



## RESEARCH ARTICLE

# REVISED Recurrence in *Plasmodium vivax* malaria: a prospective cohort study with long follow-up from a coastal region in South-West India [version 2; peer review: 2 approved]

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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 incorrect prescription of primaquine.

## Keywords

Primaquine; relapse; severe malaria

## Open Peer Review

Approval Status  

	1	2
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<b>version 1</b> 04 Mar 2022	 view	  view

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Any reports and responses or comments on the article can be found at the end of the article.



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**REVISED Amendments from Version 1**

Some changes have been introduced in the revised version of the manuscript as per the suggestion of the peer-reviewer. The methodology has been described in detail. Baseline details of *Plasmodium falciparum* and mixed infections had been removed from the manuscript to remove distraction from the main objective. Also, statistical tests to differentiate between baseline features of severe and non-severe malaria have been removed. Table 2 shows the PQ prescription patterns in a more succinct manner so Figure 1 is removed to avoid confusion. The paragraph on severe malaria has been removed, as suggested by the reviewers.

**Any further responses from the reviewers can be found at the end of the article**

**Introduction**

Malaria is a major global health problem, with around 228 million reported cases alone in 2018, most due to *Plasmodium falciparum* (*Pf*).<sup>1</sup> 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*.<sup>2,3</sup> 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.<sup>4,5</sup> 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 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 *Pv* malarial parasites on the quantitative buffy coat (QBC) or peripheral smear examination were included in the study after taking written informed consent. Those patients with *Pf* or mixed infections (*Pv* and *Pf*) were excluded. 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.<sup>6</sup>

The diagnosis of *Pv* was based on the results of peripheral smear. 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. The patients were classified as having severe disease if they met the criteria for severity laid down by World Health Organisation (WHO).<sup>7</sup> 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 physician's discretion. The enzyme activity was quantified by the manual spectrophotometric kinetic 'gold standard' method in the institutional biochemistry laboratory. G6PD deficiency was defined as less than 30% of mean G6PD activity.

Chloroquine was used in all patients for the treatment of *Pv* malaria. In an ideal situation, G6PD levels should be done prior to initiation of primaquine. If the levels are within normal range, WHO recommends 0.5 mg/kg primaquine to prevent relapse in tropical areas. The national guidelines in India, however, recommend 0.25 mg/kg according to their last available guidance. If the levels are low, weekly primaquine is recommended for 8 weeks.<sup>8,9</sup>

The treating physicians decided the dosage of antimalarials, including PQ. The details of treatment, supportive care hospitalization days and mortality during hospital stay were noted. The primary outcome was microbiologically-confirmed recurrence at the end of the study period. Individuals were followed up telephonically every two months until the end of the study period for the development of fever recurrence. Additionally, individuals were asked to report if the fever recurred and were classified as recurrence if they were microscopically proven to have malaria again.

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 p-value of less than 0.01 was considered significant.

**Table 1. Baseline clinical and laboratory features of patients with severe or non-severe vivax malaria.**

	<i>Plasmodium vivax</i> (N=206)			<i>Plasmodium falciparum</i> (N=79)		
	Non-severe (n=144)	Severe (n=62)	P-value*	Non-severe (n=56)	Severe (n=23)	P-value*
<b>Age (years)</b>	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/mm <sup>3</sup> )	5655 ± 2154	5813 ± 2978	0.008	5049 ± 1804	7632 ± 4516	0.001
Platelet count (cells/mm <sup>3</sup> )	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

