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Prevalence, Associated Factors of Peripheral and Placental Blood *Plasmodium* Infections and Effect of Antimalarial Treatment among Parturient Attending Health Facilities in Douala, Cameroon

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Abstract Malaria in pregnancy is still a public health problem in malaria endemic countries of Africa and elsewhere due to chronic outcomes on maternal health and poor perinatal outcomes related to occurrence of placental malaria. Since the implementation of specific malaria control strategies in pregnant woman by health decision makers as recommended by the World Health Organization, the extend of placental malaria is not regularly assessed. This study aimed to assess prevalence of Plasmodium carriage in peripheral and placental blood, associated and the impact of antimalarial treatment among parturient in two healthcare facilities of Douala in Cameroon. This was a hospital-based cross-sectional study undergone from 2018 and 2019 among consenting pregnant women ready to delivery which consisted in i) collecting sociodemographic and obstetrical factors, ABO blood group and hemoglobin type, any malaria and antimalarial treatment, ii) detecting and counting of Plasmodium parasites in peripheral and placental blood. Data collected were statistically analyzed to find any association between prevalence of Plasmodium carriage and factors collected using SPSS.20 software and Chi square test considering a p-value < 5% as significant. A total of 123 parturient aged between 18 and 42 years were included in the study. Only Plasmodium falciparum asexual stage was detected by microscopy and RDT in peripheral blood and placental blood. All parturient who harboured Plasmodium in peripheral blood either by microscopy or malaria RDT had placental malaria. Prevalence of Plasmodium infection was higher in placental blood (23.6%) than peripheral blood (9.8%). Placental malaria was predominantly acute-active infection (71%). Plasmodium loads were higher in placental blood than peripheral blood and ranged between low and high in both bloods. Being of young maternal age , low educational level, blood group O and B, and hemoglobin type AA were associated to higher prevalence of placental malaria and peripheral carrying P. falciparum. No significant association was found between prevalence of Plasmodium infection in placenta and any gynecological and obstetrical features. However, being primigravid, primiparous, undergoing only four antenatal visits or less, taking less than three IPT-SP, not sleeping under a mosquito net daily or not having experienced any fever during the ongoing pregnancy. Placental Plasmodium infections persisted in 29.6% of parturient who were treatment appropriately for malaria during the pregnancy. Prevalence and parasitemia of P. falciparum infection was higher in placenta than peripheral blood. Young maternal age, low educational level, blood group O and B, and hemoglobin type AA were associated to P. falciparum infection in placenta and peripheral blood. Antimalarial treatment did not prevent placental malaria despite peripheral blood negative malaria.

Keywords: *Peripheral blood, Placental blood, Plasmodium, Infection, Antimalarial treatment, Cameroon*

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1. Introduction

Malaria is a life-threatening vector borne parasitic disease endemic in tropical areas which in 2022 is estimated to about 249 million malaria cases and 608 000 deaths reported, a malaria mortality rate globally at 14.3 deaths per 100 000 population at risk and a malaria case incidence at 58 per 1000 population at risk; the WHO

African Region owing the huge burden accounting for about 94% of cases and 96% of deaths globally [1,2]. Malaria is caused by a sporozoan parasite named Plasmodium whose five species infect human beings namely *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi*. Pregnant women belong to high-risk group to malaria exposure. Malaria in pregnancy is estimated to affect more than 25 million pregnant women every year in malaria endemic areas with the huge burden carried particularly by the WHO African region where an estimated 36% of pregnancies were exposed to malaria infection in 2022 [1,2,3]. In the WHO African region, the highest prevalence of exposure by pregnant woman to malaria infection is reported in central Africa and west Africa [1,2,3].

The burden of malaria infection is particularly high in pregnant woman due to related complications always reported including poor obstetrical adverse outcomes associated to infiltration and sequestration of *Plasmodium* infected erythrocytes in the placental intervillous space and subsequent invasion by maternal monocytes/macrophages owing to placental malaria [4,5]. Infiltration of maternal phagocytic cells in the placenta by Plasmodium infected erythrocytes causes a massive chronic intervillositis, alteration of foetal and maternal anti-inflammatory of placental environment into a proinflammatory state, oxidative stress, and apoptosis therefore inducing poor pregnancy outcomes associated to placental malaria [4,5,6,7,8] [9,10,11,12]. Sequestration of *Plasmodium* infected erythrocytes in placenta has been reported in both *P. falciparum* and *P. vivax* infections [13].

