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Low malaria infection after a decade of effective implementation of preventive measures and speciation o f *Plasmodium* s pecies a mong pregnant women attending an antenatal clinic in Benue State, Nigeria

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ABSTRACT

INTRODUCTION: Malaria remains a serious public health threat to rural and urban inhabitants of tropical and subtropical poor communities. This study reassessed the malaria infection and its risk factors among pregnant women, and determined the speciation of the genus *Plasmodium*.

METHODS: A cross-sectional study was conducted a mong 3 05 pregnant women attending an antenatal clinic in Makurdi, Nigeria. Malaria infection was a ssessed u sing Giemsa-stained microscopy and nested polymerase chain reaction (PCR). Socio-demographic, obstetric, and environmental data were collected using a structured questionnaire a nd analyzed u sing chi-square and logistic regression tests (p<0.05)

RESULTS: The infection was 1 1 (3.6%). Pregnant women w ho attended secondary school significantly h ad h igher infection, 6(8.2%) (χ 2=14.49, p=0.012), as we ll as th ose in th e second trimester, 7(7.3%) (χ2=15.47, p=0.035). The significant predictors of infection were the age groups ≤ 20 years and 21-30 years with crude odds ratios (cOR) o f 1.17 (%95CI:0.55 - 5 .38; p=0.000), and 2.67 (%95CI:1.90-6.18; p=0.000) respectively. Pregnant women w ith secondary education, and those in the second trimester respectively h ad significant cO R of 5. 40 (%95CI:2.23 - 40.76; p=0.000), and 0.23 (%95CI:0.04 - 1.17; p=0.040). Pregnant women who did not have drainage around their premises had a cOR of 6.62 (%95CI:1.25- 40.65; p=0.000), as well as those who had stagnant water around premises with cOR=7.24 (%95CI:1.29- 66.55; p=0.001).

CONCLUSION: The nested PCR found *Plasmodium falciparum* with two distinct Merozoite Surface Proteins MSP-1 having K1 and MAD20 families, and MSP-2 having FC27 and 3D7 families.

Keywords: Speciation, *Plasmodium*, merozoites, implementation, prevention

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INTRODUCTION

Malaria continues to remain a serious public health concern in rural and urban areas of tropical and subtropical poor communities of the world. It is a life-threatening vector-borne parasitic infection causing morbidities and death to several children, pregnant women, and adults [1]. Currently, there is an increase in malaria burden around the world with an estimated 263 million cases [2]. In sub-Saharan Africa, most of the increased cases were found in the highland and lowland countries such as Ethiopia, Nigeria, Uganda, and the Democratic Republic of Congo. Currently, Nigeria has the highest burden of malaria among individuals in Africa and the world [2].

In sub-Saharan Africa, the health of pregnant women is adversely affected by malaria, and it remains a debilitating issue of pregnancy among the poor inhabitants [3,4]. Subsequently, it contributes to maternal anaemia, foetal and maternal mortality, low birth weight and still birth, abortion, headache, and other uncontrollable manifestations [5,6]. Pregnant women have severe malaria with higher rates of miscarriage, intrauterine demise, premature delivery, low-birth-weight neonates, and neonatal death [7].
It has been observed that there is genetic diversity of *Plasmodium* species affecting malaria treatment among individuals around the world and causing resistance to the prescribed drugs [8,9]. During laboratory examinations, the most spread technique in identifying the polymorphic genes of the *Plasmodium* species is polymerase chain reaction (PCR) amplification. It has discovered the merozoite surface proteins 1 and 2 (MSP-1 and MSP-2), and the glutamate-rich protein (GLURP) [10,11]. The merozoite surface proteins 1 and 2 have various genomic changes in different geographical endemic areas [12].

Benue State is a north-central zone area of Nigeria that had malaria endemicity with respective infections of 76.9% in Gboko Local Government Area (LGA) [13] and 65.9% in Makurdi [14]. The infection is a public health problem, particularly among vulnerable individuals such as pregnant women and infants of less than 5 years [15]. Several studies determined the infection level of malaria in pregnancy, but none have been conducted with the genetic characterization of *Plasmodium* species in Benue State, Nigeria. This study reassessed the status of malaria infection after fifteen (15) years of effective implementation of preventive measures. It also determined the molecular characterization of the *Plasmodium* species, as well as the risk factors for malaria infection among the pregnant women attending the antenatal clinic of the Federal Medical Centre, Makurdi, Benue State, Nigeria.

