UJMR, Vol. 10 No. 1, June, 2025, pp. 93 - 107 *E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668*

https://doi.org/10.47430/ujmr/25101.011

Received: 15th January, 2025 **Accepted: 1st May, 2025**

Prevalence and Implications of Plasmodium Falciparum Multidrug Resistance Gene Mutations (pfmdr1) Among Pediatric Patients with Malaria in Kano, Nigeria

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Abstract

A genetic indicator of the vulnerability of malarial parasites to anti-malarial medications is the Plasmodium falciparum multidrug resistance gene 1 (pfmdr1). In this study, malaria patients aged 0-14 who were treated at Murtala Muhammad Specialist Hospital in Kano, Nigeria, were evaluated for multidrug-resistant resistance gene 1 (MDR1) mutations. After confirming the malaria parasite density in 100 children samples, the samples were genotyped using BigDye (v3.1) terminator cycle sequencing to look for two SNPs in pfmdr1 on samples with high and moderate parasite densities. Fisher's exact (FE) tests and Pearson Chi-square were used to evaluate the data. Of the 100 samples, 57% of the patients had low (+) malaria parasite densities, 28% had moderate (++) densities, and 15% had high (+++) densities. Only seven samples were successfully amplified for the pfmdr1 gene located at codon 1246, whereas 31 were successfully amplified and processed for the pfmdr1 gene located at codon 86 with an amplicon size of 534 bp. A Pfmdr1-N86Y mutation was found in one sample (3.2%). Additionally, the results indicated no correlation (P = 0.4237) between sex and the pfmdr1SNP mutation. Nonetheless, there was a significant correlation (P = 0.0043) between the pfmdr1 mutation and the age groups. According to the current study, Kano state in Northern Nigeria may have strains of P. falciparum that are less sensitive to the artemisinin component of artemisinin-based combination therapy (ACT). The development of this resistance gene in Plasmodium falciparum puts malaria chemotherapy at serious risk because the parasite will be immune to widely prescribed anti-malarial medications, highlighting the need for measures to checkmate this worrisome trend.

Keywords: Multidrug resistance, Mutation, Malaria, Paediatric patients, pfmdr1 gene

INTRODUCTION

Globally, malaria is the primary cause of child mortality. It is a tiny *protozoan* that is a member of the *Plasmodium species* group and has multiple subspecies is the cause of malaria that primarily affects human beings (White et al., 2014; Walker et al., 2017). According to WHO (2015b), there were approximately 214 million new cases of malaria identified worldwide in 2015, with Africa accounting for 88% of these cases, South-East Asia for 10%, and the Eastern Mediterranean region for 2%. Ninety percent (394,200 deaths) of the 438,000 malaria deaths reported globally over that time period took place in Africa (WHO, 2015a). The Eastern Mediterranean Region (2%) and South-East Asia Region (7%) accounted for the remaining deaths. 95% (292,000) of the 306,000 fatalities among children under five that were reported worldwide occurred in the African region.

Malaria can kill anybody by causing anemia, high fever, and flu-like symptoms.

Twenty five percent (25%) of malaria cases worldwide occur in Nigeria, where 50% of the population is thought to get at least one episode yearly, with under-five children often experiencing two to four attacks (WHO, 2018). Globally, the malaria death rate is between 0.3% and 2.2%, and in tropical locations, the rate for severe forms of the disease is between 11% and 30% (White et al., 2014). According to several research, the rate of malaria parasite infection has risen since 2015 (Pan American Health Organization, 2018; Dhiman, 2019). Malaria killed 619,000 people worldwide, affecting an estimated 247 million people (WHO, 2021). In certain regions of the world, the high mortality rate from malarial infections makes it challenging to stable the population and economy. Plasmodium falciparum quickly develops drug resistance, which makes anti-malarial drug resistance a significant barrier to malaria treatment in sub-Saharan Africa (Cravo et al., 2015) (Blasco et al., 2017).

The southern part of Nigeria was the primary source of reports on the prevalence of pfmdr1 polymorphisms, with pfmdr1 N86, F184, and D1246 alleles positively correlated with clinical failure (Happi et al., 2009). On the other hand, the region also reported a prevalence of 62.2% and 69.0% for the pfmdr1 86Y and F184 *alleles*, respectively (Oladipo et al., 2015). This was followed by another survey that found that the prevalence of the pfmdr1 86Y and 1246Y *alleles* was 24% and 18.6%, respectively (Dokunmu et al., 2019). In order to provide genetic epidemiology data on the prevalence of N86Y-Y184F-D1246Y SNPs in *P. falciparum* multidrug resistance 1 (pfmdr1) in the malaria hotspot of Northern Nigeria, Adamu et al. (2020) planned and carried out a study. However, there is no valid baseline data involving pfmdr1 SNPs in children between the ages of 0 to 14 years in Nigeria since the withdrawal of CQ and adoption of AL in Northern Nigeria 2021. Over a century, several research has been conducted worldwide to prevent, diagnose, and treat malaria as a result of the high burden caused by malaria infection (WHO, 2018).

An amoeboid intracellular parasite belonging to the genus *Plasmodium* accumulates malaria pigment, an insoluble hemoglobin byproduct. Five of the 172 *Plasmodium* species are capable of infecting people. *P. falciparum, P. vivax, P. ovale, P. malariae, and P. knowlesi* are among them (Antinori et al., 2012; Singh and Daneshvar, 2013; Walker et al., 2017). The illness generally referred to as malaria (Latin for Malus aer, or filthy air) is caused by all of the *Plasmodium* species listed. Similarly, the biology and morphology of all species are comparable (Vuk et al., 2008; Ashley et al., 2018). An estimated 3.4 billion people, or half of the world's population, reside in areas where malaria infections are a possibility (WHO, 2013a). Just over half of all malaria deaths globally occurred in four African nations: Nigeria (31.3%), the Democratic Republic of the Congo (12.6%), the United Republic of Tanzania (4.1%), and Niger (3.9%). 31% of malaria cases worldwide occur in Nigeria, where 50% of the population is thought to get at least one episode annually, with under-five children often experiencing two to four attacks annually (Udoh et al., 2016; WHO, 2021). According to Onwuemele (2014), *P. falciparum* is consistently and permanently spread throughout the nation, with transmission increased during the wet season compared to the dry (Samdi et al., 2012; Houben et al., 2013; Rupashree et al., 2014).

They are homologues to human P-glycoproteins (P-gh ormultidrug resistance system) and members of drug metabolite transporter (DMT) family, respectively. The former mediates drifting of xenobiotics towards the DV while the latter chucks them outside. Resistance to drugs whose target site of action is intravacuolar develops when the transporters expel them outside the DVs and vice versa for those whose target is extravacuolar (Mustapha et al., 2023).

