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Research Article

Prevalence of *Plasmodium falciparum* Infection and Anti-malarial Drug-resistance Genes in Individuals with Sickle Cell Disease in Jigawa State, Nigeria

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ABSTRACT
Malaria remains a significant cause of morbidity among individuals with sickle cell disease (SCD), despite routine antimalarial prophylaxis. This study assessed the prevalence of *Plasmodium falciparum* infection and the distribution of antimalarial drug-resistance genes among homozygous SCD (HbSS) patients attending Federal Medical Centre, Birnin Kudu, Jigawa State, Nigeria. A total of 186 HbSS individuals were enrolled. Malaria infection was diagnosed using rapid diagnostic tests and confirmed by microscopy, while parasite density was determined from thick blood smears. Molecular detection of resistance-associated genes (*Pfmdr1, Pfk13, Pfdhps, Pfdhfr,* and *Pfcrt*) was performed using multiplex PCR. Overall malaria prevalence was 38.7%, with a geometric mean parasite density of 15,832 parasites/µL. Infection was most frequent among children aged 6–10 years, and no significant association was observed with sex or age. Despite high prophylaxis coverage, *Pfdhps* (66.6%) was the most prevalent resistance gene, while *Pfcrt* (6.9%) showed the lowest frequency. These findings highlight persistent malaria transmission and circulation of resistance genes among SCD patients, underscoring the need for strengthened molecular surveillance and targeted malaria control strategies among this vulnerable population.

Keywords: Malaria; Microscopy; PCR; Prevalence; Resistance; Sickle cell disease

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INTRODUCTION

Malaria remains a major public health concern and continues to impose a substantial disease burden globally, particularly in sub-Saharan Africa (WHO, 2016; Badru, 2017). The disease is caused by protozoan parasites of the genus *Plasmodium* and is transmitted through the bite of infected female *Anopheles* mosquitoes (Nguetse *et al*., 2017; Mwaiswelo *et al*., 2020). Among the five *Plasmodium* species known to infect humans, *Plasmodium falciparum* is the most virulent and accounts for the vast majority of malaria-related morbidity and mortality in Africa (Coban, 2020; Abossie *et al*., 2022). Its high transmission efficiency, capacity for severe disease, and growing resistance to antimalarial drugs pose significant challenges to malaria control efforts.

Certain population groups are disproportionately affected by malaria due to compromised or immature immune systems (Amaratunga *et al*., 2012; Abhulimhen-Iyoha *et al*., 2015; Mbako *et al*., 2017; Gupta *et al*., 2018). These include young children, pregnant women, individuals living with HIV/AIDS, and patients with sickle cell disease (WHO, 2022).

Sickle cell disease is an inherited heamoglobinopathy caused by a mutation in the β-globin gene, resulting in the production of abnormal heamoglobin S. This leads to chronic heamolytic anemia, vaso-occlusive crises, and progressive organ damage (Brousse *et al*., 2014; Uyoga *et al*., 2019; Abdullahi *et al*., 2021). Functional asplenia, which commonly develops in individuals with SCD, significantly impairs immune responses and increases susceptibility to infections, including malaria.

In malaria-endemic regions, malaria infection in individuals with SCD is associated with severe complications such as profound anemia, increased frequency of crises, hospitalization, and death, particularly among children (Eleonore *et al*., 2020; Abdullahi *et al*., 2021). Although routine antimalarial chemoprophylaxis is recommended for SCD patients, malaria infection continues to occur, suggesting possible gaps in prophylactic efficacy, adherence, or the emergence of drug-resistant parasite strains (MGEN, 2014; Nsanzabana, 2019).

The widespread and prolonged use of antimalarial drugs, including artemisinin-based combination therapies and antifolate drugs such as sulfadoxine-pyrimethamine, has led to the selection and spread of *P. falciparum* strains harbouring genetic mutations associated with drug resistance (Menard and Dondorp, 2017). Molecular markers such as *Pfmdr1*, *Pfcrt*, *Pfdhfr*, *Pfdhps*, and *Pfk13* are widely used to monitor resistance patterns and guide malaria treatment policies (Archer *et al*., 2018; Okoro and Jamiu, 2018).

