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Prevalence of multi drug resistance malaria among patients aged 0 – 14 years attending murtala muhammad specialist hospital Kano State, Nigeria

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Abstract

The *Plasmodium falciparum* multidrug resistance gene 1 (pfmdr1) is a molecular marker of parasite susceptibility to anti-malarial drugs. This study aimed to evaluate multidrug resistance resistance gene 1 (MDR1) mutation in 0-14years old malaria patients attending Murtala Muhammad Specialist Hospital, Kano, Nigeria. Samples from 100 children with malaria were examined to confirm the malaria parasite density and further genotyped via BigDye (v3.1) terminator cycle sequencing for the presence of two SNPs in pfmdr1on samples with high and moderate parasite densities. All data were analyzed using Pearson Chi square and Fisher's exact (FE) tests. Of the 100 patients, 57% had low (+) malaria parasite density, 28% had moderate (++) and 15% had high (+++) malaria parasite densities of the 100 samples, 31 samples were successfully amplified and analyzed for the pfmdr1 gene located at codon 86 with amplicon size of 534bp while only 7 samples were successfully amplified for the pfmdr1 gene located at codon 1246.Pfmdr1-N86Y mutation was detected in 1 (3.2%) sample. In addition, only 1 (3.2%) sample with allelic change at 1246Y was detected in mutant pfmdr1gene. The result also showed that sex had no significant association (P = 0.4237) with pfmdr1SNP mutation. However, significant association (P = 0.0043) between the age groups (1 month to 14 years) represented in the study and pfmdr1mutation. The present study suggests that strains of P. falciparum with reduced sensitivity to the artemisinin component of artemisinin-based combination therapy (ACT) exist in Kano state, northern Nigeria.

Methods: sample Collection

Finger prick filter paper blood samples were collected from patients below the age of 5 years. However, 2 mL of venous blood was drawn using sterile syringe and needle from children aged 6 and 14 years. Safety procedures were adopted in the collection of blood samples by swabbing the area to be sampled with disinfectant and allowing it to dry before collection.

Keywords: Drug; Malaria; Prevalence; Specialist Hospital; Kano State

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1. Introduction

Malaria affected an estimated 247 million people causing 619,000 deaths in 2021 globally (WHO, 2021). This burden of morbidity and mortality by malaria infection has resulted to more than a century of global effort on research aimed at the prevention, diagnosis, and treatment of malaria (WHO, 2018). The malaria mortality rate globally ranges from 0.3–2.2%, and in cases of severe forms of malaria in regions with tropical climate is 11–30% (White *et al*., 2014). Different studies showed that the prevalence of malaria parasite infection has increased since 2015 (Pan American Health Organization, 2018; Dhiman, 2019). The causative agent of malaria is a small protozoon belonging to the group of *Plasmodium* species, and it consists of several subspecies. Some of the *Plasmodium* species cause disease in human (White *et al*., 2014; Walker *et al*., 2017). The genus *Plasmodium* is an amoeboid intracellular parasite which accumulates malaria pigment (an insoluble metabolite of hemoglobin). Of the 172 of *Plasmodium* species, five species can infect humans. These are *P. malariae*, *P. falciparum*, *P. vivax*, *P. ovale*, and *P. knowlesi* (Antinori *et al*., 2012; Singh and Daneshvar, 2013; Walker *et al*., 2017). All the mentioned *Plasmodium* species cause the disease commonly known as malaria (Latin for *Malus aer*—bad air). Likewise, all species have similar morphology and biology (Vuk *et al*., 2008; Ashley *et al*., 2018).

Globally, an estimated half of the world population (3.4 billion people) lives in area at risk of malaria infections (WHO, 2013a). Four African countries accounted for just over half of all malaria deaths worldwide: Nigeria (31.3%), the Democratic Republic of the Congo (12.6%), United Republic of Tanzania (4.1%) and Niger (3.9%). Nigeria accounts for 31% of global cases of malaria and an estimated 50% of the country's population suffer at least one episode of malaria every year while under-five children experience an average of 2–4 attacks in a year (Udoh *et al*., 2016; WHO, 2021). *Plasmodiumfalciparum*is stably and perennially transmitted in all parts of the country (Onwuemele, 2014), with transmission increased during the wet season compared to the dry (Samdi *et al*., 2012; Houben *et al*., 2013; Rupashree *et al*., 2014).

