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RESEARCH ARTICLE

Polymorphism in the *pvcrt-o* and *pvmdr-1* genes of *Plasmodium vivax* associated with a better prognosis for malaria

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ABSTRACT

The early molecular identification of strains of *Plasmodium vivax* that have a worse prognosis is important to stratify the risk of complications and choice of conduct made by medical teams. Thus, the aim of the present study was to associate the presence of polymorphisms in the *pvmdr-1* and *pvcrt-o* resistance genes of *P. vivax* in patients with better or worse prognosis. This cross-sectional epidemiological study was conducted based on data obtained from the records of 120 patients diagnosed with malaria in the Brazilian Amazon. The T958M and F1076L mutations of the *pvmdr-1* gene had a frequency of 3.3 and 4.2%, respectively, and primo-infected patients had a 17 times greater chance of being infected with protozoa with the T958M mutation compared to patients with previous episodes. Regarding *pvcrt-o*, the C393T and T786C polymorphisms had a frequency of 14.2 and 3.3%, respectively, and self-declared white patients had a 3.1 times greater chance of being infected with protozoa with the C393T polymorphism. In addition, patients with this *pvcrt-o* polymorphism had lower concentrations of C-reactive protein, indicating a better prognosis. These data present clues of genetic indicators useful for assessing the virulence of the parasite and the prognosis of patients with vivax malaria.

Keywords: Vivax malaria; genes; clinical evolution; drug resistance.

INTRODUCTION

Plasmodium vivax infection is responsible for 89% of malaria cases in the Brazilian Amazon. Correspondingly, outside Africa, *P. vivax* is the main species that causes malaria (World Health Organization, 2018). Chloroquine and primaquine have been used since 1952 as the first-line treatments for vivax malaria worldwide. Resistance to malaria drugs is a major obstacle in disease control strategies (World Health Organization, 2015). The failure of chloroquine treatment in *P. vivax* has increased in some endemic countries. Resistance was first reported in Papua New Guinea in 1989 (Rieckmann *et al*., 1989) and was later reported in several other countries (Price *et al*., 2014; Rahimi *et al*., 2014; Garrido-Cardenas *et al*., 2019).

In Brazil, the first reported case of chloroquine resistance was reported in the Brazilian Amazon in 1999 (Alecrim *et al*., 1999). In 2014, 5.2% resistance to conventional treatment for vivax malaria was reported. In parallel with the emergence of chloroquine resistance in the Brazilian Amazon, reports of clinical severity, exclusively associated with *P. vivax* infection, have gradually increased (Alecrim *et al*., 1999; Marques *et al*., 2014; Garrido-Cardenas *et al*., 2019; Kotepui *et al*., 2020).

Plasmodium vivax infections have been associated with mild symptoms, such as fever, headache, fatigue, and chills. However, serious complications, including renal failure, jaundice, acute respiratory distress syndrome, cerebral malaria, seizures, anemia, thrombocytopenia, bleeding, pulmonary edema, splenic rupture, and death, have been reported to be exclusively associated with *P. vivax* species (Alecrim *et al*., 1999; Marques *et al*., 2014; Garrido-Cardenas *et al*., 2019; Kotepui *et al*., 2020). Recent research has shown that clinical severity may be associated with increased levels of expression of two parasitic genes likely involved in chloroquine resistance, *pvmdr-1* and *pvcrt-o* (Fernɔndez-Becerra *et al*., 2009; Melo *et al*., 2014; Anantabotla *et al*., 2019; Sɔ *et al*., 2019).

The presence of serious *P. vivax* infection is becoming more widespread, making it difficult to manage clinical cases, posing a huge threat to the health of millions of people that are at risk. The analysis of SNPs in drug-resistant genes was proven to be useful and important in monitoring the pathogenesis and resistance of *P.*

vivax in countries with endemic malaria (Suwanarusk *et al*., 2007; Hawkins *et al*., 2018; Garrido-Cardenas *et al*., 2019).

Genetic markers of *P. vivax* that demonstrate "host" susceptibility to infection and pathogenesis of this parasite can contribute to determining the prognosis of the disease. The early molecular identification of *P. vivax* strains that develop severe malaria is important to stratify the risk of mortality and choice of conduct made by medical teams. Thus, the objective of the present study was to associate the polymorphisms of *pvmdr-1* and *pvcrt-o* with the better or worse prognosis of vivax malaria, which can indicate the pathogenicity status of these parasites.

