical failure (LCF), iii) late parasitological failure (LPF), and iv) adequate clinical and parasitological response (ACPR) (Table 1). PCT was defined as the time interval (in hours) from the start of treatment until the first negative blood smear for parasites, and remained negative for the subsequent 24 hours. FCT was defined as the time (in hours) from the start of treatment until the oral temperature decreased to $<37.5^{\circ}\text{C}$ and remained so for at least 48 hours. Time 0 was defined as the time treatment was initiated. Day 0 represented the first 24 hours after treatment was started.

Table 1. WHO Classification of Treatment Outcomes*

Early treatment failure (ETF)	a) Danger signs or severe malaria on day 1, 2 or 3 in the presence of parasitaemia; or b) Parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature; or c) Parasitaemia on day 3 with axillary temperature $\geq 37.5^{\circ}\text{C}$; or d) Parasitaemia on day 3 $\geq 25\%$ of count on day 0.
Late clinical failure (LCF)	a) Danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 28 in patients who did not previously meet any of the criteria of early treatment failure; or b) Presence of parasitaemia on any day between day 4 and day 28 with axillary temperature $\geq 37.5^{\circ}\text{C}$ in patients who did not previously meet any of the criteria of early treatment failure.
Late parasitological failure (LPF)	The presence of parasitaemia on any day between day 7 and day 28 and axillary temperature $< 37.5^{\circ}\text{C}$ in patients who did not previously meet any of the criteria of early treatment failure or late clinical failure.
Adequate clinical and parasitological response (ACPR)	The absence of parasitaemia on day 28, irrespective of axillary temperature, in patients who did not previously meet any of the criteria of early treatment failure, late clinical failure or late parasitological failure.

*Source: Methods of surveillance of antimalarial drug efficacy. World Health Organisation 2009.

Data were entered from medical records onto a standard template. SPSS version 15.0 was used for data management and analysis. Data were summarised using mean or median values for continuous data, and percentages for categorical data. Continuous data were assessed for normality by Shapiro-Wilk test and by inspection of frequency distribution plots. Differences in mean or median values between each time interval for each variable were assessed using ANOVA or the Kruskal-Wallis test. General linear modelling was used to assess for differences and trends in PCT and FCT. The a priori level of statistical significance was taken as <0.05 . In posthoc analyses of significant results, the Type 1 error was adjusted using the Bonferroni approach. The study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University.

Systematic Review

A database of antimalarial clinical efficacy trials, consisting of 1157 studies published between 1960 and 2013, is available online at the Worldwide Antimalarial Resistance Network (WWARN).¹¹ We filtered this database for all studies on *P. vivax*. We also searched MEDLINE, Scopus, and Web of Science databases using the search terms 'Vivax' in combination with each of the search terms 'Efficacy', 'Resistance', 'Clearance', 'Chloroquine', and 'Primaquine', for studies published between 1 January 2013 and 30 July 2015. The reference lists of some publications were also screened for relevant studies. The inclusion criterion was any single-arm or comparative clinical study published in English that assessed the in-vivo efficacy of chloroquine alone or chloroquine in combination with primaquine for the treatment of *P. vivax* mono-infection. This included studies on *P. vivax* relapses and in-vitro studies which also presented results of the schizonticidal phase of treatment. Exclusion criteria included: i) study participants with mixed *Plasmodium* infections, ii) study participants

who were treated with chloroquine in combination with another antimalarial drug other than primaquine, iii) studies of patients with *Plasmodium* infections in which the clinical data were not presented separately for each *Plasmodium* species, and iv) studies that limited the study population to pregnant women or children <15 years of age. Information extracted from these studies included the date of publication, study site, study duration, treatment regimen, inclusion criteria, sample size, follow-up period, technique of microscopy, timing of microscopic examination, time for resolution of parasitaemia, and the treatment success rate. The data were directly extracted from the full-length articles to a standard template containing all the relevant variables and outcomes. EndNote X7 was used to manage, deduplicate and screen article references for eligibility.

Results

Clinical Study

This study included data from 335 patients who were diagnosed with uncomplicated *P. vivax* malaria during the period 2004 to 2013, all of whom completed 28-days of follow-up. A total of 332 patients were treated with a combination of chloroquine and primaquine and 3 patients were treated with chloroquine only. There were 21 patients who defaulted follow-up before the 28-day period (5 patients in 2009, 11 patients in 2010 and 5 patients in 2011) and were excluded from the data analysis. There were relatively few eligible patients in 2004, 2005, and 2007 (11, 8 and 11 patients respectively); therefore, the data from years 2004 to 2007 were combined and analysed as one group. The majority of patients were Myanmar nationals (68.9%). Other nationalities/ethnic groups represented in the study included Karen (10.8%), Thai (10.3%), Mon (4.3%), Laos (3.4%) and Cambodia (2.3%).

