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Prevalence, Associated Factors and Treatment Outcomes of Laboratory-confirmed Pregnancy Malaria at Antenatal Care in Three Healthcare Facilities of Douala, Cameroon

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Authors' contributions

This work was carried out in collaboration among all authors. Authors TK and ASE designed the study. Authors TK, HE, LBM, NA and CKM collected the data. Authors TK and HE analyzed the data. Authors TK, NA and CKM wrote the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Pregnancy malaria is a life-threatening condition to the mother, the fetus and the newborn. Since the implementation of the World Health Organization's recommendations of specific malaria control strategies to pregnant women in malaria endemic countries, evaluation studies are needed to

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assess the prevalence of malaria in pregnancy. This cross-sectional prospective study was therefore set to determine the prevalence and associated factors as well as the treatment outcomes of *Plasmodium* infection among pregnant women attending antenatal care in three hospitals of Douala in Cameroon.

Each volunteered pregnant woman received for antenatal care was questioned according to the study questionnaire to collect sociodemographic data, use of malaria prevention tools and pregnancy history. Then a laboratory test was carried for *Plasmodium* detection in the peripheral blood using microscopy and a HRP2-based rapid diagnostic test. *Plasmodium* carrying pregnant women were treated according to the national malaria program scheme followed by post-treatment follow-up. Data were processed and analyzed using SPSS.20 software. Association of *Plasmodium* 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.

A total of 487 pregnant women aged between 18 years and 42 years were included in the study. The prevalence of *Plasmodium* infection was 3.5% and 4.3% by malaria rapid diagnostic test and microscopy respectively. Only *Plasmodium falciparum* asexual stage was detected. Parasite loads were low. Factors associated with *Plasmodium falciparum* higher prevalence were being less than 21 years old (0.02), not sleeping under mosquitoe net daily (p=0.04) and having fever during the pregnancy (p=0.04). Parenteral treatment with either artesunate, artemether or quinine sulfate showed good efficacy.

Conclusion: Pregnant women attending the antenatal cares harbored low *Plasmodium* infection prevalence and loads. Being of young age, not sleeping under mosquitoe bednet daily and having fever during the pregnancy were predictive *Plasmodium falciparum. Plasmodium* infected pregnant women were successfully treated with antimalarial medications recommended by the national Ministry of Public Health. The authors recommend increasing efforts by health authorities to strengthen malaria prevention in pregnant women through more adherence to sleeping under ITNs daily and taking IPT-sp as well as systematic detection of *Plasmodium* infection at each antenatal visit.

Keywords: antimalarial treatment, factors, Plasmodium falciparum, pregnancy, Cameroon.

1. INTRODUCTION

Malaria is a preventable and treatable disease which continues to have a devastating impact on people's health and livelihoods around the world with the huge burden reported in Africa [1,2,3]. In 2017, an estimated 219 million malaria cases and about 435 000 malaria-related deaths were recorded, the sub-Saharan Africa accounted for 92% malaria cases and 93% deaths [2,3]. Pregnant women and under five years children remain the most vulnerable group for malaria infection. In moderate and high malaria transmission countries in the WHO African Region, an estimated 32% pregnancies were exposed to malaria infection, the west Africa and central Africa having the highest prevalence of exposure during pregnancy, the east and southern Africa having the least prevalence [4]. The problematic nature of malaria in pregnancy is also of concern in almost all regions of Cameroon as transmission vary from moderate to high in the country including the Littoral region [5].

Plasmodium infected pregnant woman is particularly at risk of severe and lifethreatening outcomes like its fetus and the newborn [3,6]. Complications due to *Plasmodium* infection occur both among asymptomatic and symptomatic pregnant women [7,8,9,10]. Frequently reported pregnancy malaria related outcomes in symptomatic mothers include maternal anemia, placental malaria and maternal death [7,9,10]. The fetus and the newborn born to *Plasmodium* infected mother are at high risk of intrauterine growth retardation, stillbirth, preterm delivery, low birthweight, neurodevelopmental delay like delay language development, congenital malaria and early neonatal death [6,9,11,12,13,14,15]. Peripheral blood *Plasmodium* infection have been reported both among symptomatic as well as asymptomatic pregnant women at antenatal visits in Ethiopia with higher infection prevalence among symptomatic [16]. Among the Ethiopian *Plasmodium* infected asymptomatic pregnant women, significant decreased hemoglobin level was reported [8]. Occurrence of anemia among asymptomatic pregnant women infected by *Plasmodium* called for the need implement specific interventions during pregnancy irrespective to symptomatology status to limit health consequences of malaria infection in the mother and offspring.

To overcome the high burden of malaria transmission areas, the World Health Organization (WHO) has recommended in its strategic plan three specific key interventions in vulnerable groups namely prevention of transmission, prevention of morbidity and mortality, coupled to prompt diagnostic and treatment of suspected malaria cases as part of universal health coverage [3,17]. To prevent transmission of *Plasmodium* to humans, the WHO recommended sleeping under a mosquitoe bednet. In this regard, public health ministries in malaria endemic countries proceeded through mass distribution of insecticide-treated bednet (ITNs) to all at risk groups including and under five years children coupled to sensitization for indoor residual spraying (IRS). Distribution to pregnant women took place at antenatal care units. Previous reports indicated significant reduction of pregnancy malaria in many endemic countries [3,13,17].