MATERIALS AND METHODS

Study design

This was a cross-sectional epidemiological study based on data obtained from records of patients diagnosed with malaria at the Malaria Diagnosis and Treatment Reference Center, Hospital Universitário Júlio Muller, in the city of Cuiabá, State of Mato Grosso, Brazil.

Location, population, and samples

We retrospectively included 120 patients with *P. vivax* infection from the Brazilian Amazon who sought treatment at the Reference Center, where the study was conducted from 2011 to 2017. Epidemiological, clinical, and laboratory information were obtained from patient records. There were no autochthonous cases of malaria in the study city, which allows us to differentiate between primary infection, reinfection or relapse. Patients with the first infection were classified as prime infected, patients who were in an endemic area and returned with malaria were classified as reinfection and patients who, after a diagnosis of malaria in the study city, did not return to the endemic area and presented a new infection were classified as relapsed. In this study, there was no case of return of the parasitaemia within the follow-up period (up to 30 days). Therefore, no patient can be classified as having recrudescence.

All patients who sought care were in the acute phase of the disease and had classic signs and symptoms of malaria. Those were treated with chloroquine and primaquine, with concentrations defined in accordance with WHO recommendations, for seven days and followed up at 7, 15, 21 and 30 days after the start of treatment with slides for verification of cure (World Health Organization, 2018). No patient presented severe malaria, according to the WHO criteria, and all had a diagnosis made at the time of microscopy examination, as described by Alves-Junior *et al*. (Alves-Junior *et al*., 2014a, 2014b). Simultaneously, blood samples were collected for laboratory and molecular tests to confirm the species, as described by Snounou *et al*. (1993). These blood samples are currently deposited and preserved in the malaria laboratory for possible future confirmation of the results, and aliquots were used for the molecular analysis in this study to identify polymorphisms.

Patients with comorbidities, mixed malaria, or in use of antibiotics in the last seven days prior to the care, or already undergoing treatment at the time of care, were excluded from this study.

Laboratory classification of the prognosis of vivax malaria

Currently, the laboratory parameter C-reactive protein (CRP) is the best marker for classification of the severity of malaria caused by *P. vivax*, as it demonstrates an inflammatory state from the disease that may be more or less intense (Paul *et al*., 2012; Vemula *et al*., 2016). Even in a recent study, as described by Alves-Junior (2020), among 42 parameters, CRP was the most sensitive to demonstrate the worst or best prognosis of a patient with vivax malaria. The immunoturbidimetric method was used for quantitative determination of C-Reactive Protein (CRP).

Identification of polymorphism at *pvmdr-1* and *pvcrt-o* genes of *P. vivax*

From the extracted DNA (Wizard DNA Genomic Kit, Promega®, Madison, WI, USA), the polymerase chain reaction (PCR) for the *pvmdr-1* and *pvcrt-o* genes was performed with a final volume of 25 µL, according to the method described by Garg et al., modified (Garg *et al*., 2012). These modifications were as follow: 20 µL of 1x PCR Master Mix (Promega®), 0.4 µM of each primer, and 200 ng of DNA. Amplification was performed using a Veriti thermal cycler (Applied Biosystems®, CA, USA) according to the following description: to amplify the region of interest of the gene *pvmdr-1* were used the primers: forward GGATAGTCATGCCCCAGGATTG and reverse CATCAACTTCCCGGCGTAGC. 35 cycles were used with anneling temperature at 55° Celsius, producing a 603 base pair DNA product. For the gene *pvcrt-o* were used the following primers: forward: GCGGAGCTCATGACCATCCTGAAAAAGAAGAAG e reverse: GCGGTCGACGGGGAAATGTGCACTTGAAATAT during 35 cycles with anneling temperature at 62° Celsius producing a 1193 base pair DNA product. The amplification and sequencing of a 603 base pair fragments of the *pvmdr-1* gene, between the nucleotide positions 2751 to 3354 (Brega *et al*., 2005), was performed to identify the presence of SNPs previously associated with resistance to chloroquine. The resistance results in the exchange of amino acids Y976F and F1076L, as well as the identification of T958M, which is very common in *P. vivax* (Sá *et al*., 2005; Suwanarusk *et al*., 2007; Vargas-Rodríguez *et al*., 2012). The 1193 base pair pvcrt-o fragment, corresponding to the initial 1 kb of the gene (Garg *et al*., 2012), was performed to identify SNPs from the region of a common mutation in the *pvcrt-o* called K10, which is an insertion of a lysine in the tenth codon of exon 1(Lu *et al*., 2011; Sá *et al*., 2019) and is also associated with resistance.