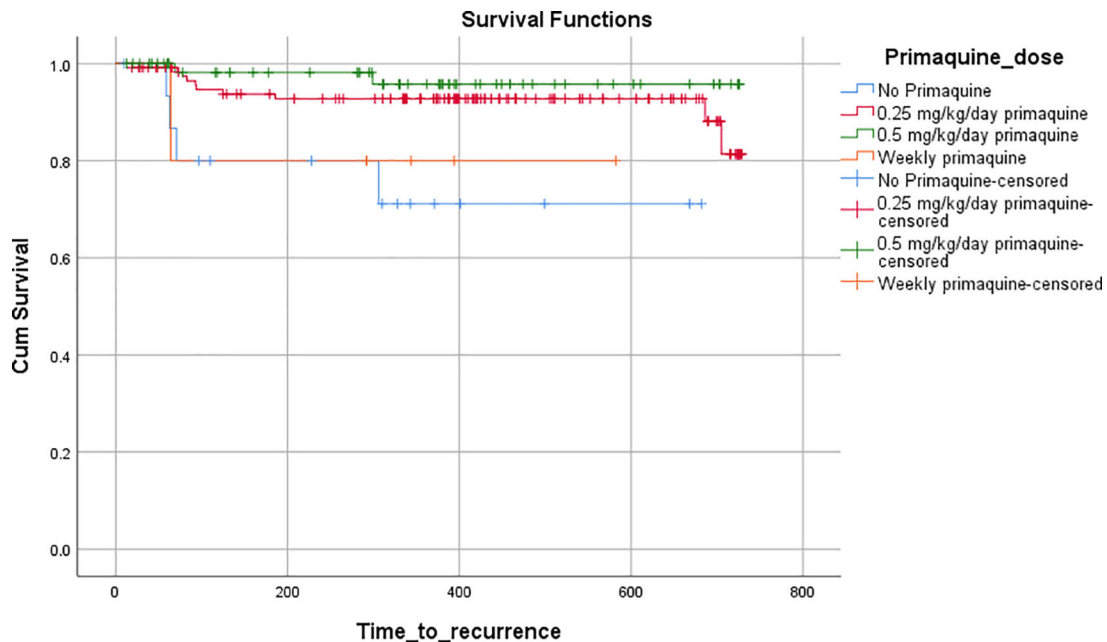
	<i>Plasmodium vivax</i> (N=206)		<i>Plasmodium falciparum</i> (N=79)		P-value*
	Non-severe (n=144)	Severe (n=62)	Non-severe (n=56)	Severe (n=23)	
Serum Creatinine (mg/dL)	0.98±0.27	1.17±0.48	1.01±0.42	1.7±2.01	0.01
Total Bilirubin (mg/dL)	1.49 ± 0.62	3.8 ± 2.9	1.5 ± 0.6	6.8 ± 7.56	<0.001
Direct Bilirubin (mg/dL)	0.6 ± 0.3	2.08 ± 2.42	0.6 ± 0.4	4.23 ± 5.16	<0.001
Aspartate transaminase (IU/L)	33.5 (24.43)	49 (30,65.5)	36 (25, 58.5)	47.5 (37.3, 96)	0.02
Alanine transaminase (IU/L)	34 (22,53)	43.5 (27.2,87.7)	43 (24, 70)	54.5 (31.7, 103.2)	0.2
Alkaline phosphatase (IU/L)	75 (60,94)	99 (76.3,144.7)	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) dose	G6PD levels low (n=9)		G6PD levels normal (n=187)		G6PD not done (n=10)	
	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.



**Figure 1.** Time to recurrence stratified according to primaquine dosing on Kaplan-Meier survival analysis.

## Results

A total of 294 malaria cases were screened 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. The baseline clinical and laboratory features of patients with *Pv* and *Pf* malaria have been summarized in [Table 1](#).

Of 206 *Pv* cases included in the study, there were 20 recurrences in 17 (8.5%) patients. The median time to follow-up was 388 (293–567) days. The median time to the first recurrence 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 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 1](#)).

## 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*.<sup>10,11</sup> The same trend is noted in other parts of India.<sup>12,13</sup>

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%).<sup>14,15</sup> Like a previous study, all recurrent cases had mild symptoms, presumably due to the development of acquired immunity from the previous episode.<sup>16</sup> The median time to recurrence was 83 days in our study, similar to previously published studies.<sup>14</sup> Those patients for whom PQ was not used had higher rates of recurrence.