Table 2 shows baseline characteristics of the study population. Information on G6PD status was not available

Table 2. Baseline Characteristics of Study Population

	2004 – 2007	2008	2009	2010	2011	2012	2013
Number of patients, n	64	46	30	46	53	45	51
Age (years)*	22 (15 – 50)	23 (15 – 45)	23 (15 – 45)	25.5 (16 – 55)	24.5 (14 – 60)	27 (16 – 76)	23 (15 – 64)
Male gender (%)	62.5	100	100	95.7	100	82.2	90.3
Height (cm)*	161 (139 – 182)	161 (151 – 174)	163 (149 – 172)	164 (153 – 176)	163 (142 – 181)	162 (141 – 176)	163 (135 – 178)
Weight (kg)*	52.0 (35 – 85)	53.0 (38 – 82)	51.5 (37 – 71)	52.8 (44 – 71)	55.0 (43 – 70)	54.0 (40 – 90)	55.6 (43 – 94)
Body surface area (m ²)†	1.54 (0.15)	1.56 (0.11)	1.54 (0.12)	1.57 (0.09)	1.58 (0.10)	1.59 (0.17)	1.62 (0.16)
G6PD deficiency‡	1/64 (1.6)	8/46 (17.4)	4/30 (13.3)	2/45 (4.4)	4/52 (7.7)	5/39 (12.8)	3/49 (6.1)
Other medical history‡	0	0	0	1 (2.2)	2 (3.8)	3 (6.7)	1 (2.0)
Concurrent antibiotic treatment‡	6 (9.4)	1 (2.2)	2 (6.7)	3 (6.5)	3 (5.7)	0	1 (2.0)
Haemoglobin (g/dL)*	12.6 (6.3 – 17.3)	12.9 (11.2 – 17.4)	13.4 (6.8 – 15.8)	12.8 (8.0 – 15.3)	13.0 (8.1 – 17.5)	12.2 (6.5 – 16.7)	13.0 (8.2 – 15.9)
Total white cell count (/mm ³)*	6.5 (2.1 – 13.7)	6.3 (3.2 – 10.8)	6.0 (2.3 – 17.5)	6.3 (2.6 – 10.0)	6.1 (2.6 – 10.9)	6.5 (3.1 – 10.9)	5.3 (3.0 – 10.2)
Platelet count (/mm ³)*	158 (25 – 568)	85 (26 – 198)	110 (30 – 267)	105 (17 – 459)	101 (12.5 – 516)	124 (33 – 542)	88 (35 – 320)

*Data represent median values (range).

†Body surface area (BSA) was calculated using Dubois formula ($BSA = \text{height}^{0.725} \times \text{weight}^{0.425} \times 0.007184$). Data represent mean values (standard deviation).

‡Data represent number of patients (%).

in 11 patients. Height measurements and information on nationality were missing in 6 and 3 patient records, respectively. For the other variables, the data were either complete or there was 1 missing data point over the 10-year period. Normality testing showed that most of the variables, except temperature on day 0 and body surface area, had a non-normal distribution. The median age for each time interval was between 22 to 27 years. Overall, males comprised 87.5% of patients. Most patients were otherwise healthy; only 7 patients were noted to have underlying medical conditions.

The temperature and parasitaemia levels before initiation of treatment and the temperature and parasitological responses to treatment are shown at Tables 3 and 4

respectively. The range of median PCT was between 46 and 59 hours and the range of median FCT was between 14 and 28 hours. The FCT was ≥ 100 hours in 8 patients, including 2 patients with FCT ≥ 200 hours. Co-infection was present in 3 patients (2 patients with dengue and 1 with enteric fever), including both patients with a FCT ≥ 200 hours, and 1 patient with a FCT of 126 hours.

Sixteen patients were also treated with antibiotics for intercurrent illnesses during the admission period. Antibiotics that were used included ceftriaxone (2 patients), amoxicillin (5 patients), cloxacillin (4 patients), metronidazole (2 patients), ciprofloxacin (1 patient), ofloxacin (1 patient), and azithromycin (1 patient). The median PCT of patients on antibiotics was 55.5 hours

Table 3. Temperature and Parasitaemia Levels before Initiation of Treatment

	2004 – 2007	2008	2009	2010	2011	2012	2013
Number of patients, n	64	46	30	46	53	45	51
Mean temperature on D0 (°C) (standard deviation)	38.5 (1.0)	38.6 (1.1)	38.2 (0.9)	38.2 (0.8)	38.5 (1.0)	38.5 (0.9)	38.4 (1.0)
Geometric mean asexual parasitaemia on D0 (/μL) [range]	7922 [150 – 115000]	6334 [98 – 58320]	7719 [162 – 101640]	6187 [129 – 55080]	11481 [132 – 90800]	7747 [150 – 70980]	5860 [150 – 38290]
Presence of gametocytes on D0, number (%)	16 (25.0)	22 (47.8)	9 (30.0)	20 (43.5)	19 (35.8)	15 (33.3)	10 (19.6)
Geometric mean gametocytaemia on D0 (/μL) [range]	16.79 [0 – 297]	53.49 [0 – 756]	50.3 [0 – 570]	46.0 [0 – 360]	53.0 [0 – 1068]	45.0 [0 – 788]	51.0 [0 – 488]