Associated obstetrical adverse outcomes related to placental malaria include adverse perinatal outcomes such as stillbirth, low birthweight, preterm birth, and small for-gestational-age neonates, higher rate of maternal anaemia, maternal death [5,13,14,15,16,17,18,19], occurrence of neurodevelopmental delay in offspring by delaying language development [20]. Another consequence of placental sequestration of *P. falciparum*-infected erythrocytes was its association with increased susceptibility to malaria infection in infants [21].

Due to the deleterious outcomes of pregnancy malaria and its correlate placental malaria, the World Health Organization (WHO) has recommended since 2012 in moderate to high transmission areas of Africa three specific key interventions in pregnant woman including i) prevention of transmission, ii) prevention of morbidity and mortality through intermittent preventive treatment (IPTp), iii) prompt diagnostic and treatment of suspected malaria cases as part of universal health coverage to prevent poor perinatal outcomes [22,23]. In Cameroon like many other African malaria endemic countries, health decision makers promote prevention of poor obstetrical outcomes related to pregnancy malaria through systematic donation free of charge of insecticide-treated mosquito net to all pregnant women to prevent transmission, intermittent preventive therapy with sulfadoxine/pyrimethamine at each scheduled antenatal visit starting as early as the second trimester of amenorrhea to prevent morbidity and mortality, prompt diagnostic and treatment of all suspected malaria cases in pregnancy [1,22,23]. Cameroon implements these strategies to pregnant women since 2011 for ITNs and 2012 for IPT-p through donation free of charge of ITNs to all pregnant women, as well sulfadoxine/pyrimethamine as IPTp at scheduled antenatal visit starting from the sixteenth week of amenorrhea [24,25]. The Ministry of Public health in Cameroon recommends that each pregnant woman takes at least four doses of IPTp during each pregnancy [25]. The WHO estimated that hundred thousand low birthweights would be averted in the three WHO Africa regions if all the pregnant women visiting antenatal care (ANC) clinics at least once received a single dose of IPTp, given that low birthweight is a strong risk factor for neonatal and childhood mortality [1]. Previous reports indicated significant reduction of pregnancy malaria [15,22,23] as well as significant reduction of frequency of placental malaria among pregnant women who were compliant to mosquito net use in some malaria endemic countries [26]. Also, increased implementation of IPTp strategy in malaria endemic countries of sub-Saharan Africa was associated to significant reduction of maternal anemia, low birthweights and perinatal mortality among pregnant women who took at least three doses of IPTp during pregnancy [1,22,27,28,29]. However, persistence of perinatal adverse outcomes was reported in *Plasmodium* infected pregnant women appropriately treated with commonly recommended antimalarial drug [30].

Since the launching of these malaria control strategies in pregnant women, few evaluation studies have so far assessed the prevalence of *Plasmodium* infection among pregnant women at delivery and placental *Plasmodium* carriage even after specific antimalarial treatment during pregnancy in many African countries. Also, more recent data are needed in stable malaria transmission areas to assess the compliance and efficacy of these strategies among pregnant women. Previous data on pregnancy malaria reported moderate to high prevalence of this disease in Cameroon [31], Nigeria [32,33], Ethiopia [34,35] and Ghana [36]. Most frequently associated factors to pregnancy malaria include early pregnancy age, primigravidae, not sleeping under insecticide treated mosquito net and insufficient IPT-SP [31,32,35,37,38].

Aim of the study. This study was set to evaluate the prevalence of *Plasmodium* infection in peripheral blood and placental blood with relationship to sociodemographic, obstetrical, mother genetic factors and specific anti-malarial treatment, among pregnant women at delivery received in the gynaecological ward of two Douala-based health facilities in Cameroon.

2. Methodology

2.1. Study Type and Period

This was a hospital-based cross-sectional study done between 2018 and 2019.

2.2. Research Settings

The study took place in the gynecology units and laboratory of two Douala-based healthcare facilities in Cameroon for recruitment of parturient and preliminary

laboratory analysis of peripheral blood and placental samples namely the Gynecology-Obstetric and Pediatric Hospital of Douala which is a second category hospital of the national health pyramid, and the Bonassama District Hospital which is a fourth category hospital. These healthcare facilities are high standing hospitals with specialized gynecologists, pediatricians, and other medical specialists as well as trained nurses, and trained laboratory technicians. Then a quality control step of microscopy examination of blood smears by checking stained slides was undergone in the parasitology laboratory of the Faculty of Medicine and Pharmaceutical Sciences of the University of Douala. Douala itself is the economic capital of Cameroon, located in a stable malaria transmission area of the Equatorial zone with four seasons during the year namely two rainy seasons and two dry seasons.

