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Prevalence and distribution of *Plasmodium falciparum* multidrug resistant 1 D1246Y allele among children in Ibadan Southwest, Nigeria

Osazuwa John Patrick[1,6]✉, Olukemi Kehinde Amodu[1], Adewale Allen Sokan-Adeaga[2], Micheal Ayodeji Sokan-Adeaga[3] & Yasuhiro Kotera[4,5]

The emergence and spread of the *Plasmodium falciparum* multidrug-resistant 1 (*Pf*mdr1) allele pose a significant setback to global efforts to control and eradicate malaria infection by diminishing the efficacy of commonly prescribed antimalarial drugs, particularly in Sub-Saharan Africa, where malaria remains endemic. The *Pf*mdr1 D1246Y mutation is of specific importance due to its potential role in modulating parasite susceptibility to antimalarial medicines and treatment outcomes. This study aimed to determine the presence and prevalence of the wild-type and mutant D1246Y alleles of *Pf*mdr1 among children in Ibadan, Southwest Nigeria. A total of 133 archived DNA samples collected between March 2016 and June 2021 from children aged 6 to 132 months with varying malaria phenotypes (asymptomatic infection, uncomplicated, and severe malaria) were analyzed. The *Pf*mdr1 D1246Y allele was amplified via nested PCR, and the mutation was detected using the restriction enzyme EcoRV. The digested nested PCR products were resolved on a 2% agarose gel and visualized under ultraviolet light. All statistical analyses were performed using SPSS version 25, and statistical significance was set at $p \leq 0.05$. Among the 133 samples, 97 (72.9%) were successfully genotyped. Of these, 50 (51.55%) carried the wild-type allele, while 47 (48.45%) had the mutant allele. Notably, the *Pf*mdr1-1246Y mutation was detected in all severe malaria cases (41/41, 100%), whereas its prevalence was significantly lower in asymptomatic (3/36, 8.3%) and uncomplicated malaria cases (3/20, 15%). The difference in mutation prevalence across the malaria phenotypes was statistically significant ($p < 0.05$). The study provided valuable insight into the coexistence of wild-type and mutant *Pf*mdr1 D1246Y alleles within the population. It revealed a significantly higher mutation rate in all severe malaria cases, while the wild-type allele remained more prevalent overall. These findings contribute to a deeper understanding of the possible role of the wild-type and mutant D1246Y alleles in the various clinical manifestations of malaria.

Keywords Malaria, Mutant, Wild-type, Multidrug resistance, *Plasmodium falciparum*, Pfmdr1-D1246Y allele

Malaria remains one of the most common vector-borne parasitic infections, inflicting substantial and long-lasting devastation on millions of lives throughout the globe since ancient times[1]. It is a significant public health issue and a growing threat to socioeconomic development, particularly in malaria-endemic countries, with children under the age of five and pregnant women being the most vulnerable groups[1,2]. It is caused by single-celled protozoan parasites of the genus *Plasmodium* and is mainly transmitted to humans through the bite of

[1]Institute of Child Health, Faculty of Public Health, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria. [2]Public Health Program, College of Health Sciences, Bowen University, Iwo, Nigeria. [3]Department of Community Health and Primary Care, Faculty of Clinical Sciences, College of Medicine, University of Lagos, Lagos, Nigeria. [4]Faculty of Medicine and Health Sciences, University of Nottingham, Nottingham NG7 2TU, UK. [5]Center for Infectious Disease Education and Research, Osaka University, Osaka 565-0871, Japan. [6]Department of Restorative Dentistry, Faculty of Dentistry, College of Medicine, University of Ibadan, Ibadan, Nigeria. ✉email: mercypoj@gmail.com

infected female Anopheles mosquitoes. At least five different species are known to cause malaria in humans, with *P. falciparum* being the most virulent causative agent[2,3].

Over the years, considerable progress has been made to reduce the global burden of malaria through various interventions, notably the use of effective antimalarial medicines. However, the development of resistance to almost all available antimalarial drugs poses a critical challenge to malaria control and eradication efforts[2,4,5]. The World Health Organization (WHO) defines antimalarial drug resistance as the ability of a parasite strain to survive and multiply despite the administration and absorption of antimalarial drugs at doses equal to or higher than those usually recommended[1].

