Liver and kidney dysfunction, hypoglycemia, and thrombocytopenia in *Plasmodium vivax* malaria patients at a Colombian Northwest region

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ABSTRACT

Plasmodium vivax has high morbidity, it is the *Plasmodium* species with the greatest worldwide distribution, and its ability to trigger severe symptoms is currently recognized. The present study aims to compare the clinical and epidemiological characteristics of patients with *P. vivax* malaria, with and without complication criteria, in an endemic area for malaria transmission in northwest Colombia. A descriptive cross-sectional study was carried out between 2017 and 2019, patients with *P.vivax* severe malaria ($n = 50$), non-severe malaria ($n = 56$) and healthy controls ($n = 50$) were included. Sociodemographic, clinical, hematological, and biochemical characteristics were analyzed. Clinical follow-up was carried out in a group of patients with severe malaria. The statistical analysis was carried out in GraphPad Prism; the Chi-square test analyzed categorical variables, comparisons of variables for the three groups were carried out by the Kruskal-Wallis test and comparison between two groups by the Mann-Whitney test. A multiple correspondence analysis described the relationship between variables, which was carried out through the R software. One hundred fifty-six individuals were linked to the study, 76 women and 80 men, between 3 and 71 years old. For 50% of the patients, it was their first malaria episode; 42% of the patients classified with severe malaria required hospitalization, compared to 7.1% of the patients with non-severe malaria. Parasitaemia was similar in both clinical groups; however, 10% of severe patients presented high parasitemia, between 20,000-135,000. The most frequent clinical characteristics in patients with severe malaria were severe thrombocytopenia in 54%, hypoglycemia in 48%, and liver and kidney failure in 30%. Biochemical and hematological parameters returned to normal in 90% of the patients with severe malaria on the third day after starting treatment. Thrombocytopenia, hypoglycemia, and liver and kidney dysfunctions were the most frequent *P. vivax* malaria complications in this study. Hemoglobin concentration and parasite count were not related to the clinical condition of patients. Thrombocytopenia was the most frequent finding in patients with malaria, and its severity presented an inverse relationship with the number of previous malaria episodes.

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1. Introduction

Malaria is the most prevalent parasitosis in the world. The most recent WHO report estimates that in 2019, there were 228 million cases and approximately 405,000 deaths worldwide (WHO, 2019). In America, 976,000 cases were reported; Venezuela, Brazil and Colombia contribute 83% of the cases for the region, of which 74.1% are attributed to *P. vivax*. In Colombia, there has been an average of 60,121 cases of non-severe malaria and 930 cases of severe malaria in the last 5 years, showing an increase of 14% and 15% in the notifications for each clinical classification for 2018, when compared to the previous year (INS, 2018).

Plasmodium vivax is prevalent in Southeast Asia and South America (Dayananda et al., 2018). In America, it is responsible for 64% of malaria cases (WHO, 2017), a percentage that historically rises to around 70% in Colombia (Rodríguez et al., 2011). However, epidemiological changes have occurred in the last three years, showing a similar behavior for *P. falciparum* and *P. vivax* regarding the number of cases. For decades, *P. vivax* was considered a clinically benign species, but currently, there is evidence that describes neurological and hematological complications (such as anemia and severe thrombocytopenia), as well as respiratory, renal and hepatic compromise (Castro-Gomes et al., 2014; Douglas et al., 2012; Howes et al., 2016; Kumar et al., 2017; Sarkar and Bhattacharya, 2008).

Severe anemia is the most frequently reported complication in Asia and in some South America countries, it affects children, adults and pregnant women. Its relationship with different comorbidities has been studied, where it may worsen, but its presentation is not exclusive of the presence of two simultaneous diseases. Respiratory distress or pulmonary compromise has been reported in *P. vivax* monoinfections; for example, different post-mortem studies in India and Brazil identified the parasite presence in tissues by molecular methods. (M. V. Lacerda et al., 2012; Valecha et al., 2009). Reports of cerebral malaria created by *P. vivax* are infrequent; coma, seizures, and altered consciousness are the most frequent manifestations. In cases of cerebral malaria caused by this species, the infection has been confirmed by microscopy or molecular biology, ruling out another type of microorganism. In the brain, findings of localized inflammation are reported (Mukhtar et al., 2019; Pinzon et al., 2013).

Pathophysiological mechanisms that mediate the different *P. vivax* malaria clinical forms are poorly understood. The low parasite load observed during infection, the restrictive invasion towards young red blood cells, the little evidence of sequestration of the parasitized blood cells in organs, has led to consider that there are different mechanisms (than those described for *P. falciparum*) that participate in *P. vivax* pathogenesis (Milner Jr., 2018). The exacerbated production of pro-inflammatory cytokines, the activation and expression of endothelial adhesion molecules and autoimmune mechanisms, are some of the mechanisms that can cause clinical complications by this species (Barber et al., 2015; Rivera-Correa et al., 2019; Wassmer et al., 2015).

There are no specific complication criteria for *P. vivax* established by the WHO. Current indications suggest the use of the same parameters as for *P. falciparum* (WHO, 2015), despite being two species with different biological and epidemiological characteristics, which it undoubtedly merits a singularity in the criteria definition by which patients would receive a certain therapeutic management. The present study aims to compare the clinical and epidemiological characteristics of *P. vivax* malaria patients, with and without complication criteria, in an endemic area for malaria transmission.

