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Distribution of Allelic Frequencies and Diversity of *Plasmodium falciparum* Merozoite Surface Proteins and Glutamate-Rich Protein Genes from Southern and Northern Nigeria

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Abstract

Background of study: Extensive genetic diversity and distribution of allelic frequencies in *P. falciparum* isolates is an indication for high malaria transmission. Therefore, Malaria control and elimination strategies may require geographic evaluation of the population genetic profile of *Plasmodium falciparum* to guide malaria interventions. The genetic diversity and allelic frequency distribution of merozoite surface proteins (*msp1* & *msp2*) and glutamate-rich protein (*glurp*) from southern and northern Nigeria was evaluated.

Methods: Dried blood spots were obtained at enrollment from febrile children aged 6 months to 10 years in Ibadan and Kaduna. The *msp1*, *msp2*, and *glurp* genes were genotyped by nested PCR and the allelic frequency distribution, expected heterozygosity (*He*) and multiplicity of infection (MOI) was evaluated.

Results: The *msp1*, *msp2* and *glurp* genes amplified in 58.6%, 98.7%, and 53.0%, respectively. The allelic frequency of *msp1* was predominant in Kaduna ($\rho = 0.0002$) while *glurp* was more in Ibadan ($\rho = 0.00001$). No significant difference was observed in msp-2 ($\rho = 0.0713$) with

respect to location. In all, 7 *msp1*, 13 *msp2* and 8 *glurp* haplotypes were detected. The K1 monoclonal allele was predominant (30.4%) in Kaduna, but multiclonal allelic haplotype (RO33/MAD20) was predominant in Ibadan (65.7%). The *msp2* haplotype distribution was similar in both locations while *glurp* allele of 1000bp was the most predominant in Ibadan (31%) and Kaduna (18%). The multiplicity of infection (MOI) for *msp1* and *msp2* was 26% and 20% monoclonal and 74% and 80% multiclonal, while *glurp* was 99% multiclonal and only 1% monoclonal in Ibadan. The expected heterozygosity (*He*) and mean MOI for *msp1*, *msp2* and *glurp* was not statistically significant in both locations with respect to gender, age, fever, parasitaemia and anemia.

Conclusion: *Plasmodium falciparum* isolates are extensively diverse with high multiplicity of infection in southern and northern Nigeria.

Keywords: *Plasmodium falciparum* genetic diversity, multiplicity of infection, merozoite surface proteins, glutamate-rich protein, Nigeria

List of Abbreviation

CAMRAB: Center for Advanced Medical Training and Biotechnology

DBS: Dried Blood Spots

DNA: Deoxyribonucleic Acid

Glurp: Glutamate-Rich Protein

kDa: Kilo Dalton

MOI: Multiplicity of Infection

Msp1: Merozoite Surface Protein 1

Msp2: Merozoite Surface Protein 2

NMEP: National Malaria Elimination Programme

PCR: Polymerase Chain Reaction

SPSS: Statistical Package for Social Sciences

WHO: World Health Organization

Introduction

A substantial reduction has been recorded in malaria related illnesses and deaths globally with malaria mortality reducing drastically from about 400.000 in the years 2010 to 260 000 in 2018, the largest reduction was recorded in Nigeria, from about 153 000 deaths in 2010 to 95 000 deaths in 2018 [1]. Despite the decrease in mortality, malaria remains the highest contributor to febrile illnesses and death with serious adverse economic effects and human wellbeing in endemic countries. According to the WHO, Nigeria accounts for 25% of malaria cases and 24% death globally [2], with about 50% of Nigeria's adult population suffering at least one attack each year while the under-five suffer between 2–4 attacks annually. Malaria is transmitted throughout Nigeria, with 76% of the population living in high transmission areas and 24% in low transmission areas [3]. *Plasmodium falciparum,* the most virulent *specie*, is predominant in Nigeria [4]. Parasite virulence and disease severity is attributed to the infecting parasite strains and their genetic profiles [5]. Notable *P. falciparum* membrane-bound proteins implicated in malaria transmission intensity, multiplicity of infection (MOI) and pathogenesis include merozoites surface proteins (*Msp1*, *Msp2*) and glutamate-rich protein (*Glurp*) [6]. Prevalence of these membrane-bound proteins distinctly contribute and influence the geographical description and variation in the genetic profile of the parasite population [7]. An understanding of the parasite genetic profile, transmission dynamics, mechanisms of adaptation to environmental and interventional pressures are essential in the development of new and innovative approaches in the global control and elimination of malaria [8].