For prevention of malaria-related morbidity and even mortality in pregnant woman, the WHO has recommended since 2012 in moderate to high transmission settings of sub-Saharan Africa chemoprevention with sulfadoxine-pyrimethamine at antenatal care starting as early as possible in the second trimester of pregnancy [3,17]. For this strategy which also named intermittent preventive treatment in pregnancy (IPTp), at least three doses IPTp should be given to all pregnant women at antenatal clinic visit before delivery. The chemoprevention with sulfadoxine-pyrimethamine strategy in sub-Saharan Africa showed significant reduction maternal anemia, low birthweight and perinatal mortality among pregnant women who took at least three doses of IPTp during pregnancy [7,17]. Increased IPT-sp coverage among pregnant women in malaria endemic countries resulted in up to 47.5% low birthweights averted [4].

The prompt diagnosis and treatment strategy of malaria cases was considered as the most effective way to prevent a mild malaria case from developing into severe disease and even death. Therefore, the WHO recommends early and accurate laboratory detection of any suspected malaria case using a combination of at least microscopy and a histidine-rich protein-2-based malaria rapid diagnostic test (HRP2-RDTm) [3,5,6,18,19,20,21]. Using the two laboratory methods simultaneously has the advantage to combine a quantitative method namely microscopy which determines *Plasmodium* species and stages with an easy to make qualitative and highly sensitive method [1,22]. Prompt diagnosis is advantageous as it enables gathering knowledge on the prevalence of malaria in pregnant women with respect to associate factors therefore helping to recommend adapted quality of care, malaria prevention strategies and treatment given to this vulnerable group during antenatal care.

The three key interventions are implemented in Cameroon to pregnant women at antenatal care by the public health ministry since more than a decade precisely since 2011 for mass distribution of insecticide treated bednets, and since 2012 for implementation of the chemoprevention of pregnancy malaria sulfadoxine/pyrimethamine [5]. Since the launching of these malaria control strategies in pregnant women, few evaluation studies have been conducted so far to assess the prevalence of *Plasmodium* infection among pregnant women as well as the treatment outcomes in respect of specific therapeutic options adopted in many African countries. Previous reports in some African malaria endemic countries reported moderate to high prevalence of pregnancy malaria including Nigeria [23,24,], Ethiopia [25,26] and Ghana [27]. Previous studies among febrile pregnant women in Cameroon before the launching of the prevention strategies in 2011 reported high prevalence of pregnancy malaria in fourth or fifth category health facilities in almost rural areas of the south-west region [28]. Data from a second category health facility in the urban area of Yaoundé reported a moderate prevalence of *Plasmodium* among febrile pregnant women [29]. In almost all studies, the most frequently reported associated factors to pregnancy malaria were early pregnancy age, primigravidae, not sleeping under insecticide treated bednet and insufficient IPT-sp [23,26,28,29]. According to a hospital-based study in an urban area of Cameroon where pregnancy malaria was a risk factor of low birth weight, taking at least three doses of IPT-sp was associated to reduced risk of low birthweight [30].

Previous data in endemic areas of Ethiopia which reported *Plasmodium* infection both among symptomatic and asymptomatic pregnant women as well as significant hemoglobin decreased among the Plasmodium infected pregnant women was indicative that a systematic accurate and specific laboratory detection of malaria parasites should be addressed to all pregnant attending antenatal visits using national

recommended techniques namely microscopy and a quality assured malaria diagnostic test. In this regard, HRP2-RDTm have shown high sensitivity and specificity in the detection of peripheral blood *P. falciparum* infection among febrile pregnant women including Cameroon [28,29], Uganda [13]. HRP2-RDTm have shown high sensitivities as diagnostic tool in the management of suspected uncomplicated malaria received in health facilities nationwide using microscopy as gold standard in Ghana [27] and Cameroon [31].

This cross-sectional study aimed to assess the prevalence of *Plasmodium* infection in pregnant women in peripheral blood using microscopy detection in Giemsa-stained blood smears and «One Step Malaria HRP-II (P.f) and the pLDH (Pan) Antigen Rapid» combination test as malaria rapid diagnostic test, as well as identify factors associated to *Plasmodium* carriage and treatment outcomes of Plasmodium carrying pregnant women.

2. METHODOLOGY

2.1 Study Type, Period and Place

This was a cross-sectional and analytical study done between 2018 and 2019 in the gynecology units of three Douala-based healthcare facilities in Cameroon including two second category hospitals of the national health pyramid namely the Laquintinie Hospital of Douala and the Gynecology-Obstetric and Pediatric Hospital of Douala, and the Bonassama District Hospital which is a fourth category hospital. These health facilities are high standing hospitals with specialized gynecologists, pediatricians, and other medical specialists as well as trained nurses, and trained laboratory technicians. Laboratory detection of *Plasmodium* infection were undergone in the laboratories of these health facilities and cross checked 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.

