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Original Article

Prevalence of *Plasmodium falciparum* parasitemia and Pfk13 gene detection among patients attending selected health facilities in Ota Metropolis, Ogun State, Nigeria

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Abstract

Introduction: Malaria remains a major public health concern in Africa, driven largely by mutations in the *kelch13* (PfKelch13) gene. This study assessed the demographic characteristics of age and gender in relation to the presence of the PfKelch13 gene among patients infected with *P. falciparum* at selected health facilities in Ota metropolis of Ogun State, Nigeria.

Methodology: Demographic information from 479 patients with suspected severe *P. falciparum* malaria was analyzed. Blood samples were examined microscopically for infective stages and parasitemia levels, while PfKelch13 was genotyped with primary and nested PCR.

Results: Out of the 479 participants, 265 (55.33%) tested positive for *P. falciparum*. The highest prevalence (33.61%) occurred in the 11–20-year age group, and males accounted for 29.44% of infections ($p < 0.05$). Higher parasitemia levels (> 200 parasites/100 μL) were frequent among males aged 0–10 and 11–20 years. The PfKelch13 gene was successfully amplified by PCR in 46.7% of the selected samples, with a slightly higher proportion among males (53.6%).

Conclusions: Age and gender affected the prevalence of *P. falciparum* parasitemia in the study population. However, no statistically significant association was observed between age and PfKelch13 positivity. There is a need for population-centered genomic surveillance, targeted public health interventions, and supportive diagnostic and therapeutic strategies to limit the spread of potential artemisinin-resistant strains.

Key words: *Plasmodium falciparum*; malaria; parasite; prevalence; PfKelch13.

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Introduction

Malaria remains one of the greatest threats to public health because of its high morbidity and mortality rates, particularly in Africa [1]. Despite the implementation of various preventive measures and therapeutic interventions, malaria continues to be a devastating public health problem and significantly affects socioeconomic development in many regions. In low-income settings, where malaria remains endemic in approximately 100 countries, more than 50% of the population is at risk of malaria infection [1]. In 2018, it is estimated that the cases of malaria have reached 228 million, with 93 percent of cases in the WHO-African Region [2]. About 405,000 malaria deaths were also reported in the world in the same year, with 67,000 among the under-five-year-olds [2]. Malaria is one of the major health challenges in the world, particularly in Asia and sub-Saharan Africa, where the disease affects 263 million individuals in 2023 and caused 597,000 deaths [3]. Important control interventions did not reduce prevalence rates or mortality, which increased by 4.1% and 8.1% in 2015 and 2023, respectively [3].

Poor access to healthcare, poverty, the lack of mosquito control, and low application of preventive measures (especially for pregnant women and children under five) contribute to high infection rates [4]. In 2024, Nigeria accounted for about 27% of all global malaria cases and approximately 31% of worldwide malaria deaths, according to the latest WHO estimates [3]. Despite considerable progress achieved through various intervention programs [5], the country continues to lag behind its national goal of malaria elimination by 2050 [6].

One of the greatest challenges to global malaria control is the emergence of artemisinin resistance, which is associated with mutations in the *Plasmodium falciparum* Kelch 13 (*PfK13*) gene. These mutations were first identified in Southeast Asia and have been

linked to delayed parasite clearance and reduced treatment efficacy [7]. Globally, more than 260 different PfK13 mutations have already been reported [8]. Although artemisinin-based combination therapies remain largely effective in sub-Saharan Africa, the detection of candidate and validated *PfK13* resistance-associated polymorphisms in several African countries highlights the urgent need for continuous molecular surveillance [9]. Despite Nigeria bearing one of the highest malaria burdens globally, there remains limited information regarding the distribution and prevalence of *PfK13* molecular markers associated with artemisinin resistance. This knowledge gap limits understanding of the distribution of *PfK13* polymorphisms, their association with parasite density, and their relationship with demographic characteristics within local populations. Addressing this gap is essential to inform treatment policies, strengthen surveillance systems, and prevent the spread of resistant parasite strains. Therefore, genomic surveillance of *P. falciparum* isolates remains critical for monitoring the prevalence of *PfK13* polymorphisms and detecting early signs of artemisinin tolerance in Nigeria. This study investigates the prevalence of *Plasmodium falciparum* infection and detection of the *Pfk13* gene, and their associations with parasitemia and demographic factors among patients attending selected health facilities in Ota Metropolis, Ogun State, Nigeria.

