

Drugs Resistance Gene Mutation (MDR1) of *Plasmodium falciparum* in East, South and Central Sudan

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Received: February 28, 2025; **Published:** March 20, 2025

Abstract

Malaria remains a major public health concern worldwide. According to the World Health Organization (WHO), in 2017, an estimated 219 million people were infected with malaria, leading to 435,000 deaths, predominantly affecting vulnerable populations in Africa. In 2004, Sudan implemented the Artesunate and Sulfadoxine/Pyrimethamine (SP) combination as its primary treatment due to the significant resistance of *falciparum* to antimalarial medications. Every year, epidemic malaria claims the lives of around one million people, the majority of whom are not African. With an estimated 7.5 million cases and 35,000 fatalities annually, malaria remains a serious health issue in Sudan. The purpose of this comprehensive study was to identify the antidrug-resistant gene (1-Pfmdr) across four states in Sudan. This cross-sectional study, conducted from July 2019 to December 2022, involved the collection of three ml blood samples from all study participants to identify the malaria parasite *falciparum* using blood smears stained with Giemsa. The study focused on 225 positive samples of *falciparum*, collected from five states: Kassala, Khartoum, Singa, Abu Hojar, and Damazin, including 50.3% males and 49.7% females, for the detection of the multidrug resistance gene (1-Pfmdr), which encompasses comprehensive, intermediate, and low endemic areas in Sudan. Of the 193 samples screened using PCR, seventeen positive results were sent for genetic sequencing to analyze genetic mutations across different areas in Sudan from ages 1 to 60 years. The highest prevalence of the mutant allele N (26.9%) was recorded in Ad-Damazin, while the lowest prevalence of N (8.8%) was found in Khartoum. The study indicated the presence of five genetic mutations of the malaria resistance gene *P. falciparum* at position 184 of the gene sequence, revealing a significant relationship between the parasite load and the study site P value = 0.000 while showing no significant relationship between parasite load and age group P value = 0.655 or sex P value = 0.148. The findings suggest the occurrence of genetic mutations in malaria resistance markers following the implementation of dual treatment.

Keywords: Malaria; Drug Resistance; Sudan; 1-Pfmdr; Prevalence

Introduction

Malaria is a disease triggered by protozoan parasites belonging to the genus *Plasmodium*. The five notable *Plasmodium* species responsible for human malaria include *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium*

knowlesi. *Plasmodium falciparum* accounts for the majority of malaria fatalities. The infection can onset rapidly and result in various life-threatening complications [1]. The ongoing prevalence of malaria poses a significant health threat and hinders economic development at local, national, and individual levels. *Plasmodium Falciparum* is responsible for the most common type of malaria in tropical countries [2], which explains why mortality and disease rates are so high, particularly among children. Africa is estimated to account for over 80% of malaria cases and deaths, especially among high-risk groups such as expectant mothers and young children [2]. Approximately half of the world's population is at risk of contracting malaria, which currently affects 500 million people globally [3]. Malaria parasites, such as *P. falciparum*, *P. malariae*, *P. ovale*, *P. vivax*, and *P. knowlesi*, belong to the genus *Plasmodium* (Phylum Apicomplexa) [4]. Approximately 90% of malaria-related deaths and 80% of all malaria cases are attributed to the parasitic *Plasmodium falciparum*, which is the most common cause of infection [5]. Other parasitic *Plasmodium* species can infect chimpanzees, birds, reptiles, primates, and rodents [6]. Several simian malaria species, including *P. knowlesi*, *P. inui*, *P. schwetzi*, *P. simiovale*, *P. brazilianum*, and *P. simium*, have been known to infect humans; however, aside from *P. knowlesi*, they are mainly limited significance. Malaria is believed to account for 16.6% of outpatient visits and 31.2% of inpatient admissions in Sudan's public and private healthcare facilities. In these facilities, malaria-a mosquito-borne disease caused by eukaryotic protozoa of the genus *Plasmodium*-is thought to be responsible for 31.2% of inpatient admissions and 16.6% of outpatient visits. The disease is most prevalent in tropical and subtropical regions, including parts of the Americas (22 countries), Asia, and Africa [7]. The global malaria burden heavily impacts the African Region of the WHO. In 2015, this area accounted for 88% of all global malaria cases and 90% of deaths worldwide. From 2000 to 2015, malaria cases decreased by 42%, and the malaria death rate fell by 66% in the African Region [8]. In Sudan, the estimated annual problem size reaches 1.5 million cases, resulting in 30,000 fatalities. Citizens allocate between 20% and 40% of their income to health services, with the mortality rate ranging from 15% to 20%. This situation leads to a 2% decrease in working days. Moreover, malaria accounts for 37.2% of maternal and child mortality, and 18.8% of underweight births can be attributed to this disease, significantly impacting the high infant mortality rates in the country. Increased morbidity and mortality are attributed to the parasite's resistance to antimalarial drugs and the mosquito vector's resistance to current pesticides [9]. Sulfadoxine/pyrimethamine resistance has been observed in Sudan since the early 1990s [10] and was recorded through *in vivo* studies in eastern Sudan shortly before the deployment of ACT. The occurrence of highly resistant pfdhfr/pfdhps quintuple mutants has also been noted in research conducted across various regions in southern and eastern Sudan [11-13]. Currently, there is no vaccine available to prevent malaria. The disease is transmitted to humans by Anopheles mosquitoes, among the four known species that cause malaria-*P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*-*Plasmodium falciparum* is the most lethal. This research examined the various resistance markers for *P. falciparum* malaria in an area of unstable malaria transmission in East, South, and Central Sudan. The primary objective was to study the drug-resistance gene mutation (MDR1) of *Plasmodium falciparum*.