2. Material and methods

2.1. Study participants, study design and data collection

The study site was the Córdoba department, which is located at the Caribbean region in Colombian Northwest, where a descriptive cross-sectional study was carried out. The study participants were recruited at the San Jerónimo de Montería Hospital and at the San José de Tierralta Hospital (Tierralta municipality), between October 2017 and March 2019. Three study groups were formed: patients with severe *P. vivax* malaria ($n = 50$, according to criteria in Table 1), non-severe *P. vivax* malaria ($n = 56$) and healthy controls from endemic area ($n = 50$) (Fig. 1).

Blood samples were taken from each patient by EDTA and dry tube venipuncture, samples were taken during the feverish period and before the start of antimalarial treatment. The following analysis were carried out: automated hemogram, thick drop, peripheral blood smear, serum glucose quantification, creatinine, total bilirubin, direct bilirubin, aspartate aminotransferase, alanine aminotransferase and species confirmation by nested PCR, as previously described by Andrade and coworkers (Andrade et al., 2009).

The classification of patients with severe malaria was carried out according to the World Health Organization's Guide to Malaria Treatment (WHO, 2015) and the National Institute of Health's Guide to Comprehensive Clinical Care for the malaria patient in Colombia (INS, 2010), as shown in Table 1. The group of healthy controls in the endemic area were afebrile people at the time of sample collection, without malaria events during the last six months and recruited at Tierralta municipality. Children under 2 years old, women in pregnancy, people with underlying diseases, mixed malaria, *P. falciparum* monoinfections, leptospirosis and dengue were excluded from the study for all groups. A survey was carried out on all the participants, in order to collect sociodemographic, clinical, and epidemiological information. Patients with severe malaria who were discharged after day three ($n = 16$) were followed up with clinical and laboratory parameters (described above).

Table 1
Classification criteria for severe *P. vivax* malaria patients.

Classification criteria
Platelet count ≤50,000/uL
Hypoglycemia ≤60 mg/dL
Metabolic acidosis (plasma bicarbonate <15 mmol/l)
Serum creatinine ≥1.5 mg/dL
Hyperlactatemia (lactate >5 mmol/l)
Indirect Bilirubin ≥1.5 mg/dL
Total Bilirubin ≥1.5 mg/dL
Transaminases ≥40 U.I.
Hemoglobin ≤7 g/dL
Parasites count ≥50.000 parasites/μL
Coma status
Seizures
Altered state of consciousness
Breathing difficulty
Pulmonary edema
Hemoglobinuria

2.2. Data analysis

GraphPad Prism version 7.00 software was used for data statistical analysis. Categorical variables between the groups were analyzed through the Chi square test, comparisons between quantitative variables in the three groups were compared using the Kruskal-Wallis test and the comparison between two groups was carried out through the Mann-Whitney test. A multiple correspondence analysis was carried out with R statistical software version 3.6.3, for the group of patients with non-severe and severe malaria, in order to observe the association trend between variables.

3. Results

3.1. Sociodemographic characteristics of the study population

A total of 156 individuals were linked to the study, 76 females and 80 males, between 3 and 71 years old. The age variable behaved similarly between the three study groups (p 0.5698, Kruskal-Wallis test). 90.5% of the individuals diagnosed with malaria came from Tierralta municipality and the remaining percentage came from Puerto Libertador and Montelíbano municipalities. The total of individuals from the control group were recruited from Tierralta municipality, 80.1% of the population lived in dispersed rural areas and 83.3% had been living for two years or more in that area. The main roles for the study population were students and people dedicated to livestock and miscellaneous activities, with 37.8%, 28.7% and 23.7% respectively. Mosquitoes sighting in the household area was reported by 94% of the participants, 63.5% used awnings, the population indicated they didn't use neither wire mosquito nets in the houses' windows nor repellent as protection measures for insect bites (Table 2).

3.2. Clinical, hematological, and biochemical characteristics of the study population

3.2.1. Clinical characteristics

For the group of patients with severe malaria, 74% of cases were having malaria for the first time or had suffered a single previous episode of the disease. This same situation occurred in the group of non-severe malaria for 57% of cases. For the healthy control group, 60% of the people indicated that they had never fallen ill with malaria. Furthermore, only 42% of patients with severe malaria required hospitalization, while only 7.1% of patients with non-severe malaria were hospitalized (Table 2).

Fever, headache and shivers were the most frequent clinical manifestations in 94% of patients with malaria; however, patients with severe malaria presented a greater diversity in terms of manifestations, such as abdominal pain, retroocular pain, vomiting, arthralgia, among others, when compared with the group of patients with non-severe malaria (Table 3).

Parasitaemia in both groups of severe and non-severe patients showed a median of 2.400 and 2.388, respedixctively. 10% of patients with severe malaria and 3.5% of non-severe malaria patients presented parasitaemia between 20,000–135,000 and for the parasite count, there were no significant differences between these two groups, suggesting that this variable does not define the compromise to other organs.