D0: Day 0

Table 4. Temperature and Parasitological Responses to Treatment

	2004 – 2007	2008	2009	2010	2011	2012	2013
Number of patients with asexual parasitaemia (%)							
Day 0	64 (100)	46 (100)	30 (100)	46 (100)	53 (100)	45 (100)	51 (100)
Day 1	60 (93.8)	41 (89.1)	23 (76.7)	36 (78.3)	41 (77.4)	37 (82.2)	43 (84.3)
Day 2	28 (43.8)	13 (28.3)	6 (20.0)	8 (17.4)	23 (43.4)	17 (37.8)	23 (45.1)
Day 3	6 (9.4)	1 (2.2)	0	1 (2.2)	4 (7.5)	1 (2.2)	6 (11.8)
Day 4	2 (3.1)	1 (2.2)	0	0	1 (1.9)	0	1 (2.0)
Day 5	0	0	0	0	0	0	0
Number of patients with gametocytaemia (%)							
Day 0	16 (25.0)	22 (47.8)	9 (30)	20 (43.5)	19 (35.8)	15 (33.3)	10 (19.6)
Day 1	3 (4.7)	6 (13.0)	2 (6.7)	5 (10.9)	7 (13.2)	5 (11.1)	4 (7.8)
Day 2	0	1 (2.2)	0	1 (2.2)	1 (1.9)	1 (2.2)	1 (2.0)
Day 3	0	0	0	0	0	0	0
Median parasite clearance time (hours) [range]	58.0 [30 – 123]	57.0 [33 – 118]	46.0 [21 – 82]	51.5 [27 – 100]	59.0 [22 – 135]	57.0 [12 – 172]	53.0 [24 – 160]
Median fever clearance time (hours) [range]	28 [0 – 126]	14 [0 – 52]	18 [0 – 36]	22 [0 – 264]	26 [0 – 144]	24 [0 – 72]	28 [0 – 200]

(range: 33 to 133 hours), which was similar to the median PCT of 56 hours (range: 12 to 172 hours) of the entire study population. The median FCT of patients on antibiotics and of the study population was 30 hours (range: 0 to 264 hours) and 24 hours (range: 0 to 264 hours) respectively. There was no significant difference in median PCT of patients who received 15 mg base of primaquine per day ($n = 182$) compared to those who received 30 mg base per day ($n = 145$) ($P = 0.122$).

In the univariate analysis, the differences in median PCT between each time interval were marginally non-significant ($P = 0.051$). The differences in median FCT values were not statistically significant ($P = 0.067$). Among the baseline demographic and clinical data, the differences in median platelet counts were statistically significant (χ^2 : 18.81, $P = 0.004$). Posthoc pairwise comparisons showed that the difference in the median platelet count between 2004 to 2007 and 2008 and between 2004 to 2007 and 2013 were significant ($P = 0.001$). There were no significant differences between time intervals in age, weight, height, body surface area, haemoglobin, haematocrit, white blood cell count, temperature on day 0, asexual parasitaemia on day 0 and gametocytaemia on day 0.

The influence of potential confounding variables on PCT and FCT values was assessed by ANCOVA testing. In view of the non-normal distributions of PCT and FCT, outliers were identified using the outlier labelling rule and removed from the dataset for the purpose of this analysis. In these modified datasets, 6 subjects with PCT >130 hours (1 in

2011, 4 in 2012, and 1 in 2013) and 8 subjects with FCT >90 hours (4 in 2004 to 2007, 2 in 2010, 1 in 2011 and 1 in 2013) were excluded from the PCT and FCT analysis, respectively. In both analyses, the year of diagnosis was entered as the factor variable, and age, body surface area, haemoglobin, white blood cell count, platelet count, asexual parasitaemia on day 0 and gametocytaemia on day 0 were entered as covariates. In the analysis of FCT, temperature on day 0 was entered as an additional covariate. The differences in adjusted mean PCT values between year of diagnosis were statistically significant ($F = 4.047$, $P = 0.001$, partial eta-squared = 0.073). Posthoc comparisons showed significant differences in adjusted mean PCT values between 2004 to 2007 and 2009 ($P < 0.001$), and between 2009 and 2013 ($P = 0.001$). The differences in adjusted mean values of FCT between time intervals were not significant ($F = 1.778$, $P = 0.103$, partial eta-squared = 0.034).

