https://dx.doi.org/10.4314/ijs.v24i1.3

GENETIC DIVERSITY IN MEROZOITE SURFACE PROTEIN 1 OF *Plasmodium falciparum* ISOLATES FROM IGBOGBO-BAYEKU, LAGOS, NIGERIA

Agomo, C. O.[1,*], Lameed, O.[1], Ajibaye, O.[2]

[1]Department of Medical Laboratory Science, University of Lagos, Lagos, Nigeria

[2]Department of Biochemistry and Nutrition, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria

*Corresponding Author's Email: coagomo@unilag.edu.ng

(Received: 17th March, 2021; Accepted: 25th January, 2022)

ABSTRACT

Mutations in merozoite surface protein 1 (MSP1) serve as indicators of genetic diversity in *Plasmodium falciparum* population in a given area. Diversity in MSP1 gene of *P. falciparum* isolates in Igbogbo-Bayeku, a peri-urban settlement of Lagos, Nigeria was assessed. Malaria was diagnosed by microscopy and polymerase chain reaction (PCR). MSP1 gene polymorphisms were analyzed in *P. falciparum*-positive samples using allele-specific primers of the three families of MSP1 block 2. Of the 63 malaria cases, 34 (54%) were microscopic while 29 (46%) were sub-microscopic cases. The three MSP1 families were present in the *P. falciparum* isolates with RO33 being the most abundant (55; 87.3%). Thirteen distinct genotypes of MSP1 were observed. There were more polyclonal infections (40; 63.5%) than monoclonal infections (23; 36.5%). The multiplicity of infection (MOI) was 1.98 and the expected heterozygosity (He) was 0.64. Participants aged >8 years had significantly higher MOI (2.26±0.98) than those aged ≤ 8 years (1.78±0.83) (P=0.04). Polyclonal infections were similar in microscopic (23/39; 67.6%) and sub-microscopic infections (17/29; 58.6%) (P=0.46). However, polyinfections were more in microscopic (26/34; 76.5%) than in submicroscopic infections (15/29; 51.7%) (P=0.04). A high level of genetic diversity was observed in the *P. falciparum* isolates in this community-based study which is an indication of intense malaria transmission.

Keywords: MSP1 families, *Plasmodium falciparum*, Multiplicity of infection, sub-microscopic malaria.

INTRODUCTION

Malaria is a parasitic disease of public health importance in Nigeria. Nigeria accounts for 27% of 229 million global malaria cases and 25% of malaria deaths globally (WHO, 2020). *Plasmodium falciparum* is the most virulent and most prevalent malaria parasite species in the WHO Africa region, accounting for 99.7% of the estimated malaria cases in 2018 (WHO, 2020). In Lagos, *P. falciparum* is also the most prevalent malaria parasite species (Agomo and Oyibo, 2013; Aina *et al.*, 2013).

The release of *P. falciparum* merozoites from infected erythrocytes elicits immune responses that are responsible for the clinical signs and symptoms associated with malaria infection (Singh and Chitnis, 2017). The *P. falciparum* merozoite surface is covered with several membrane proteins, of which merozoite surface protein 1 (MSP1) is the most abundant (Kauth *et al.*, 2006, Lin *et al.*, 2014). MSP1 is involved in the attachment of parasite to red blood cell receptors prior to invasion of red blood (Lin *et al.*, 2016). The success of *P. falciparum* in infecting red blood cells is attributed to the compatibility of its surface antigens with the receptors on the surface of red blood cells (Tilley *et al.*, 2011).

Molecular markers, such as merozoite surface protein 1 (MSP1) gene, are used to study the population structure of *P. falciparum* in a given area (Baruah *et al.*, 2009). MSP1 gene is divided into seventeen blocks of which block 2 shows extensive allelic polymorphism worldwide generated by insertion/deletion of repeats (Miller *et al.*, 1993; Wanji *et al.*, 2012). Block 2 alleles are mainly represented by three families namely K1, MAD20, and RO33 based on their characteristic tri-peptide motifs. Different allelic sequences belonging to these families show highly skewed and continent-specific geographical distribution (Aspeling-Jones and Conway, 2018). Fragment size polymorphisms in the three block 2 allelic families have commonly been used as a molecular marker in studying falciparum malaria transmission dynamics and host immunity (Branch *et al.*, 2001; Maestre *et al.*, 2013). Moreover, analysis of *P. falciparum* MSP1 gene block 2 polymorphisms are informative of malaria transmission intensity (Wanji *et al.*, 2012).

