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Original Research Article

Genetic Diversity of *Plasmodium falciparum* in Northern Taraba State, Nigeria

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ABSTRACT

Malaria remains a significant public health challenge in Nigeria, particularly in the north-eastern Nigeria. Understanding the genetic diversity of *Plasmodium falciparum* in this area is crucial for improving treatment and control strategies. This study aimed to determine the genetic diversity of P. falciparum associated with patients attending health facilities in Northern Taraba State, Nigeria. A cross-sectional prospective study involving 1,500 patients diagnosed with *P. falciparum* infection was conducted at three facilities; Federal Medical Centre, Jalingo; General Hospital, Lau; General Hospital in Zing over a period of 6 months (October, 2023- March, 2024). Polymorphic msp1 and msp2 genes were used to explore the genetic diversity and multiplicity of falciparum malaria infections. Parasite DNA was extracted and gene frequencies and diversity were analyzed using allele-specific nested PCR assay followed by sequencing. Statistical analysis was performed to evaluate the prevalence and distribution of different alleles. All the three allele types for MSP1; K1, MAD20 and RO33 and of MSP2; 3D7 and FC27 were identified in Zing and Lau, except in Jalingo where RO33 of the MSP1 family was not amplified. Mixed infection of the allele types was also observed. Analysis of msp1 alleles revealed that MAD20 allelic family was predominant with 20.0% followed by K1 at 17.8% and RO33 at 13% respectively. In msp2, the 3D7 family was most represented with 35.2% prevalence against 16.3% for FC27. Forty-nine percent were multi-clonal infections. Mean multiplicity of infections (MOI) ranged 1.57-1.77, and 1.48-1.49 for msp1 and msp2 genes respectively. The study revealed significant genetic diversity among P. falciparum isolates in the study region, indicating a complex malaria transmission pattern. These findings underscore the importance of ongoing surveillance and the need for tailored treatment strategies to address the specific challenges posed by malaria in this region. This study is the first to analyze the genetic diversity of Plasmodium falciparum in Northeast Nigeria using merozoite surface protein (msp) antigens, revealing significant polymorphism in MSP1 and MSP2 across various sites. It highlights the prevalence of different msp1 and msp2 alleles, the predominance of MAD20 and 3D7 families, and the significant multiplicity of infection (MOI), providing valuable insights for malaria surveillance and control strategies in Taraba State and similar endemic regions.

Keywords: Malaria, *Plasmodium falciparum,* genetic, merozoites surface proteins

INTRODUCTION

Malaria presents a significant public health threat, with Africa bearing 80 to 90% of its morbidity and mortality burden, affecting individuals of all ages [1-3]. It remains a major parasitic disease in tropical regions, often leading to severe cases with altered haematological and biochemical parameters, resulting in common complications [4]. Patients with malaria commonly exhibit haematological abnormalities such as anaemia, thrombocytopenia, and leukocytosis or leucopenia [5, 6].

The rise in genetic diversity within Plasmodium falciparum strains hampers malaria eradication efforts in Nigeria, necessitating a comprehensive understanding of genetic variation for effective drug development and eradication strategies [7]. This diversity complicates infection management, as individuals can harbor multiple strains simultaneously, making treatment challenging and increasing the risk of treatment failure [8, 9]. Genotyping, particularly utilizing merozoite surface protein (msp) genes, aids in assessing genetic diversity and infection dynamics, informing interventions such as drug and vaccine trials [7 - 9].

Plasmodium falciparum merozoites, the invasive stage of the parasite, trigger host immune responses and induce pathophysiological symptoms by invading and destroying red blood cells [10]. Key proteins on the parasite's plasma membrane, MSP-1 and MSP-2, play critical roles in red blood cell invasion and inflammatory responses [11, 12]. These proteins, carried in organelles called rhoptries and micronemes, facilitate evasion of host immune responses and contribute to drug resistance strategies [12, 13].

The presence of multiple genetically diverse parasites in a population can increase the complexity of infection (COI) within individuals, referred to as multiplicity of infection (MOI) or COI [8, 9]. Genetic polymorphism is used to genotype infected populations and distinguish diverse P. falciparum isolates, with merozoite surface protein (msp) genes being the most common markers due to their high variability [7,8,9,14]. Moreover, some studies have stated that certain P. falciparum genotypes can be linked with more virulent infections [15, 16] that makes it more difficult for the immune system to deal with, resulting in severe malaria [17]. Several studies have linked MOI to the severity of malaria especially in areas with high transmission rates [18, 19]. The genetic diversity of P. falciparum varies based on infection degree, host age, and geographical location, with higher diversity observed in malaria-endemic regions like Nigeria [20, 21,22,23,24]. Despite the endemicity, data on *P. falciparum*'s genetic diversity, especially in Northern Taraba Municipality, is lacking. Understanding these parameters is crucial for diagnosis, treatment, and complication prevention, thus underscoring the importance of studying genetic variation for effective malaria control measures [25].

