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Assessing the therapeutic efficacy of artemether-lumefantrine for uncomplicated malaria in Lagos, Nigeria: a comprehensive study on treatment response and resistance markers

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Abstract

Background The burden of malaria persists in sub-Saharan Africa and the emergence of artemisinin resistance has introduced complexity to control efforts. Monitoring the efficacy of artemisinin-based treatment for malaria is crucial to address this challenge. This study assessed treatment efficacy of artemether-lumefantrine (AL) and genetic diversity of *Plasmodium falciparum* isolates in a Nigerian population.

Methods Participants presenting with clinical symptoms of uncomplicated malaria at a health centre in Lagos, Nigeria, were screened for *P. falciparum*. Enrolled participants were treated with AL and monitored through scheduled check-up visits, clinical and laboratory examinations for 28 days. Parasite clearance and genetic diversity were assessed through polymerase chain reaction (PCR) analysis of merozoite surface proteins (*msp1* and *msp2*). The prevalence of drug resistance mutations was assessed by *P. falciparum* multidrug resistance gene 1 (*mdr1*) genotyping followed by *P. falciparum* ubiquitin-specific protease 1 (*ubp*1) gene sequencing.

Results The PCR-uncorrected treatment outcome revealed 94.4% adequate clinical and parasitological response (ACPR) and 5.6% late parasitological failure (LPF) rates. After PCR correction, no suspected LPF case was detected and ACPR 67/67 (100%) was achieved in all the individuals. Moreover, a high prevalence of wild-type alleles for *mdr1* N86Y (93.7%), and *mdr1* D1246Y (87.5%) was observed. Genetic diversity analysis revealed predominant K1 allelic family for *msp1* (90.2%) and FC27 for *msp2* (64.4%). Estimated multiplicity of infection (MOI) was 1.7, with the highest MOI observed in the 5–15 years age group. *ubp1* sequence analysis identified one nonsynonymous E1528D polymorphism at a low frequency (1.6%).

Conclusion The study demonstrated sustained efficacy of AL for treating uncomplicated *P. falciparum* malaria. Genetic diversity analysis revealed various allelic types, suggesting occurrences of polyclonal infections. Nonetheless, the detection of a significant *ubp1* polymorphism could have future implications for the epidemiology of anti-malarial drug resistance in the population.

Keywords Artemether-lumefantrine, Genetic diversity, Nigeria, *mdr1*, *ubp1*, ACPR

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Background

Malaria is a debilitating disease caused by the protozoan parasite *Plasmodium falciparum*. It remains a significant global health concern particularly in sub-Saharan Africa [1]. Central to anti-malarial strategies is artemisinin-based combination therapy (ACT), with artemether-lumefantrine standing out for its widely recognized efficacy in treating uncomplicated *P. falciparum* malaria [2–4]. The unique mechanism of action of ACT involves rapid clearance of circulating parasites with artemisinin, coupled with the sustained action of the partner drug, making this combination a key component in the global malaria control toolkit.

However, artemisinin resistance poses a significant obstacle to malaria control and its multifaceted genetic basis involves several pivotal molecular markers [5–7]. A nuanced understanding of these genetic factors is crucial for effective strategies to mitigate resistance and sustain the efficacy of ACT. *Plasmodium falciparum* ubiquitin-specific protease 1 (*ubp1*) gene plays a role in protein degradation and regulation within the parasite [8]. *ubp1* influences the stability and turnover of proteins in the parasite. This is a potential mechanism through which parasites can withstand the rapid action of artemisinin treatment. As such, *ubp1* polymorphism may impact the clearance of damaged or misfolded proteins. The observed decline in the prevalence of *P. falciparum* multidrug resistance gene 1 (*mdr1*) mutations, which are associated with resistance against chloroquine and other anti-malarials, represents a noteworthy trend in certain populations [9, 10]. This is a positive development in the context of malaria control efforts as it suggests positive response to reduced use of drugs like chloroquine and the adoption of alternative anti-malarial strategies. While an encouraging reduction in the prevalence of *mdr1* mutations might occur in these populations [9, 10], the potential influence of selective pressure from ACT partner drugs on the genetic landscape of these mutations requires careful consideration [11, 12]. This knowledge is crucial for refining population-targeted drug combinations and optimization of treatment regimens.

Genetic diversity of *P. falciparum* is a critical factor influencing the epidemiology, transmission dynamics and potential for the development of anti-malarial drug resistance. Merozoite surface proteins (MSPs), such as *msp1* and *msp2*, have been extensively studied as genetic markers to assess the diversity and complexity of *P. falciparum* infections [13–15]. Knowledge of the genetic characteristics of malaria parasites is essential for tailoring control strategies. This study employed a systematic approach integrating clinical observations, parasitological investigation and genetic analyses to assess the therapeutic efficacy of artemether-lumefantrine (AL) in Lagos, Nigeria. The study provided valuable insights into the status of artemether-lumefantrine as a frontline treatment for uncomplicated falciparum malaria to inform malaria control strategies in the country.