We classified the patients according to the G6PD levels because in those patients where G6PD levels were not done, we couldn't judge the correctness of the prescription choices. The idea was to show that a G6PD levels were not even offered to some patients. On top of that, many patients were given incorrect prescriptions despite G6PD levels indicating otherwise. Of the 16 patients for whom no PQ was used, only one patient had proven low levels of G6PD. This implies

that PQ was not prescribed because of possible lack of awareness. This reflects the need to reinforce the fact that G6PD levels should be done in all patients with *Pv* and the prescriptions should be guided by the G6PD levels.

Since the recurrence rates were lowest in those where primaquine was used as 0.5 mg/kg, patients with normal G6PD levels should receive 0.5 mg/kg prophylaxis. Even with a lower dose of PQ (0.25 mg/kg), the recurrences are lower when compared to those who were not given PQ. Similar results were observed in other studies as well.<sup>17</sup> 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. New *Pv* infections could be differentiated from relapses in this study. The study follow-up was of long duration so recurrences at 1-2 years may or may not be related to the PQ dose.

### Conclusions

The study concludes that, *Pv* may be associated with recurrences, especially when PQ prescription practices are not aligned with international evidence based recommendations. G6PD levels should be ascertained in all patients with *Pv* malaria, and daily PQ prophylaxis should be given to those patients with normal G6PD levels. 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.

Divya Gandrala: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing-original draft preparation, Writing-Review & Editing

Nitin Gupta: Formal analysis, Validation, Writing-original draft preparation, Writing-Review & Editing

Alekhyia Lavu: Data curation, Formal analysis, Investigation, Methodology

Vishnu Teja Nallapati: Writing-original draft preparation, Writing-Review & Editing

Vasudeva Guddattu: Formal analysis, Software, Writing-original draft preparation, Writing-Review & Editing

Kavitha Saravu: Conceptualization, Data curation, Formal analysis, Project administration, Supervision, Validation, Writing-original draft preparation, Writing-Review & Editing

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# Open Peer Review

Current Peer Review Status:  

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## Version 2

Reviewer Report 12 April 2022

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**Cindy Chu**

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The authors have improved the clarity of the manuscript. The discussion and conclusion are consistent with the study methods and results. The conclusion is clear.

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Plasmodium vivax, radical cure with 8-aminoquinolines, G6PD deficiency

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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## Version 1

Reviewer Report 18 March 2022

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**Cindy Chu**

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This manuscript describes the prevalence and some epidemiologic aspects of *Plasmodium vivax*

(*Pv*), *Plasmodium falciparum* (*Pf*), and mixed-species infection in Southwest India. The study is a prospective observational study and appears to be part of a prescription audit. There is a sub-analysis that compares the recurrence rates of *Pv* malaria among four groups: no primaquine, weekly primaquine, low dose primaquine, and high dose primaquine. Primaquine (PQ) was not supervised. The data are useful, however, the presentation of the study design, sample size, and methods need more explanation and some of the results need more precision so that the correct conclusions can be made.

#### ABSTRACT:

- The conclusion states that recurrence in *Pv* is common especially when PQ prescription is inappropriate. I suggest using a more precise word instead of 'inappropriate'.

#### INTRODUCTION:

- The last sentence of the introduction states that 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. The authors then conduct a prospective observational study. However, this analysis appears to be a part of a prescription audit (as stated in the Discussion) rather than a study specifically designed to compare drug groups. This becomes more apparent in the study methods where it is stated: "the study aimed to record the prescription practices of treating physicians". Moreover, the inclusion of *Pf* and mixed cases does not match the study objective or the sample size calculation. Please specify the study design and study outcome(s).

#### METHODS:

- In the methods section, it is stated that the treating physicians decided on the dosage of antimalarials. Since PQ is being used, can the authors describe how G6PD testing is used before prescribing PQ? Which schizonticidal treatments are prescribed?