The *Msp1* is a predominant surface protein encoded by the *msp1* gene on chromosome 9 with 17 blocks of sequences containing polymorphic, semi-conserved, and conserved regions which play an essential role in the parasite-host relationship and also mediates erythrocyte invasion. The repetitive Block 2 region is the most polymorphic domain with three allelic families (K1, MAD20, and RO33) which has been reported to play key role in the symptomatology of malaria infection [9].

The *Msp2* is a glycoprotein abundantly expressed on the surfaces of the erythrocytes encoded by the *msp2* gene on chromosome 2. It is composed of 5 blocks, a central repetitive block (block 3), which contains the two allelic families (FC27 and 3D7) has been reported to be essential during parasite invasion of the erythrocyte and is retained on the surface during the invasion and degraded soon after the invasion is completed [10].

The *Glurp* on the other hand, is a 220 kDa antigen synthesized throughout the life cycle of *P. falciparum* in an infected vertebrate host with an open reading frame of 1,271 amino acids, including a conserved region (R0) and two blocks of polymorphic regions, including RI and RII [11]. Studies have shown that anti-*Glurp* antibodies react with all stages of the parasite life cycle (hepatic, asexual and gametocyte stages) by inducing several proteolytic activities, thereby inhibiting parasite growth and conferring protective immunity to the host [12].

Malaria transmission intensity is reported to be parasite population dependent, causing marked effects on the population genetics of *P. falciparum* with multiclonal strains of infected parasites [13]. Areas of lower malaria transmission have shown to have lower disease severity in children with malaria and often characterized by lower parasitaemia [14]. Genetic profiling of *P. falciparum* has improved routine molecular epidemiological surveillance to inform malaria interventions in geographic settings with different transmission intensity [15]. An increase in transmission of malaria has been linked to an increase in the circulating parasite strains within a population [16]. Baseline information and continuous molecular surveillance of the genetic structures of these genes across geographical locations would support the national malaria elimination programme (NMEP) in scaling-up malaria control and intervention in Nigeria. This study reported the allelic frequency distribution of *P. falciparum* genetic diversity of *msp1*, *msp2* and *glurp* genes, the expected heterozygosity (*He*) and the MOI in southern and northern parts of Nigeria.

Material and Methods

Study Design and Subjects

Dried blood spots on filter paper were obtained from 236 (Ibadan, n = 86; Kaduna, n = 150) children at enrollment from children aged 6 months to 10 years presenting in study centers with symptomatic microscopy confirmed malaria during the therapeutic efficacy study of artemether-lumefantrine. Capillary blood obtained from finger-prick on the middle finger by aseptic technique was spotted on Whatman™ 3MM filter paper. The blood spots were air-dried and stored in a dust-free area and securely placed in individual zip-lock bags with silica gel for

preservation. In addition, clinical and demographic details such as history of fever, body temperature, age, weight and sex of each participant were entered into case record forms (CRFs). Ethical approval for the main study (therapeutic efficacy) was obtained from the Institutional Review Boards of University College Hospital/University, Ibadan and Ahmadu Bello University Teaching Hospital/Ahmadu Bello University Zaria.

