Infection (2023) 51:213–222
https://doi.org/10.1007/s15010-022-01905-9

ORIGINAL PAPER

Risk of hemolysis in *Plasmodium vivax* malaria patients receiving standard primaquine treatment in a population with high prevalence of G6PD deficiency

Huaie Liu[1] · Weilin Zeng[1] · Pallavi Malla[2,3] · Chengqi Wang[4] · Seetha Lakshmi[2] · Kami Kim[2,4] · Lynette Menezes[2] · Zhaoqing Yang[1] · Liwang Cui[2,4]

Received: 11 February 2022 / Accepted: 7 August 2022 / Published online: 17 August 2022

Abstract

Background Primaquine is essential for the radical cure of *Plasmodium vivax* malaria, but it poses a potential danger of severe hemolysis in G6PD-deficient (G6PDd) patients. This study aimed to determine whether primaquine is safe in a population with high G6PD prevalence but lacking G6PD diagnosis capacity.

Methods In Myanmar, 152 vivax patients were gender- and age-matched at 1:3 for G6PDd versus G6PD-normal (G6PDn). Their risk of acute hemolysis was followed for 28 days after treatment with the standard chloroquine and 14-day primaquine (0.25 mg/kg/day) regimen.

Results Patients anemic and non-anemic at enrollment showed a rising and declining trend in the mean hemoglobin level, respectively. In males, the G6PDd group showed substantially larger magnitudes of hemoglobin reduction and lower hemoglobin nadir levels than the G6PDn group, but this trend was not evident in females. Almost 1/3 of the patients experienced clinically concerning declines in hemoglobin, with five requiring blood transfusion.

Conclusions The standard 14-day primaquine regimen carries a significant risk of acute hemolytic anemia (AHA) in vivax patients without G6PD testing in a population with a high prevalence of G6PD deficiency and anemia. G6PD testing would avoid most of the clinically significant Hb reductions and AHA in male patients.

Keywords Primaquine · Anemia · Glucose-6-phosphate dehydrogenase · Gender · Parasitemia · Hemolysis

Introduction

Plasmodium vivax has become the predominant species in many endemic areas, challenging malaria elimination. The resilience of *P. vivax* to conventional malaria control measures is especially attributed to its ability to form hypnozoites in the liver, responsible for relapses [1–3]. Therefore, a radical cure of vivax infection requires the co-administration of a schizonticide to clear the blood-stage infection and a hypnozoitocide to eliminate the liver stage. In most vivax-endemic regions, chloroquine (CQ)/primaquine (PQ) remains the frontline treatment for uncomplicated *P. vivax* infections [4]. WHO recommends 14 days of PQ treatment at a daily dose of 0.5 mg/kg for Oceania and 0.25 mg/kg for other regions [5]. Although PQ has demonstrated excellent clinical efficacies in preventing malaria relapses, its association with hemolytic toxicity in glucose-6-phosphate dehydrogenase deficient (G6PDd) patients limits its widespread use [6]. G6PD deficiency is highly prevalent at about 8%

Huaie Liu and Weilin Zeng have contributed equally to this work.

✉ Zhaoqing Yang
zhaoqingy92@hotmail.com

✉ Liwang Cui
liwangcui@usf.edu

1 Department of Pathogen Biology and Immunology, Kunming Medical University, Kunming 650500, Yunnan Province, China

2 Department of Internal Medicine, Morsani College of Medicine, University of South Florida, 3720 Spectrum Boulevard, Suite 304, Tampa, FL 33612, USA

3 Present Address: Center for Disease Control and Prevention, 1825 Century Center Blvd, Atlanta, GA 30345, USA

4 Center for Global Health and Infectious Diseases, University of South Florida, 3720 Spectrum Boulevard, Suite 404, Tampa, FL 33612, USA

in the impoverished rural malaria-endemic tropics [7], hindering the implementation of this anti-relapse therapy [8]. Although knowing the G6PD status of a vivax patient is essential prior to PQ prescription, many clinics and hospitals in malaria-endemic areas do not have the resources and capacity to perform G6PD testing. In these areas, unsupervised prescription of PQ to patients with unknown G6PD status would lead to possibly serious consequences requiring compulsory clinical monitoring during the daily PQ therapy [5]. In addition, accessibility to rescue transfusion services is needed in case of acute hemolytic anemia (AHA). Recent studies aiming to enhance patients' compliance with the PQ therapy showed excellent anti-relapse efficacy of compressing the 14-day PQ regimen to 7 days of 1.0 mg/kg daily [9, 10], but this high-dose regimen requires pre-screening for the G6PD status.

In the Greater Mekong Subregion (GMS), Myanmar has the heaviest malaria burden, with areas experiencing vivax malaria outbreaks in recent years [11]. The frontline treatment of uncomplicated *P. vivax* malaria remains CQ/PQ, and in most cases, PQ is often prescribed as unsupervised treatment [5, 12]. Within the GMS, G6PD deficiency is highly prevalent with predominantly WHO class II or III variants [7, 13]. Among the Kachin ethnic residents at the China–Myanmar border, G6PD deficiency reaches almost 20%, with the Mahidol variant (class III) as the major variant [14]. Similar to the African A− variant, the Mahidol variant is expected to precipitate self-limiting anemia without progression to life-threatening complications during standard PQ treatment. In an earlier detailed observation of the hemolytic course in G6PDd Thai patients treated with the standard dosing regimen of PQ, moderate hemolytic crises were observed at treatment initiation, which were subsequently resolved [15]. Similarly, an evaluation of this regimen among 364 vivax patients in Thailand in hospital settings, including 22 G6PDd males, further indicated this standard PQ therapy was safe [16]. However, previous reports in the GMS also showed that the standard dose of PQ induced AHA [17–19]. Therefore, the uncertainty of the standard PQ regimen prescribed without G6PD testing in the GMS populations with prevalent G6PD deficiency demands further safety evaluation. Here, we evaluated the frequency of severe hemolytic events in vivax patients receiving standard PQ treatment at the China–Myanmar border.

Materials and methods

Ethical consideration

The study protocol was approved by the Institutional Review Boards of Kunming Medical University, Kunming, Yunnan Province, China (#KMC2012-01), and the Department of Health of Kachin, Myanmar. All participants provided written informed consent prior to screening and enrollment.