Sequencing

The quality and concentration of the PCR products from *pvmdr-1* and *pvcrt-o* genes were verified by electrophoresis on a 1.5% agarose gel in a source and vat PowerPac Basic Power Supply (Biorad®, CA, USA), using the photo documenter Gel Doc ™ XR (Biorad®). Purification of the amplified material was performed using the Illustra ExoProStar S kit (GE Healthcare Life Sciences®, Chicago, IL, USA) and used as a template for Sanger capillary sequencing in an ABI 3500 sequencer (Life Technologies®, CA, USA).

The sequences were analyzed using the CLC Workbench v5.5 software (Qiagen, Hilden, Germany) and were compared with the standard sequence of *pvmdr-1* (GenBank Acc. No. AY618622), and *pvcrt-o* (GenBank Acc. No. AF314649) to identify the polymorphisms.

Statistical analysis

Statistical analysis was performed using Excel 2019 (Microsoft Office Home and Student, WA, USA) and STATA Analysis and Statistical v12 (StataCorp LLC, TX, USA), considering a significance level (α) of 5% (0.05).

Based on the Shapiro-Wilk test, the data did not follow a normal curve and, correspondingly, the statistic used was non-parametric. Descriptive analysis of variables was performed as measures of central tendency (median), proportion, and measures of dispersion (interquartile range).

To compare the CRP levels between the presence or absence of polymorphisms, the Wilcoxon signed-rank test was used (Mann-Whitney U test).

Odds ratio (OR) calculations, with the respective 95% confidence intervals (95% CI), were obtained to estimate the associated risk. Similarly, infection classification variables, anthropometric variables and patient characteristics were compared in the presence of polymorphisms.

Ethical considerations

This study obeys the ethical precepts of resolution 466/12 of the National Health Council of Brazil. The collection of information only started after approval by the Research Ethics Committee, registered at the *Plataforma Brasil* of the Ministry of Health, under agreement No. 1.001.158/UFMT. Participants were instructed on the objectives of the study and signed a free and informed consent form. All participating patients were treated at the Hospital's Infectious Diseases Clinic and were guaranteed antimalarial treatments and adequate health care in accordance with the WHO recommendations (World Health Organization, 2015).

RESULTS

Patient characterization

There was a predominance of male patients (82%). Regarding skin color, patients declared themselves white (35.3%), black (12.6%), and *pardo* (52.1%). The term "*pardo*" is a complex term, more commonly used to refer to Brazilians of mixed ethnic ancestries. *Pardo* Brazilians represent a diverse range of skin colors and ethnic backgrounds. They are typically a mixture of Europeans, Sub-Saharan Africans, and/or native Brazilian (Mattos, 2006). The mean age (standard deviation) of patients was 40 (±14) years, working in high risk professions such as miner prospectors and truck drivers, all of whom came from Brazil's Legal Amazon, with a predominance of patients from the state of Pará (54%) and Rondɔnia (31%), followed by Northern Mato Grosso (12%) and Amazonas state (3%). Only 23% of the patients were prime-infected, and the remaining participants of the study reported at the time of diagnosis that they had at least one other previous episode of malaria in their lifetime (Table 1).

Frequency of *pvmdr-1* and *pvcrt-o* polymorphisms

For the *pvmdr-1* gene from 120 patients investigated, nine patients (7.5%) were identified with a polymorphism each, with four (3.3%) at position 2873, with a replacement of a thymine by a cytosine (T2873C). This replacement corresponds to the change in the codon at position 958 from methionine to threonine (T958M). And five (4.2%) were identified with polymorphisms at position 3226, with a replacement of thymine by cytosine (T3226C), corresponding to a change in the codon at position 1076 from phenylalanine to leucine (F1076L) (Table 2).

No more than one polymorphism per patient was found simultaneously in the amplified region of *pvmdr-1*. The pvmdr-1 Y976 mutation was not found in the present study.

From the *pvcrt-o* gene of 120 investigated patients, polymorphisms were identified in 21 patients (17.5%), 17 (14.2%) in position 393, with a replacement of cytosine by thymine (C393T), and four (3.3%) polymorphisms at position 786, with substitution of a thymine for cytosine (T786C) (Table 2). The C393T and T786C polymorphisms are located in introns and, therefore, in a non-coding region. In addition, no more than one polymorphism was found simultaneously within the amplified region of *pvcrt-o*.