The sexual and asexual stages of *Plasmodium's* life cycle occur in both the vertebrate hosts and the vector mosquitoes. The sexual stage of the parasite's life cycle takes place in the vectors. Humans, the intermediate host for malaria, go through the asexual phase of the life cycle (Vuk et al., 2008; Soulard et al., 2015). Only female Anopheles mosquitoes are capable of transmitting malaria to humans. Following a bite from an infected female mosquito, the parasite sporozoite enters the human bloodstream and penetrates the hepatocytes after 30 minutes of blood circulation (Josling & Llinás, 2015). Hepatocytes undergo the initial stage of *Plasmodium* asexual development, followed by erythrocytes. Every variety of *Plasmodium* causes the rupture of erythrocytes (Vuk et al., 2008; Cowman et al., 2016; Ashley et al., 2018).

The life cycle of the human malaria parasite. *Plasmodium* sporozoite, the motile infectious form, is transferred to humans when an insect bites the skin, searches for a blood vessel to feed from, releases different vasodilators to enhance the likelihood that it will find a vessel, and secretes saliva into the blood to stop clotting. The circulatory system transports the thread-like *sporozoites* to the liver within 30 to 60 minutes of injection (Krettli and Miller, 2001; NIAID, 2007). The *sporozoites* rupture the

hepatocytes after growing into *schizonts* and developing up to 30,000 *merozoites* during a However, some vivax and ovale sporozoites develop into hypnozoites, which can induce relapses in infected individuals and remain dormant in the liver for months or years (Walker et al., 2010). Recurrences of falciparum malaria have been documented in patients a few years after they left a location where the disease is endemic. According to Szmitko et al. (2008), Poilane et al. (2009), and Theunissen et al. (2009), it indicates that *P. falciparum* has a dormant stage at least periodically. The *merozoites* then invade RBC to expand by ingesting hemoglobin, starting the asexual cycle (Figure 1). After undergoing mitotic divisions, the parasite develops within the host red blood cell from the early ring stage to the late *trophozoite* and finally to the *schizont* stage, which comprises 6 to 32 *merozoites*, depending on the parasite species (Jiraprapa *et al.*, 2002). The liberated *merozoites* invade other RBCs to continue the life cycle after the erythrocytic *schizont* ruptures. When *schizonts* burst to release fresh infectious *merozoites*, cyclic fevers usually occur just prior to or during RBC lysis. This happens every 72 hours in cases of quartan malaria and every 48 hours in cases of tertian malaria. Some *merozoites* undergo this recurring cycle by differentiating into erythrocytic *gametocytes*, which are single-nucleated male and female sexual forms, and then waiting for a female Anopheles mosquito that is looking for blood to arrive (Jiraprapa et al., 2002; NIAID, 2007).

Gametogenesis is then triggered when the mosquito consumes gametocytes. The macrogametes are penetrated or fertilized by the *flagellated* microgametes produced by *exflagellation*, producing zygotes. After transforming into *ookinetes*, the zygotes mature into spherical *oocysts*. The nucleus inside the *oocyst* divides frequently, causing the *oocyst* to expand and produce a significant number of *sporozoites* (WHO, 2013c). The *oocyst* ruptures when the *sporozoites* are completely developed, discharging them into the mosquito's bodily cavity, or hemocoel. The life cycle is completed when the *sporozoites* move to the salivary glands (Figure 2.1). The malaria life cycle is sustained when *sporozoites* from the mosquito's salivary glands enter a new human host (WHO, 2013c; CDC, 2016).

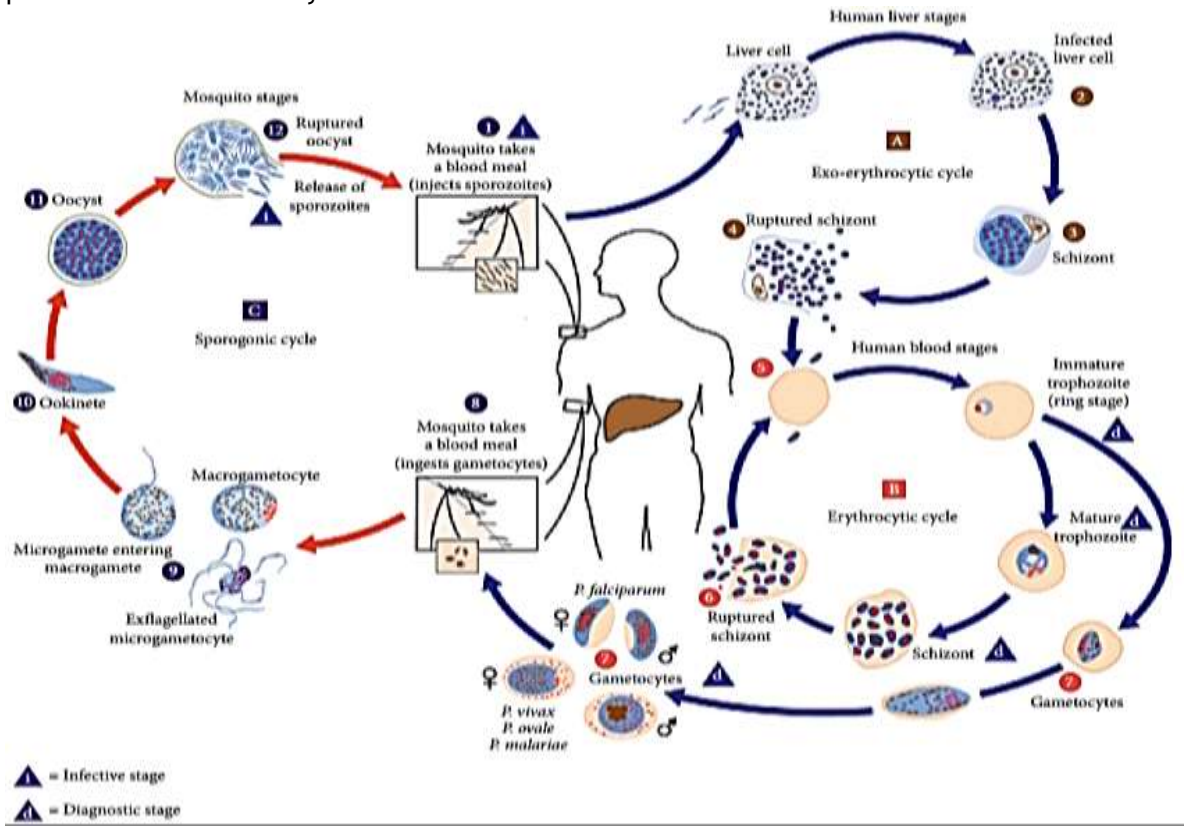

Figure 1: Life Cycle of Malaria Parasite (WHO, 2013c; CDC, 2016)

Diagnosis of Malaria:

Early and precise diagnosis of malaria is essential for successful patient care. In general, it can be divided into parasitological and clinical diagnostics. The patient's symptoms and physical examination findings are the basis for

the clinical diagnosis (WHO, 2015a; CDC, 2016). A parasitological diagnosis of probable malaria should be made in every situation (WHO, 2015a). RDTs and light microscopy are frequently used techniques for this purpose.