2.3. Ethical Statement

The protocol of this study was approved by the Institutional Ethic Committee of the University of Douala hosted by the Faculty of Medicine and Pharmaceutical Sciences which granted an ethical clearance for this study, the Ethic Committee of each of the hospitals and the Regional Delegation of Public Health of the Littoral region in Douala to secure research authorizations before starting the study.

During the study, the study investigators approached all pregnant women who attended the gynecology unit of any of the study hospitals for delivery, then presented and explained her the study protocol. Any pregnant woman with a completed medical file who volunteered to participate in the study was asked to sign the study written informed consent before her inclusion. Sociodemographic, gynecological and obstetrical as well as ABO blood group, hemoglobin type and any antimalarial treatment should have been recorded in the medical file. For those who had no ABO blood group or hemoglobin type in the medical file, they had to accept to undergo these tests before their enrolment in the study.

2.4. Study Sample

Random sampling was used for this study. All pregnant women who attended the gynecology unit of any of the study hospitals for delivery during the study period and who volunteered to participate in the study and who filled the study criteria was eligible for the study.

2.5. Data Collection

For any pregnant woman ready to delivery who filled the study criteria, data related to the participant were collected from her medical file. Sociodemographic data namely her age, educational level, blood group ABO, hemoglobin type (AA, AS or SS). Gynecological and obstetrical features collected included gravidity, parity, delivery term, number of antenatal visits performed during the pregnancy, number of IPT-SP swallowed in the pregnancy, mosquito net use daily, timing of any fever during the pregnancy and any antimalarial treatment received. Delivery terms were classified as follow: i) preterm (delivery before 38 weeks of amenorrhea), ii) normal term (delivery between 38 weeks and 40 weeks of amenorrhea), iii) post term (delivery after more than 40 weeks of amenorrhea).

Plasmodium infection detection in peripheral blood and placental blood were performed before delivery and after delivery respectively. *Plasmodium* infection testing in peripheral blood was done by microscopy examination of Giemsa-stained thick and thin blood smears, and a HRP2/pLDH combination malaria rapid diagnostic test using blood drops from a finger prick of the participant according to WHO recommendations for accurate laboratory detection of malaria [23,25,39,40]. Placental malaria was diagnosed through microscopy examination of Giemsa-stained placental impression smears method following recommendations by Ouédraogo and collaborators who described this method as an easier and cheaper alternative accurate method to the gold standard placental histopathology technique [41,42].

The «One Step Malaria HRP-II (P.f) and the pLDH (Pan) Antigen Rapid» combination test made by Standard Diagnostic (SD) RDT kit procured at the National Center for Essential Drug Control of the country Ministry of Public Health was used as malaria rapid diagnostic test according to the specifications of the manufacturer. This RDT was designed to detect specifically Histidine-Rich Protein II of *P. falciparum* (pfHRPII) and non-specifically Lactate Dehydrogenase (LDH) antigen of infections due to other *Plasmodium* species. Results of the RDT were read at spot according to the manufacturer's guidelines and recorded as positive or negative.

Freshly made peripheral blood thin and thick smears as well as placental blood impression smears were allowed to dry at spot at room temperature. Thin smears were fixed with methanol before staining. Both peripheral blood and placental blood smears were stained with a 10% Giemsa solution. Stained smears were then examined at high magnification under a light microscope by trained microscopists at high magnification according to standard laboratory guidelines [43,44,45]. Discrepant microscopic readings were cross-checked by the study investigators to monitor the examination quality. *Plasmodium* loads obtained through microscopy were expressed as number of each *Plasmodium* specie and stage per µl of blood after examining microscopic fields in the thick film for 500 white blood cells (WBC) considering the results from full blood counts as reported in the participant's file. A thick blood film was considered negative after review of 300 high-magnification microscopic fields. *Plasmodium* loads were classified as low (less than 1000 asexual or sexual stage/µl of blood), moderate (1001 to 2000 asexual or sexual stage/µl of blood) and high (greater than 2000 asexual or sexual stage/µl of blood). Prevalence of laboratory-confirmed pregnancy malaria was also classified as low (less than 10%), moderate (between 10% and less than 20%) and high (greater than 20%). Placental malaria was classified according Rogerson and collaborators as follow: i) not infected defined as no evidence of parasites or malaria hemozoin in red blood cells, ii) active-acute infection defined as presence of parasites with absence or minimal hemozoin deposition within fibrin, i_3) active-chronic infection referred to presence of parasites with substantial amounts of hemozoin in fibrin or in red blood cells, i_4) past infection

identified as presence of hemozoin with no parasite [46].