METHODS

Study Area

The study was undertaken at the antenatal clinic of the Federal Medical Centre, Makurdi, Benue State, Nigeria. It is located at latitude 7°43'N and longitude 8º32'E. It is located within the Guinea savannah region of Nigeria. The warmest period in the area is the month of February with 37.6°C, while the coldest period is the month of August with 22.0°C (https://weatherandclimate.com/nigeria/benue/makurdi). There are two distinct seasons, the wet season from April to October, and the dry season from November to March (https://weatherandclimate.com/nigeria/benue/makurdi). Most of the city dwellers are civil servants, military/police, traders, farmers, fishermen, and artisans.

The Ethical Committee of the Federal Medical Centre, Makurdi, Benue State, Nigeria granted the research approval for the study (FMH/FMC/MED.108/VOL.I/X). The study participants' anonymity was maintained and the findings were treated with utmost confidentiality. Participants who were positive by microscopy were advised to go for malaria treatment.

Study Population and Sample Size Determination

The study population consisted of pregnant women who were attending the antenatal clinic at the Federal Medical Centre, Makurdi, Benue State, Nigeria. The recruitment of the study participants was carried out after the consent of each participant in the study.
The minimum Sample size was determined using the formula described by Kramer [16].
$n=Z^2p(1-p)/d^2$, assuming a malaria prevalence of 68.3%, a 95% confidence level (Z = 1.96), and a 5% margin of error, yielding a minimum sample size of 332

Study Design

The study was cross-sectional, and a systematic random sampling was used to enroll the willing

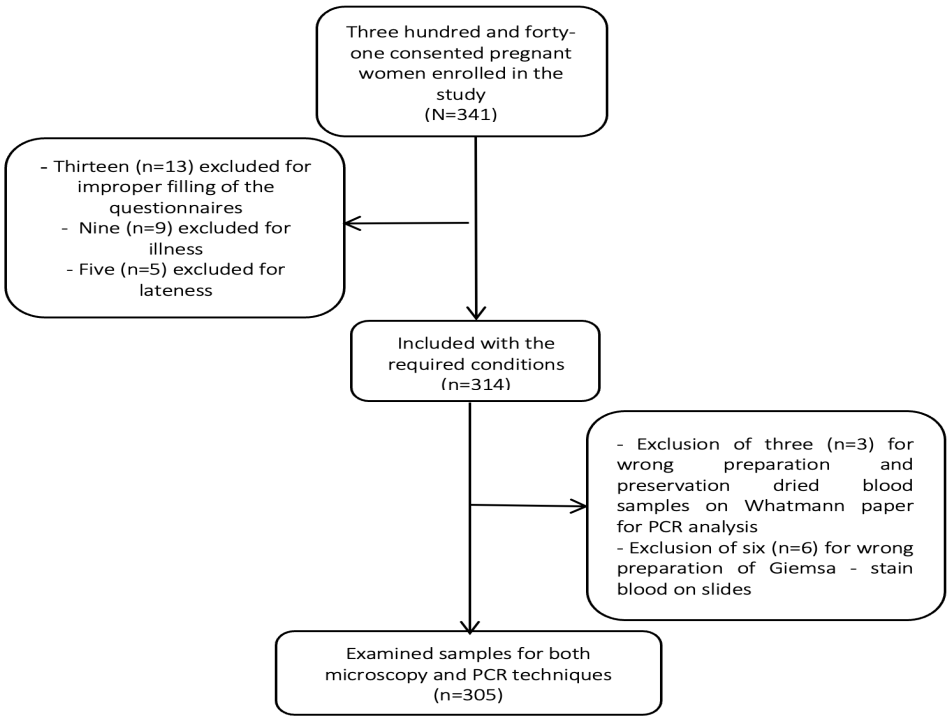

Figure 1: Flow chart showing exclusion and inclusion of the pregnant women

pregnant women who gave their consent. The study was conducted from March 2022 to June 2022. Three hundred and forty-one (341) consented pregnant women were enrolled in the study. During the interactions to fill the questionnaires, Thirteen pregnant women (n=13) who did not properly fill them were excluded. Those who were sick (n=9) were also excluded, as well as those who came late during the interview (n=5). Three hundred and fourteen (n=314)

