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Genetic Diversity of *Plasmodium falciparum* Isolated from Symptomatic and Asymptomatic Individuals in Parts of Kaduna Metropolis, Kaduna State, Nigeria

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Abstract Malaria due to *Plasmodium falciparum* is still a major public health problem and cause of high morbidity and mortality in Nigeria despite many efforts and interventions programmes put in place to control malaria, with transmission occurring throughout the year. Characterization of *P. falciparum* isolates from different geographical locations of Nigeria could provide the much needed information on the genetic composition of *P. falciparum* natural populations with regard to three polymorphic genes: *Merozoite Surface Protein 1 (MSP 1), Merozoite Surface Protein (MSP 2)* and *Glutamate-Rich Protein* (*GLURP*) genes, the findings of this study will assist in adopting more strategies and intervention programmes aim at controlling *P. falciparum* infection in Nigeria. This study was conducted to determine the genetic diversity and elucidate possible genomic variation between isolates of *P. falciparum* from symptomatic and asymptomatic malaria cases in Kaduna metropolis. *Plasmodium falciparum* genomic DNA was extracted from 42 positive blood samples collected onto whatman's filter paper using the phenol-chloroform DNA extraction method. Genotyping of *P. falciparum* was based on the amplification of *MSP* 1, *MSP* 2 and *glurp* genes. Analysis of PCR products of *P. falciparum* isolates shows the presence of 33 alleles spread across *MSP* 1, *MSP* 2 and *glurp* genes at a frequency of 5, 24 and 4 respectively. Detection of the three genes; (*MSP*1); MAD20 and KI, (*MSP*2); FC27 and 3D7/ICI and (*glurp*) gene and the occurrence of single and multiple genotypic infection recorded showed the diverse genetic composition of *P. falciparum* population, an indication of the endemicity of malaria. This information is essential for effective malaria control programme.

Keywords: *genetic diversity, P. falciparum malaria, symptomatic individuals, asymptomatic, individuals*

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1. Background

Despite many efforts and interventions programmes put in place by government and non-governmental organisations (NGOs) to control malaria in Nigeria, malaria is prevalent and remain the cause of morbidity and mortality with *P. falciparum* being the dominant species causing much malaria morbidity and mortality in Nigeria [1,2,3,4]. The incidence of malaria is still high in Kaduna state [5].This draw back has been associated to many factors which include the emergence of drug resistance parasite strain, insecticide resistance mosquitoes and genetic diversity *P. falciparum* natural parasite populations has been shown to have limitations on the efficacy of protective immunity among others [3,4,5].

Molecular epidemiological studies remains an important tool of assessing the genetic diversity of *P. falciparum* population especially in areas of intense malaria transmission, the strategy to control malaria requires an understanding of the genetic composition of *P. falciparum* as this information is essential and may facilitate the development of an effective antimalarial vaccine [3,6,7]. The diverse antigenic composition of *P. falciparum* isolates has been shown to hinder the development of effective antimalarial vaccine and play a major role in the acquisition of protective immunity to malaria [3,6].

In malaria endemic areas, *P. falciparum* natural populations exhibit diverse genetic composition [7,8,9]. *Plasmodium falciparum* genetic diversity of natural parasite populations has been characterised in Nigeria through investigation of the allelic variation in three polymorphic antigens; merozoite surface proteins

(*MSP*-1 & 2), and *glutamate-rich protein* (*GLURP*) among 47 school children in Ibadan [10] and among isolates from patients in Ogun State general hospital [3]. Genetic diversity of *P. falciparum* isolates at *Merozoite Surface Protein 2* gene alone has been characterised among children in Ibadan [11] all located in South-western Nigeria, and in naturally infected children in Nasarawa North-central Nigeria [12].

There seems to be no study conducted on the genetic composition of *P. falciparum* that involve investigation of the allelic diversity at the three polymorphic antigens from symptomatic and asymptomatic individuals in Kaduna State, North-western Nigeria. The aim of this study therefore is to Characterize *P. falciparum* isolated from symptomatic and asymptomatic individuals in parts of Kaduna metropolis. This will provide information on the genetic composition of *P. falciparum* isolates with regard to polymorphic genes (*MSP* 1, *MSP* 2 and *glutamate-rich protein* genes), the findings of this study will assist in adopting more strategies and intervention programmes aim at controlling *P. falciparum* infection in the state.

2. Materials and Methods

2.1. Study Area

This study was carried out in Kaduna metropolis, Kaduna state, Nigeria. Kaduna metropolis is the capital of Kaduna State, it is located in North-western geopolitical zone and lies geographically within latitude $10^{0}21^{1}23^{11}$N andlongitude$7^{0}26^{1}2^{11}$E, and is 608 meters above sea level. Kaduna State experiences two distinct seasons viz: Dry season, which commences in the months of November to March and a rainy season usually from April through October and last between 4-5 months in the far and northern parts of the state and 5-6 months in the southern parts of the state, with vegetation typically of guinea savannah type [13]. Relatively high temperatures are experienced during the dry season, with an annual average high temperature of 31.6°C, and a relatively lower temperatures occur during the rainy season with annual low temperatures of 18.5°C [14]. The study population comprised of two categories of people viz: symptomatic and asymptomatic individuals.