2.2 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, the Ethic Committee of each of the three hospitals, and the Regional Delegation of Public Health of the Littoral region in Douala. The Institutional Ethic Committee of the University of Douala granted an ethical clearance for this study. Each of the hospitals and the regional delegation of public health in Douala gave a research authorization to undergo the study. During the study, the participation in the study of each pregnant woman who attended gynecology units of the study hospitals for either antenatal visit was sought by a study investigator through approaching her, presenting and explaining the study protocol. Each pregnant woman who volunteered to participate in the study was asked to sign the study written informed consent before here inclusion. The study inclusion criteria included: i) all pregnant women who attended the gynecology ward for antenatal visit irrespective of any suspicion of malaria; ii) who gave a written informed consent for its participation into the study. Any pregnant woman who had taken any antimalarial treatment in the preceding two weeks was not included in the study.

2.3 Data Collection

For each pregnant who fulfilled the study criteria, data related to the participant were collected from her medical file. Then *Plasmodium* infection testing was undergone immediately using peripheral blood drops from a finger prick of the participant to make a thin and a thick blood smear for microscopy detection and a HRP2/pLDH combination malaria rapid diagnostic test. The microscopy examination of stained blood smears was chosen because it is considered as the foremost gold standard for routine diagnosis of malaria infection in medical laboratory and research settings since it has the advantages that it indicates the *Plasmodium* specie and the stages, it is quantitative and therefore provide information required for *Plasmodium* detection and follow-up of treatment efficacy [1,32]. Data collected from the file of the participant were age, parity, gravidity formula, gestational age, bednet use, number of antenatal visits undergone, number of IPT-SP taken during the pregnancy, history of any fever during the pregnancy.

Thin and thick blood smears were air dried then stained with a 10% Giemsa solution, then examined at high magnification under a light microscope according to standard laboratory guidelines [1,32]. Giemsa-stained thick and thin

blood smears were blinded examined by two trained microscopists, and discrepant readings cross-checked by the study investigators. Microscopy results were expressed as number of each *Plasmodium* stage per µl of blood after examining fields in the thick film for 500 white blood cells (WBC) considering the results from its full blood counts as reported in the patient's file. A thick blood film was considered negative after review of 300 high-magnification microscopic fields. *Plasmodium* load was then 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%).

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

2.4 Treatment Outcomes of the Laboratory-confirmed Congenital Malaria Cases

Laboratory-confirmed malaria cases were treated immediately according to national regulations trough parenteral administration of monotherapies using artemisinin derivatives or quinine sulfate [20,21]. Monotherapy treatment with artemisinin derivatives were administered according to one of the following three regimen: 1/ three intramuscular injections of artesunate as follow: 2.4 mg /kg at inclusion, 12 hours and 24 hours after inclusion, then once daily. 2/ intramuscular injection of artemether once daily in five consecutive days as follow: 80 mg twice the first day and 80 mg daily from the second day to fifth day followed by an Artemether-Lumefantrine treatment for three consecutive days. Quinine was administered by intravenous route as follow: 8.3 mg/kg of quinine each hour for 7 consecutive days. Parasitological control tests were done three days, seven days and 14 days after treatment start to assess anti-malaria treatment efficacy.

2.5 Data Analysis

Data analysis was processed using SPSS.20 software. The data was analyzed in relation to maternal age, gravidity status, parity, gestational age, mosquitoe bednet use, IPT-SP compliance and occurrence of fever 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

As indicated in the table below, a total of 487 pregnant women aged between 18 years and 42 years were included in the study. The mean age of participants was 30±4.3 years. Pregnant women aged between 20 years and 30 years were the most represented group (48.9%) and those aged between less than 20 years the least represented (3.9%). Paucigravidae were predominant (49.5%) and primigravidae were the least represented (20.3%). Pregnant women in the third trimester were predominant (61%) and those in the first trimester the least represented (4.8%). With respect to sleeping under a mosquitoe bednet daily, pregnant women who used mosquitoe bednet were predominant (70%). The proportion of pregnant women who used a bednet was higher than the 65.5% national rate in 2018 [20].

3.1 Plasmodium Infection Prevalence and Parasite Loads

Prevalence of *Plasmodium* infection was 4.3% by microscopy and 3.5% through malaria RDT. *P. falciparum* was the only *Plasmodium* specie detected using both techniques in this study. Prevalence recorded in this study was low compared to previous data in Cameroon using microscopy which reported higher prevalence of *Plasmodium* infection in symptomatic pregnant women up to 69.2% by microscopy and 77.9% by a HRP2-based RDT in a Yaoundé-based second category hospital [29], as well as data from a cohort study in fourth category hospitals in the South-West region of Cameroon with prevalence up to 43.2% by microscopy at the first antenatal visit and 6.8% at the third antenatal [28]. The low prevalence recorded herein may be related to inclusion of both symptomatic and