Methodology

Ethical Approval

The study received approval from the Covenant Health Research Ethics Committee (CHREC/432/2024) to obtain data from patients' records, and the identity of each patient remains anonymous, with confidentiality maintained given the intricacy and sensitivity of the study.

Sampling

A total of 479 patients attending both inpatient and outpatient clinics with confirmed malaria infection were recruited for this study using a random cluster sampling method, stratified by infection severity and demographic characteristics. Five milliliters of blood samples were collected from smear-confirmed *Plasmodium falciparum*-infected patients attending major health facilities in Ota, Ogun State, including General Hospital and Covenant University Medical Centre, Ota, Ogun State, Nigeria. Blood samples were transported under cold chain conditions and stored at 2–8 °C before analysis. Eligibility was determined based on standard clinical intake procedures and malaria diagnosis at the health facilities. Individuals with known chronic diseases other than malaria were excluded by the facility clinicians prior to sample collection. Only consenting participants whose routine clinical samples were made available were included in the study.

Microscopic examination and parasitemia determination

On a clean microscope slide, 2 µL of blood was spread to form a thin film and allowed to air-dry, while 6 µL of blood was used to prepare a thick film. The thin-film portion was fixed with methanol and then allowed to dry. Both thin and thick films were stained with a prepared 3% Giemsa stain, rinsed with phosphate-buffered water at pH 7.2, and allowed to air-dry. Each Giemsa-stained slide was examined under the light microscope at x100 oil immersion. The percentage of red blood cells containing malaria parasites was used to assess parasitemia [10], as described below.

Parasitemia (%) = (Total infected red blood cells/ Total red blood cells) × 100

PfK13 genotyping

Genotyping of the *pfk13* gene was conducted on the 479 collected blood samples. In accordance with Nigerian national treatment guidelines designating artemisinin-based combination therapies (ACTs) as the first-line treatment for uncomplicated malaria, this study evaluates the baseline prevalence of *pfk13* polymorphisms within the population seeking care at these selected clinical sites in Ota. Genomic DNA was extracted from *Plasmodium falciparum*-positive blood samples following the TE buffer and proteinase K-based technique, as described by Seesui *et al* [11]. Briefly, 100 µL of EDTA-anticoagulated whole blood was added to 1 mL of sterile distilled water and then vortexed vigorously. The mixture was incubated at 40 °C for 10 minutes, centrifuged at 2,400 rpm/min, and the supernatant was discarded. The resulting pellet was

Table 1. Primer sequence of the Kelch 13 gene.

Primer	PCR	Duration	Sequence (5'→3')	Reference
Kelch 13	Primary	Forward	CGGAGTGACCAAATCTGGGA	[11]
		Reverse	GGGAATCTGGTGGTAACAGC	
	Nested	Forward	GCCAAGCTGCCATTCATTTG	
		Reverse	GCCTTGTTGAAAGAAGCAGA	

resuspended in 10 µL of proteinase K solution and 40 µL of TE buffer. The solution was incubated at 56°C for 1 hour, with intermittent mixing. Following proteinase K inactivation, the solution was centrifuged, the supernatant was removed, measured with a NanoDrop 2000/2000c (Thermo Fisher Scientific Inc., USA), and stored at -20 °C. Amplification of the k13-propeller gene was performed using the nested polymerase chain reaction, and the primer sequences are listed in Table 1. The primary PCR amplification was performed in 7 µL of DNA template, 0.5 µL each of the *PfK13*-specific forward and reverse primers, 12.5 µL of 2X Easy Taq PCR Super Mix, and 4.5 µL of double-distilled water (ddH_2O), for a total reaction volume of 25 µL. Primary PCR amplification was initiated with an initial denaturation step at 95 °C for 5 minutes, followed by 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 49 °C for 2 minutes, and extension at 72 °C for 2 minutes. A final extension step was performed at 72 °C for 10 minutes, followed by a hold at 4 °C. The nested PCR reaction mixture consisted of 7 µL of the primary PCR amplicon, 0.5 µL each of the *PfK13*-specific forward and reverse primers, 12.5 µL of 2× Easy Taq PCR Super Mix, and 4.5 µL of double-distilled water (ddH_2O), resulting in a final reaction volume of 25 µL. Nested PCR amplification was initiated with an initial denaturation step at 95 °C for 5 minutes, followed by 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 47 °C for 1 minute, and extension at 72 °C for 1 minute. A final extension was performed at 72 °C for 10 minutes, followed by a hold at 4 °C.