2.2. Sample Collection

A total of 1000 blood samples comprising 500 blood samples from symptomatic patients collected in selected hospitals within the metropolis, and 500 asymptomatic blood samples collected from apparently healthy blood donors in some of the selected hospitals within the metropolis and from the National Blood Bank Kaduna were collected and examined between March and November, 2011.

Blood samples were collected from the following hospitals: Kaduna South L.G.A: Yusuf Dantsoho Memorial Hospital, Tudun Wada Kaduna; Nigerian Army Reference Hospital (44), Kaduna; Gwamna Awang Hospital, Nassarawa Kaduna; St. Gerald Hospital, Kakuri. And in Kaduna North L.G.A: Barau Dikko Specialist Hospital, Kaduna; Barau Dikko Children Hospital; Kaduna; Nigerian Defence Academy Medical Centre, Ribadu Cantonment, Kaduna; General hospital Kawo, Kaduna.

Ethical approval was obtained from the Federal Ministry of Health, Kaduna State Ministry of Health, Nigerian Army Reference Hospital (44) and other hospitals sampled prior to commencement of sample collection. At the course of sample collection, questionnaires were administered to establish information on the biodata of the individuals sampled viz; age, sex, occupation and socio-economic status of all individuals sampled, in addition, information on malaria history and type of anti-malarial drug intake by symptomatic individuals were recorded. Sample collection was conducted with the assistance of medical personnel from the respective sampled hospitals and the National Blood Transfusion Service (NBTS). Venous blood sample was collected from each person into labelled tubes (vacutainer) containing Ethylene diaminetetraacetic acid (sequestrene) anticoagulant [15].

2.3. Sample Analysis

2.3.1. Plasmodium falciparum Detection

Each Blood sample was screened for *P. falciparum* by thick and thin blood films preparation stained with 30% giemsa stain for 10 min using the procedures described by [15]. Parasites detection and parasitaemic level determination were carried out on thick blood films while parasite was identified on thin film [4,15].

Few drops of positive blood samples with high parasite density of >5000 parasites/µL of blood in symptomatic individuals and <3000 parasites/µL of blood in asymptomatic individuals were spotted on a labelled whatman's filter paper (3MM), air-dried, packaged in re-sealed polythene bags and stored at -4˚Cuntil ready for genomic DNA extraction.

2.3.2. DNA Extraction from *Plasmodium falciparum*

Plasmodium falciparum genomic DNA was extracted from positive blood sample collected onto whatman's filter paper using the phenol-chloroform DNA extraction method [16]. Three millimeter (3mm) diameter circle of dried spotted blood on each filter paper was punched and placed in a tube. To this, 200uL of lysis buffer and 20uL of proteinase K was added and vortexed. The mixture was incubated at 65°C for 1hr in heating block and again vortexed from time to time to homogenise. The pieces of filter paper in the tube were removed and 400uL phenol-chloroform added and vortexed. The tube containing the mixture was centrifuged at 13000rpm for 10 min and the supernatant was transferred into a clean tube containing 400uL chloroform and again vortexed. The mixture was spun and the supernatant was removed and added to a tube containing 1000uL ethanol and 10uL sodium acetate. It was then stored at -20°C overnight, after which it was centrifuged in a cold centrifuge at 4°C for 10 min at 12000rpm.The supernatant was removed and added to a tube containing 400uL of 70% ethanol and spun again at 4°C for 10 min at 12000rpm. Thereafter, ethanol was completely removed and the DNA pellet formed was dried in safety cabinet for about 5 min. To the DNA pellet, 30uL of ultra pure water was added. Purified DNA obtained was stored at -20°C until required for amplification [6,8].

Similarly, *Plasmodium falciparum* genomic DNA from whole blood (positive control) was extracted by using 200 uL of 5% saponin to free parasites from red blood cells. This was allowed to stand for 10 min at room temperature, spun for 10 min and the supernatant was removed. The sediment was processed by phenol-chloroform method as described previously. Purified DNA was stored at -20°C until required for amplification [6,8,16].

2.3.3. Amplification of *Merozoite Surface Protein 1* (*MSP*1) Gene

At first, the initial amplification of the outer regions (conserved) of the (*MSP*1) was carried out by conventional PCR in a total volume of 20uL each, the master mix contains 1uL of each primer (RF), 15uL of ultra-pure water, 3uL of template DNA, were all added into a ready prepared premix (Bioneerinc. USA) (dNTPs (1mM of 250uM each dNTPs), Tag polymerase (1-5 units), $Mgcl_2$ (1.5mM), Reaction buffer (5.0mM).

The reaction mixture was vortexed, spun briefly and amplified (Thermocycler; PTC 100, MJ Comp. USA) with initial denaturation of 94°C for 5 min, then 94°C for 30sec followed by annealing at 55°C for 1 min and extension at 72°C for 2 min, this was followed by additional extension (final) at 72°C for 10 min. The reaction mixture was amplified for 39 cycles. The product generated was the first round PCR product (PCR1) that was used as template to ran the nested PCR, (PCR2) for each of the alleles of *MSP*1 gene (KI, MAD20, RO33) using separate specific primers (Table 1) and protocols of Snounou *et al.*, [1999] with slight modifications [6,8].

