Factors Affecting Primaquine Combination Treatment in Malaria Patients in Selangor, Malaysia

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Introduction: Primaquine is vital for the management of liver-stage Plasmodium vivax and Plasmodium ovale malaria. However, primaquine effectiveness is dependent on various factors and differs between populations. Therefore, this study was conducted to identify factors that affect the length of stay and relapse during primaquine combination treatment in malaria-infected patients in the local setting. Materials and Methods: A retrospective study on the use of primaquine combination among P. vivax and P. ovale infected patients in Selangor, Malaysia within a 5-year period from 2011 to 2015 was obtained from the National Malaria Case Registry, Malaysia. Data collected were patient characteristics (age, gender, nationality, glucose-6-phosphate dehydrogenase, pregnancy); disease characteristics (survival, past malaria infection, parasite type, presence of gametocyte, parasite count, week onset, severity, transmission type); and treatment characteristics (type of antimalarial, treatment completion). Outcome measures were length of stay and relapse during a 1-year follow-up. Results: A total of 635 patients were included in the study. Based on a multivariate logistic regression analysis, the significant predictors for length of stay were gender ($P = 0.009$) and indigenous transmission ($P < 0.001$). Male patients had a shorter length of stay than females by 0.868 days ($P = 0.009$), and indigenous transmission took 1.82 days more compared to nonindigenous transmission ($P < 0.001$). Predictors for relapse were indigenous transmission of malaria ($P = 0.019$), which was 15.83 times more likely to relapse than nonindigenous transmission ($P < 0.01$). Conclusions: This study reveals that the effectiveness of primaquine was clinically associated with gender and indigenous transmission. To that end, vigilant monitoring of primaquine use is required to reduce relapse and future transmission.

Keywords: 8-Aminoquinoline, malaria, Plasmodium species, primaquine, Selangor

Introduction

Malaria infection has become a significant global concern and causes an estimated 13.8 million cases globally. Approximately half of all malaria cases occur outside Africa. Malaria is also found in Malaysia, although effective interventions are being implemented under the National Strategic Plan for Elimination of Malaria (2011–2020) to successfully eradicate the infection. Among the steps in reducing malaria are the use of bed nets, patient education, closer monitoring of drug resistance, and frequent monitoring of parasite elimination. However, eliminating malaria remains a challenge due to the limited number of antimalarials available to effectively treat Plasmodium infection.

The distinguished features of the Plasmodium species threaten the control of malaria globally. The Plasmodium parasite is able to develop in the anopheles mosquito vector at lower temperatures and to survive at higher...
altitudes and in cooler climates.\(^{(1)}\) *Plasmodium* infection may also cause relapse in some cases following a primary infection, due to the ability to develop into dormant liver stages known as hypnozoites in the liver,\(^{(2,4)}\) greater asymptomatic asexual carriage, and early gametocyte production. These features have appreciably affected local transmission; thus, elimination of malaria faces far greater obstacles than initially thought.\(^{(5,6)}\)

The ability to form hypnozoites and cause infections at a later date is unique to both *Plasmodium vivax* and *Plasmodium ovale*. Interestingly, *P. vivax* persists to affect many regions across all Western Pacific region countries. In Malaysia, it is one of the main infections causing malaria within the past 10 years.\(^{(6)}\) The majority of *P. vivax* is mainly found in Peninsular Malaysia, most notably in Selangor.\(^{(6)}\) On the other hand, *P. ovale* occurs at a less alarming rate in Malaysia. However, management of both *P. vivax* and *P. ovale* requires attention due to the risk of hypnozoite formation during infection.