Methods

Study design

This single-arm prospective observational study was conducted according to the modified World Health Organization (WHO) drug efficacy protocol [16]. The field study took place from September to November 2021 at Ijede General Hospital and Agura Health Centre, both in Ikorodu Local Government, Lagos, Nigeria. The study evaluated clinical and parasitological responses to directly observed treatment of uncomplicated malaria. Individuals who met the inclusion criteria were enrolled, treated on-site with artemether-lumefantrine and followed up for 28 days. The follow-up included check-up visits and corresponding clinical and laboratory examinations. Based on the assessment results, patients were classified as having therapeutic failure (early or late) or an adequate response. Parasite isolates collected during follow-up days were subjected to molecular analysis to distinguish between recrudescence (treatment failures) and reinfection (new infections), followed by drug resistance genotyping and targeted sequencing.

Participant selection and enrollment

Ethical approval was obtained from the Institutional Review Board of the Nigerian Institute of Medical Research (NIMR), Lagos (reference number IRB/21/017). Individuals aged 1–70 years presenting with symptoms of uncomplicated malaria, were screened using histidine-rich protein 2-based SD BIOLINE *P. falciparum* Ag Test (Standard Diagnostics, Inc.) rapid diagnostic tests (RDTs) followed by microscopy. RDTs were used to facilitate malaria detection after which thin and thick smears were prepared for RDT-positive samples. Additional recruitment criteria included fever in the preceding two days, no history of anti-malarial intake in the previous four weeks and *P. falciparum* parasitaemia ranging between 2000 and 100,000 parasites/μL of blood on presentation [16]. Participants with signs of complicated malaria or mixed infections were not recruited into the study, and those developing concurrent ailments during follow-up were promptly attended to and withdrawn from the study.

Sample size determination

As the study focused on non-comparative monitoring of the efficacy of a single anti-malarial agent, AL, the required sample size was the total number of patients

assignable to a clinical outcome at the end of the study. Using the formula $N = Z^2 \times P(1-P) / d^2$, where Z represents the standard normal distribution set based on the confidence level (for 95%, Z = 1.96), P is the expected treatment failure rate based on a previous study, and d is precision, a minimum of 88 patients were required. This accounted for an expected maximum artemisinin failure rate of 5% and included an additional 20% (15 patients) to accommodate potential loss to follow-up.

Outcome assessment

Patients were classified as having therapeutic failure (early or late) or an adequate response based on the outcomes of assessments. Molecular genotyping of DNA extracted from blood spots at different time points was adopted to determine the multiplicity of infections and distinguish between treatment failure and re-infections. Classification of treatment outcomes included early treatment failure (ETF), late treatment failure (LTF) or late clinical failure, late parasitological failure (LPF) and adequate clinical and parasitological response (ACPR).

18s rRNA diagnostic PCR

Diagnostic PCR was performed by targeting the 18S rRNA using an established protocol [17]. The PCR was conducted in a total volume of 25 µL with the following reaction mixture: a first round of amplification with 10 nM of each genus-specific primer (rPLU5 and rPLU6), 0.2 µM of each primer (Additional File 1: Table S1), 12.5 µL One Taq Quick-Load 2X Master Mix with Standard Buffer (containing PCR buffer, $MgCl_2$, and dNTPs) (New England Biolabs, Massachusetts, USA), nuclease-free water and 2 µL of the extracted parasite DNA. Amplification occurred with one cycle at 94°C for 30 s, and 30 cycles of 94 °C for 30 s, an annealing temperature of 45 °C for 1 min, and extension at 68 °C for 1 min, with a final extension at 68 °C for 5 min. The second round of amplification followed the same procedure with the primary reaction, although this time species-specific primers rFAL1/rFAL2 and 2 µL of the first amplification were introduced to the reaction and the annealing temperature was set at 49 °C. Lastly, 5.0 µL of PCR products were analyzed by agarose gel (1%) electrophoresis.

MSP genotyping

Genomic DNA was extracted from blood samples after which molecular genotyping was performed to determine multiplicity of infections and distinguish between treatment failure and re-infections. *msp1* and *msp2* genes were targeted, and multiplicity of infection (MOI) was estimated. The primer sequences used for MSP amplifications are described in Additional File 1: Table S2.

mdr1 genotyping

Mdr1 genotyping involved nested polymerase chain reaction followed by restriction fragment length polymorphism (RFLP) analysis https://www.medschool.umaryland.edu/malaria/Protocols/. This approach facilitated the detection of point mutations in the *mdr1* gene (Additional File 1: Table S3).