Study site and participants

This study was conducted between April 2014 and March 2017 in two settlements for internally displaced people (IDP) and four villages in Laiza, Kachin State, Myanmar, along the China–Myanmar border. In the IDP clinic, patients diagnosed with uncomplicated *P. vivax* infections by microscopy were recruited into the study. Children younger than 5 years old as well as pregnant and lactating women were excluded. Those with severe malnutrition, severe liver and kidney diseases, and those who were allergic to antimalarial drugs or had antimalarial treatment within the last 4 weeks were also excluded. Patients with hemoglobin (Hb) levels lower than 7 g/dL were also excluded. Since this study aimed to compare the risk of hemolysis between G6PDd and G6PD normal (G6PDn) patients, each G6PDd patient was age- and gender-matched to G6PDn vivax patients. The demographic information of the patients was obtained using a structured questionnaire. Symptoms were recorded, and the axillary temperature was measured using a digital thermometer. Bodyweight and height were measured to calculate the body mass index (BMI) using the formula: [weight (kg)/height squared (m^2)]. Anemia was defined using age- and gender-specific WHO thresholds. Specifically, children aged 5–11 years, children aged 12–14 years, non-pregnant women (15 years and above), and men (15 years and above) with Hb levels (g/dL) < 11.5, < 12.0, < 12.0, and < 13.0 were classified as anemic [20]. Clinically concerning decline in Hb is defined as the Hb nadir level changes of either an absolute reduction of 5 g/dL, a fractional reduction of > 25%, or both. The local threshold of Hb level used for blood transfusion is 6 g/dL.

Parasitological procedures, hematology, and screening of G6PD deficiency

Thick blood smears were read by two certified microscopists to determine parasite density as described [21]. Dried filter-paper blood spots were used to extract DNA for molecular confirmation of *P. vivax* infection by PCR targeting the 18S rRNA gene [22]. Upon enrollment and on days 7, 14, and 28, a venous blood sample (0.5–2 ml) was drawn from each patient into an EDTA tube for hematological analysis using a TEK-II Mini automated hematological analyzer (Jiangxi Tekang Technology Co. Ltd, China). Daily internal quality control and scheduled external quality assessment were performed following the instructions from the manufacturer. G6PD deficiency was diagnosed on day 0 using the G6PD Fluorescence Spot Test (FST, MICKY Inc., Guangzhou, China) following the manufacturer's instructions [14, 23].

Samples that did not result in any fluorescence after 30 min of incubation were considered G6PDd [14].

Patient treatment and follow-up

P. vivax malaria patients were treated following the WHO guidelines for vivax malaria (25 mg CQ base/kg given in 3 days and 0.25 mg PQ/kg/day for 14 days) [5, 24]. After completing the clinical examination, each patient was issued a medication card to monitor their daily medication during the 14-day treatment period. As a part of routine care, PQ was prescribed without the G6PD test, but subjects were warned about the risk of PQ toxicity and asked to closely monitor the color of their urine. Medical staff at the IDP clinic inspected the patient's medication cards, inquired about and recorded any adverse reactions to the medications, and distributed antimalarial drugs daily. Clinicians were free to stop PQ if there were clinical signs of severe hemolysis (dark urine) or other clinical reasons of concern. Medical technicians performed the hematological tests on all enrolled patients at the time of enrollment and on days 7, 14, and 28 without knowing the patient's G6PD status. A transfusion was performed at the nearby township hospital if a patient's Hb fell below 6 g/dL.

Statistical analysis

The EpiData 2.0 software was used for data entry. Data were analyzed and visualized using Graphpad Prism (version 6.0). Two-group comparisons were performed using the Mann–Whitney *U* test for continuous variables or Chi-square test, or Fisher's exact test when mandated by sparse data for categorical variables. The overall mean Hb nadir levels between G6PDn and G6PDd patients were compared using the Student's *t* test. Pearson correlation was used to detect the correlation of the baseline Hb levels with parasitemia (log-transformed), as well as with both the absolute and fractional changes of Hb levels. To test for interactions, logistic regression models were fit with the clinically significant Hb drop as the outcome variable. Gender, age, initial Hb levels (anemia and non-anemia), and initial parasitemia were used as covariates in these models. All *P* values were interpreted in a two-tailed fashion, and a $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics of the *P. vivax* patients

Out of 238 patients presenting with uncomplicated vivax malaria and subsequently evaluated for G6PD status using an FST, we selected 38 (18 male and 20 female) G6PDd *P. vivax* cases. These were then age- and gender-matched to select 114 G6PDn *P. vivax* patients in the ratio 1:3 (Table 1, Supplementary Fig. 1). All *P. vivax* infections were verified by nested PCR analysis. All patients received standard treatment with CQ/PQ without G6PD pre-screening (standard of care in this region). The median age of the vivax patients was 15 years for males and 12.5 years for females. At the time of enrollment, there were no significant differences in BMI, parasite density, or Hb level between the G6PDn and G6PDd groups. There was a high prevalence of anemia in this IDP population, with > 50% of patients being anemic at presentation. Although *P. vivax* infection is often associated with enhanced red blood cell (RBC) destruction and consequently lower baseline Hb levels [25], we did not find a significant correlation between parasitemia and the baseline Hb level (Pearson correlation, $r^2 = 0.0098$). More patients in the G6PDd group (61%) were anemic than in the G6PDn group (54%), but the difference was not statistically significant. The median parasite density in male patients was lower in the G6PDd group than in the G6PDn group, whereas it was at a similar level in female G6PDd and G6PDn patients (Table 1).

Hemoglobin dynamics, nadir levels, and recovery

After the start of the CQ/PQ treatment, the magnitude and direction of the change in the Hb level varied at the individual patient level (Fig. 1), despite that the median Hb levels remained relatively constant (Fig. S2A, Supplementary Table 1). When stratified by Hb level at presentation, the non-anemic patients showed a general decline in Hb over time, whereas anemic patients showed an increase of Hb on day 7 and a gradual increase thereafter (Fig. S2B, C). These trends were consistent for both G6PDn and G6PDd patients.