Table 1: Primary and secondary primers for Plasmodium species

PCR round	Primers' names	Sequences (5'-3')	Amplicon size (basepair)	Species
Primary reaction	rPLU1	TCAAAGATTAAGCCATGCAAGTGA	1.6-1.7 kb	*Plasmodium sp.*
	rPLU5	CCTGTTGTTGCCTTAAACTCC		
Secondary reaction (Nested PCR)	rFAL1	TTAAACTGGTTTGGGAAAACCAAATATATT	206 bp	*P. falciparum*
	rFAL2	ACACAATGAACTCAATCATGACTACCCGTC		
	rOVA1	ATCTCTTTTGCTATTTTTTAG TATTGGAGA	800 bp	*P. ovale*
	rOVA2	GGAAAAGGACACATTAATTGT ATCCTAGTG		
	rMAL1	ATAACATAGTTGTACGTTAAG AATAACCGC	144 bp	*P. malariae*
	rMAL2	AAAATTCCCATGCATAAAAAA TTATACAAA		
	rVIV1	CGCTTCTAGCTTAATCCACAT AACTGATAC	120 bp	*P. vivax*
	rVIV2	ACTTCCAAGCCGAAGCAAAGA AAGTCCTTA		

were included, but three samples (n=3) were excluded for wrong dried blood preservation on Whatmann paper for the PCR. For microscopic analysis six samples (n=6) were excluded for wrong preparation. Three hundred and five (305) blood samples were properly examined (Figure 1)

Questionnaire administration

Before the laboratory procedures, a well-structured questionnaire was issued to each participant. The socio-demographic data, information on the gestational period and parity, preventive measures and intermittent preventive drug therapy, and the risk factors for the infection were requested from each participant. The requirement was based on the voluntary participation of each pregnant woman, and a verbal briefing on the importance of the study was provided to each participant.

Laboratory procedures

Microscopy: Five (5) mL of venous blood was collected aseptically from each pregnant woman into a labelled EDTA bottle. A thick and thin smear slide was made from venous blood of each willing pregnant woman. Each slide was stained with 10% Giemsa solution and allowed to dry before microscopic examination. The films were used for malaria parasite identification using the x100 oil immersion objective of the microscope [17].

Polymerase chain reaction (PCR) procedures, amplification, and electrophoresis for *Plasmodium* species.

A 10 µL blood samples were blotted on 0.5 × 2 cm strips of 3 mm Whatman filter No. 1 and dried in a laminar flow hood. The dried blood spots (DBS) were preserved in a zip-lock bag with one to three desiccants and packed in a big brown envelope for shipment for molecular analysis.

In the molecular laboratory, the DNA was extracted from the dried blood spot (DBS) on the Whatman filter paper using the hole puncher to appropriate sizes. It was placed into 1.5 µL microcentrifuge tubes. This method was performed using the Zymo DNA extraction protocol [18]. The extraction technique was the QUICK-DNA™ Miniprep Plus Kit, and solid tissue (25 mg) protocol. Ninety-five (95) µl of water, 95 µL of solid tissue buffer, and 10 µL of protokinase K were added to the 25 mg of tissue sample and thoroughly mixed. It was then incubated at 55% for 3 hours. Two (2) volumes of genomic binding buffer were added to the supernatant and thoroughly mixed. The mixture was transferred to a Zymo-spin™ 11°C XL column in a collection tube, centrifuged for one (1) minute at 1200 G, and the collection tube was discarded with the flow through. Four hundred (400) µL of

Table 2: Sequences of primers for MSP-1 and MSP-2 and allelic family

Genotype	PCR round	Primers names	Sequence (5'-3')	Allelic families
MSP-1	Primary	M1-OF	CTAGAAGCTTTAGAAGATGCAGTATTG	
		M1-OR	CTTAAATAGTATTCTAATTCAAGTGGATCA	
	Secondary	M1-KF	AAATGAAGAAGAAATTACTACAAAAGGTGC	K1
		M1-KR	GCTTGCATCAGCTGGAGGGCTTGCACCAGA	
		M1-MF	AAATGAAGGAACAAGTGGAACAGCTGTTAC	MAD 20
		M1-MR	ATCTGAAGGATTTGTACGTCTTGAATTACC	
		M1-RF	TAAAGGATGGAGCAAATACTCAAGTTGTTG	RO33
		M1-RR	CATCTGAAGGATTTGCAGCACCTGGAGATC	
MSP-2	Primary	M2-OF	ATGAAGGTAATTAAAACATTGTCTATTATA	
		M2-OR	CTTTGTTACCATCGGTACATTCTT	
	Secondary	M2-FCF	AATACTAAGAGTGTAGGTGCARATGCTCCA	FC27
		M2-FCR	TTTTATTTGGTGCATTGCCAGAACTTGAAC	
		M2-ICF	AGAAGTATGGCAGAAAGTAACCTYCTACT	3D7
		M2-ICR	GATTGTAATTCGGGGGATTCAGTTTGTTCG	