Treatment of hypnozoites is dependent on the antimalarial primaquine. Primaquine is an 8-aminoquinoline that was introduced 60 years ago. At present, primaquine has a unique role as it is the only approved drug for eliminating hypnozoites in *P. vivax* and *P. ovale* infected patients.\(^{(7)}\) However, its effectiveness is limited by a long treatment course (14 days) and concerns over hemolytic adverse events. Interestingly, other use also includes primaquine as a gametocytocide to reduce transmission of *Plasmodium falciparum*.\(^{(8,9)}\) Indeed, despite being among the older quinoline antimalarial, primaquine plays a vital role in managing malaria infection. At present, resistance toward the drug remains minimal. However, the data on primaquine resistance must be interpreted with caution because of many confounding factors, such as geographical variations in relapse patterns, unsupervised therapy, parasite tolerance, the risk for reinfection, and the difficulty of finding a valid control group.\(^{(10-12)}\) In view of the dependency of primaquine for hypnozoite clearance, ensuring effectiveness of the drug is vital. This is especially a concern in areas where *P. vivax* occurs frequently.

Recent work has shown that effectiveness of antimalarials to be dependent on various factors such as ethnicity, gender, previous infection, and type of treatment.\(^{(13)}\) The use of primaquine in combination with a blood schizontocide such as chloroquine and artemisinin makes it difficult to determine effectiveness. However, in view of the limited availability of antimalarials recommended for hypnozoite activity, the use of primaquine must be closely monitored. To achieve sustainable use of primaquine, collective efforts should be concerted in looking for ways to minimize the failure and side effects following treatment. Understanding the factors involved in primaquine efficacy and monitoring clinical outcomes may reveal interindividual differences in response toward primaquine therapy. Therefore, this study aims in evaluating the use of primaquine in *P. vivax* and *P. ovale* infection in the local population in an attempt to understand factors involved in efficacy of the drug.

**MATERIALS AND METHODS**

**Study design**

This was a retrospective study on the usage of primaquine among *P. vivax* and *P. ovale* infected patients in Selangor, Malaysia within 5-year period from 2011 to 2015. Data were extracted from the National Malaria Case Registry (NMCR) under the Ministry of Health. NMCR is a central database that registers all malaria cases notified by the different health sectors, including public and private health institutions. All reported cases from various states in Malaysia are compiled under the NMCR.

**Ethics**

The study was registered under the National Medical Research Registration and ethical approval obtained from the National Medical Research Ethics Committee (ID: NMRR-14-1308-23222). Ethical procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000.

**Study criteria**

The data were screened for patients with *P. vivax* and *P. ovale* infected patients of which primaquine is routinely prescribed as therapy. Data collected were patient characteristics (age, gender, nationality, pregnancy, and glucose-6-diphosphate (G6PD) status), disease characteristics (relapse, survival, severity, length of stay, previous malaria infection, type of malaria parasite, sexual parasite counts on admission, asexual parasite counts on admission, malaria transmission type, gametocyte presence on admission, and week onset), and treatment characteristics (primaquine combination either with chloroquine or artemisinin combination therapy). Malaria transmission type is categorized into imported, indigenous, introduced, or relapse.\(^{(14)}\) Indigenous transmission is defined as locally acquired malaria infection. Infections acquired outside Malaysia are termed imported transmission. Introduced transmission is defined as malaria contracted locally, with strong epidemiological evidence linking it directly to a known imported case (first-generation local transmission).
Relapse is recurrence of parasite infection from dormant hypnozoites in liver cells.\(^4\) Week onset of infection is based on a 52-week calendar. Incomplete data on type of malaria parasite were excluded from the study.

**Study outcome**
Clinical outcome was measured as the length of hospital stay for each patient and occurrence of relapse in 1-year follow-up. As part of the clinical practice on malaria management, patients are monitored for parasitemia on a daily basis.\(^{15}\) Blood samples are collected and blood film malaria parasite investigations are performed. Patients are discharged when both parasitemia and gametocytemia levels are zero on two separate occasions within 48 h. This has become the standard practice in handling malaria infection during admission.\(^{15}\) Therefore, the length of stay is therefore defined as the 1st day the patient is admitted for malaria infection until the patient is discharged after 48 h of zero parasitemia and gametocytemia.

Relapse is defined as recurrence of asexual parasitemia following primary.\(^{4}\) Based on the Ministry of Health, Malaysia protocol, patients will be followed up within a year after infection to identify relapse. The occurrence of relapse was determined from positive blood film malaria parasite slides during the 1-year follow-up after discharge.