Nested PCR was carried out for each of the alleles of the *MSP*1 gene (K1, MAD20, and RO33) by using 3 uL of the first round PCR product (PCR1)) for each of the alleles as follows:

The KI allele was amplified by nested PCR in a total volume of 20uL containing 1uL of each of the K1 specific primer (RF), 15uL of ultra pure water, 3uL of first round PCR product (PCR1) and premix. The reaction mixture was amplified for 39 cycles with denaturation at 94°C for 5 min, then 94°C for 30sec, followed by annealing at 55°C for 1 min and extension at 72°C for 2 min, followed by an additional extension (final) at 72°C for 10 min, this generated the second PCR product (PCR 2).

The two other alleles of *MSP*1, (MAD20 and RO33) were also amplified separately by nested PCR with separate specific primer for each of the allele and first PCR product (PCR1) using similar reaction conditions (protocol) as for the K1 allele, to generate the second round PCR product (PCR2) [6,8]

2.3.4. Amplification of *Merozoite Surface Protein 2* (*MSP*2) Gene

The outer regions (conserved) of the (*MSP*2) was similarly amplified by conventional PCR in a total volume of 20uL reaction containing 1uL of each primer (RF), 15uL of ultra pure water, 3uL of template DNA and a ready prepared premix using similar reaction conditions as for the *MSP*1 outer region. The reaction mixture was vortexed, spun briefly and amplified with initial denaturation at 94°C for 5 min, then 94°C for 30sec followed by annealing at 55°C for 1 min and extension at 72°C for 2 min, this was followed by additional extension (final) at 72°C for 10 min. The reaction mixture was amplified for 39 cycles. The product generated was the first round PCR product (PCR1) that was used as template to ran the nested PCR (PCR2) for each of the alleles of *MSP* 2 alleles (FC 27 and 3D/IC) using separate primers described previously that are specific to each allele [6,8].

Table 1. Primer sequences for alleles of *MSP1*, *MSP 2* and *Glutamate-rich protein (glurp.)* genes

Genes	Primers Ref. [8].	Sequences	Expected amplicons (Band sizes)	Cycling parameter
MSP1				
CONSERVED	MI-OF	5'-CTAGAAGCTTTAGAAGATGCAGTATTG-3'	-	
CONSERVED	MI-OR	5'-CTTAAATAGTATTCTAATTCAAGTGGATCA-3'	-	
MAD20	MI-KF	5'-AAATGAAGAAGAAATTACTACAAAAGGTGC-3'	130-220	
MAD20	MI-KR	5'-GCTTGCATCAGCTGGAGGGCTTGCACCAGA-3'	130-220	a
KI	MI-MF	5'-AAATGAAGGAACAAGTGGAACAGCTGTTAC-3'	160-225	
KI	MI-MR	5'-ATCTGAAGGATTTGTACGTCTTGAATTACC-3'	160-225	
RO33	MI-RF	5'-TAAAGGATGGAGCAAATACTCAAGTTGTTG-3'	150-250	
RO33	MI-RR	5'-CATCTGAAGGATTTGCAGCACCTGGAGATC-3'	160	
MSP 2				
CONSERVED	M2-OF	5'-ATGAAGGTAATTAAAACATTGTCTATTATA-3'	-	
CONSERVED	M2-OR	5'-CTTTGTTACCATCGGTACATTCTT-3'	-	
FC27	M2-FCF	5'-AATACTAAGAGTGTAGGTGCARATGCTCCA-3'	290-420	
FC27	M2-FCR	5'-TTTTATTTGGTGCATTGCCAGAACTTGAAC-3'	290-420	a
3D7/ICI	M2-ICF	5'-AGAAGTATGGCAGAAAGTAAKCCTYCTACT-3'	500-700	
3D7/ICI	M2-ICR	5'-GATTGTAATTCGGGGGATTCAGTTTGTTCG-3'	500-700	
Glurp.				
CONSERVED	G-OF	5'-TGAATTTGAAGATGTTCACACTGAAC-3'	-	
CONSERVED	G-OR	5'-GTGGAATTGCTTTTTCTTCAACACTAA-3'	-	b
	G-NF	5'-TGTTCACACTGAACAATTAGATTTAGATCA-3'	660-1100	

Key-Cycling parameter
a: 94°C for 5 min, 94°C for 30sec, 55°C for 1 min, 72°C for 2 min, 72°C for 10 min (39 cycles) [6].
b: 95°C for 5 min, 94°C for 1 min, 58°C for 2 min, 72°C for 2 min, 72°C for 5 min (30 cycles) [17].

Nested PCR was also ran separately for each of the two alleles of *MSP*2 (FC27 and 3D7/ICI) and generated second round PCR product (PCR2). The FC27 was similarly amplified by nested PCR in a total volume of 20uL containing 1uL each of the FC27 specific primer (RF), 15uL of ultra pure water, 3uL of first PCR product (PCR1) and premix. The reaction mixture was amplified for 39 cycles with denaturation at 94°C for 5 min, then 94°C for 30 sec followed by annealing at 55°C for 1min and extension at 72°C for 2 min, this was followed by an final extension at 72°C for 10 min and generated the second PCR product (PCR2).

Similarly, 3D7/ICI allele was also amplified by nested PCR using its specific primer and the same reaction conditions as for the FC 27 allele in a total volume of 20 uL reaction to generate second PCR product (PCR2) [6,8].