#### RESULTS:

- In the results section, 294 malaria cases were enrolled. The sample size is specified to be 206. Do the authors mean that 294 malaria cases were screened?
- Figure 1 describes the data by malaria species but the analysis is by drug group. Please modify so that the trial diagram is consistent with the data analysis.
- The chi-squared test would normally be used to analyse 2x2 groups. The chi-squared test as used in the results section is for 2x4 groups (*Pv* yes/no and 4 different drug regimens). The Kaplan Meier figure is the correct analytic tool so the chi-squared result could be removed.

#### DISCUSSION:

- There is one paragraph dedicated to severe malaria, however, to this point in the manuscript, the outcome of the study was *Pv* recurrence. This paragraph (the *Pf* part) does not seem related the stated study outcome. Please clarify.
- What is the relevance of G6PD activity levels in this study for *Pv* recurrence? I understand the G6PD test would be used to determine which PQ regimen should be prescribed, but that has not yet been described in the manuscript and it should be added. There is a statement that only one patient in the no PQ group had low G6PD levels. Presumably any participant

with low G6PD activity should have been in the no PQ or weekly PQ groups. Please explain.

- The following sentence “This reflects the need to reinforce the importance of primaquine prescription in patients with *Pv*”, needs more specific language. Do the authors mean low G6PD levels reflect the need to reinforce the importance of PQ prescription? Or the need for G6PD testing before prescribing PQ?
- The following sentence “The rates were lower in the daily PQ group even when they were used at a lower dose”, needs more specific language. What rates were lower? What is 'they' - the patients or PQ?

#### STUDY LIMITATIONS:

- New *Pv* infections cannot be differentiated from relapses in this study. The study follow-up has a very long duration so recurrences at 1-2 years may or may not be related to the PQ dose. This should be included in study limitations and appropriate citations included.

#### CONCLUSION:

- What do the authors mean by inappropriate PQ prescription? How do they determine whether recurrences are associated with inappropriate prescription when new infections cannot be differentiated from relapses or if results are affected by non-compliance? What prescription practices need to be improved? It seems that if the study design and methods are clearer, then the results and conclusion will be better supported.

#### **Is the work clearly and accurately presented and does it cite the current literature?**

Partly

#### **Is the study design appropriate and is the work technically sound?**

Partly

#### **Are sufficient details of methods and analysis provided to allow replication by others?**

No

#### **If applicable, is the statistical analysis and its interpretation appropriate?**

Partly

#### **Are all the source data underlying the results available to ensure full reproducibility?**

No

#### **Are the conclusions drawn adequately supported by the results?**

Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Plasmodium vivax, radical cure with 8-aminoquinolines, G6PD deficiency

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have**

significant reservations, as outlined above.

Author Response 31 Mar 2022

**Kavitha Saravu**, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, India

**This manuscript describes the prevalence and some epidemiologic aspects of *Plasmodium vivax* (Pv), *Plasmodium falciparum* (Pf), and mixed-species infection in Southwest India. The study is a prospective observational study and appears to be part of a prescription audit. There is a sub-analysis that compares the recurrence rates of Pv malaria among four groups: no primaquine, weekly primaquine, low dose primaquine, and high dose primaquine. Primaquine (PQ) was not supervised. The data are useful, however, the presentation of the study design, sample size, and methods need more explanation and some of the results need more precision so that the correct conclusions can be made.**

**Reply:** We appreciate the constructive suggestions by the reviewer. We have modified our manuscript as per the suggestions. A point-wise reply, explanation, and modification are as follows.