Antimalarial drugs target different parts of the parasite's cell. Most of them affect the digestive food vacuoles (DVs) of *P. falciparum*, while others target other organelles such as the cytosol, apicoplast, mitochondria, and parasite membrane[6]. The intracellular distribution of antimalarial drugs is influenced by factors such as the solubility of the antimalarial drug, its ability to permeate the cell membrane, and its binding affinity to transporter proteins that regulate drug movement within the cells[6]. Access of antimalarial drugs to their specific target site is crucial for their action; however, *P. falciparum* has developed biological processes to prevent drug accumulation or access to the target site, thereby contributing to drug resistance[2,6,7]. The development of resistance to available antimalarial drugs is influenced by several factors. These include the parasite mutation rate, parasite density, efficacy of the antimalarial drug of choice, poor adherence to malaria treatment guidelines, presumptive use of antimalarial drugs without diagnostic confirmation, improper doses, poor pharmacokinetic properties, and counterfeit drugs[1].

P. falciparum possesses a number of transporter proteins, one of which is the *P. falciparum* multidrug-resistant 1 gene (*Pf*mdr1) located in the digestive food vacuole membrane of the parasite[6,7].*Pf*mdr1, first reported in 1989, encodes a transmembrane glycoprotein structurally homologous to the multidrug-resistant gene found in human tumor cell lines and certain yeast cells such as *Candida albican*[8–10]. *Pf*mdr1 is an adenosine triphosphate-binding cassette (ABC) protein located on Chromosome 5, consisting of one exon coding for P-glycoprotein homologue 1 with 1419 amino acids and 162.25 kDa[10,11]. They function as a group of energy-mediated carriers that translocate a wide range of structurally and functionally diverse substrates across extracellular and intracellular membranes, including metabolic products, amino acids, inorganic ions, metals, lipids, sterols, and drugs[6,7,12].

Genetic variations at specific amino acid positions (N86Y, Y184F, S1034C, N1042D, and D1246Y) of *Pf*mdr1 have been reported to alter the transport of different antimalarial drugs, such as lumefantrine andartemisinin, in or out of the target site of the parasite's digestive food vacuole, leading to sensitivity or resistance to antimalarial drugs[10,11]. Genetic variations such as single nucleotide polymorphisms (SNPs), copy number variations (CNVs), and mutations in *Pf*mdr1 alleles can lead to alterations in amino acid sequence, structural conformation, function, or quantity of proteins[10,11]. These changes can potentially diminish the efficacy of antimalarial drugs by reducing their binding affinity or disrupting the molecular transport system, thereby promoting parasite survival[4,5,7,11].

The mutation of the *Pf*mdr1 allele is a significant public health concern due to its potential role in antimalarial drug resistance and its impact on malaria treatment efficacy. The distribution and prevalence of the *Pf*mdr1 allele vary geographically due to different selective pressures on the parasite over time. Notably, the NH2 terminal mutations (N86Y and Y184F) are more common in Asian and African parasites, while COOH terminal mutations (S1034C, N1042D, and D1246Y) are predominantly found in South American parasites[6,13–15]. Despite being less common in African than in South American parasites, the D1246Y allele mutation has been reported in Nigeria, with documented prevalence rates of 3.66% in Southeast Nigeria[11], 1.93% in North-West Nigeria[14], 3.00% in North-East Nigeria[14], and 18.6% in Ogun State, Southwest Nigeria[16]. Moreover, there are limited data on the prevalence and distribution of wild-type and mutant *Pf*mdr1 D1246Y alleles, particularly in Ibadan, Southwestern Nigeria. The increasing resistance of *P. falciparum* to virtually all antimalarial drugs has intensified the malaria burden, presenting a persistent challenge to global efforts to control and eradicate malaria[17]. Understanding the genetic basis and allelic frequency of *the Pfmdr1* D1246Y allele in *P. falciparum* in this population is crucial for comprehending resistance mechanisms and conducting parasite surveillance and drug efficacy assessments in the population. Therefore, the aim of this study was to determine the presence and prevalence of the wild-type and mutant *Pf*mdr1 D1246Y alleles among children in Ibadan, Southwest Nigeria.

Materials and methods

Study area

The study was conducted in Ibadan, Oyo State, Southwest, Nigeria. Ibadan is predominantly an urban settlement and serves as the capital and most populous city of Oyo State, Nigeria with over 6 million people[18]. Situated in the southeastern part of Oyo State, it is approximately 119 km northeast of Lagos and 120 km east of the Nigerian border with the Republic of Benin. Ibadan has a tropical climate, characterized by two distinct seasons: the rainy (wet) season, spanning from.