Materials and Methods

Study design

A descriptive cross-sectional study is an observational study that analyzes data collected from a population or a representative subset at a single point in time.

Study area

This study was conducted in various malarial regions of Sudan, specifically in Senga, Abo Hagar, Al Damazin, Sinnar, and Khartoum State (a meso-endemic area).

Study population

These studies focused on malaria patients from hospitals and clinics across Sudan. A doctor completed a systematic questionnaire regarding each patient's medical history and sociodemographic details. The study was carried out following the WHO's guidelines for anti-malarial drug efficacy surveillance procedures [14].

DNA extraction

1.5 ml tube (Eppendorf), blue tips, yellow tips, spectrophotometer RBC lysis buffer, lysis buffer, protein PPT buffer, DNA rehydration buffer, 70% ethanol, isopropanol (2-propanol). Added 300 microliters of whole blood and 900 microliters of RBC lysis buffer; incubated at room temperature for 15 to 20 minutes, then centrifuged for 2 minutes at 10,000 rpm. Repeat this step until a clear supernatant is obtained, then 300 microliters of cell lysis solution are added to the resuspended cells and pipetted up and down to lyse the cells. Cooled the sample to room temperature and added 100 PPT buffer to the cell lysate, vortexing vigorously at high speed for 20 seconds. Placed samples on ice for 5 minutes (at -20°C) and centrifuged at 13000 rpm for 3 minutes (the precipitated proteins formed a light white pellet). Transferred 300 to 400 microliters of the supernatant containing DNA (leaving behind the precipitated protein pellet) into a 1.5 ml tube.

DNA precipitation step

Add 300 microliters of 100% isopropanol and mix the sample by gently inverting it several times. Centrifuge at 13000 rpm for 1 minute. As a result of your precise work, the DNA will be visible as a small white pellet. Pour off the supernatant and add 1 ml of 70% ethanol, then invert the tube several times to wash the DNA pellet. Centrifuge at 13000 rpm for 1 minute and carefully pour off the ethanol. Allow to dry for 15 minutes.

DNA rehydration

One hundred microliters of DNA rehydration buffer were added. Rehydrate the DNA by incubating at 65°C for 30 minutes or at 4°C overnight. Store the DNA at -20°C.

Molecular methods

Polymerase chain reaction fragment length polymorphism (PCR) was utilized to identify resistance genes and investigate the genetic diversity (genetic variation) of antimalarial-resistant *Plasmodium*. Gene segments spanning codon 86 of the Pmdr1 gene were amplified in a 20 µl standard PCR mixture containing 3 µl of extracted DNA and 0.5 µl of the primers MDR1 5'-TGTTGAAAGATGGGTAAAGAGCAGAAA-GAG-3' and MDR3 5'-TACTTTCTTATTACTATGACACCACAAACA-3'. The PCR amplification stages included 94°C for 2 minutes, followed by 35 cycles at 94°C for 10-30 seconds, 55°C for 45 seconds, and 72°C for 1-15 minutes. Nested amplification from this segment was then performed under the same PCR conditions using 1µl of the outer PCR product and primers MDR2 5'-GTCAAACGTGCATTTTTTATTAAT-GACCATTTA-3' and MDR4 5'-AAAGATGGTAACCTCAGTATCAAAGAAGAG-3'. The digests were separated by electrophoresis on a 1.5% agarose gel and detected by staining with ethidium bromide.