3.2.2. Hematological characteristics

The number of red blood cells and leukocytes was significantly lower in patients with severe malaria, compared to the other two study groups, while the hemoglobin concentration was similar among patients diagnosed with malaria, regardless of their classification criteria. However, this parameter concentration was found decreased in individuals diagnosed with malaria, when compared to the group of healthy controls. Hemoglobin concentration in patients with malaria ranged between mild
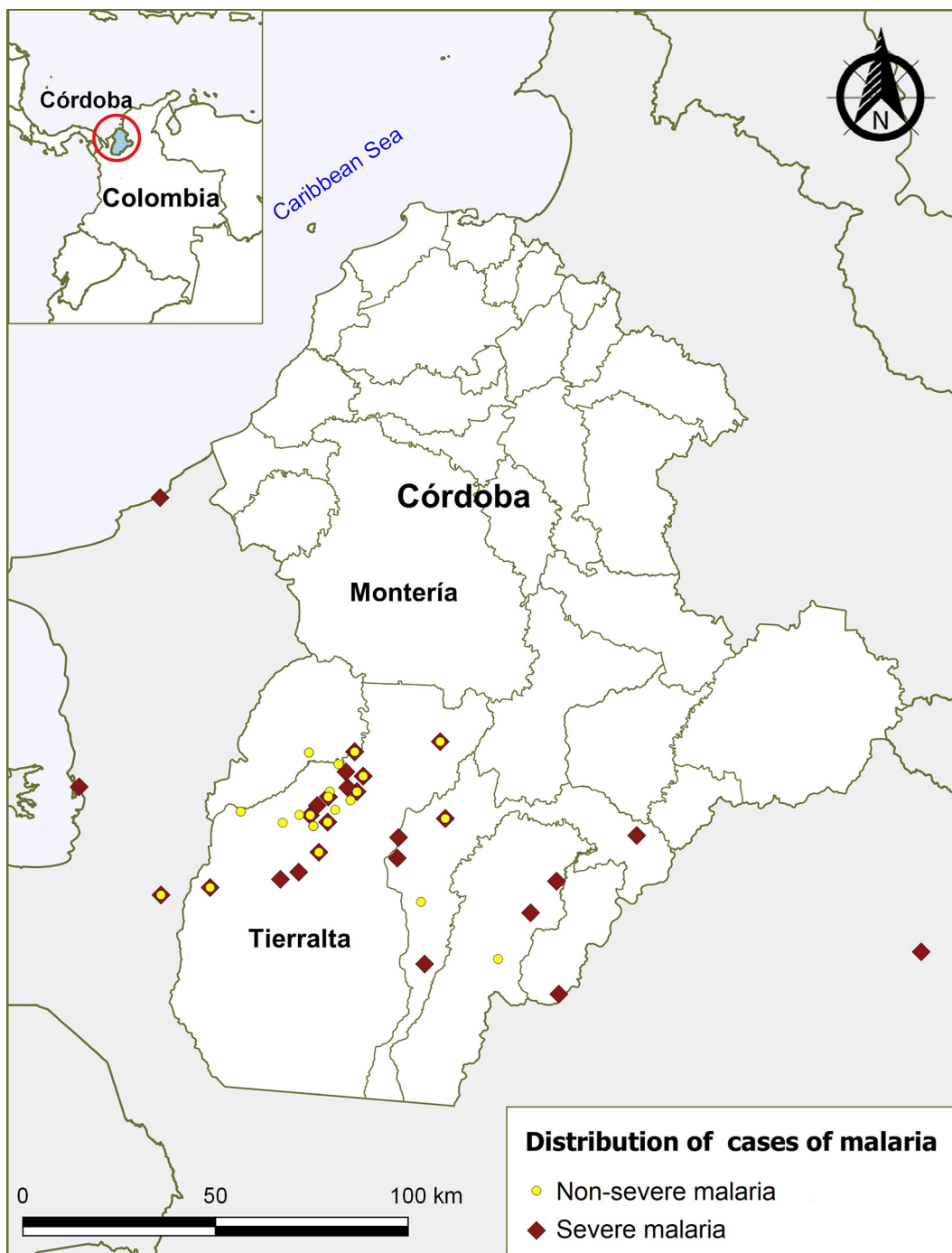
Fig. 1. Geographical distribution for severe and non-severe *P. vivax* malaria cases in the study area. Source: Own.

(10.1–10.9 g/dL) and moderate anemia (7.1–10.0 g/dL), only one patient had a hemoglobin value of 6.8 g/dL, which is considered as severe anemia (7 g/dL). For which reason, it was included in the group of severe patients (See Table 4).

Platelet count showed significant differences between the three study groups. In the severe malaria group, 54% of the patients presented severe thrombocytopenia ($<$50,000 platelets/mm^3), 28% presented moderate thrombocytopenia (50,000–99,999 platelets/mm^3) and 8% presented mild thrombocytopenia (100,000–149,000 platelets/mm^3) (Table 5), while the platelet count in patients with non-severe malaria presented similar degrees of moderate and mild thrombocytopenia, found in 35.7% and 32.1%, respectively.

Table 2
Demographic characteristics for the study population.

Description	Severe	Non-severe	Healthy Controls
Gender [n (%)]			
Female	24 (48,0)	22 (39,3)	30 (60)
Male	26 (52,0)	34 (60,7)	20 (40)
Age [(Median (IQR)]	16 (11–26)	17 (12–35)	20 (12–34)
N° of previous malaria episodes [n (%)]			
0	25 (50)	23 (41)	30 (60)
≤ 2	18 (36)	23 (41)	17 (34)
≥ 3	6 (12)	9 (16)	3 (6)
Hospitalized [n (%)]			
Yes	21 (42)	4 (7,1)	N/A
No	29 (58)	52 (92,9)	N/A
Presence of mosquitoes	46 (92)	56 (100)	45 (90)
Use of wire mosquito net for windows	0 (0)	0 (0)	0 (0)
Insecticide use	4 (8)	3 (5,4)	5 (10)
Use of repellents	0 (0)	0 (0)	0 (0)
Awning use	39 (78)	39 (69,6)	21 (42)
Use of long-lasting insecticide nets	11 (22)	2 (3,6)	3 (6)
Spraying insecticides around the house	13 (26)	1 (1,8)	3 (6)
Crops near the house	35 (70)	54 (96,4)	32 (64)

IQR = Interquartile range, N/A = Does not apply.

