Recurrence in *Plasmodium vivax* malaria: a prospective cohort study with long follow-up from a coastal region in South-West India [version 1; peer review: awaiting peer review]

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Abstract

**Background:** India is endemic for *Plasmodium vivax* (*Pv*) malaria. Despite a decrease in incidence, its elimination is hampered by recurrences. This study aimed to characterize recurrences in *Pv* malaria and study its association with primaquine (PQ) usage.

**Methods:** Symptomatic adult *Pv* patients were followed-up for up to 23 months for recurrences. The time to recurrence was compared by the PQ dosage they received using a log-rank test.

**Results:** Of the 294 malaria patients, 206 (70%) patients had *Pv* infection during the study period. A total of 20 (9.7%) recurrences were seen in 17 (8.2%) patients of *Pv*. The percentage of first-time recurrences were highest in the no PQ group (25%), followed by the weekly PQ group (20%), low dose daily PQ (8.2%) group, and high dose daily PQ group (3.1%).

**Conclusions:** Recurrence in *Pv* malaria is common, especially in those who receive an inappropriate prescription of primaquine.

**Keywords**
Primaquine; relapse; severe malaria
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Introduction

Malaria is a major global health problem, with around 228 million reported cases alone in 2018, most due to *Plasmodium falciparum* (*Pf*). Consequently, most reports on malaria concentrate on *Pf*. Traditionally, *Pf* has been described as the causative agent for severe malaria. However, recent reports have shown that malaria caused by *Plasmodium vivax* (*Pv*) can also be severe. Although India represents a small percentage of the overall global malaria cases, it is responsible for nearly half of the total cases of *Pv*. Despite a decline in the number of Malaria cases in India, the major roadblock to elimination is the tendency of *Pv* to relapse frequently, mainly when primaquine (PQ) is not prescribed or prescribed in sub-therapeutic dosage. Therefore, the objective of the study was to calculate the incidence of recurrence in patients with *Pv* malaria and find the impact of PQ prescription practices on recurrence.

Methods

A prospective observational cohort study was conducted at Kasturba Hospital, Manipal in Udupi district of Karnataka State, India, for two years, from October 2016 to August 2018. The study was commenced after taking approval from the Institute's Ethical Committee (IEC 636/2016). All patients of either sex above 18 years of age who presented during the study period with fever and had malarial parasites on the quantitative buffy coat (QBC) examination were included in the study after taking written informed consent. The article was reported according to the STROBE guidelines and all the criteria in the STROBE checklist were met. The sample size was calculated as 206 cases of *Pv*, considering recurrence prevalence as 31.5%, 95% level of confidence and 6.5% precision.

They were categorized into *Pv*, *Pf* or mixed based on the results of peripheral smear. The patients were classified as having severe disease if they met the criteria for severity laid down by World Health Organisation. A detailed history (including comorbidities), physical examination, and laboratory parameters were noted in a predefined case study form. In addition, the worst value of the variables during hospitalization was recorded. Since the study aimed to record the prescription practices of treating physicians, the study objectives were not disclosed to them to avoid bias. The diagnosed cases were treated by the treating team. Glucose-6 Phosphate dehydrogenase (G6PD) levels were requested by the treating team. The details of treatment, supportive care need, requirement of supportive care, and mortality were comparable in both groups and summarized in Figure 1. The baseline clinical and laboratory features of patients with *Pv* and *Pf* malaria have been summarized in Table 1.

Statistical analysis was performed using Statistical Package for the Social Sciences version 23.0 (SPSS, RRID: SCR_002865, http://www-01.ibm.com/software/uk/analytics/spss/). Continuous variables were summarized as mean with standard deviation (SD) or median with interquartile range (IQR) (in skewed data). Categorical variables were summarized as the frequency with proportion. Overall, patients with *Pv* were divided into four groups according to PQ dosage-no PQ, weekly PQ, low dose daily PQ (0.25 mg/kg/day), and high dose daily PQ (0.5 mg/kg/day). The number of recurrences in each group were calculated. A Kaplan-Meier survival plot was generated to determine the survival function of recurrences according to PQ categories until 23 months’ follow-up duration. Log-rank test was used to compare the survival function. A Chi-square test was used to compare quantitative variables, whereas an independent t-test was used to compare quantitative variables. A p-value of less than 0.01 was considered significant.