The variable genetic structure of the malaria parasites has also been related to the development of antimalarial immunity in endemic areas. Individuals are infected with multiple parasite clones in a malaria-endemic area leading to development of antimalarial immunity; while the immune response leads to selection or dominance of certain genotypes in a given area (Takala *et al.*, 2006; Noranate *et al.*, 2009).

Limited reports are available on the genetic diversity in *P. falciparum* populations in Lagos, Nigeria. This study determined multiplicity of infection and distribution of MSP1 families in *P. falciparum* infections in a peri-urban community in Ikorodu, Lagos, Nigeria.

MATERIALS AND METHODS

Study Area: This study was carried out in Igbogbo-Bayeku, Ikorodu Local Government Area of Lagos State. Ikorodu is located in the North-East region of Lagos State. The latitude of Ikorodu is 6.616865, and the longitude is 3.508072 (Latlong, 2021). It is located along the Lagos Lagoon and shares a boundary with Ogun State. Participants in the study were residents of Igbogbo Bayeku who volunteered to be screened for malaria, irrespective of their ages and gender.

Sample Collection and Processing

Finger prick blood sample was used for the preparation of malaria blood films and dried blood spots on 3mm Whatman filter paper. The blood films were stained with Giemsa stain and examined as previously described (Agomo *et al.*, 2009). The body temperature of the participants was measured with a non-contact infra-red thermometer (Unioncare Model IT-122, Taiwan). Fever was defined as body temperature ≥37.5°C.

MSP 1 Gene Analyses

Malaria parasite genomic DNA was extracted from dried blood spots by phenol-chloroform - isoamyl alcohol (PCI) DNA extraction method (Panda *et al.*, 2019). Dried Blood Spots were cut into small circular discs using a clean paper punch and were placed in Eppendorf tube with 250 µL of lysis buffer (containing KCl, $MgCl_2$, Tris Cl, and Tween-20). This was incubated overnight with Proteinase K and 250 µL of PCI was added to the DNA solution in a 1.5 mL micro-centrifuge tube. In between samples, the paper punch was sterilized by dipping in ethanol and flaming with a Bunsen burner to prevent cross contamination of samples. The extracted DNA solution was vortexed briefly and centrifuged for 10 min at 13,000 rpm. The top aqueous layer containing the DNA was carefully transferred to a new tube. Thereafter, 500 µL of ice-cold ethanol was added to the aqueous phase and mixed gently. It was spun at 13,000 rpm for 10 min and the supernatant was decanted. The precipitate was washed with 500 µL of 70% ethanol at room-temperature by centrifuging at 13,000 rpm for 10 min. The supernatant was removed and the pellet was dried at room temperature. The pellet was dissolved in 50 µL of Tris-EDTA buffer solution (pH 8.0).

The *P. falciparum* MSP1 gene was amplified using primers previously described (Mwingira *et al.*, 2011). Briefly, all reactions were carried out in a final volume of 20 µL which included 2 µL of DNA template (primary PCR product). Oligonucleotide primers were used at final concentrations of 0.3 mM. The reaction mixture contained PCR buffer, 0.2 mM of each of the four deoxynucleoside triphosphates, and 0.75 Units of AmpliTaq polymerase. Cycling conditions for primary reaction were: initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 61 °C for 45 s and elongation at 72 °C for 90 s and a final elongation at 72 °C for 5 min. Two microliters of primary PCR product were used as DNA template in the secondary PCR. The reaction mixture of the secondary PCR had similar concentrations as the primary PCR. The cycling conditions for the secondary PCR were: initial denaturation at 95°C for 5 min, 40 cycles of 94 °C for 45 s; 61 °C for 30 s and 72 °C for 1 min followed by final elongation step at 72 °C for 10 min. Each genotype was identified based on the size of the PCR products resolved on 1.5% agarose gel. A 0.5 μg/mL of ethidium bromide was used for the UV detection. Electrophoresis conditions were 100 mV for 30 min.

Data Analysis

Data analysis was performed using SPSS 25.0 statistical software. Frequency tables were used to summarize categorical variables. Analysis of variance or Student's T-test was used for

comparison of continuous variables conforming to normal distribution depending on which is appropriate. Pearson's Chi-square or Fisher Exact Test was used to compare association between two categorical variables. Spearman's rank correlation coefficients were calculated to assess association between multiplicity of infection (MOI) and geometric mean parasite density. Statistical significance was set at $P<0.05$.