Despite the high burden of malaria and SCD in northern Nigeria, data on genomic surveillance and distribution of antimalarial resistance genes among individuals with SCD in Jigawa State remains limited. This study therefore assessed the prevalence of *Plasmodium falciparum* infection among homozygous SCD patients attending Federal Medical Centre Birnin Kudu, Jigawa State, and characterized the distribution of selected molecular markers associated with antimalarial drug resistance.

MATERIALS AND METHODS

Study Design and Location

This hospital-based cross-sectional study was conducted at the sickle cell clinic of Federal Medical Centre Birnin Kudu, Jigawa State, located in north-western Nigeria. The region experiences seasonal malaria transmission, with peak transmission occurring during the rainy season (Mustapha and Salisu, 2018).

Ethical Considerations

Ethical approval for the study was obtained from the Human Research Ethics Committee (HREC) Jigawa State Ministry of Health. Written informed consent was obtained from adult participants, while assent and parental consent were secured for minors prior to sample collection.

Study Population

The study population comprised individuals diagnosed with homozygous sickle cell disease (HbSS) who were attending routine clinical follow-up at the study site. Participants of both sexes and all age groups were eligible for inclusion.

Inclusion and Exclusion Criteria

Only confirmed HbSS patients who consented to participate were enrolled. Individuals with known HIV infection, renal failure, severe malnutrition, or those discharged against medical advice were excluded from the study.

Sample Size and Sampling Technique

A total of 186 HbSS individuals were recruited using a convenience sampling approach over a four-month period (August–November 2024), corresponding to the high malaria transmission season.

Data Collection

Sociodemographic data including age and sex were collected using interviewer-administered structured questionnaires.

Blood Sample Collection

Approximately, 2 mL of venous blood was collected aseptically into EDTA tubes by trained healthcare personnel. The samples were used for heamoglobin phenotype confirmation, malaria diagnosis, and molecular analysis.

Heamoglobin Phenotype Determination

Heamoglobin electrophoresis was performed using cellulose acetate membranes at alkaline pH to confirm HbSS status following standard laboratory procedures (Egsie *et al*., 2008; Abhulimhen-Iyoha *et al*., 2015).

Malaria Diagnosis

Malaria infection was initially screened using an HRP-2 based malaria rapid diagnostic test (MRDT). Positive results were confirmed by light microscopy using Giemsa-stained thick and thin blood smears. Parasite density was estimated by counting parasites per 200 white blood cells and expressed as parasites per microliter of blood (Cheesbrough, 2009; Chibuta and Acar, 2020; Orimadegun *et al*., 2023).

DNA Extraction and PCR Analysis

Genomic DNA was extracted from microscopy-confirmed malaria-positive samples using the QIAamp DNA Mini Kit according to the manufacturer's protocol (Ariey *et al*., 2014; Ouji *et al*., 2018; Lloyd *et al*., 2018; Gigris *et al*., 2023). Nested multiplex PCR assays were performed to amplify *Pfmdr1*, *Pfcrt*, *Pfdhfr*, *Pfdhps*, and *Pfk13* genes associated with antimalarial drug resistance. The PCR products were visualized using 2% agarose gel electrophoresis under ultraviolet illumination (Ariey *et al*., 2014; JID, 2016).

Table 1: List of Primers

Name of the Gene	Primer Sequences	Size (bp)
*Pf*dhfr	F: GTTTTCGATATTTATGCCATATGTG	490
	R: TGATAAACAACGGAACCTCC	
*Pf*dhps	F: TTTGTTGAACCTAAACGTGC	640
	R: AACATTTTGATCATTCATGCAAT	
*Pf*kelch13	F: AAGCCTTGTTGAAAGAAGCA	860
	R: GGGAACTAATAAAGATGGGCC	
Pfcrt	F: CGCTGTGCTCATCTGCTTG	180
	R: GGAATCTGGTGGTAAACGG	
*Pfmdr*1	F: TGGGAAACAGGAAAATTGGTAT	360
	R: TACGACATTAAACACACTGGAA	

Data Analysis

Data were analysed using appropriate statistical software. Descriptive statistics were used to summarize prevalence, while chi-square tests assessed associations between malaria infection and sociodemographic variables. Statistical significance was set at $p \leq 0.05$.