March to October, and the dry season, covering November to February. The mean total annual rainfall is recorded at 1,420.06 mm, distributed over approximately 109 days per year. The maximum temperature reaches 26.46 °C, while the minimum temperature is 21 °C, and the relative humidity averages 74.55%. These climatic conditions create a favorable environment for mosquito breeding and malaria transmission. Malaria remains a major public health concern in Ibadan, with high transmission rates recorded throughout the year[18] The city lies within Nigeria's rainforest belt, where abundant rainfall and stagnant water bodies provide suitable breeding sites for *Anopheles* mosquitoes, the primary vectors of malaria[18]. The prevalence of malaria is significantly high, and studies have shown that over half of the population experiences at least one episode annually, while children under five years old suffer from an average of two to four episodes per year[18,19].

Study location

The study was conducted in the molecular laboratory at the Institute of Child Health, College of Medicine, University of Ibadan. The hospital is a foremost tertiary healthcare facility located in Ibadan, Southwest Nigeria, that provides medical care to diverse patients from across the entire country, especially the south-southwest. This hospital also provides training for undergraduate and postgraduate students.

Study design and study participants

The study was carried out using a descriptive cross-sectional study design. A total of 133 archived DNA samples collected between March 2016 and June 2021 from children aged 6 to 132 months with varying malaria phenotypes who presented at the clinic in the Institute of Child Health, College of Medicine, University of Ibadan, were used for the study. The study samples were classified into three distinct malaria phenotypes: asymptomatic (56), uncomplicated (36), and severe (41) malaria phenotypes, respectively. The criteria used were based on standard malaria testing and treatment guidelines[20]. Children confirmed microscopically of *P. falciparum* infection, but no apparent symptoms of malaria were classified as asymptomatic. Those with mild symptoms and confirmed *P. falciparum* infection, but without severe manifestations or organ dysfunction, were categorized as having uncomplicated malaria. Severe malaria was defined by the presence of *P. falciparum* in the blood alongside one or more of the following symptoms: impaired consciousness, generalized weakness, inability to feed, respiratory distress, or severe anemia with a packed cell volume (PCV) of less than 15%. Moreover, other data, such as the demographic and clinical characteristics of the study participants, were obtained from their medical records.

Sample size determination

The number of archived DNA samples analyzed in this study was determined using the Kish and Leslie sample size calculation formula for a cross-sectional study; $N = Z^2 (pq)/d^2$, where N represents the sample size, Z is the confidence level at 95% (standard value of 1.96), p is the prevalence rate (78%)[18] and d is the margin of error at 5%. The minimum sample size was calculated to be 264 participants. However, due to a limited number of samples that did not meet the inclusion criteria, the final sample size was reduced to 133.

Collection of blood sample

Sterile techniques and disposable, single-use materials were used at all times during the collection of the blood sample. The study participant was asked to sit and remain in a resting position for at least two minutes prior to sample collection. The skin over the area where the sample was to be collected was disinfected with 70% ethanol, and a tourniquet was tied around the arm. The phlebotomist palpated the arm to identify the vein. A 23-gauge hypodermic syringe was aseptically inserted into the vein, and the tourniquet was removed. The appropriate amount of five milliliters (5 ml) of whole blood was drawn into EDTA tubes by trained and licensed medical laboratory technologists. The puncture site was dressed with cotton and covered with a phlebotomy plaster.

DNA extraction

The archived DNA used for the study was extracted from dried blood spots using the Chelex method. A specific drop of blood from each participant who met the inclusion criteria was spotted onto the designated circular areas of Whatman 903 Protein Saver Cards, allowed to air-dry at room temperature, and formed a stable dried blood spot (DBS). The DBS samples were stored with a desiccant in a dry, cool environment until processing. A small cutting or punch was taken from the DBS and placed into a microcentrifuge tube. A 5% saponin solution prepared in 1× phosphate-buffered saline (PBS) was then added, and the sample was incubated at 4 °C overnight. After incubation, the samples were briefly centrifuged, and the saponin solution, along with cellular debris, was removed using a vacuum pump. The remaining pellet was washed twice with PBS to eliminate residual saponin, followed by another centrifugation step to remove the wash buffer. The samples were then resuspended in a 5% Chelex-100 resin solution prepared with nuclease-free water. The tube was heated to 99–100 °C for 15 min in a block heater to lyse cells, denature proteins, and inactivate nucleases. It was then vortexed for 30 s and centrifuged at 10,000 rpm for 2–3 min to separate the DNA-containing supernatant from the Chelex beads. The DNA-containing supernatant was aseptically and carefully transferred to a sterile, labeled tube using a pipette. The purified DNA extract was then stored at -20 °C until further analysis.