The *Plasmodium* life cycle is very complex and takes place in two phases; sexual and asexual, the vector mosquitoes and the vertebrate hosts. In the vectors, the sexual phase of theparasite's life cycle occurs. The asexual phase of the life cycle occurs in humans, the intermediate host for malaria (Vuk *et al*., 2008; Soulard *et al*., 2015). Human malaria is transmitted only by female mosquitoes of the genus *Anopheles*. The parasite, in the form of sporozoite, after a bite by an infected female mosquito, enters the human blood and after half an hour of blood circulation, enters the hepatocytes (Josling and Llinás, 2015). The first phase of *Plasmodium*a sexual development occurs in the hepatocytes, and then in the erythrocytes. All *Plasmodium* species lead to the rupture of erythrocytes (Vuk *et al*., 2008; Cowman *et al*., 2016; Ashley *et al*., 2018).

1.1. Malaria

The term *malaria* was derived from the Italian word "*mala aria*" meaning foul air (WHO, 2018). It is a protozoal blood infection caused by a mosquito-borne apicomplexan parasite, which is transmitted to humans during the bite of an infected female *Anopheles* mosquito species (White *et al*., 2014; Pan American Health Organization, 2018). The United States National Institute of Allergy and Infectious Diseases (NIAID) defined malaria as a disease caused by a parasite that lives part of its life in humans and part in mosquitoes (Dhiman, 2019). This review aims to present all aspects of malaria in a coherent and comprehensive manner. An attempt was made to give introductory concepts regarding history, causative agents, prevalence, and incidence of malaria (Adamu *et al*., 2020). It also provides old and new notions about the cell biology, pathophysiology, diagnosis, and management of malaria in one umbrella including some tips from Ethiopia. In advance, we seek to summarize recent developments in drug, vaccine, and control measures of malaria (Adamu *et al*., 2020).

Malaria is an ancient disease that could be traced back to the very earliest human history. It was accepted as a disease by Hippocrates in the fourth century BC (Walker *et al*., 2017). In the early seventeenth Century, the Peruvian bark of Cinchona tree was known to treat fever (Antinori *et al*., 2012). Othmer Zeidler synthesized Dichloro-Diphenyl-Trichloroethane (DDT) in 1874 for his thesis. Alphonse Laveran noticed parasites, which he called *Oscillaria malariae*, in the blood of a malaria patient in 1880 (Singh and Daneshvar, 2013). The genus *Plasmodium* was portrayed by Ettore Marchiafava and Angelo Celli in 1885 (Vuk *et al*., 2008).

1.2. Global Distribution of Malaria

Globally, about 214 million new cases of malaria was diagnosed in 2015 of which Africaaccounted for 88%, South-East Asia (10%) and the Eastern Mediterranean region (2%) (WHO,2015). Within the same period, a total of 438,000 malaria deaths were recorded worldwide of which 90 % (394,200 deaths) occurred in Africa (WHO, 2015a). The remaining

deaths were recorded in South-East Asia Region (7%) and the Eastern Mediterranean Region (2%). Of the 306,000 deaths recorded globally among children under-fives, 95% (292,000 deaths) was from the African Region.

1.3. Lifecycle of Malaria Parasite

The human malaria parasite has a complex life cycle as shown in Figure 2.1. The motile infectious form, *Plasmodium* sporozoite, is passed to individuals when the insect bites the skin, probes for a blood vessel from which to feed, releases various vasodilators to increase its chance of finding a vessel and salivate into the blood to prevent clotting. Within 30–60 min of inoculation, the thread-like sporozoites are carried to the liver by the circulatory system (Krettli and Miller, 2001; NIAID, 2007). Over a period of 7–12 days, the sporozoites grow into schizonts and can develop up to 30,000 merozoites, which rupture the hepatocytes (WHO, 2013b; Ricardo *et al*., 2014). On the other hand, some *vivax* and *ovale* sporozoites turn into hypnozoites, a form that can remain latent in the liver for months or years and cause relapses in infected people (Walker *et al*., 2010). Interestingly, recurrence of *falciparum* malaria was reported in patients some years after leaving an endemic area. It tells that, at least occasionally, *falciparum* has a dormant stage (Szmitko *et al*., 2008; Poilane *et al*., 2009; Theunissen *et al*., 2009). Then, the asexual cycle begins (Figure 2.1), with the merozoites invading RBC to grow by consuming hemoglobin. Within the host RBC, the parasite undergoes development from the early ring stage to late trophozoite and then after mitotic divisions to the schizont stage, which contains 6 to 32 merozoites, depending on the parasite species (Jiraprapa *et al*., 2002).