Light microscopy is the gold standard for diagnosing malaria, which finds the parasites on peripheral blood smears stained with Giemsa. Because of their very identical morphologies, *knowlesi* cannot be diagnosed with microscopy alone (Murray, 2009; WHO, 2012). All stages of development after the hepatic cycle are seen in the peripheral blood in the cases of vivax, ovale, and malariae. However, because mature parasites sequester, only ring forms, and banana-like gametocytes are typically found in the peripheral blood of falciparum (Nicoletta et al., 2015).

Mechanism of Artemisinin action and resistance in *P. falciparum:*

ACTs have been the first-line treatment for malaria since the early 2000s and have gained rapid global adoption (White, 2014). Artemether + lumefantrine (Coartem) and artesunate + amodiaquine are used in Africa, while artesunate + mefloquine and DHA + piperaquine are used in Southeast Asia. These are the four primary medication combinations that contain artemisinin derivatives. Dr. Youyou Tu won a Nobel Prize in Medicine in the 1970s for her team's work defining the medicinal properties of artemisinin and figuring out its chemical structure (Figure 2) (Tu, 2016). Despite having a short half-life of less than one hour, the compound's extraordinary properties include the rapid drug-activated death of P. falciparum parasites in both the asexual blood stage and early sexual gametocyte forms within hours of exposure at low Nano molar concentrations.

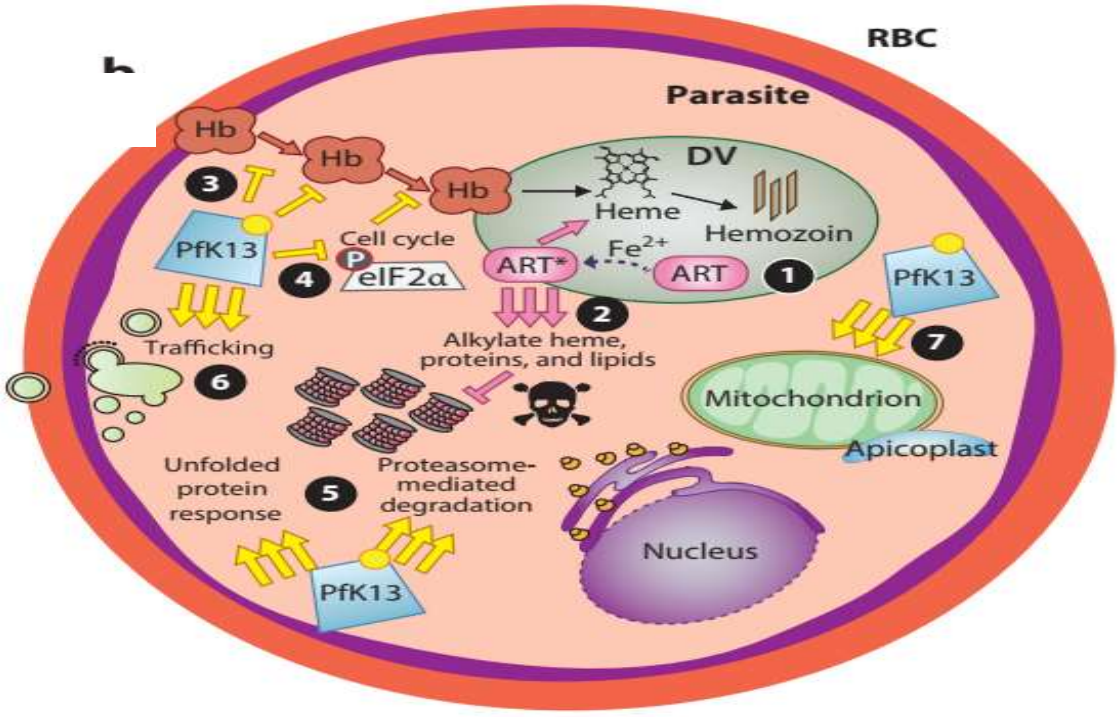

Figure 2: Mechanism of action of artemisinins and resistance mechanisms mediated by *pfmdr1*, and K13 in *P. falciparum* asexual blood stage parasites.

However, artemisinin has been shown to bind to a wide range of parasite proteins and appears to affect many organellar and cellular processes, including hemoglobin endocytosis, glycolysis, protein synthesis and degradation, and cell cycle regulation. In contrast, the majority of anti-malarial medications, such as Sulfadoxine-Pyrimethamine (SP), Atovaquone, and Chloroquine, inhibit either a single target or a single pathway, such as DHFR-mediated folate synthesis by SP, Atovaquone inhibition of cytochrome bc1, and heme detoxification by Chloroquine (Wanget al., 2015; Ismail et al., 2016; Bridgford et al., 2018). Its distinctive characteristic is caused by the cleavage of its endoperoxide bridge by free Fe 2^{+} PPIX released from digested hemoglobin.

The heme-drug carbon-centered radical, once activated, alkylates heme, proteins, and lipids, accelerating the production of more cytotoxic reactive oxygen species through a cluster bomb effect that ultimately results in cell death (Ismail et al., 2016).

Other research has indicated that artemisinins may also target mitochondrial function by depolarizing the membrane potential of this organelle (Wanget al., 2010; Li et al., 2015).

MATERIALS AND METHODS

Apparatus and Equipment

Microscope (Olympia, Japan), Gel documentation system (Syngene, UK), water bath (Grant JB, Nova UK), EDTA container, cotton wool, spirit, syringe, glass slide, DNA extraction kit, QIAquick purification kit (QIAGEN, Germany), microcentrifuge (Prism – mini, Labnet, Korea), and so on

Reagents

Methanol (BDH, England), Giemsa (BDH, England), ethidium bromide (Sigma, USA), agarose powder (Bioline, Japan), DNA ladder (Thermo Fisher, USA), sodium acetate (Thermo Fisher, USA), Tris Acetate EDTA (TAE), buffer (Bioline, Japan), Nuclease free water (Pomega, USA), etc.

Study Population

This study consists of all febrile patients presenting symptoms of malaria of both sexes and aged between 1 month and 14 years attending Murtala Muhammad Specialist Hospital (MMSH) Kano.

Methods

Sample Collection

Blood samples were taken from patients under five years old using finger prick filter paper. However, children between the ages of 6 and 14 had 2 mL of venous blood extracted using a sterile syringe and needle. When collecting blood samples, safety precautions were taken, such as swabbing the area to be sampled with disinfectant and letting it dry before collecting. Blood samples were collected only after obtaining a parent or guardian's informed consent. Additionally, a structured questionnaire was given to parents or guardians of patients who were referred to the hospital's laboratory department to gather their demographic data.