Patients who had the disease for the first time in the severe malaria group presented decreased platelet concentrations when compared to patients who had the disease for the first time, but that were categorized in the non-severe malaria group ($p < 0.0001$). For the severe patients' group, the mean platelet concentration was 39.12 ± 16.21, while for the non-severe malaria group, it was 116.8 ± 44.42. For 66% of patients with severe thrombocytopenia, it was the first time they had a malaria episode (Fig. 2).

3.2.3. Biochemical characteristics

For the group of patients with severe malaria, biochemical parameters behaved as follows: 28% ($n = 14/50$) presented an increased serum creatinine concentration (≥1.5 mg/dL); within this group, 2 individuals presented values higher than 3.0 mg/dL. In general, creatinine concentration was higher in the group of severe patients than in the other two study groups. 40% ($n = 20/50$) of the patients in this group presented decreased glycemia concentrations, according to what was established by INS (<60 mg/dL), and 6% ($n = 3/50$) showed concentrations below 40 mg/dL (the latter corresponds to the WHO criterion). Thirty percent of patients with severe malaria presented increased levels of both types of transaminases (> 40 U/L) (Table 4).

Forty-eight percent of patients categorized in the severe malaria group registered a single complication criterion; for 24% of these patients, hypoglycemia was the only complication criterion ($n = 12/50$). This was followed by severe thrombocytopenia in 16% ($n = 8/50$) of severe cases, while 52% of patients in this group presented between 2 and 6 complication criteria, being severe thrombocytopenia and increased transaminases the most frequently altered parameters (Table 5).

Table 3
Clinical characteristics of patients with severe and non-severe *P. vivax* malaria.*

Clinical manifestations	Severe malaria n = 50	%	Non-severe malaria n = 56	%
Fever	50	100	55	98,2
Headache	49	98	55	98,2
Shivers	47	94	53	94,6
Sweating	0	0	5	8,9
Jaundice	0	0	3	5,3
Arthralgia	6	12	3	5,3
Myalgia	3	6	0	0
Drowsiness	2	4	0	0
Nausea	4	8	0	0
Diarrhea	3	6	0	0
Retroocular pain	6	12	0	0
Abdominal pain	12	24	1	1,8
Fatigue	2	4	0	0
Appetite loss	8	16	1	1,8
Dizziness	5	1	2	3,6
Vomiting	17	34	9	16,1

* The control group had no symptoms.

Table 4
Hematologic and biochemical profile of patients with severe and non-severe *P. vivax* malaria.

	SM N = 50 Median (IQR)	NM N = 56 Median (IQR)	Healthy Controls *N* = 50 Median (IQR)	SM vs NM vs HC	SM vs NM	SM vs HC	NM vs HC
Parasite count/μL	2400 (1400–5393)	2388 (1800–4150)	N/A	0,6510	0,6510	N/A	N/A
RBC x10^6/μL	4053 ± 0,0956	4402 ± 0,08103	4753 (4408–5058)	<0,0001	**0,0061**	<0,0001	0,0021
Leukocytes x10^3/ μL	5,28 (4,59–6,39)	5,93 (4,62–7,3)	7,55 (6,9–9,47)	<0,0001	**0,0320**	<0,0001	<0,0001
Hemoglobin g/dL (Mean ± SD)	11,21 ± 1,81	11, 83 ± 1,78	12,66 ± 1,51	0,0002	0,0757	<0,0001	0,0122
Platelets x10^3/ μL	46,5 (31,75–89,5)	118 (85,5–154,8)	146 (245,3–326,3)	<0,0001	**<0,0001**	<0,0001	<0,0001
Glycemia mg/dL	67,95 (53,73–79,6)	79,9 (71,7–110))	76,6 (67,65–82,7)	0,0010	**0,0003**	0,013	0,13
Creatinine mg/dL	0.96 (0,72–1,51)	0,79 (0,67–1,0)	0,73 (0,60–0,84)	0,002	**0,0125**	<0,0001	0,0168
Total Bilirubin mg/dL	0.71 (0,43–1,08)	0,75 (0,59–0,84)	0,71 (0,55–0,87)	0,8223	0,8984	0.7457	0,4947
Alanine aminotransferase (GPT) U/L	34,65 (16,6–52,18)	28,8 (20,78–45,1)	23 (16,25–30,53)	0,0026	**0,0475**	<0,0001	0,0179
Aspartate aminotransferase (GOT) U/L	26,05 (14,18–42,7)	20,15 (14,85–26,55)	16,8 (11,7–22,9	0,0022	**0,0337**	<0,0001	0,0482

IQR = Interquartile range. SM: Severe malaria; NM: Non-severe malaria; HC: Healthy Controls. Comparisons between the three groups were carried out through Kruskal Wallis, and comparisons between two groups through Mann Whitney.

Table 5
Clinical characterization of patients with severe malaria.