Thirty-nine (25.7%) patients showed higher Hb levels at all three time points after PQ treatment than baseline levels (Supplementary Table 2). Notably, 87.2% (34/39) of these patients were anemic at enrollment, consistent with the rising Hb trend in anemic patients. Conversely, 113 (74.3%) patients exhibited Hb decreases at one or more time points during the follow-up (Table S2). Also, there were significant negative correlations between absolute or fractional changes of Hb levels on day 7 and day 14 with the baseline Hb levels ($P < 0.001$, Pearson correlation, Fig. 2). In addition, among male patients, the G6PDd group had substantially larger magnitudes of Hb reduction than the G6PDn group at each time point, with the absolute Hb reduction on day 7 being statistically significant between G6PDd (−3.3 g/dL) and G6PDn (−1.2 g/dL) patients ($P < 0.05$) (Table 2). However, among female patients, there were no significant differences in the magnitude of Hb reduction between the G6PDd and G6PDn groups ($P > 0.05$). Collectively, these data indicated that the most substantial Hb reduction in the

Table 1 Baseline characteristics of *P. vivax* patients stratified by sex and G6PD status at the time of enrollment

Parameters	All patients ($N=152$)	G6PD normal ($N=114$)	G6PD deficient ($N=38$)
Male[†]	72	54	18
Age (year)	15.0 (11.0–20.0)	15.00 (11.0–20.3)	15.50 (11.0–20.3)
Body mass index	19.0 (16.7–20.8)	19.0 (16.9–20.8)	18.07 (16.7–20.7)
Parasite density (/μL)	2189 (893–4877)	2372 (699–5528)	2040 (1004–2961)
Hemoglobin (g/dL)	12.05 (10.53–13.50)	12.20 (10.75–13.50)	11.75 (10.08–14.23)
RBC (10^{12}/L)	4.37 (3.63–4.77)	4.45 (3.74–4.90)	4.25 (3.47–4.63)
WBC (10^9/L)	5.65 (4.20–8.00)	6.00 (4.15–7.80)	5.40 (4.08–8.28)
Platelets (10^9/L)	98.50 (83.25–142.00)	102.50 (84.75–144.25)	87.00 (73.00–118.75)
Anemic patients [n (%)][#]	42 (58.3%)	31 (57.4%)	11 (61.1%)
Female[†]	80	60	20
Age (year)	12.5 (10.3–16.0)	12.5 (10.0–16.0)	12.5 (11.0–15.5)
Body mass index	17.2 (15.9–20.9)	17.2 (15.8–20.4)	18.9 (16.1–22.7)
Parasite density (/μL)	2165 (867–4135)	2169 (867–4619)	2165 (890–3355)
Hemoglobin (g/dL)	11.75 (10.25–12.80)	11.90 (10.33–13.15)	11.40 (10.25–12.48)
RBC (10^{12}/L)	3.93 (3.51–4.391)	4.04 (3.41–4.49)	3.885 (3.53–4.18)
WBC (10^9/L)	5.40 (3.83–8.73)	5.40 (3.83–9.28)	5.400 (3.90–7.90)
Platelets (10^9/L)	102.00 (78.50–138.0)	89.50 (78.50–135.80)	112.00 (79.50–155.80)
Anemic patients [n (%)][#]	42 (52.5%)	30 (50.0%)	12 (60.0%)

All data except anemic patients are expressed as median (interquartile range)

For each parameter, there was not statistical difference between G6PDn and G6PDd patients ($P>0.05$)

[†]G6PD-deficient patients and G6PD-normal patients, defined based on the FST results, were age-matched as 1:3

[#]Anemia was determined based on the WHO classification

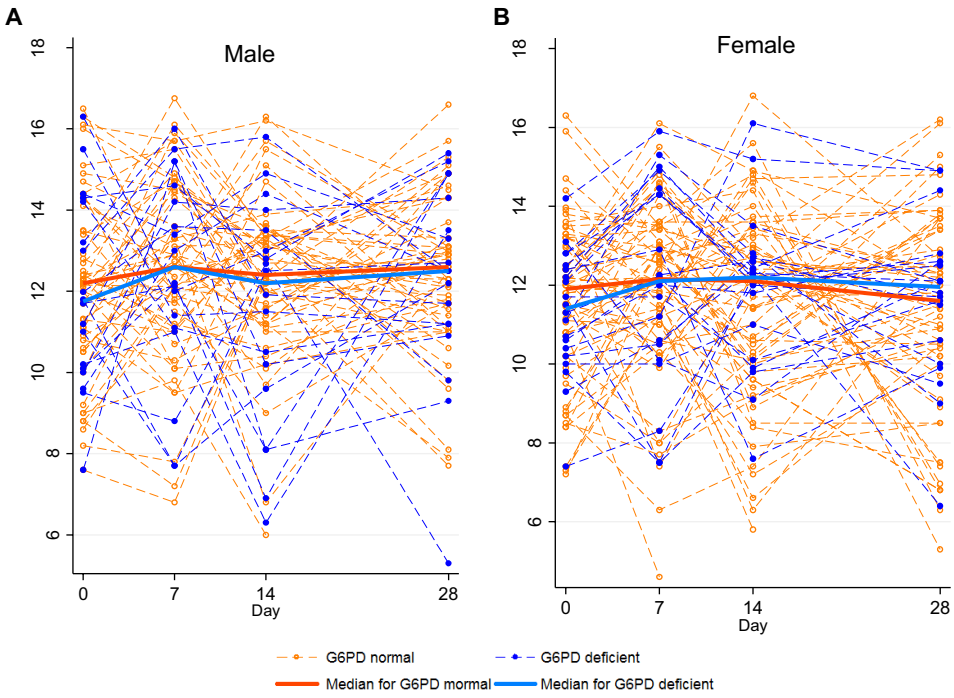

Fig. 1 Changes in hemoglobin levels (g/dL) over time in male (**A**) and female (**B**) *P. vivax* patients. Red and blue colors refer to G6PD-normal and -deficient individuals, respectively. Thick lines show the median levels

G6PDd patients occurred during the first 2 weeks of PQ intake. Only the G6PDd male group showed relatively large magnitudes of absolute Hb decreases (−2.2 to −3.3 g/dL) at all the time points.