Study area and sites

The study was conducted in Ibadan, Southwest Nigeria and Kaduna, Northwest Nigeria between 15th June 2017 and 16th August 2018. Ibadan is the capital city of Oyo State in Southwestern Nigeria, located between latitudes 7°05'N and 7°25'N and longitudes 3°40'E and 3°55'E. Ibadan is a densely populated city with over 3,875,000 million people (https://www.macrotrends.net/) and covers an area of 3,080 square kilometers. Two seasons, comprising a raining season between April and October and dry season between November and March occur in Ibadan. The mean annual rainfall is 1420mm, while the average annual temperature is 26°C, with a relative humidity of 74.5%. Kaduna, on the other hand, is the capital city of Kaduna state and one of the largest and most populated cities in Northern Nigeria with about 1,187,000 million people (https://www.macrotrends.net/). Its latitudinal location is 10°31'08" N, 7°26'35" E and 10.519 longitudinally placed with 644 meters above sea level. It has a tropical savanna climate with a warm weather year-round and a wet season from May to September, and a drier season from October to April.

The dried blood spots collected from the two study sites were analyzed at the Center for Advanced Medical Research and Biotechnology (CAMRAB), Babcock University, Ogun state, Nigeria.

DNA Isolation

Genomic DNA was extracted from dried blood spots (DBS) using QIAamp DNA Mini kit blood and tissue (QIAGEN Germany) kit according to the manufacturer's instructions. The extracted DNA was stored at -20^{0}C for PCR amplification.

Plasmodium Specie Amplification

A nested PCR amplification protocol targeting the 18SrRNA gene of *Plasmodium* species was carried out using primary genus-specific primers and a secondary *species*-specific primer sequences (*P. falciparum, P. malaria, P. ovale, and P. vivax*) as previously described(Funwei et al., 2018; Snounou et al., 1993). In a 20µL reaction volume, PCR mixture containing 5X PCR Master Mix (Solis Biodyne, Estonia), 0.5mM each of forward and reverse primers, nuclease-free PCR grade water, and 100ng DNA template was prepared for the primary reaction. In the Nested reaction, 2µL of the primary PCR product was used as a template in 18µL PCR mixture, containing the same reagents as the primary reaction except with a *species*-specific forward and reverse primers. The PCR thermal cycling conditions were previously described [15].

Allelic Genotyping of *msp1*, *msp2* & *glurp* Genes

PCR amplification targeting the polymorphic regions of *msp1* (block 2), *msp2* (block 3) and *glurp* (R2) regions were genotyped with allelic specific primers as previously described [15]. The antigenic allelic families of *msp1* (K1, MAD20, & RO33) and *msp2* (FC27 & 3D7) were amplified in a final reaction volume of 20µL, containing 5X PCR Master mix (Solis Biodyne, Estonia), primers (forward and reverse) 0.5mM each and 100ng DNA template in the primary reaction. 2µL of the first PCR product was used as template in the nested reaction. The PCR cycling conditions for both primary and nested reactions were as previously described [15]. The nested PCR products were electrophoresed on 1.5% agarose gel pre-stained with EZ-vision blue light DNA dye (EZ-Vision® Bluelight DNA Dye, VWR Chemicals USA) and were run at 100volt for 60 minutes for fragment size differentiation.

The gel was visualized and photographed under a 312 nm UV trans-illuminator documentation system. Amplified fragments were grouped into allelic types and base-pair "bins" (bands within the same gel image). Fragments within the limits of the bin were considered as having a similar allelic genotype. Analysis of the allelic frequencies was carried out as a proportion of the total alleles detected among all positive samples analyzed.

Multiplicity of infection

The MOI was calculated by dividing the number of detected alleles of *msp1*, *msp2* and *glurp* fragments by the number of PCR-positive samples for the individual marker. Isolates with more than one genotype were classified as multiclonal infections, while a single allele was considered as monoclonal infection [5].

Statistical Analysis

Data was entered with Microsoft Excel and exported to SPSS (IBM version 20) for statistical analysis. The allelic frequency of *msp1*, *msp2* and *glurp* was calculated as the proportion of the allele detected for each allelic family out of the total of alleles detected in the individual gene. The frequency of multiclonal infection was calculated using number of samples with more than one amplified fragment divided by the total samples. The mean MOI was determined by dividing the total number of alleles detected in both *msp1*, *msp2* and *glurp* by the total number of positive samples for each marker. The expected heterozygosity (*He*) was calculated to estimate the fraction of all parasites that would be heterozygous for any of the loci. The Chi-square test was used to compare proportions. Statistical significance was set at $P < 0.05$.