Determination of malaria parasite infection:

Prior to staining, thin blood films were fixed for 10 seconds with 100% methanol (BDH, England) and left to dry at room temperature. As outlined in Basic Malaria Microscopy (1991), thick blood films were stained for 30 minutes using 3% Giemsa (BDH, England) in Gurr® buffered water, pH 7.2 (BDH, England). With immersion oil, all blood films, thick and thin, were seen under a microscope at a magnification of × 100. Before a sample was declared "negative," or "no malaria parasites seen," at least 200 fields had to be inspected. The "plus" system scale was used to assess positive results on the thick smear: + (1 to 9 trophozoites in 100 fields); ++ (1 to 10 trophozoites in 10 fields); +++ (1 to 10 trophozoites per field); ++++ (>10 trophozoites per field). Assuming a white blood cell count of 8,000/µl, the following scores were used to predict parasite densities: + = 10 to 90 parasites/µl; ++ = 100 to 1,000 parasites/µl, +++ = 1,000 to 10,000 parasites/µl; ++++ = >10,000 parasites/µl.

Statistical Analysis

Sequences were analyzed using CLUSTAL W available on the EBI website (www.ebi.ac.uk), while *pfmdr1* SNPs were determined using CLC sequence viewer (version 8.0) in reference to the *pfmdr1* sequence of *P. falciparum* deposited at the NCBI database [Accession Number X56851] (Adamu *et al.*, 2020). All data were statistically analyzed using Excel Spreadsheet and Fisher´s exact (FE) test of Graph-Pad Prism. Chi-square test was performed to determine the relationship between variables and *P* values < 0.05 were considered to be statistically significant.

Ethical Approval

Ethical approval for this study was obtained from the Kano State Ministry of Health (MOH/Off/797/T.I/1611). Blood samples were only taken after full informed consent from a parent/guardian, and also assent from the child had been obtained. During sample collection, the purpose of the survey was once again explained to the parent/guardian on a one-to-one basis. A copy of the consent form in the local language was also provided and explained to the patients. In addition, semi-structured questionnaires were administered to consenting guardians of the children who met the inclusion criteria. The samples were collected between August and September 2019.

Determination of the Plasmodium falciparum multidrug resistance gene 1 (*pfmdr1*)

There can be no doubt that point mutations in *pfmdr1* are modulating the response to 4-quinolinemethanols, other class 2 blood schizontocides, artemisinins, and chloroquine, but the role of polymorphisms remains unclear. *Pfmdr1* is situated on chromosome 5 of *P. falciparum* and encodes a 162 kDa transmembrane protein with 12 transmembrane domains organized in two symmetrical units (Figure 3).

Mutations in *pfmdr1* modulate resistance to chloroquine and increase sensitivity to chloroquine (Al-Koofee and Mubarak, 2020). This function seems to be located at 86^{Y} (Al-Koofee and Mubarak, 2020). Transfection experiments showed that mutations in 1034^{C}, 1042^{D}, and 1246^{Y} are associated with mefloquine resistance (Al-Koofee and Mubarak, 2020).

Association with chloroquine resistance has also been reported for mutants 184^{F}, 1034^{C}, $1042^{D,}$ and 1246^{Y}, especially from South America (Wong et al., 2017). Observations in Peru indicate that neither the 1246^{Y} nor an association of 1034^{C} and 1246^{Y} compromised the clinical-parasitological efficacy of mefloquine in *falciparum* malaria (Wong et al., 2017). The absence or presence of mutations in codons 86 and 184 did not influence the in-vitro response of *P. falciparum* to chloroquine, arylaminoalcohols, and artemisinins (Al-Koofee and Mubarak, 2020). Similarly, mutations on codons 86, 184, 1034, 1042 and 1246 failed to influence the outcome of ultra-low-dose mefloquine treatment in Gabon (Mawili-Mboumba *et al.*, 2013). Observations with fresh, predominantly (96%) chloroquine-resistant *P. falciparum* isolates in Brazil, where 86^{TYR} does not occur, demonstrated the presence of 1042^{D} and 1246^{Y} mutations (Po´voa *et al.*, 1998). Surprisingly, all isolates were sensitive to amodiaquine, indirectly suggesting a role of 86^{Y} in the modulation of amodiaquine resistance, a phenomenon encountered in tropical Africa where 86^{Y} is known to occur.

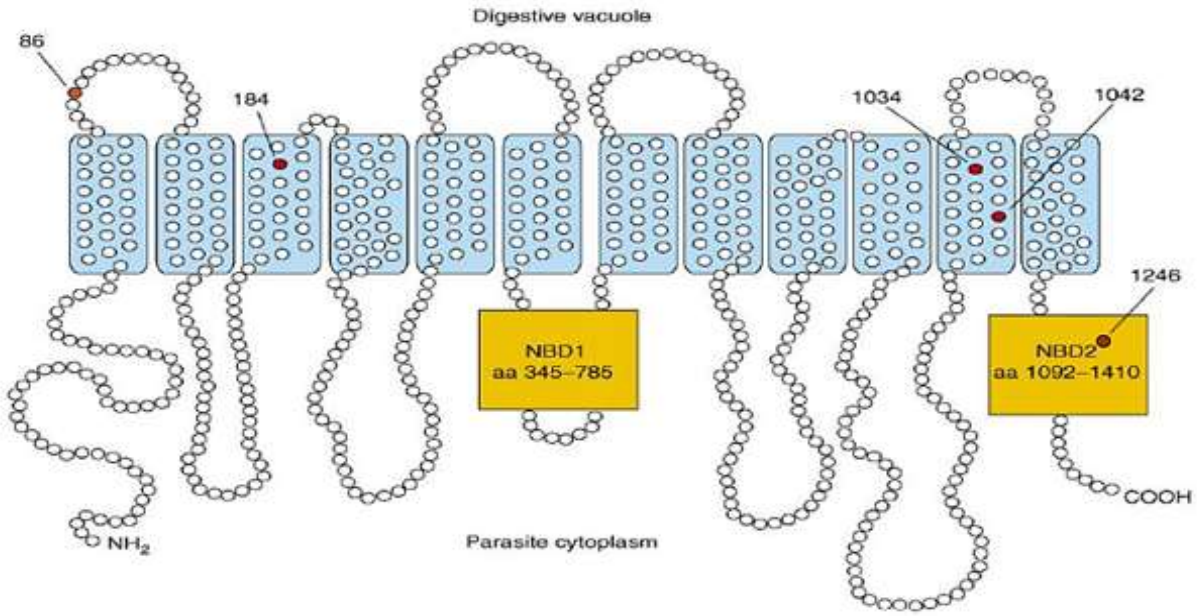

Figure 3: *PFMDR1* – a multidrug resistance protein (Valderramos and Fidock, 2006)

RESULTS

Age distribution of the study subjects

According to the study population's age distribution, children between the ages of 10 and 14 made up nearly one-third of the study population (32%), followed by those between the ages of 5 and 9 (30%). The frequency of occurrence was 21% for children aged 1–4 years (12 males and 9 females), whereas the frequency of occurrence was 17 (7 males and 10 females) for children aged 1–12 months (Figure 4).

