Review

Primaquine treatment and relapse in *Plasmodium vivax* malaria

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The relapsing peculiarity of *Plasmodium vivax* is one of the prime reasons for sustained global malaria transmission. Global containment of *P. vivax* is more challenging and crucial compared to other species for achieving total malaria control/elimination. Primaquine (PQ) failure and *P. vivax* relapse is a major global public health concern. Identification and characterization of different relapse strains of *P. vivax* prevalent across the globe should be one of the thrust areas in malaria research. Despite renewed and rising global concern by researchers on this once 'neglected' species, research and development on the very topic of *P. vivax* reappearance remains inadequate. Many malaria endemic countries have not mandated routine glucose-6-phosphate dehydrogenase (G6PD) testing before initiating PQ radical cure in *P. vivax* malaria. This results in either no PQ prescription or thoughtless prescription and administration of PQ to *P. vivax* patients by healthcare providers without being concerned about patients' G6PD status and associated complications. It is imperative to ascertain the G6PD status and optimum dissemination of PQ radical cure in all cases of *P. vivax* malaria across the globe. There persists a compelling need to develop/validate a rapid, easy-to-perform, easy-to-interpret, quality controllable, robust, and cost-effective G6PD assay. High-dose PQ of both standard and short duration appears to be safe and more effective for preventing relapses and should be practiced among patients with normal G6PD activity. Multicentric studies involving adequately representative populations across the globe with reference PQ dose must be carried out to determine the true distribution of PQ failure. Study proving role of cytochrome P450-2D6 gene in PQ metabolism and association of CYP2D6 metabolizer phenotypes and *P. vivax* relapse is of prime importance and should be carried forward in multicentric systems across the globe.

Keywords: Malaria, *Plasmodium vivax*, Primaquine, Relapse, Recurrence, G6PD

Introduction

Control and elimination of *Plasmodium vivax* remains hindered mainly due to relapses by the hypnozoites activation. After inoculation by the vector, a sporozoite population of *P. vivax* undergoes dormancy in the hepatocytes and survives during seasons while even mosquito vectors do not thrive to continue transmission. These dormant stages also known as 'hypnozoites' give rise to relapses. Relapse may occur due to either insufficient dosage or cumulative primaquine (PQ) drug concentration achieved within the body. Potential risk factors viz., baseline anti-malarial immunity, patients' body weight, and quality of PQ drug being used might play crucial role in determining the PQ treatment outcome/*P. vivax* relapse. Identifying the determinants of relapse and molecular markers of PQ efficacy

in *P. vivax* malaria is a major topic in malaria research, as the global containment of this menace significantly depends upon preserving the PQ's efficacy intact.

Method

We present herewith a narrative review of the relevant literature accessed from the PubMed and Google Scholar databases by using the terms *P. vivax* and 'relapse' and 'recurrence' and 'reappearance' and 'G6PD' in combination with the limiting term 'human' for papers published in English language from the year 2001 to 2015.

Review

Magnitude of P. vivax and its relapse

Of the five known *Plasmodium* species malariogenic to humans, *P. vivax* and *P. falciparum* are the most important ones as they contribute most to the global malaria burden, its related morbidity and mortality. There are

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about 3 billion people at risk of *P. vivax* infection across the globe.¹ According to the World Health Organization (WHO), there were a total 198 million (95% uncertainty interval, 124–283 million) estimated global malaria cases in the year 2013, about 8% cases were due to *P. vivax*, of which 47% cases were from outside the African continent. That 8% estimate was equivalent to 15.8 million (95% uncertainty interval 11.9–22.0 million) *P. vivax* cases globally.² Notably, WHO's estimates of global malaria burden have been controversial as the methods adopted to derive the estimates have not been scientifically vetted by peer reviews.^{3–6} There seems a gross underestimation of global *P. vivax* burden in estimates of the WHO. Global *P. vivax* burden has been reported to vary from 70 to 390 million cases per year.⁷ India together with Ethiopia, Indonesia, and Pakistan accounts for more than 80% of global *P. vivax* burden by this estimation. *P. vivax* imparts about 50% of total annual malaria cases in India.⁸

Notably, *P. vivax* is progressively being documented to cause complicated malaria and mortality.^{9–11} According to the World Malaria Report 2013, there has been relatively lesser decrease in the incidence of *P. vivax* cases than *P. falciparum*. It has been postulated that *P. vivax* is resilient to control measures due to its distinct biological nature including relapses, early gametocyte formation, and ability to survive at lower temperatures.⁸ Due to a slower rate of decrease in *P. vivax* incidence, it is important to give greater attention to its control programs. A relapse rate exceeding 50% would become the principal source of *P. vivax* malaria.¹² Length of follow-up greatly determines the overall rate of recurrences observed in a study. The quantum of relapse burden is subject to the method of determination of relapse. Studies on unsupervised treatment efficacy of chloroquine (CQ, 1500 mg over 3 days) and PQ (210 mg over 14 days) regimen have documented *P. vivax* relapse rate varying from 8.1¹³ to 38%.¹⁴ From India, *P. vivax* relapse rate has been documented to be ranging from 1.55% as per month of recurrence method, to 2.0% as per PCR-RFLP (PvMSP3 α and PvMSP3 β) and 1.47% by PCR sequencing (PvMSP1) for 15 mg/day over 14 days PQ treatment.¹³ Notably, in the same study,

concordance among the three methods in differentiating relapse/reinfection was estimated to be only 45% (15/33). Table 1 summarizes several recent studies on *P. vivax* relapse from across the globe.