2.3.5. Amplification of *Glutamate-rich protein* (*Glurp*) Gene

Glutamate-rich protein (*glurp*) gene outer region (conserved) was amplified by PCR. The reaction was carried out in a total volume of 20uL, made up of 1uL of primer (RF), 15uL of ultra pure water,3uL of template DNA and a ready prepared premix. The mixture was vortexed and spun briefly. The reaction mixture was amplified for 30 cycles with denaturation at 95°C for 5 min, then 94°C for 1 min followed by annealing at 58°C for 2 min, extension at 72°C for 2 min and final extension at 72°C for 5 min and generated the first PCR product (PCR1) that was used as template in the second round nested PCR.

The region II of *glutamate-rich protein* gene was amplified by nested PCR. This was carried out also in a total volume of 20uL containing 1uL each of the *glurp* specific primer (RF), 15uL of ultra pure water, 3uL of the first PCR product (PCR1), and premix. The reaction mixture was amplified for 30 cycles with initial denaturation at 95°C for 5 min, followed by 94°C for 1 min, annealing at 58°C for 2 min and extension at 72°C for 2 min and final extension at 72°C for 5 min and yielded the second and final PCR product (PCR2) [8,17]. All the six amplified PCR products generated (PCR2) were visualized in 2% agarose gel.

2.3.6. Agarose Gel Electrophoresis

All the six amplified PCR products generated (PCR2) were visualized in 2% agarose gel. Briefly, 2gm of agarose powder was weighed and 100 mL of TAE buffer (tris acetate) buffer was added in a flat bottom flask. The mixture was place on an oven and heated gently until the agarose dissolves completely. The mixture was removed from the oven and 6 uL of ethidium bromide was added and mix gently. The gel containing ethidium bromide was poured into a gel cast with an inserted comb and allowed to solidify. The comb was removed and gel cast transferred to an electrophoretic tank. To the wells, 15 uL each of PCR products (PCR 2) and 10 uL of ladder were loaded, and the tank closed and ran for 40 min at 400mA (current). Thereafter, the resultant products were visualized by UV trans-illumination. The number and size of resulting DNA bands were analysed using molecular weight ladder of 1000bp [6,8].

3. Results

A total of 42 parasitologically diagnosed positive blood samples that were spotted on whatman's filter paper consisting of 30 from symptomatic and 12 from asymptomatic individuals were analysed using PCR, 19out of 42 (45.24%) of the *P. falciparum* isolates showed DNA bands for the polymorphic genes *MSP*1, *MSP*2 and *glutamate-rich protein* genes. *Plasmodium falciparum* isolates from symptomatic patients accounted for 89.45% of the polymorphic genes, while only 10.53% of the isolates from asymptomatic individuals showed bands for these genes. Molecular characterization of *P. falciparum* isolates from symptomatic and asymptomatic individuals indicated the presence of 33 allelic variants spread across *MSP*1, *MSP*2 and *glutamate-rich protein* genes (Table 2).

The same table shows the distribution of *P. falciparum* genes detected with their respective alleles and base pair ranges in symptomatic and asymptomatic individuals. Five allelic variants of *MSP*1 gene were detected in isolates from symptomatic individuals. Out of these alleles, MAD20 (Figure 1) and KI alleles (Figure 2) from symptomatic patients accounted for 3(60.0 %) and 1 (20.0 %) with estimated band size range of 200-220bp and 200bp respectively. However, only 1MAD20 (20.0%) allele was detected from isolates of asymptomatic individuals.

In *MSP*2 gene, 24 allelic variants were detected, out of which 21 alleles consisting of 6 (25.0%) FC27 (Figure 3) and 15 (62.5%) 3D7/ICI (Figure 4) of band sizes ranging between 290-300bp and 500-600bp respectively were detected in isolates from symptomatic individuals, while 3 alleles consisting of 2(8.33%) FC27 and 1(4.17%) 3D7/ICI were detected in isolates from asymptomatic individuals.

Table 2. The distribution of *Plasmodium falciparum* genes detected with their respective alleles and base pair ranges in symptomatic and asymptomatic individuals

		Malaria Status		
		Symptomatic	Asymptomatic	
Genotypes/ Alleles	Total alleles	No. of alleles Detected (%)	No. of alleles detected (%)	Band sizes (bp)
***MSP*1**				
RO33	**5**	0	0	0
MAD20		3(60.0%)	1 (20.0%)	200-220bp
KI		1(20.0%)	0	200bp
***MSP*2**				
FC27	24	6(25.0%)	2(8.33%)	290-300bp
3D7/ICI		15(62.5%)	1(4.17%)	500-600bp
GLURP	4	3 (75.0%)	1(25.0%)	700-800bp
Total	**33**	**28 (84.85%)**	**5(15.15%)**	**-**

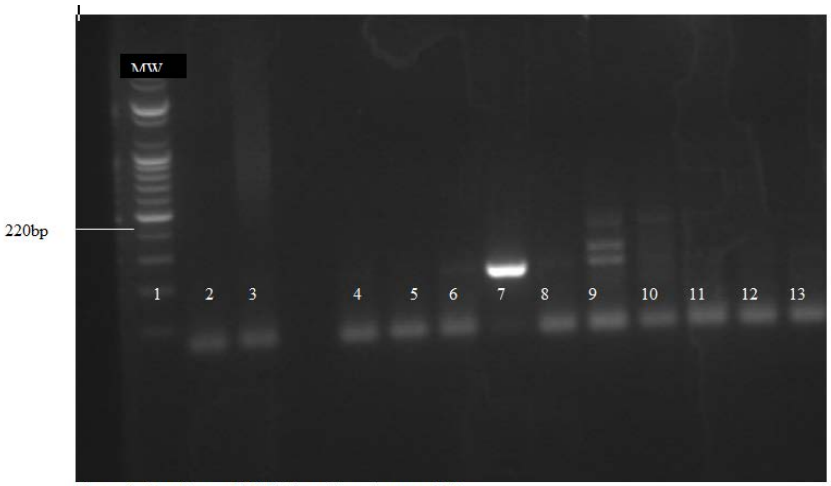

Figure 1: Amplicon of *MSP1* Gene Size: Approx.220bp
Lane 1 Molecular Weight ladder (1000bp), Lane 2 Negative Control,Lanes 3,4,5,6,8,10,11,12,13 Samples from other individuals, Lane 7, 9 *MSP1* MAD20.