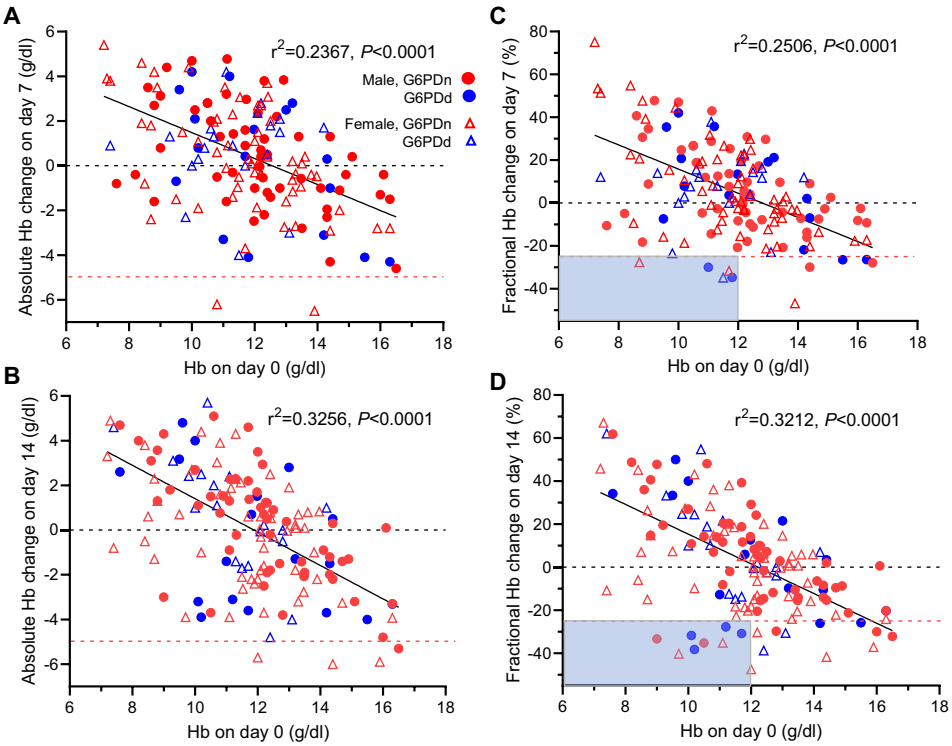

Fig. 2 Correlation between the baseline hemoglobin levels and absolute changes (**A**, **B**) or fractional changes (**C**, **D**) on day 7 (**A**, **C**) and day 14 (**B**, **D**). Dots indicate male patients, while triangles indicate female patients. G6PD-normal (G6PDn) and G6PD-deficient (G6PDd) patients are indicated in red and blue, respectively. The baseline Hb levels correlated negatively with the Hb changes (both absolute and fractional) on day 7 and 14 ($r^2 = 0.2367$–0.3256, $P < 0.0001$, Pearson's correlation). The dashed red lines mark the absolute fall of 5 g/dL (**A**, **B**) or fractional fall of 25% (**C**, **D**) compared to the baseline levels. Shaded areas in **C** and **D** indicate patients anemic at enrollment who experienced > 25% fractional Hb fall on day 7 (**C**) and day 14 (**D**)

Table 2 The dynamics and magnitude of hemoglobin reduction during primaquine treatment among *P. vivax* patients experiencing hemoglobin reduction stratified by sex and G6PD status

Sex	Day	Hb reduction [median (IQR), g/dL]		
		G6PDn	G6PDd	*P**
Male	0	0	0	
	7	−1.20 (−0.55 to −2.05)	−3.30 (−1.00 to −4.10)	**0.0414**
	14	−1.90 (−1.30 to −3.20)	−3.25 (−1.48 to −3.75)	0.1252
	28	−1.40 (−0.70 to −2.50)	−2.20 (−1.10 to −3.60)	0.2851
Female	0	0	0	
	7	−1.45 (−0.50 to − 2.70)	−3.00 (−2.30 to −4.00)	0.0632
	14	−2.20 (−1.00 to −3.70)	−1.50 (−0.35 to − 3.43)	0.2314
	28	−2.10 (−1.05 to −5.00)	−0.80 (−0.50 to −3.18)	0.1072

Hb reduction refers to comparison to the day 0 level

*Mann–Whitney test. Significance was highlighted in bold

G6PDn, G6PD normal; G6PDd, G6PD deficient

Hb nadir levels were used to determine whether PQ treatment should be discontinued. For the 113 patients showing any Hb decreases during the follow-up period, 22.4%, 28.3%, and 23.0% reached the nadir Hb levels on days 7, 14, and 28, respectively (Supplementary Table 3). Apparent disparities existed between the genders. In male patients, the G6PDn group had a higher mean Hb nadir level (10.53 g/dL) than the G6PDd group (9.51 g/dL), but the female patients showed the opposite, higher in the G6PDd group (10.17 g/dL) than the G6PDn group (9.35 g/dL), though the differences were not statistically significant.

A total of 76 (50%) patients achieved hematological recovery, with their Hb levels on day 28 exceeding the baseline levels at enrollment. There were no significant differences in the recovery rates between the male and female groups or between the G6PDn and G6PDd groups (Supplementary Table 1). Compared to the Hb levels at enrollment, the day 28 Hb levels in all groups experienced slight increases (0.21–0.56 g/dL) except for the G6PDn female patients, which showed a slight decrease (−0.1 g/dL) (Supplementary Table 1).

Severity outcomes

Over the course of the study, 45 patients experienced clinically concerning declines in Hb (an absolute reduction of

5 g/dL, a fractional reduction of > 25%, or both). While 62.2% of these clinically concerning Hb declines occurred during PQ treatment, 37.8% happened on day 28. A significantly larger proportion of G6PDd males (55.5%) experienced clinically concerning Hb decreases than G6PDn males (16.7%) (Table 3, $P < 0.01$, χ^2 test). Although this trend was reversed in females, the difference was insignificant.

We first evaluated the independent effects of age, gender, G6PD status, the initial Hb, and the initial parasitemia on the risks of clinically concerning Hb reductions. Although the analysis did not reveal significant impacts of these factors on the risks of clinically concerning Hb drop ($P > 0.05$, Table 4), G6PDd patients had a high OR (1.826). In contrast, those who were anemic and had a high parasitemia level at enrollment had a lower OR in experiencing a clinically concerning drop in Hb.

Since G6PD status may be modified by other factors such as parasitemia [26], a stratified analysis was conducted. While we observed higher ORs of the G6PDd group than the G6PDn group in males, anemic patients at enrollment, and between different initial parasitemias, only the OR for G6PDd males (OR = 6.25, 95% CI 1.93–20.20) was statistically significant (Table 4).