| Clinical characteristics	Severe patients	%
Severe thrombocytopenia <50.000	27	54
Moderate thrombocytopenia 50.000–99.000	14	28
Mild thrombocytopenia 100.000–149.000	4	8,0
Hypoglycemia (< 40 mg/dL) OMS	4	8,0
Hypoglycemia (<60 mg/dL) INS	20	40,0
Renal failure (> 1.5 mg/dL) INS	13	26,0
Renal failure (> 3.0 mg/dL) OMS	2	4,0
Mild to moderate anemia (Hb < 11 g/dL)	20	40,0
Liver failure	15	30,0
(GPT/GOT > 40 U/L or Total bilirubin >1.5 mg/dL)		
Hiperparasitaemia >50.000 par/uL	2	4,0
With 1 complication criterion	24	48,0
With 2 complication criteria	11	22,0
With 3 complication criteria	7	14,0
With 4 complication criteria	4	8,0
With 5 complication criteria	3	6,0
With 6 complication criteria	1	1,0
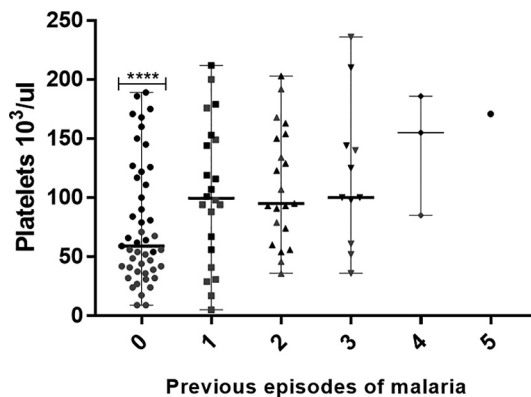
Fig. 2. Platelet concentration vs. number of previous episodes of malaria in patients with *P. vivax* malaria. *Red: patients with severe malaria; black: patients without severe malaria. ****p ≤0.0001. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)*

3.3. Association between clinical variables of patients with severe and non-severe P. vivax malaria

For the principal component analysis (PCA), in both the group of patients with severe and non-severe malaria, an expected positive correlation was observed between hematological variables such as hemoglobin, hematocrit, and red blood cells, considering the differences already established regarding the gender. In both groups, there is little difference between the symptoms presented by males and females.

For the group of patients with severe malaria, there was a positive and simultaneous correlation between total bilirubin, direct bilirubin, GPT and GOT, and in turn, these same variables are negatively associated with age, previous episodes of malaria and platelet and eosinophils count. This analysis shows that people who have suffered more malaria episodes have fewer symptoms than people with fewer episodes suffered from the disease. In severe patients, a positive correlation is evident between hemoglobin, hematocrit, red blood cells, leukocytes, and basophils, which is a correlation not observed in patients with non-severe malaria. These parameters could be an essential marker of a possible complication (Fig. 3).
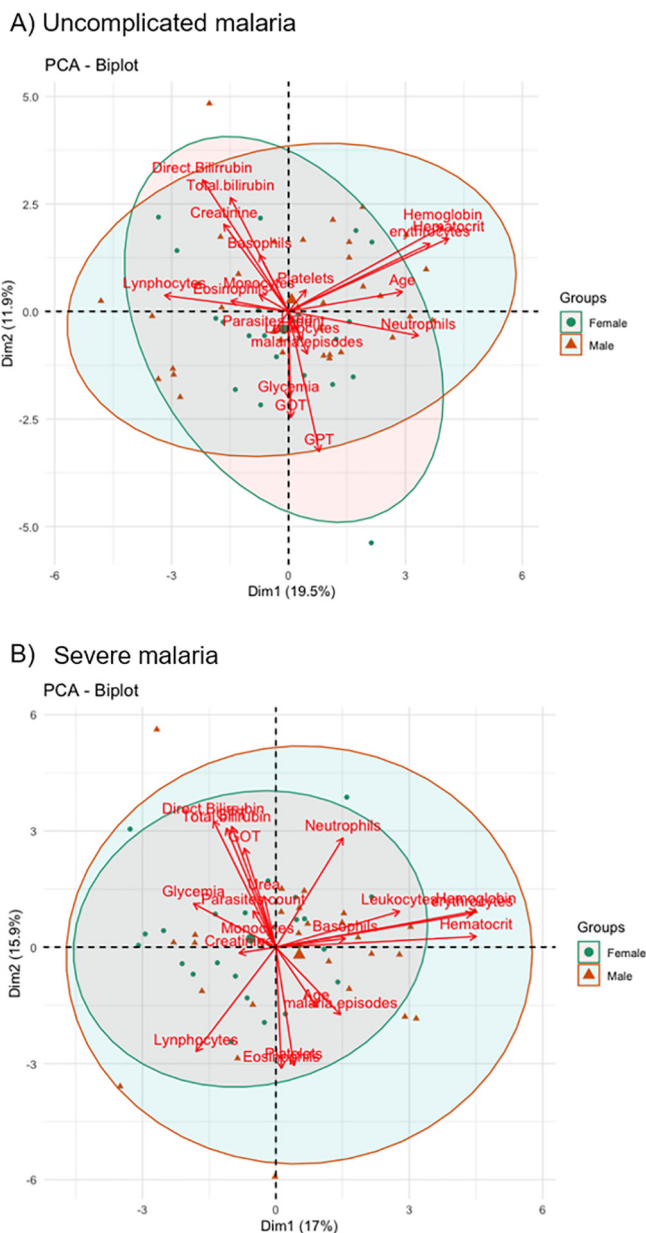
Fig. 3. Relationship analysis for epidemiological, hematological and biochemical variables by study groups A) Patients with non-severe malaria, B) Patients with severe malaria.

3.4. Follow-up of severe P. vivax malaria patients

From the group of severe malaria, 32% ($n = 16/50$) of patients were followed up; from these, 87.5% ($n = 14/16$) were discharged on day three after admission. The remaining percentage ($n = 2/16$) were discharged on day seven. On day one, 43.7% ($n = 7/16$) of patients presented mild to moderate anemia and at the time of hospital discharge, 50% presented anemia within these same ranges. Platelets concentration showed an increase regarding the value found on day one.