**ABSTRACT:**

- **The conclusion states that recurrence in Pv is common especially when PQ prescription is inappropriate. I suggest using a more precise word instead of 'inappropriate'.**

**Reply:** The inappropriate word was changed to 'Incorrect'.

**INTRODUCTION:**

- **The last sentence of the introduction states that 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. The authors then conduct a prospective observational study. However, this analysis appears to be a part of a prescription audit (as stated in the Discussion) rather than a study specifically designed to compare drug groups. This becomes more apparent in the study methods where it is stated: "the study aimed to record the prescription practices of treating physicians". Moreover, the inclusion of Pf and mixed cases does not match the study objective or the sample size calculation. Please specify the study design and study outcome(s).**

**Reply:** The study was designed as a prospective observational study where patients with malaria were enrolled at baseline. A follow-up component was added to estimate the percentage of patients with recurrence. Prescription audit was a main component of the study which is a type of observational study. Since there is a follow-up component built in the study to estimate the percentage of recurrences, we described our study as a prospective observational study.

We included all malaria cases but the analysis was restricted to Pv as recurrences in Pf are not common. The primary objective was to study the recurrences in Pv. The sample size was

also calculated based on that primary objective. Baseline parameters were collected at presentation and prescription patterns were recorded. The patients were followed up for recurrences. The inclusion of other cases was shown for the sake of comprehensiveness but as the reviewer suggests, they appear to be distractors. We have therefore removed all data on Pf and mixed malaria from this study.

#### **METHODS:**

- **In the methods section, it is stated that the treating physicians decided on the dosage of antimalarials. Since PQ is being used, can the authors describe how G6PD testing is used before prescribing PQ? Which schizonticidal treatments are prescribed?**

**Reply:** We appreciate the suggestion and have incorporated the following statement, "Chloroquine was used in all patients for the treatment of Pv malaria. In an ideal situation, G6PD levels should be done prior to initiation of primaquine. If the levels are within normal range, WHO recommends 0.5 mg/kg primaquine to prevent relapse in tropical areas. The national guidelines in India, however, recommend 0.25 mg/kg according to their last available guidance. If the levels are low, weekly primaquine is recommended for 8 weeks."

#### **RESULTS:**

- **In the results section, 294 malaria cases were enrolled. The sample size is specified to be 206. Do the authors mean that 294 malaria cases were screened?**

**Reply:** We enrolled 294 cases with 206 being Pv. For the other patients who had Pf or mixed, the baseline parameters were recorded. However, as mentioned earlier, we have removed the data on Pf and mixed malaria for clarity as per the reviewer's suggestion.

- **Figure 1 describes the data by malaria species, but the analysis is by drug group. Please modify so that the trial diagram is consistent with the data analysis.**

**Reply:** The table shows the trial diagram in a more succinct manner so Figure 1 is removed to avoid confusion. Since we have removed Pf and mixed malaria, Figure 1 seemed redundant anyways.

- **The chi-squared test would normally be used to analyse 2x2 groups. The chi-squared test as used in the results section is for 2x4 groups (Pv yes/no and 4 different drug regimens). The Kaplan Meier figure is the correct analytic tool so the chi-squared result could be removed.**

**Reply:** Chi-square test was meant for comparing severe and non-severe malaria at baseline. However, we have removed that from the main table as suggested.

#### **DISCUSSION:**

- **There is one paragraph dedicated to severe malaria, however, to this point in the manuscript, the outcome of the study was Pv recurrence. This paragraph (the Pf part) does not seem related the stated study outcome. Please clarify.**

**Reply:** Since we classified the baseline features into severe and non-severe, we had discussed this in the discussion section. The paragraph has now been removed as

suggested.

- **What is the relevance of G6PD activity levels in this study for *Pv* recurrence? I understand the G6PD test would be used to determine which PQ regimen should be prescribed, but that has not yet been described in the manuscript and it should be added. There is a statement that only one patient in the no PQ group had low G6PD levels. Presumably, any participant with low G6PD activity should have been in the no PQ or weekly PQ groups. Please explain.**

**Reply:** We agree with the suggestion and have added the following statement. "If the levels are within normal range, WHO recommends 0.5 mg/kg primaquine to prevent relapse in tropical areas. The national guidelines in India, however, recommend 0.25 mg/kg according to their last available guidance. If the levels are low, weekly primaquine is recommended for 8 weeks."