Geographical location of the study respondents

According to the current study (Figure 5), the number of patients (26%; 14 males, 12 females) were found in Dala Local Government Area (LGA), followed by Gwale LGA (24%; 13 males, 11 females) and Kano Municipal LGA (23%; 13 males, 10 females). The proportion distribution of males (9, 60%) in Nassarawa LGA was higher than that of females (6, 40%). The findings also revealed that the percentage distributions of male and female patients from the other LGAs—Fagge, Kumbotso, and Tarauni LGAs—were 4 (33.3%), 5 (41.7%), and 3 (25%) correspondingly.

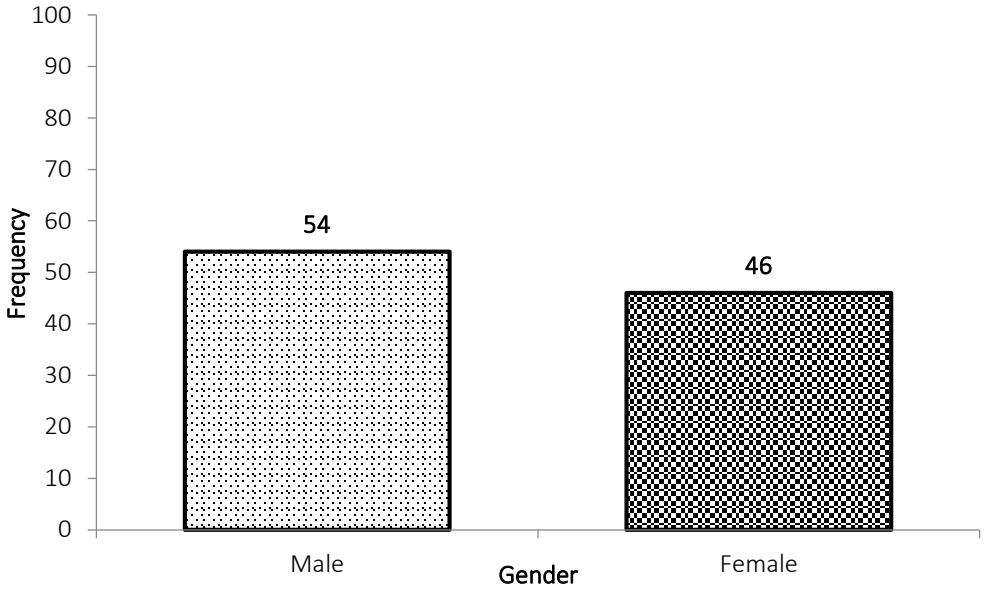

Figure 4: Gender distribution of malaria patients 0-14 years old attending Murtala Muhammad Specialist Hospital, Kano (*N* = 100)

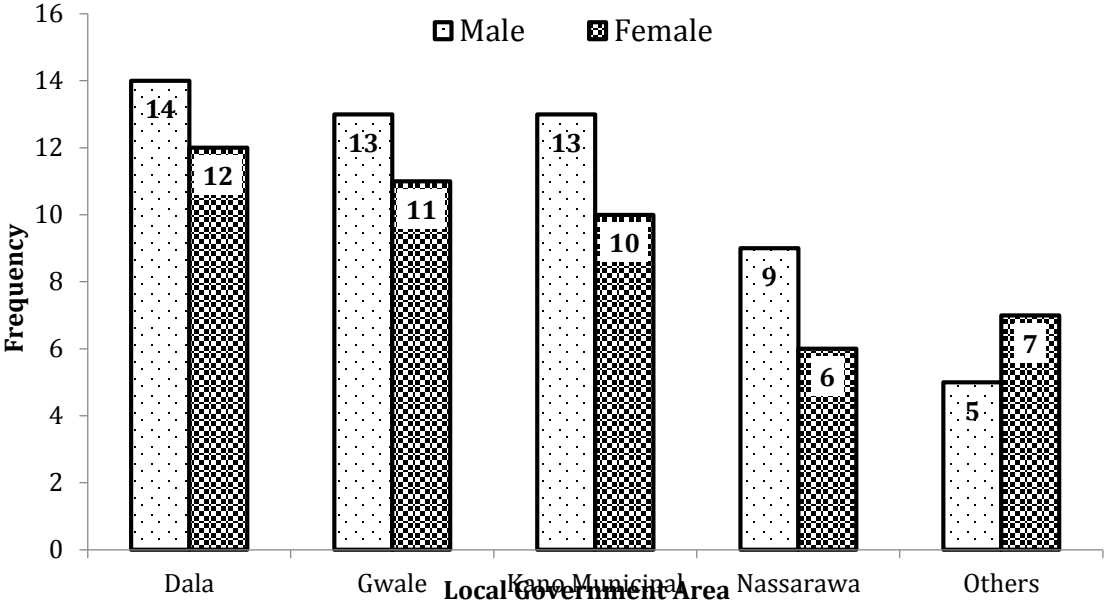

Figure 5: Population distribution of malaria patients 0-14 years old attending Murtala Muhammad Specialist Hospital, Kano according to Local Government Area *N* = 100.

Table 1: Malaria Parasite Density of the Subject according to Age Group

Age Group	+		++		+++		Total (%)
	Male	Female	Male	Female	Male	Female	
1 - 12 months	4	5	2	3	1	2	**17**
1 - 4 years	7	5	3	3	2	1	**21**
5 - 9 years	9	9	4	4	2	2	**30**
10 - 14 years	11	7	4	5	3	2	**32**
Total	**31**	**26**	**13**	**15**	**8**	**7**	**100**

+ = positive for malarial infection (low parasite density)
++ = positive for malarial infection (moderate parasite density)
+++ = positive for malarial infection (high parasite density)

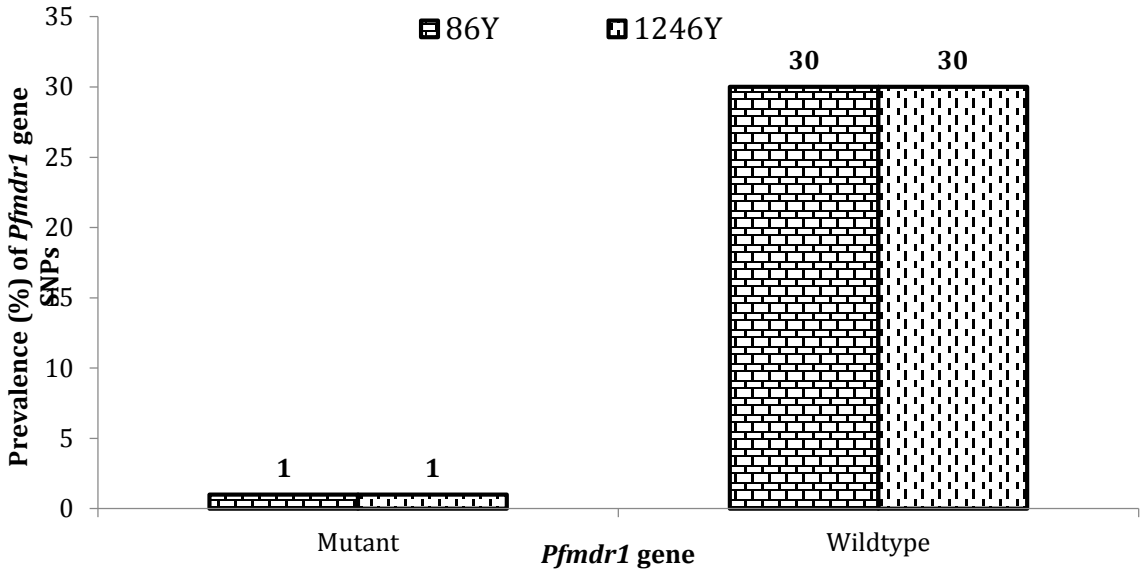

Figure 6: Prevalence of mutant alleles at 86Y and 1246Y of *Pfmdr1* gene in parasite isolates SNP Mutation