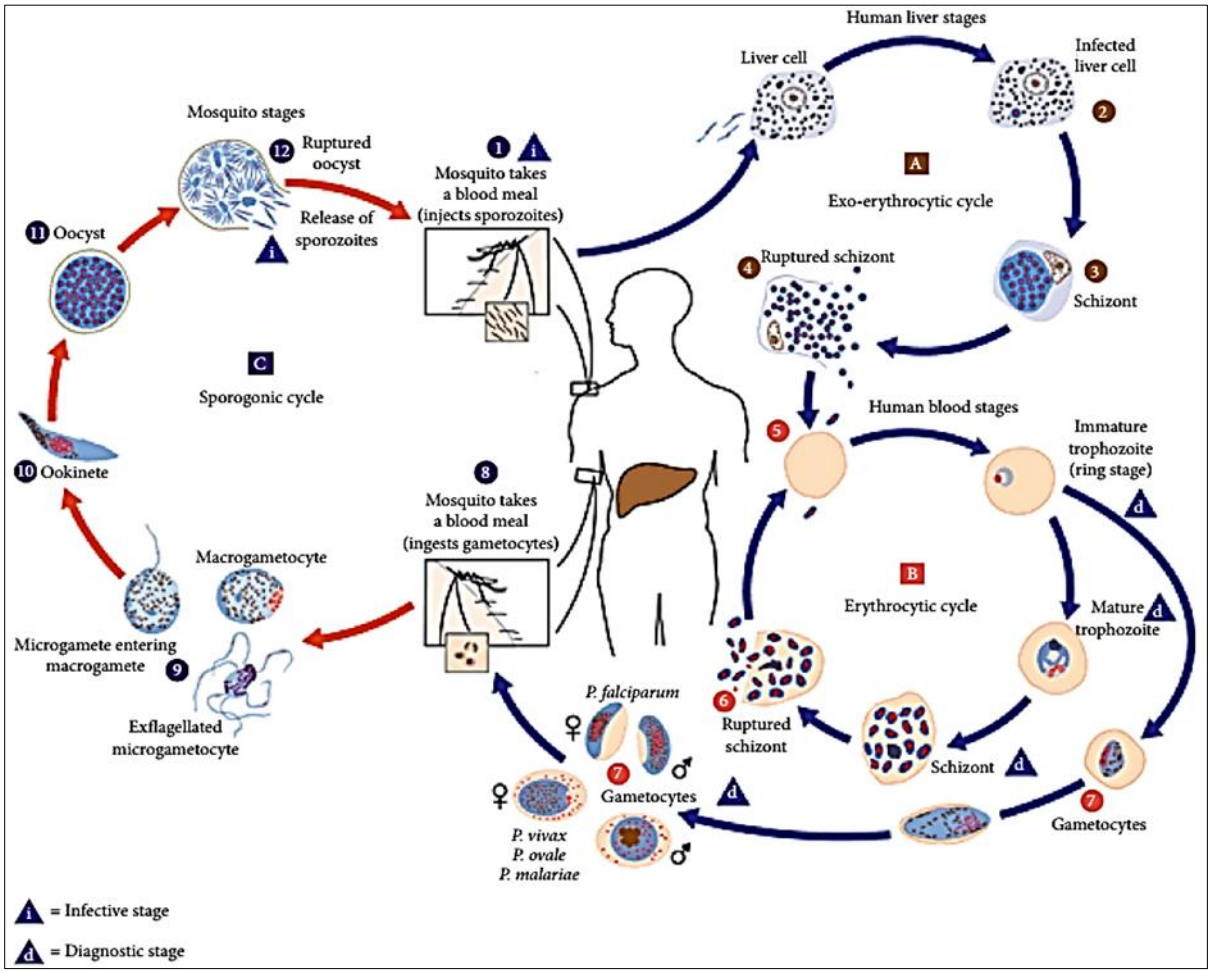

Figure 1 Life Cycle of Malaria Parasite (WHO, 2013c; CDC, 2016)

When the erythrocytic schizont ruptures, the released merozoites continue the life cycle by invading other RBCs. Cyclical fevers are typically happening shortly before or at the time of RBC lysis as schizonts rupture to release new infectious merozoites. This occurs every 48 h in tertian malaria and every 72 h in quartan malaria infection. During this repeated cycle, some merozoites differentiate into male and female sexual forms known as erythrocytic gametocytes with one nucleus and then awaiting the arrival of a blood-seeking female *Anopheles* mosquito (Jiraprapa *et al*., 2002; NIAID, 2007).