Figure 1.

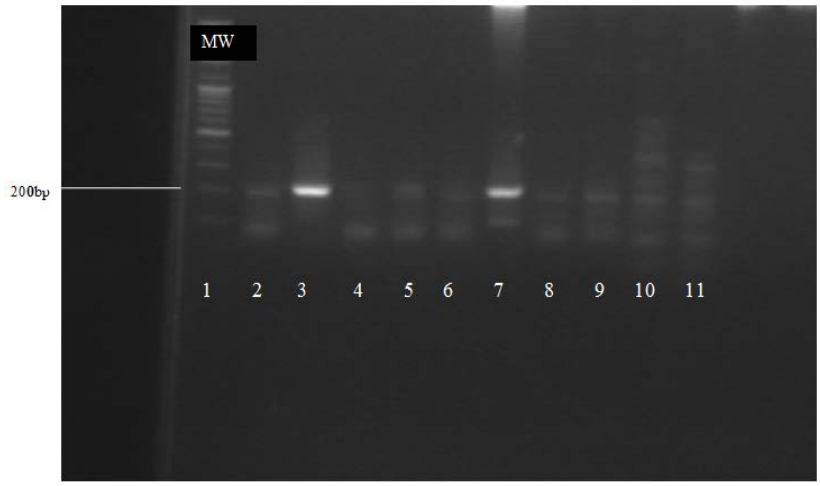

Figure 2: Amplicon of *MSP1* Gene Size: Approx. 200bp
Lane 1 Molecular Weight Ladder (1000-bp), Lane 2 Negative Control, Lane 3 Positive Control, Lanes 4,5,6,8,9,10,11 Samples from other individuals, Lane 7 *MSP1* KI.

Figure 2.

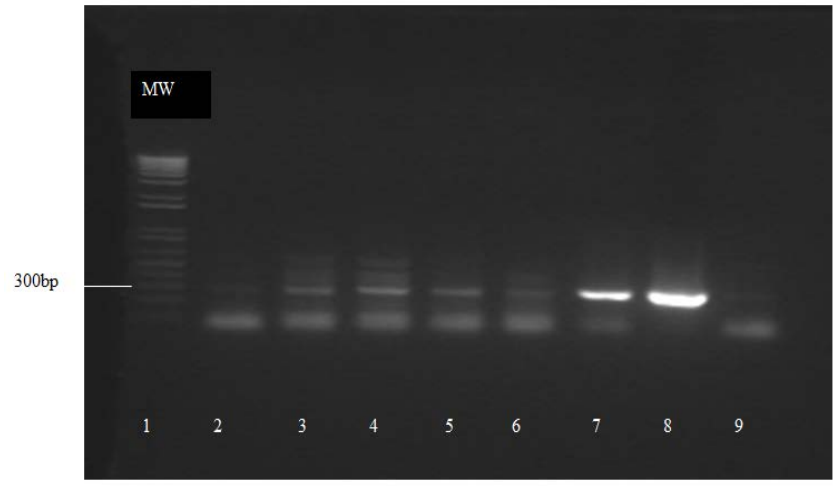

Figure 3: Amplicon of *MSP 2* gene Size: Approx.300bp
Lane 1 Molecular Weight Ladder (1000-bp), Lane 2 Negative Control, Lane 3 Positive Control, Lanes 4, 5, 7 and 8 *MSP 2* FC27, Lanes 6, 9 Samples from other individuals,

Figure 3.

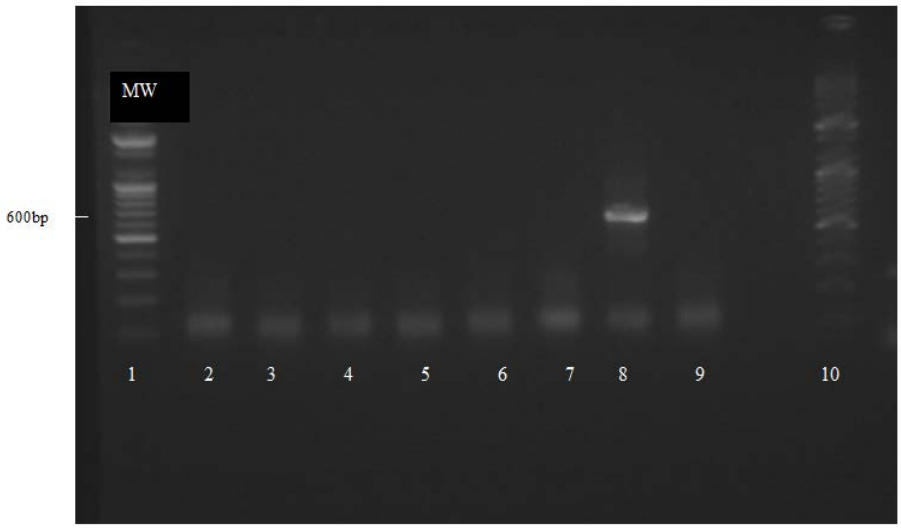

Figure 4: Amplicon of *MSP 2* gene Size: Approx. 600bp
Lane 1 and 10 Molecular Weight Ladder (1000-bp), Lane 2 Negative Control, Lanes 3, 4, 5, 6, 7, 9, Samples from other individuals, Lane 8 *MSP2* 3D7/ICI,

Figure 4.