Malaria's public health threat, exacerbated by the genetic diversity of P. falciparum, necessitates comprehensive research to inform effective control strategies and reduce mortality rates, particularly in vulnerable populations. The aim and objective of the study are to assess the genetic diversity of Plasmodium falciparum among patients seeking care in health facilities in Northern Taraba State.

MATERIALS AND METHODS

Ethical Considerations

The Ethical approval was sought and obtained from the Taraba State Ministry of Health Research Ethical Committee (TRSHREC/2023/018) and presented to the health facilities where the blood samples were collected in compliance with the Declaration on the Right of the Patient [26].

Written informed consent for participation in the study was sought from the patients in accordance with standards for human experimentation and the Helsinki

Declaration. Before enrolment for the study, the patients/subjects involved signed an informed consent form. Guardian/Parent signed the consent form on behalf of participants below the age of 18 years old.

Study Area

Taraba state is the second largest state in terms of landmass in Nigeria. It is located in the Southern part of northeastern Nigeria along the eastern borderland between Nigeria and Cameroon. The state lies roughly between latitude 6° 25'N and 9° 30'N and between longitude 9°30'E and 11° 45'E. The state covers a land area of about 60,291 km2 with a population of about 2,300,736 people according to the 2006 census. Taraba state is made up of 16 Local Government Areas (LGAs) and 3 Senatorial districts (northern Taraba, southern Taraba, and Taraba central). Northern Taraba consists of six LGAs, Ardo Kola, Karim Lamido, Lau, Jalingo, Yorro and Zing. The residents of the local governments are predominantly Mumuye, Yandang, Fulani and Hausa among others and they are mostly farmers. The climate in the area can be described as tropical sub-humid type with two distinct (wet and dry seasons). It has an average rainfall of 7 months annually with total range between 1,200mm and 2000mm in the months of April and October. The temperature is relatively high throughout the year averaging 28-32°C with occasional peak at 44.0 °C between March and April.

Figure 1: Map of study area
Source: Britannica

Research Design

This is a prospective cross-sectional study involving subjects with *Plasmodium falciparum* (*P. falciparum*) malaria infection serving as tests while *P. falciparum* negative subjects serving as controls.

Data Collection

The study sites include Federal Medical Centre in Jalingo, General Hospital in Lau, and General Hospital in Zing, selected randomly.

Data were collected for a period of 6 months, each study centre was allocated a period of 4 months; Federal Medical Centre, Jalingo: October, 2023 – January, 2024, General Hospital, Lau: November, 2023 – February, 2024, and General Hospital in Zing: December, 2023 – March, 2024. A total of 1,500 patients who were diagnosed for the presence of *Plasmodium species*, by the routine laboratory methods as per WHO protocol [27] were included in this study. Sample collected was given a coded number without the patient's name and labelled appropriately taking note of the centre of collection, Local Government Area, gender, age group, marital status, educational status etc.

Sample Size Estimation

The sample size was determined from a standard formula for the calculation of sample size.

Sample size $n = \frac{(Z_{1-a})^2 \times P(1-P)}{d^2}$

Where n = minimum sample size,

Z_{1-a} value of standard normal deviation which is at 95% confidence level has been found to be 1.96.

P=best estimate of the population prevalence obtained from the literature review.

d=proportion of sample error in a given population.

At prevalence rate of plasmodium malaria of 19.2% [28], using 5% precision at 95% confidence level, the minimum sample size n for this study is calculated as follows:

Sample size $n = \frac{(Z_{1-a})^2 \times P(1-P)}{d^2}$

Where Z=1.96,

P=19.2%, 0.192 d= 5%, 0.05

Therefore, $n = \frac{(1.96) \times 0.192 \times (1-0.192)}{0.05^2}$

n=238

The minimum sample size is 238

Added attrition was = 110% of 238

Total sample size=500 x 3 =1500

Study population and Selection of subjects

Participant between the ages of 5 and 100 years was considered for this study after signing a written consent.

Inclusion criteria

Only subjects who consent to the study were included. Only individuals with *P. falciparum* infection were included as test subjects

Exclusion criteria

Subjects who refuse to give consent were excluded. Subjects who are infected with or who have co-infection with other types of *Plasmodium* species were also excluded.

Sample Handling and Disposals

All the samples collected for this research work was handled discretely by professional Laboratory Technicians or scientist who determine the infection status of participants. After testing, the samples were incinerated with the aid of Kerosene. The Data collected was kept confidential and only use for research purpose only. The test result of each individual was made known to them following request.

Collection and preparation of blood specimen

Blood samples were collected aseptically, with 5ml drawn from adults' cubital veins or children's hands/legs. Samples were divided into plain bottles for coagulation and EDTA bottles for malaria parasite tests and hematological studies. After centrifugation, serum samples were stored for biochemical analyses. Thick and thin blood films were prepared on the same slide for each subject and stained with 10% Giemsa stain. Stained slides were flooded with diluted Giemsa stain, washed, and examined under a microscope (Nikon, Japan) for malaria parasites as described by [29].