**Statistical analyses**
Statistical analyses were performed using IBM Statistical Package for the Social Sciences software package (version 23) and were used to determine whether there was a difference in length of hospital stay and occurrence of relapse between the independent variables. Independent \(t\)-test and analysis of variances were tested for equal variances between groups. Mann-Whitney and Kruskal–Wallis were used for unequal variances between groups. Mann–Whitney and Kruskal–Wallis were used for unequal variances between groups. Missing values were treated as pairwise deletion. \(P < 0.05\) was considered statistically significant.

**Results**

**Patient characteristics**
A total of 635 malaria patients treated with primaquine combination drugs between the years 2011 and 2015 were included in the study. There were 393 adults aged 25 years and above which constituted more than 60%. The mean age of the study population was 28.5 ± 9.9 years (from <1 to 80 years). There was a total of 4.6% (29) patients between the age of <1 and 14 years (children), 33.5% (213) patients between the age of 15 and 24 years (adolescents), 61.6% (391) patients between the age of 25 and 59 years, and 0.3% (2) patients above 60 years old (elderly). Most patients were noted to be from South East Asia region: Indonesia (27.8%, 177), Eastern Mediterranean region: Pakistan (n = 188, 29.6%) and Western Pacific region: Malaysia (14.6%, 93) as presented in Table 1a.

The main malaria parasite in the study was \(P.\) vivax (99.5%, 632) compared to \(P.\) ovale (0.5%, 3). An average of 5.46 ± 2.81 (range 0–19) days was reported for both parasitemia and gametocytemia to clear as presented in Table 1b. The treatment guideline was followed and adhered to at all health institutions.\(^{14,16}\) Treatment of infection was either with a combination of chloroquine/primaquine (65.67%, 471) or artemisinin/primaquine (34.33%, 164). Majority of the patients in the study population completed the recommended primaquine treatment on follow-up (96.7%, 614) compared to those that did not complete treatment (3.3%, 21) as summarized in Table 1c. Relapse within 1 year was observed in 0.79% (5) patients.

Table 1a: Characteristics of the patients in the study population \((n=635)\) from 2011 to 2015

| Patient characteristics | \(n=635\) |
|-------------------------|----------|
| Age                     | Mean±SD  |
|                         | 28.50±9.9|
|                         | Range    |
|                         | <1-80    |
| Gender, \(n\) (%)       | Male     |
|                         | 557 (87.72)|
|                         | Female   |
|                         | 78 (12.28)|
| Nationality, \(n\) (%)  | Malaysian|
|                         | 93 (14.65)|
|                         | Non-Malaysian |
|                         | 542 (85.35)|
| WHO regions, \(n\) (%)  | Africa   |
|                         | 7 (1.10)  |
|                         | South East Asia\(^a\) |
|                         | 348 (54.80)|
|                         | Western Pacific\(^b\) |
|                         | 97 (15.28) |
|                         | European  |
|                         | 2 (0.31)  |
|                         | Eastern Mediterranean\(^c\) |
|                         | 181 (28.50)|
| G6PD, \(n\) (%)         | Deficient|
|                         | 248 (39.06)|
|                         | Intermediate |
|                         | 2 (0.31)  |
|                         | Normal    |
|                         | 289 (45.51)|
|                         | Unknown   |
|                         | 96 (15.12)|
| Pregnancy, \(n=78\), \(n\) (%) | No |
|                         | 76 (97.44)|
|                         | Yes       |
|                         | 2 (2.56)  |