Then intake of gametocytes by the mosquito induces gametogenesis. The flagellated forms of microgametes, formed by exflagellation, penetrate or fertilize the macrogametes generating zygotes. The zygotes change into ookinetes and then become a round oocyst. Inside the oocyst, the nucleus divides repeatedly, with the formation of a large number of sporozoites and enlargement of the oocyst (WHO, 2013c). When the sporozoites are fully formed, the oocyst bursts, releasing the sporozoites into the haemocoel (the mosquito's body cavity). The sporozoites migrate to the salivary glands, thus completing the life cycle (Figure 2.1). Entrance of the sporozoites from the mosquito's salivary glands into a new human host perpetuates the malaria life cycle (WHO, 2013c; CDC, 2016).

1.4. Diagnosis of Malaria

Malaria must be diagnosed early and accurately to end up with an effective management of patients. Broadly, one can classify it into clinical and parasitological diagnoses. Clinical diagnosis is based on the patient's symptoms and on signs at physical examination (WHO, 2015a, CDC, 2016).

All of the suspected malaria should be confirmed with a parasitological diagnosis in all settings (WHO, 2015a). Light microscopy and RDTs are routinely employed methods for parasitological diagnosis of malaria. Detection of the parasites on giemsa-stained peripheral blood smears by light microscopy is used as the gold standard for diagnosis of malaria. As *knowlesi* and *malariae* have almost similar morphology, microscopy alone is insufficient to diagnose *knowlesi* (Murray, 2009; WHO, 2012). In case of *vivax, ovale*, and *malariae*, all development stages subsequent to the liver cycle can be seen in the peripheral blood. However, in *falciparum*, only ring forms and banana-like gametocytes are usually present in the peripheral blood since mature parasites become sequestered (Nicoletta *et al*., 2015).

1.5. Malarial Vector

Insects are major vectors that transmit several diseases especially in the tropics (Bassey and Izah, 2017). Mosquito,a blood sucking vectors (Kamaraj *et al*., 2011), transmits many life threating diseases caused by some viruses andprotozoa including malaria, filariasis, yellow fever, dengue fever, encephalitis etc. especially in the tropical andSub tropical regions (Borah *et al*., 2010; Okigbo *et al*., 2010; Bagavan andAbdul Rahuman, 2011; Ghose *et al*., 2012; Bhattacharya *et al*., 2014a; Bhattacharya *et al*., 2014b; Alayo *et al*.,2015 Mukherjee *et al*., 2015; Ndiok *et al*., 2016; Pal *et al*., 2016; El Maghrbi, 2016; Keziah *et al*., 2016).

Typically, Aedes, Culex, Anopheles and Mansonia transmit diseases in humans and animals (Ivoke *et al*., 2009).Each of the genera causes specific disease conditions. For instance, Anopheles, specifically female Anopheles,transmits malaria. Several species of Anopheles exist but some notable species include *A. gambiae*, *A. funestus*, *A.arabiensis and A. melas* which are the major vectors of human malaria (Nmadu *et al*., 2015). Owoeye *et al*. (2016), Hamza *et al*. (2014) further reported that *A. gambiae* and *A. arabiensis* are the main species that transmit malaria. Bhattacharya *et al*. (2014b), Ndiok *et al*. (2016) also reported that *Culex quinquefasciatus* transmits lymphatic filariasis. Adebajo *et al*. (2014) reported that *Aedes aegypti* transmits chikungunya, yellow and dengue fevers. The occurrence of the vectors may be attributed to climatic conditions. For instance, Ojo and Mafiana (2005) reported that the equatorial region favours the growth of mosquitoes, and the low incidence in Northern Africa may be due to the dry Sahara desert. Malaria infection is mostly acquired in areas where human hosts carrying the *Plasmodium*parasites are found in addition to enough anopheline mosquitoes under suitable environmental conditions, especially temperature and humidity (Ani, 2004).

1.6. Plasmodiumfalciparum Chloroquine Resistance Transporter Gene, pfcrt

Plasmodium falciparum Chloroquine Resistance Transporter (*pfcrt*) protein is made up of 422 amino acids distributed over 10 transmembrane domains. Inside the structure there are 32 candidate codons for having point mutations that confer for changing *pfcrt* function (Figure 2.2). The majority of them occur at the site that faces the DV media. Binding of substrates to *pfcrt* does not require ATP activation as in P-glycoprotein molecules.The replacement of lysine by threonine on codon 76 (76^{T}) has been recognized as the key factor of chloroquine resistance in *P. falciparum* (Durand *et al*., 2012). Although observations in Uganda and Senegal (Thomas *et al*., 2002; Talisuna *et al*., 2012) have shown that the presence of 76^{T} on its own is not necessarily predictive of chloroquine resistance, all resistant parasites carried this mutant. Usually the 76^{T} mutation in *pfcrt* does not stand alone, but is accompanied by mutations on other codons (74^{I}, 75^{E}, 220^{S}, 271^{E}, 326^{S}, 356^{T} and 371^{I}) in African or South East Asian isolates. South American isolates may carry, besides 76^{T}, the mutations 72^{S}, 75^{E}, 220^{S}, 326^{S}, 356^{T} and 371^{I}(Al-Koofee and Mubarak, 2020). All resistant isolates carry at least the 76^{T} and 220^{S} mutations, and usually in addition the 86^{Y} mutation on *pfmdr1*(Al-Koofee and Mubarak, 2020).

