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Molecular prevalence and risk factors of plasmodial infection among parturients in Abengourou, Ivory Coast

Bedia-Tanoh A. V.[1,2]*, Konaté-Touré A.[1,2], Kiki-Barro P. C. M.[1], Gnagne A. P.[2], Miezan A. J. S.[1], Koné E. G. M.[1], Kassi K. F.[1], Angora K. E.[1], Bosson V. H.[1], Djohan V.[1], Menan E. I. H.[1] and Yavo W.[1,2]

[1]Department of Parasitology, Mycology, Animal Biology and, Zoology, Faculty of Pharmaceutical and Biological Sciences (UFR), Félix Houphouët-Boigny University, BPV 34, Abidjan, Ivory Coast.
[2]Malaria Research and Control Center, National Institute of Public Health, PBV 47 Abidjan, Ivory Coast.

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This study aimed to determine the molecular prevalence and risk factors of plasmodial infection in parturients in Abengourou. This cross-sectional study included pregnant women who were presented for delivery at the maternity ward in Abengourou. Plasmodial DNA was extracted from venous blood, placental, and cord samples, dried on Whatman filter papers, using the Chelex 100 method. The extracts were subsequently amplified by nested PCR using specific primer pairs for each *Plasmodium* species. The average age of women was 27.25 years (standard deviation = 6.2 years). The coverage rate for IPTp3 was 46%. Most women were multigravida (58%) and multiparous (49.5%). Over half of the women had undergone at least 4 antenatal care visits (53%), and they were more compliant with IPTp (IPTp3; 70.8%, p=<0.0001). Placental, venous, and cord *Plasmodium* infection rates were 22.5, 16.5, and 2.2%, respectively. There was a significant relationship between age (p=0.018), gravidity (p=0.02), and plasmodial infection. Primigravidae were 2.5 times more likely to have a plasmodial infection. This study, confirming the presence of plasmodial infection in both the parturient and the newborn, underscores the need to strengthen malaria prevention measures during pregnancy.

Key words: Parturients, *plasmodium* infection, Abengourou, Ivory Coast.

INTRODUCTION

Malaria remains a public health concern, with pregnant women and children under 5 years being the most vulnerable populations (WHO, 2022; World Health Organization, 2021). According to the World Health Organization (WHO), 30 million pregnant women are exposed to malaria in the endemic areas of Africa. Each year, 200,000 infants and 10,000 pregnant women die from malaria annually (World Health Organization, 2019). Malaria and pregnancy are two conditions that exacerbate each other. Malaria is more severe in the

*Corresponding author. E-mail: akouaval@yahoo.fr.

third trimester and during childbirth, leading to maternal, fetal, and perinatal morbidity and mortality (Bourée, 2013). In endemic areas, placental infection is very common during pregnancy and may exist even in the absence of peripheral parasitemia (Tahirou et al., 2020). Malaria constitutes a perpetual threat to the mother-child dyad in these areas. One in four pregnant women presents evidence of placental infection during childbirth (Tahirou et al., 2020).