Results

Demographic and Clinical Characteristics of study enrollees

Two hundred and thirty-six (236) dried blood spots (DBS); 86 and 150 from Ibadan and Kaduna respectively, were analyzed in this study. Overall, there was more malaria infections in males (60.3%) and significantly more in children above 36 months (27.4%; p=0.000) in the two locations. Mean temperature was significantly higher among the children in Kaduna (ρ<0.0001) while heamatocrit level was similar in both locations. The geometric mean parasite density was higher among study participants from Ibadan compared to those from Kaduna.

Table 1: Patient's profiles from Southern (Ibadan) and Northern (Kaduna) Nigeria

Variables	Ibadan	Kaduna	Total (%)	ρ-value
Number of patients Male (%): Female (%):	86 53 (61.6) 33 (38.4)	150 89 (59.3) 61 (40.9)	237 143 (60.3) 94 (39.7)	0.000
Age (months): 0 – 36 >36	 15 (17.4) 71 (82.6)	 50 (33.3) 100 (66.7)	 65 (27.4) 172 (72.6)	0.000*
Temperature (°C) Mean ± SD Range	 37.6 ± 1.25 35 -40.5	 38.6 ± 0.66 37.5 – 40.5		0.0001*
Haematocrit (%) Mean ± SD Range	 31.4 ±5.06 19 – 43	 30.9 ± 6.9 15-42		0.558
Day0 Parasite density/μL (Geometric mean)	 28,459.01 ± 7, 989	 6,885 ± 1432.79		0.0001*

Prevalence of *Plasmodium species* in Ibadan and Kaduna

All the blood spots collected in Ibadan and Kaduna were initially positive by microscopy and was confirmed by nested PCR. After PCR speciation, *P. falciparum* was 100% for both study locations. However, *P. malariae* (2.3%; 2/86) was detected in Ibadan as mixed infection but none in Kaduna. Similarly, *Plasmodium ovale* was detected in Kaduna (1.3%; 2/150) and Ibadan (1.2%; 1/86) as mixed infections with *P. falciparum*. No *P. vivax* was detected in the two study sites.

Table 2: Prevalence of *Plasmodium species* in Ibadan and Kaduna

Plasmodium specie	**Ibadan: N = 86 (%)**	**Kaduna: N = 150 (%)**
falciparum	86 (100)	150 (100)
Malariae	2 (2.3)	0 (0)
Ovale	1(1.2)	2 (1.3)
Vivax	0 (0)	0 (0)
falciparum + malariae	2 (2.3)	0 (0)
falciparum + ovale	1 (1.2)	2 (1.3)

Amplification of *msp1*, *msp2* and *glurp* genes in Ibadan and Kaduna

The *msp1*, *msp2* and *glurp* genes were successfully amplified from both location, accounting for 58.6% (138/236), 98.7% (233/236), and 53.0% (125/236), respectively. Comparison of the percentage amplification between the two sites showed significantly higher *msp1* ($\rho = 0.0002$) amplification in Kaduna and *glurp* in Ibadan ($\rho = 0.00001$). No significant difference was observed in *msp2* amplification in the both locations ($\rho = 0.0713$).

Table 3: Variants of *msp1*, *msp2* and *glurp* genes amplification between Ibadan and Kaduna

Gene	**Amplified samples (Ibadan) n= 86 (%)**	**Amplified samples (Kaduna) n=150 (%)**	**Total (N= 236) (%)**	**ρ-value**
msp1	36 (41.8)	102 (68.0)	138 (58.5)	0.0002*
msp2	84 (97.7)	149 (99.3)	233 (98.7)	0.0713
glurp	70 (81.4)	55 (36.7)	125 (53.0)	0.0001*

Genetic diversity and haplotype frequency distribution in Ibadan and Kaduna

A total of 28 *msp1*, *msp2* and *glurp* genotypes were detected in both Ibadan and Kaduna.