Data Analysis

Data obtained from demographic questionnaires, microscopy, parasitemia assessment, and *PfK13* genotyping were analyzed using GraphPad Prism version 9 and SPSS version 26. The demographic features, prevalence of malaria, parasitemia level, and PfKelch13 detection rate were summarized by descriptive statistics. The Chi-square (χ^2) test was used to compare categorical variables, including the prevalence of malaria by age group and gender, with a $p < 0.05$ considered significant. The association between parasitemia levels and age was evaluated using Pearson's correlation coefficient, and scatterplots were generated to visualize the relationship. Differences in mean parasitemia between genders were assessed using an independent-samples *t*-test.

Results

Age and gender demography of P. falciparum prevalence

Out of the 479 blood samples obtained from malaria-suspected patients, 55.33.% (265/479) were *Plasmodium falciparum* positive (Figure 1). Based on the gender demography of the *Plasmodium falciparum*-infected patients (n = 265), male subjects had a significantly higher malaria prevalence of 29.44% compared to *Plasmodium falciparum*-infected female subjects ($p = 0.034$) (Table 2). Age-based analysis revealed a significantly higher prevalence of *Plasmodium falciparum* infection among individuals aged 11–20 years (33.61%), followed by those aged 21–30 years (7.31%) and 0–10 years (6.47%) ($p < 0.001$; Table 3).

Plasmodium falciparum parasitemia across age groups and gender

The relationship between *Plasmodium falciparum* parasitemia level and the age distribution of male-infected populations was estimated. Scatterplot analysis showed higher parasitemia levels among younger male participants, suggesting an inverse relationship between

Table 2. Gender demography in *P. falciparum* prevalence rate.

Gender	*P. falciparum* prevalence		χ^2	*p*
	Negative, n (%)	Positive, n (%)		
F	121 (25.26)	124 (25.89)		
M	93 (19.41)	141 (29.44)	4.50	0.034
Total	214 (44.67)	265 (55.333)		

Table 3. Age distribution in *P. falciparum* prevalence rate.

Age Group (Years)	*P. falciparum* prevalence		χ^2	*p*
	Negative, n (%)	Positive, n (%)		
0–10	42 (8.77)	31 (6.47)		
11–20	68 (14.20)	161 (33.61)		
21–30	21 (4.38)	35 (7.31)		
31–40	29 (6.05)	12 (2.51)	56.77	< 0.0001
41–50	20 (4.18)	14 (2.92)		
Above 50	34 (7.09)	12 (2.51)		
Total	**214 (44.67)**	**265 (55.33)**		

age and parasitemia (Figure 2A). Similarly, among female participants, parasitemia levels tended to increase with decreasing age, although the observed linear relationship was weak (Figure 2B). Comparative analysis of parasitemia distribution by age and gender among *P. falciparum*-infected participants further revealed higher parasitemia levels in males than in females. A noticeable increase in parasitemia levels was observed among both males and females within the 11–20-year age group, whereas relatively lower and more consistent parasitemia levels were observed in the older age groups (Figure 2C).