However, 56.25% ($n = 9/16$) were discharged with moderate thrombocytopenia, and only 18% ($n = 3/16$) had normal platelet counts when they were discharged. The remaining hematological parameters were kept within normal. Creatinine was found increased on the day of recruitment in 31.25% ($n = 5/16$) of the patients, at the time they were discharged, only in 20% of the patients ($n = 1/5$) concentrations returned to normal. Bilirubin and transaminase concentrations were normal at discharge in this group of patients (Fig. 4).

Creatinine was found increased on the recruitment day in 31.25% (n $= 5/16$) of the patients; at the time they were discharged, only in 20% of the patients (n $= 1/5$) these concentrations returned to normal. Bilirubin and transaminase concentrations were normal at the time of discharge in this group of patients (Fig. 4).

4. Discussion & Conclusions

This study aimed to assess the clinical and epidemiological characteristics of patients diagnosed with severe and non-severe *P. vivax* malaria in an endemic area in the Colombian northern region. Despite the existence of a global policy led by the WHO, aimed at reducing and eliminating areas of malaria transmission, some South American countries such as Brazil, Venezuela and Colombia have increased their reporting cases in recent years (WHO, 2017).

Currently, *P. vivax* is recognized as a species that can cause severe malaria cases than can even lead to death; however, there are no specific complication criteria defined for this species (Baird, 2013b; Naing et al., 2014). The WHO indicates that the same parameters described for complications in *P. falciparum* malaria should be applied, despite knowing that they are species with different biological and epidemiological behaviors. In Colombia, complication criteria for malaria patients contemplate these parameters established by the WHO, but include some variations according to the country's own experiences. Nevertheless, these general parameters are used regardless of the species.

The systematic review of Rahimi and coworkers about severe *P. vivax* malaria reflects the clinical variability of infections concerning geographic conditions, endemicity, and even the underreporting of severe *P. vivax* malaria cases due to a lack of interest in some cases (Rahimi et al., 2014). The clinical variability reasons are still unknown; studies are required to determine the influence of both genetic factors of the parasite and the host, as well as ecological factors in the clinical outcome. Likewise, the need to evaluate the severity criteria for *Plasmodium vivax* according to the epidemiological parameters for different geographic areas is evident.

The information collected shows that there is no adherence to conventional measures in order to avoid mosquito bites, such as wire mosquito nets in windows, repellents, awning and re-impregnation, pest control program, among others. This situation is similar in the three study groups.

Parasitaemia presented a median of 2400 parasites/µl; regardless of the clinical category, sex or age, the count was lower than that reported in other endemic areas, where the median is frequently around 10,000 parasites (Arévalo-Herrera et al., 2017; Baird, 2013a). In the group of patients with severe malaria, two patients with parasitaemia greater than 50,000 parasites/mm^3 were admitted; the rest of the patients in this group, despite not having high parasite counts, they presented criteria for hematological, renal, and hepatic complications. No correlation was found between the parasites' concentration and the different complications that patients presented during *P. vivax* infection. The hospitalization criterion was a variable that was not associated with the complication criteria, since less than half of the patients in this group were hospitalized at the time of being included in this study. Thrombocytopenia, hypoglycemia, liver and kidney dysfunction were the complications found in the group of patients with severe malaria, no cases of pulmonary or brain disorders were observed.

Hematological compromises found in this study were anemia and thrombocytopenia. Anemia is one of the most frequent alterations in *P. vivax* infections, severe anemia is considered a complication that mainly affects adolescents and pregnant women (Castro-Gomes et al., 2014). In this study, 33.9% of the patients with malaria had moderate or mild anemia and the analysis of this variable within each clinical group indicated that 38% of the patients with severe malaria had anemia, compared to 30.4% of the patients with non-severe malaria. For the severe malaria group, only one patient presented severe anemia, which corresponded to a 2% within this clinical group. The behavior of this variable was similar to what has been described by other authors for *P. vivax* endemic areas in Latin America (Arevalo-Herrera et al., 2015). However, the behavior of this variable presents variations that have been associated with other variables, such as geographic areas, age, among others (Douglas et al., 2012).

The appearance of anemia during the infection by this protozoan is not fully understood, the limited ability of this species to parasitize red blood cells of all ages shows that the destruction of parasitized cells is not a reflection of the resulting anemia in the host. This fact has led to suggest that there are other mechanisms involved in mediating this alteration. One of the most robust hypotheses is the autoantibodies generation against proteins in the red blood cell membrane, which mediate the destruction of parasitized and non-parasitized red blood cells (Rivera-Correa et al., 2017).