Seven genotypes for *msp1* was detected, K1 (100 bp) was present in both locations. The 400 bp genotype of MAD20 was only detected in Kaduna, RO33 (200 bp) was detected in both locations while 300bp and 150bp was present in Ibadan and Kaduna respectively.

Similarly, 3 of the 6 genotype (300 bp, 400 bp) detected for FC27 were present in both locations. However, the 500bp, 700bp and 350bp, 600bp genotype was present only in Ibadan and Kaduna respectively. In addition, seven genotypes were detected in the 3D7 family ranging from 200bp to 900bp. While 400bp and 800bp genotype was distinct in Ibadan, 500bp, 700bp and 900bp was present in Kaduna.

The *glurp* gene amplified 8 different genotypes; 800 – 1100bp and 400 – 1100bp from Ibadan and Kaduna respectively.

Table 4: Number of detected genotypes and Base pair range of *msp1*, *msp2* and *glurp* gene

Note: Identical allelic fragment size from the same marker was counted as one

Marker	Ibadan Allelic size range (bp)	Kaduna Allelic size range (bp)	Ibadan + Kaduna No. of Genotypes per allele
msp1 RO33 MAD20 K1 Total	 200, 300 200, 300 100	 150, 200 200, 400 100	 3 3 1 **7**
Msp2 FC27 3D7 **Total**	 300, 400, 500, 700 200, 400, 800	 300, 350 600 200, 500, 700, 900	 6 7 **13**
glurp **(RII)**	800, 900, 1000, 1100	400, 500, 600, 700, 900, 1100	**8**
Total			**28**

Clonality of infection

The K1 monoclonal allele was predominant (30.4%) in Kaduna, unlike in Ibadan, where the multiclonal RO3+MAD20 was the main haplotype (65.7%) detected. The K1+RO33 and K1+MAD20 haplotype were completely absent in Ibadan. For the *msp2* gene, both study locations recorded similar distribution of the haplotypes with 3D7/FC27 the predominant, however, the 3D7 monoclonal infection was 9.6% and 0.7% while FC27 was 10.8% and 14.0% in Ibadan and Kaduna respectively (Figure 1 and 2).

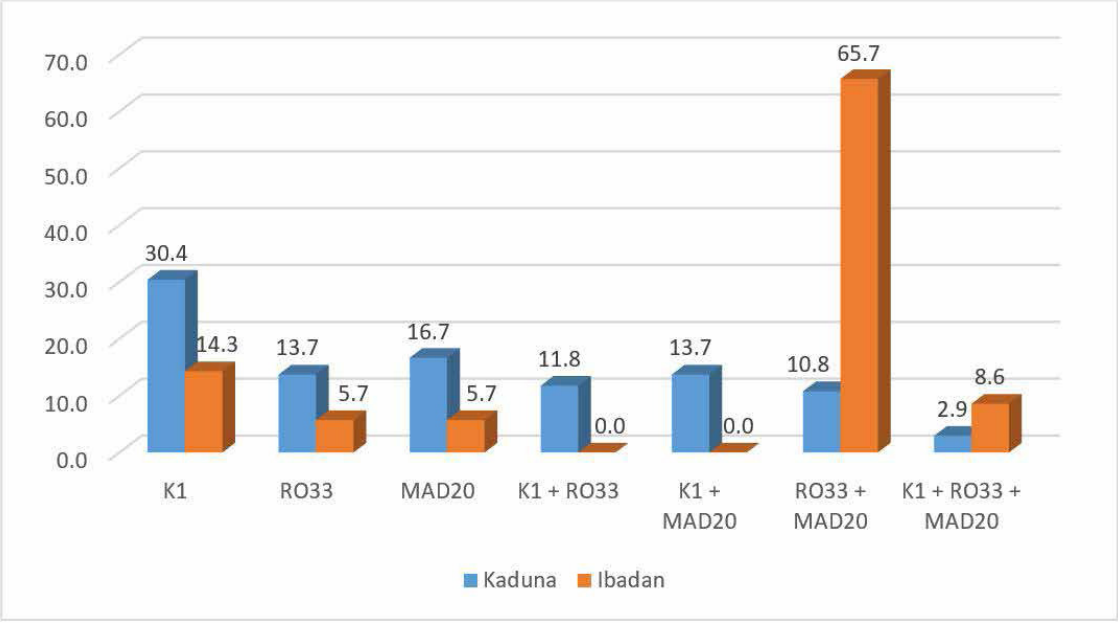

Figure 1: Haplotypes distribution for *msp1* allelic family stratified by location