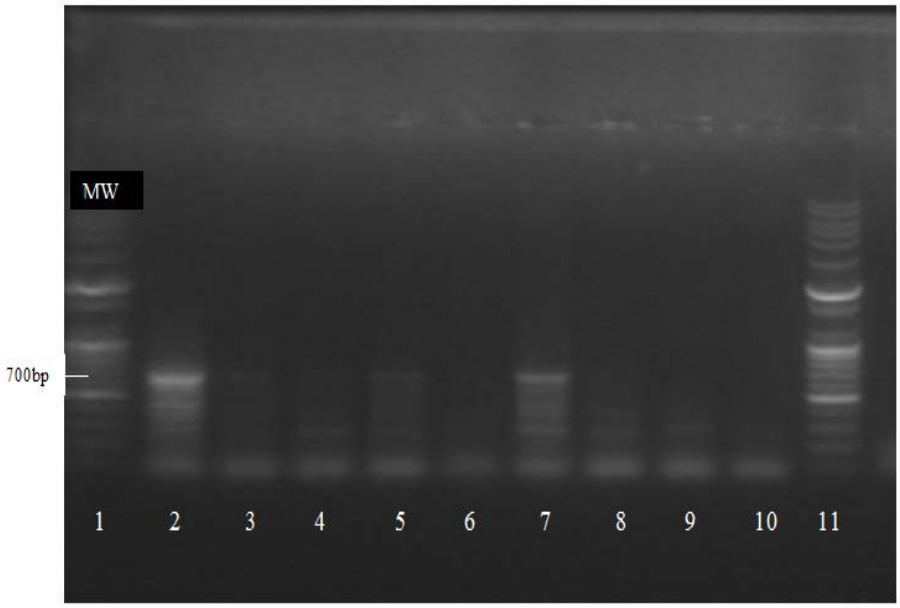

Figure 5: Amplicon of *glurp* gene, Size: Approx.700bp
Lane 1 and 11 Molecular Weight Ladder (1000-bp); Lane 2 Positive Control, Lanes 3, 4, 5, 6, 8, 9,10 Samples from other individuals, Lane 7 *glurp* gene

Figure 5.

However, 4 samples showed bands for *glutamate-rich protein* gene with estimated band size ranges between 700-800bp (Figure 5), out of which 3 (75.0%) were detected in isolates from symptomatic individuals, while only 1 (25.0%) was detected in isolates of asymptomatic individuals.

Distribution of genotypes detected in relation to age of infected symptomatic and asymptomatic individuals is presented in Table 3. *Plasmodium falciparum* genotypes were detected in 10 (76.9%) symptomatic individuals of age group 0-5 yr, 3 (75.0%) in age group 6-10 yrs, 3(60.0%) in age group >40 yr and 1(16.6%) in age group 21-30yr, while in asymptomatic individuals, *Plasmodium falciparum* genotypes were detected in only 2 individuals within the age groups 31-40 yr and >40 yrs with frequencies of 33.3% and 20.0% respectively.

Distribution of *P. falciparum* genotypes detected in relation to gender of infected symptomatic and asymptomatic individuals is presented in Figure 6 *Plasmodium falciparum* genotypes were detected in 11(84.6%) females and 6 (35.2%) males that were in the symptomatic group, while among the asymptomatic individuals, *P. falciparum* genotypes were detected only in 2 (20.0%) isolates from males.

Table 3. *Plasmodium falciparum* genotypes detected in relation to age of infected symptomatic and asymptomatic individuals

	Malaria status					
	Symptomatic			Asymptomatic		
Age group (yr)	Number examined	Number positive	% positive	Number examined	Number Positive	% positive
0-5	13	10	76.9	0	0	0
6-10	4	3	75.0	0	0	0
11-20	1	0	0	0	0	0
21-30	6	1	16.6	4	0	0
31-40	1	0	0	3	1	33.3
>40	5	3	60.0	5	1	20.0
Total	**30**	**17**	**56.7**	**12**	**2**	**16.7**