Statistical analysis

The statistical software used for this study's data analysis was the Statistical Package for Social Sciences (SPSS) version 17. The data was collected on a hard drive and analyzed using chi-square tests with 95% confidence intervals. P-values less than 0.05 and t-test tabulation correlation were considered statistically significant.

Results

The study conducted on 225 *P. falciparum* samples collected from five states (Kassala, Khartoum, Cenga, Abohogar, and Al-Damazin), which include holo-endemic, meso-endemic, and hypo-endemic malarial areas in Sudan, examined 193 samples using a PCR 20 device, resulting in positive outcomes. These samples were then sent for genetic sequencing, where genetic mutations were successfully identified from various regions of Sudan. Twenty samples were analyzed, and 17 were successfully sequenced from various regions in Sudan. Figure 1 shows that all samples were already known to exhibit Chloroquine resistance. The reference strain (GenBank accession number X56851) displayed wild-type N and Y at positions 86 and 184, respectively. No point mutation was detected at position 86 in any of the samples. Regarding position 184, four distinct mutations were identified (Y184F, Y184L, Y184W, Y184M) in 13 samples, while four sam-

ples were excluded. Figure 2 illustrates the frequency of the pfmdr1 mutation among the 17 human blood samples. Thirty pfmdr1 samples were successfully extracted, sequenced, and analyzed for protein polymorphisms at positions 86 and 184, as shown in figure 3. (A) shows the alignments of all samples with the wild-type sequence at position 86, while (B) displays each sample’s alignment with the wild-type sequencing at position 184. Although “aa” stands for amino acid, the lengths of these sequences are indicated on the left side of both A and B. While no mutations were found at location 86, various types of point mutations were discovered at location 184, as illustrated in figure 3.

Gene name	Codon positions	Frequency
Pfmdr1	Wild (N86)	100% (17)
	Mutant (Y86)	0
	Wild 184	0
	Mutant (184F)	69.2% (9)
	Mutant (184W)	7.6% (1)
	Mutant (184M)	7.6% (1)
	Mutant (184L)	15.3% (2)

Table 1: Frequency of codon positions of Pfmdr1 mutation among 17 blood samples from Sudan.

Area	Frequency	Percent
DMZ	52	26.9
SNG	50	25.9
HGR	43	22.3
Kassla	31	16.1
KRM/O	17	8.8
Total	193	100.0

Table 2: The frequency of the pfmdr1 mutation of the 17 human blood samples.

Age ranges	Frequency	Percent
1-20	88	45.6
21-40	72	37.3
41-60	29	15.0
>60	4	2.1
Total	193	100.0

Table 3: The range of the sample’s age frequencies and their percentages.

Variables		Count					Total
		+	++	+++	++++	+++++	
DMZ	Count	14	25	9	4	0	52
	% within Area	26.9%	48.1%	17.3%	7.7%	0.0%	100.0%
SNG	Count	14	23	12	0	1	50
	% within Area	28.0%	46.0%	24.0%	0.0%	2.0%	100.0%
HGR	Count	10	12	4	1	16	43
	% within Area	23.3%	27.9%	9.3%	2.3%	37.2%	100.0%
	Count	11	15	5	0	0	31
	% within Area	35.5%	48.4%	16.1%	0.0%	0.0%	100.0%
KRM/O	Count	3	8	3	0	3	17
	% within Area	17.6%	47.1%	17.6%	0.0%	17.6%	100.0%
Total	Count	52	83	33	5	20	193
	% within Area	26.9%	43.0%	17.1%	2.6%	10.4%	100.0%

Table 4: Area according to the parasite count.