Polymerase Chain Reaction

DNA extraction

Parasite DNA was extracted from 150 µL of venous blood using the phenol/chloroform method [30]. Briefly, the blood samples was thawed and treated using proteinase K. DNA was re-suspended in 50 µL of buffer, therefore, 1 µL of the DNA template corresponded to 3 µL of whole blood [31].

Design of synthetic oligonucleotides and DNA amplification

Specific primers were designed using the Primer Express Program (Applied Biosystems, Foster City, CA, USA) to hybridize the cytochrome c oxidase genes of the mitochondrial genome, based on the sequence of *P. falciparum*, cox III (GenBank accession nos. GI8346992 and M76611). The primers were compared with reference sequences (indicated by the GI above) and with all the sequences registered in GenBank by BLAST algorithms and databases from the National Center for Biotechnology (http://ncbi.nlm.nih.gov). For *P. falciparum*, the sequences that were used as primers (Invitrogen, Life Technologies, Carlsbad, CA, USA) include; Pf1 (5′-CCTGCATTAACATCATTATATGGTACATCT-3′) and Pf2 (5′ GATTAACATTCTTGATGAAGTAATGATAATACCTT-3′). The amplifications were generated fragments of 273 and 290 bp, respectively. A total volume of 20 μL was used as a reaction mixture [2 μL of DNA, 1.8 mM $MgCl_2$, 250 μM of deoxynucleotide triphosphate, 250 pMol of primer, 2 μL of PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl), 1.0 unit of Taq DNA polymerase (Invitrogen, Life Technologies, Carlsbad, CA, USA) and 11 μL of water]. The amplification conditions were: one denaturation cycle at 96 ˚C for 10 min; 30 cycles at 95 ˚C for 1 min and 60 ˚C (*P. falciparum*) for 5 min; and a final extension at 60 ˚C for 1h. All amplifications were performed in an Eppendorf thermal cycler (Eppendorf, Hamburg, Germany). The amplified products were resolved by 2% agarose gel electrophoresis and visualized by ethidium bromide staining. Genomic DNA from healthy individuals with no history of malaria were included as negative control in all PCR assays performed. To establish the minimum number of parasites that could be detected, samples with known numbers of *P. falciparum* parasites were employed. They were obtained from a tenfold serial dilution using non-infected whole blood as diluent [31].

Nucleotide sequence analysis of PCR products

The PCR products of two samples (2 *P. falciparum*) were sequenced. Direct sequencing of mtDNA (cox I and cox III) was based on biochemical synthesis of the DNA chain using the Big Dye^{TM} Terminator Cycle Sequence kit, v. 3.1, in both forward and reverse directions. Vertical electrophoresis was performed with the POP-7^{TM} polymer in a 36 cm Avant Capillary Array using the Applied Biosystems ABI prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The nucleotide sequences produced was directly edited with sample manager software BioEstat 4.0. The resulting sequences were aligned and compared with all sequences registered in the GenBank [31].

Multiplicity of infection

Multiplicity of infection (MOI) was determined by calculating the number of different alleles at each locus; single infections were those with only one allele per locus at all of the genotyped loci. [32, 33, 34, 35]. Multiclonal/ mixed infections were defined as those having more than one allele in at least one locus out of the loci genotyped.

Statistical analysis

The data obtained was analyzed using Statistical Package for Social Sciences (SPSS) version 28 (SPSS Inc., Chicago, IL, USA). Results are presented simple frequencies in tables and charts.

The MSP1 and MSP2 allelic frequency was calculated. The mean multiplicity of infection (MOI) was calculated for MSP 1 and MSP2.

RESULTS

Table 1 shows the distribution of malaria cases by age and gender within the study area. In the 1-5 years age group, 93.3% of 15 males and 45.9% of 37 females were infected, totaling 59.6% of 52 individuals. For ages 6-10, 75.0% of 4 males and 45.4% of 11 females were infected, totaling 53.3% of 15 individuals. Among those 11-17 years, 25.6% of 78 males and 13.1% of 198 females were infected, totaling 16.6% of 276

individuals. In the 18-30 age group, 19.3% of 429 males and 24.7% of 263 females were infected, totaling 21.4% of 692 individuals. For ages 31-40, 13.2% of 113 males and 15.1% of 132 females were infected, totaling 14.2% of 245 individuals. Among those 41-50 years, 14.1% of 71 males and 15.7% of 70 females were infected, totaling 14.8% of 141 individuals. For individuals over 50, 38.1% of 21 males and 12.1% of 58 females were infected, totaling 18.9% of 79 individuals.