2.6. Data Analysis

Data collected were analyzed using SPSS.20 software in relation to maternal age, gravidity status, parity, mosquito net use, IPT-SP compliance and occurrence of fever and any antimalarial treatment received during the pregnancy. Association of malaria infection with risk factors was analyzed as univariate using Pearson Chi-square and Fisher Exact Tests considering a *P-value* < 0.05 as statistically significant.

3. Results and Discussion

A total of 123 pregnant women at delivery were included in the study. The distribution of study participants according to sociodemographic, gynecologic and obstetrical features is given in Table 1 and Table 2 below.

3.1. Sample Distribution According to Study Factors

As indicated in Table 1, participants were aged between 18 years and 42 years with a mean age of 28.9 ± 5.3 years, participants aged at least 30 years being most represented (48.78%). Participants who attended secondary or higher educational level predominant (84.55%). Also, participants of blood group O and those of hemoglobin AA were predominant (35.8% and 51.2% respectively).

Gynecologic and obstetric features of the study participants as presented in Table 2 indicated that study participants were predominantly paucigravidae (53.6%), primiparous (49.6%), normal term delivery participants (89.5%), those who underwent at least four antenatal visits (71.5%), parturient who swallowed at least four doses of IPT-SP during the pregnancy (74.8%), participants who used a mosquito net daily (88.6%) and women at delivery who had no fever during the pregnancy were also most represented (78.1%). Among those who had fever during the ongoing pregnancy, those who experienced fever in the first trimester and the second trimester were most represented (8.9% and 11.4% respectively).

3.2. Prevalence and Loads of Plasmodium Infection in Peripheral Blood and Placental Blood

Plasmodium falciparum was the only specie found in both peripheral and placental bloods by microscopy as well as by malaria RDT. *Plasmodium* found were asexual stages. *Plasmodium* loads ranged between 475 and 3050 asexual stage/µl in the peripheral blood whereas in placenta blood, *Plasmodium* loads were greater and ranged between 700 to 13050 asexual stage/µl. The prevalence of *Plasmodium* infection was higher in the placenta (23.6%) than in the peripheral blood (9.8%).

Detection of only *P. falciparum* was in accordance with previous data which reported only *P. falciparum* in both placenta and peripheral bloods among pregnant women in Cameroon [47] and South Sudan [48,49]. A similar result was also earlier reported in Cameroon in a study on pregnancy malaria by combining microscopy and a HRPII/PLDH combination malaria rapid diagnostic test [50]. This trend confirmed the leading place of *P. falciparum* among *Plasmodium* species in Cameroon as earlier reported [51]. However, more *Plasmodium* species could have been detected with more sensitive methods like molecular techniques as previously reported with PCR technique which detected in placental and peripheral bloods of pregnant women *P. malariae* and *P. ovale* as mono-infections or mixed infections in addition to *P. falciparum* with younger women (under 20 years old) and paucigravidae having the significantly higher prevalence in Cameroon [47] and South Sudan [49].

Concerning the higher prevalence of placental malaria than peripheral blood *Plasmodium* infection, results gathered were consistent with previous reports in other malaria endemic countries of Africa including Uganda [52], Tanzania [14,53], South Sudan [48,49] and in Columbia [54]. Using more sensitive methods like histopathology or molecular diagnostic techniques could yield higher prevalence as earlier reported in Uganda with quantitative PCR (qPCR) [52], in Columbia with loop-mediated isothermal amplification (LAMP) [54,55] or histopathology combined with nPCR in the diagnosis of either gestational, congenital and placental malaria due to either *P. falciparum* or *P. vivax* in Columbia [55]. The LAMP technique has advantage that it detects low-density infections and asymptomatic malaria cases both in placental and peripheral blood in pregnant women [54].

3.3. Prevalence of Placental Malaria Types

As indicated in Table 1, microscopy examination of placental impressions smears identified 74.8% as «non infected», 1.6% as «past infection» and 23.6% as «active infection». Active infections detected were predominantly acute-active infection (75.9%).