asymptomatic pregnant participants or to a better adherence of pregnant women to prevention measures recommended for prevention of malaria. In fact, all participants had taken IPTp at least once. Also, 70% of the participants used a mosquitoe net, a proportion higher than the national rate (65.5%) reported by the national malaria control program [20,21]. However, higher prevalence obtained by microscopy than RDTm was of concern since the latest was recommended by the WHO due to its higher sensitivity compared to microscopy. Data recorded was not in line with previous reports in Senegal [33], in a Yaoundé-based second category hospital [29] and in Ghana [27] which indicated higher prevalence with HRP2-based RDTm than microscopy in laboratory detection of malaria parasites among symptomatic pregnant women. The difference in prevalence between microscopy and RDTs with the studies mentioned above might be related to either the fact that pregnant women included harbored predominantly low *P. falciparum* loads, or to any parasite lineages deletions in genes coding for HRP2 antigens. In this regard, many HRP2-based malaria RDTs have been reportedly low sensitive in the detection of low-density infections therefore leading to frequent false negative results [34]. Existence of *Plasmodium* lineages deletions in genes coding for HRP2 antigens could also well explain the least sensitivity of the HRP2-based RDTm. In fact, previous data in Erithrea detected *Plasmodium* lineages deletions in genes encoding for HRP2 as the main factor of increasing HRP2/pLDH failure rate to detect microscopically confirmed *P. falciparum* infections and *P. vivax* infections in the country [35].

However, data from this study corroborated previous report in Indonesia, a country co-endemic for *P. falciparum, P. vivax* and *P. malariae* where microscopy detected higher but not significant prevalence of *Plasmodium* infection in asymptomatic pregnant women than RDTm, and higher sensitivity of microscopy than RDTm using PCR as reference [36]. In a mixed *P. falciparum* and *P. malariae* mixed transmission area in Central African Republic, the most common used HRP2 antigen detecting RDTm namely CareStart™Malaria (Pf)®, SD Bioline Malaria Antigen (Pf)® and Paracheck (Pf)®, showed lower sensitivity than microscopy and that given by the manufacturer [34]. Similar report of higher prevalence of microscopy than RDTs in the detection of *Plasmodium* infections in pregnant women were reported in Columbia [37] and Nigeria [24]. However, our data recorded false negative results with RDTm as also previously reported in symptomatic pregnant women in Cameroon who harbored high parasitemia [29]. Data gathered in this study corroborated previous data in Eritrea where high false-negativity rate were recorded with HRP2-based RDTm among monospecific microscopically confirmed *P. falciparum* and *P. vivax* up to 80% and 7.7% respectively [35]. Occurrence of such false negative in microscopically confirmed *P. falciparum* infected participants may be due either HRP2 deletions as reported in Eritrea [35], or to storage conditions as previously reported in remote areas in Brazilian amazon Region [38]. Further studies will enable confirm the main cause of such false negative results with HRP2-based RDTm.

Regarding the presence of only monospecific *P. falciparum* infection recorded by both microscopy and HRP2-based RDT in peripheral blood samples of pregnant women at antenatal clinic, similar result has been reported in previous studies with microscopy among Cameroonian pregnant women [28,29,39]. However, using more sensitive techniques like molecular biology may have detected more species as reported earlier in a study among Cameroonian pregnant women in which PCR detected in addition to *P. falciparum* as the leading specie, *P. malariae* and *P. ovale* [39].

Plasmodium parasites loads were predominantly low (range: 325-1375 asexual stages/µl of blood). Of the infected participants, 71.4% had a *Plasmodium* load less than 1000 trophozoites/µl of blood, the remaining having more than 1000 trophozoites/µl of blood. These low parasitemia may be due to use of IPT-SP and could explain low prevalence recorded with RDT as indicated in a previous report which in a multi-country study, found CareStart Malaria HRP-2/pLDH(pf /pan) Combo Test to be less sensitive than microscopy in blood samples with *Plasmodium* loads less than 1000 trophozoites/µl of blood [40].

3.2 Associated Factors to *Plasmodium* Infection Prevalence

According to table below, age significantly influenced prevalence of *Plasmodium* infection. *Plasmodium* infection was recorded only among under 35 years old pregnant women with significant higher prevalence found among less than 21 years old participants both by

Table 1. Prevalence of *Plasmodium* infection among pregnant women attending antenatal care in Douala

Factors		Sample size (%)	Rapid Diagnostic Test		Microscopy	
			Positive	Negative	Positive	Negative
Age groups	<21 years	19(3.9)	3(15.8)	16(84.2)	4(21.1)	15(78.9)
	21-30 years	238(48.9)	9(3.8)	229(96.2)	11(4.6)	227(95.4)
	31-35 years	201(41.3)	5 (2.5)	196(97.5)	6(3.0)	195(97.0)
	≥35 years	29(5.9)	0 (0)	29(100)	0(0)	29(100)
	Total	487(100)	17(3.5)	470(96.5)	21(4.3)	466(95.7)
	P		*0.01**		*0.02**	
Gravidity	Primigravidae	99(20.3)	9(9.1)	92(90.9)	9(9.1)	90(90.9)
	Paucigravidae	241(49.5)	5(2.1)	237(97.9)	7 (2.9)	234(97.1)
	Multigravidae	147(30.2)	3(2.1)	145(97.9)	5(3.4)	142(96.6)
	P		*0.7*		*0.1*	
Parity	Primiparous	258(53)	12(4.6)	246(95.4)	15(5.8)	243(94.2)
	Pauciparous	135(27.7)	4(2.9)	134(97.1)	5(3.7)	130(96.3)
	Multiparous	94(19.3)	1(1.1)	94(98.9)	1(1.1)	93(98.9)
	P		*0.9*		*0.8*	
ITN use daily	Yes	341(70.0)	9(2.6)	332(97.4)	9(2.6)	332(97.4)
	No	146(30.0)	8(5.5)	140(94.5)	12(8.2)	134(91.8)
	P		*0.05*		*0.04**	
IPT-SP	1	214(44.0)	10(4.7)	204(95.3)	11(5.1)	203(94.9)
	2	102(20.9)	5(4.9)	97(95.1)	6(5.9)	96(94.1)
	≥3	171(35.1)	2(1.2)	169(98.8)	4(2.3)	167(97.7)
	P		*0.5*		*0.8*	
Gestational age	1st trimester	62(12.7)	3(4.8)	59(95.2)	3(4.8)	59(95.2)
	2nd trimester	128(26.3)	5(3.9)	123(96.1)	6(4.7)	122(95.3)
	3rd trimester	297(61.0)	9(3.0)	288(97.0)	12(4.0)	285(96.0)
	P		*0.3*		*0.7*	
Fever in pregnancy	Yes	16(3.3)	6(37.5)	10(62.5)	5(31.3)	11(68.7)
	No	471(96.7)	11(2.3)	460(97.7)	16(3.4)	455(96.6)
	P		0.03*		0.04*	