Amplification of Pfmdr1 (D1246Y) allele

The detection of the *Pf*mdr1 D1246Y allele was performed using a nested PCR method followed by restriction fragment length polymorphism (RFLP) according to an established protocol[16,21]. The amplification of the *Pf*mdr1 D1246Y allele involved two rounds of PCR. The initial round targeted the outer region of the gene, followed by a nested PCR using specific primers. The PCR primer sequences and thermocycling conditions used for amplifying the target sequence in *Pfmdr1* D1246Y allele are shown in Table 1.

Restriction digestion with EcoRV

For the PCR-RFLP methodology, the restriction enzyme, EcoRV was used, designed to cleave the DNA into 210 bp and 199 bp fragments in the presence of a mutation, resulting in a double band. Conversely, in the absence of a mutation (wild type), the DNA would remain uncut, producing a single band of 409b[16,21]. Laboratory adapted Genomic DNA of Dd2 strains of *Plasmodium falciparum* provided by Malaria Research and Reference Reagents Resources Centre (MR4, USA) was used as positive controls for wild and mutant types. The RFLP procedure was performed by adding 5 µl of restriction buffer, 4 µl of nuclease-free water, and 1 µl of the enzyme (EcoRV) to 10 µl of each of the nested PCR products, to achieve a final volume of 20 µl. The plate was properly capped and incubated at 37 °C overnight in an incubator (DNP 9022 A, SANFA) to facilitate normal digestion, adhering to the manufacturer's instructions (New England Biolabs). The digested nested PCR products were

Gene fragment	Sequence (5′ → 3′)	Direction	Cycling condition
Pfmdr1 D1246Y	CAG GAA GCA TTT TAT AAT ATG CAT	Forward	94 °C for 5 min, 40 cycles of 94 oC for 30s, 56 °C for 1 min and 72 °C for 1 min 30s, and a final 10 min extension step at 72 °C
	CGT TTA ACA TCT TCC AAT GTT GCA	Reverse	
	GACTTGAAAAATGATCACATT	Forward nested	94 °C for 5 min, 35 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min, A final 10 min extension step at 72 °C
	GTCCACCTGATAAGCTTTT	Reverse nested	

Table 1. PCR oligonucleotide primer sequences and thermocycling condition for target sequence in Pfmdr1 (D1246Y).

Parameter	Asymptomatic	Uncomplicated	Severe	*P* value
Number of participants	56	36	41	0.579
Gender (Frequency)				
Male	32(57.1%)	17(47.2%)	20(48.8%)	
Female	24(42.9%)	19(52.8%)	21(51.2%)	
Mean age (Months)	56.52 ± 4.10	45.90 ± 5.59	39.61 ± 4.19	0.024**
Mean packed cell volume (%)	32.52 ± 1.05	31.36 ± 0.75	18.37 ± 1.23	0.0001**
Mean temperature (°C)	36.80 ± 0.23	37.60 ± 0.13	38.11 ± 0.12	0.0001**
Geometric mean parasite density(SD)	97.59(1400.90)	71.01(2686.10)	1639.28 (66746.60)	0.001**
Mean white blood cell count	7082.14 ± 366.97	9594.83 ± 523.70	8739.02 ± 612.78	0.001**

Table 2. The demographic and clinical parameters among children in Ibadan, Southwest, Nigeria. **Significant result $p \leq 0.05$.

subsequently resolved on a 2% agarose gel containing 0.5 µg/mL ethidium bromide and visualized by the Gel Documentation System (Gel Doc TMEZ Imager, Bio-Raid, USA) to read the DNA band formed.

Data analysis

All statistical analyses were carried out using SPSS software, version 25. The means of the parasite density, PCV, WBC, age, and temperature among various malaria groups were compared using analysis of variance (ANOVA). An independent samples t-test was used to compare age (in months) between the wild-type and mutant D1246Y alleles. The distribution of gender and the frequency of wild-type and mutant *P. falciparum* multidrug-resistant 1 (D1246Y) alleles in the malaria groups were examined using the Pearson chi-square (χ^2) test. A p-value of 0.05 was considered statistically significant.