ubp1 sequencing

Ubp1 gene was amplified using nested polymerase chain reaction (nPCR) as described by Adams et al. [18] with minor modifications. The primer sequences and cycling parameters are described in Additional File 1: Table S4. PCR was performed in a total volume of 25 µL with the following reaction mixture: 0.2 µM of each primer, 12.5 µL One *Taq* Quick-Load 2X Master Mix with Standard Buffer (contains PCR buffer, $MgCl_2$, and dNTPs) (New England Biolabs, Massachusetts, USA), nuclease free water and 2 µL of the extracted parasite DNA. Two microlitres of the first-round product was used as a template in a 25 µL inner PCR reaction. A DNA sample extracted from the 3D7 parasite strain was used as a positive control. The PCR thermal conditions were the same for both steps, but different annealing temperatures (Additional File 1: Table S4). The thermal cycling program for the 1st amplification was 94 °C for 30 s and 30 cycles of 94 °C for 30 s, annealing temperature for 30 s and 68 °C for 1 min with a final extension of 68°C for 5 min. The second round of PCR consisted of initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, annealing temperature for 30 s and 68 °C for 1 min with a final extension of 68°C for 5 min. Lastly, 5.0 µL PCR products were analysed by agarose gel (1%) electrophoresis. The remaining PCR products were purified prior to Sanger sequencing.

Data analysis

Therapeutic assessment data were recorded in the field using WHO-standard template to calculate per-protocol analysis results [16]. Treatment outcomes for each participant patient were defined, according to WHO guidelines, as early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF), or adequate clinical and parasitological response (ACPR). ETF was described as signs of severe malaria on day 1, 2, or 3 after initiation of treatment, or if parasites were still present with the presence of symptoms. LCF was defined as initial improvement with a return of malaria-related symptoms between day 4 and day 28, without evidence of a new infection. LPF is characterized by the reappearance of parasites in the blood between day 7 and day 28, without the presence of symptoms. ACPR

was the desired treatment outcome. It indicated that the patient had cleared the parasites from their bloodstream and remained symptom-free for at least 28 days after the start of treatment. The occurrence of each class of treatment outcome, prevalence of parasitaemia at day 3, and patients lost to follow-up were evaluated descriptively. The efficacy of artemether-lumefantrine was assessed using PCR-uncorrected and PCR-corrected methods. For PCR-uncorrected efficacy, the proportion of patients who were parasitologically cured (i.e., no detectable *P. falciparum* parasites) after treatment was calculated, without accounting for genetic variations. PCR-corrected efficacy involved re-evaluating cure rates by considering the presence of persistent parasites that were initially undetectable but later confirmed through PCR as resistant strains. The corrected efficacy thus adjusted the cure rate by accounting for these false negatives to provide a more accurate measure of drug effectiveness. The frequency of *msp1* and *msp2* allelic families was calculated as a proportion of all detected alleles in the isolates. Spearman's rank correlation coefficient was calculated to assess the association between MOI and mean parasite density and age. The *ubp1* sequence data were converted from ab1 to fastq using a web server http://sequenceconversion.bugaco.com/converter/biology/sequences/abi_to_fastq.php. FastQC was used to check the sequence quality. PF3D7_0104300 (from PlasmoDB) was used as reference sequences for alignment to detect polymorphisms in UBP1 gene. The sequences were mapped with the reference using Burrows-Wheeler Aligner (BWA) after which SAM files were converted to BAM and sorted using the Samtools. Variant calling was done using GATK-Haplotype caller to generate the gVCF file. The sequences were merged into one VCF file using CombineGVCFs followed by annotation which was done using SnpEff version 4.0. Python 3.9.18 was used for data analysis and visualization. P-value < 0.05 was considered statistically significant.

Results

Demographic characteristics

A total of 972 outpatients were screened for malaria, of which 197 (20.3%) were RDT positive. Ninety-eight (49.7%) of the RDT positive cases were confirmed by microscopy to be *P. falciparum* positive (Fig. 1) of which eighty-eight patients were invited to participate in the drug assessment. However, only eighty-four (85.7%) patients with RDT and microscopy positive results with at least 2000 parasites/uL were enrolled and followed up, of which seventy-one (84.5%) of the enrolled participants completed follow-up. The enrolled patients were categorized into three age groups; < 5 years (8 patients; 9.5%), 5–15 years (57 patients; 67.9%), and > 15 years (19 patients; 22.6%) (Table 1). The positive samples recorded by microscopy on days 0, 1, 3, 7, 14, 21 and 28 were 84/84 (100%), 36/80 (45%), 0/78 (0%), 2/76 (3%), 0/75 (0%), 0/72 (0%), and 2/71 (3%), respectively, given that thirteen (16%) participants were lost at different times to follow-up (Fig. 1). Geometric mean parasitaemia at baseline was 42,727 parasites/μL (95% CI 14,522–26,599) while the