DNA pre-wash buffer was added to the column in a new collection tube and centrifuged for one (1) minute and the collection tube was emptied. Seven hundred (700) µL of g-DNA wash buffer was added and centrifuged for one (1) minute and the collection tube was emptied. Two hundred (200) µL of g-DNA wash buffer was added and centrifuged for one (1) minute and the collection tube discarded with the flow through. It was transferred to a clean microcentrifuge tube to elute the DNA. Fifty (50) µL of DNA elution buffer was added and incubated for five (5) minutes and then centrifuged for one (1) minute.

The polymerase chain reaction (PCR) adopted the procedures of Snounou and Balbir [23]. The PCR amplification was carried out in two phases. For the genus *Plasmodium*, the primary malaria PCR was amplified using 1.6-1.7 kilobases (kb) amplicons using rPLU1 and rPLU5. The secondary PCR was performed for the *Plasmodium* species using the genus-specific primers rFAL1 and rFAL2 under the same cyclic conditions to yield a 206 base pair fragment for *Plasmodium falciparum* (Table 1).

The PCR procedures for Merozoite Surface Protein (MSP) 1 and 2 used the surface antigen loci. The primer M1-OF was the specific sequence for the primary MSP-1 genotype with M1-OR as the variable size. M2-OF was the primary MSP-2 genotype, and M2-OR was the variable size. The DNA template was amplified using Nested PCR with second-round primers specific to allelic families. The primers M1-KF and M1-KR secondary to MSP-1 were used to detect K1 allelic family, while M1-MF and M1-MR secondary to MSP-1 detected MAD 20. The primers M1-RF and M1-RR secondary to MSP-1 detected the RO33 allelic family. The M2-FCF and M2-FCR secondary to MSP-2 detected FC27 allelic family, while the primers M2-ICF and M2-ICR secondary to MSP-2 detected 3D7 allelic family (Table 2).

The PCR products were separated by gel electrophoresis on a 2.5% agarose gel stained with ethidium bromide along with a 100 bp DNA size marker. Upon completion of the gel electrophoresis, gels were placed in a gel imaging cabinet and digitally photographed under UV light.

Data Analysis

The qualitative variables from the questionnaires were attributed to the ordinal numbers and were entered into Microsoft Excel 2016 and then exported to IBM SPSS Statistics for Windows version 26 (IBM Corp., Armonk, N.Y., USA). The variables of socio-demographic, gestational, and parity were categorized with an interval of 10 years for the age groups; 1-3 months as first trimester, 4-6 months as second trimester, and 7-9 months as third trimester for the gestational period of pregnancy. For parity, the pregnant women were classified as primiparous for one child, secundiparous for two children, and multiparous for more than two children.

The association of the categorized variables and the malaria infection was analyzed using the Chi-square ($\chi 2$) test. The multinomial regression model with an equation: $f(x)=b_0+b_1x_1+b_2x_2+b_3x_3+ ..+ bnxn$, where f(x) is known as the dependent variable. The independent variables known as predictors of malaria in the area were socio-demographic status, gestation and parity, intermittent preventive therapy, sanitation, hygiene, and drainage around houses. The significant difference level was at $p\leq 0.05$.

RESULTS

Description of the participants

Table 3 describes the variables of the pregnant women attending the antenatal clinic in Federal Medical Centre, Makurdi, Benue State, Nigeria. Participants living in urban areas were 286 (93.7%), while those in rural areas were 19 (6.2%). Pregnant women who had ≤ 20 years were 6 (1.9%), while those having 21-30 years were 235 (77.0%). Pregnant women with primary education were 9(2.9%), while those with tertiary education were 158 (51.8%). Traders were 145 (47.5%), while students were 50 (16.4%). Married pregnant women were 283 (92.7%) and non-married were 22 (7.2%). Pregnant women in the first trimester were 51 (16.7%), while those in the third trimester were 158 (51.8%). The primiparous pregnant women were 114(37.3%), while the multiparous were 65 (21.3%).