ITN: insecticide treated bednet. IPT-SP: intermittent preventive treatment with sulfadoxine/pyrimethamine

RDTm (p=0.01) or microscopy (p=0.02). These data were in line with previous report in Ghana where young age pregnant women were the most infected group among women with pregnancy malaria as well as occurrence of anemia [9], at Plateau Joss in Nigeria with both microscopy and HRP2-RDTm among symptomatic pregnant women attending antenatal clinic [24] and in Tanzania among asymptomatic pregnant women by microscopy [10]. A similar trend was reported in West Ethiopia where early pregnancy age was associated with pregnancy malaria [26]. Highest vulnerability recorded in young than older pregnant women may be related to an acquired immunity with age. Results from this however contrasted data from Ekiti state in Nigeria where oldest pregnant women had the highest prevalence [41].

Gravidity did not significantly influenced prevalence of *Plasmodium* infection though primigravidae had the highest prevalence both by RDTm (7.1%) or microscopy (13.1%) while multigravidae had the least prevalence irrespective to the diagnostic technique (p= 0.1). Data gathered in this study were in accordance with previous reports among symptomatic pregnant women at Benin city and Imo state in Nigeria [23,42] and West Ethiopia [26], and in Southeast Tanzania among asymptomatic pregnant women [10] which found primigravidae as a main risk factor of pregnancy malaria and anemia. However, these data contrasted with an earlier report at Plateau Joss and Ekiti state in Nigeria which found highest prevalence among multigravidae [24,41].

According to parity, Primiparous were the most represented (53%) and multiparous the least represented (19.3%). *Plasmodium* infection prevalence was highest among primiparous irrespective to the diagnostic technique than pauciparous and multiparous (p=0.8). This

observation may be related to an acquired partial immunity in multiparous. A previous report from Ghana indicated that being nulliparous at the first antenatal visit as a major risk factor for pregnancy malaria among pregnant women attending antenatal visit in health facilities in Ghana [9].

Gestational age had no significant influence on the prevalence of *Plasmodium* infection both by microscopy (p=0.7) and by RDTm (p=0.3). However, *Plasmodium* infection prevalence showed an increase with gestational age according to results with RDTm whereas microscopy detected a decrease. Data gathered with RDT corroborated previous reports in Nigeria which found increasing prevalence of *P. falciparum* with gestational age among pregnant women attending antenatal care in Nigeria either using microscopy alone [23,41,43] or when combining microscopy and HRP2-RDTm [24].

Data gathered with microscopy corroborated previous reports with microscopy alone detected a sharp decrease of malaria prevalence with increasing antenatal visits or gestational age among pregnant women attending antenatal care in the South-West Cameroon [28] and Nigeria [23,43]. A similar decrease trend of *Plasmodium* infection prevalence with gestational age was reported in Nigeria using both microcopy and a HRP2-based RDTm [24]. Such a decrease may be due to previous treatment of malaria cases found at the first antenatal visits.

In this study, each of the participating pregnant woman had taken IPT-sp at least once. The *Plasmodium* infection prevalence decreased with increasing IPT-sp doses by RDTm (p=0.5), while microscopy detected an increased in *Plasmodium* infection prevalence in pregnant women who had swallowed two IPT-sp followed by a decrease with increasing IPT-sp doses (p=0.8). This result encourages to improve the quality of malaria prevention as earlier reported in Benin where pregnant women who took at least three doses of IPT-sp during pregnancy had significantly reduced chances to born underweight child [7]. A similar benefit of IPT-sp on morbidity was reported in Ghana where six years after of implementation of IPT-sp, a decrease of 33% and between 43 to 57% was noticed in anemia and placental malaria respectively among pregnant women [44].

Prevalence of *Plasmodium* infection was higher though not significantly among pregnant women who did not sleep under an ITN daily irrespective to the diagnostic test. This finding corroborated previous reports in Ghana [9], Plateau Joss in Nigeria [24] and in West Ethiopia [26] where nonuse of a mosquitoe net was a major associated factor to pregnancy malaria among pregnant women attending antenatal visits. This indicated that, mosquitoe bednet use remain a powerful tool against transmission of malaria parasites, therefore sustaining WHO recommendations. However, a study at Benin City in Nigeria found no association between malaria para8sitemia in pregnant women and use of any malaria prevention method [42].