RESULTS

A total of 186 samples were collected for a period of 4 months out of which 67 samples were positive for malaria by MRDT (36.0%), while 72 samples were also positive by light microscopy. This shows that more *Plasmodium* infections were detected by microscopy than MRDT. The sensitivity of malaria rapid diagnostic test (MRDT) using microscopy test as standard is 94.5 % as calculated from Table 2.

The mean parasite density of *Plasmodium falciparum* recorded at the Federal Medical Centre, Birnin Kudu (FMCBK) Jigawa, was 15,832 (7,448–30,232) parasites/µl as shown on Table 3.

At the Federal Medical Centre, Birnin Kudu (FMCBK), Jigawa, 91 females and 95 males were examined, among whom 34 females (37.3%) and 38 males (40.0%) were infected Table 4. Although, statistically there is no significant association between prevalence of malaria and gender across study sites.

The prevalence of *Plasmodium falciparum* infection varied across different age groups in the study hospital, as shown in Table 5. Among children aged 0–5 years, prevalence was 19.4%. In the 6–10 years age group, highest prevalence (23.6%) was recorded. Iindividuals aged 16–20 years, recorded 18.1% prevalence. Among those aged 21–25 years, prevalence of 13.8% was recorded. The 26–30 years group, prevalence was 11.1% while the 31–35 years age group recorded the lowest prevalence across sites, ranging from 1.4% to 5.5%. In the 31–above years group, had a prevalence of 1.3%.

Out of the 72 DNA samples amplified using multiplex PCR and analyzed by agarose gel electrophoresis as shown on Table 6 *Pfdhps* gene has the highest prevalence recorded at the sampled hospital (66.6%) while *Pfcrt* gene has the lowest prevalence across the sample hospital (6.9%).

The distribution of *Plasmodium falciparum* resistance genes on table 7 showed that all five genes *Pfmdr1*, *Pfk13*, *Pfdhps*, *Pfdhfr*, and *Pfcrt* were detected across all age groups, with the highest frequencies observed in the 6–10 years age group**.** This age category accounted for over 22% of gene occurrences across most markers. The prevalence slightly declined in the 0–5 years group and continued to decrease progressively with increasing age, with the lowest detection in individuals aged 31 years and above**.** Among the genes, *Pfk13* and *Pfdhps* appeared most widespread across all ages, while *Pfcrt* showed relatively lower percentages overall.

The gel electrophoresis of the resultant multiplex PCR products shows that they were visualized at expected amplicon sizes as presented in Plate 1.

Table 2: The Prevalence of Malaria among the Study Participants by Microscopy and Malaria Rapid Diagnostic Test

Test method	Number Exam	Number Infected	% Infected
Microscopy	186	72	38.7
*MRDT	186	67	36.0

*Sensitivity; 94.5%

Table 3: Mean parasite density of *Plasmodium falciparum* among individuals with sickle cell disease in Jigawa

Hospital	N	Mean Parasitaemia	95%CI
FMCBK	186	15,832	7448-30,232

Keys: significant at p≤0.05, CI=Confidence interval, x^2 = Chi square, FMCBK: Federal Medical Centre Birnin Kudu

Table 4: Gender base Prevalence of *Plasmodium falciparum* amongst study population

Hospital	Male			Female						
	No. Examined	No. Infected	% Infected	No. Examined	No. Infected	% Infected	x^2	OR	CI	P value
FMCBK	95	38	40.0	91	34	37.3	0.78	0.87	0.63-1.19	0.38
TOTAL	283	123	43.5	321	151	47.				