Table 1: Demographic characteristics of study participants

Age (Years)	Male		Female		Total	
	No. Examined	No. Infected (%)	No. Examined	No. Infected (%)	No. Examined	No. Infected (%)
1-5	15	14 (93.3)	37	17 (45.9)	52	31 (59.6)
6-10	4	3 (75.0)	11	5 (45.4)	15	8 (53.3)
11-17	78	20 (25.6)	198	26 (13.1)	276	46 (16.6)
18-30	429	83 (19.3)	263	65 (24.7)	692	148 (21.4)
31-40	113	15 (13.2)	132	20 (15.1)	245	35 (14.2)
41-50	71	10 (14.1)	70	11 (15.7)	141	21 (14.8)
>50	21	8 (38.1)	58	7 (12.1)	79	15 (18.9)
Total	**731**	**153 (20.9)**	**769**	**151 (19.6)**	**1500**	**304 (20.3)**

Allelic distribution and Multiplicity of Infection (MOI) of the MSP1 and MSP2 families from Study sites

Allele typing analysis displayed the high polymorphic nature of *P. falciparum* in the different study sites with respect to MSP1 and MSP2. All the three allele types for MSP1- K1, MAD20 and RO33 and of MSP2-3D7 and FC27 were identified in Zing and Lau, except in Jalingo where RO33 of the MSP1 family was not amplified.

Table 2 shows the distribution of allele and multiplicity of infection (MOI) of the MSP1 and MSP2 families in three facilities. In Jalingo, mono-infection was observed in K1 with the highest frequency of 28 (25%) and MAD20 3 (2.7%). RO33 mono-infection was not seen. Mixed infection was seen in K1+MAD20, 13 (11.6%), K1+RO33, 12 (10.7%) and K1+MAD20+RO33, 15 (13.4%).

In Zing, unlike Jalingo, K1 has the lowest allele frequency 3 (2.6%) for mono-infections, MAD20 23 (19.8%) and RO33 26 (22.4%) with the highest frequency. Mixed infections include K1+MAD20 4 (3.4%), K1+RO33 1 (0.9%), MAD20+RO33 11 (9.5%) and K1+MAD20+RO33 17 (14.7%).

In Lau, 10 (9.2%) alleles were found for K1, 20 (18.3%) for MAD20 and 4 (3.7%) RO33 mono-infections. Mixed infections include 32 (29.4%) K1+MAD20 with highest frequency, 2 (1.8%) k1+RO33, 4 (3.7%) MAD20+RO33 and 2 (1.8%) K1+MAD20+RO33.

Table 2: Allelic distribution and Multiplicity of Infection (MOI) of the MSP1 and MSP2 families from Taraba, Northeast Nigeria

	FAMILY	HAPLOTYPE	FREQUENCY	MOI
Jalingo	MSP1	K1	28	1.77
		MAD20	3	
		RO33	0	
		K1+MAD20	13	
		K1+RO33	12	
		MAD20+RO33	0	
		K1+MAD20+RO33	15	
		TOTAL	71	
	MSP2	3D7	24	1.49
		FC27	11	
		3D7+FC27	33	

		TOTAL	68	
Zing	MSP1	K1	3	1.59
		MAD20	23	
		RO33	26	
		K1+MAD20	4	
		K1+RO33	1	
		MAD20+RO33	11	
		K1+MAD20+RO33	17	
		TOTAL	85	
	MSP2	3D7	28	1.49
		FC27	15	
		3D7+FC27	42	
		TOTAL	85	
Lau	MSP1	K1	10	1.57
		MAD20	20	
		RO33	4	
		K1+MAD20	32	
		K1+RO33	2	
		MAD20+RO33	4	
		K1+MAD20+RO33	2	
		TOTAL	74	
	MSP2	3D7	30	1.48
		FC27	12	
		3D7+FC27	38	
		TOTAL	80	
	MSP1	K1	41	
		MAD20	46	
		RO33	30	
		K1+MAD20	49	
Total		K1+RO33	15	
		MAD20+RO33	15	
		K1+MAD20+RO33	34	
		TOTAL	230	
	MSP2	3D7	82	
		FC27	38	
		3D7+FC27	115	
		TOTAL	233	

MOI - Multiplicity of Infection, MSP1 - merozoite surface protein 1, MSP2 - merozoite surface protein 2

For the MSP2 family, 24 (21.4%) 3D7, 11 (9.8%) FC27 and 33 (29.5%) mixed infection of 3D7+FC27 in Jalingo. In Zing, 28 (24.1%) 3D7, 15 (12.9%) FC27 and 42 (36.2%) mixed infection of 3D7+FC27. Also, in Lau, 30 (27.5%) 3D7, 12 (11.0%) FC27 and 38 (34.9%) mixed infection of 3D7+FC27.

DISCUSSION

Few studies have investigated the genetic diversity and multiplicity of infection of P. falciparum in Northern Nigeria. To our knowledge, this is the pioneer study on the genetic diversity of P. falciparum isolates based on utilizing merozoite surface protein (msp) antigen in Northeast Nigeria.

In the current study, allele typing analysis displayed the high polymorphic nature of *P. falciparum* in the different study sites with respect to MSP1 and MSP2. PCR amplification of the 18s-rRNA gene of the samples showed that 75.9% samples were positive from samples collected from Jalingo, 73.3% samples were also positive from Zing and 75.2% samples from Lau. A total of 74.8% samples were positive from the three sites all together. MSP1 amplification was successful for 83.5% while 80.2% samples were positive for the

MSP2 allelic families of *P. falciparum*-positive samples from Jalingo. From Lau, 90.2% of samples positive for MSP1 and 97.6% samples were positive for MSP2. Meanwhile, all the samples positive for 18s-rRNA from Zing were all 100% successfully amplified for MSP1 and MSP2 which is in line with the other reports [11, 12].