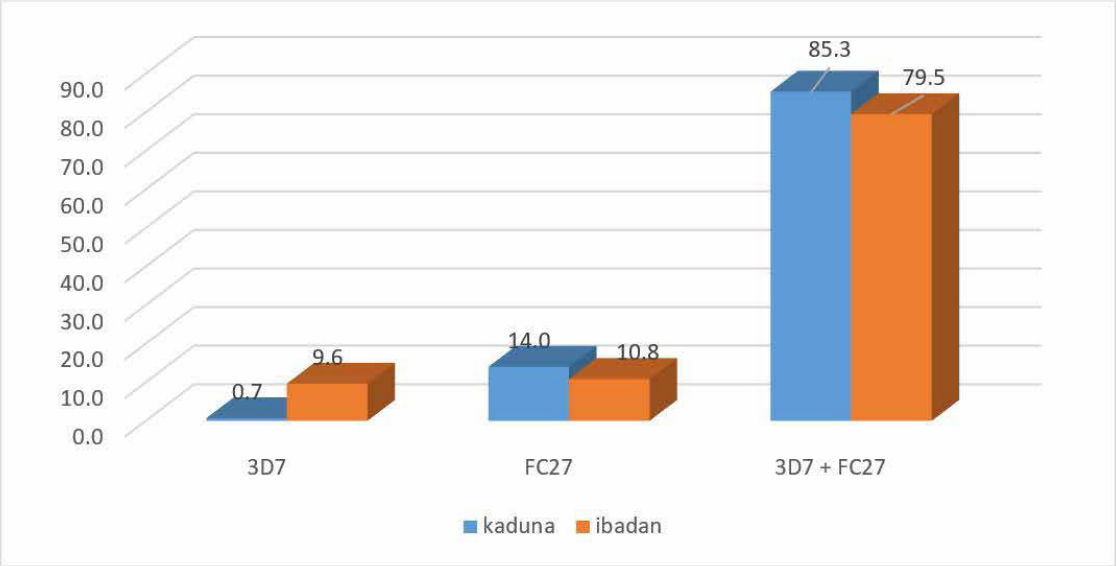

Figure 2: Haplotypes distribution for *msp2* allelic family stratified by location

The *glurp* allele of 1000bp was the most predominant in both Ibadan (31%) and Kaduna (18%). The 400-700bp allele was completely absent in Kaduna, whereas it recorded a frequency of 11% in Ibadan.

Table 5: Distribution of allelic variants of *Glurp* (RII) repeat region of P. falciparum in Ibadan and Kaduna.

Genotypes	Allelic size variants (100 bp bin)	Ibadan (N= 87), N (%)	Kaduna (N = 150), N (%)
I	400	-	1 (0.7)
II	500	-	10 (6.7)
III	600	-	1 (0.7)
IV	700	-	2 (1.3)
V	800	12 (17.1)	5 (3.3)
VI	900	23 (32.9)	7 (4.7)
VII	1000	31 (44.3)	18 (12.0)
VIII	1100	3 (4.3)	2 (1.3)
I + VII	400; 1000	-	1 (0.7)
II + VII	500; 1000	-	6 (4.0)
II + VIII	500,1100	-	2 (1.3)
V + VI	800; 900	1 (1.4)	-
II + IV +VII	500; 700; 1000	-	1 (0.7)
MOI		**1.01**	**1.2**

Multiplicity of infection and expected heterozygosity

The results for multiplicity of infection shows that *msp1* and *msp2* recorded 26% and 20% monoclonal and 74% and 80% multiclonal infection while *glurp* was 99% multiclonal with only 1% monoclonal infection in Ibadan. However, the MOI for *msp1* and *glurp* in Kaduna was more monoclonal; 70% and 82% respectively, while *msp2* recorded very significant multiclonal (85%) infection. The expected heterozygosity (*He*) and mean MOI was similar in both locations.