As a measure of parasite genetic diversity, the expected heterozygosity (HE) which represents the probability of being infected by two parasites with different alleles at a given locus and ranging between 0 and 1 was calculated using the formula: $HE= [n/ (n-1)] [(1-\sum Pi2)]$; where n = sample size, Pi = allele frequency (Kloosterman *et al*., 1993; Mwingira *et al*., 2011). The MOI was determined by dividing the total number of distinct DNA fragments of *P. falciparum* MSP1 detected by the number of positive samples (Oyebola *et al*., 2014). Infection with a single allelic family per infection was considered as mono-infection while presence of more than one family per infection was defined as polyinfection. The presence of a single genotype per infection was regarded as monoclonal infection while more than one genotype per infection was regarded as polyclonal infection.

Ethical Consideration

Consent form containing information on the study protocol, benefits and risk was given to each participant or their care-giver. Informed consent and assent (where applicable) were obtained from study participants before they were allowed to participate in the study. All the information collected in this study was treated as confidential and assess was limited to investigators only. Personal identifiers were removed from electronic version of the data obtained from this study. The study was approved by the Health Research Ethics Committee of College of Medicine of the University of Lagos, Lagos, Nigeria (CMUL/HREC/09/19/654).

RESULTS

Of the 183 blood samples analyzed by microscopy and PCR, 34 (18.6%) and 63 (34.4%) were positive for malaria. Therefore, 63 samples were analyzed for genetic diversity in *Plasmodium falciparum* MSP1 block 2. The median age of the study participants was 8 years (Table 1) of which most of the participants were aged 5 to 9 years (28; 44.4%). The male to female ratio was 1:1.1. Only 6 (10%) of the participants had body temperature ≥37.5 °C. Of the 63 samples, 29 (46%) were submicroscopic malaria infection. The geometric mean parasite density was 2,985 parasites/μL (range: 91 – 160,753 parasites/μL) (Table 1).

Merozoite Surface Protein 1 (MSP1) Gene Families

The three MSP1 block 2 families (K1, MAD20 and RO33) were present in *Plasmodium falciparum* isolates from the study site. The families were observed alone per infection and in combination with other families. Triple families per infection were found in 9 (14%) of the malaria cases, while double and single families per infection were found in 32 (50.8%) and 22 (34.9%) of the malaria cases, respectively. Infections with RO33 alone (18; 28.6%) and in combination with K1 (28.6%) were the commonest observed infections, while the combination of K1 and MAD20 had the least frequency 1 (1.6%) (Figure 1). Overall, RO33 was the predominant family occurring in 55 (87%) of the malaria cases (Table 2), while K1 and MAD20 were detected in 33 (52%) and 22 (35%) of the malaria cases, respectively.

MSP1 Genotypes

Thirteen distinct genotypes of MSP1 gene block 2 were observed in this study. The number of genotypes of K1, MAD20 and RO33 were 7, 4 and 2, respectively. The number of genotypes per infection ranged from 1 to 4 genotypes (Figure 2). There were more polyclonal infections (40; 63.5%) than monoclonal infections (23; 36.5%). The frequency of single, double, triple and quadruple clones per infection were 23 (36.5%), 22 (34.9%), 14 (22.2%) and 4 (6.3%), respectively (Figure 2).

Overall, the mean MOI was 1.98±0.92 (Table 3) and the expected heterozygosity (HE) was 0.64. An association between MOI and age was observed. The MOI of study participants aged >8 years 2.26±0.98 was significantly higher than the MOI of participants aged 8 years and below 1.78±0.83 ($P=0.04$). The MOI was not dependent on gender, body temperature or malaria type

(submicroscopic or microscopic) (P>0.05) (Table 3).

The proportion of polyclonal infections were similar in microscopic and submicroscopic infections (P=0.458). Majority of the microscopic and submicroscopic infections were polyclonal infections (23/34; 67.6%) and (17/29; 58.6%), respectively. There were significantly more polyinfections in microscopic (26/34; 76.5%) than in submicroscopic malaria infections (15/29; 51.7%), (P=0.04) (Table 4).

Reclassification of the number of families into single and multiple families/infection showed that a significantly higher proportion of the malaria cases in microscopic infections had multiple MSP1 families (26/34; 76.5%) than sub-microscopic infections in which the proportion of multiple MSP1 families was (15/29; 51.7%) (P=0.04). The proportion of multiple genotypes in microscopic infections (23/39; 67.6%) was similar to the proportion of multiple genotypes in sub-microscopic infections (17/29; 58.6%) (P=0.46) (Table 4).

Multiplicity of Infection and Parasite Density

The correlation between parasite density and MOI was not significant [r=-0.159; P=0.40]. Although the geometric mean parasite density of monoclonal infections 3,780 [range: 186 – 160,753] parasites/μL was higher than that of polyclonal infections 2698 [range: 91 – 138, 560] parasites/μL, the difference was not significant (P=0.722).