All three alleles of the msp1 (K1, MAD20, RO33) were successfully genotyped. For the samples from Jalingo, K1 has the high number of alleles for the MSP1 allelic families, with majority of the alleles 41.2% existing as mono-infection, which is consistent with infections reported in South-Western Nigeria [36]. However, Zing had the highest frequency of RO33 mono-infection (30.6%) which corroborates a previous study [37]. Different from what was observed in the samples from Jalingo and Zing for MSP1 genotyping, samples from Lau had highest allelic frequency and mono-infection of MAD20 (27%), then K1 (13.5%) and least RO33 infections (5.4%) as reported in Minna, Nigeria [23].

Overall, the analysis of msp1 alleles revealed that MAD20 allelic family was predominant with 20.0% followed by K1 at 17.8% and RO33 at 13% respectively. This is similar to reports in Northwest Nigeria [23] and western Ethiopia [38]. However it contradicts reports from other parts of Nigeria which identified K1 as the most common msp1 allele [39, 40]. Dikwa et al. [41] observed MAD20 and K1 alleles in symptomatic individuals, with a single MAD20 allele in asymptomatic cases. Another study in India reported K1 as predominant msp1 allele followed by Mad20, and RO33 [42].

In msp2 family, both 3D7 and FC27 were genotyped successfully from the three sites. Similar to a report in Northwest Nigeria which observed 3D7 and FC27 alleles in their study [41]. Overall in this study, the 3D7 family was most represented with 35.2% prevalence against 16.3% for FC27. This is in agreement with reports in Lagos, Nigeria [43], Senegal [44] and replicates the infection pattern found in Cameroon [45]. However, this is contrary to several studies that reported a high prevalence of FC27 among different populations in Nigeria [23,39,40,46,47]. Another study [48] reported more msp2 alleles in asymptomatic malaria cases compared to uncomplicated and severe malaria cases.

The allelic diversity of P. falciparum MSP1 and MSP2 is mostly due to meiotic recombination events involving genetically distinct parasite clones that infect the same mosquito vector, and hence, human host [49,50]. Therefore, the proportion of mixed infections and the number of clones per individual is one of the prerequisites to generate new genotypes and to increase the diversity of the parasitic population. Multiple clonal infections with different genotypes of P. falciparum were identified among the P. falciparum isolates in the study locations with a significant multiplicity of infection (MOI). Several studies have found that the genetic diversity of this parasite varies depending on a variety of parameters, including the degree of infection, the age of the infected persons, and their geographic location [20-23]. In malaria-endemic countries like Nigeria, where transmission rates are higher, diversity is usually greater [20].

Mean MOI ranged 1.57 – 1.77, and 1.48 – 1.49 for msp1 and msp2 genes respectively. This lower than reports in Sudan [51], Mauritania [52], Burkina Faso [53], but higher than studies in Gadarif state, Sudan [54]. The slight variation in our report could be due to similar population profile and season, with the current study carried out during the dry season. It has been suggested that MOI varies with seasons, with higher values in the rainy season than in the dry season reflecting periods of higher malaria transmission [53]. However, other studies have reported higher MOI values in the dry season [55, 56]. The differences in MOI can be attributed to variations in geographical areas, malaria transmission intensity, and factors such as the age and mean parasite density of the study population [20-23]. The low MOI observed in our study may also

result from malaria control measures, including the seasonal malaria chemotherapy and the large-scale distribution of LLINs across the country.

LIMITATIONS

The study did not correlate genetic diversity or MOI with age or gender and was limited to a single season, preventing exploration of seasonal variations' impact on these variables. Additionally, using agarose gel electrophoresis for PCR detection, while effective, is considered a limitation. More advanced techniques, such as capillary electrophoresis and genome-wide studies, could more accurately identify genetic peculiarities in malaria-infected populations.

CONCLUSION

Understanding Nigeria's malaria situation in relation to Plasmodium falciparum genetic variation can enhance treatment and immunization strategies. The multiplicity of infection (MOI) values observed in the study area indicate the endemic nature and transmission intensity of malaria in Taraba State's northern senatorial zone. These findings offer crucial insights for surveillance and evaluating current malaria control measures, guiding future treatment strategies tailored to Taraba and Africa's malaria-endemic populations. Recommendations include strengthening malaria eradication efforts, conducting further research on the impact of allelic distributions on treatment response, an area that has been underexplored in Nigerian studies, and conducting periodic genetic diversity studies in Taraba State to better understand the overall genetic diversity of P. falciparum in Nigeria.

Declaration by Authors

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Conflict of Interest: The authors declare no conflict of interest.