The insertion mutation of a lysine at the 10th position of the amino acid sequence (K10) was not found in the present study.

***pvmdr-1* and *pvcrt-o* polymorphisms and anthropometric characteristics**

Anthropometric and laboratory characteristics were compared with the presence of polymorphisms in *pvmdr-1* and *pvcrt-o* to investigate the relationship between mutations and the probability of infection.

The patient characteristics included were sex, age, skin color, occupation and classification of malaria. There was association between the presence of the C393T polymorphism in the *pvcrt-o* gene and skin color. Positive cases for this polymorphism in patients declared as white was 3.1 times (OR: 3.1; p 0.028) higher than the proportion of patients declared as black or *pardo*. In contrast, the proportion of this polymorphism in *pardo* patients was lower (OR: 0.3; p = 0.043) (Table 3). There was no association between polymorphisms and other socio-demographic or anthropometric parameters analyzed.

Table 1. Absolute and relative frequency of characteristics of 120 patients with *P. vivax* malaria from the Brazilian Amazon

Characteristics		(%)
Sex	Male	82
	Female	18
Age (years)	0–5	03
	6–11	01
	12–17	02
	18–39	44
	≥ 40	50
State	Pará	54
	Rondônia	31
	Matogrosso	12
	Amazonas	03
Profession	Miner prospectors	22
	Truck drivers	17
	Others (46 professions)	61
Previous episode of malaria	0	23
	1–2	34
	3–4	12
	≥ 5	31
Parasite density (/µL)	< 5,000	57
	5,000–10,000	18
	10,000–50,000	23
	> 50,000	2

Table 2. Frequency of polymorphisms for *pvmdr-1* and *pvcrt-o* genes from 120 patients from the Brazilian Amazon region suffering *P. vivax* malaria

Gene	Polymorphisms / Mutations	n	Frequency %
pvmdr-1	T2873C / T958M	4	3,3
	T3226C / F1076L	5	4,2
pvcrt-o	C393T / [a]	17	14,2
	T786C / [a]	4	3,3

[a] This polymorphism does not comprise a coding region, so there is no amino acid swap.

Table 3. Comparison by Odds Ratio of the skin color characteristics between the positive and negative presence of the *pvcrt-o*/C393T polymorphism from 120 patients with *P. vivax* malaria from the Brazilian Amazon

Skin color	*pvcrt-o* (n)		Odds Ratio (95% CI)	*p* [a]
	Positive C393T	Negative C393T		
White	10	7	**3,1** (1,0–10,5)	**0,028**
Black and *Pardo*	32	70		
Black	2	13	0,9 (0,1–4,7)	0,910
White and *Pardo*	15	89		
Pardo	5	57	**0,3** (0,1–1,1)	**0,043**
White and Black	12	45		

[a] Odds Ratio significance Test by χ^2; 95% CI: 95% Confidence Interval.

pvmdr-1 and _pvcrt-o_ polymorphisms and the classification of malaria

Among the polymorphisms detected, there was only an association between the T958M mutation on *pvmdr-1* with primo-infection occurrences, as the primo-infected patients presented 17 times more (OR: 17.5; p 0.001) chances of having infection with parasites with this mutation than patients with other reinfections (Table 4). There was no association between the classification of malaria and other polymorphisms.

Polymorphisms of _pvmdr-1_ and _pvcrt-o_ and the prognosis of vivax malaria

There was association between the presence of the C393T polymorphism in *pvcrt-o* and serum CRP concentrations, with lower CRP levels in patients with parasitic infections (p = 0.039) (Table 5).

Table 4. Comparison by Odds Ratio of the classification of malaria between positive and negative polymorphisms of *pvmdr-1* T958M from 120 patients with malaria by *P. vivax* from the Brazilian Amazon

Classification	*pvmdr-1* (n)		Odds Ratio (95% CI)	*p* [a]
	Positive T958M	Negative T958M		
Primo-infection	3	1	**17,5** (1,3–925,8)	**0,001**
Relapse and Reinfection	17	99		
Relapse	1	49	0,45 (0,0–5,9)	0,492
Primo-infection and Reinfection	3	67		
Reinfection	0	49	[b]	0,091
Relapse and Primo-infection	4	67		

[a] Odds Ratio significance Test by χ^2; 95% CI: 95% Confidence Interval.
[b] It is not possible to calculate the OR when there is a "zero" value in one of the categories.