Plasmodium infection was recorded both among asymptomatic and febrile pregnant women with highest prevalence occurring among the latest group both by RDTm (P=0.03) and microscopy (p=0.04). The prevalence of *Plasmodium* infection both by microscopy and RDT was higher among symptomatic pregnant women than asymptomatic. This finding was in line with previous reports in Ethiopia [8,16]. In a longitudinal study carried out in a malaria perennial transmission area in Cameroon, few *P. falciparum* parasites carrying pregnant women in peripheral blood were found symptomatic, all pregnant women had placental malaria, and paucigravidity was the main associated factor to *P. falciparum* infection and anemia [45]. Presence of *Plasmodium* infection among asymptomatic pregnant called for implementation of systematic screening for detection of *Plasmodium* infection in all pregnant women at antenatal visit irrespective to existence of any malaria like symptom.

3.3 Antimalarial Treatment Outcomes among *Plasmodium* infected Pregnant Mothers

After antimalarial treatment of *Plasmodium* infected pregnant, subsequent microscopy as well RDTm were negative at the seventh day follow-up. Although *Plasmodium* infected mothers were successfully with recommended antimalarials, prevention of the infection should be strengthened to prevent occurrence of any malaria related adverse event. In fact, as indicated in a systematic review, pregnancy malaria associated adverse outcomes including stillbirth, preterm delivery, low birthweight and placental malaria were still reported in pregnant women who harbored higher parasitaemia by WWARN among *Plasmodium* infected pregnant women appropriately treated with commonly

recommended antimalarial drug namely quinine and artemisinin-based combination therapies [46]. The magnitude of such adverse outcomes was not mentioned by the authors. Persistence of malaria related adverse advents after an appropriate treatment of detected pregnancy malaria indicated that effective malaria prevention in pregnant women remains the main way to reduce malaria-associated adverse birth outcomes.

4. CONCLUSION

Prevalence of pregnancy malaria in the antenatal care unit of the three hospitals of Douala was low. Infected pregnant women harbored monospecific asexual *Plasmodium falciparum* with predominantly low parasite loads. Young age, not using insecticide treated bednet and having fever during pregnancy were associated with pregnancy malaria. *Plasmodium* infected pregnant women were effectively treated using the national recommended antimalarial. This study calls for increased awareness of pregnant women regarding prevention and control measures of malaria in the study hospitals.

CONSENT AND ETHICAL APPROVAL

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 and each pregnant woman who volunteered to participate in the study was asked to sign the study written informed consent before here inclusion.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization. Severe falciparum malaria. World Health Organization, Communicable Diseases Cluster. Trans R Soc Trop Med Hyg 2000; 94(Suppl 1):1–90
2. World Health Organization. World malaria report 2018. World Health Organization 2019a;210.
3. World Health Organization. Global technical strategy for malaria 2016–2030, 2021 update. World Health Organization. 2021; 40. Licence: CC BY-NC-SA 3. 0 IGO
4. World Health Organization. WHO malaria report 2022. World Health Organization. 2022;372.
5. Ministry of Public Health of Cameroon. Profil sanitaire analytique 2016–Cameroun; 2018. [In French]. Available:http:/www.afro.who.int/fr/Cameroun/ consulted
6. World Health Organization. Report on malaria in the world 2016. WHO/HTM/GMP.2017;4: 24.
7. Biaou COA, Kpozehouen A, Glèlè-Ahanhanzo Y, Ayivi-Vinz G, Ouro-Koura A-R, Azandjèmé C. Sulfadoxine-pyrimethamine-based intermittent preventive treatment in pregnant women and its effect on birth weight: application of 3-dosing regimen in the urban area of South Benin in 2017. PAMJ. 2019;34: 155. DOI:10.11604/pamj.2019.34.155.19357 [article in French]
8. Feleke DG, Adamu A, Gebreweld A, Tesfaye M, Demisiss W, Molla G. Asymptomatic malaria infection among pregnant women attending antenatal care in malaria endemic areas of North-Shoa, Ethiopia: a cross-sectional study. Malar J. 2020;19:67. Available:https://doi.org/10.1186/s12936-020-3152-9
9. Fondjo LA, Addai-Mensah O, Annani-Akollor ME, Quarshie JT, Boateng AA, Assafuah SE et al. A multicenter study of the prevalence and risk factors of malaria and anemia among pregnant women at first antenatal care visit in Ghana. PLoS One. 2020;15(8):e0238077. Available:https://doi.org/10.1371/journal.pone.0238077
10. Mlugu EM, Minzi O, Kamuhabwa AAR, Aklillu. Prevalence and correlates of asymptomatic malaria and anemia on first antenatal care visit among pregnant women in Southeast, Tanzania. Int J Environ Res Public Health. 2020;17(9):3123.

DOI: 10.3390/ijerph17093123.
PMCID: PMC7246851.