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REFERENCES

1. Afolabi BM. (2001). Malaria: The global scourge. Nigeria Clinic. 5:9-12.
2. Ikekpeazu EJ, Neboh EE, Maduka IC, Nwagbara IJ, Nwobodo MW (2010). Type-2 diabetes mellitus and malaria parasitemia: Effect on liver function tests. Asian J. Med. Sci. 2(5):214-217.
3. Ogbodo, SO, Okeke, SC, Obu HA, Shu EN, Chukwurah EF. (2010) Nutritional status of parasitemic children from malaria endemic rural communities in Eastern Nigeria. Curr. Paediatric Res., 14(2): 131-135
4. Khuraiya P, Sharma SS, Thakur AS, Pandey VP, Verma S. (2016) The study of clinical, biochemical and hematological profile in malaria patients. Int J Adv Med.;3(2):209–217. doi: 10.18203/2349-3933.ijam20160685.
5. Maina RN, Walsh D, Gaddy C, Hongo G, Waitumbi J, Otieno L, *et al*. Impact of Plasmodium falciparum infection on haematological parameters in children living in Western Kenya. Malar J. 2010; 9(Suppl 3):S4.
6. Abukari, Z., Okonu, R., Nyarko, S. B., Lo, A. C., Dieng, C. C., Salifu, S. P., Gyan, B. A., Lo, E., & Amoah, L. E. (2019). The Diversity, Multiplicity of Infection and Population Structure of P. falciparum Parasites Circulating in Asymptomatic Carriers Living in High and Low Malaria Transmission Settings of Ghana. Genes, 10(6), 434. https://doi.org/10.3390/genes10060434

7. Conway, D. J., Roper, C., Oduola, A. M., Arnot, D. E., Kremsner, P. G., Grobusch, M. P., Curtis, C. F., & Greenwood, B. M. (1999). High recombination rate in natural populations of Plasmodium falciparum. Proceedings of the National Academy of Sciences of the United States of America, 96(8), 4506–4511. https://doi.org/10.1073/pnas.96.8.4506
8. Kolakovich KA, Ssengoba A, Wojcik K, Tsuboi T, Al-Yaman F, et al. Plasmodium vivax: favored gene frequencies of the merozoite surface protein-1 and the multiplicity of infection in a malaria endemic region. Exp Parasitol. 1996;83(1):11–19.
9. Woehlbier, U., Epp, C., Hackett, F., Blackman, M. J., & Bujard, H. (2010). Antibodies against multiple merozoite surface antigens of the human malaria parasite Plasmodium falciparum inhibit parasite maturation and red blood cell invasion. Malaria journal, 9, 77. https://doi.org/10.1186/1475-2875-9-77
10. Deitsch, K. W., & Chitnis, C. E. (2012). Molecular basis of severe malaria. Proceedings of the National Academy of Sciences of the United States of America, 109(26), 10130–10131. https://doi.org/10.1073/pnas.1207174109
11. Beeson, J. G., Rogerson, S.J., Cooke, B.M., Reeder, J.C., Chai, W., Lawson, A.M., Molyneux, M.E. and Brown, G.V. (2000). Adhesion of *Plasmodium falciparum*-infected erythrocytes to hyaluronic acid in placental malaria. *Nat. Med.* 6, 86–90.
12. Peyerl-Hoffmann G., Jelinek T., Kilian A., Kabagambe G., Metzger W., Von Sonnenburg F. Genetic diversity of Plasmodium falciparum and its relationship to parasite density in an area with different malaria endemicities in West Uganda. Trop. Med. Int. Health. 2001;6:607–613. doi: 10.1046/j.1365-3156.2001.00761.x.
13. White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, et al. Malaria. Lancet. 2014;383(9918):723–735.
14. Engelbrecht F, Felger I, Genton B, Alpers M, Beck HP. Plasmodium falciparum malaria morbidity is associated with specific merozoite surface antigen 2 genotypes. Exp Parasitol. 1995;81:90– 6.
15. Ariey F, Hommel D, Le Scanf C, Duchemin JB, Peneau C, Hulin A. Association of severe malaria with a specific Plasmodium falciparum genotype in French Guiana. J Infect Dis. 2001;15:237 – 41.
16. John CC, Park GS, Sam-Agudu N, Opoka RO, Boivin MJ. Elevated serum levels of IL-1ra in children with Plasmodium falciparum malaria are associated with increased severity of disease. Cytokine. 2008;41:204 – 8.
17. Kiwuwa MS, Ribacke U, Moll K, Byarugaba J, Lundblom K, Farnert A, et al. Genetic diversity of Plasmodium falciparum infections in mild and severe malaria of children from Kampala. Uganda Parasitol Res. 2013; 112:1691– 700.
18. Rout R, Mohapatra BN, Kar SK, Ranjit M. Genetic complexity and transmissibility of Plasmodium falciparum parasites causing severe malaria in central-east coast India. Trop Biomed. 2009;26:165 –72.
19. Ashley, E. A., Pyae Phyo, A., & Woodrow, C. J. (2018). Malaria. Lancet (London, England), 391(10130), 1608–1621. https://doi.org/10.1016/S0140-6736(18)30324-6
20. Mohammed, H., Hassen, K., Assefa, A. *et al*. Genetic diversity of Plasmodium falciparum isolates from patients with uncomplicated and severe malaria based on msp-1 and msp-2 genes in Gublak, North West Ethiopia. Malar J 18, 413 (2019). https://doi.org/10.1186/s12936-019-3039-9
21. Ajogbasile, F.V., Kayode, A.T., Oluniyi, P.E. *et al*. Genetic diversity and population structure of Plasmodium falciparum in Nigeria: insights from microsatellite loci analysis. Malar J 20, 236 (2021). https://doi.org/10.1186/s12936-021-03734-x
22. Usman-Yamman, H., Omalu C J, I., Abubakar, A., Abolarinwa S O, Eke S, S., & Otuu, C. A. (2021). Genetic diversity of plasmodium falciparum isolates in Minna, North Central Nigeria inferred by PCR genotyping of Merozoite surface protein 1 and 2. Infection, genetics and evolution: journal of molecular epidemiology and evolutionary genetics in infectious diseases, 96, 105143. https://doi.org/10.1016/j.meegid.2021.105143
23. Udosen, EO. (2003). Malaria treatment using oral Medkafin: Changes in biochemical and haematological parameters in Nigerian children with uncomplicated falciparum malaria. Orient j. Med., 15:12-22