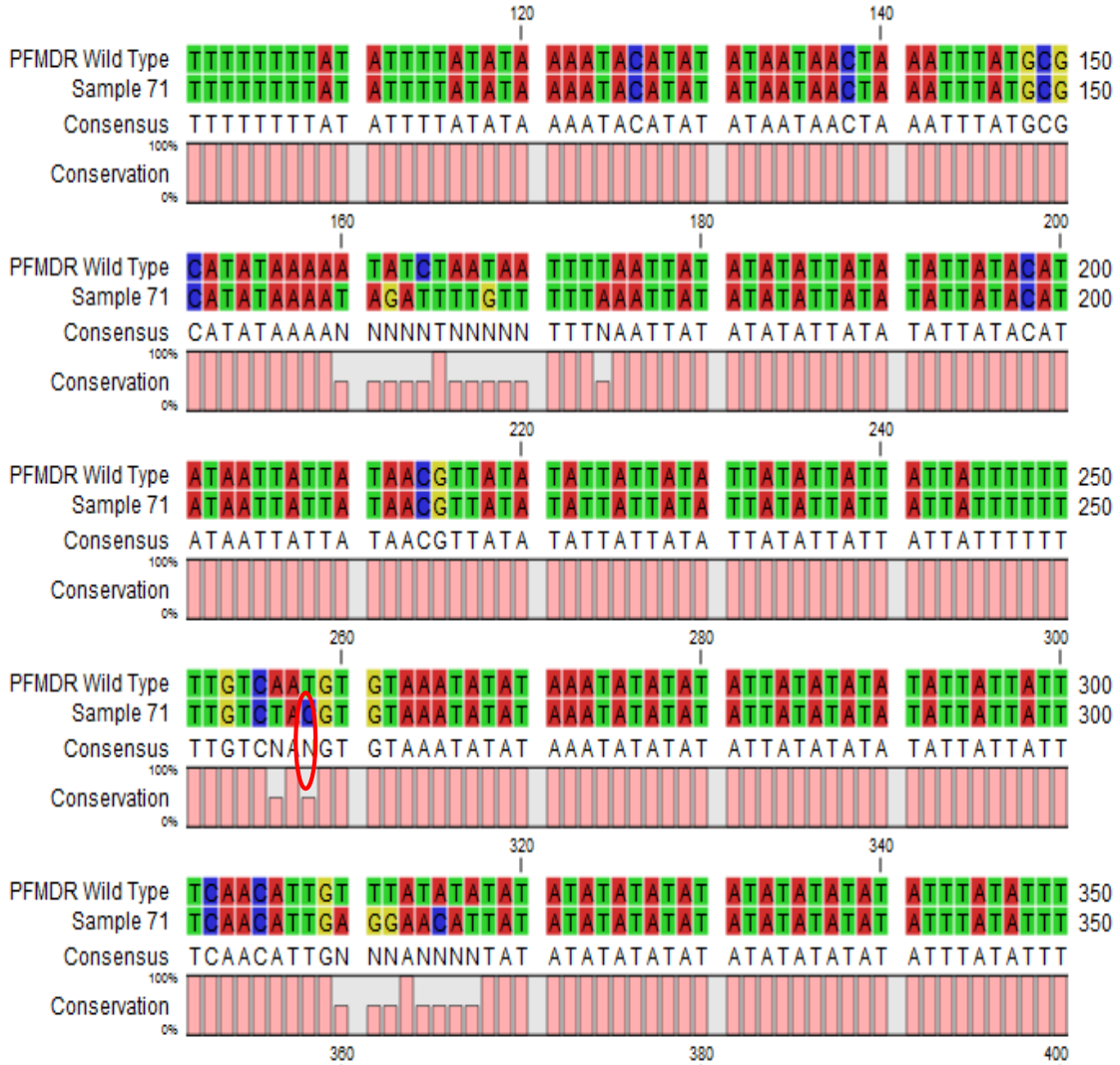

Figure 7: Alignment of samples with the *pfmdr1* reference gene in malaria patients 0-14 years old attending Murtala Muhammad Specialist Hospital, Kano