Table 1. Classification of placental malaria infections detected

Infection status of placenta		N	Prevalence
Plasmodium free placenta		92	74.8%
Past infection		2	1.6%
Plasmodium active infected placenta		29	23.6%
Frequencies of placenta infected stage			
	Acute-active infection	22	75.9%
	Chronic-active infection	7	24.1%

3.4. Association Between *Plasmodium* Infection and Sociodemographic Factors

As indicated in Table 1, the prevalence of *Plasmodium* infection was associated to most study sociodemographic data including young maternal age, low educational level, blood group O and B, and hemoglobin type AA.

Increasing maternal age was negatively associated with prevalence of peripheral blood Plasmodium infection both by microscopy (P=0.02) and malaria RDT (p=0.001). However, *Plasmodium* infections in placental blood were detected only among under 30 years old pregnant women through malaria RDT, while microscopy detected

Plasmodium parasites in all age groups with higher prevalence recorded among under 30 years participants both in peripheral and placental blood (p=0.014). Findings in this study were in accordance with previous reports in Cameroon [47,56] and South Sudan [48,49] which identified maternal age as a major risk factor for placental malaria, younger and first-time mothers being more likely to have placental malaria. Higher malaria prevalence among young age mothers might be due to rapid decrease of partial immunity to *Plasmodium* infection in this age group.

Plasmodium infection prevalence decreased as the educational level increased with highest prevalence occurring in parturient who had primary educational level both in peripheral blood (p=0.04) as well as in placental blood (p=0.003). This finding might be due to low compliance of least educated participants to malaria prevention tools recommended the national ministry of public health and the World Health organization.

3.5. Influence of ABO Blood Group and Hemoglobin Types on Peripheral and Placental Blood Infection by *Plasmodium*

ABO blood groups significantly influenced prevalence of placental blood *Plasmodium* infection (p=0.02) as well as peripheral blood *P. falciparum* infection (p=0.01); higher prevalence being recorded among blood group O and B mothers. Nevertheless, blood group AB had the lowest *Plasmodium* infection prevalence in peripheral and placental blood, while blood groups O and B women had the highest *Plasmodium* infection prevalence. We did not find any previous study which reported an association of pregnancy malaria and blood groups ABO. However, data recorded were in general accordance with *Plasmodium* infection trend previously reported among outpatients pregnant or not who attended Douala-based healthcare facilities in Cameroon [57].

Hemoglobin type AA, As and SS significantly influenced *Plasmodium* carriage both in peripheral blood and placental blood with a decreasing prevalence from AA, to AS and SS (P = 003). This trend indicated a higher susceptibility of hemoglobin AA subjects over AS and SS in peripheral blood and placental blood. The higher prevalence of hemoglobin AA subjects over other hemoglobin type carriers was consistent with a previous report among Nigerian parturient which indicated significant high *P. falciparum* in hemoglobin AA blood group compared to hemoglobin genotype AS [58].

As presented in Table 2, results concerning any influence of gynecologic and obstetric factors of the parturient indicated a significant influence of gravidity, delivery terms, and antenatal visits performed. Primigravidae had the highest prevalence of *P. falciparum* infection while multigravida had the lowest prevalence in both placental blood (p=0.1) and peripheral blood (p=0.04). The results gathered corroborated previous reports in Cameroon which found paucigravidae as the most vulnerable groups [47,56,59]. Similar trend was also reported earlier among parturient in Nigeria [58], Cameroon [13] and Sudan [48]. The higher *Plasmodium* infection risk in primigravidae may be due to lack of premunition which may occur during subsequent pregnancies therefore explaining the gradual decreased of malaria in paucigravida and multigravida. The higher frequency of frequency of peripheral blood and placental blood *Plasmodium* infection in primigravida as well as paucigravidae indicates that systematic testing and management of malaria in these groups should be highly recommended by malaria control program of endemic areas as priority.