Table 1: Demographic characteristics of the study participants

Character	Frequency	%
SEX		
Male	33	52.4
Female	30	47.6
Age (years)		
Median (Range)	8.0	0.6 – 72.0
<5	14	22.2
5-9	28	44.4
≥10	21	33.3
Temperature (°C)		
≤37.5	6	10.0
<37.5	54	90.0
Malaria Type		
Microscopic	34	54.0
Submicroscopic	29	46.0
Parasite density (parasites/μL)		
Geometric mean	2,985	
Range	91-160,753	

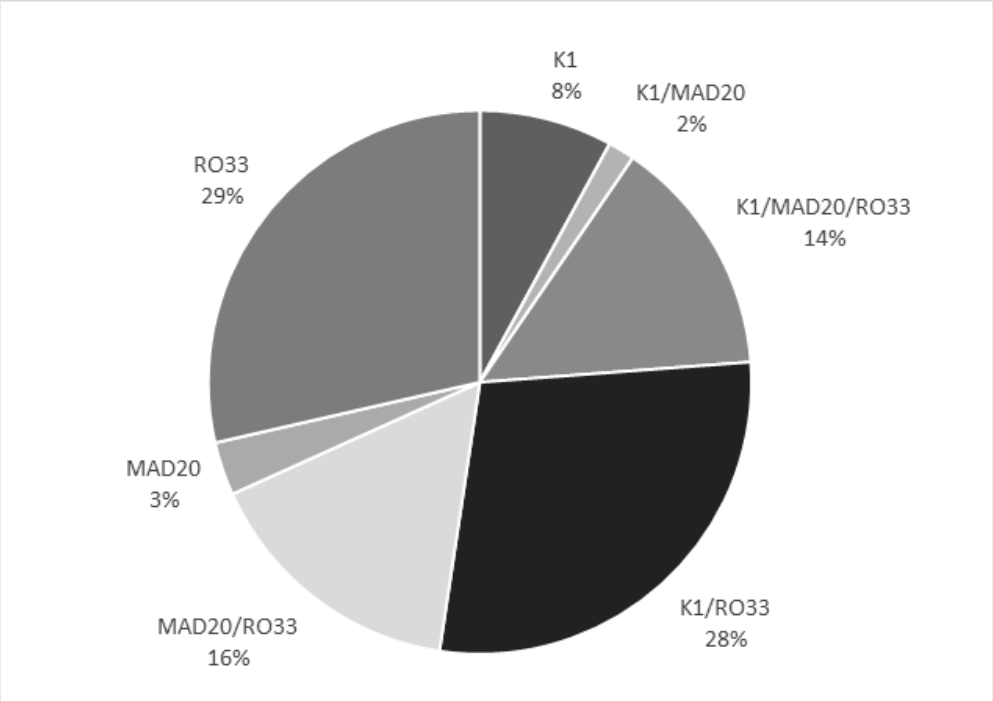

Figure 1: Distribution of *Plasmodium falciparum* Merozoite Surface Protein 1 families per infection

Table 2: Distribution of Merozoite Surface Protein 1 families and genotypes

MSP1 FAMILY	Frequency (%)	Genotypes	Size range (bp)
KI	33 (52.4)	7	125-220
MAD20	22 (34.9)	4	125-240
RO33	55 (87.3)	2	110-175