Malaria infection in relation to participants' characteristics.

Table 4 shows malaria infection in relation to socio-demographic, gestational, and parity of pregnant women attending the antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria.

Table 3: Socio-demographic, gestational stage and parity

Variables	Number (N=305), n (%)
Infection	11 (3.6)
Location	
▪ Urban	286 (93.7)
▪ Rural	19 (6.2)
Age (years)	
▪ ≤ 20	6 (1.9)
▪ 21-30	235 (77.0)
▪ 31-40	63 (20.6)
▪ 41-50	1 (0.3)
Education	
▪ Primary school	9 (2.9)
▪ Secondary school	129 (42.3)
▪ Tertiary school	158 (51.8)
Occupation	
▪ Student	50 (16.4)
▪ Business	130 (42.6)
▪ Civil servant	37 (12.1)
▪ Trader	15 (4.9)
▪ Others	73 (23.9)
Marital status	
▪ Married	283 (92.7)
▪ Non-married	22 (7.2)
Gestational stage	
▪ First trimester	51 (16.7)
▪ Second trimester	96 (31.4)
▪ Third trimester	158 (51.8)
Parity	
▪ Primiparous	114 (37.3)
▪ Secundiparous	126 (41.3)
▪ Multiparous	65 (21.3)

An infection of 11 (3.6%) was reported among the pregnant women. Those who had secondary education significantly had higher malaria infection, 6 (8.2%) ($\chi2=14.49$, p=0.012). Pregnant women who were in their second trimester of gestation had 7 (7.3%) of malaria ($\chi2=15.47$, p=0.035).

Predictors of malaria infection among studied pregnant women

The predictors of malaria among pregnant women attending the antenatal clinic at Federal Medical Centre, Makurdi, Benue State, Nigeria are shown in Table 5.

The age groups ≤ 20 years, and 21-30 years exposed pregnant women to malaria infection with crude odds ratios (cOR) of 1.17 (95%CI: 0.55 - 5.38; p=0.000,) and 2.67 (95%CI: 1.90-6.18; p=0.000) respectively. The educational status and gestational age showed that pregnant women with secondary education had a cOR of 5.40 (95%CI:2.23-40.76; p=0.000), and those in the second trimester had 0.23 (95%CI:0.04-1.17; p=0.043) respectively. Pregnant women who did not have drainage around their premises had a cOR of 6.62 (95%CI: 1.25-40.65; p=0.000), as well as those who had stagnant water around their premises with cOR=7.24 (95%CI: 1.29 - 66.55; p=0.001).

Genetic diversity of *Plasmodium falciparum* Merozoites Surface Protein-1 (MSP-1) and Merozoite Surface Protein-2 (MSP-2) and the allelic families in studied pregnant women

The nested PCR indicated that *Plasmodium falciparum* was the only species found with MSP-1 and MSP-2 families during amplification. MSP-1 had two (2) allelic families and was classified as K1 with 100 bp (18.2%, 2/11), 250 bp (9.1%, 1/11), and 275 bp (9.1%, 1/11), and MAD with 200 bp (9.1%, 1/11) (Figure 2). MSP-2 had two (2) allelic families and classified as FC 27 with 320 bp (9.1%, 1/11) and 350 bp (18.2%, 2/11), and 3D7 with 430 bp (9.1%, 1/11), 450 bp (27.3%, 3/11) and 510 bp (36.4%, 4/11) (Figure 3).

DISCUSSION

In sub-Saharan Africa, pregnant women are usually vulnerable to malaria which has consequential issues on their immune systems and foetuses. This study reassessed malaria in pregnancy a

Table 4: Malaria infection in relation to socio-demographic, gestational, and parity of pregnant women