Table 6: Distribution of heterogeneity and Multiplicity of Infection by Location

Study sites	Gene	Monoclonal Infection (%)	Multiclonal Infection (%)	*HE*	MOI	p-value
Ibadan	*msp1*	9/35 (26)	26/35 (74)	0.1	1.82	**<0.0001**
	msp2	17/83 (20)	66/83 (80)	0.7	2.10	
	glurp	1/70 (1)	69/70 (99)	0.7	1.01	
Kaduna	*msp1*	71/102 (70)	31/102 (30)	0.2	1.42	
	msp2	22/150 (15)	128/150 (85)	0.6	2.25	
	glurp	45/55 (82)	10/55 (18)	0.8	1.2	

There was no significant association of gender, age, fever, parasitaemia and anemia on MOI in the two study locations.

Table 7: Association of demographic and clinical characteristics of *msp1* and *msp2* on MOI

		Ibadan		Kaduna	
Variable		**MOI (*msp1* + *msp2*)**	**p-value**	**MOI (*msp1* + *msp2*)**	**p-value**
Gender	Male	2.53	0.758	3.16	0.972
	Female	2.90		3.16	
Age (months)	> 60	2.86	0.562	3.07	0.231
	60 – 120	2.67		3.30	
Fever (Temp >37.4°C)	Yes	2.58	0.353	3.16	-
	No	2.86		-	
Anemia (PCV > 30%)	Yes	2.50	0.340	3.21	0.639
	No	2.82		3.12	
Day0 Parasite density	1000 -5000	2.30	0.587	1.13	0.443
	5001 - 10000	2.71		1.14	
	>10,000	2.72		1.14	

Discussion

Continuous assessment of the genetic diversity and multiplicity of *P. falciparum* infection is essential in understanding malaria transmission dynamics and pathogenesis across varying geographical locations with transmission intensity. This study evaluated and compared the frequency distribution of allelic diversity, heterozygosity (*He*) and multiplicity of infection (MOI) in *P. falciparum* isolates from Ibadan (Southern) and Kaduna (Northern) Nigeria.

Baseline demographic and clinical characteristics of enrollees indicated more malaria infection in males and children above 36 months. Clinical malaria is now increasingly reported in older children [29] which could be due to giving more priority to under-five with more efficient malaria control and intervention strategies in endemic areas [16]. The focus of malaria intervention programmes in under-five children have yielded tremendous reduction in malaria morbidity and mortality amongst this most vulnerable population. There is need to review current malaria intervention policies to extend similar attention to older children to reduce malaria transmission. The finding of *P. falciparum* as the predominant *specie* in Nigeria is consistent with earlier report. Other *Plasmodium species* (*malariae* and *ovale*) rarely occur as mono-infection but rather, as mixed infection with low prevalence from both locations. This finding corroborates earlier reports from Nigeria [17] and other west African countries include Burkina Faso, Gambia, Ghana and Mali [18].

There was more *msp1* allelic family amplified in northern Nigeria while *glurp* gene was more in southern Nigeria. The implication of the haplotype difference between the evaluated genes in the two regions are unclear. Although, variant of allelic frequencies across geographic settings have been reported to define malaria transmission intensity [19].