Keys: n= number, %= proportion, x^2 = chi square, OR = odds ratio, CI = confidence interval

Table 5: Prevalence of *Plasmodium* parasite by age group

Age Group(years)	No. Examined	No. Infected	% Infected	x^2	P-value
0-5	37	14	19.4	11.09	0.086
6-10	43	17	23.6		
11-15	24	9	12.5		
16-20	36	13	18.1		
21-25	22	10	13.8		
26-30	21	8	11.1		
30-above	3	1	1.3		
Total	**186**	**72**	**38.7**		

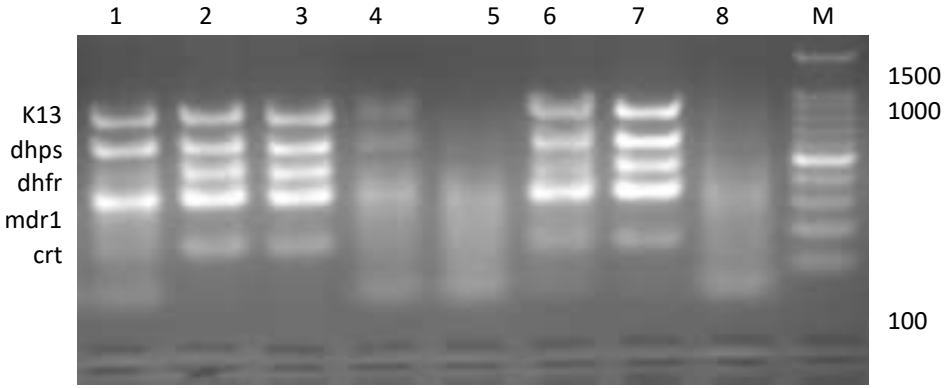

Plate 1; Gel Electrophoregram of Multiplex PCR (*Pfmdr1*, *Pfk13*, *Pfdhr* and *Pfdhps*) for drug resistance genes of samples with high and low parasitemia count
Keys: M: DNA Ladder; BP: Base pair; *Pfk13*: 860; *Pfdhps*: 640; *Pfdhr*: 490; *Pfmdr1*: 360; *Pfcrt*: 180

Table 6: Prevalence of *Plasmodium falciparum* genes detected using Gel electrophoresis

Hospital	Genes	N= 72	%
FMCBK	Pfmdr1	19	26.4
	Pfk13	28	38.9
	Pfdhps	48	66.6
	Pfdhfr	34	47.2
	Pfcrt	5	6.9

Table 7: Distribution of *Plasmodium falciparum* resistance genes by age group

Age (years)	Genes									
	Pfmdr1	%	**Pfk13**	%	**Pfdhps**	%	**Pfdhfr**	%	**Pfcrt**	**%**
0-5	2	10.5	3	10.7	4	8.3	3	8.8	0	0.00
6-10	6	31.7	7	25.0	19	39.6	13	38.2	2	40.0
11-15	2	10.5	4	14.3	5	10.4	5	14.7	1	20.0
16-20	3	15.8	4	14.3	7	14.6	2	5.9	0	0.00
21-25	2	10.5	6	21.4	6	12.5	3	8.8	1	20.0
26-30	3	15.8	2	7.1	4	8.3	6	17.6	0	0.00
31-above	2	10.5	2	7.1	5	10.4	2	5.9	1	20.0
Total	19	100	28	100	48	100	34	100	5	100

DISCUSSION

This study demonstrated that *Plasmodium falciparum* remains the predominant malaria parasite infecting individuals with sickle cell disease attending Federal Medical Centre Birnin Kudu. The exclusive detection of *P. falciparum* aligns with existing reports (Pondei *et al*., 2012; Abah and Temple, 2015; Nyirakanani *et al*., 2018; Wogu and Nduka, 2018) from northern Nigeria and other malaria-endemic regions of sub-Saharan Africa, where this species accounts for the overwhelming majority of malaria infections.