The plots of adjusted mean values of PCT and FCT appear to show a decreasing trend in PCT and FCT between 2004 and 2009, and an increasing trend from 2009 onwards (Figs. 1 and 2). To further assess the possibility of a linear time-trend in PCT and FCT, we also fitted linear regression models on the modified datasets using PCT and FCT as the response variable. The independent variables in both models included the year of diagnosis, gender, and the covariates used in the ANCOVA analysis. When the analyses were limited to data from 2009 and subsequent years only, there was a statistically significant linear relationship between PCT and year of diagnosis ($\beta = 2.340$, $P = 0.013$, adjusted

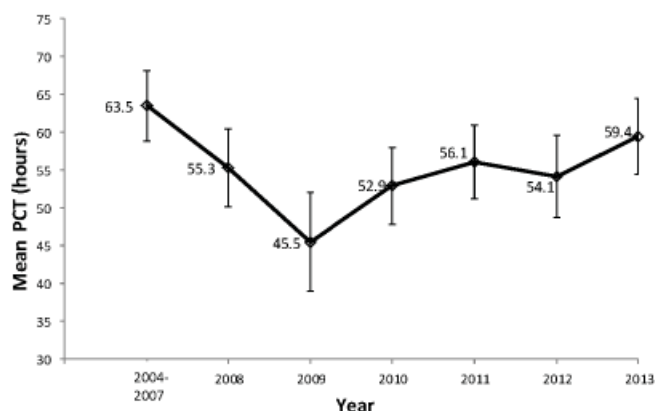


Fig. 1. Graph showing time-trends in adjusted mean parasite clearance time. PCT: Parasite clearance time. Data labels represent the adjusted mean PCT values for each time interval. Vertical bars represent the confidence interval for each adjusted mean value.

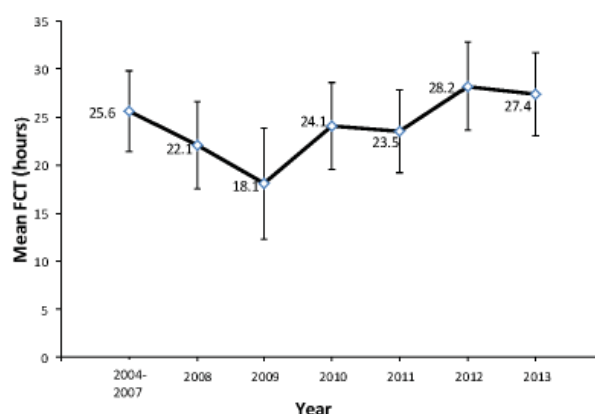


Fig. 2. Graph showing time-trends in adjusted mean fever clearance time. FCT: Fever clearance time. Data labels represent the adjusted mean FCT values for each time interval. Vertical bars represent the confidence interval for each adjusted mean value.

$R^2=0.164$), but no linear relationship between FCT and year of diagnosis ($\beta = 1.440$, $P = 0.072$, adjusted $R^2 = 0.312$).

A total of 332 patients (99.1%) showed an ACPR. There were 3 patients who had parasitaemia on day 3 together with temperature $\geq 37.5^\circ\text{C}$ (1 case each in 2004 to 2007, 2011 and 2013); thus, these patients met the WHO criteria for early treatment failure. All 3 patients received primaquine in addition to chloroquine. There were 16 other patients with parasitaemia on day 3, and 5 patients with parasitaemia on day 4; however, these patients were afebrile at that time and therefore did not meet the criteria for early treatment failure or late clinical failure. All patients with parasitaemia on and beyond day 3 responded well clinically and did not receive any additional treatment.

Systematic Review

Through review of study abstracts, 152 articles were selected for full-text retrieval (Fig. 3). Seven full-text articles were not available. Among 104 studies that met the inclusion criteria, a point estimate of parasite clearance was presented in hours in 26 studies, and in days in 6 studies. In 44 studies, there were other descriptions of parasite clearance, e.g. the proportion of patients who had cleared parasitaemia or failed to clear parasitaemia by a particular day. In 2 of the 26 studies which expressed PCT in hours, chloroquine was administered as a single dose of 300 mg or 450 mg,¹² and a weekly dose of 300 mg for 8 weeks.¹³ In the other 24 studies, a standard regimen of chloroquine was administered in divided doses over 48 to 72 hours at a total dose of ≥ 1500 mg base or a total dose of 25 mg base/kg bodyweight. Table 5 shows some study parameters and results from these 24 studies.¹⁴⁻³⁷ There was variation in

study design, including the sample size, parasite density on enrolment, primaquine regimen and the technique and timing of microscopy. Primaquine was administered concurrently with chloroquine in 4 studies,^{18,21,29,34} and was commenced on completion of chloroquine or later in the follow-up period in 12 studies.^{15-17,19-20,22,26,28,30-33} Parasite counts were initially obtained 6-hourly in 4 studies,^{20,24,29,34} and 8-hourly in 4 studies.^{27-28,35-36} In the remaining studies, parasite counts were obtained every 12 hours or less frequently. Both thick and thin films were used to assess for parasitaemia in 15 studies.^{15-22,25-26,29,30,33-34,36} In 4 studies, thick blood films only were used,^{27,32,35,37} and in 5 studies, the technique of microscopy was not specified.^{14,23-24,28,31} In studies which defined PCT, the beginning of the interval was most commonly defined as the time from initiation of treatment.^{15-16,18-20,22,25,27,31,35} The endpoint of the interval was defined as the time for blood films to become negative or to fall below the level of microscopic detection, and 4 studies also specified that blood films should remain negative for at least 24 hours.^{19,22,27,31} Among these 24 studies, the mean and median values of PCT varied from 36.7 to 70 hours and 20 to 32 hours respectively. Eighteen of these 24 studies also reported a mean or median FCT in hours.^{14-16,18-20,22,24,27,29-37}