Five patients (2 males and 3 females) experienced Hb falls below 6 g/dL, the local threshold for transfusion (Table 3, Supplementary Table 4). Four patients were anemic at enrollment (Hb 9.0–10.8 g/dL), and one male was G6PDd. One G6PDn male patient had a reduction of Hb level from 9 g/dL at enrollment to 6 g/dL on day 14, while the other G6PDd male patient with a Hb level of 9.5 g/dL at enrollment showed an initial increase during the first 2 weeks, but exhibited a subsequent drop in Hb to 5.3 g/dL on day 28. The three females with the reduced Hb level to ≤ 6 g/dL were all G6PDn, and transfusion referral was made on days 7, 14, and 28, respectively (Fig. 1). None of the patients showed signs of AHA or had dark urine.

Discussion

G6PD deficiency occurs at a relatively high frequency in the GMS, with the class III variants Mahidol and Viangchan being the predominant G6PD variants [13, 14, 27]. Previous studies have shown that the standard treatment regimen for vivax malaria was generally safe without requiring blood transfusion, even though significant falls in Hb were detected in G6PDd patients and heterozygous females with 40–60% residual activity [16, 28, 29]. However, recent studies conducted in the GMS have demonstrated elevated risks of clinically concerning Hb declines in G6PDd patients treated with weekly PQ [30], or in G6PD heterozygous but phenotypically normal female patients treated with higher daily PQ doses [9, 31]. Our study is the first to evaluate the risk of severe hemolysis in vivax patients treated with the standard CQ/PQ regimen in vivax-endemic eastern Myanmar. We observed that the overall Hb levels after PQ treatment in both male and female G6PDn and G6PDd groups were relatively stable, with a slight increase by day 28, suggesting a general Hb recovery. However, significant variations were detected among individual patients. Almost one-third (29.6%) of the patients experienced clinically concerning falls in Hb, including five patients with Hb levels dropping below 6 g/dL requiring blood transfusion, although the two occurring on day 28 may be unrelated to PQ since both had day-14 Hb levels above 12 g/dL. The Mahidol variant is the most common in the study area [14, 32]. Though the Mahidol variant is classified as a mild deficiency, undiagnosed patients can sometimes develop life-threatening AHA after receiving the standard PQ treatment [19]. Similarly, unsupervised PQ treatment in the Brazilian Amazon with a prevalence of ~ 5% G6PD African A- class III variant has led to life-threatening anemia and acute renal failure [33]. Our study reinforces the need for implementing G6PD testing in areas with prevalent class III G6PD variants.

The clinical outcomes of PQ treatment reveal differences across genders, consistent with G6PD deficiency being a sex-linked trait. Male G6PDd patients recorded a much larger magnitude (an absolute reduction of 3.25–3.30 g/dL) of Hb reduction than the G6PDn patients in the first 2 weeks

Table 3 Patients with clinically concerning reduction in hemoglobin and severe hemolysis

Sex	Clinically concerning Hb reduction			Severe hemolysis (Hb < 6 g/dL)		
	G6PDn	G6PDd	*P**	G6PDn	G6PDd	*P***
Male (*n* = 72)	9/54 (16.7%)	10/18 (55.5%)	**0.0012**	1/54 (1.9%)	1/18 (5.6%)	0.4401
Female (*n* = 80)	21/60 (35.0%)	5/20 (25.0%)	0.4083	3/60 (5%)	0	0.569
Total (*n* = 152)	30/114 (26.3%)	15/38 (39.5%)	0.1239	4/114 (3.5%)	1/38 (2.6%)	1.0

Definition of clinically concerning decrease: absolute reduction of > 5 g/dl or/and fractional reduction of > 25% from baseline levels

*Chi-square test; **Fisher's exact test

Table 4 Risk factors analysis for patients with clinically concerning decrease in hemoglobin

	Clinically concerning decrease ($N=45$)	No clinically concerning decrease ($N=107$)	OR (95% CI)	P value[#]
Age, year [N (%)]				0.765
< 15	26/85 (30.6%)	59/85 (69.4%)	1	
≥ 15	19/67 (28.4%)	48/67 (71.6%)	0.90 (0.44–1.82)	
Sex [N (%)]				0.410
Female	26/80 (32.5%)	54/80 (67.5%)	1	
Male	19/72 (26.4%)	53/72 (73.6%)	0.75 (0.37–1.50)	
G6PD [N (%)]				0.124
G6PD normal	30/114 (26.3%)	84/114 (73.7%)	1	
G6PD deficient	15/38 (39.5%)	23/38 (60.5%)	1.83 (0.84–3.95)	
Initial parasitemia (/µl), [N (%)]				0.121
< 4000 (low)	37/112 (33.0%)	75/112 (67.0%)	1	
≥ 4000 (high)	8/40 (20.0%)	32/40 (80.0%)	0.51 (0.21–1.21)	
Initial Hb (g/dl) [N (%)]				0.167
Non-anemic	24/68 (35.3%)	44/68 (64.7%)	1	
Anemic	21/84 (25.0%)	63/84 (75.0%)	0.61 (0.30–1.23)	
Age, ≥ 15 [N (%)]				0.117
G6PD normal	12/51 (23.5%)	39/51 (76.5%)	1	
G6PD deficient	7/16 (43.8%)	9/16 (56.3%)	2.53 (0.78–8.23)	
Age, < 15 [N (%)]				0.495
G6PD normal	18/63 (28.6%)	45/63 (71.4%)	1	
G6PD deficient	8/22 (36.4%)	14/22 (63.6%)	1.43 (0.51–4.00)	
Male [N (%)]				**0.001***
G6PD normal	9/54 (16.7%)	45/54 (83.3%)	1	
G6PD deficient	10/18 (55.6%)	8/18 (44.4%)	6.25 (1.93–20.20)	
Female [N (%)]				0.408
G6PD normal	21/60 (35.0%)	39/60 (65.0%)	1	
G6PD deficient	5/20 (25.0%)	15/20 (75.0%)	0.62 (0.20–1.94)	
Initial Hb level non-anemic [N (%)]				0.666
G6PD normal	18/53 (34.0%)	35/53 (66.0%)	1	
G6PD deficient	6/15 (40.0%)	9/15 (60.0%)	1.30 (0.40–4.22)	
Initial Hb level anemic [N (%)]				0.066
G6PD normal	12/61 (19.7%)	49/61 (80.3%)	1	
G6PD deficient	9/23 (39.1%)	14/23 (60.9%)	2.63 (0.92–7.49)	
Initial parasitemia—low [N (%)]				0.215
G6PD normal	24/81 (29.6%)	57/81 (70.4%)	1	
G6PD deficient	13/31 (41.9%)	18/31 (58.1%)	1.72 (0.73–4.05)	
Initial parasitemia—high [N (%)]				0.533
G6PD normal	6/33 (18.2%)	27/33 (81.8%)	1	
G6PD deficient	2/7 (28.6%)	5/7 (71.4%)	1.80 (0.28–11.60)	