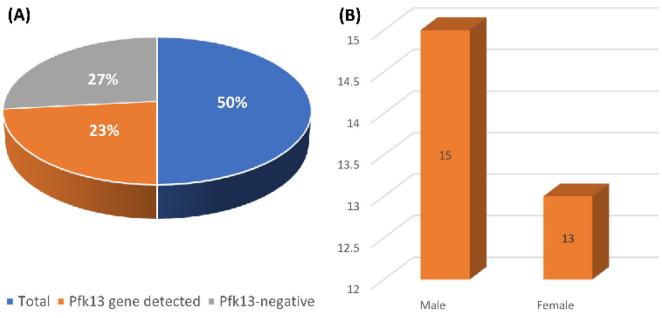
Population PfK13 occurrence

Among the malaria-infected participants screened for *PfK13*, the gene was detected in 23% (28/60) of the samples analyzed (Figure 3A). A higher detection rate was observed among male participants (53.6%) compared with female participants (Figure 3B). Age-based distribution showed that *PfK13* detection was highest among participants aged 11–20 years (26.66%), followed by those aged 31–40 years (8.33%) and 0–10 years (6.67%). A detection rate of 1.67% was observed in the remaining age groups (Table 4).

Discussion

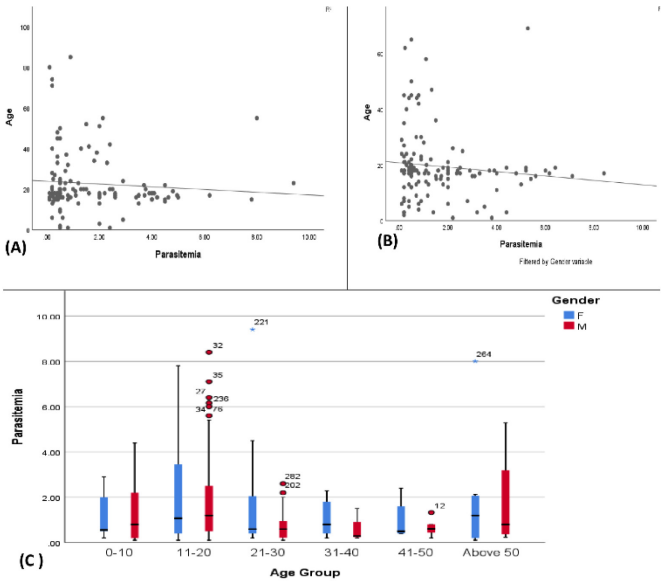
Over the years, significant progress has been made in reducing the global burden of malaria; however, transmission remains high across sub-Saharan Africa,

Figure 3. *PfK13* genotype for gender.



with evidence suggesting a recent resurgence in several settings [10]. Continuous monitoring of molecular

Figure 2A. Simple Scatter with Fit Line of Age by Parasitemia-Female; **B.** Simple Scatter with Fit Line of Age by Parasitemia-Male; **C.** Clustered Boxplot of Parasitemia by Age Group by Gender.



markers, particularly mutations in the *Plasmodium falciparum* Kelch 13 (*PfK13*) gene, is essential for detecting and tracking artemisinin resistance in malaria-endemic regions [12]. Such surveillance is critical for understanding the emergence and spread of resistance in endemic populations

This study reported a high prevalence (55.33%) of malaria infection in Ota, Ogun State, and confirmed the presence of the *PfK13* gene in local *P. falciparum* isolates, indicating the need for sustained molecular surveillance. The high malaria prevalence observed in this study is consistent with previous reports, which identified the area as a high-burden setting for malaria among patients attending health facilities [13,14]. The studies found that *Plasmodium falciparum* served as the primary malaria species in that region. Collectively, the findings confirm Ota as an active malaria transmission hotspot, in line with reports from other parts of Nigeria [14–17].

The gender disparities reveal that the malaria prevalence in males and the parasite level (parasitemia)

Table 4. Distribution of *P. falciparum* k13 among different age groups.

Age Group (Years)	Pfk13		χ^2	*p*
	Negative, n (%)	Positive, n (%)		
0–10	3 (5.0)	4 (6.67)		
11–20	18 (30.0)	16 (26.66)		
21–30	7 (11.67)	1 (1.67)		
31–40	1 (1.67)	5 (8.33)	7.53	0.184
41–50	1 (1.67)	1 (1.67)		
Above 50	2 (3.33)	1 (1.67)		
Total	32 (53.33)	28 (46.67)		

were higher when compared to females. This has also been the case with other African communities where malaria is common [18–20]. This difference could be attributed to increased occupational or outdoor exposure in the evening among males and the lack of adherence to preventive measures. It is known that females have hormonal and genetic factors that boost immune responses [21]. It has also been suggested that females may clear asymptomatic infections more efficiently, leading to lower observed parasitemia [18].