Recognition of hypnozoites as source of relapse

Evidence of exoerythrocytic schizogony of *P. gallinaceum* appeared at first in the year 1937 through the work of James and Tate with avian malaria.¹⁵ During that era, relapse was considered to occur due to either upsurge of sustained subpatent erythrocytic parasitaemia upon host immune impairment, or from parthenogenesis of microgametocyte, or through sustenance of obscured form of parasite in the inner organs further invading erythrocytes upon host immune deficiency. Subsequently, Shortt and Garnham^{16,17} in the year 1948 demonstrated for the first time the existence of schizonts of *P. cynomolgi* in hepatic parenchyma of *Macaca mulatta*. They postulated that hepatic schizonts cause relapse in simian malaria and due to similarities with *P. cynomolgi*, *P. vivax* relapse in human malaria could also be due to hepatic schizonts. Thereafter in the same year, Shortt & colleagues¹⁸ did demonstrate pre-erythrocytic *P. vivax* stage in sections made from liver biopsy after seven-day experimental inoculation of *P. vivax* infection in a volunteer requiring the then famous malaria treatment for neurosyphilis. Despite this evidence of existence of pre-erythrocytic stage of *P. vivax*, pre-erythrocytic liver stage causing relapse did not gain wide recognition until the year 1982. Meanwhile, the incubation/latency in *P. vivax* was elucidated in terms of some postulates viz., deviation of some merozoites from the cyclic process of exoerythrocytic schizogony leading to relapse; or innately (both metabolic and genetic) different strains having short latency (tachysporozoites) and long latency (bradysporozoites) characteristics.¹⁹ Finally, Krotoski *et al.*^{20,21} in the year 1982 confirmed the dormant liver stage i.e. 'hypnozoite' of both *P. cynomolgi* and *P. vivax* responsible for relapses. Later on, the cyclic process of exoerythrocytic schizogony leading to relapse was asserted.²² However, the exact mechanism of hypnozoites' evolution and their periodic activation remains elusive till date.

Table 1 Pattern of *P. vivax* reappearance with CQ (25 mg/kg body weight divided over 3 days) and PQ (0.25 mg/kg body weight daily for 2 weeks) standard treatment in studies across the globe

Study references	Place of study	Population size	Age range (years)	Follow-up duration (months)	Reappearance rate
Llanos-Cuentas <i>et al.</i> ⁵⁶	Peru, Brazil, Thailand and India	50	16–72	6	22% (11/50)
Durand <i>et al.</i> ²⁷	Amazon Basin, Peru	180	1–70	7	13.6% (22/162)
Rajgor <i>et al.</i> ¹³	Mumbai, India	398	18–76	6	8.1% (26/322)
Kim <i>et al.</i> ¹⁴	Kolkata, India	42	31 $\pm$ 12*	15	38% (16/42)
Maneeboonyang <i>et al.</i> ⁶¹	Thailand	46	2–70	3	Nil
Yeshiwondim <i>et al.</i> ⁷⁹	Debre Zeit and Nazareth in East Shoa, Ethiopia	145	4–60	5	3% (4/132)
Alvarez <i>et al.</i> ²⁶	Colombia	68	29.4 $\pm$ 11.1*	6	17.6% (12/68)
Duarte <i>et al.</i> ⁸⁰	Brazilian Amazon	50	14–77	8	14% (7/50)

*Mean age with standard deviation, age range were not mentioned in these articles.

Biologic implications of *P. vivax* relapse

There exists an astonishing periodicity in *P. vivax* relapse. The majority of *P. vivax* strain show a short incubation period (8–9 days) before primary illness, but their pre-relapse latency period varies enormously. Traditionally, based on the time interval between any relapse (i.e. hypnozoites' latency period), and geographical climate there have been two distinct strains of *P. vivax*. A short time interval (about 1 month) relapsing strain prevailing across the tropical regions is known as the 'Chesson' strain, whereas the long time interval (about 6–15 months) relapsing strain prevailing across temperate globe is called as 'St. Elizabeth' strain. However, this dichotomization discords with evidence of existence of both short and long latency *P. vivax* in tropical regions as in Kolkata, India.¹⁴ In much of the malarious world across Asia (except southeast Asia), Europe, North America, North Africa, and the horn of Africa, a long time interval relapsing *P. vivax* prevails.¹² Whereas a short time interval relapsing *P. vivax* is spread across entire southeast Asia, India, and South America.¹² Coexistence of both long and short time interval relapsing *P. vivax* is confined across South America and India.^{12,14} Co-existence of both strains is a matter of concern for malaria epidemiologists trying to discern the temporal configuration of illness due to individual parasite strain as it makes the distinction of the two strain extremely difficult without genotyping.¹⁴

Gametocyte development of *P. vivax* happens in concurrence with asexual parasite stages. This results in high probability of mosquito infections with multiple genotypes ensuing further recombination among those genetically diverse parasites. Genetic diversity in *P. vivax* by areas of diverse endemicity possesses a significant implication for relapse studies. Simultaneous occurrence of multiple genotypes in *P. vivax* relapse descends from hypnozoites activation from previous infections. It is assumed that the illness associated with malaria itself might be the activator of subsequent relapses in *P. vivax*.¹² Relapse has been proposed to have developed as an acclimatization to manipulate the seasonal transformations in vector endurance and thereby enabling transmission.²³ The probability of relapse is a function of size of sporozoite inoculum, which could vary from 20 to 80%.¹²

Recently, a first characterization of *P. vivax* liver stage antigen using synthetic peptides has been achieved.²⁴ Similar future efforts in this direction are required to better understand the liver stage biology of *P. vivax*, to find potential vaccine candidate to prevent relapse, immunodiagnostic test to diagnose the presence of hypnozoites, and to assess the efficacy of tissue hypnozoitocides.

P. vivax and primaquine therapy

The need of optimum and robust delivery of radical treatment is augmented with the revelation that most acute cases of *P. vivax* originate from secondary rather than primary infections, i.e. from hypnozoites rather than

sporozoites.²⁵ PQ remains the only hypnozoitocidal therapy approved by the Food and Drug Administration for the clinical use. There persists a dilemma over the standard duration of PQ regimen and its relation to treatment outcome(s). Rationally, PQ cumulative dosage seems to be more deterministic for treatment outcome(s) than its duration. A standard PQ dose of 15 mg/day over 14 days (210 mg) has been proven to have up to 30% better protective efficacy than over 7 day (105 mg) or 3 day (45 mg) treatments.²⁶