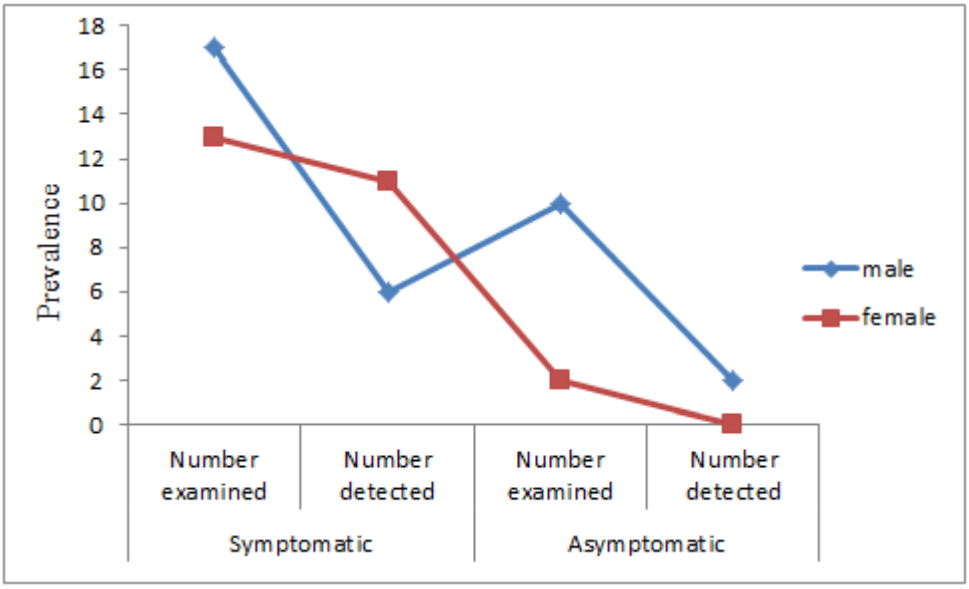

Figure 6. Prevalence of P. *falciparum* genotype in relation to gender of infected symptomatic and asymptomatic individuals

Table 4. Frequency of multiple alleles detected in symptomatic and asymptomatic individuals

	Multiple Alleles		
Symptomatic	Frequency	Asymptomatic	Frequency
MAD20/3D7ICI/FC27	1	MAD20/*GLURP*./FC27/3D7ICI	1
MAD20/3D7ICI/FC27/*GLURP*.	1	-	-
KI/MAD20/3D7ICI/FC27	1	-	-
GLURP./3D7ICI	1	-	-

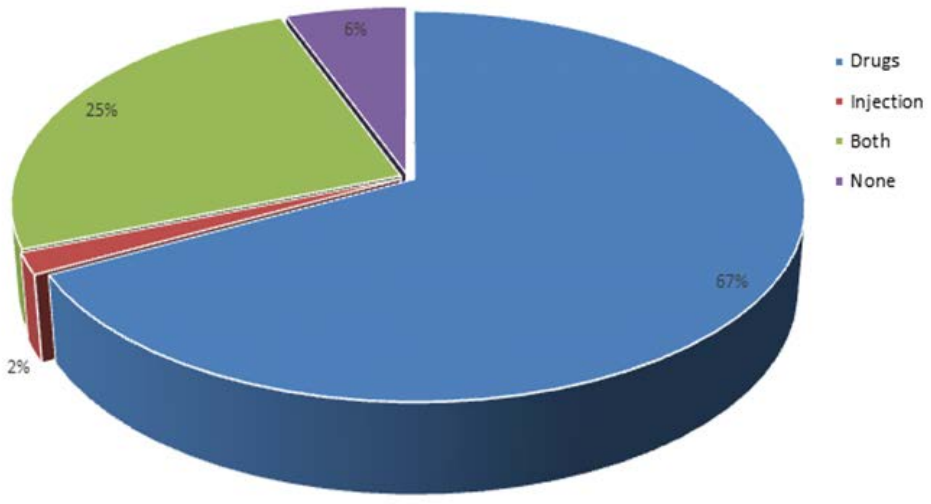

Figure 7. Anti-malarial administration by symptomatic individuals

In both symptomatic and asymptomatic individuals, alleles detected occurred as single and/or multiple alleles, although, multiple genotypes were detected more in symptomatic individuals (4), compared to asymptomatic individual (1) (Table 4).

Anti-malarial drug administration by symptomatic Individuals showed that 67% takes oral drugs, 25% takes anti-malarial drugs orally or parenterally (injectables) and 2% takes anti-malarial drugs parenterally (Figure 7).

4. Discussion

The genetic composition of *P. falciparum* isolated from symptomatic and asymptomatic individuals in Kaduna metropolis, revealed the presence of the three genes and their alleles viz: *MSP*1: MAD20 (130-220bp) and KI (250bp); *MSP*2: FC27 (290-300bp) and 3D7/ICI (470-700bp) and the *glurp*. gene (600-800bp). These genotypes and their alleles occurred more common in *P. falciparum* isolates from symptomatic individuals than isolates from asymptomatic individuals.

Diverse genetic composition of *P. falciparum* population has been suggested as an indication of malarial transmission intensity in an area [18]. Highly endemic areas have been generally characterised by extensive parasite diversity and infected individuals are reported to carry multiple genotypes while the reverse is the case in areas of low transmission intensity [6,9,18]. The findings in this study therefore are suggestive of the level of malaria transmission in Kaduna metropolis. In a related study, out of the 144 *P. falciparum* isolated in 3 endemic areas of Thailand, 96.5% had multiple genotypes [9]. Similarly in Uganda, of the 164 patients comprising 82 with severe malaria and 82 with mild malaria, 97% had *MSP*1 gene, 98.8% *MSP*2 gene and 87.2% *glurp* gene were recorded [7].