Malaria is responsible for the persistently high rate of maternal and neonatal mortality (Botolahy et al., 2011; Chiabi et al., 2012; Diallo et al., 2019) due to its impact on the mother's health (e.g., anemia). It also affects the fetus (e.g., abortion, growth retardation), and the newborn (e.g., prematurity, low birth weight) (Chiabi et al., 2012; Fitri et al., 2014; Kuete et al., 2022). Considering 11 million pregnant women affected by Malaria in Africa, approximately 900,000 of their newborns had low birth weight. Therefore, preventing malaria in pregnant women is a public health priority in Africa. The Ivorian National Malaria Control Program (NMCP) recommends several control strategies, including the use of long-lasting insecticide-treated nets (LLINs), intermittent preventive treatment (IPT) with sulfadoxine-pyrimethamine (SP), and the prompt and free management of uncomplicated malaria cases in pregnant women and children under 5 years. These efforts face resistance from *Plasmodium falciparum* against commonly used antimalarial drugs used for chemoprevention. Some authors have also reported relatively high prevalence levels of mutations in the dhfr and dhps resistance genes to SP in the country (Ako et al., 2012). Despite the presence of these mutations, studies on the effectiveness of intermittent preventive treatment (IPTp) with SP have shown that the latter remains effective (Djaman et al., 2004; Offianan et al., 2012). The WHO recommends monitoring the effectiveness of this control strategy to adjust interventions at the appropriate time (WHO, 2013). Therefore, every two years, the NMCP, in collaboration with research institutions, conducts studies to assess the effectiveness of IPT-SP. However, these studies rely on microscopy (thick smear/thin smear), a method with lower sensitivity compared to newer molecular biology methods (Dicko-Traoré et al., 2011). Indeed, a study using polymerase chain reaction (PCR) among asymptomatic individuals in low transmission areas showed a prevalence of sub-microscopic parasitic carriage of 12.44% (90/723), and 6.41% (45/702) in 2014, and 2015 respectively, while the prevalence based on *Plasmodium* microscopy was only 0.27% (2/723) and 0.14% (1/702) in 2014 and 2015 (Niang et al., 2017). Indeed, a study using PCR among asymptomatic individuals in low-transmission areas revealed a sub-microscopic parasitic carriage prevalence of 12.44% (90/723) and 6.41% (45/702) in 2014 and 2015, respectively. In contrast, the prevalence based on *Plasmodium* microscopy was significantly lower, at 0.27% (2/723) in 2014 and 0.14% (1/702) in 2015 (Niang et al., 2017).

Therefore, conventional techniques (that is, thick smear and blood smear) have limitations in terms of the detection of low parasitemia (Niang et al., 2017; Otambo et al., 2022; Uneke, 2008; Yentur Doni et al., 2016). Nested PCR can be an appropriate diagnostic technique to overcome the limitations of microscopy. This observation led to the formulation of the hypothesis that the prevalence of malaria in parturients, often underestimated by conventional methods and resulting in an overestimation of the effectiveness of IPT, could be more accurately detected using molecular tools. Hence, the use of PCR in this study to determine the molecular prevalence and risk factors of plasmodial infestation in parturients in Abengourou, in order to obtain accurate data on the true prevalence of malaria.

MATERIALS AND METHODS

Study type and population

A cross-sectional study was conducted using dried blood samples on Whatman paper. The samples were obtained from 200 pregnant women who came to deliver at the Henri Konan Bédié health maternity in Abengourou, a sentinel site for malaria surveillance in Côte d'Ivoire. Women were recruited during a study evaluating the effectiveness of intermittent preventive treatment with SP in pregnant women in 2017.

The required sample size was determined to assess the impact of IPT on birth weight. The objective was to compare the prevalence of low birth weight (LBW) between groups of pregnant women, those who received IPT (IPT+) and those who did not receive IPT (IPTp-). B Preliminary survey data indicated that the prevalence of LBW was 12% among women who did not receive IPT (IPTp-) compared to 3% among those who received IPT (IPTp+). To detect a statistically significant difference between these two groups with 90% power, a sample size calculation was performed. Given only 30% of pregnant women in the general population typically receive IPT, a total of 1,043 women was required, distributed as follows:

1) 695 IPT- who did not receive IPT (IPTp-)
2) 348 who received IPT (IPTp+).

To achieve this target, a total of 1200 women were recruited across the six sentinel sites in the country, including the Abengourou site, with 200 women per site to ensure optimal representativeness of the findings. All parturients who came to deliver at the Henri Konan Bédié health maternity in Abengourou provided informed consent before inclusion in the study. However, certain cases led to exclusion based on the following criteria:

1) limited comprehension of the questions, which could compromise the accuracy and reliability of the responses provided,
2) Withdrawal of consent at any stage of the study, as participation was entirely voluntary
3) Inability to collect placental samples, either due to logistical constraints, medical complications, or other unforeseen circumstances that made sampling unfeasible.