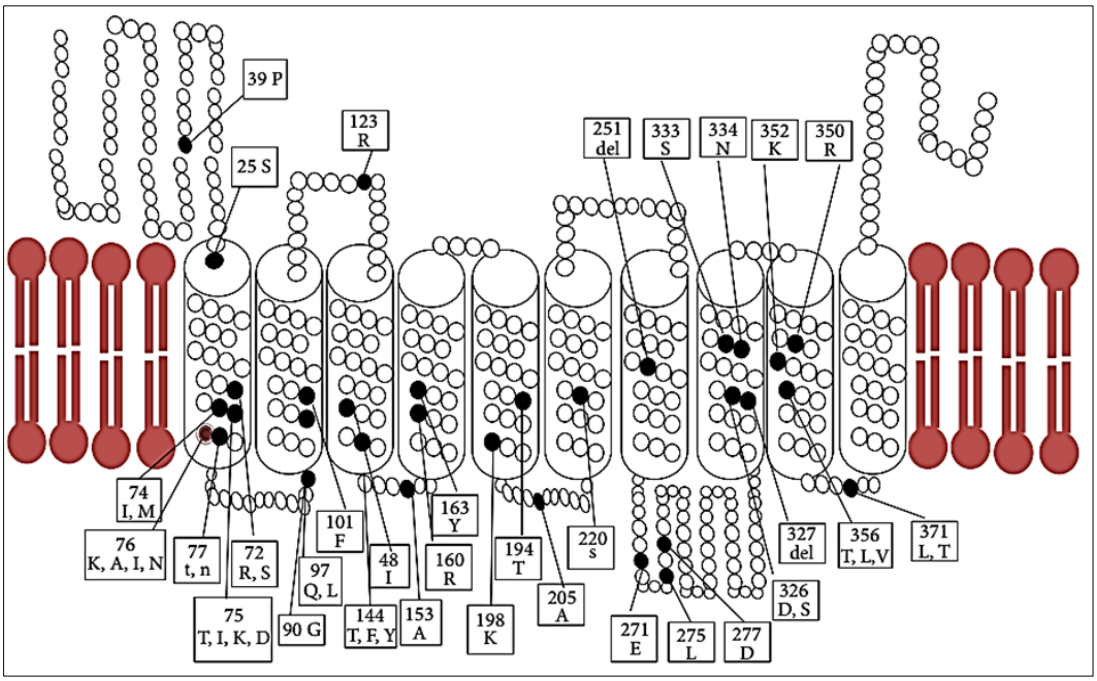

Figure 2 Protein structure of *pfcrt*(Ibraheem *et al*., 2014)

1.7. *Plasmodiumfalciparum* Dihydrofolate Reductase and Dihydropteroate Synthase Genes, pfdhfr and pfdhps