[#]Chi-square test. *$P < 0.05$

of PQ treatment. They also carried a significantly higher risk of clinically relevant Hb reductions. Given that the G6PDn and G6PDd phenotypes in male patients are clearly separate, implementing G6PD screening before PQ administration and not treating G6PDd males would reduce the number of male patients with clinically relevant Hb reductions by > 50%. In females, we also observed a much larger magnitude of Hb reductions in G6PDd patients than the G6PDn patients in the first week of PQ treatment, but the hemolytic effects observed in males were not evident in females (Table 3). This may be due to the misclassification of heterozygous females with intermediate G6PD activity to the G6PDn group. The FST typically detects G6PD deficiency at < 30% of normal activity [31, 34], which more reliably identifies

G6PDd hemizygous males and homozygous females, but often misses the heterozygous females with intermediate G6PD activity. Our data indicate that some female patients carried a significant hemolytic risk even with the low-dose daily PQ regimen (0.25 mg/kg), necessitating a more sensitive G6PD test for female patients.

Despite normally low parasitemia, acute vivax malaria is often associated with anemia, partly due to the destruction of parasitized RBCs, enhanced elimination of non-parasitized RBCs, and compromised erythropoiesis [35–37]. In malaria-endemic areas of Southeast Asia, hemoglobinopathies such as thalassemia and Hb E are highly prevalent and can also contribute to lower Hb levels [38, 39]. In the IDP camps, anemia is prevalent among school-age children, partially linked to malnutrition and infection with soil-transmitted helminths [40]. Similarly, 52% of the 152 patients were anemic at enrollment, and 19 patients had Hb levels of ≤ 9 g/dL. Unlike many clinical PQ studies that excluded patients with moderate-to-high levels of anemia [41], this study included all *P. vivax* patients regardless of their G6PD status or Hb levels (if it was above 7 g/dL), which provided us the opportunity to follow the risk of AHA in anemic patients. Consistent with earlier hematological studies in vivax malaria patients [42], we found that both absolute and fractional Hb reductions after PQ treatment were inversely correlated with the initial Hb levels at enrollment. Consequently, the increase in Hb levels in some anemic patients would have masked the low Hb levels often observed around day 7 [16, 29]. Despite this trend, 42.2% of the 45 patients showing clinically relevant Hb reductions after PQ treatment were anemic at enrollment. Thus, a substantial drop of Hb in these patients would be concerning. Indeed, in four such patients, Hb fell to ≤ 6 g/dL, requiring transfusion. Thus, measuring Hb levels in combination with G6PD testing before initiating PQ treatment should be considered. Although the high prevalence of anemia in the IDP camps may be a unique situation, the presence of anemia in almost 50% of healthy school children elsewhere in Myanmar suggests that this may be a more common phenomenon [43].

This study had several limitations. Since children (< 18 years) made up 75.7% of the study population, findings from this study may not be fully extended to adult patients. While the FST is reliable in identifying male G6PDd hemizygotes, it is not sensitive to identify female heterozygotes, thus making the comparison within female patients less accurate. Unfortunately, even with the quantification of G6PD activity in female heterozygotes of the Mahidol variant, the risk of PQ-induced AHA cannot be reasonably predicted [31]. In this study, PQ safety outcome was primarily measured by comparing nadir Hb levels with baseline Hb levels. Since a meta-analysis of CQ/PQ studies showed that the nadir Hb typically occurs on day 3 [42], we might have missed some patients' nadir Hb levels occurring during the first week. Future studies with more frequent follow-ups may more accurately detect PQ-induced hemolysis occurring during the first week of PQ treatment [31, 39, 42]. Also, we did not account for the prevalent hemoglobinopathies such as thalassemias and HbE in this region [32]. Vivax patients with these hemoglobinopathies combined with G6PD deficiency are at the greatest risk of PQ-induced AHA resulting from a weekly PQ regimen [39].

Conclusions

This study demonstrated that the standard CQ/PQ treatment regimen (low-dose PQ) in the absence of G6PD diagnosis carried a significant risk of AHA in vivax patients where the G6PD Mahidol variant (mild) is highly prevalent—the treatment is dangerous in about one of three patients treated. Whereas G6PD pre-screening with a less-sensitive FST can prevent most clinically significant falls in Hb and AHA in male patients, determining the G6PD status in females demands a more sensitive G6PD test. Regardless of the treatment regimen, PQ treatment of vivax patients in the GMS requires medical supervision, especially in populations with prevalent anemia. For the patient population with generally low Hb levels at the time of presentation for care, PQ-induced AHA could be life-threatening. Thus, closely monitoring patients with anemia at admission also helps to reduce and prevent clinically dangerous AHA.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s15010-022-01905-9.

Acknowledgements We want to thank the medical staff at the study clinics for assisting with the study.

Author contributions ZY and LC conceived the design of this study. HL and WZ facilitated sample collection and data curation. HZ, ZY, PM, CW, SL, KK, LM, and LC performed data analysis and drafted the manuscript. HL, LM, ZY, KK, and LC reviewed and revised the manuscript. All authors read and approved the final manuscript.

Funding This study was supported by the National Institute of Allergy and Infectious Diseases, and National Institutes of Health USA (U19AI089672). ZY was also supported by the National Natural Science Foundation of China (31860604 and U1802286), the Science and Technology Project of Yunnan (2018ZF0081), and International Science and Technology Cooperation-Yunnan International Science and Technology Cooperation Base (202003AE140004). WZ was supported by the Education Department Fund of Yunnan Province (2019J1184). HL was supported by the High-level Health Talents Project of Yunnan Province (H-2017071).