The higher malaria prevalence observed among individuals aged 11–20 years in this study is consistent with findings from Ado-Odo/Ota, Ogun State, where age groups below 20 years were significantly associated with malaria infection [17]. School-age children and adolescents in endemic African settings serve as a crucial reservoir for malaria transmission, according to research evidence [22]. This finding also corroborates Lalloo *et al.* [23] report regarding the neglect of adolescents by malaria control programs, primarily focused on children less than five years old and pregnant mothers. The observed inverse relationship between parasitemia and age may reflect the gradual development of partial immunity following repeated exposure. Younger individuals, particularly those transitioning from childhood to adulthood, may therefore experience higher parasite densities [24].

Molecular analyses demonstrated successful amplification of the *PfK13* gene across the population, confirming its presence in local isolates of *P. falciparum*. Although sequencing was not performed to identify specific mutations, these findings provide a baseline for future studies aimed at characterizing *PfK13* polymorphisms and assessing potential artemisinin resistance. In addition, males were found to have a slightly higher percentage of PfK13-positive isolates than females, consistent with the greater parasitemia present in males. This finding is similar to the report by Afolabi *et al.* [25], who observed a high prevalence of *PfK13* in males compared to females. Individuals aged 11–20 years recorded the highest Pfk13 positivity rate. However, the differences across age groups were not statistically significant. Larger sample sizes may give more valuable information concerning the age distribution of *PfK13* gene detection. Furthermore, the information presented in this study will toinform future strategies for malaria control, both regionally and nationally, in mitigate the effects of developing drug resistance, thereby helping eliminate the burden of malaria across the globe.

The continued presence of high levels of malaria is a clear indicator that there are still a large number of people who are vulnerable to the malaria parasite, and thus, there is an urgent need to focus on population-based implementation and improvement of the current malaria control programs. The rising number of adolescents with malaria infection and high parasitemia suggests an important shortcoming in malaria control initiatives that currently focus on young children and pregnant women. Since adolescents serve as reservoirs for the transmission of malaria parasites, parasites may continue to be transmitted from these individuals with high-level morbidity [24]. The relatively low detection rate of PfK13 in this study does not eliminate the possibility of emerging artemisinin resistance but rather emphasizes the importance of continuous molecular surveillance. Early detection of resistance-associated markers is critical to preserving the efficacy of artemisinin-based combination therapies, which remain the cornerstone of malaria treatment [26]. Strengthening routine monitoring of PfK13 polymorphisms will therefore be vital for informing national malaria treatment policies and preventing the spread of resistant parasite strains. The increased incidence of malaria in males compared to females indicates the need for gender-oriented education, behaviour change promotion, and better access to guidelines to prevent malaria.

Future Perspectives

This study highlights important opportunities for improving malaria control, particularly among adolescents and young adults, who are disproportionately affected by high malaria prevalence and elevated parasitemia levels. The evidence indicates that health education campaigns among adolescents and young adults should be carried out through school and community-based programs, such as the provision of insecticide-treated nets (ITNs) to curb infection rates and the institution of regular screening of children and youth at risk. Furthermore, regular molecular surveillance of PfK13 mutations and other emerging antimalarial resistance markers is essential for early detection of potential artemisinin resistance. Strengthening surveillance systems will support the timely identification of drug-resistant parasite strains and inform evidence-based updates to national malaria treatment guidelines.

Conclusions

The research study has provided evidence of a high prevalence of *Plasmodium falciparum* infection among patients attending selected health facilities in Ota Metropolis, Ogun State, Nigeria. Distribution of

infections and parasitemia was also different in the demographic groups, with the greater burden being recorded among the young people and males. The *Pfk13* gene was detected in a significant percentage of the samples, but there was no significant difference between age and Pfk13 positivity. These results emphasize the role of demographics in malaria transmission dynamics and the necessity to further monitor antimalarial resistance markers with the help of molecular surveillance and enhance the targeted control strategies within the study area.

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Conflict of interest

No conflict of interest is declared.

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