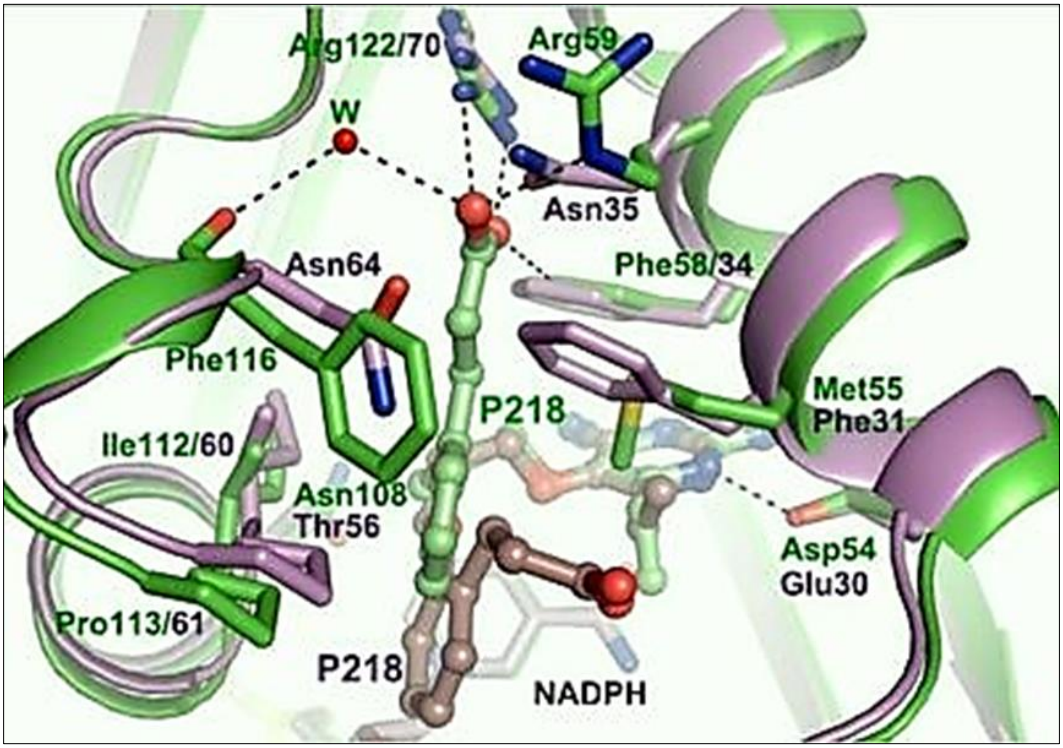

Figure 3 Structure of the *P. falciparum* dihydrofolate reductase (*Pf*DHFR) domain (Huang *et al*., 2012)

In view of their role in resistance to the sulfonamide/ pyrimethamine combinations, polymorphisms on the *pfdhfs* and *pfdhps* genes are here considered together. In sulfonamide/ pyrimethamine resistant *P. falciparum* numerous mutations on the *pfdhfr* gene have been observed, among which those at codons 108^{D} and 164^{L} are strong determinants of pyrimethamine resistance, usually complemented by the modulating mutations 51^{I} and 59^{R}(Figure 2.3) (Urdaneta *et al*., 1999; Thaithong *et al*., 2001). Other mutations that probably modulate pyrimethamine resistance are codons 16^{S}, 50^{R} and 164^{M} (Thaithong *et al*., 2001). Mutations 16^{V} and 108^{T} apparently modulate resistance to cycloguanil

(Thaithong *et al*., 2001; Schmider *et al*., 2003). The combination of 51^{I}, 59^{R} and 108^{D} is considered a reliable marker of pyrimethamine resistance (Schmider *et al*., 2003). Long-term *in-vitro* observations in *P. falciparum* under pyrimethamine pressure suggest that, quite apart from the aforementioned mutations, *pfdhfr* gene amplification is also an important mechanism of pyrimethamine resistance (Wernsdorfer and Noedl, 2003).

2. Material and method

2.1. Materials

2.1.1. Apparatus and Equipment

Refrigerated centrifuge (Centurion, UK), QIAquick purification kit (QIAGEN, Germany), Micro centrifuge (Prism –mini, Labnet, Korea), Vortex IR mixer (IR, Italy), 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, etc.

2.1.2. 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.

2.1.3. Design: Experimental Design

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 Kano.

2.2. Methods

2.2.1. Sample Collection

Finger prick filter paper blood samples were collected from patients below the age of 5 years. However, 2 mL of venous blood was drawn using sterile syringe and needle from children aged 6 and 14 years. Safety procedures were adopted in the collection of blood samples by swabbing the area to be sampled with disinfectant and allowing it to dry before collection. Blood samples were only taken after informed consent from a parent/guardian had been obtained. In addition, a structured questionnaire was administered to obtain the demographic information from parent/guardian of patients that were referred to the laboratory section of the hospital.

2.2.2. Determination of malaria parasite infection

Thin blood films were fixed with absolute methanol (BDH, England) for 10 seconds and were allowed to dry at room temperature before staining. Thick blood films were stained with 3% Giemsa (BDH, England), in Gurr® buffered water, pH 7.2 (BDH, England) for 30 minutes, as described previously in *Basic malaria microscopy* (1991). All thick and thin blood films were examined under the microscope at a magnification of × 100 with immersion oil.At least 200 fields had to be examined before designating a sample 'negative', or more accurately, 'no malaria parasites seen'. Positive findings were graded on the thick smear using the 'plus' system scale: + (1 to 9 trophozoites in 100 fields); ++ (1 to 10 trophozoites in 10 fields); +++ (1 to 10 trophozoites per field); ++++ (>10 trophozoites per field). These scores were used to estimate parasite densities: + = 10 to 90 parasites/µl; ++ = 100 to 1,000 parasites/µl, +++ = 1,000 to 10,000 parasites/µl; ++++ = >10,000 parasites/µl, assuming a white blood cell count of 8,000/µl.