Results

A total of 294 malaria cases were enrolled during the study period, of which 206 (70%) were *Pv*, 79 (27%) were *Pf*, and 9 (3%) were mixed (*pv+pf*). A total of 29.6% (87/294) cases had severe malaria. The proportion of severity, the requirement of supportive care, and mortality were comparable in both groups and summarized in Figure 1. The baseline clinical and laboratory features of patients with *Pv* and *Pf* malaria have been summarized in Table 1.

Of 294 cases included in the study, there were 21 recurrences in 18 (6.12%) patients. All patients with recurrent disease had non-severe malaria with good clinical recovery. Twenty recurrences (20/206, 9.7%) belong to the *Pv* group and 1 (1/79, 1.3%) patient from the *Pf* group. Of the 20 recurrences in the 17 patients were in the *Pv* group, three patients had a recurrence for the second time. The median time to follow-up was 388 (293–567) days. The median time to the first recurrence in the *Pv* group was 83 (66.5–242.5) days.

Of the 206 patients with *Pv*, G6PD levels could be done in 196 patients only, out of which nine patients were found to have low G6PD levels (Table 2). No case of PQ-induced hemolysis was noted in our cohort. The dose of PQ was significantly higher...
associated with recurrences on the Chi-square test (p<0.001). The percentage of first-time recurrences were highest in the no PQ group (25%), followed by the weekly PQ group (20%), low dose daily PQ (8.2%) group, and high dose daily PQ group (3.1%) (Table 2). A Kaplan-Meier curve was plotted to compare the median time to recurrence in each of the PQ-based groups, and the difference was found to be significant on the log-rank test (p=0.009) (Figure 2).