Key:
MSP1 = Merozoite Surface Protein 1
bp = base pair

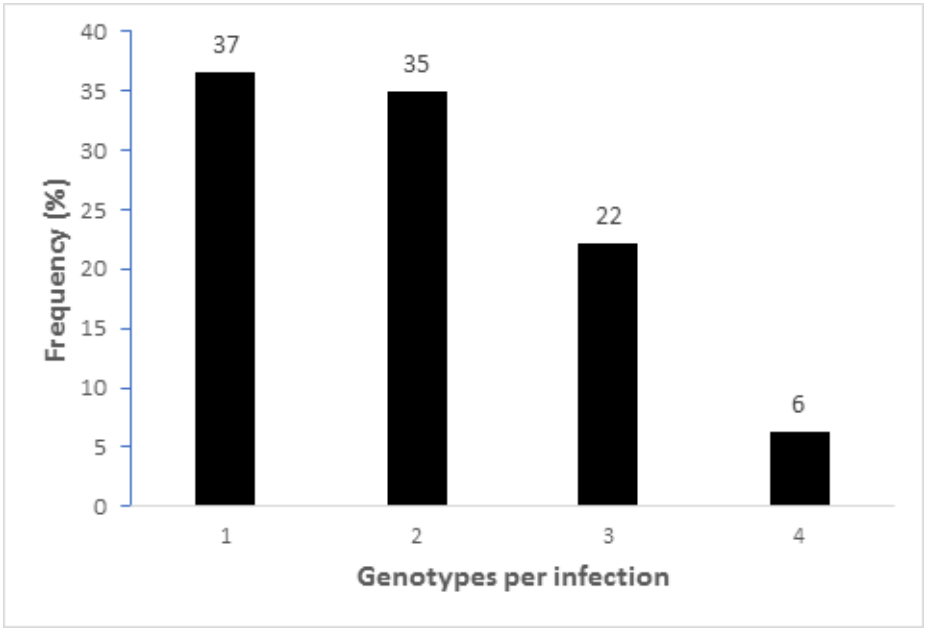

Figure 2: Distribution of the number of *Plasmodium falciparum* Merozoite Surface Protein 1 genotypes per infection.

Table 3: Comparison of Multiplicity of Infection with Gender, Age, Body Temperature and Type of Malaria Infection

			MOI		
.Variables		**Frequency**	**Mean**	**SD**	**P**
Gender	Male	33	1.94	0.86	0.690
	Female	30	2.03	1.00	
Age (years)	1-8	36	1.78	0.83	0.040
	>8	27	2.26	0.98	
Temperature (°C)	>=37.5	6	1.83	0.75	0.749
	<37.5	54	1.96	0.95	
Malaria type	Microscopic	34	2.15	1.02	0.131
	Submicroscopic	29	1.79	0.77	
	Total	**63**	**1.98**	**0.92**	

Key:
MOI = Multiplicity of infection
SD = Standard deviation
P = Probability value

Table 4: Comparison of clones and families per infection with type of malaria infection

Variable		**Type of Malaria**		**Total n(%)**	**P**
		Microscopic n (%)	**Submicroscopic n (%)**		
Clones/ infection	Monoclonal	11 (32.4)	12 (41.4)	23 (36.5)	0.458
	Polyclonal	23 (67.6)	17 (58.6)	40 (63.5)	
Families/ infection	Monoinfection	8 (23.5)	14 (48.3)	22 (34.9)	0.04
	Polyinfection	26 (76.5)	15 (51.7)	41 (65.1)	
	Total	**34 (100.0)**	**29 (100.0)**	**63 (100.0)**	

Key:
n = Frequency
P= Probability value

DISCUSSION

This study was conducted in a community in Lagos, Nigeria. Low prevalence rates of malaria have been reported in Lagos State by a number of studies (Agomo *et al*., 2009; Aina *et al*., 2013; Udoh *et al*., 2020). The high proportion of submicroscopic malaria cases reported in this study shows that reliance on malaria microscopy as the gold standard of malaria diagnosis may be underestimating the prevalence of malaria in Nigeria. In this study, malaria prevalence was underestimated by about 46% using microscopy. Increased malaria prevalence rates are obtained when diagnostic tests that are more sensitive than microscopy are used (Niang *et al*., 2017). Deployment of more sensitive malaria diagnostic tests will enable the detection and treatment of as many malaria cases as possible.

Poor treatment outcomes have been associated with polyclonal infections (Gosi *et al*., 2013). In this study, the proportion of polyclonal infections were similar in microscopic and submicroscopic infections. The predominance of polyclonal infections in these mostly asymptomatic malaria cases suggests that these malaria infections have the potential of causing severe morbidity and mortality. It is therefore important to target both microscopic and submicroscopic malaria cases to significantly reduce malaria morbidity and mortality.

The MSP1 allelic family RO33 was the most predominant in this study site. It was observed alone and in combination with K1 and MAD 20 in 87% of the infections. This is consistent with the findings of Ghoshal and co-workers who reported a predominance of RO33 in mild malaria cases (Ghoshal *et al*., 2018). All the cases in this study were mild malaria cases, mostly asymptomatic cases. Only 10% of the study participants were febrile at presentation. In some studies in Nigeria, where the study participants were clinical cases of malaria, K1 was the predominant MSP1 family in the *P. falciparum* infections (Olasehinde *et al*., 2012; Oyebola *et al*., 2014).

The lack of association between parasite density and polyclonal infections as observed in this study indicates competition between the different clones within a host which suppresses the expected exponential growth of individual clones (Sondo *et al*., 2019). This finding is however contrary to the report by Diouf *et al.* (2019), who observed a significantly higher parasitaemia in polyclonal than in monoclonal infections. Also, parasite density was not associated with MOI in this study. Lack of association between parasite density and multiplicity of infection have been reported by other workers (Happi *et al*., 2004; Mohammed *et al*., 2015).