2.3. Age distribution of the study respondents

The result of the age distribution of the study population revealed that the study population consisted more of children within the age group of 10 – 14 years (32%) followed by children between the ages of 5 – 9 years (30%). Children that aged between 1 – 4 years had frequency 21% (12 male and 9 female) while those that aged 1 – 12 months had frequency of occurrence of 17 (7 males and 10 females).

2.4. Geographical Location of the study respondents

The present study showed that Dala Local Government Area (LGA) had the highest number of patients (26%; 14 males, 12 females) followed by Gwale LGA, (24%; 13 males, 11 females) and Kano Municipal LGA (23%; 13 males, 10 females).

In NassarawaLGA, the males (9, 60%) had higher percentage distribution compared to the females (6, 40%). The result also showed that male and female patients from other LGAs Fagge, Kumbotso and Taurauni LGAhad percentage distribution of 4 (33.3%), 5 (41.7%) and 3 (25%) respectively(Figure 4.3).

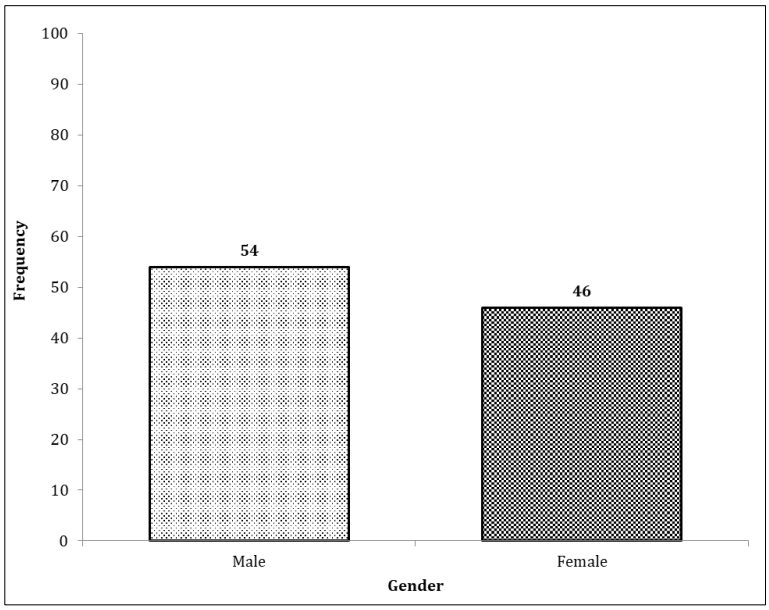

Figure 4 Gender Distribution of malaria patients 0-14 years old attending Murtala Muhammad Specialist Hospital, Kano (*N* = 100)

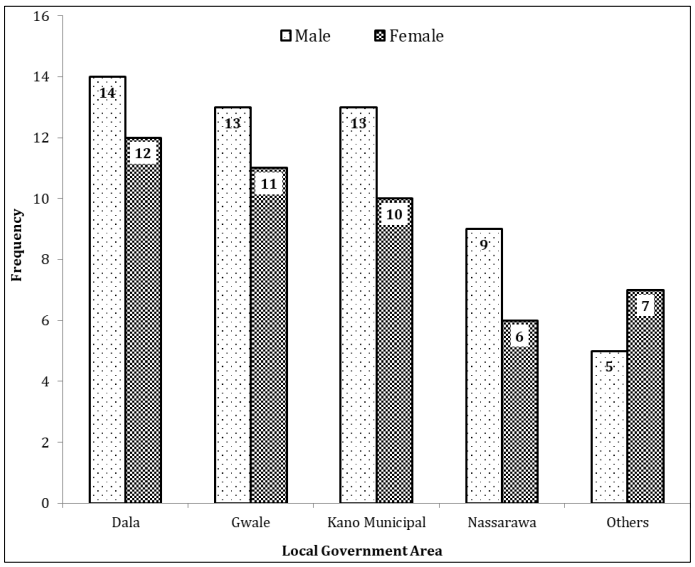

Figure 5 Population distribution of malaria patients 0-14 years old attending Murtala Muhammad Specialist Hospital, Kano according to Local Government Area *N* = 100

Table 1 Malaria Parasite Density of the Respondents 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)