Data collection

Demographic, clinical, and therapeutic data were gathered either

from the mother-child health record, the delivery room register, or through participant interviews.

Biological examinations conducted

Each included woman underwent peripheral, placental, and cord blood sampling. These blood samples were used for the preparation of smears (thick and thin blood films) and dried blood spots on Whatman paper (Whatman International Ltd., Maidstone, England). The DBS were used for molecular biology tests, including *Plasmodium* DNA extraction and Nested−PCR, at the Malaria Research and Control Center of the National Institute of Public Health (CRLP/INSP). *Plasmodium* DNA was extracted using the Chelex 100 method (Sigma Aldrich, St. Louis, MA, USA) (Bedia-Tanoh et al., 2021). The obtained extracts were stored at -20 °C until use.

The target fragment (*Plasmodium* 18S ssrRNA) was amplified through nested PCR using a SimpliAmp™ 96-well thermal cycler (Thermo Fisher Scientific, Waltham, MA, USA). A preliminary non-specific amplification was performed with the primers rPlu5 and rPlu6 (Table 1), using 1 µL of the DNA extract. This was followed with a specific second amplification using 1 µL of the first PCR product as a template, with species-specific primers for the four *Plasmodium* species (Table 1). All amplifications were carried out in a total volume of 25 µL. The PCR-amplified DNA fragments were visualized through electrophoresis (Kuro GEL Midi 13 VWR*) on 1.5% agarose gel.

Statistical analysis of data

The data collected from the study were systematically coded and entered into the database using Excel 2016 and Word 2010 software for documentation and organization. The Statistical analysis was then performed using the SPSS version 21.0 for Windows. One of the key measures calculated was the Plasmodial Index, which represents the percentage of individuals carrying *Plasmodium* in the study population. This index is an important indicator for understanding the prevalence of the sample group. To compare the proportions of various outcomes between groups, statistical tests such as the Chi-square and Fischer's exact test were applied. A significance level (α) of 5% was set at the threshold to determine whether the observed differences were statistically significant, meaning that results with a p-value below 0.05 were considered unlikely to have occurred by chance.

Ethics approval

Prior to the implementation of this study, the protocol was submitted and received approval (041/MSLS/CNER-kp) from the National Ethics and Research Committee of Côte d'Ivoire (CNER-CI).

RESULTS

The result of the study shows that the mean age of women was 27.25 years (sd = 6.2 years), with a range from 18 to 46 years. The age group of 20-30 years was the most represented (57%). The majority of women were housewives and had never received formal education, and a significant proportion were in a relationship. Nearly all women included in the study received SP as part of intermittent preventive treatment (96%), with approximately half of the participants receiving the full IPT3 regimen.

The majority of women (85%) reported receiving an LLIN; however, only half of them slept under the mosquito net every night. Only 27% of women used Insecticide spray as a malaria prevention method (Table 1). Most women were multigravida (58%) and multiparous (49.5%). Over half of the women had attended at least four antenatal care (ANC) visits. Approximately 12% of newborns had low birth weight (Table 1). A statistically significant correlation was observed between the level of education, parity, number of antenatal care, and compliance with IPT-SP (Table 1). There was also a significant association between age, gravidity, and *Plasmodium* infection (Table 2). The results of this study indicated that primigravidae had a 2.5 times higher risk of presenting with *Plasmodium* infection (P=0.020; OR= 2,5; IC=1.18-5.46) (Table 2). A higher placental plasmodial index (22.5%) was observed compared to venous blood (16.5%) and cord blood (2.2%) in the included women (Figure 1). In comparison to microscopy results (placenta=17%; venous=15.5%; cord=1.5%), submicroscopic infection rates of 5.5, 1, and 0.5% were observed in placental, venous, and cord blood, respectively (Figure 1). Mothers with newborns exhibiting low birth weight had taken less than three doses of IPTp-SP and were as equally infected as those whose children had normal weight. No statistically significant difference was found. Similarly, those who had undergone at least four ANC visits demonstrated better compliance to IPTp (IPTp3; 70.8%) (Table 1). A significant correlation was established between the number of ANC visits and IPTp compliance. A statistically significant difference was observed between venous and placental infection (p=0.0001), as well as between placental and cord infection (p=0.010) (Table 3). Women who took fewer than three doses of IPTp were the least infected in terms of venous, placental, and cord blood, however, no significant difference was observed (Table 2).