Variables			Count					Total	
			+	++	+++	++++	+++++		
Age	1-20	Count	26	41	12	2	7	88	
		% within Age	29.5%	46.6%	13.6%	2.3%	8.0%	100.0%	
	21-40	Count	19	26	18	2	7	72	
		% within Age	26.4%	36.1%	25.0%	2.8%	9.7%	100.0%	
	41-60	Count	6	14	3	1	5	29	
		% within Age	20.7%	48.3%	10.3%	3.4%	17.2%	100.0%	
	>60	Count	1	2	0	0	1	4	
		% within Age	25.0%	50.0%	0.0%	0.0%	25.0%	100.0%	
	Total		Count	52	83	33	5	20	193
	% within Age			26.9%	43.0%	17.1%	2.6%	10.4%	100.0%

Table 5: Age according to the parasite count.

Variables	Count					Total
	+	++	+++	++++	+++++	
Count	26	39	13	4	14	96
% within Gender	27.1%	40.6%	13.5%	4.2%	14.6%	100.0%
Count	26	44	20	1	6	97
% within Gender	26.8%	45.4%	20.6%	1.0%	6.2%	100.0%
Count	52	83	33	5	20	193
% within Gender	26.9%	43.0%	17.1%	2.6%	10.4%	100.0%

Table 6: Gender according to the parasite count.

Variables	Mutation	Frequency	Percent
Valid	L	3	10.0
	F	16	53.3
	M	2	6.7
	W	1	3.3
	P	1	3.3
	G	1	3.3
	Total	24	80.0
Missing	System	6	20.0
Total		30	100.0

Table 7: The polymorphism at position 184.

Variables		Position 184						Total
		L	F	M	W	P	G	
Area	DMZ	2	3	0	0	0	1	6
	SNG	1	4	0	0	0	0	5
	HGR	0	3	0	1	0	0	4
	Kassla	0	3	1	0	0	0	4
	KRM/O	0	3	1	0	1	0	5
Total		3	16	2	1	1	1	24

Table 8: Area according to the position 184.

Variables		Position 184						Total
		L	F	M	W	P	G	
Age	1-20	2	9	0	0	0	1	12
	21-40	1	5	1	0	1	0	8
	41-60	0	2	1	1	0	0	4
Total		3	16	2	1	1	1	24

Table 9: Age according to the position 184.

Variables		Position 184						Total
		F	M	W	P	G		
Gender	F	1	9	0	0	1	1	12
	M	2	7	2	1	0	0	12
Total		3	16	2	1	1	1	24

Table 10: Gender according to the position 184.

Variables		Position 184						Total
		F	M	W	P	G		
Parasite Count	+	1	2	1	0	0	1	5
	++	2	6	1	0	1	0	10
	+++	0	4	0	0	0	0	4
	++++	0	1	0	0	0	0	1
	+++++	0	3	0	1	0	0	4
Total		3	16	2	1	1	1	24

Table 11: Parasite count according to the position 184.

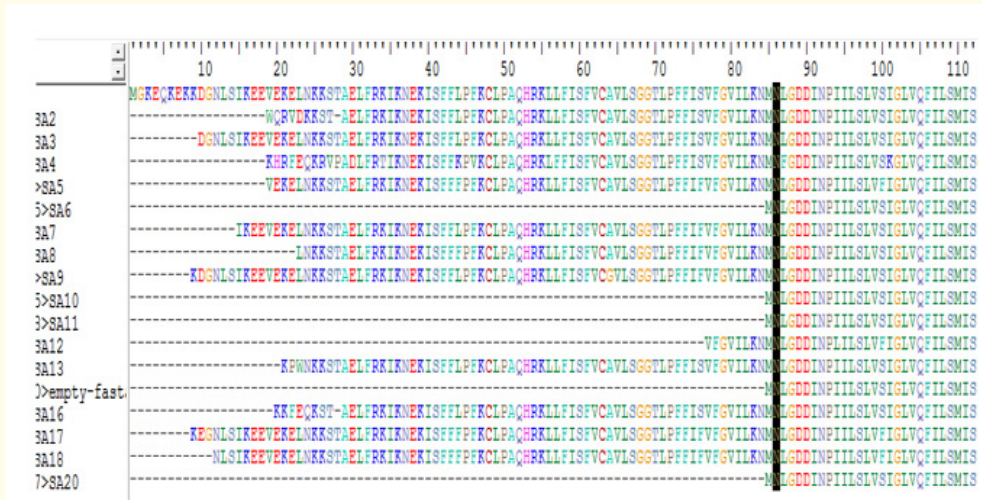


Figure 1: Clustal W multiple alignments of 18 different pfmdr1 sequences.