Ethical consideration

Ethical clearance was obtained from the Oyo State Ethical Review Committee at the Ministry of Health, with the assigned approval number: AD13/4347[B]. Permission was obtained from the Director of the Institute of Child Health, College of Medicine, University of Ibadan, where the study was carried out. Written informed consent was obtained from each child's parent or legal guardian before beginning the study. The study's objectives, methodology, anticipated benefits, and potential risk were thoroughly explained to the parents or guardian. The privacy and confidentiality of the data of the children collected were strictly adhered to. All methods were performed in accordance with Helsinki's relevant guidelines and regulations.

Results

Demographic and clinical parameters of participants

The demographic and clinical characteristics of the study population are presented in Table 2. The study comprised 133 children, with 69 (51.9%) males and 64 (48.1%) females. The study samples were grouped into asymptomatic 56 (42.1%), uncomplicated 36 (27.1%), and severe malaria 41 (30.8%), respectively. The age of the study population ranged from 6 to 132 months, with a mean age of 48.43 ± 2.69 months.

The geometric mean of parasite density was notably higher among severe cases compared to uncomplicated and asymptomatic groups. A statistically significant difference in parasitemia was observed across the groups ($P < 0.05$). Additionally, the mean packed cell volume, body temperature, age, parasite density, and white blood cell count exhibited significant variations across all malaria phenotypes ($P < 0.05$).However, there was no statistical significant difference in distribution of gender among the three malaria clinical phenotypes ($p = 0.579$).

The Chi-Square test results (Table 3) indicate that there was no statistically significant association between *Pf*mdr1 D1246Y allele (Wild-type vs. Mutant) and gender (Male vs. Female) in this sample ($p = 0.493$). Therefore, based on this analysis, it revealed that gender does not appear to influence the distribution of *Pf*mdr1D1246Y alleles in the population.

Of the 133 archived DNA samples randomly selected from the three malaria groups, 72.9% (97) were successfully genotyped. Among these, 51.55% (50) of the samples exhibited the wild-type allele of *Pf*mdr1-D1246, while 48.45% (47) possessed the mutant *Pf*mdr1-1246Y allele. The highest frequency of the *Pf*mdr1 1246Y

Pfmdr1 D1246Y	Male (n = 53)	Female (n = 44)	Total (n = 97)	P value
Wild-type	29 (54.7%)	21 (47.7%)	50 (51.5%)	0.493
Mutant	24 (45.3%)	23 (52.3%)	47 (48.5%)	
Total	53 (54.6%)	44 (45.4%)	97 (100%)	

Table 3. Distribution of *Pfmdr*1 D1246Y allele by gender among children in Ibadan, Southwest, Nigeria. Significant $p \leq 0.05$.

	Asymptomatic	Uncomplicated	Severe	Total	χ^2	P value
	%(N)	%(N)	%(N)	%(N)	109.925	0.0001**
Wild type	91.7%(33)	85%(17)	0%(0)	51.55%(50)		
Mutation	8.3%(3)	15%(3)	100%(41)	48.45%(47)		
Total	37.1%(36)	20.6%(20)	42.3%(41)	100%(97)		

Table 4. Frequency of D1246Y allele across the three malaria clinical phenotypes among children in Ibadan, Southwest, Nigeria. **Significant result $p \leq 0.05$. N = Number of *Pf*mdr1-D1246Y allele found across the malaria phenotypes. % = Percentage of total number of allele. χ^2 = Chi-square value.

Pfmdr1 D1246Y	N	Mean age (Months)	Std. deviation	p-value
Wild-type	50	55.64	32.41	0.004**
Mutant	47	38.02	25.75	

Table 5. Comparison of age (in months) between wild-type and mutant alleles. **Significant result $p \leq 0.05$.

mutation was observed in the severe malaria cases, accounting for 100% (41), whereas in the asymptomatic and uncomplicated groups, the frequencies were 8.3% (3) and 15% (3), respectively (Table 4).

The findings revealed a significant association between the *Pf*mdr1 D1246Y allele (wild-type and mutant D1246Y alleles) and the age of the children ($p = 0.004$), with the wild-type allele having a higher mean age (55.64 months) compared to the mutant allele (38.02 months) (Table 5).