The debate on the efficacy of daily dose of 15 mg PQ has been long standing. Various studies have attempted to address the best dose and duration of PQ administration that decrease *P. vivax* relapses.^{13,14,27–29} The usually prescribed PQ dose (0.25 mg/kg body weight/day) for a normal glucose-6-phosphate dehydrogenase (G6PD) individual is 15 mg/day over 14 days i.e. 210 mg cumulative dosage often remains at sub therapeutic/suboptimal for persons weighing over 60 kgs and do not prevent relapses.³⁰ Indeed, this dose is known to be much less effective for radical cure of Chesson strain.³¹ Significance of high dose PQ in preventing relapses has been evidenced by a study²⁸ administering one week of PQ alone of 30 mg/day and 60 mg/day, wherein cumulative relapse proportion over 28 days was noted to be 7% with PQ 60 mg/day and 29% with 30 mg/day. Similarly, studies having treatment arms as: artesunate (600 mg) plus PQ 30 mg/day for 14 days³² and artesunate (600 mg) plus PQ 30 mg twice a day for 7 days²⁹ have proven the safe and comparable efficacy for preventing relapses up to ~100% till 28 days follow-up. However, this follow-up duration is too short to assess the true difference between the used regimens. Recently, in Indonesia anti-relapse efficacy up to 12 months of follow-up with 30 mg/day PQ dose for 14 days in combination with quinine (1.8 gm/day for 7 days) and 'alone' after 25 days of prior treatment with dihydroartemisinin (120 mg) plus piperazine (960 mg) daily for 3 days was found to be 92 and 98%, respectively.³³ As per a recent COCHRANE review, the relative risk of *P. vivax* parasitaemia detection more than 30 days after starting PQ is 10.05 (95% confidence interval, 2.82–35.86) for 5 days PQ regimen against 14 days PQ regimen.³⁴ So, it is apparent that higher dose PQ regimen is more effective in preventing relapse of *P. vivax* infection. Moreover, WHO recommends an essential use of high-dose PQ (0.5–0.75 mg base/kg body weight per day for 14 days), with any combination anti-malarial drug, among normal G6PD patients to prevent relapses of PQ resistant or tolerant *P. vivax*.³⁰ The evidence of good tolerability and safety of the higher PQ dosage with food rather than on an empty stomach dates back to the early 1990s.^{35,36} Notably PQ is known to have better anti-relapse efficacy, when administered in combination with other anti-malarial drugs. Serial administration of PQ following any other anti-malarials may result in relapse/PQ failure.³⁶ Conversely, a safe and synergistic antirelapse efficacy of PQ with blood was proven with

quinine and CQ combinations only.³⁷ The WHO's advice to combine PQ with any combination blood schizontocide needs a thorough reassessment. Simultaneous administration of some anti-malarials could result in dangerous interactions.³⁸ Moreover, some drugs (e.g. lumefantrine) inhibit cytochrome P450-2D6 (CYP2D6) and may disable PQ efficacy against hypnozoites,^{39,40} and pharma company (Novartis pharmaceuticals corporation – Coartem®) clearly warns against using lumefantrine with drugs that are metabolized to active forms via CYP2D6 viz., PQ. Thus, advice on combination of PQ with any other anti-malarial must be made evidence based.³⁸

G6PD deficiency (G6PDd) and primaquine: a therapeutic dilemma

The recognition of the association of G6PDd with PQ induced hemolysis dates back to the year 1956.⁴¹ Global burden of G6PDd has been estimated over 400×10^6 including ~8% of global population comprising $\sim 220 \times 10^6$ men.^{42,43} Unfortunately, global spread of G6PDd is highly skewed toward malaria endemic regions.^{30,42} Innately X-linked recessive nature of G6PDd gets further complicated by its enormous genotypes each expressing varied extent of deficiency and associated risks of hemolysis. Mediterranean G6PDd variants are known to pose profoundly greater risk of hemolysis than African G6PDd variants (A-).^{30,44,45} Moreover, the extent of PQ induced hemolysis is proportionate to the PQ dose/dosage and variations in G6PD phenotypes. In *P. vivax* cases with mild to moderate G6PDd, a 0.75 mg/kg body weight PQ weekly dose for eight weeks is recommended.³⁰ In Cambodia, even weekly dose of PQ (0.75 mg/kg for 8 weeks) in adults resulted in significant fall in hemoglobin among G6PDd individuals.⁴⁶ Notably, PQ radical treatment is contra-indicated in pregnancy, age < 4 years and severely deficient G6PD individuals.³⁰ Despite these contra-indications for PQ radical treatment, a short half-life and swift body clearance remains to be its providential feature. Remarkably, PQ-induced hemolysis wanes rapidly upon immediate discontinuation of PQ treatment. Hence, the constant vigilance and the prompt medical attention are the prime requisites for a successful PQ radical therapy in G6PDd individuals.

It is known that *P. vivax* can result in severe disease manifestations similar to *P. falciparum*.^{9–11} One of the worse probable outcomes of *P. vivax* malaria is severe anemia, particularly among infants,^{47,48} and inadequate treatment with frequent relapses may aggravate morbidity and rate of mortality.⁴⁹ Frequently relapsing *P. vivax* malaria has been implicated for occurrence of severe anemia among 20% of hospitalized individuals in Papua.¹¹

Many malaria endemic countries^{50–52} have not mandated a routine G6PD testing before initiating PQ radical cure in *P. vivax* malaria. This results in either no PQ prescription or thoughtless prescription and administration of PQ to

P. vivax patients by healthcare providers without being concerned about patients' G6PD status and associated complications. National drug policy on malaria in India⁵² emphasizes to instruct the *P. vivax* patients receiving PQ therapy to report back in case of adverse outcomes viz., hematuria/cyanosis. Nonetheless, neither *P. vivax* patients undergoing PQ treatment are actively supervised nor any PQ induced adverse events are recorded during routine surveillances. In the Brazilian Amazon, the extent of hospitalization and mortality in G6PDd *P. vivax* infected males after PQ treatment has been reported as 94.28% (19974/21185) and 2.70% (572/21185), respectively, and this imposes a hefty fiscal obligation on the public health sector;⁵¹ however, these are derived estimates and not a real finding. Surprisingly, from India and other south-east Asian regions, there is a dearth of precise data on the occurrences of PQ induced adverse events in G6PDd *P. vivax* population. A standard 14-day PQ dosage poses a definite risk of hemolysis/adverse events in a G6PDd patient with *P. vivax* infection⁵³ and hence justifies the mandatory routine screening of G6PDd. Currently, the spectrophotometric kinetic assay manually quantifying activity of erythrocytes' G6PD enzyme is the 'gold standard' method. The enzyme activity is determined by the measurement of increase in NADPH concentration, that strongly absorbs UV light (340 nm). However, this 'gold standard' method requires trained laboratory personnel, a laboratory setting, and at least 3–4 h time. These prerequisites limit the availability of this test in resource poor settings of almost all the malaria endemic countries and also limit the straightforward adoptability in even well-resourced settings. The NADPH fluorescence spot test (FST) based on Beutler's method^{54,55} is the WHO recommended current reference standard.⁵³ However, the availability and adoptability of the FST kits are limited owing to the requirement for a cold chain for the FST kit's reagents, UV lamp, high cost, trained personnel to efficiently perform the test, and mandatory quality control.⁵³ Thus, there persists a compelling need to develop and validate a rapid, easy-to-perform, easy-to-interpret, quality controllable, robust, and cost-effective G6PD assay.⁵³ More detailed insights into the G6PDd and PQ-induced hemolysis can be obtained from the WHO Evidence Review Group meeting reports^{43,53} and elsewhere.⁵⁰

Future of primaquine: how long is it likely to stand alone?