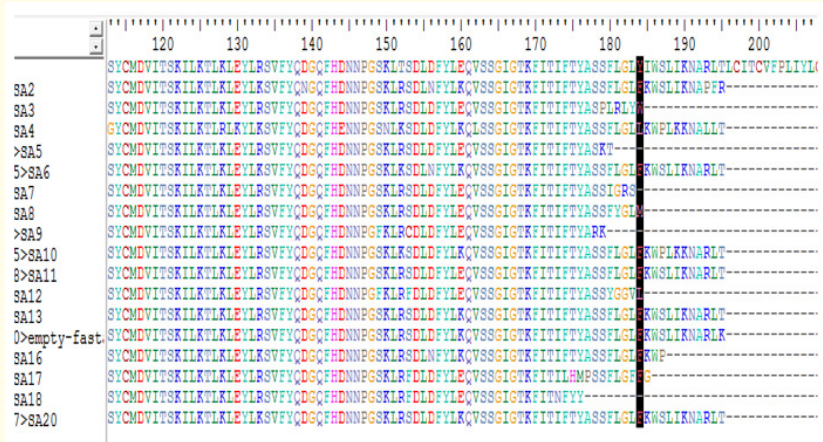


Figure 2: Clustal W multiple alignments of 18 different pfmdr1 sequences.

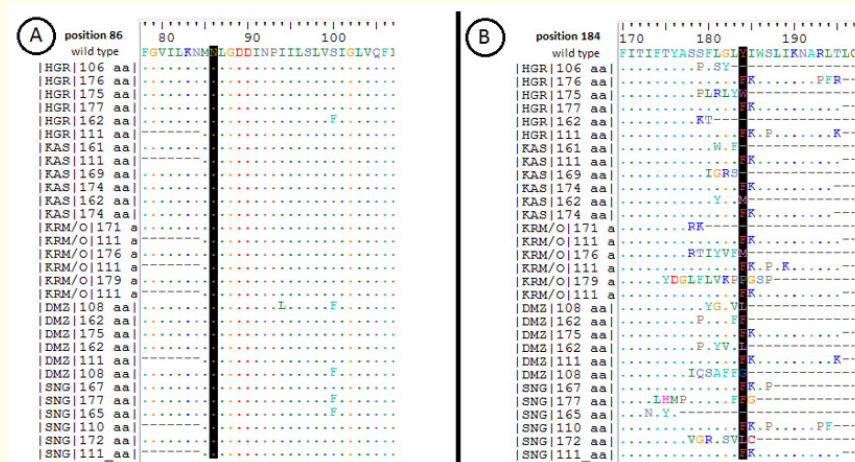


Figure 3: The alignments of all samples with the wild-type sequence at position 86.

Discussion

The analysis of 225 samples revealed a significant correlation between the parasite count on the thin film slide and the area, gender, and age. 48.1% of DMZ individuals had ++ (11–100 parasites per 100 thick film fields), equivalent to 26.9%, 25.9%, and 22.3% in Dmazin, Senga, Abohogar, and Kassla, respectively. However, 8.8% of Khartoum residents had the lowest parasite count, which is lower than any other area, while 7.7% of DMZ residents had the highest parasite count. This suggests that the DMZ region contains malaria parasite