DISCUSSION

Given the burden of malaria, particularly on vulnerable populations such as pregnant women and children under 5 years old, this study aimed to contribute to the improvement of malaria prevention and elimination. The study population was young (mean = 27.25 years; sd = 6.2 years). This observation can be explained by the fact that the reproductive age for women is typically between 15 and 49 years worldwide (World Health Organization 2019). However, it is important to note that, due to certain cultural norms, limited access to education, and availability of contraception, the age of parturients may still be relatively young in some countries (World Health Organization, 2019). Despite their young age, more than half of the parturients underwent at least four ANC visits. In Côte d'Ivoire, the recommended minimum number of

Table 1. Distribution of IPT use according to socio-demographic, parasitological, and obstetrical characteristics of women

Characteristics	Number n (%)	<3	≥ 3	P value
Age n (%)				
<20	29 (14.5)	16 (55.2)	13 (44.8)	0.281
]20-30]	114 (57)	66 (57.9)	48 (42.1)	
]30-40]	53 (26.5)	23 (43.4)	30 (56.6)	
>40	4 (2)	3 (75)	1 (25)	
Profession				
Homemaker	99 (49.5)	62 (62.6)	37 (37.3)	0.161
Trader	40 (20)	20 (50)	20 (50)	
Student	21 (10.5)	10 (47.6)	12 (57.1)	
Official	9 (4.5)	4 (44.4)	5 (55.5)	
Informal sector	31 (15.5)	12 (38.7)	18 (58.1)	
Marital status				
Married	10 (5)	7 (70)	3 (30)	0.424
In a relationship	154 (77)	84 (54.5)	70 (45.5)	
Single or Bachelor	36(18)	17 (47.2)	19 (52.8)	
Education level				
Never schooled	91 (45.5)	56 (61.5)	35 (38.5)	0.048
Primary	61 (30.5)	33 (54.1)	27 (44.3)	
Secondary	36 (18)	16(44.4)	21 (58.3)	
Superior	12 (6)	3 (25)	9 (75)	
Use LLTNs_night				
Yes	109 (54.5)	60 (55)	49 (45)	0.745
No	91 (45.5)	48 (52.7)	43 (47.3)	
Insecticide use				
No	146 (73)	77	9	0.632
Yes	54 (27)	31	23	
Gravidity				
≤ 2	83 (41.5)	43 (51.8)	40 (48.2)	0.6
>2	117 (58.5)	65 (56.6)	52 (44.4)	
Parity				
≤ 2	133 (66.5)	65 (48.9)	68 (51.1)	0.040
>2	67 (33.5)	43 (64.2)	24 (35.8)	
Number of antenatal care				
<4	94 (47)	77 (81.9)	17 (18.1)	<0.0001
≥4	106 (53)	31 (29.2)	75 (70.8)	
Newborn weight				
<2500	24 (12)	14 (58.3)	10 (41.7)	0.550
≥2500	176 (88%)	94 (53.4)	82 (46.6)	
Placental infestation				
Positive	45 (22.5)	26 (57.8)	19 (42.2)	0.564
Negative	155 (77.5)	82 (52.9)	73 (47.1)	
Cord infestation				
Positive	5 (2.5)	4 (80)	1 (20)	0.377
Negative	195 (97.5)	104 (53.3)	91 (46.7)	