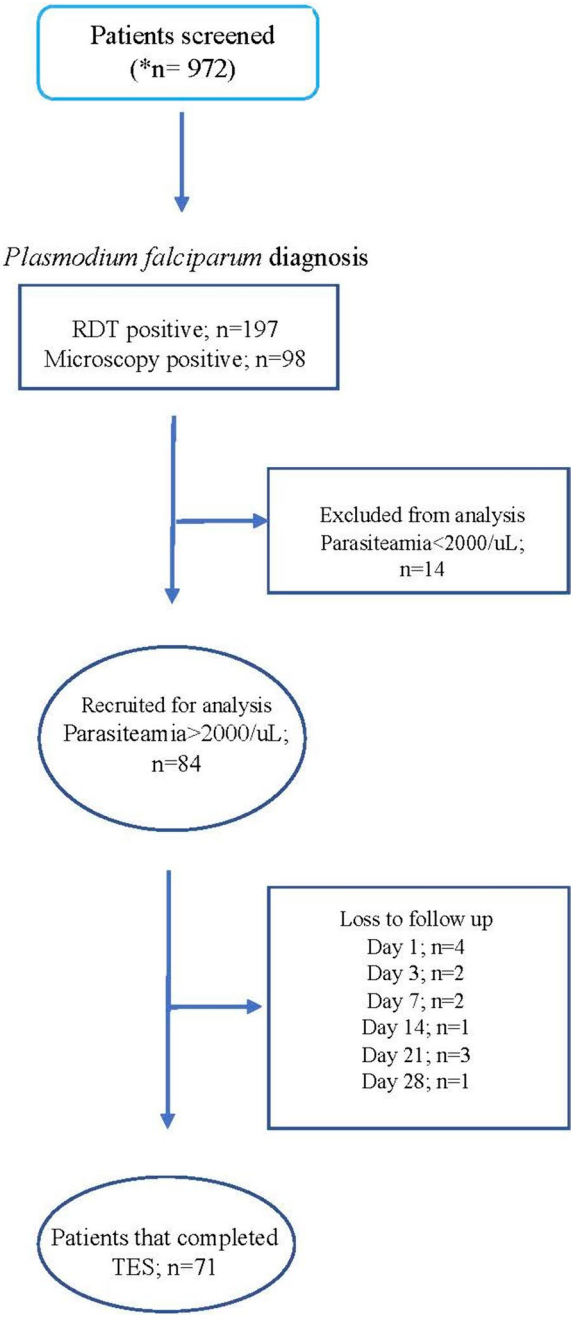

Fig. 1 Study participants' enrolment flow chart (*n = number of cases)

Table 1 Demographic characteristics of study participants

	Total
Enrolled, n	84
Age distribution (years)	
< 5	8 (9.5%)
5–15	57 (67.9%)
> 15 years	19 (22.6%)
Gender	
Male	43 (51%)
Female	41 (49%)
Mean body temperature (°C)	
Mean ± sd	37.5 ± 1.1
Height, cm	
Mean ± sd	136.42 ± 24.9
Weight, kg	
Mean ± sd	33.46 ± 17.9
Parasite density, parasites per µL at enrollment, median (range)	19,407 (2,200–196,521)

mean axillary temperature was 37.4 °C (95% CI 37.1–37.7). There was an inverse relationship between age and parasite density (correlation coefficient = − 0.2072, P = 0.0586) (Fig. 2; Additional File 1: Fig. S1). The same relationship was observed for age vs axillary temperature (correlation coefficient = − 0.1626, P = 0.1394) (Additional File 1: Fig. S2).

18S rRNA diagnostic PCR and treatment outcome

Eighty-four day 0 samples from the enrolled patients were screened by 18S rRNA PCR, of which 82 (97.6%) were confirmed *P. falciparum*-positive (Additional File 1: Plate S1). On follow-up days, 31/78 (39.8%), 23/76 (30.3%), 5/74 (6.8%), 4/73 (5.5%), 1/70 (1.4%) and 3/69 (4.3%) were 18S rRNA PCR-positive on days 1, 3, 7, 14, 21 and 28 respectively (Table 2). According to the PCR-uncorrected data, 4/71 (5.6%) patients were classified as LPF, while 67/71 (94.4%) patients achieved ACPR in the per-protocol analysis (Table 3). However, after PCR correction, no suspected LPF case was detected and ACPR was observed in 67/67 (100%) individuals (Table 3; Additional File 2: Sheet S2).

msp1 and *msp2* allelic diversity

Msp1 genotyping was achieved for 61 isolates while 45 isolates were typed for *msp2* locus. For MSP1 isolates, three K1 (160–500 base pairs), five MAD20 (220–500 bp) and five RO33 (160–600 bp) alleles were identified (Additional File 1: Plate S2; Additional File 2: Sheet S7). The predominant *msp1* allelic family was K1 55/61 (90.2%), followed by RO33 38/61 (62.3%) and