\(^a\)Country: Indonesia \((n=177, 27.8\%)\), \(^b\)Country: Malaysia \((n=93, 14.65\%)\), \(^c\)Country: Pakistan \((n=188, 29.6\%\). SD: Standard deviation, WHO: World Health Organization, G6PD: Glucose-6-phosphate-dehydrogenase.
A univariate analysis was performed to identify variables affecting length of stay. Male patients had a shorter length of stay than females by 1.064 days ($P = 0.002$). Malaysians took 1.308 days more than non-Malaysians for parasite to clear ($P = 0.001$). Patients from the Western Pacific took 1.192 days more than non-Western Pacific patients for parasites to clear ($P < 0.0001$). In addition, indigenous infections took 2 days more for parasites to clear than nonindigenous infections ($P = 0.001$). When all four variables were tested in a multivariate analysis, only two variables were found to be factors that affected the length of stay when controlling for other confounding factors. The model was statistically significant ($P < 0.001$, $R^2 = 0.085$), indicating that 8.5% variance in length of stay was accounted for by this model when other predictors were taken into account. Both gender (male) and transmission type (indigenous) were found to be statistically significant. Male patients had a shorter length of stay than females by 0.868 days ($P = 0.009$), and indigenous transmission took 1.82 days more for parasites to clear compared to nonindigenous transmission ($P < 0.001$) as presented in Table 2.

**Factors affecting relapse during primaquine combination treatment**

Further analysis was then performed to identify factors that affected relapse during primaquine treatment. A total of 0.79% (5) patients were found to have relapse after primaquine combination treatment during a 1-year follow-up. A univariate analysis demonstrated that time taken for the length of stay and transmission type affected relapse outcome. An increase in 1 day taken for the length of stay increases relapse by 1.265 times ($P = 0.002$). On the other hand, indigenous transmission was 21.71 times more likely to relapse than nonindigenous transmission ($P < 0.01$). When both variables were analyzed using a multivariate logistic regression, it was found that transmission type was a significant factor when controlling for other confounding factors. The model was statistically significant ($P = 0.03$, $R^2 = 0.202$), indicating that 20.2% variance in relapse was accounted for by this model when other predictors were taken into account. Indigenous transmission was 15.83 times more likely to relapse than nonindigenous transmission ($P = 0.019$) as presented in Table 3.

**DISCUSSION**

Despite the gradual decline of overall malaria infection in this country, the prevalence of malaria infection in the state of Selangor remains a health concern. Selangor is situated on the west coast of Peninsular Malaysia and...
has been well known as a gateway and industrial hub. It is the most populous state with a total population of 5.46 million inhabitants and has a high level of urbanization compared to other states in Malaysia.\textsuperscript{[17]} As such, this study highlighted the most common malaria infection, \textit{P. vivax}, was mainly attributed by the influx of foreigners, as previously described in other work.\textsuperscript{[18]} Malaria was also observed to predominantly affect adult patients between 25 and 59 years. This causes a major socioeconomic impact, as those that are affected tend to be among the agricultural and outdoor workers.\textsuperscript{[19]} In view of this, ensuring appropriate management is vital especially in developing countries such as Malaysia.

Both primaquine combinations such as chloroquine/primaquine and artemisinin/primquine were largely used during the study duration. The large use of both drugs is mainly due to the change in national protocol that recommended older quinoline combinations: chloroquine/primaquine, before 2012 for treatment of \textit{P. vivax} malaria.\textsuperscript{[16,20]} Following the recommendation of the World Health Organization, artemisinin replaced the use of chloroquine in chloroquine-resistant malaria.\textsuperscript{[21]} The duration of primaquine treatment, however, remains the same, daily management for 14 days in normal G6PD patients and weekly doses for 8 weeks in G6PD-deficient patients.\textsuperscript{[11,21]} Primaquine is extensively

### Table 2: Univariate and multivariate regression analysis on length of stay (days) during treatment with primaquine combination (n=635) from 2011 to 2015