Primaquine [8-(4-amino-1-methylbutylamino)-6-methoxyquinoline] remains the only available treatment for the radical cure of *P. vivax*. Of course, there have been some promising results for a probable alternative candidate to PQ in the research and development pipeline. Tafenoquine (TQ), an 8-aminoquinoline, is the most promising future alternative to PQ.⁵⁶ Unlike PQ 14-day course of radical cure, TQ with a longer half-life of two to three weeks could

possibly result in radical cure with a single dose, eliminating the drawback of patient's noncompliance with longer duration radical treatment. But the availability of any such PQ alternative in routine clinical practice is roughly 5–7 years ahead from now. Like PQ, TQ also induces hemolysis in G6PD deficient persons. Considering these facts, it is unlikely that PQ will be totally and/or abruptly replaced across the globe in the near future. Despite possible availability of TQ for clinical use within next few years, PQ is likely to co-exist in practice.

Distinguishing *P. vivax* reappearances/recurrences

Precise differentiation and determination of relapse, recrudescence and reinfection of *P. vivax* remains elusive. Till date there does not exist a single exclusive method to differentiate reappearances in *P. vivax* precisely. Out of the existing methods, PCR sequencing involving multiple candidate genes having highly polymorphic regions seems more reliable and reproducible. Following the treatment of *P. vivax* infection with CQ–PQ, there could be three potential modes of reappearance of parasitaemia. Reappearance from the residual blood stage parasite due to chloroquine failure is called 'recrudescence,' while another type arising from the dormant liver stage due to PQ failure is known as 'relapse.' 'Reinfection' may also happen by new parasite from fresh mosquito inoculation and reappearance from unknown origin is called as 'recurrence.'³⁶ In a non-endemic region, recurrence of parasitaemia within 16 days of treatment is unlikely to be a relapse, whereas after that time, a relapse cannot be distinguished from a recrudescence.⁵⁷ But a similar consideration cannot be made for any endemic region due to possible reinfections. However, these time frames might be a function of transmission intensity, prevailing strain of parasites, climatic and seasonal variations. Most of the relapses do present with low patent parasitaemia. Microscopy cannot differentiate a relapse from reinfection. A case of reinfection during initial period may be present similarly with low parasitaemia as relapse or coincide with reinfection leading to false identification as 'relapse.' Having the assumption that a relapsing parasite descends from a previous infecting parasite brood, the distinction between relapse/recrudescence and reinfection can be made by genotyping *Pvcsp*, *Pvmsp1*, *Pvmsp3α*, and other genes. However, making a reliable distinction between relapse and recrudescence is cumbersome. Furthermore, immense genetic variations in relapses arise due to reactivation of either the previous parasite brood i.e. homologous, or genetically different brood i.e. heterologous, and due to infection by multiple *P. vivax* strains.^{14,58} Occurrence of these variations limits the reliability of present genotyping approach with *P. vivax*. The probability of finding multiple broods of relapse rises with increasing number of molecular markers. Genotyping microsatellites loci seem reliable for differentiating *P. vivax* relapse/recrudescence or reinfection.^{14,59,60}

Plausible determinants of *P. vivax* relapse

Per se 'resistance' to PQ in *P. vivax* has not been confirmed to date. Any precise determination of PQ resistance could be confounded by either subtherapeutic PQ dosage (due to poor compliance by patients, poor drug quality and incorrect medical prescription) or parasite or/and human host factors hindering penetration/attainment of optimal lethal drug concentration. Patients' poor compliance to the two-week long PQ standard regimen is most frequent and seemingly difficult to curb. Globally out of 58 countries with ongoing *P. vivax* transmission, 52 countries including India have implemented a strategy of treating *P. vivax* cases with PQ. Furthermore, on mere basis of national malaria control program reports, in the 'World Malaria Report, 2013' the WHO claims that 26 countries out of 52 have implemented directly observed therapy (DOT) with PQ into practice.⁸ However, there does not exist any literature to support this claim except a few trials^{61–63} assessing the efficacy of DOT method of PQ delivery for preventing the relapse. Further, as per the same WHO report,⁸ the information on the extent to which *P. vivax* patients get PQ treatment has been lacking. Notably, the proportion of *P. vivax* cases possibly treated with PQ varies extensively and associates with the prevalence of *P. vivax* cases in each nation. In the year 2012, only about 10% of all patients with *P. vivax* in the 24 nations reporting on PQ therapy could be possibly treated with the PQ doses distributed.⁸ Off late, Nacher *et al.*,⁶⁴ have brought to notice the non-prescription/availability of PQ in Camopi, an Amerindian village in the Amazon forest due to local logistical and legal litigations. They have reported that only 4.5% subjects of total 622 subjects had received PQ prescriptions.⁶⁴ It is clear, that among most settings very few patients requiring PQ treatment actually receive it and take it. The single greatest obstacle to PQ effectiveness is the rational fear of its prescription. Only G6PD screening will mitigate that fear.

Also, due to 14-day long course of PQ treatment regimen many patients fail to comply to complete the full dosage, and it results in inadequate blood drug concentration. The importance of inadequate dosage of PQ resulting in relapse is evident from studies comparing the outcomes of directly observed CQ–PQ therapy with unobserved CQ–PQ therapy.^{61,65} Over the 3 months follow-up period, the directly observed treatment group remained devoid of relapses whereas the unobserved treatment group had a 10.9% relapse rate.⁶¹ With longer follow-up duration up to 647 days, even supervised PQ treatment group had 36.6% relapses but it was substantially less than the unsupervised treatment group (71.5%).⁶⁵ *In vivo* baseline PQ drug concentration may vary from person to person and among different ethnicities. Also, the optimum PQ concentration to prevent the relapse varies across the globe depending on the prevailing parasite strain in respective regions. Other potential risk factors viz., baseline anti-malarial immunity, patients' body weight, and quality of PQ drug being used

might play crucial role in determining the PQ treatment outcome/*P. vivax* relapse.