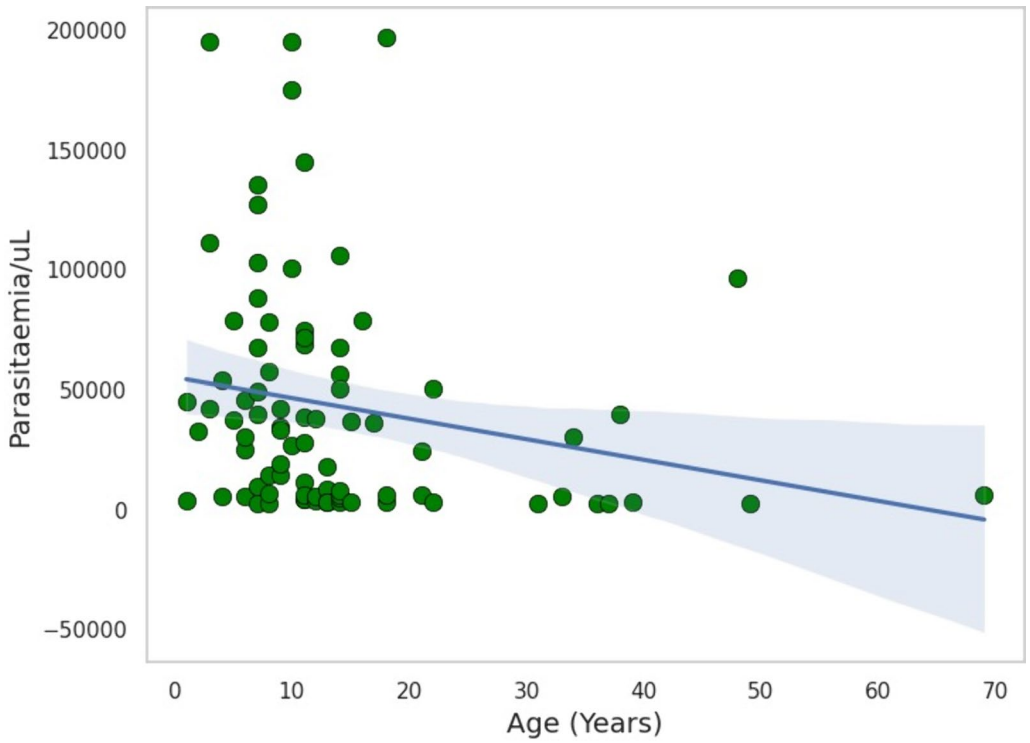

Fig. 2 Relationship between (day 0) parasitaemia and age (years) of participants

Table 2 *P. falciparum* parasite detection on day 0 and follow-up days

Day	Microscopy, n (%)	18S rRNA, n (%)
0	84 (100)	82 (97.6)
1	36/80 (45)	31/78 (39.8)
3	0/78 (0)	23/76 (30.3)
7	2/76 (3)	5/74 (6.8)
14	0/75 (0)	4/73 (5.5)
21	0/72 (0)	1/70 (1.4)
28	2/71 (3)	3/69 (4.3)

MAD20 31/61 (50.8%). Specifically, 19/61 (31.1%) of *msp1* positive samples had only K1 family, 9/61 (14.8%) had MAD20, and 7/61 (11.5%) had RO33 allelic family. Monoclonal infections were 35/61 (57.3%), while the remaining 26 (42.6%) were polyclonal, with K1/MAD20, K1/RO33, and MAD20/RO33 representing 6/61 (9.8%), 11/61 (18.0%), and 1/61 (1.6%), respectively (Fig. 3). Furthermore, 8/61 (13.1%) samples possessed three *msp1* allelic types (i.e., trimorphic). Eight *msp2* alleles were detected, including three FC27 and five 3D7 alleles, with sizes ranging between 400 to 800 bp for FC27 allelic family and 300 to 800 bp for 3D7 (Additional File 1: Plate S3). The frequency of FC27 and 3D7 were 29/45 (64.4%) and 26/45 (57.8%), respectively (Additional File 1: Table S5); 20/45 (44.4%) had only FC27, 17/45 (37.8%) had only 3D7 while 8/45 (17.8%) possessed both *msp2* allelic families (Fig. 4). The estimated MOIs were 2.0 and 1.2 for *msp1* and *msp2* loci, respectively (Additional File 1: Table S5). The overall mean MOI was 1.7. There was no statistically significant correlation between age and MOI estimated for *msp1* and *msp2* (Spearman rank coefficient = 0.5000; P > 0.9999) loci (Table 4). However, the highest MOI was observed in the 5 – 15 years group (2.1 for *msp1* and 1.2 for *msp2*). Also, no significant correlation was observed between MOI and the parasitaemia for *msp1* (Spearman rank coefficient = 0.5000; P > 0.9999) and *msp2* (Spearman rank coefficient = 1.0000; P = 0.3333) markers (Table 5).

Prevalence of *mdr1* mutations

The prevalence of N86Y and D1246Y mutations was determined for 64 PCR positive samples. For *mdr1*-N86Y locus, a prevalence of 60/64 (93.7%) was observed for wild-type *mdr1*-N86 (Table 6). No mutant or mixed *mdr1*-86Y allele was found. With respect to *mdr1*-D1246Y locus, the prevalence of mixed mutant and