Declarations

Conflict of interest The authors have no conflicts of interest.

Consent for publication Not applicable.

References

1. Adams JH, Mueller I. The biology of Plasmodium vivax. Cold Spring Harb Perspect Med. 2017;7: a025585.
2. Betuela I, Rosanas-Urgell A, Kiniboro B, Stanisic DI, Samol L, de Lazzari E, Del Portillo HA, Siba P, Alonso PL, Bassat Q, Mueller I. Relapses contribute significantly to the risk of Plasmodium vivax infection and disease in Papua New Guinean children 1–5 years of age. J Infect Dis. 2012;206:1771–80.
3. Robinson LJ, Wampfler R, Betuela I, Karl S, White MT, Li Wai Suen CS, Hofmann NE, Kinboro B, Waltmann A, Brewster J, et al: Strategies for understanding and reducing the Plasmodium vivax and Plasmodium ovale hypnozoite reservoir in Papua New Guinean children: a randomised placebo-controlled trial and mathematical model. PLoS Med. 2015;12:e1001891.
4. Chu CS, White NJ. The prevention and treatment of Plasmodium vivax malaria. PLoS Med. 2021;18: e1003561.
5. WHO: Guidelines for the treatment of malaria. Third edition. 2015:pp. 316.
6. Baird JK. Primaquine toxicity forestalls effective therapeutic management of the endemic malarias. Int J Parasitol. 2012;42:1049–54.
7. Howes RE, Piel FB, Patil AP, Nyangiri OA, Gething PW, Dewi M, Hogg MM, Battle KE, Padilla CD, Baird JK, Hay SI. G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: a geostatistical model-based map. PLoS Med. 2012;9: e1001339.
8. John GK, Douglas NM, von Seidlein L, Nosten F, Baird JK, White NJ, Price RN. Primaquine radical cure of Plasmodium vivax: a critical review of the literature. Malar J. 2012;11:280.
9. Chu CS, Phyo AP, Turner C, Win HH, Poe NP, Yotyingaphiram W, Thinraow S, Wilairisak P, Raksapraidee R, Carrara VI, et al. Chloroquine versus dihydroartemisinin-piperaquine with standard high-dose primaquine given either for 7 days or 14 days in Plasmodium vivax malaria. Clin Infect Dis. 2019;68:1311–9.
10. Taylor WRJ, Thriemer K, von Seidlein L, Yuentrakul P, Assawariyathipat T, Assefa A, Auburn S, Chand K, Chau NH, Cheah PY, et al: Short-course primaquine for the radical cure of Plasmodium vivax malaria: a multicentre, randomised, placebo-controlled non-inferiority trial. Lancet. 2019.
11. Geng J, Malla P, Zhang J, Xu S, Li C, Zhao Y, Wang Q, Kyaw MP, Cao Y, Yang Z, Cui L. Increasing trends of malaria in a border area of the Greater Mekong Subregion. Malar J. 2019;18:309.
12. Guidelines for malaria diagnosis and treatment in Myanmar. [https://www.eliminatemalaria.net/wp-content/uploads/2018/08/Myanmar-NTG-2015.pdf]
13. Howes RE, Dewi M, Piel FB, Monteiro WM, Battle KE, Messina JP, Sakuntabhai A, Satyagraha AW, Williams TN, Baird JK, Hay SI. Spatial distribution of G6PD deficiency variants across malaria-endemic regions. Malar J. 2013;12:418.
14. Li Q, Yang F, Liu R, Luo L, Yang Y, Zhang L, Liu H, Zhang W, Fan Z, Yang Z, et al. Prevalence and molecular characterization of glucose-6-phosphate dehydrogenase deficiency at the China-Myanmar border. PLoS ONE. 2015;10: e0134593.
15. Charoenlarp P, Areekul S, Pholpothi T, Harinasuta T. The course of primaquine-induced haemolysis in G-6-PD-deficient Thais. J Med Assoc Thai. 1973;56:392–7.
16. Buchachart K, Krudsood S, Singhasivanon P, Treeprasertsuk S, Phophak N, Srivilairit S, Chalermrut K, Rattanapong Y, Supeeranuntha L, Wilairatana P, et al. Effect of primaquine standard dose (15 mg/day for 14 days) in the treatment of vivax malaria patients in Thailand. Southeast Asian J Trop Med Public Health. 2001;32:720–6.
17. Aung-Than-Batu H-P. U, Thein-Than: Primaquine induced haemolysis in G-6-PD deficient Burmese. Trans R Soc Trop Med Hyg. 1970;64:785–6.
18. Karwacki JJ, Shanks GD, Kummalue T, Watanasook C. Primaquine induced hemolysis in a Thai soldier. Southeast Asian J Trop Med Public Health. 1989;20:555–6.
19. Chen X, He Y, Miao Y, Yang Z, Cui L. A young man with severe acute haemolytic anaemia. BMJ. 2017;359: j4263.
20. WHO: Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. 2011.
21. Yuan L, Wang Y, Parker DM, Gupta B, Yang Z, Liu H, Fan Q, Cao Y, Xiao Y, Lee MC, et al. Therapeutic responses of Plasmodium vivax malaria to chloroquine and primaquine treatment in northeastern Myanmar. Antimicrob Agents Chemother. 2015;59:1230–5.
22. Zhao Y, Wang L, Soe MT, Aung PL, Wei H, Liu Z, Ma T, Huang Y, Menezes LJ, Wang Q, et al. Molecular surveillance for drug resistance markers in Plasmodium vivax isolates from symptomatic and asymptomatic infections at the China-Myanmar border. Malar J. 2020;19:281.
23. Rao Y, Fang X, Yang Z, Wan Z. Fluorescence spot test and G6PD/6PGD rate test detecting of G6PD. Chinese J Birth Health Heredity. 2010;18:78–9.
24. Xu S, Zeng W, Ngassa Mbenda HG, Liu H, Chen X, Xiang Z, Li C, Zhang Y, Baird JK, Yang Z, Cui L. Efficacy of directly-observed chloroquine-primaquine treatment for uncomplicated acute Plasmodium vivax malaria in northeast Myanmar: A prospective open-label efficacy trial. Travel Med Infect Dis. 2020;36: 101499.
25. Barber BE, William T, Grigg MJ, Parameswaran U, Piera KA, Price RN, Yeo TW, Anstey NM. Parasite biomass-related inflammation, endothelial activation, microvascular dysfunction and disease severity in vivax malaria. PLoS Pathog. 2015;11: e1004558.
26. Ley B, Alam MS, Kibria MG, Marfurt J, Phru CS, Ami JQ, Thriemer K, Auburn S, Jahan N, Johora FT, et al. Glucose-6-phosphate dehydrogenase activity in individuals with and without malaria: Analysis of clinical trial, cross-sectional and case-control data from Bangladesh. PLoS Med. 2021;18: e1003576.
27. Bancone G, Chu CS, Somsakchaicharoen R, Chowwiwat N, Parker DM, Charunwatthana P, White NJ, Nosten FH. Characterization of G6PD genotypes and phenotypes on the northwestern Thailand-Myanmar border. PLoS ONE. 2014;9: e116063.
28. Krudsood S, Wilairatana P, Tangpukdee N, Chalermrut K, Srivilairit S, Thanachartwet V, Muangnoicharoen S, Luplertlop N, Brittenham GM, Looareesuwan S. Safety and tolerability of elubaquine (bulaquine, CDRI 80/53) for treatment of Plasmidium vivax malaria in Thailand. Korean J Parasitol. 2006;44:221–8.
29. Rueangweerayut R, Bancone G, Harrell EJ, Beelen AP, Kongpatanakul S, Mohrle JJ, Rousell V, Mohamed K, Qureshi A, Narayan S, et al. Hemolytic potential of tafenoquine in female volunteers heterozygous for glucose-6-phosphate dehydrogenase (G6PD) deficiency (G6PD Mahidol variant) versus G6PD-normal volunteers. Am J Trop Med Hyg. 2017;97:702–11.
30. Kheng S, Muth S, Taylor WR, Tops N, Kosal K, Sothea K, Souy P, Kim S, Char CM, Vanna C, et al. Tolerability and safety of weekly primaquine against relapse of Plasmodium vivax in Cambodians with glucose-6-phosphate dehydrogenase deficiency. BMC Med. 2015;13:203.
31. Chu CS, Bancone G, Moore KA, Win HH, Thitipanawan N, Po C, Chowwiwat N, Raksapraidee R, Wilairisak P, Phyo AP, et al. Haemolysis in G6PD heterozygous females treated with primaquine for Plasmodium vivax malaria: a nested cohort in a trial of radical curative regimens. PLoS Med. 2017;14: e1002224.
32. Deng Z, Yang F, Bai Y, He L, Li Q, Wu Y, Luo L, Li H, Ma L, Yang Z, et al. Co-inheritance of glucose-6-phosphate