There was similar heterogeneity in studies that reported other measures of parasite clearance. Overall, 73% (76/104) of studies provided some measure of parasite clearance; however, with the exception of a few studies that used parasite clearance measurements to assess the relative efficacies of different antimalarial regimens or treatment groups,^{15-16,20,22} these measurements were not used to interpret the efficacy of the treatment regimen. The 28-day cure rate was the primary measure of efficacy. We did not identify any studies that assessed time-trends in parasite clearance.

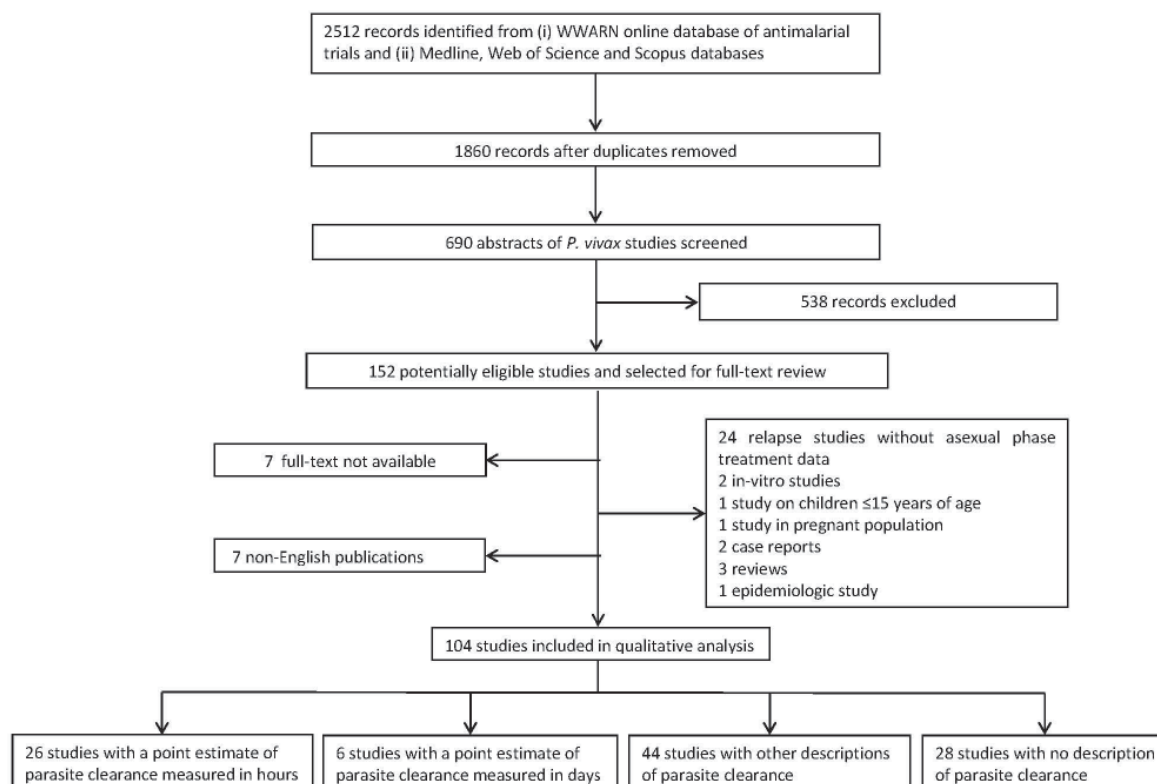


Fig. 3. Chart showing the systematic review search strategy and results.

Discussion

Of the total 335 patients, 332 (99.1%) showed an ACPR. Thus, the treatment of *P. vivax* infection with a combination of chloroquine and primaquine has remained efficacious over the study period. These findings are consistent with other studies on the in-vivo efficacy of chloroquine for treatment of *P. vivax* infection in Thailand.^{19,29-31,34,38-40} There were 16 patients on antibiotics concurrently with antimalarial treatment but, with the exception of azithromycin, these antibiotics are not known to have any antimalarial effects and would not have influenced the rate of parasite clearance. The range of median PCT in our study was 46 to 59 hours. Previous studies of chloroquine treatment for *P. vivax* have recorded PCT values ranging from 20 to 70 hours. However, there was heterogeneity among these studies in study parameters which may influence the rate of parasite clearance, and significant variation in the reporting of parasite clearance. In view of this heterogeneity, we did not consider it meaningful to perform a meta-analysis of PCT from previously published studies. Since almost all patients in our study showed an adequate clinical and parasitological response, the PCT values obtained in this study may provide a reasonably valid interval estimate of the expected median PCT values in a population where *P. vivax* retains susceptibility to chloroquine.