Table 3 Artemether-lumefantrine treatment outcomes

PCR-uncorrected	Treatment outcome	number (%)	lower 95%CI	upper 95% CI
	ETF	0 (0.0%)	0	5.1
	LCF	0 (0.0%)	0	5.1
	LPF	4 (5.6%)	1.6	13.8
	ACPR	67 (94.4%)	86.2	98.4
	Total patients per protocol	71		
	WTH	0		
	LFU	13		
	Total patients LFU/WTH	13 (15.5)		
	Total patients at baseline	84		
PCR-corrected		number (%)	lower 95%CI	upper 95% CI
	ETF	0 (0)	0	5.4
	LCF	0 (0)	0	5.4
	LPF	0 (0)	0	5.4
	ACPR	67 (100)	94.6	100
	Total patients per protocol	67		
	WTH	2		
	LFU	13		
	Total patients LFU/WTH	15 (18.3)		
	[a]Total patients at baseline	82		

ETF early treatment failure, *LCF* late clinical failure, *LPF* late parasitological failure, *ACPR* adequate clinical parasitological response, *LFU* Loss to follow-up, *n*, number, *WTH* withdrawn

[a] This is the total number (n = 82) of viable samples for PCR analysis

This was less than the total number (n = 84) of patients enrolled for the study

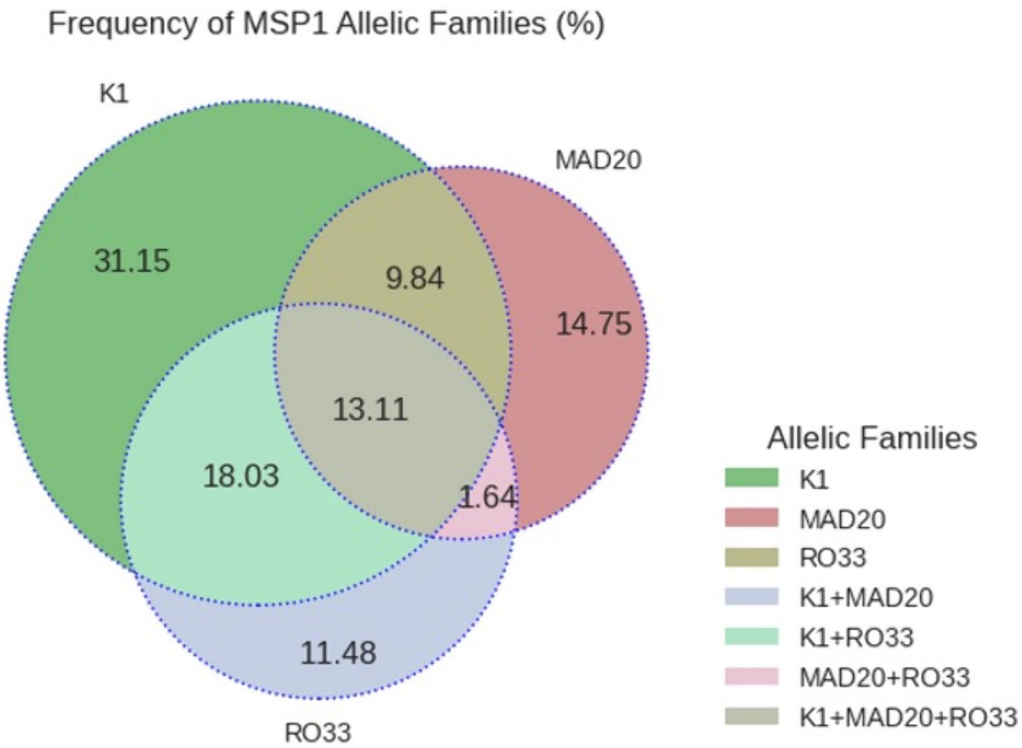

Fig. 3 Distribution of MSP1 allelic families in the parasite population

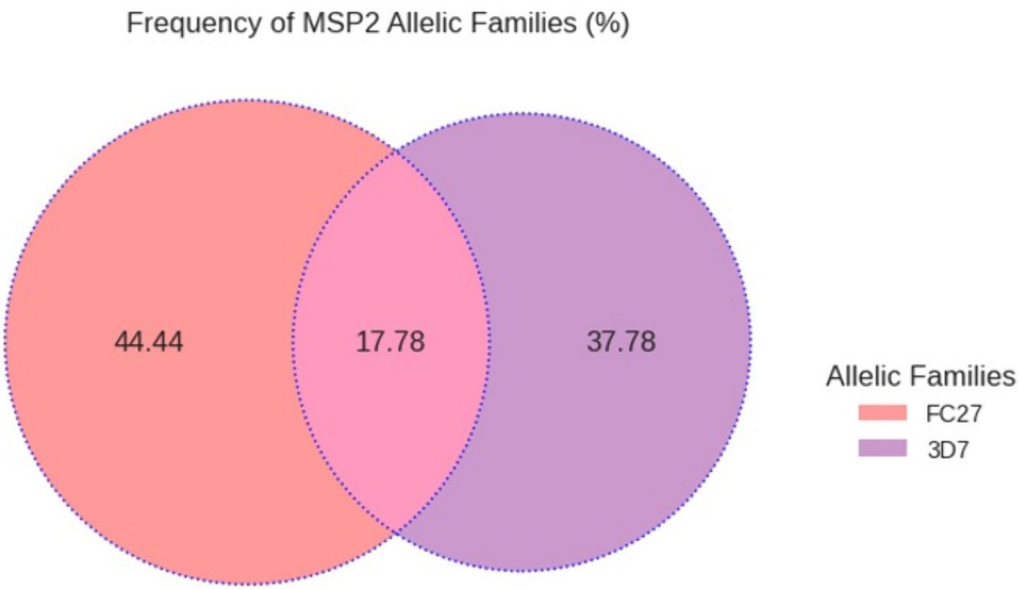

Fig. 4 Distribution of MSP1 allelic families in the parasite population

wildtype genotype was 4/64 (6.2%), 58/64 (87.5%) wildtype D1246, and 1/64 (1.6%) had 1246Y mutant allele. Overall, there was a high proportion of circulating parasites with the wild type alleles for *mdr1* genes.

ubp1 polymorphisms

Sixty-one *P. falciparum* isolates with positive PCR amplification of the 304-bp region were Sanger sequenced (Additional File 1: Plate S4). Among these,

Table 4 Distribution of MSP1 and MSP2 allelic types in different age groups *of P. falciparum* infected patients

	< 5 years	5–15 years	> 15 years
MSP1			
K1	1	16	2
MAD20	1	5	3
RO33	2	3	1
K1 + MAD20	1	4	1
K1 + RO33	1	8	2
MAD20 + RO33	0	1	0
K1 + MAD20 + RO33	0	6	2
Total	6	43	11
Total K1	3	43	9
Total MAD20	2	22	7
Total RO33	3	27	5
MOI	1.3	2.1	1.9
MSP2			
FC27	3	12	4
3D7	1	14	3
FC27 + 3D7	0	7	1
Total	4	33	8
Total FC27	3	22	5
Total 3D7	1	20	4
MOI	1.0	1.2	1.1

MOI Multiplicity of Infection

Table 5 Distribution of MSP1 and MSP2 allelic types in different parasite densities