Discussion

The increasing resistance of *P. falciparum* to nearly all available antimalarial drugs, particularly in malaria-endemic regions, presents a significant global health challenge. This resistance, driven by genetic mutations and the spread of multidrug-resistant strains, threatens malaria control and eradication efforts[4]. Understanding the genetic markers associated with drug resistance is crucial for the effective management of malaria infections. This present study investigated the prevalence of wild-type and mutant *P. falciparum* multi-drug resistant 1 (*Pf*mdr1) alleles (D1246Y) across different malaria phenotypes among children in Ibadan, Southwest Nigeria. It also assessed the potential role of this mutation and its correlation with demographic parameters in the different clinical malaria phenotypes.

The distribution of gender among the three malaria clinical phenotypes did not show a statistically significant difference ($p = 0.579$) (Table 2), suggesting that sex was not a determinant of malaria severity in this population. This is in line with other studies which reported that gender is not a major risk factor in malaria pathogenesis[22,23]. However, a significant difference was observed in the mean age of participants across the clinical categories ($p = 0.024$). Children with severe malaria were younger (39.61 ± 4.19 months) compared to those with uncomplicated (45.90 ± 5.59 months) and asymptomatic malaria (56.52 ± 4.10 months).This finding suggests that younger children are more susceptible to severe malaria due to their relatively immature immune systems and reduced prior exposure to malaria infection, which may lead to lower acquired immunity. Our results are consistent with the previous studies conducted in Mali, which reported that the average age of a child with any form of severe malaria ranges between 21 and 39 months[24].

Similarly, significant differences were also found in key haematological and physiological parameters across the asymptomatic, uncomplicated, and severe malaria phenotypes. The mean packed cell volume (PCV) showed a distinct decline in the severe malaria phenotype (18.37 ± 1.23%) compared to the uncomplicated (31.36 ± 0.75%) and asymptomatic phenotypes (32.52 ± 1.05%) ($p < 0.0001$) (Table 2). This result substantiates the well-established correlation between severe malaria and haemolytic anaemia, a significant contributor to malaria morbidity and mortality[25,26]. Additionally, the mean body temperature was significantly elevated in the severe malaria cases (38.11 ± 0.12 °C), compared to the uncomplicated (37.60 ± 0.13 °C) and asymptomatic malaria phenotypes (36.80 ± 0.23 °C) ($p < 0.0001$), which further confirmed the role of fever as a substantial clinical symptom of malaria severity. The elevated white blood cell (WBC) count in the uncomplicated (9594.83 ± 523.70) and severe cases (8739.02 ± 612.78) compared to the asymptomatic group (7082.14 ± 366.97) suggests a continuous immune response to the rapid genomic replication of the parasite during the clinical disease.

Geometric mean (SD) of the parasite density was significantly different across the three malaria phenotypes (p = 0.001), with the highest parasite burden found in children with severe malaria 1639.28 (66746.60), followed by the uncomplicated 71.01 (2686.10) and asymptomatic groups 97.59 (1400.90) (Table 2). This finding is indicative of a direct relationship between parasite density and the clinical outcome of malaria infection, and this corroborates previous reports that higher parasitaemia is a significant predictor of severe malaria outcomes[1,27]. The significantly high variance observed in the severe malaria cases may reflect differences in host immune responses and genetic factors that influence parasite clearance[27–30].

The analysis of the distribution of the *Pf*mdr1 D1246Y allele revealed no significant gender-based differences (p = 0.493) (Table 3), indicating that the allele is evenly distributed among male and female children. However, a strong correlation was observed between the prevalence of the wild-type and mutant D1246Y alleles and the severity of malaria (p = 0.0001) (Table 4). The overall frequency of the wild-type and mutant D1246Y alleles for *Pf*mdr1 was 51.55% and 48.45%, respectively (Table 4). The frequency of the 1246Y mutation (48.45%) observed in this study was significantly higher than previously reported findings in Southeast Nigeria (3.66%)[11], Yobe State, Northeast Nigeria (5.26%)[14] and Ogun State, Southwest Nigeria (18.6%)[16]. This finding also buttresses previous observations from other scholars that the distribution of *Pf*mdr1 alleles' mutations is inconsistent and varies from one region to another, possibly due to different unfavorable environmental and biological conditions or selective drug pressure that each strain of the parasite had been exposed to over time[6,13,14]. The wild-type allele was predominant in asymptomatic (91.7%) and uncomplicated malaria cases (85%), whereas the mutant 1246Y allele was mainly associated with severe malaria (100%) (Table 4). This association strongly suggests that the D1246Y mutation may contribute to malaria severity, potentially through its impact on parasite resistance mechanisms to anti-malarial drugs.