24. Ibekwe, A. C. (2004). Baseline Salmonella agglutinin titers and Plasmodium load investigationin apparently healthy freshmen in Nnamdi Azikwe University, Akwa. B.Sc. Project in the Department of Applied Microbiology and Brewing, Faculty of Natural Sciences, Nnamdi Azikwe University, Akwa. pp. 41
25. World Medical Association (2013). World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA, 310(20), 2191–2194. https://doi.org/10.1001/jama.2013.281053
26. World Health Organization, editor. Basic malaria microscopy. 2. Geneva: WHO; 2010.
27. Yakubu B., Longdet I., Tony H., Davou D., Obishakin E. High-complexity Plasmodium falciparum infections, North Central Nigeria, 2015-2018. Emerg. Infect. Dis. 2019;25(7):1330–1338.
28. Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries Part 2. New York: Cambridge University Press.
29. Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. Plainview, N.Y.: Cold Spring Harbor Laboratory Press.
30. Cunha M. G., Medina T. S., Oliveira S. G., Marinho A. N., Povoa M. M., Ribeiro-dos-Santos A. K. (2009). Development of a Polymerase Chain Reaction (PCR) Method Based on Amplification of Mitochondrial DNA to Detect Plasmodium falciparum and Plasmodium vivax. Acta Trop. 111 (1), 35–38. 10.1016/j.actatropica.2009.02.003
31. Snounou G, Zhu X, Siripoon N, Jarra W, Thaithong S, Brown KN, et al. Biased distribution of msp1 and msp2 allelic variants in Plasmodium falciparum populations in Thailand. Trans Roy Soc Trop Med Hyg. 1999;93:369–74.
32. Viriyakosol S, Siripoon N, Petcharapirat C, Petcharapirat P, Jarra W, Thaithong S, et. al. Genotyping of Plasmodium falciparum isolates by the polymerase chain reaction and potential uses in epidemiological studies. Bull World Health Organ. 1995;73:85.
33. Tusting LS, Bousema T, Smith DL, Drakeley C. Measuring changes in Plasmodium falciparum transmission: precision, accuracy and costs of metrics. Adv Parasitol. 2014;84:151–208.
34. Escalante AA, Pacheco MA. Malaria molecular epidemiology: an evolutionary genetics perspective. Microbiol Spectr. 2019;7:AME-0010-2019.
35. Olasehinde, G. I., Yah, C. S., Singh, R., Ojuronbge, O. O., Ajayi, A. A., Valecha, N., ... & Adeyeba, A. O. (2012). Genetic diversity of Plasmodium falciparum field isolates from south western Nigeria. African Health Sciences, 12(3), 355-361.
36. Funwei, R. I., Thomas, B. N., Falade, C. O., & Ojurongbe, O. (2018). Extensive diversity in the allelic frequency of Plasmodium falciparum merozoite surface proteins and glutamate-rich protein in rural and urban settings of southwestern Nigeria. Malaria journal, 17, 1-8.
37. Tadele, G., Jaiteh, F. K., Oboh, M., Eniyou O., Sisay D., Alfred A. and Lemu G. Low genetic diversity of Plasmodium falciparum merozoite surface protein 1 and 2 and multiplicity of infections in western Ethiopia following efective malaria interventions. Malaria Journal (2022) 21:383. https://doi.org/10.1186/s12936-022-04394-1
38. Idakwo J, Idowu, ET, Oyebola KM, Otubanjo OA (2018) Low levels of Plasmodium falciparum genetic diversity in two Nigerian communities bordering the Niger River. Pan Afri J life Sci. https://doi.org/10.36108/pajols/8102/10(0130).
39. Kolawole OM, Mokuolu OA, Olukosi YA, Oloyede TO (2016) Population genomics diversity of Plasmodium falciparum in malaria patients attend -ing Okelele health centre, Okelele, Ilorin, Kwara State. Nigeria Afr Health Sci 16(3):704
40. Dikwa KB, Yahaya UA, Suleiman MDB, AB, (2020) Diversity of Plasmodium falciparum isolated from symptomatic and asymptomatic individuals in parts of kaduna metropolis. Kaduna state Am J Microbiol Res 8(3):83–9237
41. Ahmed, R. and Sharma, I. Genetic diversity and multiplicity of infection in Plasmodium falciparum isolates causing uncomplicated malaria infections in North-East India. Proceedings of the National Conference on Innovations in Biological Sciences (NCIBS), January 10, 2020. Pp 62-77.
42. Agomo CO, Chijioke LO, Ajibaye O. Plasmodium falciparum Merozoite Surface Protein 2 Genetic Diversity and Multiplicity of Infection in Asymptomatic Malaria Cases in Lagos, Nigeria. University of Lagos