The term ‘resistance’ in context with PQ remains subjective and needs validation. Like many other anti-biotics/anti-malarials, the development of resistance to PQ in *P. vivax* has been postulated to be under selection pressure due to erratic therapeutic practices.³⁶ PQ resistance in asexual blood stages has got negligible curative significance even though there is linkage for PQ resistance in blood and liver stages. Here, we are concerned solely with PQ resistance to the liver stages. The idea of PQ resistance in *P. vivax* arose with the inception of ‘Chesson strain’ during the World War II. Historically, Chesson strain has been considered the most resilient and difficult to contain by the PQ radical cure.³¹ There have been few successful efforts on devising methods of *P. vivax* propagation *in vitro*.^{66–71} However, lack of an unsophisticated *in vitro* culture and susceptibility testing methods for *P. vivax* renders therapeutic drug efficacy studies to be the sole source of evidence on the status of anti-malarial drug susceptibility. Therapeutic efficacy studies remain the gold standard for guiding drug policy and such studies should be undertaken periodically. However, such studies ascertain the treatment failures only, but the ‘resistance’ and the underlying mechanisms remain equivocal.

Determining a reference point sensitivity of *P. vivax* to PQ is required to define PQ resistance. The 15 mg base (or 0.25 mg/kg) recommended standard therapy of PQ among adults once daily for 14 days, could be an apparent standard. Tangible evaluation of PQ failure among all studies across the globe remains inconclusive. Multicentric studies involving adequately representative populations across the globe with varying but clinically suitable reference PQ dose must be carried out by simultaneous use of therapeutic, pharmacokinetic, and genetic tools to determine the true distribution of PQ failure.

Primaquine and CYP2D6 gene

Metabolism of 80–90% of all clinically used drugs takes place through cytochrome P450 dependent pathways. In spite of representing only 2–4% of hepatic CYP450 content, CYP2D6 metabolizes approximately 25% of all drugs in clinical use and is one of the most polymorphic CYP450 isoenzymes. Metabolism of PQ mediated through the cytochrome gene family has been postulated, but tangible evidence to this assumption has come only recently.^{72–74} PQ metabolism occurs in hepatocytes and the CYP2D6-dependent pathway serves as the prime elimination route. PQ phenolic metabolites able to undergo redox cycling via quinoneimine intermediate get generated primarily by the CYP2D6 mediated mechanism.⁷⁴ PQ is freely absorbed across gastrointestinal epithelia and apex plasma attainment takes about 1–2 h after administration. The elimination half-life is reported to be about 3–6 h.^{30,75} PQ gets extensively dispersed across body tissues. The major metabolite of PQ is carboxyprimaquine,

which accumulates in the plasma upon repeated administration. Since true resistance to PQ has not been reported till now, it may be postulated that host factors might have a crucial role in PQ failure.⁷³ CYP2D6 genotyping of *P. vivax* patients treated with CQ–PQ combined therapy have revealed three distinct metabolizer genotypes viz., poor, intermediate, and extensive. Both poor metabolizer (PM, two non-functional alleles) and intermediate metabolizer (IM, heterozygous with one null and another reduced function allele) show significantly decreased PQ plasma clearance than extensive metabolizer (EM, at least one allele coding for normal enzyme). Corroborating with metabolizer genotypes, poor and intermediate genotypes are associated with relapses, unlike the extensive metabolizer genotype.^{39,73} Nonetheless, these findings are based upon the relapses among merely three individuals and their CYP2D6 phenotype and hence severely lack in strength and generalizability. Similar studies including a statistically significant number of relapse cases and metabolizer phenotypes among various ethnicities are required to determine any precise and globally applicable conclusions in this regard. Notably, PM and IM individuals exhibit different pharmacokinetics than EMs. Hypnozoicidal metabolites of PQ are generated through the CYP2D6 metabolism pathway and polymorphism in the CYP2D6 gene is associated with reduced PQ metabolism and *P. vivax* relapse.

P. vivax strains prevailing across southeast Asia and the Pacific Islands are known to have developed PQ tolerance. This may be due to underlying genetic factors viz., prominently prevailing PM or IM genotypes. The PM phenotype is the commonest (8–10%) among Northern European Caucasians.⁷⁶ Regardless of the high occurrence of the PM phenotype, operational alleles characterize 71% of the polymorphisms in the population. Contrastingly, Eastern and south-eastern Asians and Pacific Islanders have a lower occurrence rate of the PM phenotype i.e. up to 1.2%. However, among them the occurrence of diminished operational alleles is more than 40%.⁷⁷ Overall, this results in a significantly diminished rate of CYP2D6-dependent metabolism among Asian EMs with respect to Caucasians rendering the same operational phenotype numbers.⁷⁷ The prevalence of poor metabolizer phenotypes of CYP2D6 gene across India ranges from 1.8 to 4.8%.⁷⁸ These implications of genetic polymorphisms and CYP2D6 phenotypes in PQ metabolism are tantalizing.

Conclusions

PQ failure and *P. vivax* relapse is a major global public health concerns. Optimum dissemination of PQ radical cure is lacking in a substantial proportion worldwide. It is imperative to ascertain the G6PD status in all cases of *P. vivax* malaria before administration of PQ radical treatment. Existing tools/methods to estimate G6PDd require improvements to overcome their limited availability and adoptability. Identification and characterization of different

relapse strains of *P. vivax* prevalent across the globe should be one of the priority areas in malaria research. Multicentric studies involving adequately representative populations with reference PQ dose must be carried out to determine the true distribution of PQ failure. Candidate molecular markers for the assessment of PQ efficacy should be verified and further developed into a screening tool for the determination of customized PQ dosage.

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Conflict of Interest

Authors declare that there is no conflict of interest.