A significant association was also observed between age and the prevalence of the D1246Y allele (p = 0.004) (Table 5). Children harboring the wild-type allele had a higher mean age (55.64 months) compared to those with the mutant allele (38.02 months) (Table 5). This finding implies that younger children are more likely to harbor the resistant allele, which could be due to selective pressures imposed by the frequent use of antimalarial medicines in this population. Younger children, having had less repeated exposure to malaria and potentially weaker immunity may be more susceptible to infections caused by resistant mutant strains, leading to more severe disease outcomes. The presence of the *Pf*mdr1 D1246Y mutation in all the severe malaria cases has significant implications for malaria treatment and control strategies. This mutation has been associated with resistance to several frontline anti-malarial drugs, including lumefantrine and amodiaquine, and may contribute to reduced efficacy of artemisinin-based combination therapies (ACTs)[14,15]. Continuous surveillance of *Pf*mdr1 mutations is therefore critical in monitoring emerging drug resistance patterns and informing treatment policies.

In addition, the higher prevalence of the mutation in younger children stresses the need for targeted interventions in this age group, such as improved access to preventive therapies, enhanced vector control measures, and the reinforcement of appropriate anti-malarial drug use to mitigate the selective pressure for resistant strains. Our findings from this study provide significant insights into the prevalence and distribution of *Pf*mdr1 D1246Y allele among children in Ibadan, Southwest Nigeria, and its association with malaria severity. The results indicate that children under the age of five are at a higher risk of severe malaria, and the findings align with previous studies[1,2,18,24].

Furthermore, the presence of the mutant 1246Y allele in all severe cases underscores its potential role in malaria pathogenesis and resistance mechanisms. These findings point out the urgency of continuous molecular surveillance and targeted interventions to reduce the impact of drug-resistant malaria strains in this population studied and other malaria endemic regions.

Limitation of the study

While this study offered novel insights, several limitations need to be noted. First, due to the limited number of archived DNA samples meeting the inclusion criteria, the sample size for the study was reduced to 133 (N = 133), which is smaller than initially calculated. Second, the required DNA kits and reagents for the molecular techniques were not readily available locally, necessitating importation and causing delays in the study's commencement. Third, as this is a cross-sectional study, causality of the studied variables was not assessed[31]. Moreover, possible seasonal effects were not eliminated[32]. Additionally, we acknowledge that the gel electrophoresis results presented in our manuscript are not of optimal quality. However, we have taken steps to optimize the experiment and improve the resolution of the bands. Unfortunately, due to possible DNA degradation, limited DNA quantity, inadequate gel electrophoresis conditions, limited resolution of the gel, and instrumentation limitations, we were unable to obtain clearer results. We believe that the results presented are still valid and support our conclusions, but we understand the importance of high-quality data and will strive to improve our experimental techniques in future studies. The outcomes of the nested PCR and digested PCR products are provided in Supplementary Information S1, S2 and S3, respectively. Lastly, sequencing of the *P. falciparum* multidrug resistance gene (D1246Y) was not conducted.

Conclusions

The findings provided valuable insight into the intricate dynamics of malaria in this population, highlighting the critical need for comprehensive strategies to understand and combat the disease. The coexistence of wild-type and mutant *Pf*mdr1 D1246Y alleles, particularly the significantly higher mutation rate observed in severe malaria cases, underscores the importance of genetic monitoring in informing treatment approaches. In light of the findings from this study, further studies with a larger sample size should be conducted. Also, to enhance the efficacy of malaria management and control efforts, there should be regular surveillance of molecular markers of D1246Y and other *Pf*mdr1 alleles associated with antimalarial drug resistance in this population and other

regions of the country. This continuous assessment will provide valuable insights into the evolving dynamic of drug resistance, promoting more effective public health interventions.

Data availability

The dataset presented in the study is available on request from the corresponding author during submission or after publication. The data are not publicly available due to confidentiality and ethical purpose.

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Declarations

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to O.J.P.

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