

Increased Primaquine Total Dose in Patients with Multiple Plasmodium vivax Relapses Associated with Impaired CYP2D6 Activity: Report of Three Cases

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Case report

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Abstract

Background

The relapsing nature of *Plasmodium vivax* infection is a major barrier to its control and elimination. Factors such as adequate dosing, adherence, drug quality, and pharmacogenetics can impact the effectiveness of radical cure of *P. vivax* and need to be properly evaluated. CYP2D6 pathway mediates the activation of primaquine (PQ) into an active metabolite(s) in hepatocytes, and impaired activity has been linked to higher risk of relapse.

Cases presentation

Three patients diagnosed with *P. vivax* malaria presented repeated relapses after being initially treated with chloroquine (25mg/kg) and primaquine (3.5mg/kg in 14 days) at a non-endemic travel clinic. Recurring episodes were subsequently treated with higher dose of primaquine (7mg/kg in 14 days), which prevented further relapses in two patients. However, one patient still presented 2 episodes after higher primaquine dose and was prescribed 300mg of chloroquine weekly to prevent further episodes. Impaired CYP2D6 function was observed in all of them.

Conclusion

Lack of response to PQ was associated with impaired CYP2D6 activity in three patients presenting multiple relapses followed in a non-endemic setting. Higher PQ dosage was a safe and effectively prevented relapses in two patients and should be further investigated as an option in Latin America. It is important to investigate the factors associated with unsuccessful radical cure and alternative therapeutic options.

Background

Plasmodium vivax is the most geographically widespread species causing human malaria, with approximately 40% of the world's population at risk of infection¹⁻³. There was near 157,000 new cases in Brazil in 2019, most due *P. vivax* (89.1%), of which around 21% being classified as recurrences within 60 days⁴. Relapses, that accounted for around 33.000 episodes that year, and earlier gametocyte production make this species especially challenging for treatment and control, but the mechanisms leading to activating hypnozoites remain unknown⁵.

Radical cure of vivax malaria requires antimalarial drugs that target both blood and liver stages. Primaquine (PQ) is the most available drug to eliminate hypnozoites^{6,7}. PQ's clinical effectiveness is limited by the toxicity and potential hemolytic adverse events in patients with glucose-6 phosphate deficiency (G6PD) that is why, the drug is contraindicated during pregnancy and for infants less than six months.

There is no definitive method to differentiate recurrences of *P. vivax* as recrudescence, especially with increasing evidence of resistance to chloroquine (CQ)⁸, relapses, and reinfection in areas with active transmission. The possibility of following patients in non-endemic areas provides an advantage where at least reinfection could be excluded. Recent studies in Brazil showed that recurrences rates in the Amazon setting range from 29.4% to 39.6% in Amazon and non-Amazon areas⁹⁻¹¹, despite PQ's routine prescription. It was recently described that cytochrome P450 2D6 (CYP2D6) pathway mediates the activation of primaquine into active phenolic metabolite(s) in hepatocytes^{12,13} and some genetic polymorphisms implied in reduced PQ metabolism have been associated with higher risk of relapse¹⁴. Individuals with specific CYP2D6 polymorphic alleles fail to metabolize PQ and may experience treatment failure, leading to false assumptions of PQ efficacy and tolerance.

CYP2D6 gene is highly polymorphic with over 150 alleles categorized in no, decreased, normal, and increased function alleles based on enzyme activity¹⁵. The CYP2D6 allele combinations give rise to different predicted metabolizer phenotypes: poor (gPM), intermediate (gIM), normal (gNM), and ultrarapid (gUM) metabolizers¹⁵. Therefore, vivax malaria patients with the defective CYP2D6 function would be at increased risk for therapeutic failure (relapses) regardless of proper treatment regimens with PQ^{14,16-18}. Thereat, to identify patients at a higher risk for recurrences CYP2D6 metabolizer status provides valuable information in improving the interpretation of treatment failure in *P. vivax* and strengthening the efforts to control this parasite. Herein, we describe three cases of multiple vivax malaria relapses in individuals with impaired CYP2D6 metabolic activity followed-up at a non-endemic area in Brazil. The individual responses to different drug schemes varied related to CYP2D6 metabolic status and showed to be complex.