Table 2. Prevalence of *Plasmodium* parasites infection in peripheral blood and placental blood according to maternal sociodemographic factors

Factors		Sample size (%)	Peripheral blood		Placental blood	
			N	%	N	%
Study participants		123(100)	12	9.8	29	23.6
Maternal age	<20 years	9(7.3)	2	22.2	3	33.3
	20-30 years	54(43.9)	9	16.7	20	37
	≥30 years	60(48.8)	1	1.7	6	10
	P		*0.02*		*0.014*	
Educational level	Primary	19(15.4)	5	26.3	10	52.6
	Secondary	59(48.0)	5	8.5	12	20.3
	University	45(36.6)	2	4.4	7	15.6
	P		*0.048*		*0.003*	
Blood group	A	35(28.5)	2	5.7	7	20
	AB	6(4.9)	0	0	1	16.7
	B	38(30.9)	4	10.5	10	26.3
	O	44(35.8)	6	13.6	11	25
	P		*0.01*		*0.02*	
Hemoglobin type	AA	63(51.2)	7	11.1	21	33.3
	AS	37(30.1)	4	10.8	4	10.8
	SS	23(18.7)	0	0	4	17.4
	P		*0.04*		*0.03*	

N: number of *Plasmodium* positive participants. %: prevalence.

3.6. Association of Gynecological and Obstetrical Factors with Peripheral and Placental Blood Infection by *Plasmodium*

Undergoing less than four antenatal visits was a risk factor of *Plasmodium* carriage in peripheral blood (p=0.001) but not in placental blood (p=0.12). Prevalence of *Plasmodium* infection were highest among pregnant women who underwent one to three antenatal visits during the pregnancy compared to those who underwent at least four antenatal visits. All participants in this study attended at least one antenatal visit. A previous report in malaria endemic area of Sudan Blue pointed absence of antenatal care as a risk factor of placental malaria [48].

Delivery term did not influence *Plasmodium* infection prevalence either in peripheral blood (p=0.75) or placental blood (p=0.25). *Plasmodium* infection in placental blood was recorded irrespective to the delivery term whereas *Plasmodium* carriage in peripheral blood was recorded only among parturient who delivered in normal term and post term.

Plasmodium falciparum infections were detected in placental smears both among participants who did not experience malaria during the pregnancy as well as those had malaria. Of participants who experienced malaria during their pregnancy, *P. falciparum* infection was detected in either trimester of pregnancy with higher frequency in the first trimester. Analysis of peripheral

blood detected *P. falciparum* infections only among women who did not experience any malaria episode during their pregnancy indicating that pregnant women with no malaria-like symptom during their pregnancy need also special attention and should systematically be screened also for malaria at antenatal visits. This observation was in accordance with previous report in Cameroon which indicated that in a malaria perennial transmission area in Cameroon, few *P. falciparum* parasites carrying pregnant women were symptomatic [59]. Our results were however not in line with a previous report among Nigerian parturient which indicated no significant influence of having fever in a rural area on placental malaria [58]. However, absence of *Plasmodium* parasites in the peripheral blood might be due to antimalarial treatment received during the pregnancy after a positive malaria diagnostic as a general recommendation by the national program for malaria control concerning the prompt diagnosis and treatment strategy of any suspected malaria case [1,41,51].

Table 3. Prevalence of *Plasmodium* parasites infection in peripheral blood and placental blood according to gynecological and obstetrical features

Factors		Sample size (%)	Peripheral blood		Placental blood	
			N	%	N	%
Study participants		123(100)	12	9.8	29	23.6
Gravidity	Multigravida	28(22.8)	1	3.6	3	10.7
	Paucigravida	66(53.6)	4	6.1	15	22.7
	Primigravida	29(23.6)	7	24.1	11	37.9
	P		*0.04*		*0.11*	
Parity	Multiparous	20(16.3)	1	5	3	15.0
	Pauciparous	61(49.6)	3	4.9	12	19.7
	Primiparous	42(34.1)	8	19	14	33.3
	P		*0.012*		*0.2*	
Delivery term	Preterm	1(0.8)	0	0	1	100
	Normal term	110(89.5)	11	10	25	22.7
	Post-term	12(9.8)	1	8.3	3	25.0
	P		*0.75*		*0.25*	
Antenatal visits	1-3	35(28.5)	7	20	12	34.3
	≥ 4	88(71.5)	5	5.7	17	19.3
	P		*0.012*		*0.12*	
IPT-SP	<4	31(25.2)	8	25.8	14	45.2
	≥4	92(74.8)	4	4.3	15	16.3
	P		*0.001*		*0.016*	
Mosquito net use	No	14(11.4)	3	21.4	4	28.6
	Yes	109(88.6)	9	8.2	25	22.9
	P		*0.25*		*0.8*	
Timing of fever during the pregnancy	No	96(78.1)	12	12.5	21	21.9
	1st trimester	11(8.9)	0	0	4	36.4
	2nd trimester	14(11.4)	0	0	3	21.4
	3rd trimester	2(1.6)	0	0	1	50
	P		*0.000*		*0.003*	
Antimalarial treatment	Yes	27 (100)	0	0	8	29.6