Table 2. Distribution of plasmodial infestation according to socio-demographic characteristics and obstetric history of women

Parameters	Effective (%)	Venous PCR			
		Positive n (%)	Negative n (%)	P value	Odds Ratio (95% CI)
Age					
<20	29 (14.5)	10 (52.6)	19 (65.5)		
[20-30]	114 (57)	15 (13.2)	99 (88.8)	0.018	-
[30-40]	53 (26.5)	6 (11.3)	47 (88.7)		
>40	4 (2)	2 (50)	2 (50)		
IPTp					
Yes	192 (96)	32 (16.7)	160 (83.3)	1	0.71 (0.08-6)
No	8 (4)	1 (12.5)	7 (87.5)		
IPTp Number o					
<3	108 (4)	17 (15.7)	91 (84.3)	0.754	0.89 (0.42-1.87)
≥3	92 (23)	16 (17.4)	76 (82.6)		
Use LLTNs_night					
Yes	109 (54.5)	22 (20.2)	87 (79.8)	0.132	1.84 (0.84 -4.03)
No	91 (45.5)	11 (12.1)	80 (87.9)		
Insecticide use					
Yes	54 (27)	9 (16.7)	45 (83.3)	1	0.984 (0.425-2.3)
No	146 (73)	24 (16.4)	122 (83.6)		
Gravidity					
≤ 2	83 (41.5)	20 (24.1)	63 (75.9)	0.020	2.54 (1.18-5.46)
>2	117 (58.5)	13 (11.1)	104 (88.9)		
Parity					
≤ 2	133 (66.5)	23 (17.3)	110 (82.7)	0.840	119 (0.53 2.67)
>2	67 (33.5)	10 (14.9)	57 (85.1)		
Number of antenatal care					
<4	94 (47)	16 (17)	78 (83)	0.851	1.07 (0.50 -2.27)
≥4	106 (53)	17 (16)	89 (84)		
Newborn Weight					
<2500	24 (12)	4 (16.7)	20 (83.3)	1	1 (0.32-3.19)
≥2500	176 (88%)	29 (16.5)	147 (83.5)		

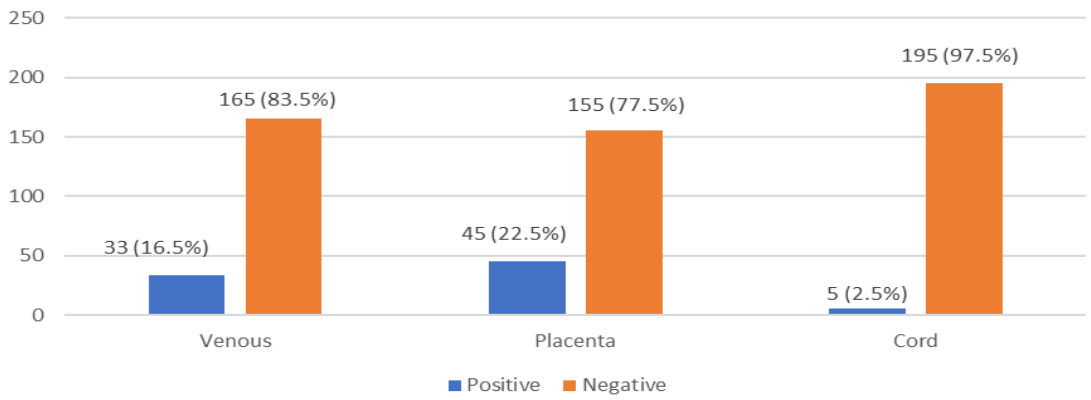

Figure 1. Distribution of *Plasmodium* infection at the venous, placental, and cord levels.