The most commonly used measure of parasite clearance in the literature is the cumulative proportion of patients who are aparasitaemic or who have failed to clear parasitaemia by a particular day. This information is also provided in Table 3 which shows that for each time interval, >96% of patients were aparasitaemic at the end of day 4, and all patients were aparasitaemic by day 5. However, this provides little information about the initial changes in parasitaemia, and in some patients, especially those with high initial parasitaemia, low numbers of parasites may persist for several days.⁴¹

There are some potential limitations to this study. First, the quality of information collected was reliant on the accuracy and completeness of information in patients' medical records. Second, there were only 11, 8 and 11 eligible patients in 2004, 2005 and 2007 respectively. In order to avoid any possible biases resulting from the small number of patients, the data from 2004 to 2007 were combined and analysed as a single group. Since the primary aim of this study was to assess trends in PCT and therapeutic efficacy over a 10-year period, combining the data from the earlier years would likely not have distorted the conclusions of the study. Third, information on adverse events to drug treatment was not available from the medical records. There were records of symptoms experienced by patients during hospital

Table 5. Data from Studies that Reported Parasite Clearance as a Point Estimate in Hours

Study	Treatment Groups [*]	Sample Size [†]	Initial Parasitaemia [‡] (/μL Blood)	Microscopy	Parasite Clearance Time		
					Mean/Median Value	Point Estimate (Hours)	Interval Estimate (Hours)
Dixon (1985) ¹⁴	CQ only	11	<100,000	12 hourly	Mean	48	SD: 20
	CQ + PQ	14				50	SD: 19
Pukrittayakamee (1994) ¹⁵	CQ + PQ	30	NS	12 hourly	Mean	62	SD: 22
Pukrittayakamee (1994) ¹⁶	CQ only	30	NS	12 hourly	Mean	55 – 60 [§]	-
	CQ + PQ	25				55 – 60 [§]	-
Tan-ariya (1995) ¹⁷	CQ + PQ	57	Any level	Once daily	Mean	48	SD: 15
Fryauff (1997) ¹⁸	CQ + PQ	27	0.001% – 0.1%	NS	Mean	53	R: 24 – 120
Looareesuan (1999) ¹⁹	CQ only	445	NS	12 hourly	Mean	61.3	SD: 20.2
	CQ + PQ	441				59.1	SD: 17.1
Pukrittayakamee (2000) ²⁰	CQ only	30	NS	6 hourly	Mean	65	SD: 18
	CQ + PQ	30				64	SD: 22
Villalobos-Salcedo (2000) ²¹	CQ + PQ	39	>100	Once daily	Mean	56.2	SD: 20.1
	CQ + short PQ regimen	40				59.0	SD: 20.4
	CQ + PQ (G6PD +ve)	342				59.4	SD: 17.5
Buchachart (2001) ²²	CQ + PQ (G6PD +ve)	342	NS	12 hourly	Mean	59.4	SD: 17.5
	CQ + PQ (G6PD –ve)	22				59.8	SD: 15.0
Soto (2001) ²³	CQ only	28	500 – 25,000	12 hourly	Mean	70	NR
Congpuong 2002) ²⁴	CQ + PQ	26	NS	6 hourly	Mean	49	R: 18 – 72
Fryauff (2002) ²⁵	CQ only	62	NS	Once daily	Mean	36.7	95% CI: +/-5.6
Hamedi (2002) ²⁶	CQ + PQ	40	2000 – 35,000	Once daily	Mean	67.2	SD: 22.5
Phan (2002) ²⁷	CQ + PQ	113	Any level	8 hourly	Median	24	R: 8 – 64
Dunne (2005) ²⁸	CQ + PQ	102	≤100,000	8 hourly	Median	20	NR
Tasanor (2006) ²⁹	CQ + PQ	31	1000 – 22000	6 hourly	Median	30	95% CI: 12 – 48
Krudsood (2006) ³⁰	CQ + PQ	71	Any level	12 hourly	Mean	43.68	SD: 17.29
Krudsood (2007) ³¹	CQ + PQ	51	Any level	12 hourly	Mean	55.8	R: 23 – 106
Nateghpour (2007) ³²	CQ + PQ	195	250 – 100,000	Once daily	Mean	63.5	SD: 15.84
Yeshiwondim (2010) ³³	CQ only	145	≥250	Once daily	Mean	48.34	SD: 17.68
	CQ + PQ	145				50.67	SD: 15.70
Muhamad (2011) ³⁴	CQ + PQ	130	1000 – 100,000	6 hourly	Median	24	95% CI: 12 – 40
Poravuth (2011) ³⁵	CQ only	228	≥250/μL	8 hourly	Median	32	R: 7.5 – 63.9
Liu (2013) ³⁶	CQ + PQ	128	400 – 100,000	8 hourly	Mean	37.4	SD: 11.9
Liu (2014) ³⁷	CQ only	750	50 – 120,000	12 hourly	NR	38.1	± 12.6 hours