	< 5000/μL	5000–10,000/μL	> 10,000/μL
MSP1			
K1	7	3	11
MAD20	5	2	4
RO33	0	0	7
K1 + MAD20	1	0	3
K1 + RO33	1	1	7
MAD20 + RO33	1	1	1
K1 + MAD20 + RO33	2	0	4
Total	17	7	37
Total K1	11	8	37
Total MAD20	14	2	14
Total RO33	7	2	26
MOI	1.9	1.7	2.1
MSP2			
FC27	4	3	13
3D7	8	1	8
FC27 + 3D7	2	1	5
Total	14	5	26
Total FC27	6	4	19
Total 3D7	10	2	14
MOI	1.1	1.2	1.3

MOI Multiplicity of Infection

Table 6 Frequency of the wild and mutant alleles for MDR1 genes in the study population

	MDR1 (N86Y) n (%)	MDR1 (D1246Y) n (%)
Negative	4 (6.3)	3 (4.7)
Wildtype	60 (93.7)	56 (87.5)
Mutant	0 (0)	1 (1.6)
Hybrid	0 (0)	4 (6.2)

Table 7 UBP1 polymorphisms in the parasite population

Sample ID	Codon	BioType	Amino acid	Nucleotide sequence
IM342	1528	SNP (non-synonymous)	Glu-Asp	GAA-GAC
B542	1532–1524	Deletion	Lys, Tyr, Asp	AAA TAT GAT
IM139	1532–1524	Deletion	Lys, Tyr, Asp	AAA TAT GAT
IM188	1532–1524	Deletion	Lys, Tyr, Asp	AAA TAT GAT
IM059	1535	Insertion	Lys, Tyr, Asp	AAA TAT GAT
IM115	1535	Insertion	Lys, Tyr, Asp	AAA TAT GAT
B056	1528	Insertion	Lys, Tyr, Glu	AAA TAT GAA
B400	1532	Insertion	Lys, Tyr, Glu	AAA TAT GAA

Table 8 Prevalence of UBP1 polymorphisms in the parasite population

Biotype	Codon	Prevalence (%)	Amino acid changes
SNP	1528[a]	1.64	Glu-Asp
Deletion	1532–1524	4.92	Lys, Tyr, Asp
Insertion	1535	3.33	Lys, Tyr, Asp
Insertion	1532	1.67	Lys, Tyr, Glu
Insertion	1528	1.67	Lys, Tyr, Glu

[a] Associated with delayed parasite clearance[27]

one isolate (1.6%) was found to harbour one non-synonymous mutation (Tables 7, 8). In total, seven indels were identified, with 11.5% (7/61) causing a frameshift in the sequence reading frame. Multiple nucleotide sequence alignments revealed that three isolates (4.9%) had AAATATGAT deletions (encoding KYD) at amino acid residues 1532 to 1534. Furthermore, two isolates had insertions of AAATATGAT (encoding KYD) at amino acid residues 1535 to 1537, one insertion at amino acid residues 1528 to 1530 and one at 1532–1534 (Table 7). A single nucleotide substitution (1/61; 1.6%) was also observed in 1528 locus (Table 7).