Variales	Malaria infection			
	No. exam	Positive (%)	χ^2	P-value
Infection	305	11 (3.6)		
Age (years)			5.09	0.165
▪ ≤ 20	6	1 (16.9)		
▪ 21-30	235	6 (2.6)		
▪ 31-40	64	4 (6.3)		
▪ 41-50	1	0 (0.0)		
Education			4.49	0.212
▪ Primary	9	0(0.0)		
▪ Secondary	129	8(6.2)		
▪ Tertiary	158	3(1.9)		
Occupation			1.99	0.736
▪ Student	37	3(6.0)		
▪ Business	130	3(2.3)		
▪ Civil servant	37	1(2.7)		
▪ Others	73	3(4.1)		
Marital status			2.05	0.152
▪ Married	283	9(3.2)		
▪ Unmarried	22	2(9.1)		
Gestational stage			15.47	0.035
▪ First trimester	51	1(2.0)		
▪ Second trimester	96	7(7.3)		
▪ Third trimester	158	3(1.9)		
Parity			3.11	0.211
▪ Primiparous	114	5(4.4)		
▪ Secundiparous	126	6(4.8)		
▪ Multiparous	65	0(0.0)		

LLTINs = Long-Lasting Treated Insecticide Nets. First trimester: 1-3 months. Second trimester: 4-6 months. Third trimester: 7-9 months

decade after a first assessment [14]. The low malaria infection observed was an application and implementation of the preventive and control measures by the pregnant women in the areas. Such measures were implemented by the Federal Government of Nigeria around 2010 after the

Table 5: Multinomial logistic regression analysis showing important predictors as socio-demographic, gestational, parity, intermittent preventive therapy and drainages around houses

Independent variables	crude OR (%95CI)	p-value
Socio-demographic		
Age (years)		
≤ 20	1.17 (0.55- 5.38)	0.000
21-30	2.67 (1.90- 6.18)	0.000
31-40	-	-
Education		
Primary	4.32 (1.01- 23.71)	0.101
Secondary	5.40 (2.23- 40.76)	0.003
Tertiary	-	-
Occupation		
Student	0.98 (0.11- 8.56)	0.990
Business	1.86 (0.26- 13.27)	0.533
Civil servant	0.71 (0.04- 12.66)	0.814
Others	-	-
Gestational stage		
First trimester	1.10 (0.08- 14.66)	0.931
Second trimester	0.23 (0.04- 1.17)	0.047
Third trimester	-	-
Parity		
Primiparous	7.64 (2.31- 57.12)	0.977
Secundiparous	1.05 (0.19- 4.67)	0.974
Multiparous	-	-
Intermittent preventive therapy		
No	0.25 (0.04- 1.32)	0.413
Yes	-	-
Sanitation and hygiene around houses		
with provision of drainage or not		
No	3.32 (0.08- 6.77)	0.000
Yes	-	-
Presence of stagnant water around houses		
Yes	7.24 (1.29- 66.55)	0.001
No	-	-

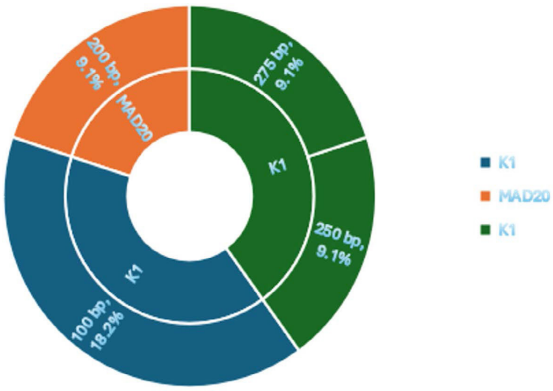

Figure 2: Genetic diversity of *Plasmodium falciparum* MSP-1 and allelic families among studied pregnant women

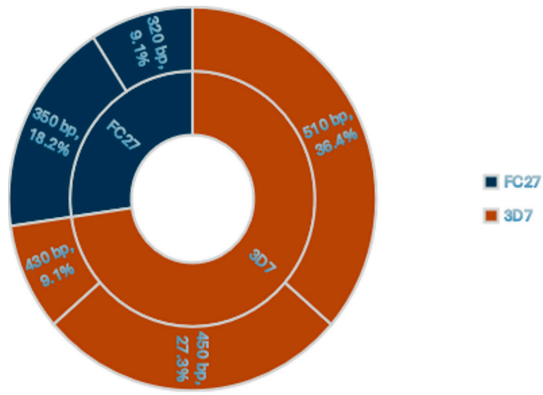

Figure 3: Genetic diversity of *Plasmodium falciparum* MSP-2 and allelic families among studied pregnant women

recommendations of the preventive and control program in pregnancy by the World Health Organization policies [19]. The application of such rules reduced the high rate of morbidities and deaths among infected pregnant women in Benue State, Nigeria. The reduced infection level has also been reported in other areas in Nigeria such as Lagos State [20] and Bauchi State [21]. This low level of infection among pregnant women was the effective use of the long-lasting treated nets against mosquito bites. They were also instructed to adhere to the use of Sulfadoxine-Pyrimethamine (SP) in pregnancy as recommended by the World Health Organization [22].