Site and standard procedures:

The *Instituto Nacional de Infectologia Evandro Chagas* (INI) is a reference center for diagnosing and treating infectious diseases at Fundação Oswaldo Cruz (Fiocruz), in Rio de Janeiro, Brazil. Patients with suspicion of malaria are evaluated by infectious disease physicians and follow the national malaria treatment guidelines. The guidelines state that vivax malaria should be treated with CQ (25mg/kg during three days) and PQ (3.5mg/kg during seven or 14 days). Blood slides were collected by experienced microscopists and malaria species confirmed by polymerase chain reaction (PCR)^{19,20}. Patients were followed until parasitological clearance and routinely at days 3, 7, 14, 21, 28, 40, and 60 post-treatment and at any time in case of recurring fever. All patients were tested for G6PDd. PQ was adjusted for body weight (bw) when necessary. None of the patients returned to the endemic area.

CYP2D6 genotyping

Genotyping of one tri-nucleotide deletion (2615-2617delAAG [rs5030656]), eight single-nucleotide polymorphisms (SNPs) (-1584C>G [rs1080985], 100C>T [rs1065852], 1023C>T [rs28371706], 1846G>A [rs3892097], 2850C>T [rs16947], 2988G>A [rs28371725], 3183G>A [rs59421388], 4180G>C [rs1135840]) and *CYP2D6* copy number analysis were performed by Real-Time PCR, according to protocols previously

described^{16,22}. CYP2D6 haplotypes were inferred from genotypes using the software PHASE v.2.1^{23,24} and phenotypes were predicted based on activity score (AS) model¹⁵. Patients were categorized into five predicted phenotype classes: poor metabolizer (gPM; AS score = 0), intermediate metabolizer (gIM; AS score = 0.5), normal-slow metabolizer (gNM-S; AS score = 1), normal-fast metabolizer (gNM-F; AS score 1.5-2.0), and ultrarapid metabolizer (gUM ; AS score > 2).

Ethical approval was obtained from the INI-Fiocruz Ethical Board (number 0020.0.009.000-07), and all participants provided informed written consent.

Case Presentation

Herewith we describe three cases of *P. vivax* malaria infection which presented multiple recurrences. All patients remained in the non-transmission area throughout the follow-up. Due to the lack of tools for differentiating relapses from recrudescence, we applied the proposed criteria of classifying recurrences as recrudescence if happening with less than 28 days post-treatment and as relapses if occurring after this period. There was no risk of reinfection for the cases.

Case 1: Male, 32 years-old (yo), 78.5 kg of body weight (bw), resided in São Gabriel da Cachoeira (Amazon state - AM) for two years (until 18 Dec 2015) where he had a diagnosis of vivax malaria on 1st Nov 2015 being treated with chloroquine (CQ) and primaquine (PQ) for seven days (Figure 1). On 25 Jan 2016, 38 days after having moved to Rio de Janeiro city (and 85 days after the initial diagnosis), he sought care at INI and was diagnosed with *P. vivax* infection (18.320 parasites/mm³). G6PD activity was tested normal. He was treated with CQ and PQ (total dose of PQ: 3.44 mg base/kg given during nine days). On 16 Apr 2016 (81-days interval), he presented another malaria episode diagnosed as *P. vivax* (6.000 parasites/mm³). He was then treated with CQ and higher-dose PQ (total dose of PQ: 7.03 mg base/kg bw given in 22 days). *CYP2D6* genotype was performed and classified as intermediate metabolizer (Table 1). He was followed-up for more than one year and has not presented new episodes until 20 Jun 2017.