There have been various reports on the relationship between multiplicity of infection and age. Some workers did not observe any relationship between MOI and age (Happi *et al*., 2004; Mohammed *et al*., 2015). Yavo *et al.* (2016) reported that MOI decreased with age in Cote d'Ivoire. In this study, we observed that MOI was higher in study participants older than 8 years compared to those aged 8 years and below. The differences in these study findings suggests that population structure of *P. falciparum* varies from location to location.

A high level of genetic diversity was observed in *P. falciparum* isolates in this study. For a malaria vaccine based on MSP1 to be efficacious in Lagos, all the distinct allelic variants will have to be incorporated into the vaccine. Genetic diversity in *Plasmodium falciparum* has been a major obstacle to the development of malaria vaccines (Genton *et al*., 2002).

The mean MOI and the number of distinct genotypes of K1, MAD20 and RO33 reported in this study are greater than previous reports in Lagos and Ogun (a state that shares geographical border with Lagos State) (Olasehinde *et al*., 2012; Oyebola *et al*., 2014). This is an indication of stable malaria transmission in spite of the low malaria prevalence in Lagos compared to other states in Nigeria.

CONCLUSION

The high level of genetic diversity and multiplicity of *P. falciparum* infections suggests intense malaria transmission in this study area. Polyclonal infections were more than monoclonal infections. Polyinfection was associated with microscopic infections. Multiplicity of infection was age dependent but not associated with submicroscopic or microscopic malaria infections.

ACKNOWLEDGEMENTS

We thank the traditional ruler of Igbogbo Bayeku Community and the Malaria Program Manager of Ikorodu L.G.A. for their support in facilitating the study. We are grateful to the persons that participated in the study.

AUTHORS' CONTRIBUTIONS

COA and LO designed the study. LO, OA collected sample and performed laboratory studies. COA, LO and OA participated in the data analysis. COA, LO and OA drafted and revised the manuscript. All authors read and approved the final manuscript.

FUNDING

No funding was obtained for the study.

REFERENCES

Agomo, C. O. and Oyibo, W. A. 2013. Factors associated with risk of malaria infection among pregnant women in Lagos, Nigeria. *Infectious Disease of Poverty,* 2: 19.

Agomo, C. O., Oyibo, W. A., Anorlu, R. I. and Agomo, P. U. 2009. Prevalence of malaria in pregnant women in Lagos, South-West Nigeria. *Korean Journal of Parasitology,* 47: 179-183.

Aina, O. O., Agomo, C. O., Olukosi, Y. A., Okoh, H. I., Iwalokun, B. A., Egbuna, K. N., Orok, A. B., Ajibaye, O., Enya, V. N., Akindele, S. K., Akinyele, M. O. and Agomo, P. U. 2013. Malariometric survey of Ibeshe community in Ikorodu, Lagos state: dry season. *Malaria Research and Treatment*, 2013: 487250.

Aspeling-Jones, H. and Conway, D. J. 2018. An expanded global inventory of allelic variation in the most extremely polymorphic region of *Plasmodium falciparum* merozoite surface protein 1 provided by short read sequence data. *Malaria Journal,* 17: 345.

Baruah, S., Lourembam, S. D., Sawian, C. E., Baruah, I. and Goswami, D. 2009. Temporal and spatial variation in MSP1 clonal composition of *Plasmodium falciparum* in districts of Assam, Northeast India. *Infection Genetics and Evolution,* 9: 853-859.

Branch, O. H., Takala, S., Kariuki, S., Nahlen, B. L., Kolczak, M., Hawley, W. and Lal, A. A. 2001. *Plasmodium falciparum* genotypes, low complexity of infection, and resistance to subsequent malaria in participants in the Asembo Bay Cohort Project. *Infection and Immunity,* 69: 7783-7792.

Diouf, B., Diop, F., Dieye, Y., Loucoubar, C., Dia, I., Faye, J., Sembene, M., Perraut, R., Niang, M. and Toure-Balde, A. 2019. Association of high *Plasmodium falciparum* parasite densities with polyclonal microscopic infections in asymptomatic children from Toubacouta, Senegal. *Malaria Journal,* 18: 48.