11. Omer SA, Idress HE, Adam I, Abdelrahim M, Noureldein AN, Abdelrazig AM *et al*. Placental malaria and its effect on pregnancy outcomes in Sudanese women from Blue Nile State. Malar J. 2017;16(1):374.
DOI: 10.1186/s12936-017-2028-0.
12. Oladeinde BH, Omoregie R, Odia I, Oladeinde OB. Prevalence of malaria and anemia among pregnant women attending a traditional birth home in Benin City, Nigeria. Oman Medical Journal. 2012 ; 27(3): 232-236.
DOI 10. 5001/omj.2012.52.
13. Kyabayinze D, Tibenderana JK, Nassali M, Tumwine LK, Riches C, Montague M *et al*. Placental *Plasmodium falciparum* malaria infection: operational accuracy of HRP2 rapid diagnostic tests in a malaria endemic setting. Malaria Journal. 2011;10:306. Available:http://www.malariajournal.com/content/10/1/306
14. Mahamar A, Andemel N, Swihart B, Sidibe Y, Gaoussou S, Barry A *et al*. Malaria infection is common and associated with perinatal mortality and preterm delivery despite widespread use of chemoprevention in Mali: an observational study 2010 to 2014. Clin Infect Dis. 2021;73(8):1355-1361.
DOI:10.1093/cid/ciab301
PMCID: PMC8528392 PMID: 33846719
15. Weckman AM, Conroy AL, Madanitsa M, Gnaneswaran B, McDonald CR, Kalilani-Phiri L et al. Neurocognitive outcomes in Malawian children exposed to malaria during pregnancy: An observational birth cohort study. PLoSMed. 2021;18(9): e1003701.
DOI: 10.1371/journal.pmed.1003701.
PMCID: PMC8478258. PMID: 34582452
16. Tegegne Y, Asmelash D, Ambachew S, Eshetie S, Addisu A, Zeleke AJ. The prevalence of 2malaria among pregnant women in Ethiopia: A systematic review and meta-analysis. Journal of Parasitology Research. 2019;8396091:9.
Available:https://doi.org/10.1155/2019/8396091
17. World health organization. World malaria report; 2017.
18. World Health Organization. World Malaria Report. 2014;28.
19. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). Am J Trop Med Hyg. 2007;77(6 Suppl):119-127.
20. Ministry of Public Health. National strategic plan for control of malaria in Cameroon 2019-2023. National Program for Malaria Control. 2019;103.
21. Ministry of Public Health. Guide de prise en charge du paludisme au Cameroun à l'usage du personnel soignant. National Program for Malaria Control. 2019;71.
22. World Health Organization. Guidelines for the Treatment of Malaria–2nd edition. Geneva: World Health Organization; 2010.
23. Frank MD, Robinson–Bassey GC, Akaeze GO. Prevalence of malaria parasitaemia among pregnant women attending three selected health centers in Ideato south local government area, Imo state. Obstet Gynecol Int J. 2016;4(3):103–106.
24. Yakubu DP, Kamji NB, Dawet A. Prevalence of malaria among pregnant women attending antenatal care at Faith Alive Foundation, Jos Plateau State, Nigeria. Noble International Journal of Scientific Research. 2018;2(4):19-26. ISSN(e): 2521-0246 ISSN(p): 2523-0573
25. Gontie G B, Wolde H F, Baraki A G. Prevalence and associated factors of malaria among pregnant women in Sherkole district, Benishangul Gumuz regional state, West Ethiopia. BMC Infectious Diseases (2020) 20:573. Available:https://doi.org/10.1186/s12879-020-05289-9
26. Almaw A, Yimer M, Alemu M, Tegegne B. Prevalence of malaria and associated factors among symptomatic pregnant women attending antenatal care at three health centers in north-west Ethiopia. PLoS ONE. 2022;17(4):e0266477. Available:https://doi.org/10.1371/journal.pone.0266477
27. Abuaku B, Amoah LE, Peprah NY, Asamoah A, Amoako EO, Donu D et al. Malaria parasitaemia and mRDT diagnostic performances among symptomatic individuals in selected health care facilities across Ghana. BMC Public Health. 2021;21:239.
DOI: 10.1186/s12889-021-10290-1.
PMCID: PMC7844948. PMID: 33509161
28. Asoba GN, Ndamukong KJN, Achidi EA. Prevalence of malaria parasite infection in pregnant women in three towns of the

South West Region of Cameroon. Journal of the Cameroon Academy of Sciences. 2009;8(2/3):71-77.