CI: Confidence interval; CQ: Chloroquine, NS: Not specified; NR: Not recorded; PQ: Primaquine; R: Range; SD: Standard deviation

[†]In all studies, chloroquine was administered as a total dose of 25 mg base/kg or a total dose of ≥1500 mg base over 2 – 3 days.[‡]In studies that compared a chloroquine regimen with a non-chloroquine regimen, sample size refers to size of the chloroquine treatment arm.[§]Parasite density enrolment criterion.[§]Data were presented in graphical form.

admission, but it was not possible to ascertain whether these symptoms were drug-related or a consequence of the malarial infection. However, there were no occurrences of severe drug reactions which required discontinuation of treatment or switching to an alternative drug regimen. Fourth, information on PCT and treatment outcomes was

not available for 21 patients who did not complete 28 days of follow-up (11 patients in 2010, and 5 patients in each of the years 2009 and 2011). A sensitivity analysis showed that the ACPR would decrease to 86%, 81% and 90% for 2009, 2010 and 2011 respectively, had all dropouts been included in the analysis and assuming all of them had failed

treatment. Fifth, oral temperature measurements were obtained in this study, whereas WHO criteria for treatment failure are based on axillary temperature measurements. However, axillary temperatures are generally lower than oral temperatures by approximately 1°C.⁴² Thus, the use of oral temperature instead of axillary temperature would not have increased the number of treatment failures or altered the study conclusions. Lastly, the recommended dose of primaquine for radical cure of vivax malaria is 0.5 mg base/kg bodyweight per day for tropical strain infections.⁴³ Thus, a dose of 30 mg base per day would have been inadequate for patients whose bodyweight was greater than 60 kg. All 3 patients who met the criteria for early treatment failure had a bodyweight of less than 60 kg.

The results from the regression analysis suggest that the covariates accounted for a relatively small proportion of the total variation in PCT and FCT. Therefore, there may be other variables not accounted for in this analysis that may influence the rates of parasite and fever clearance. We observed a statistically significant increase in PCT between 2009 and 2013. In our analyses, we applied the conventional value of 0.05 as a cutoff point for statistical significance, which does not necessarily imply clinical relevance. In order to use parasite clearance as a marker to guide policy, it would be necessary to develop guidelines for determining clinically significant values of parasite clearance rates and changes in these rates over time.

The reporting of the systematic review adhered to the recommendations of the PRISMA statement.⁴⁴ Seven full-text articles that were not available and 7 foreign language publications were excluded from the list of eligible studies. We retrospectively reviewed these studies to assess the possibility of selection bias. Of the 7 unavailable full-text articles, the abstracts were also not available for 5 studies. These studies were published prior to 1980.^{45–49} The other 2 studies evaluated the efficacy of chloroquine and other antimalarials for treatment of *P. vivax* and other *Plasmodium* species.^{50–51} These abstracts did not make any reference to parasite clearance. We also obtained the English language abstracts and a limited translation of the foreign language publications which comprised 7 chloroquine efficacy studies. Chloroquine was administered in a single dose of 600 mg in one study.⁵² In the other studies which utilised a standard regimen of chloroquine, parasite clearance was expressed as the cumulative proportion of patients who were aparasitaemic by a particular day of treatment.^{53–58} As the main focus of the review was to assess standards in measurement and reporting of parasite clearance in the scientific literature, we do not consider publication bias to be a significant concern in our review.

WHO has published guidelines on methods for surveillance of antimalarial drug efficacy.¹⁰ These

recommend establishing sentinel sites where data can be collected and which represent all the epidemiologic strata in the country. There are a number of potential difficulties in conducting and interpreting results from these studies. Each study is a single-arm or randomised clinical trial, and WHO recommends that all firstline and secondline medicines be evaluated at least once every 24 months at all sites.⁵⁹ Thus, these studies require adequate and ongoing preparation, financial support and human resources. Due to the prospective nature of the study and the need to achieve an adequate sample size, there is a lag period between the commencement of the study and availability of results. Thus, the results of the study may not represent the actual situation regarding drug resistance at a given time. In countries where the area of malaria transmission is very large, it may be necessary to establish a large number of sentinel sites in order for information collected to be representative of the disease in the entire region or country. This may not be practical due to resource constraints.

There are a few factors that may affect the reproducibility, accuracy and interpretation of parasite clearance measurements. These include i) Diagnostic protocols, i.e., the type of blood films obtained, and the frequency with which parasite counts are obtained. Thick blood films give counts approximately 30% lower than the corresponding thin film.^{41,60} Increasing the frequency of microscopic examinations will improve the precision of parasite clearance estimates; ii) Concurrent use of chloroquine and primaquine. Since primaquine has blood schizonticidal activity against *P. vivax* at therapeutic doses,^{16,61} administration of primaquine early in the disease course may accelerate PCT; iii) Interindividual variability in parasite clearance. This may be due to differences between individuals in splenic function and other host defences, and levels of acquired immunity;⁴¹ iv) Initial parasitaemia. It is possible that patients with high parasite counts prior to treatment will take longer to clear their parasitaemia compared to patients with low initial counts; and v) Variation in estimation of parasitaemia between different laboratory personnel, especially between microscopists with different levels of skills and experience.