Microscopy detected a slightly higher prevalence (38.7%) of malaria compared to MRDT, highlighting its superior sensitivity, particularly in cases of low parasitaemia. The reduced performance of MRDT observed in this study may be attributable to deletions or mutations in the histidine-rich protein-2 gene, which has been increasingly reported in African parasite populations. This is in agreement with the work of Idoko *et al*. (2015), Wogu and Nduka (2018) and Akindele *et al*. (2023) who reported higher prevalence with microscopy than MRDT. The false negative results and lower prevalence recorded in this study by MRDT as compared to microscopy may be as a result of mutation of the Histidine-Rich Protein-II gene (Oyeyemi *et al*., 2015; Beshir *et al*., 2017).

Despite the high proportion of participants receiving routine antimalarial prophylaxis, a considerable prevalence of malaria infection was recorded. This finding underscores the vulnerability of individuals with SCD to malaria infection, likely due to impaired splenic function and altered immune responses. Persistent parasitaemia among this population suggests that chemoprophylaxis alone may be insufficient, particularly in high-transmission settings (Okiro, 2019).

Age-specific analysis revealed the highest malaria prevalence among children aged 6–10 years. This observation is consistent with existing report (Geleta and Ketema, 2016) indicating that children represent a high-risk group due to incomplete acquisition of protective immunity. In SCD patients, this vulnerability is further amplified by underlying immunological deficits, placing school-aged children at increased risk of infection and complications (Snow *et al*., 2017).

No significant association was observed between malaria prevalence and sex, indicating comparable exposure risk among male and female participants. This finding supports earlier studies reporting minimal or inconsistent gender differences in malaria infection rates (Ghebreyesus *et al*., 2000; Brooker *et al*., 2004). The result is in contrast to Otajevwo, (2013) and Jenkins *et al*. (2015) who reported higher infection in females than males. In addition, the findings are also in contrast to that of Nyarko and Cobblah (2014) and Nmadu *et al*. (2015) who reported higher malaria prevalence in males than females.

Molecular analysis revealed a high prevalence of antifolate (66.6%) genes associated with resistance, particularly the *Pfdhps* gene, suggesting sustained drug pressure from the continued use of sulfadoxine-pyrimethamine for chemoprevention. In contrast, the low prevalence of *Pfcrt* (6.9%) gene is indicative of declining chloroquine resistance following its withdrawal from first-line malaria treatment This aligns with previous studies that reported higher prevalence of *Pfdhps* gene in Northern, Nigeria (Maiga, 2016; 2021; Wang *et al*., 2022; Enato *et al*., 2022; Hossain *et al*., 2023). The detection of *Pfk13* gene associated with resistance across age groups is of particular concern, as it may signal emerging tolerance to artemisinin derivatives.

The widespread distribution of resistance genes across age categories, with higher frequencies among school-aged children, highlights this group as a potential reservoir for resistant *P. falciparum* strains. This aligns with findings of Martin-Ramirez *et al*. (2025) and Roh *et al*. (2023). These findings emphasize the importance of continuous molecular surveillance and the need for targeted malaria control strategies tailored to high-risk populations such as individuals with sickle cell disease.

CONCLUSION

Plasmodium falciparum infection remains prevalent among individuals with sickle cell disease despite widespread use of antimalarial prophylaxis. Microscopy proved more sensitive than rapid diagnostic testing for parasite detection. Molecular analysis confirmed the circulation of antimalarial drug resistance–associated genes, with a high prevalence of *Pfdhps* and a low prevalence of *Pfcrt*, reflecting sustained sulfadoxine–pyrimethamine drug pressure and declining chloroquine resistance. Children aged 6–10 years bore the highest burden of infection and resistance markers. These findings highlight the need for continuous parasitological and molecular surveillance, alongside strengthened, targeted malaria control strategies for sickle cell populations in endemic settings.

Conflict Of Interest: Authors declare no conflict of interest.

Ethics Approval and Informed Consent: Ethical approval for this study was obtained from the ethical review board FMC/HREC/APP/CLN/001/1/240. All participants were duly informed of the objectives of the study and the protocol for sample collection. All participants signed an informed consent form were signed. Participation was voluntary.

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