PFMDR Wild Type 86: Sequence of susceptible strain (reference gene)
SNP Mutation

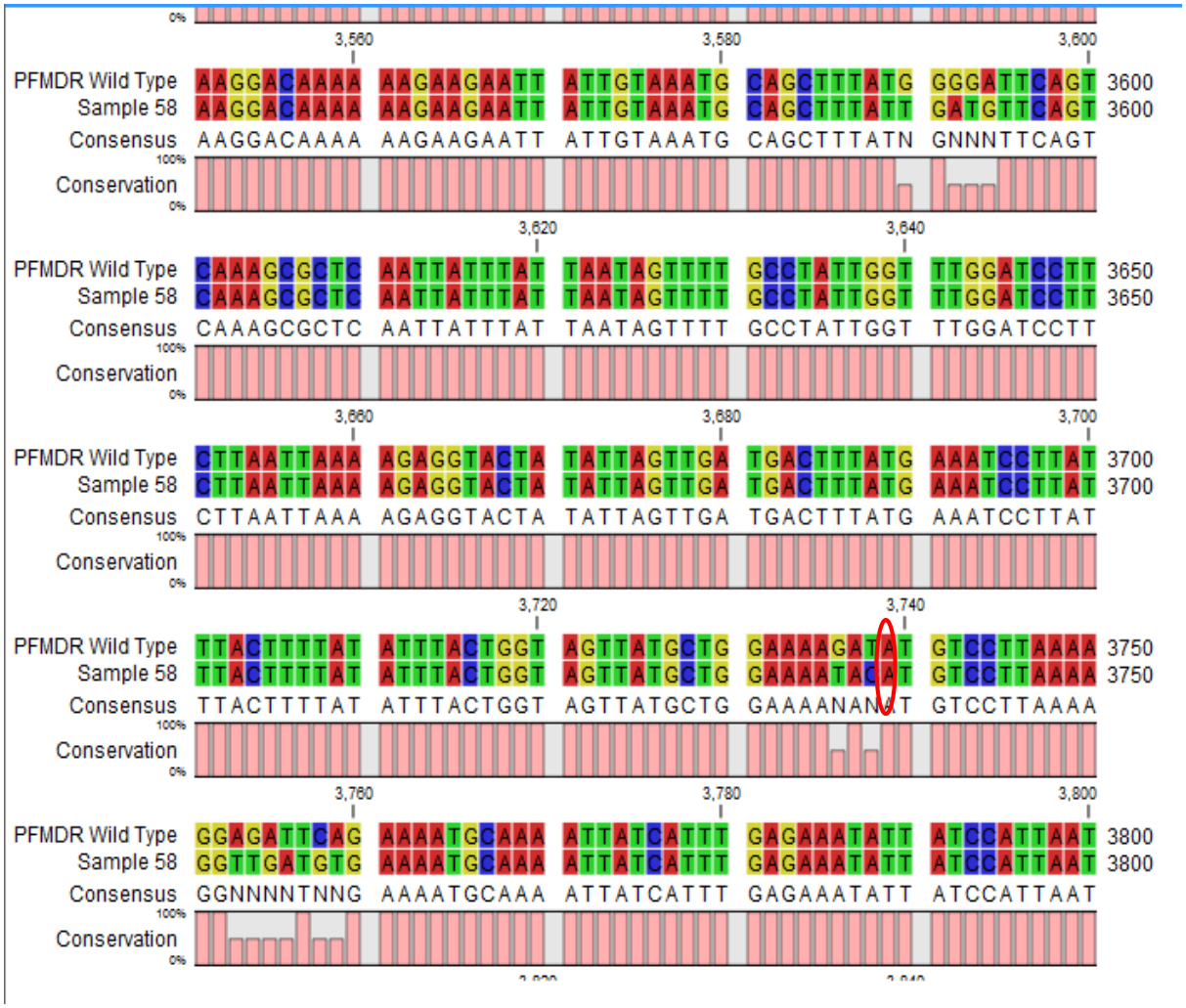

Figure 8: Alignment of Sample with the *pfmdr1* Reference Genein Malaria Patients 0-14 years old Attending Murtala Muhammad Specialist Hospital, Kano
PFMDR Wild Type 1246: Sequence of susceptible strain (reference gene)

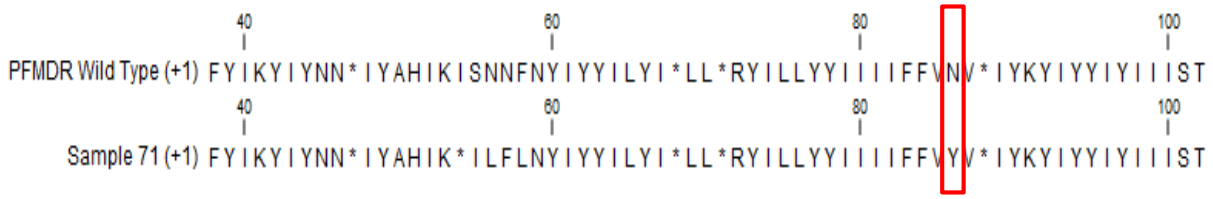

Figure 9: Translated Amino acid sequence showing the substitution of Asparagine (N) for Tyrosine (Y) at codon 86 (red mark)

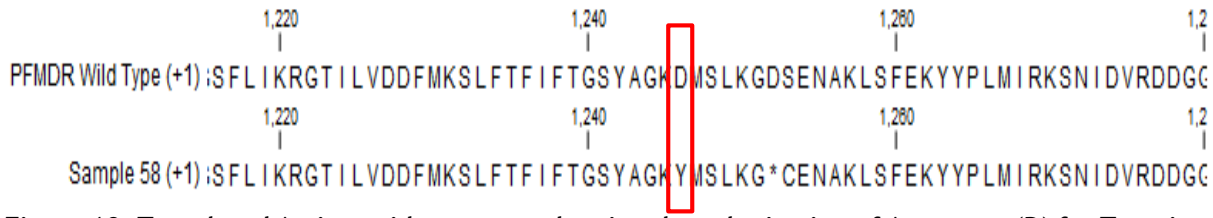

Figure 10: Translated Amino acid sequence showing the substitution of Aspartate (D) for Tyrosine (Y) at codon 1246 (red mark)

DISCUSSION

In the various age groups, the prevalence of malaria varied greatly and increased with age. Compared to children under the age of five, the recent findings showed that 62% of the malaria-infected patients were between the ages of five and fourteen. Recent domestic and international research has documented similar patterns (Ceesay et al., 2008; Mawili-Mboumba et al., 2013; Ogah et al., 2013; Noland et al., 2014). The frequency of malaria shifted from children under five to older children, according to a 6-year cross-sectional study conducted in Gabon and a related study conducted in the Gambia (Ceesay et al., 2008; Ogah et al., 2013). In a study conducted in Ghana using archived filter paper blood spots from under-five children with uncomplicated malaria in 2003–2010, the prevalence of *pfmdr1* 184F was reported to increase steadily as 26–78%, 35–82%, 48–70%, and 40–80% for 2003–2004, 2005–2006, 2007–2008, and 2010, respectively. Indeed, reports of the high occurrence of the mutant *pfmdr1* 184F in West Africa were corroborated by the present findings in which it was observed that the prevalence of *pfmdr1* 184F was high in all the states, and especially in Kano and that its prevalence is higher in North-West compared to North-East Nigeria (Duah et al., 2013).

The observed improvements in malaria control efforts that focused on this anagraphic group may cause decreased prevalence in these patients. They may have been more vulnerable to malaria later in life due to their decreased exposure to the parasite in this category due to a delayed development of functional immunity. According to the current study, 56% of patients adhered to the anti-malaria treatment below recommended levels. The high adherence (90-100%) documented in related research (Dondorp et al., 2010; Bello et al., 2019) substantially differed from this finding, while the prevalence of *Pfcrt* was observed to be higher than *Pfmdr 1*. The high prevalence of the *Pfcrt* mutant gene in this present study could be a result of the treatment of malaria infection with the use of chloroquine in which long-term exposure of the parasite to the drug could bring about mutation, leading to the development of the *Pfcrt* mutant genes (Sa et al., 2009). While among other factors, reports of chloroquine-resistant *P. falciparum* treatment failure rate of up to 53.6% in South Eastern Nigeria and up to 37% in South Western Nigeria (Umotong et al., 1991), with a drop to 15% in 1992 (Simon-Oke et al., 2017). This gives reason for the necessity of continuous monitoring of resistance to chloroquine and other anti-malarial drugs. This could be a significant issue in terms of clinical results as well as the emergence of artemisinin resistance (Dondorp et al., 2010), and It was also observed that the *Pfcrt* (95%) mutant gene recorded a higher prevalence than *Pfmdr 1* (45%) mutant gene. However, the prevalence recorded is higher than the result of those who recorded 24% and 18.9% for *Pfcrt* and *Pfmdr 1* , respectively (Okungbowa and Mordi, 2013). The high prevalence of these mutant genes in the study area could be a result of the high indiscriminate use of drugs (drug abuse) for the treatment of malaria by people in the study area. Also it could also be as a result of long time use of chloroquine as anti-malarial drugs. Constant exposure of the parasite to drugs could lead to the development of these resistance genes (Sa et al., 2009).