N: number of *P. falciparum* positive participants. %: prevalence

3.7. Influence of IPT-SP on Prevalence of Peripheral and Placental Blood Infection by *Plasmodium*

According to number of IPT-SP swallowed during the pregnancy, parturient women took one to five doses of IPT-SP during antenatal visits. Pregnant Women who underwent at least four IPT-SP during pregnancy had the lowest *Plasmodium* infection prevalence in in placental blood (p=0.016) as well as peripheral blood by microscopy (p=0.001) and by RDT (p=0.002). Results gathered in this study were in accordance with those gathered among Nigerian parturient which reported non-use of ITN and not receiving IPT-SP among predisposing factors to malaria parasitemia [58]. This tendency encouraged more sensitization towards women of childbearing age to undergo at least three IPT-SP during their pregnancy as recommended by the WHO [23] as well as the country national malaria control program in Cameroon [25,51]. Persistence of a remnant placental malaria among women who had undergone at least three IPT-SP during the pregnancy was in accordance with data from southern Ghana where despite a 6-year implementation of IPT-SP, some pregnant women at delivery had malaria parasites in placental by both microscopy, malaria RDT and PCR despite their prevalence among them was lesser than among those who underwent less than three IPT-SP doses [26]. Swallowing IPT-SP significantly reduce mean placental *Plasmodium falciparum* densities among Nigerian parturient [58].The efficiency of IPT-SP among delivery women was demonstrated not only by a significant reduction in prevalence of placental malaria but also significant reduction in maternal anemia and increased birth weight [26]. However, microscopy and PCR detectable placental *P. falciparum* infection was still recorded in placental blood of parturient who had taken three doses of IPTp-SP in Ghana [26].

3.8. Influence of Insecticide-treated Mosquito Use Daily on Peripheral and Placental Blood Infection by *Plasmodium*

Using ITN during pregnancy did not significantly influence the prevalence of *P. falciparum* infection in placental blood smears (p=0.8) as well as in peripheral blood (p=0.25). Prevalence of malaria infection was however significantly high in placenta than peripheral blood among those who used a mosquito net than those who did not use any mosquito net. Higher prevalence of malaria infection recorded among nonusers of a net was in line with a previous report in Blue Nile state of Sudan and malaria endemic countries of East Africa where not using bed nets was found a risk factor for placental malaria [29,48].

3.9. Influence of Malaria Infection During Pregnancy and Antimalarial Treatment on Peripheral and Placental Blood Infection by *Plasmodium* at Delivery

Examination of placental blood detected *Plasmodium* infections both among women who experienced malaria symptoms and those who were asymptomatic during pregnancy. All women who had malaria during their last pregnancy were treated accordingly in the antenatal clinic, indicating that presence of malaria parasites in their placenta may be due to partial placenta parasitemia

clearance or a reinfection which could have occur after the said treatment and that though they were treated, there exist a risk of congenital malaria to their offspring though their peripheral blood was *Plasmodium* free.

Among the 27 parturient who were treated with an antimalarial during the pregnancy, 29.6% had placental malaria. The prevalence of *Plasmodium* infection in peripheral blood was 0%. This result indicates that placental malaria may remain in pregnant woman at delivery despite antimalarial treatment.

4. Conclusion

Data from this study indicated that one out of four pregnant women at delivery had placental malaria whereas this ratio was one out of ten pregnant women in peripheral blood testing. Being of young maternal age, low educational level, blood group O and haemoglobin type O were associated to peripheral blood and placental infection by Plasmodium. Having swallowed less than four IPT-SP and not experiencing fever during pregnancy was associated with Plasmodium infection in peripheral and placental blood. Being primigravida, primiparous and having undergone less than four antenatal visits were associated with peripheral blood infection only. The authors of study recommend systematic malaria testing for malaria during pregnancy up to delivery.

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Competing Interest

The authors of this manuscript declared that no competing interests exist for this study. This study was fully financed by the research team.

Authors' Contributions

TK, ASE designed the study. TK, NA, DCKM collected data. TK and NA analyzed data. TK, NA, DCKM and ASE wrote the manuscript. All authors read and approved the final manuscript.

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