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References

- 1 Arnott A, Barry AE, Reeder JC. Understanding the population genetics of *Plasmodium vivax* is essential for malaria control and elimination. *Malar J*. 2012;11:14.
- 2 World Health Organization. World malaria report. Geneva: World Health Organization; 2014.
- 3 Murray CJ, Ortblad KF, Guinovart C, Lim SS, Wolock TM, Roberts DA, et al. Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2014;384(9947):1005–70.
- 4 Guerra CA, Snow RW, Hay SI. Mapping the global extent of malaria in 2005. *Trends Parasitol*. 2006;22(8):353–8.
- 5 Hay SI, Guerra CA, Tatem AJ, Noor AM, Snow RW. The global distribution and population at risk of malaria: past, present, and future. *Lancet Infect Dis*. 2004;4(6):327–36.
- 6 Mendis K, Sina BJ, Marchesini P, Carter R. The neglected burden of *Plasmodium vivax* malaria. *Am J Trop Med Hyg*. 2001;64(1–2 Suppl):97–106.
- 7 Price RN, Tjitra E, Guerra CA, Yeung S, White NJ, Anstey NM. Vivax malaria: neglected and not benign. *Am J Trop Med Hyg*. 2007;77(6 Suppl):79–87.
- 8 World Health Organization. World malaria report. Geneva: World Health Organization; 2013.
- 9 Saravu K, Rishikesh K, Kamath A, Shastry AB. Severity in *Plasmodium vivax* malaria claiming global vigilance and exploration—a tertiary care centre-based cohort study. *Malar J*. 2014;13(1):304.
- 10 Saravu K, Rishikesh K, Kamath A. Determinants of mortality, intensive care requirement and prolonged hospitalization in malaria – a tertiary care hospital based cohort study from South-Western India. *Malar J*. 2014;13:370.
- 11 Tjitra E, Anstey NM, Sugiarto P, Warikar N, Kenangalem E, Karyana M, et al. Multidrug-resistant *Plasmodium vivax* associated with severe and fatal malaria: a prospective study in Papua, Indonesia. *PLoS Med*. 2008;5(6):e128.
- 12 White NJ. Determinants of relapse periodicity in *Plasmodium vivax* malaria. *Malar J*. 2011;10(1):297.
- 13 Rajgor D, Gogtay N, Kadam V, Knocharekar M, Parulekar M, Dalvi S, et al. Antirelapse Efficacy of Various Primaquine Regimens for *Plasmodium vivax*. *Malar Res Treat*. 2014;2014.
- 14 Kim J-R, Nandy A, Maji AK, Addy M, Dondorp AM, Day NP, et al. Genotyping of *Plasmodium vivax* reveals both short and long latency relapse patterns in Kolkata. *PLoS ONE*. 2012;7(7):e39645.
- 15 James S, Tate P. New knowledge of the life-cycle of malaria parasites. *Nature*. 1937;139:545.
- 16 Shortt HE, Garnham PC. Pre-erythrocytic stage in mammalian malaria parasites. *Nature*. 1948;161(4082):126.
- 17 Shortt HE, Garnham PCC. Persisting exo-erythrocytic cycle in *Plasmodium cynomolgi*. *BMJ*. 1948;1(4564):1225–8.
- 18 Shortt HE, Garnham PC, et al. Pre-erythrocytic stage of human malaria, *Plasmodium vivax*. *BMJ*. 1948;1(4550):547.
- 19 Lysenko AJ, Beljaev AE, Rybalka VM. Population studies of *Plasmodium vivax*. 1. The theory of polymorphism of sporozoites and epidemiological phenomena of tertian malaria. *Bull World Health Organ*. 1977;55(5):541–9.
- 20 Krotoski WA, Krotoski DM, Garnham PC, Bray RS, Killick-Kendrick R, Draper CC, et al. Relapses in primate malaria: discovery of two populations of exoerythrocytic stages. Preliminary note. *BMJ*. 1980;280(6208):153–4.
- 21 Krotoski WA, Collins WE, Bray RS, Garnham PC, Cogswell FB, Gwadz RW, et al. Demonstration of hypnozoites in sporozoite-transmitted *Plasmodium vivax* infection. *Am J Trop Med Hyg*. 1982;31(6):1291–3.
- 22 Schmidt LH. Compatibility of relapse patterns of *Plasmodium cynomolgi* infections in rhesus monkeys with continuous cyclical development and hypnozoite concepts of relapse. *Am J Trop Med Hyg*. 1986;35(6):1077–99.
- 23 Battle KE, Karhunen MS, Bhatt S, Gething PW, Howes RE, Golding N, et al. Geographical variation in *Plasmodium vivax* relapse. *Malaria Journal*. 2014;13:144.
- 24 Goo YK, Seo EJ, Choi YK, Shin HI, Sattabongkot J, Ji SY, et al. First characterization of *Plasmodium vivax* liver stage antigen (PvLSA) using synthetic peptides. *Parasit Vectors*. 2014;7:64.
- 25 White MT, Karl S, Battle KE, Hay SI, Mueller I, Ghani AC. Modelling the contribution of the hypnozoite reservoir to *Plasmodium vivax* transmission. *eLife*. 2014;3.
- 26 Alvarez G, Pineros JG, Tobon A, Rios A, Maestre A, Blair S, et al. Efficacy of three chloroquine-primaquine regimens for treatment of *Plasmodium vivax* malaria in Colombia. *Am J Trop Med Hyg*. 2006;75(4):605–9.
- 27 Durand S, Cabezas C, Lescano AG, Galvez M, Gutierrez S, Arrospe N, et al. Efficacy of three different regimens of primaquine for the prevention of relapses of *Plasmodium vivax* malaria in the Amazon Basin of Peru. *Am J Trop Med Hyg*. 2014;91(1):18–26.
- 28 Pukrittayakamee S, Imwong M, Chotivanich K, Singhasivanon P, Day NP, White NJ. A comparison of two short-course primaquine regimens for the treatment and radical cure of *Plasmodium vivax* malaria in Thailand. *Am J Trop Med Hyg*. 2010;82(4):542–7.
- 29 Krudsood S, Tangpukdee N, Wilairatana P, Phophak N, Baird JK, Brittenham GM, et al. High-dose primaquine regimens against relapse of *Plasmodium vivax* malaria. *Am J Trop Med Hyg*. 2008;78(5):736–40.
- 30 World Health Organization. Guidelines for the treatment of malaria. 2nd ed. Geneva: World Health Organization; 2010.
- 31 Alving AS, Johnson CF, Tarlov AR, Brewer GJ, Kellermeyer RW, Carson PE. Mitigation of the haemolytic effect of primaquine and enhancement of its action against exoerythrocytic forms of the Chesson strain of *Plasmodium vivax* by intermittent regimens of drug administration: a preliminary report. *Bull World Health Organ*. 1960;22(6):621–31.
- 32 Silachamroon U, Krudsood S, Treeprasertsuk S, Wilairatana P, Chalearmrult K, Mint HY, et al. Clinical trial of oral artesunate with or without high-dose primaquine for the treatment of vivax malaria in Thailand. *Am J Trop Med Hyg*. 2003;69(1):14–8.
- 33 Sutanto I, Tjahjono B, Basri H, Taylor WR, Putri FA, Meilia RA, et al. Randomized, open-label trial of primaquine against vivax malaria relapse in Indonesia. *Antimicrob Agents Chemother*. 2013;57(3):1128–35.
- 34 Galappaththy GN, Tharyan P, Kirubakaran R. Primaquine for preventing relapse in people with *Plasmodium vivax* malaria treated with chloroquine. *Cochrane Database Syst Rev*. 2013;10:CD004389.
- 35 Baird JK, Fryauff DJ, Basri H, Bangs MJ, Subianto B, Wiady I, et al. Primaquine for prophylaxis against malaria among nonimmune transmigrants in Irian Jaya, Indonesia. *Am J Trop Med Hyg*. 1995;52(6):479–84.
- 36 Baird JK. Resistance to therapies for infection by *Plasmodium vivax*. *Clin Microbiol Rev*. 2009;22(3):508–34.
- 37 Alving AS, Arnold J, Hockwald RS, Clayman CB, Dern RJ, Beutler E, et al. Potentiation of the curative action of primaquine in vivax malaria by quinine and chloroquine. *J Lab Clin Med*. 1955;46(2):301–6.