Genton, B., Betuela, I., Felger, I., Al-Yaman, F., Anders, R. F., Saul, A., Rare, L., Baisor, M., Lorry, K., Brown, G. V., Pye, D., Irving, D. O., Smith, T. A., Beck, H. P. and Alpers, M. P. 2002. A recombinant blood-stage malaria vaccine reduces *Plasmodium falciparum* density and exerts selective pressure on parasite populations in a phase 1-2b trial in Papua New Guinea. *Journal of Infectious Diseases*, 185: 820-827.

Ghoshal, S., Gajendra, P., Datta Kanjilal, S., Mitra, M. and Sengupta, S. 2018. Diversity analysis of MSP1 identifies conserved epitope organization in block 2 amidst high sequence variability in Indian *Plasmodium falciparum* isolates. *Malaria Journal*, 17: 447.

Gosi, P., Lanteri, C. A., Tyner, S. D., Se, Y., Lon, C., Spring, M., Char, M., Sea, D., Sriwichai, S., Surasri, S., Wongarunkochakorn, S., Pidtana, K., Walsh, D. S., Fukuda, M. M., Manning, J., Saunders, D. L. and Bethell, D. 2013. Evaluation of parasite subpopulations and genetic diversity of the msp1, msp2 and glurp genes during and following artesunate monotherapy treatment of *Plasmodium falciparum* malaria in Western Cambodia. *Malaria Journal*, 12: 403.

Happi, C. T., Gbotosho, G. O., Sowunmi, A., Falade, C. O., Akinboye, D. O., Gerena, L., Kyle, D. E., Milhous, W., Wirth, D. F. and Oduola, A. M. 2004. Molecular analysis of *Plasmodium falciparum* recrudescent malaria infections in children treated with chloroquine in Nigeria. *American Journal of Tropical Medicine and Hygiene*, 70: 20-26.

Kauth, C. W., Woehlbier, U., Kern, M., Mekonnen, Z., Lutz, R., Mücke, N., Langowski, J. and Bujard, H. 2006. Interactions between merozoite surface proteins 1, 6, and 7 of the malaria parasite *Plasmodium falciparum*. *Journal of Biological Chemistry*, 281: 31517-31527.

Kloosterman, A. D., Budowle, B. and Daselaar, P. 1993. PCR-amplification and detection of the human D1S80 VNTR locus. Amplification conditions, population genetics and application in forensic analysis. *International Journal of Legal Medicine*, 105: 257-264.

LatLong 2021. Where is Ikorodu on the map? https://www.latlong.net/place/ikorodu-nigeria-14163.html. Accessed Nov 26, 2021

Lin, C. S., Uboldi, A. D., Epp, C., Bujard, H., Tsuboi, T., Czabotar, P. E. and Cowman, A. F. 2016. Multiple *Plasmodium falciparum* Merozoite Surface Protein 1 Complexes Mediate Merozoite Binding to Human Erythrocytes. *Journal of Biological Chemistry*, 291: 7703-7715.

Lin, C. S., Uboldi, A. D., Marapana, D., Czabotar, P. E., Epp, C., Bujard, H., Taylor, N. L., Perugini, M. A., Hodder, A. N. and

Cowman, A. F. 2014. The merozoite surface protein 1 complex is a platform for binding to human erythrocytes by *Plasmodium falciparum*. *Journal of Biological Chemistry*, 289: 25655-25669.

Maestre, A., Arango, E. and Carmona-Fonseca, J. 2013. Status of allele frequency and diversity of *Plasmodium falciparum* msp1, msp2 and glurp before implementation of an artemisinin-based combined therapy in Northwestern Colombia. *Colombia Medica,* 44: 208-212.

Miller, L. H., Roberts, T., Shahabuddin, M. and McCutchan, T. F. 1993. Analysis of sequence diversity in the *Plasmodium falciparum* merozoite surface protein-1 (MSP-1). *Molecular and Biochemical Parasitology,* 59: 1-14.

Mohammed, H., Mindaye, T., Belayneh, M., Kassa, M., Assefa, A., Tadesse, M., Woyessa, A., Mengesha, T. and Kebede, A. 2015. Genetic diversity of *Plasmodium falciparum* isolates based on MSP-1 and MSP-2 genes from Kolla-Shele area, Arbaminch Zuria District, southwest Ethiopia. *Malaria Journal,* 14: 73.

Mwingira, F., Nkwengulila, G., Schoepflin, S., Sumari, D., Beck, H. P., Snounou, G., Felger, I., Olliaro, P. and Mugittu, K. 2011. *Plasmodium falciparum* msp1, msp2 and glurp allele frequency and diversity in sub-Saharan Africa. *Malaria Journal,* 10: 79.