We classified the patients according to the G6PD levels because, in those patients where G6PD levels were not done, we couldn't judge the correctness of the prescription choices. The idea was to show that G6PD levels were not even offered to some patients. On top of that, many patients were given incorrect prescriptions despite G6PD levels indicating otherwise. Since the treatment plans were not decided by the study team and the objective of the study was to study the correctness or incorrectness of the prescription, we couldn't presume that the participant with low G6PD activity would be in the no PQ group or weekly PQ group. Therefore, we mentioned in the statement that only one patient in the no PQ group had low G6PD levels. PQ prescription pattern in patients with low G6PD has been depicted in Table 2.

- **The following sentence "This reflects the need to reinforce the importance of primaquine prescription in patients with *Pv*", needs more specific language. Do the authors mean low G6PD levels reflect the need to reinforce the importance of PQ prescription? Or the need for G6PD testing before prescribing PQ?**

**Reply:** We meant that G6PD levels should be done in all patients and the prescriptions should be guided by the G6PD levels. Those with low levels should be given weekly prescriptions and those with normal levels should receive 0.5 mg/kg prophylaxis.

- **The following sentence "The rates were lower in the daily PQ group even when they were used at a lower dose", needs more specific language. What rates were lower? What is 'they' - the patients or PQ?**

**Reply:** The recurrence rates were lower in the daily PQ group even when the PQ was used at a lower dose i.e 0.25 mg/kg.

#### **STUDY LIMITATIONS:**

- **New *Pv* infections cannot be differentiated from relapses in this study. The study follow-up has a very long duration so recurrences at 1-2 years may or may not be related to the PQ dose. This should be included in study limitations and appropriate citations included.**

**Reply:** We agree with the suggestion. The same has been added.

**CONCLUSION:**

- **What do the authors mean by inappropriate PQ prescription? How do they determine whether recurrences are associated with inappropriate prescription when new infections cannot be differentiated from relapses or if results are affected by non-compliance? What prescription practices need to be improved? It seems that if the study design and methods are clearer, then the results and conclusion will be better supported.**

**Reply:** We have revised the paper so that the conclusion makes more sense. The meaning of inappropriate prescription practices has been described in the discussion section. Examples included, not giving PQ or giving them at a lower dosage. The significant log-rank test between the groups suggests that poor prescription practices may have some role. Prescription practices are required to be aligned with the current evidence-based recommendations.

**Competing Interests:** No competing interests were disclosed.

Reviewer Report 16 March 2022

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**Vettakkara Kandy Muhammed Niyas** 

Department of Infectious Diseases, Kerala Institute of Medical Sciences, Thiruvananthapuram, Kerala, India

Gandrala *et al.* has performed a prospective cohort study in which malaria patients were followed up for a median duration of 388 days. There were 20 cases (9.7%) of recurrences in 206 cases of *P. vivax* malaria, occurring at a median time of 83 days. The authors observed that recurrences were highest in the no primaquine (PQ) group and were least in the high dose daily PQ group. The authors also use a Kaplan-Mier analysis to show that there was a significant difference in the median time to recurrence among various PQ-based groups. However, the median time to recurrence in each of these groups is not mentioned.

The authors mention that no PQ-related haemolysis occurred in the cohort. However, it may be noted that no patient with low G6PD levels received a high dose of daily primaquine (0.5mg/kg). It is interesting to note that despite normal G6PD levels majority of patients did not receive a high dose PQ regimen. This data calls for awareness programmes among physicians regarding the optimal dosing of primaquine to prevent relapse. The authors rightly conclude that the results of the study cannot be generalised to primary care settings due to limited access to G6PD levels.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Yes

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Infectious Diseases, Tropical Infections

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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