Thrombocytopenia occurred in 78.3% ($n = 83/106$) of people diagnosed with malaria, thrombocytopenia percentages in patients with *P. falciparum* and *P. vivax* malaria vary between 40%–78%. In most cases, it presents a low association with bleeding (Gupta, Bansal, Jain, & Sahare, 2013). Thrombocytopenia percentage was higher than what has been previously reported by
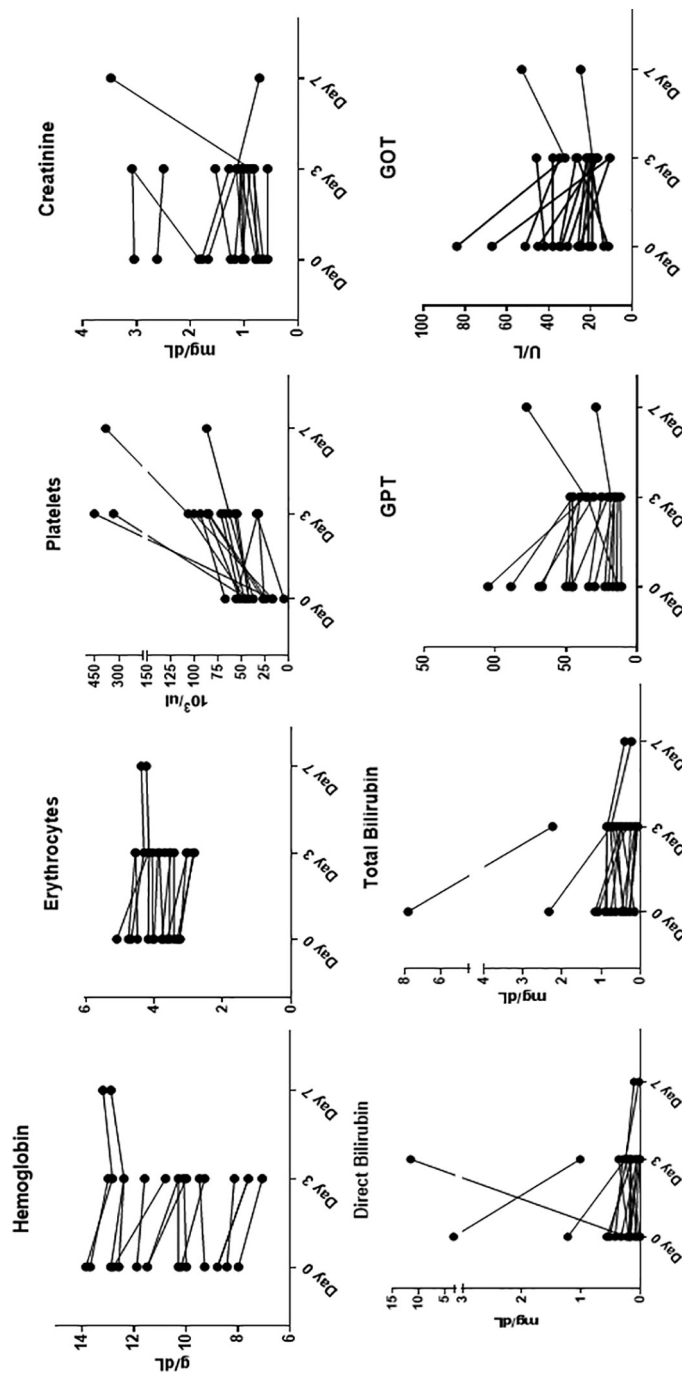
Fig. 4. Laboratory parameters profile during the follow-up of severe patients with *P. vivax* malaria.

other authors for South America endemic areas in patients with malaria (Martínez-Salazar and Tobón-Castaño, 2014), but similar to studies carried out in India, that reported 88% thrombocytopenia in infections by this species (Saravu et al., 2011).

In this study, 90% of patients with severe malaria manifested this condition. The group in which severe thrombocytopenia was considered a complication criterion presented 54% of individuals with this condition ($n = 27/50$); in turn, 62.9% ($n = 17/27$) of people with severe malaria and severe thrombocytopenia presented malaria for the first time, a situation that has been previously reported in individuals with *P. falciparum* malaria. This is the case of a study carried out in Papua Indonesia, whose patients with *P. falciparum* infection without a history of previous malaria presented lower levels of platelets, a finding that was not reported for *P. vivax* infections in that study population (Lampah et al., 2014). For this study, thrombocytopenia presented a negative correlation with parasitaemia ($r − 0.2223$, p 0.0220, data not shown), and was the most frequent finding in patients with liver dysfunction, similar to what was previously reported by other researchers (Fazil et al., 2013; Martínez-Salazar and Tobón-Castaño, 2014). The mechanism involved in thrombocytopenia that is observed in patients with malaria is not clear, there are a variety of hypotheses that include platelet sequestration by the spleen during the removal of parasitized cells, platelet auto-aggregation, circulation of anti-platelet antibodies and the alteration in thrombopoietin synthesis that derives from the cytokines action (M. V. G. Lacerda et al., 2011; Punnath et al., 2019).

Hypoglycemia is a frequent manifestation in *Plasmodium* infections. For this study, 48% of patients with severe malaria presented this condition. Hypoglycemia prevalence associated with malaria varies between countries, in ranges between 6 and 50% for *P. vivax* (Rahimi et al., 2014). For *P. falciparum*, prevalence can increase up to 70% (Dhangadamajhi et al., 2019). In addition, percentages may vary depending on age, being more frequent in children than in adults, excluding pregnant women whose physiological condition has an additional predisposing factor. Medicines such as quinine affect glucose metabolism for their ability to stimulate insulin production (Bartoloni and Zammarchi, 2012), favoring hypoglycemia in patients. It is one of the main mechanisms that explain this condition and, on the other hand, glucose consumption during severe *Plasmodium* infections is greater regarding to non-severe clinical courses of the disease. Alterations in glucose production and prolonged fasting are triggers for hypoglycemia. The decreased glucose concentration in blood is associated with a poor prognosis in malaria, mainly in children, where neurological disorders and manifestations that can lead to death are present (Madrid et al., 2015).

Acute kidney damage from *P. vivax* is reported in children and adults. Histological studies indicate the finding of thrombotic microangiopathy, characterized by endothelial injury, microvascular occlusion by platelets or fibrin thrombi and glomerular ischemia. In other cases, the presence of necrosis foci with cortical or glomerular location is reported (Sinha et al., 2013). The pathological mechanism that leads to kidney damage is not clear; however, it is known that vascular lesions can be triggered by microorganisms, toxins and medications, which lead to an imbalance in the production of complement molecules, endothelial adhesion and cytokines. It has been recognized that in *P. vivax* infections, a higher production of molecules that mediate the immune response is observed regarding to what is observed in *P. falciparum* malaria. For this reason, it is estimated that the inflammatory response imbalance plays a fundamental role in this organ alteration (Yeo et al., 2010).