Table 5. Distribution of medians and interquartile range for C-reactive protein concentrations between positive and negative polymorphisms of *pvmdr-1* and *pvcrt-o* from 120 patients with *P. vivax* malaria from the Brazilian Amazon

Gene	Polymorphism	CRP mg/dL (percentis)			*p* [a]
		P25	Median	P75	
pvmdr-1	T958M (+)	10	136	137	0,384
	T958M (–)	34	83	111	
	F1076L (+)	71	100	430	0,344
	F1076L (–)	33	83	112	
pvcrt-o	C393T (+)	13	50	85	**0,039**
	C393T (–)	37	94	117	
	T786C (+)	17	74	94	0,460
	T786C (–)	34	84	112	

[a] U-Mann-Whitney test.

DISCUSSION

Plasmodium resistance is a complex characteristic in which multiple mutations in a single parasite are necessary for the development of a resistant phenotype. In addition, the frequency of a haplotype is a useful measure to assess susceptibility in the parasite-host relationship. In areas of high endemicity, there is a high genetic diversity of *Plasmodium*, with several haplotypes circulating in the exposed population (Taylor *et al*., 2014; Winter *et al*., 2015).

The analysis of SNPs in genes responsible for drug resistance proved to be useful and important in monitoring resistance in countries with endemic malaria (Garrido-Cardenas *et al*., 2019; Hawkins *et al*., 2018; Suwanarusk *et al*., 2007). The mutations found in the present study in the *pvmdr-1* gene were T958M and F1076L, and the most frequently used to monitor resistance to chloroquine by *P. vivax* worldwide are Y976F and F1076L (Brega *et al*., 2005; Rungsihirunrat *et al*., 2015; Sá *et al*., 2019).

Although there may be variations in frequencies within the same region, our results partially agreed with two studies published in 2018 with patients from the Colombian and Brazilian Amazon region, where no Y976F mutation was found, except for F1076L (Cubides *et al*., 2018; Silva *et al*., 2018). Nevertheless, a study of Mexican patients published in 2017 showed variations in the Y976F mutation from 0% in the south, 63% in the northeast, and 88% in northwest of Mexico (González-Cerón *et al*., 2017). These differences suggest that this mutation resulted from a recent diversification, which may also occur in the Brazilian Amazon with the T958M mutation and would explain our divergences.

In Papua (Indonesia) and Ethiopia, where the resistance of *P. vivax* to chloroquine is more frequent and where the failure to treat with chloroquine reaches 65% and 13% of cases, respectively, the Y976F mutation showed a high prevalence of 39% and 75%, respectively, and was always found to be associated with the F1076L mutations (Barnadas *et al*., 2011; Ketema *et al*., 2011; Suwanarusk *et al*., 2007). The fact that all parasites with the Y976F mutation in Papua and Ethiopia also had the F1076L mutation, as originally described by Brega *et al*. (2005), may indicate that F1076L may be a background mutation that precedes the Y976F substitution and, in that case, would be indicative of an early warning for an emerging resistance to chloroquine. Therefore, considering the presence of the F1076L mutation in our study, and as a preventive measure, the use of alternative drugs should be considered in the future for the treatment of vivax malaria in the Brazilian Amazon region.

Although the mutation T958M of *pvmdr-1* has also been reported with variable frequencies in several endemic regions worldwide, it has not been associated with *P. vivax* resistance to treatment (Nomura *et al*., 2001; Brega *et al*., 2005; Cubides *et al*., 2018; Joy *et al*., 2018; Tantiamornkul *et al*., 2018; Anantabotla *et al*., 2019; Sá *et al*., 2019). Even so, in our study, primo-infection was associated with the T958M mutation; that is, primo-infected patients had a higher proportion of infections triggered by parasites carrying this mutation.

At the time of submission of this study, there are no other studies showing a greater susceptibility to any polymorphism of *P. vivax* in primary infections. We suggest that this observed behavior may be related to a differentiated response of the host's immunity according to the genetic diversity of *P. vivax*, leading to a higher predominance of parasites carrying the T958M mutation in primary infections. Therefore, as an example of a possible differentiated response, a recent article suggested that primary *P. vivax* infections induce potent immunosuppression mediated by dendritic cells, which may result in a better immune response for subsequent infections (Vallejo *et al*., 2018).