However, it's unclear what aspects of adherence, like timing dose intervals correctly or taking each dose with a fatty meal, are most important and how excellent adherence is required for ACTs to be effective. Studies from West Africa have connected the predominance of the pfmdr1 N86 allele to selection by artemether-lumefantrine (AL) after the adoption of ACTs in numerous African nations (Okell et al., 2018). Consequently, the current study reported 3.2% pfmdr1 N86 allele prevalence may indicate potential AL pressure in Kano State. Furthermore, the results may indicate that the effectiveness of ACTs' LMF component is vulnerable to the development of reduced tolerance in the local *P. falciparum* populations, as the presence of *pfmdr1* 86Y is critical in the initiation of resistance to LMF *in vivo* and that its selection primarily follows reinfection and recrudescence events associated with the elimination stage of LMF, 4–5 days after artemether clearance (Sisowath *et al*., 2005).

This indicates that the mutant genes are wildly spread throughout the country. The spread of these mutant genes, which is associated with the presence of the causative agent (*P. falciparum*) of the disease across the country, may have been facilitated by the migration of people carrying the parasite having the resistant genes from one place to another for recreational activities, employment or education pursuits and due to disasters (Okungbowa and Mordi, 2013) while Mutations in several genes, including *pfmdr1*, *pfcrt* and *pfk13* are associated with variation in parasite sensitivity to a range of drugs. The *pfmdr1* mutations N86Y, Y184F, and D1246Y SNPs are thought to modulate susceptibility to CQ, AL, and AS-AQ. It was observed that, *pfmdr1* 184F and 86Y alleles predominated in North-West Nigeria while 1246Y was higher in the North-East.

Alleles of *pfmdr1* carrying the wild-type N86 residue are associated with higher IC_{50} and IC_{90} values for LMF, MFQ, and DHA, while the Similarly, there are varying epidemiological reports on the prevalence and consequences of *pfmdr1* N86Y polymorphisms from different parts of the world (Ibraheem et al., 2014), suggesting that *pfmdr1* mutations are geographically confined and have inconsistent distributions from one geographic region to another

Ibraheem et al. (2014) claimed that the rise of drug-resistant Plasmodium falciparum strains has made malaria even more of a problem in endemic regions. Several intracellular targets are affected by anti-malarial medications. Most of them disrupt digestive vacuoles (DVs), although some also impact other organelles, such as mitochondria and apicoplasts. Plasmodium develops resistance through various methods, one of which is the prevention of drug accumulation or access to the target site. Plasmodium falciparum multidrug resistance transporter (pf mdr) and Plasmodium falciparum chloroquine resistance transporter (pfcrt), which are found in the DV membrane and are thought to be putative indicators of CQ resistance, are two of the transporters that Plasmodia are equipped with and which move medications away from the target site. They belong to the drug metabolite transporter (DMT) family and are homologues of human P-glycoproteins (P-gh or multidrug resistance system), respectively. While the latter throws them outside, the former facilitates the drift of xenobiotics in the direction of the DV. When the transporters eject medicines outside the DVs, resistance to pharmaceuticals with an intravacuolar site of action develops, and vice versa for drugs with an extravacuolar target.

The low prevalence of *pfmdr1* 86Y in Adamawa and Yobe raises the possibility that CQ may be effective against *P. falciparum* malaria in North-Eastern Nigeria once again, although this would be presumably tempered by CQ-resistance associated mutations in *pfcrt*, which was not assayed here. It is possible that the selection of *pfmdr1* 86Y allele in this region was aided by the cessation of CQ usage due to the emergence of resistance. The high prevalence of this mutation across Northern Nigeria may indicate that the efficacy of AL is at risk in this region but raises the possibility that CQ may be effective in the chemotherapy of uncomplicated malaria here. The IC_{50} of some anti-malarial drugs was shown to vary on the sole acquisition of either *pfmdr1* N86 or 86Y alleles by parasite lines expressing wild-type *pfmdr1*-Y184 or mutant 184F alleles (Veiga et al., 2016). Several alternative 86Y residue seems to confer increased resistance against CQ and AQ.

epidemiological studies on the prevalence of *pfmdr1* Y184F polymorphisms have shown that the Y184 allele is predominantly confined to East and Central Africa, while the mutant 184F allele predominates in West Africa (Okell et al., 2018). In Cameroon, the prevalence of *pfmdr1* 184F allele was shown to reduce drastically from 97.3 to 56% in 2003–2013 (Moyeh et al., 2018). In other findings following the adoption of ACT in many African countries, some studies from West Africa have linked the prevalence of the *pfmdr1* N86 allele to selection by AL (Okell et al., 2018). Therefore, the high prevalence of *pfmdr1* N86 allele observed in the present study, even with the absence of clinical data of patients, might be suggestive of possible AL pressure in all the states. In addition, the finding might also suggest that the efficacy of the LMF component of ACT is susceptible to the emergence of tolerance in the local *P. falciparum* populations, as the presence of *pfmdr1* N86 is critical in the initiation of resistance to LMF in vivo and that its selection primarily follows reinfection and recrudescence events associated with the elimination stage of LMF, 4–5 days after artemether clearance (Sisowath et al., 2005) and another study showed that between 1995 and 2003 prior to the introduction of ACT in Kenya, the prevalence of *pfmdr1* Y184 was 100%, but declined to 99.3% between 2008 and 2014 post ACT introduction (Achieng et al., 2015). The *pfmdr1* D1246Y mutation affects *P. falciparum* susceptibility to various anti-malarials, including QN, MFQ, (HF), CQ, and ART, with the latter two drugs affected in a strain-specific manner. The observed low prevalence of mutant *pfmdr1* 1246Y alleles compared to the wild type in this study is consistent with reports from Southern Nigeria (Oladipo et al., 2015).

CONCLUSION

The drug resistance gene *pfmdr1* is present in the *Plasmodium falciparum* parasites of the pediatric malaria patients treated at Murtala Muhammad Specialist Hospital Kano at codons 86Y and 1246Y. The development of this resistance among the *Plasmodium falciparum* parasites threatens to put malaria chemotherapy in serious jeopardy, because the parasite will be immune to widely prescribed anti-malarial medications.

Recommendations

The following recommendations have been made based on the findings of the current study;

1. The current study found that the pfmdr1 gene was prevalent in the study group, contributing to resistance to artemisinin combination therapy (ACT). More research should be done to ascertain the relationship between rising chloroquine (CQ) resistance and the frequency of pfmdr1 86Y alleles.
2. The current study's cross-sectional design made it difficult to determine a relationship between patient clinical and treatment outcomes and the molecular resistance marker (pfmdr1). Therefore, more research should assess the relationship between molecular resistance indicators and patient clinical and therapeutic results.

Conflict of Interest

The authors declare that there was no conflict of interest.

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