| Patient characteristics | β     | 95% CI       | P   |
|-------------------------|-------|--------------|-----|
| Age                     | −0.015| −0.037-0.007 | 0.185|
| Gender                  | −1.064| −1.727–0.40  | 0.002|
| Nationality             | 1.308 | 0.695-1.92   | 0.001|
| WHO regions             | 1.192 | 5.0469-5.5182| <0.0001|
| G6PD                    | −0.312| −0.762-0.137 | 0.173|
| Pregnancy               | 0.537 | −3.379-4.453 | 0.788|
| Disease characteristics  |       |              |     |
| Survival                | 0.656 | −0.101-1.414 | 0.089|
| Previous infection      | −0.147| −0.723-0.429 | 0.616|
| Type of malaria         | 2.476 | −0.718-5.671 | 0.128|
| Presence of gametocyte  | 0.337 | −0.152-0.827 | 0.177|
| Sexual                  | 0.000 | 0.000-0.000  | 0.36 |
| Asexual                 | 0.000 | 0.000-0.000  | 0.91 |
| Week onset (week)       | −0.004| 0.021-0.012  | 0.612|
| Severity                | −0.626| −1.562-0.31  | 0.189|
| Malaria transmission type| 2.016 | 1.44-2.593   | 0.001|
| Relapse before          | 0.545 | −1.137-2.226 | 0.525|
| Treatment characteristics|       |              |     |
| CQ plus PQ versus ACT plus PQ| −0.358| −0.82-0.103 | 0.128|

### Multivariate analysis

| Patient characteristics | β     | 95% CI       | P   |
|-------------------------|-------|--------------|-----|
| Gender                  | −0.868| −1.522–0.215 | 0.009|
| Nationality             | 0.483 | −0.165-1.131 | 0.144|
| WHO                     | −0.152| −2.04-1.736  | 0.874|
| Disease characteristics  |       |              |     |
| Malaria transmission type| 1.817 | 1.205-2.429  | <0.001|

### Table 3: Univariate and multivariate logistic regression on relapse during 1-year follow-up after treatment with primaquine combination (n=5) from 2011 to 2015

| Patient characteristics | OR  | 95% CI       | P   |
|-------------------------|-----|--------------|-----|
| Age                     | 1.044| 0.972-1.122  | 0.235|
| Gender                  | 4.860| 0.799-29.553 | 0.086|
| Nationality             | 3.949| 0.651-23.960 | 0.135|
| WHO region countries    | 1.391| 0.154-12.580 | 0.769|
| G6PD                    | 2.357| 0.391-14.208 | 0.350|
| Pregnancy               | 0.000| 0.000-0.000  | 1.000|
| Disease characteristics  |       |              |     |
| Length of stay (days)   | 1.265| 1.050-1.523  | 0.013|
| Survival                | 0.000| 0.000-0.000  | 0.999|
| Previous infection      | 0.980| 0.109-8.848  | 0.986|
| Type of malaria         | 0.000| 0.000-0.000  | 1.000|
| Presence of gametocyte  | 0.522| 0.058-4.700  | 0.562|
| Sexual                  | 1.000| 1.000-1.000  | 0.670|
| Asexual                 | 1.000| 1.000-1.000  | 0.456|
| Week onset (week)       | 1.003| 0.940-1.071  | 0.920|
| Severity                | 0.000| 0.000-0.000  | 0.998|
| Indigenous transmission | 21.714| 2.401−0.01   |     |
| Relapse before          | 0.000| 0.000-0.000  | 0.100|
| Treatment characteristics|       |              |     |
| CQ plus PQ versus ACT plus PQ| 0.480| 0.053-4.000 | 0.510|
| Status of treatment     | 0.000| 1.000        |     |

### Multivariate analysis

| Disease characteristics | OR  | 95% CI       | P   |
|-------------------------|-----|--------------|-----|
| Length of stay (days)   | 1.124| 0.927-1.364  | 0.235|
| Malaria transmission type| 15.827| 1.580-158.556| 0.019|