Table 3. Distribution of venous *Plasmodium* infection according to that of the placenta and cord

Sample type	Venous blood			Cord blood		
	Positive	Negative	p-value	Positive	Negative	p-value
Placental blood						
Positive	27	18	<0.0001	4	41	0.010
Negative	6	149		1	154	
Cord blood						
Positive	2	2	0.191	-	-	-
Negative	31	164		-	-	-

ANC visits is four (Yavo et al., 2015). Adequate attendance and follow-up of appointments and consultations by pregnant women facilitate monitoring the proper progress of pregnancy. Additionally, it enables the detection, treatment, and prevention of potential pathologies that could lead to complications in pregnancy. Results were low compared to those found by Biaou et al. (2019) in Benin in 2017, who reported that 72.68% of pregnant women had undergone at least 4 ANC visits. Indeed, the World Health Organization (WHO) has established a new model for antenatal care, increasing the number of recommended visits from 4 to 8 (World Health Organization, 2016).

The rate of ANC visits obtained in this study indicates a need for improvement in the quality of care and communication between healthcare providers and pregnant women. In addition to adhering to ANC, vector control remains an essential means of reducing the transmission of malaria. This involves avoiding mosquito bites through the use of insecticide-treated bed nets or residual insecticide spraying (Pryce et al., 2018). It was observed that approximately half of the parturients in this study used treated mosquito nets. As for IPTp, less than half of the women had taken at least three doses of IPTp (49.7%). Furthermore, parturients who used LLINs and received more than three doses were more likely to be infected in venous, placental, and cord blood.

This could be explained, on one hand, by the low utilization rates of SP and LLINs. On the other hand, in this study, the concept of taking SP was based on self-reporting or the mother-child health record, without any evidence provided through chemodetection of SP metabolites in the blood or urine. Therefore, it is likely that some women who claimed regular SP prophylaxis may not have actually adhered to it.

Additionally, it should be noted that no statistically significant relationship was observed between the number of IPTp doses and malaria infection. Several factors related to services and the community can impede the implementation of IPTp-SP. These include the unavailability of SP and insecticide-treated mosquito nets (Wanzira et al., 2016), unfavorable attitudes of healthcare personnel, and the distance to travel to health facilities (Salomão et al., 2017).

In some cases, women may receive the drug without actually taking it, as it is not always administered under direct observation [Directly Observed Treatment strategy (DOT)] (Ayubu and Kidima, 2017).

Reports indicating the low coverage of IPTp in many endemic countries in Africa (Bedia-Tanoh et al., 2021; Offianan et al., 2012; Salomão et al., 2017; Wanzira et al., 2016) have raised concerns about achieving the higher targets set in the new WHO policy (Owusu-Boateng and Anto, 2017) and, consequently, in the strategic malaria control plan in Côte d'Ivoire (which aim to achieve an 80% coverage rate for IPTp3) (National Malaria Control Program, 2016). These results highlight the need for relevant authorities to implement awareness campaigns emphasizing the importance of using LLINs and IPTp-SP. Additionally, there is a call for strengthening the free distribution of treated mosquito nets and SP to pregnant women at healthcare centers.

The results of this study contradict those obtained by Konaté et al. (2012) and Oumarou et al. (2020) in Mali and Niger respectively, who highlighted a lower rate of malaria infection in parturients using preventive measures, including LLINs. These differences may be explained by the interrogative nature regarding the use of ITNs in this study. However, there was no association between the use of ITNs and *Plasmodium* infection. Poor adherence to preventive measures can have serious consequences for the health of both the mother and the newborn. Thus, the present study assessed the presence of *Plasmodium* infection among parturients and newborns. The venous, placental, and cord blood *Plasmodium* rates were 17.4, 22, and 2.2%, respectively, almost equal to those previously found in San Pedro, of the Ivory Coast, with rates of 15.5, 16.2, and 3.6%. The prevalence of plasmodial infestation in placental blood in this series was higher compared to venous and even cord blood, a finding consistent with the existing literature (Bedia-Tanoh et al., 2021).