Discussion

Udupi district has a population of 1,177,908 with an area of 3,582 sq. km and is located 13°32′ 24.43″ N latitude and 74°52′26.78″E longitude, with typical tropical climatic conditions. The monsoon in this region starts in June and extends till October, with an average rainfall of more than 4000mm every year. The catchment area of our hospital encompasses both the rural and urban populations of coastal and interior Karnataka, Goa and Kerala. *Pv* is the largest infecting species in this region, followed by *Pf*. The same trend is noted in other parts of India. In this study, around 30% of the cases had severe malaria with a similar incidences of severity in *Pv* and *Pf*. In the last decade, the severity of malaria has ranged from 6.5% to 48% across the world. Classically, *Pf* is supposed to be one with a higher frequency of severe manifestation, whereas *Pv* is apparently the benign form. This dogma has been challenged more and more as reports emerge from *Pv* endemic areas. Like other reports, hepatic and renal dysfunction were the commonest manifestation of severity in our study. Central nervous system (CNS) manifestations, which were initially thought to be exclusive to *Pf*, were seen in *Pv* and *Pf* in our study. This study reiterates that severe *Pv* malaria
| Table 1. Baseline clinical and laboratory features of patients with severe or non-severe vivax and falciparum malaria. |
|---------------------------------------------------------------|
| **Plasmodium vivax** (N=206)                                  |
| Non-severe (n=144) | Severe (n=62) | P-value* | Non-severe (n=56) | Severe (n=23) | P-value* |
| Age (years) | 36.1±14.2 | 40.6±14.1 | 0.76 | 34.4±14.6 | 38.59±13.1 | 0.41 |
| Male gender | 121 (84%) | 55 (88.5%) | 0.38 | 48 (85.7%) | 21 (95.5%) | 0.22 |
| Fever in days | 4 (3.7) | 4 (3.6) | 0.83 | 4 (3.6) | 6 (4.7) | 0.01 |
| Diabetes mellitus | 15 (10.45%) | 13 (21%) | 0.04 | 5 (9%) | 1 (4.3%) | 0.48 |
| Hypertension | 14 (9.72%) | 10 (16.1%) | 0.18 | 5 (9%) | 3 (13.04%) | 0.58 |
| Pulse rate (beats/min) | 88±14 | 92±16 | 0.22 | 88±11 | 87±12 | 0.47 |
| Respiratory rate (breaths/min) | 19±2 | 20±5 | 0.007 | 18±2 | 21±6 | 0.001 |
| ARDS | 5 (3.5%) | 0 | 0.001 | 3 (5.4%) | 0 | 0.005 |
| Systolic blood pressure (mmHg) | 120±14 | 114±20 | 0.001 | 121±17 | 113±15 | 0.8 |
| Diastolic blood pressure (mmHg) | 77±8 | 73±12 | 0.002 | 77±9 | 73±13 | 0.15 |
| Shock | 7 (3.4%) | 0 | <0.001 | 4 (7.1%) | 0 | 0.001 |
| Pallor | 5 (3.5%) | 6 (9.8%) | 0.07 | 5 (8.9%) | 4 (18.2%) | 0.28 |
| Icterus | 44 (30.6%) | 0 | <0.001 | 17 (30.4%) | 0 | <0.001 |
| Impaired consciousness | 3 (2.1%) | 0 | 0.009 | 1 (1.8%) | 0 | 0.108 |
| Convulsion | 1 (0.7%) | 0 | 0.136 | 1 (1.8%) | 0 | 0.108 |
| Metabolic acidosis | 3 (2.1%) | 0 | 0.010 | 1 (1.8%) | 0 | 0.108 |
| Renal failure | 10 (6.9%) | 0 | <0.001 | 3 (5.4%) | 0 | 0.005 |
| Splenomegaly | 17 (11.8%) | 14 (23%) | 0.04 | 11 (19.6%) | 8 (36.4%) | 0.15 |
| Hepatomegaly | 8 (5.6%) | 15 (24.6%) | <0.001 | 6 (10.7%) | 4 (18.2%) | 0.41 |
| Hemoglobin (g/dL) | 13.4±1.9 | 12.8±2.5 | 0.01 | 12.9±2.1 | 12.2±3.2 | 0.03 |
| Hematocrit (%) | 39.7±5.6 | 37.8±7.3 | 0.02 | 38±6.5 | 35.7±9 | 0.12 |
| Total Leukocyte count (cells/mm³) | 5655±2154 | 5813±2978 | 0.008 | 5049±1804 | 7632±4516 | 0.001 |
| Platelet count (cells/mm³) | 74500 (49250,113250) | 47000 (30750,79500) | 0.001 | 75000 (48500,136250) | 39000 (16000,96500) | 0.007 |
| Plasma Glucose (mg/dL) | 132±54 | 149±60.1 | 0.13 | 139±70.8 | 132±44.5 | 0.15 |
| Blood Urea (mg/dL) | 25 (20,31) | 32 (23,45.5) | <0.001 | 24 (19, 30) | 32 (22, 65) | 0.01 |
Table 1.  
Continued

|                          | Plasmodium vivax (N=206) |                      | Plasmodium falciparum (N=79) |                      |
|--------------------------|---------------------------|----------------------|-----------------------------|----------------------|
|                          | Non-severe (n=144)        | Severe (n=62)        | P-value*                    | Non-severe (n=56)    | Severe (n=23) | P-value*          |
| Serum Creatinine (mg/dL) | 0.98 ± 0.27               | 1.17 ± 0.48          | <0.001                      | 1.01 ± 0.42          | 1.7 ± 2.01    | 0.01              |
| Total Bilirubin (mg/dL)  | 1.49 ± 0.62               | 3.8 ± 2.9            | <0.001                      | 1.5 ± 0.6            | 6.8 ± 7.56    | <0.001            |
| Direct Bilirubin (mg/dL) | 0.6 ± 0.3                 | 2.08 ± 2.42          | <0.001                      | 0.6 ± 0.4            | 4.23 ± 5.16   | <0.001            |
| Aspartate transaminase (IU/L) | 33.5 (24.43)      | 49 (30,65.5)         | <0.001                      | 36 (25, 58.5)        | 47.5 (37.3, 96) | 0.02              |
| Alanine transaminase (IU/L) | 34 (22,53)              | 43.5 (27,287.7)      | 0.01                        | 43 (24, 70)          | 54.5 (31.7, 103.2) | 0.2              |
| Alkaline phosphatase (IU/L) | 75 (60,94)             | 99 (76,144.7)        | <0.001                      | 93 (61, 115.8)       | 122.5 (76.3, 181.5) | 0.02              |