Case 2: Female, 33 yo, 62.3 kg of bw, resided in São Gabriel da Cachoeira/AM until 14 Nov 2015 where she was diagnosed and treated for vivax malaria on 1st Oct 2015 (Figure 1). In Rio de Janeiro, she was diagnosed with *P. vivax* on 18 Feb 2016 (12.480 parasites/mm³) and was treated with CQ and PQ (total dose of PQ: 3.37 mg base/ kg bw given in seven days). G6PD activity was normal. On 5 Apr 2016, she presented to INI with a vivax relapse (46-days interval). She received CQ and PQ (total dose of PQ: 7.02 mg base/ kg bw given in seven days) and she remained without further episodes (updated 20 Jun 2017). Her *CYP2D6* was classified as poor metabolizer (Table 1).

Case 3: Male, 56 yo, 82 kg of bw, resided in Machadinho do Oeste/Rondônia State (Amazon region) for two months until 4 Aug 2016 where he received treatment for vivax malaria on 1st Jul 2016 (Figure 1). After returning to Rio de Janeiro, he presented four episodes of malaria, with roughly similar intervals. For these episodes, the respective treatments were administered: CQ + PQ (total dose: 3.21mg/kg); CQ + PQ

(total dose: 3.21mg/kg); CQ + PQ (total dose: 7 mg/kg); Artemether-Lumefantrine (AL) + PQ (total dose: 7 mg/kg) – AL was administered due to CQ-induced pruritus. *CYP2D6* genotype was classified as normal-slow metabolizer (Table 1). On 8 May 2017, after discussion with the patient a decision to perform CQ prophylaxis (300mg per week for eight weeks) was taken. The patient is still under follow-up (update 20 Jun 2018) and has not had new episodes.

Discussion And Conclusions

We report three individuals who presented a varied number of *P. vivax* relapses for which an impaired CYP2D6 activity was observed, suggesting that those abnormalities are implicated in the risk of *P. vivax* malaria recurrence after treatment with chloroquine/primaquine. Our data corroborate with a developing body of knowledge that supports host genetics as a cause for PQ drug failure in individuals who experience *P. vivax* relapse. Whether routine screening of CYP2D6 alleles in patients who experience *P. vivax* malaria relapse in endemic settings is feasible and cost-effective is a matter that should be investigated. Next, more robust evidence is needed to identify the alternative treatment regimens in CYP2D6 impaired patients. To address those issues, we are planning the largest, multicenter Brazilian cohort study to elucidate the relationship between CYP2D6 activity, geographic regional dosing requirements, and clinical failure of primaquine for radical cure of vivax malaria.

P. vivax is the most geographically widespread species causing human malaria. its resilience to control and elimination efforts mainly result from its complex biology. The origin of a recurring parasitemia following a primary infection by *P. vivax* can be a result of (i) recrudescence due to resistance to the blood schizonticidal drug – usually chloroquine; (ii) relapse from activated hypnozoites – which is a particularity of *P. vivax* and *P. ovale* amongst human malaria; or (iii) reinfection in areas where active transmission exist⁷. Relapses can be responsible for up to 80% of the malaria burden in given settings²⁵, with evidence suggesting that its relative contribution increases in declining transmission intensity⁹. The factors that trigger the hypnozoite activation are not completely understood and, strain-specific patterns, environmental factors, and host characteristics have been implicated as potential contributors. For the last six decades, PQ, an 8-aminoquinoline derivative, has been the only drug with anti-relapse activity available. Its use was restricted due to the hemolytic potential in individuals with G6PD²⁷. Although the exact mechanism throughout PQ exerts its anti-relapse activity is not known, the recent finding the slow metabolizers have higher rates of relapses has added stronger evidence that a still unknown metabolite is responsible for its effect^{14,16}.

There are no molecular methods to reliably distinguish amongst the causes of recurrence for *P. vivax* as there are for *P. falciparum*. Standardized molecular methods allow differentiation between recrudescence and reinfection²⁸. In our study relapses occurred in a non-endemic area and all subjects did not travel to any *P. vivax* endemic region after the initial episodes, thereby reducing the possibility that confounding variables were responsible for the observed relapse infection. Recrudescence due to erythrocytic parasites was not probable since the parasitemia decreased in the blood and therapeutic failure in the presence of

drug was not reported. The minimum interval between episodes was 52 days (median = 91, maximum = 136), which supports the classification of these recurrences as relapses, since recrudescence due to erythrocytic stage parasites usually occur within 28 days after treatment with CQ²⁷.