The higher rate of placental infestation compared to venous blood could be explained by the fact that the placenta serves as an important reservoir of parasites, even without detectable parasitemia in peripheral blood

(Bourée et al., 2008). This phenomenon is made possible through the sequestration of parasites, facilitated by the particular cytoadherence capacity of parasitized red blood cells to the syncytiotrophoblast layer of the placenta through the chondroitin sulfate A receptor (Bourée et al., 2008).

Moreover, placental parasitemia without peripheral parasitemia may be a feature in women who have previously undergone treatment with peripheral parasite clearance, while peripheral parasitemia without placental infection can occur early in the malaria infection, especially if parasitemia is low (Cisse et al., 2016). Transplacental transmission could be attributed to the low quantity of maternal IgG antibodies against malaria transferred to the fetus during pregnancy due to low malaria immunity in mothers (Cisse et al., 2016). This transmission of malaria to the fetus can have serious consequences for its health, as observed in this series. Mothers whose newborns had low birth weight had received fewer than three doses of IPTp-SP and, despite their small sample size, they were as equally infected as those whose infants had a normal birth weight. Therefore, it is important to raise awareness among women about the use of malaria prevention measures during pregnancy, which are sleeping under ITNs and receiving intermittent preventive treatment.

Compared to the results from microscopy (placenta=17%; venous=15.5%; cord=1.5%), submicroscopic infection rates of 5.5, 1, and 0.5% were observed in placental, venous, and cord blood, respectively. The control, elimination, and eradication of malaria are closely linked to asymptomatic and submicroscopic infections (Ap et al., 2018; Niang et al., 2017). Low-density parasitemia serve as reservoirs for malaria infection (Niang et al., 2017). Microscopy detects only 54% of all *Plasmodium* infections detectable through PCR (Carmona-Fonseca and Arango, 2017). An expert microscopist can detect only 50 parasites/µL of blood, whereas PCR can detect at a rate of less than 1 parasite/µL of blood (Carmona-Fonseca and Arango, 2017; Niang et al., 2017). Consequently, this results in cases of false negatives, which can lead to an underestimation of the burden of malaria infection or, on the other hand, an overestimation of the efficacy of IPT-SP in pregnant women. This is the reason why PCR was chosen in this study.

Several studies have demonstrated the considerable and high potential of detecting low parasitemia through PCR (Siala et al., 2015). PCR has been shown to be a highly sensitive technique, allowing for the detection of submicroscopic infections and mixed infections that cannot be identified through microscopy (Mvumbi et al., 2016). Therefore, PCR is the ideal diagnostic test for malaria. However, this technique is less commonly used in routine practice due to a lack of resources, as it requires expensive equipment and reagents.

A statistically significant correlation was observed between the level of education, parity, number of antenatal care, and compliance with IPT-SP. There was also a significant association between age, gravidity, and *Plasmodium* infection. However, as this is a cross-sectional study, certain confounding factors could not always be eliminated. A study conducted in San-Pédro in 2017 in Côte d'Ivoire also demonstrated a statistically significant association between the number of prenatal consultations and IPT uptake, as well as between parity and *Plasmodium* infection (Bedia-Tanoh et al., 2021). One limitation of this study is the lack of follow-up of women throughout their pregnancy. Monitoring pregnant women would allow for the evaluation of the progression of infections and maternal-fetal complications, establish causal links between exposure to risk factors and *Plasmodium* infection, and provide accurate data on IPT-SP uptake.

Conclusion

Antenatal care and the multigravida status of parturients largely contributed to a relatively low rate of plasmodial infestation. Chemoprophylaxis with SP and the use of LLINs appeared to have no effect; however, no statistically significant association was observed. The risk of malaria infection persists, emphasizing the need to strengthen malaria prevention measures during pregnancy and to educate women about the simultaneous use of multiple prevention methods. Additionally, it would be wise to explore alternative medications for malaria prevention in pregnant women. It would also be valuable to investigate mutations associated with resistance to SP by studying the *dhfr* and *dhps* genes to anticipate the emergence of resistance and adapt control strategies accordingly.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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