The findings in this study are also consistent with the finding of other workers in Nigeria. In a related study, the genetic composition of *P. falciparum* isolates in Ogun State, South-western Nigeria were characterised, the frequency of these genes in 100 *P. falciparum* isolates were as follows: *MSP*1 K1: 4(68%), MAD20 3(40%), RO33 1(20%); *MSP*2 FC27 3(76%), 3D7/ICI 3(56%) and *glurp*. 80% [3]. Similarly, the work of Oyedeji and Awobode [12] showed that of the 320 children screened for *Merozoite Surface Protein 2* (*MSP*2) alone, 34 and 31 had distinct *MSP*2 alleles detected in the uncomplicated and asymptomatic malaria groups respectively in Lafia, Nassarawa State, in North-Central Nigeria. These however are contrary to the work of Happi *et al. [10]* and Ojurongbe *et al.* [22] who detected RO33 allele among the people screened in Ibadan and Oshogbo, Nigeria, and that of Abdel Hamid *et al.* [1] who detected RO33 allele as the most predominant allele detected in Kosti region, Sudan,. The RO33 allele was not detected in *MSP*1 in the present study.

The number of genotypes detected in symptomatic individuals in the present study could probably be associated to anti-malarial treatment since majority of them have reported sick to the hospitals and are on drugs given that the number of genotypes in an infected individuals is likely to reduce in an area due to antimalarial treatment [18].

Although, the influence of the different alleles on clinical malaria is not known [19], the most predominant allele detected in this study is the *MSP* 2 3D7/ICI allele (16), followed by 8 FC27, while in the *MSP*1 gene; the most predominant allele detected was the MAD20 allele (4). The allele all occurred in the symptomatic individuals, while in asymptomatic individuals, FC27 was the predominant allele detected. This finding is in line with the work of Oyedeji and Awobode [12] in which 3D7/ICI was dominant in symptomatic uncomplicated malaria individuals while FC27 occurred more in asymptomatic individuals screened in Lafia, Nassarawa State. The work of Kidima and Nkwengulila [20] also revealed 3D7/ICI as the most frequently detected allele compared to FC27 allele among children with uncomplicated malaria in Tanzania.

Contrary to these findings, Engelbrecht *et al.* [21] detected FC27 allele more in symptomatic malaria cases than in asymptomatic individuals and that *MSP* 2 was involved more in morbidity of malaria in Papua, New Guinea. According to Amodu *et al.* [11] the presence of FC27 and 3D7/ICI are significant indicators of severe malaria.

The bands sizes of the various amplicons detected in the study are within the ranges reported by authors [6,8,18]. *Plasmodium falciparum* genotypes were detected more in the younger age group <5yr and least in the age group 21-30 yr in symptomatic individuals sampled in this study. This observation is in line with the work of Ojurongbe *et al. [19]*, in which the number of *MSP* 2 alleles per isolate detected was found to be lower in the older age group. In endemic areas, prevalence of *P. falciparum* was high in children under five years. This implies that lower age group particularly children below one year acquire protections due to maternal and neonatal factors, but such protections are lowered as the age increases and become more susceptible [18,22,23].

Plasmodium falciparum genotypes detection was high in symptomatic females compared to males. The results further showed no significant association between infection, age and sex. Previous studies showed that genotypes detection was neither age nor sex dependent but correlate with parasite density [6].

The study has also showed that single genotypes are common in the study area although four isolates from symptomatic individuals were found to harboured multiple genotypes and one in asymptomatic group. This is in line with the work of Ghanchi *et al.* [18], were single genotypes were detected more frequent than multiple genotypes in a study in Pakistan. The detection of multiple genotypes in symptomatic individuals in this study is therefore consistent with the work of Ghanchi *et al. [18]*. Reports from several studies regarding the association of multiple genotypes and severity of malaria have been inconsistent. Kiwuwa *et al*. [7] showed that severe malaria cases were likely to have more than three genotypes (multiple) per infected individual. Similarly, multiple genotypes were also detected to be more frequent among children with uncomplicated malaria in Gabon [24]. The work of Amodu *et al. [11]*, revealed the detection of

single infection more frequently and in association with the development of severe malaria in Ibadan, South-western Nigeria.

The result of genotyping showed that allelic variants from all the three genes; (*Merozoite Surface Protein 1*(*MSP*1); MAD20 and KI, *Merozoite Surface Protein 2* (*MSP*2); FC27 and 3D7/ICI and *glutamate-rich protein* gene) are common in Kaduna Metropolis. All the 3 genotypes were detected in symptomatic and asymptomatic individuals. In addition, both single and multiple genotypic infection were recorded in the present study.

5. Conclusion

The diverse genetic composition of *P. falciparum* isolates from symptomatic and asymptomatic individuals recorded in this study shows the endemicity of malaria due to *P. falciparum* in the study area, this information are essential and necessary for a successful malaria control programme.

Limitation of the study included lack of medical history and anti- malarial intake by asymptomatic individuals which may alter the interpretation of the results.

Ethical Approval

Ethical approvals were obtained from Kaduna State Ministry Of Health (MOH/ADM/744/T/9) for the sampling of symptomatic and asymptomatic individuals in the selected hospitals within the state. Approval for the sampling of asymptomatic individuals in the National Blood Transfusion Service was obtained from the Federal Ministry Of Health (NBTS/HQ/058/04). Approvals were also obtained from the Nigerian Army Reference Hospital (44) (44/NARHK/GI/300/60) Kaduna and confirmation was obtained from Nigerian Defence Academy Medical Centre and Saint Gerald Hospital respectively.

Consent of publication,

Not applicable.

Competing Interests

Authors have no competing interest.

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Availability of Data

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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