The high infection at the second trimester could be due to the *Plasmodium* sp. immune disorders at various pregnancy stages. *Plasmodium* sp has been known for the sequestration of the immune system by inducing an inflammatory response, as well as a lack of optimal placental function, and a reduction in foetal nutrient transportation [23]. The malaria-infected pregnant women in the second trimester are also affected by the complications caused by the cytokines. It is also known that malaria in pregnancy promotes the expression of IL-1β, IL-2, IL-8, IFN-γ, TNF-α, and TGF-β [24,25]. The findings of other previous studies reported high malaria infection in the second trimester of pregnancy in Nigeria and other West African countries [26,27].
The residency of pregnant women around the swampy environment was also displayed during the focus group interactions. This might also be a result of *Plasmodium falciparum* resistance of the immune system to the antimalarial drug given. The negligence of pregnant women towards the use of the intermittent preventive treatment nets could be another cause of the infection. Such issues have been reported in Uganda [28]. It could also be that the pregnant women were not complying with the prescription of the drugs [29].

The pregnant women of the age groups ≤ 20 years and 21-30 years, and those with a secondary level of education were predicted to be more exposed to malaria infection. The young women within those age groups may lack the awareness of using preventive measures against malaria at the onset of the pregnancy. It might be also an immune suppression, or loss of acquired immunity to malaria at those ages [30]. For the secondary educational level, the non-application of wearing protective clothes, and not use of long-lasting treated bed nets, and windows/doors' nets against mosquitoes became an attitudinal exposure risk of the pregnant women to malaria in Benue State, Nigeria. The daily farming of vegetable crops along the agricultural riverine sites of the River Benue was another predictive exposure attitude of the pregnant women to mosquitoes' bites.
The absence of fluent drainage and the presence of stagnant water were predicted as breeding sites of mosquitoes. The drainages were not well evacuated as people dump their refuse thereby allowing very good breeding sites for Anopheles. It is pertinent to note that mosquitoes breed freely in such gutters and drainages as observed in Nigeria [31] and Ghana [32].

In this study, the molecular characterization found only *Plasmodium falciparum* among the pregnant women in Makurdi metropolis, Benue State, Nigeria. The polymerase chain reaction (PCR) was highly sensitive and consistent in the detection of *Plasmodium falciparum*. Previous studies found that P. falciparum was the predominant strain for individuals in Nigeria [10,33], and sub-Saharan Africa [34, 35]. P. falciparum isolates showed genetic diversity in this study with highly polymorphic markers such as the merozoites' surface protein-1 (MSP-1) and merozoites' surface protein-2 (MSP-2). The amplification of MSP-1 found K1 and MAD20 families, while MSP-2 found FC27 and 3D7 at different frequencies. This effect could be the challenges to the resistance of the parasite, and complexity to the treatment among the pregnant women on Sulfadoxine-Pyrimethamine. A previous study reported such issues of various polymorphic markers of *Plasmodium falciparum* [8,11,32].

The unprompted and unenthusiastic action for the blood preservation during packaging for evacuation to the molecular laboratory was a limitation to the study.

CONCLUSION

A low malaria infection was found among the pregnant women attending the antenatal clinic of the Federal Medical Centre, Makurdi, Benue State, Nigeria. The age groups ≤ 20 and 21-30 years, secondary school education, second trimester, provision of drainage, and presence of stagnant water were the risk factors for malaria infection among the pregnant women in Makurdi, Benue State, Nigeria. The nested PCR indicated that pregnant women were infected with only *Plasmodium falciparum*. Two distinct Merozoite Surface Proteins MSP-1 with K1 and MAD20 clones, and MSP-2 with FC27 and 3D7 clones were observed. The study recommended that inhabitants and pregnant women should be more enlightened on keeping the drainage clean, clearing of bushes around the houses, and wearing protective clothing especially at night against mosquito bites. Pregnant women should be encouraged to comply with the laid-down strategies of the malaria control programs. It has been observed in this study that non-compliance was the reason why some of the pregnant women were infected with malaria.

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