Journal of Basic Medical Sciences, (2022), 10(1&2):42-48.

43. Diouf B, Diop F, Dieye Y, Loucoubar C, Dia I, Faye J, et al. Association of high Plasmodium falciparum parasite densities with polyclonal microscopic infections in asymptomatic children from Toubacouta, Senegal. Malar J. 2019; 18(1): 48.
44. Basco LK, Tahar R, Escalante A. (2004) Molecular epidemiology of malaria in Cameroon. XVIII. Polymorphisms of the Plasmodium falciparum merozoite surface antigen-2 gene in isolates from symptomatic patients. The American Journal of Tropical Medicine and Hygiene 70238–244.
45. Oyedeji SI, Bassi PU, Oyedeji SA, Ojurongbe O, Awobode HO (2020) Genetic diversity and complexity of Plasmodium falciparum infections in the microenvironment among siblings of the same household in North-central Nigeria. Malar J 19:338
46. Amodu OK, Olaniyan SA, Omotade OO (2015) Changes in Plasmodium falciparum population dynamics in two populations at diferent time periods in Ibadan, Southwest Nigeria. Afr J Biomed Res 18:17–22
47. Amodu OK, Adeyemo OO, Ayoola OO, Gbadegesin AE, Orimadegun AK et al (2005) Genetic diversity of msp-1 locus and symptomatic malaria in southwest Nigeria. Acta Trop 95(3):226–232
48. Hongying J., Na L., Vivek G., Martine M.Z., Sudhir V., Vijayaraj N. (2011): High Recombination Rates and Hotspots in a Plasmodium falciparum Genetic Cross. Genome Biology; 12:33
49. Nyamngee A., Edogun H.A., Sulaiman M.K., Ikpe R.T. and Njan AA. Genetic Diversity of Plasmodium falciparum Based on Merozoite Surface Proteins 1 and 2 Genes. Afr. J. Biomed. Res. Vol. 25 (May, 2022); 163 - 167
50. Hamid, MMA, Arwa F.E., Albsheer, MMA, Abdelmohaymin, NSM, Mustafa, S.O., Mohamed, SEM, and Mutaz A. Multiplicity of infection and genetic diversity of Plasmodium falciparum isolates from patients with uncomplicated and severe malaria in Gezira State, Sudan. Parasites & Vectors (2016) 9:362. DOI 10.1186/s13071-016-1641-z
51. Ahmedou MS, Ndiaye M, Abdallahi MO, Lekweiry KM, Bogreau H, Konaté L, et al. Polymorphism of the merozoite surface protein-1 block 2 region in Plasmodium falciparum isolates from Mauritania. Malaria J. 2014;13:26.
52. Sondo, P., Karim D., Toussaint R., Seydou N.D., Paul T., Adama K., Hamidou I., et al. Determinants of Plasmodium falciparum multiplicity of infection and genetic diversity in Burkina Faso. Parasites Vectors (2020) 13:427. https://doi.org/10.1186/s13071-020-04302-z
53. A-Elbasit IE, ElGhazali G, A-Elgadir TM, Hamad AA, Babiker HA, Elbashir MI, Giha HA. Allelic polymorphism of MSP2 gene in severe P. Falciparum malaria in an area of low and seasonal transmission. Parasitol Res. 2007;102:29–34.
54. Adjah J, Fiadzoe B, Ayanful-Torgby R, Amoah LE. Seasonal variations in Plasmodium falciparum genetic diversity and multiplicity of infection in asymptomatic children living in southern Ghana. BMC Infect Dis. 2018;18:432
55. Agyeman-Budu A, Brown C, Adjei G, Adams M, Dosoo D, Dery D, et al. Trends in multiplicity of Plasmodium falciparum infections among asymptomatic residents in the middle belt of Ghana. Malar J. 2013;12:22
56. Britannica, The Editors of Encyclopaedia. "Taraba". Encyclopedia Britannica, 30 Oct. 2023, https://www.britannica.com/place/Taraba. Accessed 27 May 2024.

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