Previous studies have explored the correlation between parasite clearance and treatment response in individuals,^{62,63} but in view of inherent variability in parasite clearance and the potential random and systematic errors in parasite clearance measurements, it may be difficult to interpret the significance of PCT in any particular individual. However, it is likely that the average PCT among all infected individuals treated with a particular drug regimen will remain fairly constant over time if that drug regimen remains efficacious, a sufficient number of patients are evaluated at each time interval, and the baseline demographic and clinical

characteristics of patients at each time interval are similar. An increasing population trend in parasite clearance rates over time, even amongst individuals who achieve an adequate clinical and parasitological cure, may provide early warning of drug resistance. Thus, time-trends in population rates of parasite clearance may be useful as a sentinel surveillance mechanism to detect emerging resistance of *P. vivax* to chloroquine, as random and systematic errors in parasite clearance measurements are expected to be approximately similar over different time periods. If increasing trends in PCT values are noted, clinical trials and in-vitro studies could be performed in the region of interest to determine if drug resistance exists and to characterise the level and nature of resistance.

An advantage of parasite clearance as a surrogate marker for resistance is that it is not confounded by relapse or reinfection.⁷ Parasite clearance measurements can also be obtained as part of routine clinical practice and therefore do not incur any additional costs and other resources required to conduct a formal clinical trial. The parasite clearance estimator is an online tool developed to estimate parasite clearance rates in the treatment of falciparum malaria with artemisinin derivatives.¹¹ A similar tool validated for the treatment of *P. vivax* with chloroquine may help to provide robust estimates of PCT and facilitate routine monitoring of chloroquine efficacy. Information on parasite clearance could be transmitted to a central agency and pooled from different health centres across a region, allowing for real-time monitoring of trends in parasite clearance in that region, and also for comparisons between different geographical locations. However, estimation of parasite clearance is critically dependent on the ready availability of trained microscopists, and in resource-limited settings, manpower constraints may preclude highly precise measurements. Thus, measurement of PCT in hours may be relatively easy to obtain in hospitalised patients but less so in outpatient settings in endemic regions.

Temperature measurements are routinely obtained as part of clinical management of patients, and fever clearance is a useful indicator for monitoring the clinical progress in individual patients. However, measurement of fever clearance may be confounded by a number of factors including the frequency of temperature monitoring, the ambient temperature, use of antipyretics or cold compresses, use of intravenous fluids, cutoff temperature for definition of fever, site of temperature measurement, wrongly calibrated thermometers and interindividual variability in temperature. In addition, fever may be prolonged in patients for reasons other than malaria infection, such as co-infection or drug reactions. For these reasons, trends in fever clearance rates are unlikely to be helpful as a tool to monitor the efficacy of or the development of resistance to chloroquine or other drugs used to treat *P. vivax* malaria.

The use of alternative drugs to treat *P. vivax* infection would obviate the need to monitor for emerging resistance of *P. vivax* to chloroquine. Douglas et al (2010)² have reviewed the use of artemisinin combination therapies (ACTs) for the treatment of both *P. vivax* and *P. falciparum* infections. The advantages of this approach are that various ACTs are effective against chloroquine-resistant strains of *P. vivax*, and a unified treatment strategy would not depend on correct identification of malaria species.² However, ACTs are more expensive than chloroquine,⁴³ and the widespread use of ACTs for all malaria infections may also accelerate the development of resistance to ACTs in all malaria species. This may reduce the efficacy of ACTs in the treatment of severe malaria, with a resultant increase in the morbidity and mortality from malaria infections. Thus, chloroquine is still a viable and useful firstline treatment for chloroquine-sensitive *P. vivax* infections, and ongoing surveillance for emerging resistance to chloroquine would continue to be an important component of malaria control programmes.

Conclusion

The combination of chloroquine and primaquine has remained effective for the treatment of uncomplicated *P. vivax* malaria in Thailand over the period 2004 to 2013. There was some evidence for an increase in PCT between 2009 and 2013, which may warrant further evaluation for developing resistance. Relying on clinical efficacy studies alone to monitor drug resistance may result in a significant time-lag before resistance is detected, and monitoring trends in average population PCT over time may therefore be a useful sentinel surveillance mechanism to detect emerging resistance of *P. vivax* to chloroquine. Heterogeneity was observed among prior studies in study parameters which may influence the rate of parasite clearance. In order for PCT to be a useful marker for drug resistance, it would be necessary to standardise various aspects of measurement including the precise definition of PCT, the type of blood films used for assessment of parasitaemia, and the frequency of microscopy. If consensus guidelines on methodology, reporting, analysis and interpretation of parasite clearance rates and trends are developed and adopted by the scientific community worldwide, then cross-country comparisons of PCT may also be possible, thereby allowing for effective monitoring of emerging resistance globally.

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