- 38 Baird JK. Resistance to chloroquine unhinges vivax malaria therapeutics. *Antimicrob Agents Chemother*. 2011;55(5):1827–30.
- 39 Ingram RJ, Crenna-Darusallam C, Soebianto S, Noviyanti R, Baird JK. The clinical and public health problem of relapse despite primaquine therapy: case review of repeated relapses of *Plasmodium vivax* acquired in Papua New Guinea. *Malar J*. 2014;13:488.
- 40 Ehrhardt S, Meyer CG. Artemether-lumefantrine in the treatment of uncomplicated *Plasmodium falciparum* malaria. *Ther Clin Risk Manag*. 2009;5:805–15.
- 41 Alving AS, Carson PE, Flanagan CL, Ickes CE. Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science*. 1956;124(3220):484–5.
- 42 Howes RE, Piel FB, Patil AP, Nyangiri OA, Gething PW, Dewi M, *et al*. G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: a geostatistical model-based map. *PLoS Med*. 2012;9(11):e1001339.
- 43 World Health Organization. Point-of-care G6PD testing to support safe use of primaquine for the treatment of vivax malaria: WHO Evidence Review Group meeting report. Geneva: WHO/UNAIDS Building; 2015.
- 44 Pannacciulli I, Tizianello A, Ajmar F, Salvadio E. The course of experimentally induced hemolytic anemia in a primaquine-sensitive caucasian. A case study. *Blood*. 1965;25:92–5.
- 45 Salvadio E, Pannacciulli I, Tizianello A, Ajmar F. Nature of hemolytic crises and the fate of G6PD deficient, drug-damaged erythrocytes in Sardinians. *N Engl J Med*. 1967;276(24):1339–44.
- 46 Kheng S, Muth S, Taylor WR, Tops N, Kosal K, Sothea K, *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.
- 47 Poespoprodjo JR, Fobia W, Kenangalem E, Lampah DA, Hasanuddin A, Warikar N, *et al*. Vivax malaria: a major cause of morbidity in early infancy. *Clin Infect Dis*. 2009;48(12):1704–12.
- 48 Douglas NM, Lampah DA, Kenangalem E, Simpson JA, Poespoprodjo JR, Sugiarto P, *et al*. Major burden of severe anemia from non-falciparum malaria species in Southern Papua: a hospital-based surveillance study. *PLoS Med*. 2013;10(12):e1001575; discussion e.
- 49 Baird JK. Evidence and implications of mortality associated with acute *Plasmodium vivax* malaria. *Clin Microbiol Rev*. 2013;26(1):36–57.
- 50 Baird K. Origins and implications of neglect of G6PD deficiency and primaquine toxicity in *Plasmodium vivax* malaria. *Pathog Glob Health*. 2015;109(3):93–106.
- 51 Peixoto HM, Brito MA, Romero GA, Monteiro WM, de Lacerda MV, de Oliveira MR. G6PD deficiency in male individuals infected by *Plasmodium vivax* malaria in the Brazilian Amazon: a cost study. *Malar J*. 2015;14(1):126.
- 52 Directorate General of Health Services. National drug policy on malaria. New Delhi: Directorate of National Vector Borne Disease Control Programme; 2013.
- 53 World Health Organization. WHO evidence review group: the safety and effectiveness of single dose primaquine as a *P. falciparum* gametocytocide. Bangkok: Pullman Hotel, 2012.
- 54 Beutler E. A series of new screening procedures for pyruvate kinase deficiency, glucose-6-phosphate dehydrogenase deficiency, and glutathione reductase deficiency. *Blood*. 1966;28(4):553–62.
- 55 Beutler E, Mitchell M. Special modifications of the fluorescent screening method for glucose-6-phosphate dehydrogenase deficiency. *Blood*. 1968;32(5):816–18.
- 56 Llanos-Cuentas A, Lacerda MV, Rueangweeraayut R, Krudsood S, Gupta SK, Kochar SK, *et al*. Tafenoquine plus chloroquine for the treatment and relapse prevention of *Plasmodium vivax* malaria (DETECTIVE): a multicentre, double-blind, randomised, phase 2b dose-selection study. *Lancet*. 2014;383(9922):1049–58.
- 57 Looareesuwan S, Buchachart K, Wilairatana P, Chalermrut K, Rattanapong Y, Amradee S, *et al*. Primaquine-tolerant vivax malaria in Thailand. *Ann Trop Med Parasitol*. 1997;91(8):939–43.
- 58 Imwong M, Snounou G, Pukrittayakamee S, Tanomsing N, Kim JR, Nandy A, *et al*. Relapses of *Plasmodium vivax* infection usually result from activation of heterologous hypnozoites. *J Infect Dis*. 2007;195(7):927–33.
- 59 Imwong M, Nair S, Pukrittayakamee S, Sudimack D, Williams JT, Mayxay M, *et al*. Contrasting genetic structure in *Plasmodium vivax* populations from Asia and South America. *Int J Parasitol*. 2007;37(8–9):1013–22.