The significantly higher allelic diversity in the northern region could be linked to intense malaria transmission which may in parts due to climatic condition and transmission intensity. The worsening security challenges in the northern part of Nigeria has rendered many people homeless and are internally displaced in the region. This may hamper malaria intervention programmes such as distribution of long-lasting insecticide treated nets in the area. Also, a major contributory factor that may be responsible for the variation in malaria transmission in the northern part of Nigeria could be the disparity in the educational level and economic power of mothers and caregivers. It is believed that mothers and caregivers in northern Nigeria are educationally disadvantaged with little or no personal financial income compared to their peers in the southern part, which could afford a complete dose of artemisinin combination therapy (ACT) without waiting for financial support from their spouses. Also, the transmission intensity of *P. falciparum* has been proposed to have a simple linear relationship with genetic diversity with areas of low transmission having low genetic diversity, while areas with high transmission show high genetic diversity [20]. However, other studies have shown that the relationship between diversity and transmission intensity may not be simply linear because of other competing factors such as population size, the mutation rate of the loci under study, and the genotyping method [21]. The inconsistency of data from different geographic locations emphasize the need for this study and more studies are recommended which could guide our understanding on the precise impact of geographical influence on genetic diversity in order to proffer more lasting solutions to malaria control, intervention and elimination in Nigeria.

Similarly, the haplotypes distribution for *msp1* allelic family by location was higher in Kaduna than Ibadan except for RO33 + MAD20 and K1 + RO33 + MAD20 allelic combinations which was predominant in Ibadan. This distribution pattern was similar with reports from Northwest Ethiopia [22] and Bobo-Dioulasso, Burkina Faso [23]. A pooled analysis of the highest allelic frequency distribution in both region shows MAD20 as the predominant allele in *msp1*. This trend was also reported in *P. falciparum* genetic diversity from the China–Myanmar border region [24].

The expression of *msp2* allelic frequency distribution was not specific. The *msp2* allelic family amplification by region shows that isolates from Kaduna were predominated with FC27 compared to Ibadan. This finding was similar to reports from other sub-Saharan countries [25] but contrary to isolates from Bioko Island, Equatorial Guinea [26]. However, the combined allelic frequency distribution of 3D7 and FC27 was predominant in Kaduna.

The *glurp* allelic frequency distribution was more in Kaduna compared to Ibadan but the predominant allelic size (1000 bp) was more in Ibadan. Overall, the three polymorphic markers are extensively diverse in their genetic profile across the two geographic populations with higher allelic frequency distribution in Kaduna, confirming the geographical variation in transmission intensity and seasonality of malaria in southern and northern Nigeria.

Both regions exhibited similar expected heterozygosity (*He*) and multiplicity of infection as previously reported from different geographic locations with varying transmission intensities [27]. The three polymorphic markers show that many of the isolates harboured multiclonal infection in both regions. The *msp1* and *glurp* multiclonal infections were more in Ibadan while *msp2* was more in Kaduna. The high prevalence of multiclonal infections are indicative of intense malaria transmission in these regions despite the increasing scale-up activities of malaria intervention carried out by the National Elimination of Malaria Programmes (NEMP). Demographic (gender and age) and clinical (temperature, PCV and parasite density) characteristics did not significantly influence multiplicity of infection in the evaluated parasite strains with population. This finding corroborates Brice *et.al* from the Republic of Congo [28] and Funwei *et.al* [15].

Therefore, it is of note that the success of malaria control and elimination programmes can be monitored by the allelic frequency distribution, multiplicity of infection and expected heterozygosity of these essential biomarkers to monitor and evaluate the gains of the global efforts on malaria eliminate in Nigeria. The data presented in this study and our earlier study from Ibadan [15] may be useful as baseline information to guide the Nigeria National Elimination of Malaria Programmes (NEMP) as part of continuous molecular epidemiological surveillance of circulating parasite clones and profiles. This is important as genetic characterization and mapping of these genes are important in understanding the parasite dynamics. Furthermore, the impact of therapeutic interventions on *P. falciparum* transmission intensity varies across different geographical areas

and overtime. Therefore, a spatio-temporal study of the parasite's population structure in Nigeria is recommended which will help to formulate better malaria control strategies for the country.

Conclusion

Data from this study provides evidence of geographical difference in *Plasmodium falciparum* genetic diversity and multiplicity of infection from southern and northern Nigeria. This finding could be useful for the National Elimination of Malaria Programmes in designing a more effective strategy to control and eliminate malaria in Nigeria.

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Conflict of interest declaration

None declared.

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