species that evolve to become more virulent and that there is a close relationship. Unexpectedly, the elderly population (those over sixty) had the lowest parasite count, but overall, the study found no correlation between the parasite count and age categories. Males are somewhat more likely than females to experience more severe malaria, even though the study found no correlation between gender type and the parasite count. Mutations in the *P. falciparum* multidrug resistance gene (pfmdr1) have been connected to various resistances in *P. falciparum* species, including resistance to chloroquine. Different types of mutations may develop as a result of prolonged use of a particular medication or adverse environmental conditions. Numerous investigations worldwide have reported frequent point mutations at positions 86, 184, 1034, 1042, and 1246. Research has indicated that the pfmdr1 mutations N86Y and Y184F play a significant role in chloroquine resistance [15]. By sequencing 225 randomly selected samples from five different areas in Sudan-DMZ, SNG, HGR, Kassla, and KRM/O-N86Y and Y184F were successfully analyzed. The results show that no mutation at position 86 was found, and all samples in all areas displayed the wild-type asparagine, indicating that *P. falciparum* chloroquine resistance is most likely unrelated to the N86Y mutation. This is because the Sudanese Ministry of Health has claimed that chloroquine resistance has spread throughout the entire country and will no longer be used to treat malaria. This study has demonstrated that the Y184 mutation is likely the cause of chloroquine resistance in Sudan, as six different types of mutations-including Y184F-have been identified at this position. Furthermore, no wild-type tyrosine was found at position 184 in any of the following areas: DMZ, SNG, HGR, Kassla, and KRM/O. Instead, Y184F was more common than other types of mutations, with percentages ranging from 26.9% in the DMZ to 8.8% in Al Khartoum. Statistical analysis revealed no differences in the types of mutations, as all displayed a high rate of Y184F mutation. Additionally, statistical analysis indicated that there is no discernible difference in the type of mutation at position 184 between age groups and gender types, suggesting that neither sex nor age influences the prevalence or development of mutations at this position. The parasite count did not depend on the type of mutation at position 86 of pfmdr1, according to statistical analysis. This implies that the virulence of *P. falciparum* is unaffected by mutations at position 184. Previous studies in high malaria transmission areas of Sudan (such as Darfur and Kordofan) reported a low mutation rate at position 86, with limited association with chloroquine resistance. In contrast, in other African countries (e.g. Nigeria and Kenya), the Y86 mutation has been reported in areas where chloroquine is commonly used, indicating a link between the mutation and resistance. This contrasts with Sudan, where the stability at position 86 suggests differences in drug pressure or treatment policies.

Overall, the study demonstrated that *P. falciparum* in Sudan has evolved at position 184 of the pfmdr1 protein into six different types of polymorphisms rather than at position 86, as seen in many other parts of the world. Previous studies in Sudan showed moderate prevalence of the Y184F mutation in the central and northern regions, with slight geographic variation. These studies also indicated an association with the effectiveness of artemisinin-based combination therapies (ACTs), which aligns with the findings of the current study. Outside Sudan, in regions such as Southeast Asia and South America, the Y184F mutation is more common and linked to increased sensitivity to artemisinin treatments, particularly in areas with extensive use of these drugs. The higher mutation rate in Sudan may suggest a similar effect, potentially related to variations in drug policies or the parasite's genetic patterns. This is likely due to widespread chloroquine resistance in these regions, as demonstrated by Ahmed Bakheet and colleagues in their 2017 study [16]. Depending on the genetic background, pfmdr1 point mutations can significantly impact susceptibility to various antimalarial drugs. The association between this mutation and chloroquine resistance is supported by some field research but not by others. Findings from a few pfmdr1 SNP investigations suggest that the N86Y mutation plays a major role in chloroquine resistance. *P. falciparum* isolates from Guinea-Bissau, Nigeria, Malaysia, Indonesia, and Sub-Saharan Africa have been found to carry the N86Y mutation, conferring resistance to chloroquine. The isolates from Cambodia show a high frequency of pfmdr1 mutations. Recent research indicates that chloroquine resistance dropped from high to moderate levels when mutant pfmdr-1 was replaced with wild-type sequence-resistant parasites. In particular, it has been observed that CQ resistance traits are linked to the combined influence of Y184F haplotype isolates from Africa, Asia, and South America. According to the data collected for this study, Y184F is more widely distributed in Sudan than other types of polymorphisms. Additionally, the study found a strong correlation (69.2%) between the Y184F mutation and chloroquine resistance, suggesting that it could be