Reference: Gender: Female, Nationality: Non-Malaysian, WHO region: Non-Western Pacific, G6PD: Nondeficient, Pregnancy: No, Survival: No, Previous infection: No, Type of malaria: \textit{Plasmodium ovale}, Presence of gametocyte: Negative, Severity: None severe, Malaria transmission type: Nonindigenous, Relapse before: No, Type of antimalarial: CQ plus PQ, P<0.05 in univariate analysis and multivariate analysis. P<0.05 is considered significant. WHO: World Health Organization, G6PD: Glucose-6-phosphate dehydrogenase, CQ: Chloroquine, PQ: Primaquine, ACT: Artemisinin combination therapy, CI: Confidence interval.
metabolized to an inert carboxyprimaquine, which is then further metabolized into toxic hydroxylated metabolites responsible for hematologic harmful events associated with this drug.\textsuperscript{[22,23]} It remains a vital antimalarial in both \textit{P. vivax} and \textit{P. ovale} management due to its effectiveness in targeting hypnozoites in the liver when combined with blood schizontocidal agents.\textsuperscript{[31]} Ensuring primaquine effectiveness is therefore vital, as alternative drugs remain scarce.

In the present work, gender was found to be a predictor for a longer hospitalization during primaquine combination treatment for \textit{P. vivax} and \textit{P. ovale} infections. Gender-related differences in antimalarials have been shown to be predictors of primaquine effectiveness in other work.\textsuperscript{[24,25]} Slower clearance and higher drug concentrations were observed in female compared to male participants, mainly due to differences in metabolism of primaquine through the enzyme CYP1A2.\textsuperscript{[24-26]} Work has also shown that interaction of chloroquine and primaquine further increases primaquine plasma concentrations.\textsuperscript{[27]} In contrast, it was shown that female patients took longer for parasites to clear in the current work, which demonstrates the complex interplay between drug effectiveness in the clinical setting. Studies have also suggested genetic differences in metabolism of primaquine do occur such as that observed in CYP3A4, CYP2D6, and MAO-A that may to some extent affect our current findings on gender differences.\textsuperscript{[28,29]}

Indigenous infection, on the other hand, was a predictor for both length of stay and relapse. Indigenous transmission is classified as a locally acquired malaria infection in an area where malaria is a regular occurrence, with no evidence of importation and no direct link to transmission from an imported case.\textsuperscript{[14]} The longer length of stay identified in indigenous infection could be attributed to the aborigines that are usually discharged after the 14-day primaquine treatment has been completed despite zero parasitemia. This has been similarly noted in previous findings, specifically in the Gombak area within Selangor. Despite easy access to basic healthcare among these people, lack of knowledge, attitude, and the practice of traditional measures against malaria\textsuperscript{[31]} have led to closer monitoring of primaquine use in the local setting.

Indigenous patients have been identified as relapse predictors similar to previous work.\textsuperscript{[10]} The higher risk of relapse occurring among indigenous patients despite closer monitoring of primaquine use could be attributed to differences in metabolism of the drug.\textsuperscript{[25,28,29,32,33]} This may in fact give rise to variation in dose requirements.\textsuperscript{[32]} This is especially a concern as studies have shown that differences in primaquine doses may also give rise to differences in clinical outcome in infected patients.\textsuperscript{[34]} Furthermore, primaquine efficacy has been linked to the CYP-mediated metabolism pathway in particular CYP2D6. Clinical primaquine failure was observed to be linked to poor and intermediate metabolizers of CYP2D6 genotype,\textsuperscript{[36]} with a geographical distribution of intermediate metabolizer most commonly observed in this region.\textsuperscript{[37]} However, it is important to note that the number of relapse patients were few. To that end, understanding the effectiveness of primaquine treatment in reducing relapse is vital to ensure optimum use of the drug. With interindividual differences in metabolism observed during primaquine use, further investigation of how this occurs in our local population is recommended.

**Conclusions**

In general, the main aim of this study was successfully achieved. It should be noted that data were collected from the registry database and therefore validity of the study depends on the degree of completeness of the data. Details of each patient that may contribute toward their illness were also not available. Furthermore, data on relapse were few in numbers. Therefore, generalization of the results should be done with caution. It was revealed that gender and indigenous infections were predictors of length of stay and the latter in relapse occurrence. Although the reason for relapse is difficult to determine, work has suggested that differences in metabolism of the drug contribute to inefficacy of the drug. The findings thus demonstrate the need for further investigations to identify interindividual differences in effectiveness of primaquine in the local population.

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**Conflicts of interest**

There are no conflicts of interest.
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