In relation to *pvcrt-o*, the most common genetic change associated with resistance to chloroquine is the insertion of a lysine at the 10th position of the amino acid sequence (K10) (Lu *et al*., 2011; Joy *et al*., 2018; Anantabotla *et al*., 2019). This insertion was not found in the present study, as has also been demonstrated in India (Ganguly *et al*., 2013), Thailand (Rungsihirunrat *et al*., 2015), Ethiopia (Golassa *et al*., 2015), Madagascar (Barnadas *et al*., 2008), China (Lu *et al*., 2012), and the Colombian Amazon (Cubides *et al*., 2018). On the other hand, a study published in 2018 with patients from the Brazilian Amazon showed a 25 to 60% occurrence of K10 insertion in the *pvcrt-o*, although in this case, it was not found to be

associated with chloroquine resistance (Silva *et al*., 2018). Similar results were also found in Thailand and Myanmar (Lu *et al*., 2011). These differences reinforce the fact that there is a polymorphic diversity in nearby regions, and that these diversifications may result from a recent propagation of mutated *P. vivax* in the Brazilian Amazon, as suggested above.

The C393T and T786C polymorphisms for *pvcrt-o* found in the present study are included in an intron, a non-coding region, and are located respectively after exon 1 and exon 2, as it appears in the GenBank XP_001613457.1 amino acid sequence used as reference for this identification (Reference Sequence). To the best of in our knowledge, they were not previously described at the time of submission of this study.

Pardo is a term used in the former Portuguese and Spanish colonies in the Americas to refer to multiracial descendants of Europeans, Native Americans, and West Africans (Mattos, 2006), and is still used today for censuses purposes in Brazil. The self-reported white patients were more susceptible to infection by parasites carrying the C393T polymorphism in *pvcrt-o*. Given that C393T is located in a non-coding region, it could be suggested that the presence of the C393T polymorphism would indicate the existence of other polymorphisms that would favor the infectivity of *P. vivax* in white patients or discourage infectivity in *pardo* patients (Table 3). The literature suggests that susceptibility to malaria is mainly due to human factors related to invasion and survival of the parasite in red blood cells (de Mendonna *et al*., 2012), as the Duffy-blood-group-negative genotype found in African and American black people, which are resistant to infection. However, to date, there are no studies demonstrating the association of *P. vivax* polymorphisms with differentiated infectivity in ethnic groups; therefore, this result merits further study.

In the studies by Lima-Junior *et al.* (2012), Alves-Junior *et al.* (2020), and Paul *et al.* (2012), higher CRP levels were found in the plasma of individuals infected with *P. vivax,* suggesting a more robust inflammatory profile for infection by *P. vivax,* and can be used as a marker of prognosis, severity and follow-up (Lima-Junior *et al*., 2012; Paul *et al*., 2012; Vemula *et al*., 2016, Alves-Junior *et al*., 2020).

There was also an association between the C393T polymorphism of *pvcrt-o* with serum concentrations of CRP. That is, patients with vivax malaria infected with parasites carrying this polymorphism had lower concentrations of CRP at the time of diagnosis, which would be indicative of a better prognosis (Alves-Junior *et al*., 2020; Lima-Junior *et al*., 2012; Paul *et al*., 2012; Vemula *et al*., 2016).

The regions where the frequency of mutations that confer resistance to *P. vivax* is high had a greater number of cases of severity at the time of diagnosis, and these severities are not necessarily a consequence of resistance, since they occur even before any treatment, and therefore, before the occurrence of the possible resistance (Garrido-Cardenas *et al*., 2019; Kotepui *et al*., 2020). However, the opposite was observed in cases of polymorphism associated with a better prognosis. We cannot affirm that this polymorphism is related as a cause or effect of a better prognosis of vivax malaria; however, this polymorphism may suggest the existence of a haplotype that gives *P. vivax* less pathogenicity for the human host (Garg *et al*., 2012; Milner, 2018; Read, 1994). Mutations in microorganisms can be harmful or beneficial to the host. Thus, among the theories of pathogen/host co-evolution, it is assumed that virulence is largely a consequence of selection, optimizing pathogen fitness, and that less pathogenic parasite strains are maintained by selection because they are less rapidly cleared by the host, which is beneficial for the pathogen. In addition, less pathogenic parasites that promote host survival ensure their own survival because, by increasing the length of the life of the host, they increase the time for their dissemination, so, their virulence increases (Read, 1994).

The present study, as it is cross-sectional, cannot infer causality, but points to an association not yet reported in the scientific literature for *P. vivax*, in which polymorphisms may indicate greater or lesser pathogenicity. These findings contribute to further research that may corroborate for the possibility of individualized treatment.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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