- 60 Imwong M, Boel ME, Pagornrat W, Pimanpanarak M, McGready R, Day NP, *et al*. The first *Plasmodium vivax* relapses of life are usually genetically homologous. *J Infect Dis*. 2012;205(4):680–3.
- 61 Maneeboonyang W, Lawpoolsri S, Puangsa-Art S, Yimsamran S, Thanyavanich N, Wuthisen P, *et al*. Directly observed therapy with primaquine to reduce the recurrence rate of *Plasmodium vivax* infection along the Thai-Myanmar border. *Southeast Asian J Trop Med Public Health*. 2011;42(1):9–18.
- 62 Takeuchi R, Lawpoolsri S, Imwong M, Kobayashi J, Kaewkungwal J, Pukrittayakamee S, *et al*. Directly-observed therapy (DOT) for the radical 14-day primaquine treatment of *Plasmodium vivax* malaria on the Thai-Myanmar border. *Malar J*. 2010;9:308.
- 63 Leslie T, Rab MA, Ahmadzai H, Durrani N, Fayaz M, Kolaczinski J, *et al*. Compliance with 14-day primaquine therapy for radical cure of vivax malaria—a randomized placebo-controlled trial comparing unsupervised with supervised treatment. *Trans R Soc Trop Med Hyg*. 2004;98(3):168–73.
- 64 Nacher M, Stefani A, Basurko C, Lemonnier D, Djossou F, Demar M, *et al*. The burden of *Plasmodium vivax* relapses in an Amerindian village in French Guiana. *Malar J*. 2013;12(1):367.
- 65 Orjuela-Sanchez P, da Silva NS, da Silva-Nunes M, Ferreira MU. Recurrent parasitemias and population dynamics of *Plasmodium vivax* polymorphisms in rural Amazonia. *Am J Trop Med Hyg*. 2009;81(6):961–8.
- 66 Chotivanich K, Silamut K, Udomsangpetch R, Stepniewska KA, Pukrittayakamee S, Looareesuwan S, *et al*. *Ex-vivo* short-term culture and developmental assessment of *Plasmodium vivax*. *Trans R Soc Trop Med Hyg*. 2001;95(6):677–80.
- 67 Golenda CF, Li J, Rosenberg R. Continuous *in vitro* propagation of the malaria parasite *Plasmodium vivax*. *Proc Natl Acad Sci U.S.A.* 1997;94(13):6786–91.
- 68 Mons B, Collins WE, Skinner JC, van der Star W, Croon JJ, van der Kaay HJ. *Plasmodium vivax*: *in vitro* growth and reinvasion in red blood cells of *Aotus nancymai*. *Exp Parasitol*. 1988;66(2):183–8.
- 69 Udomsangpetch R, Somsri S, Panichakul T, Chotivanich K, Sirichaisinthop J, Yang Z, *et al*. Short-term *in vitro* culture of field isolates of *Plasmodium vivax* using umbilical cord blood. *Parasitol Int*. 2007;56(1):65–9.
- 70 Russell B, Suwanarusk R, Borlon C, Costa FT, Chu CS, Rijken MJ, *et al*. A reliable *ex vivo* invasion assay of human reticulocytes by *Plasmodium vivax*. *Blood*. 2011;118(13):e74–e81.
- 71 Borlon C, Russell B, Sriprawat K, Suwanarusk R, Erhart A, Renia L, *et al*. Cryopreserved *Plasmodium vivax* and cord blood reticulocytes can be used for invasion and short term culture. *Int J Parasitol*. 2012;42(2):155–60.
- 72 Pybus BS, Marcisin SR, Jin X, Deye G, Sousa JC, Li Q, *et al*. The metabolism of primaquine to its active metabolite is dependent on CYP2D6. *Malar J*. 2013;12(1):212.
- 73 Bennett JW, Pybus BS, Yadava A, Tosh D, Sousa JC, McCarthy WF, *et al*. Primaquine failure and cytochrome P-450 2D6 in *Plasmodium vivax* malaria. *N Engl J Med*. 2013;369(14):1381–2.
- 74 Pybus BS, Sousa JC, Jin X, Ferguson JA, Christian RE, Barnhart R, *et al*. CYP450 phenotyping and accurate mass identification of metabolites of the 8-aminoquinoline, anti-malarial drug primaquine. *Malar J*. 2012;11:259.
- 75 Mihaly G, Ward S, Edwards G, Orme M, Breckenridge A. Pharmacokinetics of primaquine in man: identification of the carboxylic acid derivative as a major plasma metabolite. *Br J Clin Pharmacol*. 1984;17(4):441–6.
- 76 Zhou S-F. Polymorphism of human cytochrome P450 2D6 and its clinical significance: Part I. *Clin Pharmacokinet*. 2009;48(11):689–723.
- 77 Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics*. 2002;3(2):229–43.
- 78 Gogtay NJ, Mali NB, Iyer K, Kadam PP, Sridharan K, Shrima D, *et al*. Evaluation of cytochrome P450 2D6 phenotyping in healthy adult Western Indians. *Indian J Pharmacol*. 2014;46(3):266–9.
- 79 Yeshiwondim AK, Tekle AH, Dengela DO, Yohannes AM, Teklehaimanot A. Therapeutic efficacy of chloroquine and chloroquine plus primaquine for the treatment of *Plasmodium vivax* in Ethiopia. *Acta Trop*. 2010;113(2):105–13.
- 80 Duarte EC, Pang LW, Ribeiro LC, Fontes CJ. Association of subtherapeutic dosages of a standard drug regimen with failures in preventing relapses of vivax malaria. *Am J Trop Med Hyg*. 2001;65(5):471–6.