dehydrogenase deficiency mutations and hemoglobin E in a Kachin population in a malaria-endemic region of Southeast Asia. PLoS ONE. 2017;12: e0177917.
33. Brito-Sousa JD, Santos TC, Avalos S, Fontecha G, Melo GC, Val F, Siqueira AM, Alecrim GC, Bassat Q, Lacerda MVG, Monteiro WM. Clinical spectrum of primaquine-induced hemolysis in G6PD deficiency: A nine-year hospitalization-based study from the Brazilian Amazon. Clin Infect Dis. 2019;69:1440–2.
34. Oakley MS, Majam V, Mahajan B, Gerald N, Anantharaman V, Ward JM, Faucette LJ, McCutchan TF, Zheng H, Terabe M, et al. Pathogenic roles of CD14, galectin-3, and OX40 during experimental cerebral malaria in mice. PLoS ONE. 2009;4: e6793.
35. Anstey NM, Douglas NM, Poespoprodjo JR, Price RN. Plasmodium vivax: clinical spectrum, risk factors and pathogenesis. Adv Parasitol. 2012;80:151–201.
36. Clark MA, Kanjee U, Rangel GW, Chery L, Mascarenhas A, Gomes E, Rathod PK, Brugnara C, Ferreira MU, Duraisingh MT. Plasmodium vivax infection compromises reticulocyte stability. Nat Commun. 2021;12:1629.
37. Douglas NM, Anstey NM, Buffet PA, Poespoprodjo JR, Yeo TW, White NJ, Price RN. The anaemia of Plasmodium vivax malaria. Malar J. 2012;11:135.
38. Deng Z, Li Q, Yi H, Zhang Y, Yang F, Li H, Luo L, Ma L, Yang Z, He Y, Cui L. Hemoglobin E protects against acute Plasmodium vivax infections in a Kachin population at the China-Myanmar border. J Infect. 2018;77:435–9.
39. Taylor WRJ, Kheng S, Muth S, Tor P, Kim S, Bjorge S, Topps N, Kosal K, Sothea K, Souy P, et al. Hemolytic dynamics of weekly primaquine antirelapse therapy among Cambodians with acute plasmodium vivax malaria with or without glucose-6-phosphate dehydrogenase deficiency. J Infect Dis. 2019;220:1750–60.
40. Zeng W, Malla P, Xu X, Pi L, Zhao L, He X, He Y, Menezes LJ, Cui L, Yang Z. Associations among soil-transmitted helminths, G6PD deficiency and asymptomatic malaria parasitemia, and anemia in schoolchildren from a conflict zone of northeast Myanmar. Am J Trop Med Hyg. 2020;102:851–6.
41. Rajgor DD, Gogtay NJ, Kadam VS, Kocharekar MM, Parulekar MS, Dalvi SS, Vaidya AB, Kshirsagar NA. Antirelapse Efficacy of Various Primaquine Regimens for Plasmodium vivax. Malar Res Treat. 2014;2014: 347018.
42. Commons RJ, Simpson JA, Thriemer K, Chu CS, Douglas NM, Abreha T, Alemu SG, Anez A, Anstey NM, Aseffa A, et al. The haematological consequences of Plasmodium vivax malaria after chloroquine treatment with and without primaquine: a WorldWide Antimalarial Resistance Network systematic review and individual patient data meta-analysis. BMC Med. 2019;17:151.
43. Wah ST, Yi YS, Khin AA, Plabplueng C, Nuchnoi P. Prevalence of anemia and hemoglobin disorders among school children in Myanmar. Hemoglobin. 2017;41:26–31.