* Categorical variables are summarized as the frequency with proportion whereas continuous variables are summarized as either mean (±SD) or median (IQR). Chi-square or Fischer’s exact test and Independent sample t-test or Mann Whitney U test were performed, p-value less than 0.05 shows the statistically significant difference and shown in bold font. ARDS: Acute Respiratory Distress Syndrome.

Table 2.  
Recurrences in Plasmodium vivax cases stratified according to G6PD levels and primaquine prescription patterns.

| Primaquine (PQ) | G6PD levels low (n=9) | G6PD levels normal (n=187) | G6PD not done (n=10) |
|-----------------|-----------------------|----------------------------|----------------------|
| PQ dose         | Total prescribed      | Recurrences                | Total prescribed     | Recurrences         | Total prescribed | Recurrences |
| No PQ           | 1                     | 0                          | 10                   | 2                   | 5                 | 2          |
| Weekly PQ       | 5                     | 1                          | 0                    | 0                   | 0                 | 0          |
| Daily PQ (0.25 mg/kg) | 3                   | 1                          | 114                  | 8                   | 4                 | 1          |
| Daily PQ (0.5 mg/kg) | 0                   | 0                          | 63                   | 2                   | 1                 | 0          |

PQ: Primaquine; G6PD: Glucose 6 Phosphate dehydrogenase.
cases presented with similar phenotypic features as *Pf* malaria. Although previous studies have reported variable mortality with malaria cases, mortality in our study was low, with one mortality each in *Pv* and *Pf* patients. As expected, all but one recurrence were seen in patients with *Pv*. The percentage recurrence in *Pv* cases was close to 10%, which was considerably lower than recurrences reported in the previous series (24–38%). Like a previous study, all recurrent cases had mild symptoms, presumably due to the development of acquired immunity from the previous episode. The median time to recurrence was 83 days in our study, similar to previously published studies. Those patients for whom PQ was not used had higher rates of recurrence. Interestingly, the 16 patients for whom no PQ was used, only one patient had proven low levels of G6PD. In all the other cases, the levels were either not done or were normal. This reflects the need to reinforce the importance of PQ prescription in patients with *Pv*. The rates were lower in the daily PQ group even when they were used at a lower dose. Similar results were observed in other studies as well. Since the study was done in a tertiary care hospital where G6PD levels and specialist referrals are available, the study cannot be generalized to primary care settings. Similar widespread prescription audits are required all over the country to understand the practices and pattern of recurrences in patients with *Pv*.

Limitations of the study
Self-limiting intermittent recurrences that are asymptomatic could not be ruled out as symptom-based screening for recurrence was done. The genotyping of recurrences could not be done to discern relapse and reinfection. The possibility of non-compliance cannot be ruled out as PQ therapy was unsupervised.

Conclusions
The study reiterates that *Pv* is the dominant species in this part of India with similar frequencies of severity. Moreover, it is associated with recurrences, especially when PQ prescription is inappropriate. Therefore, there is a need for improving prescription practices amongst primary care physicians through regular educational interventions.

Data availability
Data cannot be shared due to ethical and security concerns, however a de-identified dataset with all the details can be shared with reviewer or readers at reasonable request to corresponding author.

Author’s contributions
All authors have read and approved the final manuscript. The requirements for authorship have been met, and each author believes that the manuscript represents honest work.
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