Renal dysfunction cases associated with *P. vivax* range around 1–20% (Miranda-Arboleda et al., 2014; Toshan et al., 2016); for the present study, renal dysfunction was a cause of severe malaria in 30% of patients in this group. In 60% of the cases, in addition to the elevated creatinine concentration, they presented elevated transaminases, and in 28.5% of patients, it was accompanied by an increase in total bilirubin (Naqvi, 2015). Some reports show renal alterations more frequently in adult patients; however, for this study, no difference was found in the age range between patients with elevated serum creatinine concentrations compared to those patients who had normal values. For the group of patients with severe malaria, no difference in parasite count was observed according to creatinine concentration (Miranda-Arboleda et al., 2014).

In a study with characteristics similar to ours that was carried out in Brazil, they considered renal dysfunction for those patients who had creatinine values greater than 1.3 mg/dL; from this group, all patients required hospitalization for therapeutic management (Cruz et al., 2018). For our study, 71.5% of patients with creatinine elevation were hospitalized. Another factor that can cause an increase in serum creatinine is hemolysis, which leads to an increase in total bilirubin; however, not all renal dysfunction studies due to *P. vivax* show an increase in creatinine and total bilirubin at the same time, or other parameters such as severe anemia and increased BUN, as observed in this study. In spite of not finding variety in the values that indicate an increase in serum creatinine, the fact of having WHO values as a reference, which are high, might not allow an early identification of kidney dysfunctions (Miranda-Arboleda et al., 2014).

For the severe malaria group, 38% of the affected patients showed increased Alanine aminotransferase concentrations (as stated by the WHO) and 30% showed increased Aspartate aminotransferase (AST) concentrations. Only 6% had a two-fold increase regarding the normal value of these enzymes (40 U/L). During a *Plasmodium* infection, the parallel increase in bilirubin and transaminases is considered as an indicative of liver dysfunction. This situation occurred in 10% of patients in this group, who additionally presented other alterations, such as severe thrombocytopenia (except one case with moderate thrombocytopenia), increase in creatinine levels, and one case presented a count of 42,000 parasites/mm^3. The reported values of these molecules in blood related to liver dysfunction or damage in *Plasmodium* infections show variations, some authors even consider values to categorize a mild or severe compromise. The latter range has values of >3 mg/dL for bilirubin and an increase for each transaminase in three-fold ($\sim > 120$ u/L); however, some authors consider these figures to be high and to show low sensitivity for the prediction of an alteration in the organ (Shwetha, 2014).

In Colombia, the Comprehensive Clinical Care Guide for patients with malaria indicates that a bilirubin concentration of 1.5 mg/dL and an increase in transaminases> to 40 U/L are indicative of liver damage. Liver compromise is reported to a lesser extent for infections caused by *P. vivax*, which occurs with a slight alteration in bilirubin and transaminase concentrations, and in some cases accompanied by jaundice (Shwetha, 2014). These findings are similar to what was found in our study population.

There is little information available on liver pathophysiology for *P. vivax* malaria, the concentration increase of these molecules in blood is related to the parasite's liver cycle. There, it involves cells rupture in the organ and the consequent release of molecules to the circulatory system, in the case of transaminases, as well as an alteration of liver functions, which can lead to loss of this organ capacity to excrete bilirubin, which is reflected in the increase of this substance in the blood (Poles et al., 2012). Liver disorders associated with *P. falciparum* are more studied and involve pathogenic mechanisms, such as cytoadhesion of parasitized cells, microvasculature obstruction and red blood cells hemolysis (Fazil et al., 2013).

The follow-up carried out on patients who had a hospital stay of three days or more, showed that in most of the patients, biochemical parameters quantified returned to values within the reference ranges. However, hematological variables such as hemoglobin or platelet concentration, despite showing a slight increase in its concentration, did not manage in all cases to increase to normal concentrations at the time of discharge. The number of days at hospital stay was close to the ranges indicated by other studies, which report between 4 and 5 days for discharge (González et al., 2000; Iborra et al., 2013). A limitation of the study is the low number of follow-up in patients with severe malaria. The follow-up was carried out during the hospital stay; once they were discharged, they returned to their homes, which in most cases were located on sidewalks far from the hospital and even the central city. Access is limited to these areas due to armed conflicts, road conditions, and financial resources for monitoring.

5. Conclusions

Thrombocytopenia, hypoglycemia, and liver and kidney dysfunction were the most frequent complications observed in *P. vivax* malaria for the study population. Hemoglobin concentration and parasite count were similar in individuals with malaria, regardless of their clinical condition. In its different degrees, thrombocytopenia was the most frequent finding in malaria patients, and its severity was related to fewer previous episodes of the disease per individual. It is necessary to inquire more about the biological mechanisms that mediate platelets decrease and their relationship with a compromise in different organs during *P. vivax* malaria. It is necessary to review the severity criteria for *Plasmodium vivax* malaria and adjust them according to the country's epidemiological context.

Ethics statement

The inclusion and participation process for subjects was carried out voluntarily and in accordance with the national (Resolution No. 008430 from October 4th, 1993, Colombian Republic, Ministry of Health) and international guidelines (Helsinki Declaration and its amendments, World Medical Association (WMA), Edinburgh, Scotland, October 2000). Participants gave their consent by signing the informed consent or assent, according to the situation of each individual. The project received ethical endorsement from the Human Ethics Committee of the Health Sciences Faculty from Universidad de Córdoba.

Declaration of Competing Interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as fees; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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