used as a resistance marker to investigate chloroquine resistance in Sudan. The N86Y mutation (0.00%) is not significant in chloroquine resistance. Thus, its use as a marker for chloroquine resistance is not recommended. The study also identified three distinct pfmdr1 polymorphism types: Y184L, Y184W, and Y184M. All of these polymorphisms have also shown resistance to chloroquine. The association of mutations with chloroquine resistance has been noted in the *P. falciparum* multidrug resistance gene (pfmdr1). Point mutations in pfmdr1 depend on the genetic background and can significantly affect vulnerability to a range of antimalarial drugs. The association of this mutation with chloroquine resistance is supported by some field research but is contradicted by others. Findings from several pfmdr1 SNP investigations suggest that the N86Y mutation is significantly involved in chloroquine resistance. *P. falciparum* isolates from Guinea-Bissau, Nigeria, Malaysia, Indonesia, and Sub-Saharan Africa have been shown to carry the N86Y mutation, which leads to chloroquine resistance. The isolates from Cambodia have a notable frequency of pfmdr1 mutations. Recent research indicates that chloroquine resistance declined from high to moderate levels when mutant pfmdr-1 was replaced with wild-type sequence-resistant parasites. It has been noted that CQ resistance traits are connected to the combined impact of Y184F haplotype isolates from Africa, Asia, and South America. The data collected for this study shows that Y184F is more prevalent in Sudan compared to other polymorphism types. Furthermore, the study discovered a strong correlation (69.2%) between the Y184F mutation and chloroquine resistance, indicating its potential utility as a resistance marker in the study of chloroquine resistance in Sudan. The N86Y mutation (0.00%) is deemed insignificant in chloroquine resistance, and therefore, its application as a chloroquine resistance marker is not recommended. The study identified three different pfmdr1 polymorphism types: Y184L, Y184W, and Y184M. Each of these polymorphisms has also exhibited resistance to chloroquine. The study revealed geographical variation in mutation distribution, showing a higher prevalence in the DMZ and SNG regions. However, no significant statistical correlation between geographic regions and mutation patterns was identified. Additional studies in Sudan confirmed that regions with high malaria transmission rates (such as the eastern endemic areas) often exhibit resistance mutations, supporting the findings of the current study. In countries like Uganda and Tanzania, mutations are strongly linked to high transmission areas, indicating a robust connection between geography and parasite pressure. However, the geographic variation in Sudan is less pronounced than in these regions. The relationship between mutations, gender, and age: The study did not reveal significant gender differences, but mutations were more prevalent in the 1-20 age group. Studies inside Sudan: Some local studies reported similar findings, noting that mutations tend to be more common in younger age groups, possibly due to greater exposure to the parasite or weaker acquired immunity. Studies outside Sudan: External studies also indicated that mutations are generally not influenced by gender but may vary with age, with younger populations being more susceptible to malaria in areas of partial immunity. The results within Sudan are largely consistent with international studies, particularly regarding the Y184F mutation and its role in increasing sensitivity to ACT treatments. A notable difference is the relative stability at position 86 in Sudan compared to other regions that exhibit higher mutation rates, reflecting possible differences in policies or drug treatment.

Conclusion

In Sudan, artemisinin remains a safe and effective first-line treatment for uncomplicated *falciparum* malaria. However, the presence of this mutation could lead to treatment failure. The detection of the malaria medication resistance gene mutation allele in various locations indicates that the gene has spread across Sudan and may continue to do so in the future. Indeed, the presence of these drug-resistant genes could complicate the establishment and implementation of control measures to combat malaria in different regions of Sudan. A correlation was noted between the area and the number of malaria parasites, while no correlation was observed between gender and the Pfmdr-1 gene polymorphism. The mutant allele of each of these genes does not influence the level of parasitemia. Position 184 showed no correlation with either gender or age. At position 184, multiple mutation types (L, F, M, W, P, and G) were identified. An association was found between the malaria parasite count and the Pand appearance in the PCR test.

Recommendation

Monitoring the efficacy of artemisinin should continue because the country's underutilization of anti-malarial medications heightens the risk of drug failure due to sub-therapeutic doses. It is also crucial to keep an eye out for molecular markers associated with artemisinin

resistance as a supplementary measure for *in vivo* effectiveness. In Sudan, the second line of treatment, which is the optimal choice for treating uncomplicated *falciparum* malaria, is being monitored. However, more research is urgently needed in various parts of Sudan to observe any medication failures. This includes detecting all drug resistance gene mutations through sequencing. Your role in this research is crucial. Developing effective strategies and thorough research is essential to prevent the spread of the parasite. Establishing genetic sequencing tests in Sudan, identifying genes, and conducting additional analyses to uncover genetic variants that confer drug resistance is necessary.

Consent

The patient's written consent has been collected.

Ethical Approval

Omdurman Islamic University granted permission for this investigation. All study participants were informed about the study's purpose. Permission for this study was obtained from the local authorities in the area. This study's aims and benefits were explained with the assurance of confidentiality. All protocols in this study were done according to the Declaration of Helsinki (1964).

Sources of Funding

There was no specific grant for this research from any funding organization in the public, private, or nonprofit sectors.

Conflict of Interest

The authors have declared that no competing interests exist.

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Volume 21 Issue 4 April 2025

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