Niang, M., Thiam, L. G., Sane, R., Diagne, N., Talla, C., Doucoure, S., Faye, J., Diop, F., Badiane, A., Diouf, B., Camara, D., Diene-Sarr, F., Sokhna, C., Richard, V. and Toure-Balde, A. 2017. Substantial asymptomatic submicroscopic Plasmodium carriage during dry season in low transmission areas in Senegal: Implications for malaria control and elimination. *PLoS One,* 12: e0182189.

Noranate, N., Prugnolle, F., Jouin, H., Tall, A., Marrama, L., Sokhna, C., Ekala, M. T., Guillotte, M., Bischoff, E., Bouchier, C., Patarapotikul, J., Ohashi, J., Trape, J. F., Rogier, C. and Mercereau-Puijalon, O. 2009. Population diversity and antibody selective pressure to *Plasmodium falciparum* MSP1 block2 locus in an African malaria-endemic setting. *BMC Microbiology,* 9: 219.

Olasehinde, G. I., Yah, C. S., Singh, R., Ojuronbge, O. O., Ajayi, A. A., Valecha, N., Abolaji, A. O. and Adeyeba, A. O. 2012. Genetic diversity of *Plasmodium falciparum* field isolates from south western Nigeria. *African Health Sciences*, 12: 355-361.

Oyebola, M. K., Idowu, E. T., Olukosi, Y. A., Iwalokun, B. A., Agomo, C. O., Ajibaye, O. O., Tola, M. and Otubanjo, A. O. 2014. Genetic diversity and complexity of *Plasmodium falciparum* infections in Lagos, Nigeria. *Asian Pacific Journal of Tropical Biomedicine*, 4: S87-S91.

Panda, B. B., Meher, A. S. and Hazra, R. K. 2019. Comparison between different methods of DNA isolation from dried blood spots for determination of malaria to determine specificity and cost effectiveness. *Journal of Parasitic Diseases,* 43: 337-342.

Singh, S. and Chitnis, C. E. 2017. Molecular Signaling Involved in Entry and Exit of Malaria Parasites from Host Erythrocytes. *Cold Spring Harbor Perspectives in Medicine*, 7(10): a026815.

Sondo, P., Derra, K., Lefevre, T., Diallo-Nakanabo, S., Tarnagda, Z., Zampa, O., Kazienga, A., Valea, I., Sorgho, H., Ouedraogo, J. B., Guiguemde, T. R. and Tinto, H. 2019. Genetically diverse *Plasmodium falciparum* infections, within-host competition and symptomatic malaria in humans. *Scientific Reports,* 9: 127.

Takala, S. L., Escalante, A. A., Branch, O. H., Kariuki, S., Biswas, S., Chaiyaroj, S. C. and Lal, A. A. 2006. Genetic diversity in the Block 2 region of the merozoite surface protein 1 (MSP-1) of *Plasmodium falciparum*: additional complexity and selection and convergence in fragment size polymorphism. *Infection Genetics and Evolution*, 6: 417-424.

Tilley, L., Dixon, M. W. and Kirk, K. 2011. The *Plasmodium falciparum*-infected red blood cell. *International Journal of Biochemistry and Cell Biology* 43: 839-842.

Udoh, B. E., Iwalokun, B. A., Etukumana, E. and Amoo, J. 2020. Asymptomatic falciparum Malaria and its Effects on Type 2 Diabetes Mellitus Patients in Lagos, Nigeria. *Saudi Journal of Medicine and Medical Sciences,* 8: 32-40.

Wanji, S., Kengne-Ouafo, A. J., Eyong, E. E., Kimbi, H. K., Tendongfor, N., Ndamukong-Nyanga, J. L., Nana-Djeunga, H. C., Bourguinat, C., Sofeu-Feugaing, D. D. and Charvet, C. L. 2012. Genetic diversity of *Plasmodium falciparum* merozoite surface protein-1 block 2 in sites of contrasting altitudes and malaria endemicities in the Mount Cameroon region. *American Journal of Tropical Medicine and Hygiene*, 86: 764-774.

World Health Organization (2020). World malaria report 2020: 20 years of global progress and challenges. World Health Organization, Geneva. 247pp.

Yavo, W., Konate, A., Mawili-Mboumba, D. P., Kassi, F. K., Tshibola Mbuyi, M. L., Angora, E. K., Menan, E. I. and Bouyou-Akotet, M. K. 2016. Genetic Polymorphism of msp1 and msp2 in *Plasmodium falciparum* Isolates from Cote d'Ivoire versus Gabon. *Journal of Parasitology Research*, 2016: 3074803.