Patients were oriented about the importance of treatment and of reporting adverse events during the follow-up period as well as returning to the clinic in case of symptoms. None of them returned to an endemic area and presented new symptoms after the malaria therapeutic period. The three subjects were tested negative for G6PDd, and the woman was not pregnant or breastfeeding. Consequently, the patients were eligible to receive primaquine, discarding contraindication to PQ use. The results reported here corroborate partially with Deepika et al. study²⁹, indicating the use of higher total doses of primaquine to prevent relapses. Drugs weight-adjusted during the treatment are essential and all patients were treated with high dose of primaquine. Two of them did not present relapses anymore. However, for one of the cases (case #3) a decision to institute weekly chloroquine prophylaxis was taken because relapses occurred even with primaquine in high doses. Of importance, none of the subjects had comorbidities or were using any non-antimalarial medication. Therefore, it is unlikely that host factors such as drug-drug and drug-CYP2D6 interactions influenced the pharmacokinetics and metabolism of PQ by CYP2D6.

Our study has limitations. First, individual CYP2D6 phenotype was inferred from genotyping data, according to activity scores of CYP2D6 diplotypes and there is evidence for considerable range of variation in CYP2D6 function within genotype-inferred phenotype categories. Second, although true primaquine resistance could not be rule out, but considering the early parasitological cure observed after the combined CQ/PQ treatment, the most likely explanation is that the cause of the successive recurrences was due to PQ failure and not PQ resistance. Third, PQ administration was not supervised and the possibility of non-adherence may not be excluded. Nevertheless, all the patients reinforced that the full dosage of PQ has been completed in all episodes, and all attended the follow-up appointments.

This case-series, along with previous studies, points out that CYP2D6 as a possible important determinant of efficacy of primaquine against relapse. A relevant issue for clinical management and consequently control and elimination is how to achieve better radical cure and to classify and treat recurring episodes. Considering the burden of relapses and its public health implications for the elimination of vivax malaria in Latin America, we are conducting a 1-year cohort, multicenter, therapeutic efficacy study of CQ and PQ in distinct malaria transmission intensity locations in Brazil to estimate the frequency, timing, and associated risk factors for the developing of recurrences (ABRACAMAL project, Gates foundation grant INV-003970). Thus, we aim, to provide a comprehensive framework for estimate the radical curative failure rate and thereby contribute to an improved understanding of the biology, epidemiology, and treatment of *P. vivax* malaria that may lead to more effective management policies.

Abbreviations

AL - artemether-lumefantrine

AS - activity score

bw - body weight

CQ – chloroquine

CYP2D6- cytochrome P450 2D6

Fiocruz - Fundação Oswaldo Cruz

G6PDd - glucose-6 phosphate deficiency

gIM - intermediate metabolizer

gNM - normal metabolizer

gPM – poor metabolizer

gUM – ultrarapid metabolizer

INI - Instituto Nacional de Infectologia Evandro Chagas

PCR - polymerase chain reaction

PQ – primaquine

SNPs - single-nucleotide polymorphisms

yo - years-old

Declarations

COMPETING INTERESTS

The authors declare that they have no competing interests related to this study.

AUTHORS' CONTRIBUTIONS

The authors confirm contribution to the paper as follows: study conception and design: APC, CTDR, PB, TNS and AMS; data collection: APC, ACRS, EMS, RSP, JM, GLU, ADTS, OHLRS, KMDH and AMS; analysis and interpretation of results: APC, ACRS, EMS, CTDR, ; draft manuscript preparation: APC, RSP, JM, CTDR, PB, TNS and AMS. All authors reviewed the results and approved the final version of the manuscript.

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Tables

Table 1 not available with this version

Figures



Figure 1

Dates and treatment regimens prescribed for the three cases.