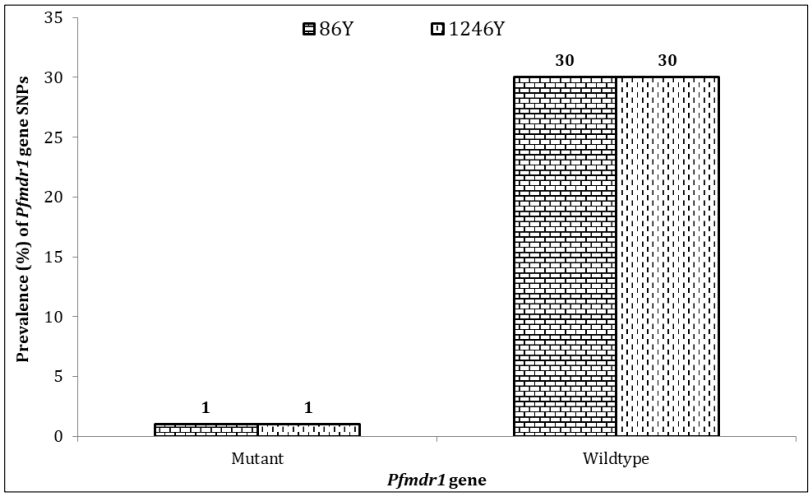

Figure 6 Prevalence of mutant alleles at 86Y and 1246Y of *Pfmdr1* gene in parasite isolates

3. Discussion

The prevalence of malaria significantly differed in the different age groups and is higher with increasing age. The current finding revealed that 62% of the malaria infected patients were within the age range of 5 – 14 years as compared to children below the age of 5 years. Such trends have been recently reported in both foreign and local studies (Ceesay *et al*., 2008; Mawili-Mboumba *et al*., 2013; Ogah *et al*., 2013; Noland *et al*., 2014). A 6-year cross sectional study done in Gabon and a similar study in Gambia observed that there was a shift in the prevalence of malaria from the kids under five years of age to older children (Ceesay *et al*., 2008; Ogah *et al*., 2013). The lower prevalence in such patients may be due to the recorded gains in the malaria control programs which had emphasized on this anagraphic category. This reduced exposure to the parasite in such category may have led to delayed acquisition of functional immunity making them more susceptible to malaria at an older age.The finding of the present study showed suboptimal adherence of 56% to the anti-malaria therapy. This finding significantly varied from the high adherence (90–100%) reported in similar studies (Dondorp *et al*., 2010; Bello *et al*., 2019). This may be an important problem, both in terms of clinical outcomes and also in the context of the development of resistance to artemisinin (Dondorp *et al*., 2010). However, it is unclear how good adherence must be for ACTs to be efficacious, and which features of adherence (such as correct timing of dose intervals or taking each dose with a fatty meal) matter most Following adoption of ACTs in many African countries, some studies from West Africa have linked the prevalence of the *pfmdr1* N86 allele to selection by artemether-lumefantrine (AL) (Okell *et al*., 2018). Therefore, the 3.2% prevalence of *pfmdr1* N86 allele observed in the present study might be suggestive of possible AL pressure in Kano State. In addition, the finding might also suggest that the efficacy of the LMF component of ACTs is susceptible to the emergence of decreased 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).

According to Ibraheem *et al*. (2014),emergence of drugs resistant strains of *Plasmodium falciparum* has augmented the scourge of malaria in endemic areas. Antimalarial drugs act on different intracellular targets. The majority of them interfere with digestive vacuoles (DVs) while others affect other organelles, namely, apicoplast and mitochondria. Prevention of drug accumulation or access into the target site is one of the mechanisms that plasmodium adopts to develop resistance. Plasmodia are endowed with series of transporters that shuffle drugs away from the target site, namely, *pf* mdr (*Plasmodium falciparum* multidrug resistance transporter) and *pf*crt (*Plasmodium falciparum* chloroquine resistance transporter) which exist in DV membrane and are considered as putative markers of CQ

resistance. 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.

4. Conclusion

The *Plasmodium falciparum* parasites in the paediatric malaria patients attending Murtala Muhammad Specialist Hospital Kanohave the drug resistance gene; *pfmdr1* at codons 86Y and 1246Y. The expression of this resistant gene by the *Plasmodiumfalciparum*parasites pose a significant danger to malaria chemotherapy as the parasite will be resistant tothe commonly used antimalarial drugs. However, socio-demographic characteristics of the participants does not influence the development of mutations in *P.falciparum* parasites.

Recommendations

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

- The present study observed that the resistance to artemisinin combination therapy (ACT) was caused by the prevalence of *pfmdr1* gene in the study population. However, further studies should be carried out to determine the association between increasing chloroquine (CQ) resistance and prevalence of *pfmdr1* 86Y alleles.
- Since the design of the current study was cross sectional, there was a limitation in establishing the association between the molecular resistance marker (*pfmdr1*) and clinical/treatment outcomes of patients. Hence, further study should evaluate the association between molecular resistance markers and clinical/ treatment outcomes of patients.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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