29. Ebong CE, Ali IM, Fouedjio HJ, Essangui E, Achu DF, Eyong L, Sama D. Diagnosis of malaria in pregnancy: accuracy of CareStart™ malaria Pf/PAN against light microscopy among symptomatic pregnant women at the Central Hospital in Yaoundé, Cameroon. Malar J.2022;21:78. DOI: 10.1186/s12936-022-04109-6. PMCID: PMC8905860. PMID: 35264170
30. Ebode Ela M, Nambile Cumber S, Djouedjon Dakenyo R, Djuissi Tekam D, Biyong Heumou PC, Lowe Marvin G et al. Association between malaria and low birth weight in Yaoundé, Cameroon. Pan African Medical Journal. 2019;33: 127. DOI: 10.11604/pamj.2019.33.127.18101 [Article in French].
31. Ali I, Bigoga J, Forsah A, Cho-Ngwa F, Tchinda V, *et al*. Field evaluation of the 22 rapid diagnostic tests for community management of malaria with artemisinin combination therapy in Cameroon. Malaria Journal. 2016;15:31.
32. World Health Organization. Malaria microscopy quality assurance manual. Version 2. Geneva: World Health Organization. 2015;140.
33. Faye B, Nath-Chowdhury M, Tine RC, Ndiaye JL, Sylla K, Camargo FW et al. Accuracy of HRP2 RDT (Malaria Antigen P.f H) compared to microscopy and PCR for malaria diagnosis in Senegal. Pathogens and Global Health; 2013. DOI: 10.1179/2047773213Y.0000000102·
34. Pamatika CM, Tepka G, Balekouzou A, Nambei WS, Moyen JM, Abrou-Pionendji F et al. Comparative study of the performance of three rapid screening tests for Plasmodium falciparum malaria at the Center for prevention and treatment of malaria, Friendship Hospital in the Central Republic of Africa. Médecine d'Afrique Noire. 2017;64(11):537-543. [In French]
35. Berhane A, Russom M, Bahta I, Hagos F, Ghirmai M, Uqubay S. Rapid diagnostic tests failing to detect *Plasmodium falciparum* infections in Eritrea: an investigation of reported false negative RDT results. Malaria Journal. 2017 ;16: 105. Available:https://doi.org/10.1186/s12936-017-1752-9
36. Ahmed R, Levy EI, Maratina SS, de Jong JJ, Asih PBS, Rosi IE et al. Performance of four HRP-2/pLDH combination rapid diagnostic tests and field microscopy as screening tests for malaria in pregnancy in Indonesia: a cross-sectional study. Malar J. 2015;14:420. DOI: 10.1186/s12936-015-0943-5 PMCID: PMC4625567 PMID: 26511932
37. Vasquez AM, Zuluaga L, Tobon A, Posada M, Vélez G, Gonzalez IJ et al. Diagnostic accuracy of loop-mediated isothermal amplification (LAMP) for screening malaria in peripheral and placental blood samples from pregnant women in Colombia. Malar J July.2018;17:262. DOI: 10.1186/s12936-018-2403-5. PMCID: PMC6044080. PMID: 30005616
38. Gomes LT, Tada MS, Katsuragawa TH, Póvoa MM, Viana GMR, Alecrim MGC et al. Low sensitivity of malaria rapid diagnostic tests stored at room temperature in the Brazilian Amazon Region. J Infect Dev Ctries. 2013; 7(3):243-252.
39. Walker-Abbey A, Djokam RRT, Eno A, Leke RFG, Titanji VPK, Fogako J *et al*. Malaria in pregnant Cameroonian women: The effect of age and gravidity on submicroscopic and mixed-species infections and multiple parasite genotypes. Am. J. Trop. Med. Hyg. 2005;72(3):229–235.
40. Maltha J, Gillet P, Bottieau E, Cnops L, Van Esbroeck M, Jacobs J. Evaluation of a rapid diagnostic test (CareStart™ Malaria HRP-2/pLDH(pf/pan) Combo Test) for the diagnosis of malaria in a reference setting. Malaria Journal. 2010;9:171. DOI:10.1186/1475-2875-9-1716
41. Simon-Oke IA, Ogunseemi MF, Afolabi OJ, Awosolu OB. Prevalence of malaria parasites among pregnant women and children under five years in Ekiti State, Southwest Nigeria. J Biomed Transl Res 2019;5(1):5-11.
42. Oladeinde BH, Omoregie R, Odia I, Oladeinde OB. Prevalence of malaria and anemia among pregnant women attending a traditional birth home in Benin City, Nigeria. Oman Medical Journal. 2012; 27(3): 232-236. DOI 10. 5001/omj.2012.52
43. Idowu OA, Mafiana CF, Dapo S. Malaria among pregnant women in Abeokuta,

Nigeria. Tanzania Health Research Bulletin. 2006;8(1):28-31.

44. Hommerich L, von Oertzen C, Bedu-Addo G, Holmberg V, Acquah PA, Eggelte TA et al. Decline of placental malaria in southern Ghana after the implementation of intermittent preventive treatment in pregnancy. Malaria Journal. 2007;6:144. DOI:10.1186/1475-2875-6-144.

45. Leke RFG, Bigoga JD, Zhou J, Fouda GG, Leke RJI, Tchinda V et al. Longitudinal studies of *Plasmodium falciparum* malaria in pregnant women living in a rural Cameroonian village with high perennial transmission. Am J Trop Med Hyg. 2010; 83(5):996–1004. DOI:10.4269/ajtmh.2010.10-0249

46. Saito M, Mansoor R, Kennon K, Anvikar AR, Ashley EA, Chandramohan D *et al*. Pregnancy outcomes and risk of placental malaria after artemisinin-based and quinine-based treatment for uncomplicated falciparum malaria in pregnancy: A WorldWide Antimalarial Resistance Network systematic review and individual patient data meta-analysis. BMC Med. 2020; 18:138. DOI:10.1186/s12916-020-01592-z. PMCID: PMC7263905. PMID: 32482173

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