Discussion

This study adopted clinical, parasitological and genetic techniques to evaluate the therapeutic efficacy of artemisinin-based treatment of malaria in Lagos, Nigeria. The demographic profile of the patients revealed a diverse distribution across age groups, with a significant representation of children between 5 and 15 years old, reflecting the vulnerability of this age group to malaria infection. An inverse relationship was observed between age and parasite density, corroborating an age-dependent susceptibility to infection across different age cohorts. This underscores the importance of considering age-specific interventions in malaria control strategies [19, 20].

Moreover, the high concordance between microscopy and PCR results stresses the reliability of these methods in diagnosing malaria and assessing treatment outcomes. These findings indicate a rapid decline in parasitaemia following treatment initiation, with a substantial proportion of patients achieving PCR-confirmed parasite clearance by day 7. In addition, ACPR was observed in all the individuals after PCR-correction, implying sustained efficacy of ACT treatment of uncomplicated *P. falciparum* in the population. This high cure rate is consistent with reports from other West African populations [21–23].

Understanding the genetic diversity of circulating parasites is crucial for malaria control strategies, including vaccine development and the design of effective antimalarial interventions. The dominance of the K1 allelic family in *msp1* highlights its prevalence in the study population. The identification of various allelic combinations, such as K1/MAD20, K1/RO33, and MAD20/RO33, suggests a diverse array of co-infection patterns, with trimorphic infections further emphasizing the genetic heterogeneity present in the parasite population. Moreover, the *msp2* locus demonstrated considerable allelic diversity, with the detection of three FC27 and five 3D7 alleles. The high MOI provided addition insight into the complexity of malaria infections in the population. While no statistically significant correlation was found between age and MOI for *msp1* and *msp2* loci, the highest MOI observed in the 5–15 years age group suggests a potential age-related pattern in infection complexity. However, the absence of a significant correlation between MOI and parasitaemia emphasizes the complex dynamics of malaria transmission and suggests that factors beyond parasite density influence the multiplicity of infections. Further research exploring the relationship between genetic diversity, transmission intensity and clinical outcomes will be instrumental in guiding targeted malaria control efforts and advancing efforts towards malaria elimination.

Analysis of *mdr1* mutations, particularly N86Y and D1246Y, unveiled a striking predominance of the wild-type *mdr1* alleles. These findings underscore the persistence of wildtype *mdr1* alleles in the circulating parasite populations, potentially influencing the efficacy of anti-malarial drugs targeting these molecular pathways [24–26]. Through Sanger sequencing, a spectrum of genetic variations within the 304-bp region of interest was identified. Specifically, one isolate (1.6%) harboured a non-synonymous mutation. Furthermore, the analysis unveiled a diverse array of indels, with 11.5% causing frameshift mutations in the sequence reading frame. Of particular interest were the AAATATGAT deletions and insertions observed at specific amino acid residues, highlighting the dynamic nature of *ubp1* polymorphisms and their potential implications for parasite biology and drug response. Identification of E1528D, albeit at a low frequency, which has been linked with delayed parasite clearance [27], underscores the dynamic nature of drug resistance and the importance of continuous surveillance.

Conclusions

This study contributes crucial information to steer local malaria control initiatives, stressing the importance of continuous surveillance and research to tackle evolving challenges, especially those associated with drug resistance. The observation of therapeutic effectiveness and genetic diversity of the parasites offers valuable insights for the deployment of malaria prevention and control strategies within the population.

Abbreviations

ACPR	Adequate clinical and parasitological response
ACT	Artemisinin-based combination therapy
AL	Artemether-Lumefantrine
BWA	Burrows-Wheeler Aligner
ETF	Early treatment failure
LPF	Late parasitological failure
LTF	Late treatment failure
MDR1	Multidrug resistance gene 1
MOI	Multiplicity of infection
MSP	Merozoite surface protein
PCR	Polymerase chain reaction
RDT	Rapid diagnostic test
UBP1	Ubiquitin-specific protease 1
WHO	World Health Organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12936-024-05088-6.

Additional file 1

Additional file 2

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Author contributions
K.M.O conceived and designed the study. K.M.O, F.C.L, A.J.O, Y.M.A, O.O.A and M.J.O implemented field study. K.O, F.C.L, Y.M.A and B.E carried out laboratory experiments. K.M.O, F.C.L and A.J.O carried out data analysis. K.M.O and F.C.L drafted the manuscript. All authors revised and approved the final manuscript.

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Availability of data and materials
The dataset(s) supporting the conclusions of this article are within the manuscript and its supplementary information. UBP1 sequence files have been deposited in National Centre for Biotechnology Information (NCBI) https://dataview.ncbi.nlm.nih.gov/object/PRJNA1078983.

Declarations

Ethics approval and consent to participate
Ethical approval was obtained from the Institutional Review Board of the Nigerian Institute of Medical Research (IRB/21/017) in accordance with the Declaration of Helsinki [28]. Before enrollment, all the participants signed Informed consent forms.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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