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A REVIEW ON THE MECHANISM OF DRUG RESISTANCE IN *PLASMODIUM FALCIPARUM*

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Abstract

Malaria is a life threatening parasitic disease that is caused by four parasites specie in the genus Plasmodium. Despite various efforts to prevent, control and eradicate the malaria at all levels, the disease continue to cause serious health problem especially in sub-saharan Africa. For decades chemoprophylaxis and chemotherapy using synthetic drugs remain the only options for prevention and management of the disease, as up to now there is no single effective vaccine for the disease. One major set-back in using these drugs is that, three*(P.falcifarum,P.vivax and P.ovale)* of the five species have developed resistance to most commonly and affordable chemoprophylactic and chemotherapeutic drugs, but the geographic distribution of resistance to any given drugs varies greatly. Therefore, the aim of this paper is to review the mechanism of drugs resistance in the three classes of antimalarial drugs: Quinoline (Chloroquine), Antifolate (Sulphadoxine Pyramethamine) and Artemisinin. One of the major causes of antimalarial drug resistance is spontaneous mutations that confer reduced sensitivity to a given

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drug or class of drugs. Other causes include use of sub standard drugs and sub-curative doses, drug pressure and accumulation of less than the lethal dose in the digestive vacuole of the parasite through high rate of efflux. Generally, resistance *in P.facifarum is associated* with mutations in adverse drug reaction (ADR) genes. For chloroquine, resistance in *Plasmodium falcifarum* is associated with mutation in *Plasmodium falcifarum chloroquine transporter* gene*(pfcrt)* at position C72S/R,73,74,N75E/D/K/I ,K76T, Q271E,N326S,I356T and R371I. For *Plasmodium falcifarum multidrug resistance-1gene (pfmdr-1)* mutations at position N86Y, Y184F, 1034, N1042D,and D1246Y are linked with drug resistance. For antifolate resistance, mutations in *pfdhfr* at position A16V, N51I, C59R, S108N and I164L are associated with antimalarial drug resistance, while mutations at position S436A, A437G, K540E/N, A581G and A613T/S are associated with drug resistance in *pfdhs.* For artemisinin mutations on *k13* propeller gene at position Y493H, R539T, I543T and C580Y are linked with antimalarial resistance. Use of sub-standard and sub-curative doses of antimalarial drugs should be avoided and monitoring of genetic mutations should be carried out regularly in order to halt the spread of resistant gene in a population.

Introduction

Malaria is a life threatening parasitic disease and it is regarded as a complex and overwhelming public health problem. The disease is caused by four species of Plasmodium parasites (*P. falcifarum, P.vivax, P.malariae and P.ovale*) and is transmitted through the bite of an infected female mosquito during blood meal (White, 2004). Majority of the infections and deaths are caused by *P. falciparum* (Ross and Fidock, 2019). Pregnant women and children under the ages of five are more vulnerable to the disease due to a very weak immunity. Due to the endemic nature of malaria in Nigeria, partial immunity to malaria is acquired among older children. However, severe forms of malaria can be seen in children less than five years of age who have not yet acquired the immunity. In 2015, 69% of

the malaria deaths that occurred worldwide were among children aged below 5 years (WHO, 2016).

The disease remain the most widespread infectious disease in the world, affecting nearly 214 million people in more than 64 countries, 45% of the world's population, with 438,000 deaths (Sarmah *et al.*, 2017). In 2015, Sub-Saharan Africa alone accounted for 90% of the malaria cases and 92% of the malaria deaths worldwide. Nigeria and Republic of Congo are two major African Countries contributing to the high malaria burden, as 36% of the malaria cases worldwide occurred in these countries (WHO, 2015). Malaria varies commonly in epidemiology and level of transmission in different parts of the world. This happens due to some factors such as malaria parasites species that occur in a given area (Bloland, 2001).

Despite various efforts to prevent, control and eradicate malaria at all levels, the disease continue to cause serious health problem especially in sub-Saharan Africa. For decades chemoprophylaxis and chemotherapy remain the only option for prevention, treatment and management of the disease. All these depend solely on synthetic Antimalarial drugs, as up to now there is no single effective vaccine for the disease. One major set-back in using these drugs is that, three *(P.falcifarum,P.vivax and P.ovale)* of the five species that cause the disease have developed resistance to the most commonly and affordable chemoprophylactic and chemotherapeutic drugs, which result in consistent changes in antimalarial drug policy (Nsanzabana *et al.,* 2018). This has also leads to the implementation of artemesinin-based combination therapy (ACT) by the World Health Organisation as a recommended drug for malaria treatment (Henry and Sanjeev, 2012).

Therefore, the aim of this paper was to review the mechanism of resistance in *Plasmodium falcifarum* in relation to quinoline (Chloroquine), antifolate (Sulphadoxine/Pyramethamine) and Artemesinin.

METHODOLOGY

For the purpose of this review, authentic and reliable data bases such as PubMed,Web of Knowledge, Web of Science, Scopus, Google scholar,

Sci.Hub and some other reliable source of published articles were consulted in order to access relevant, authentic and reliable published articles that are directly or in directly connected to the biology and mechanism of resistance in *Plasmudium falcifarum*. Different search terms like *Plasmudium falcifarum* biology, antimalarial drugs and their mode of action, drug resistance in plasmodium, Quinoline, Antifolate and Artemesinin resistance, mechanism of resistance, *Plasmudium falcifarum* life cylcle, and malaria treatment were used for the search of relevant published articles. All published articles and full text of the of the searched literatures were read and critically reviewed and evaluated. After which, all documents and published articles that were directly or indirectly relevant to the mechanism of resistance in P*lasmodium falcifarum* were grouped and arranged according to the review component.

RESULT AND DISCUSSION

Life cycle of *Plasmodium falcifarum*

Plasmodium parasites have a sexual life cycle in Anopheles mosquitoes and an asexual cycle in vertebrates (Figure 1). In humans, sporozoites transmitted in a mosquito blood meal travel through the skin into the bloodstream, where they quickly move in to the liver and invade hepatocytes (Ross and Fidock 2019). After 1 week of development, tens of thousands of *P. falciparum* merozoites are released from each infected hepatocyte into the blood-stream, where they rapidly invade red blood cells (RBCs)(Kheir, 2011). Intra-erythrocytic residence limits exposure to the immune system, and parasites also suppress the immune response through processes including antigenic variation, cytoadherence to the vascular endothelium to prevent splenic clearance, and induction of immunosuppressive cytokines.

P. falciparum parasites progress through an asexual blood stage (ABS) cycle lasting approximately 48 hrs. An invading merozoite matures to the ring stage, then trophozoite, then schizont, and finally bursts out of the host RBC as 8–24 daughter merozoites that can reinitiate further Asexual Blood Stage cycles. 1%–2% of the Asexual Blood Stage parasites will undergo sexual differentiation into male and female gametocytes. Gametocytes are taken

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up in a mosquito blood meal, where they undergo sexual recombination and develop into sporozoites, ready to infect another person (Phillips *et al.*, 2017).The majority of current antimalarials focus on the Asexual Blood Stage, and drug discovery efforts increasingly focus on compounds that also act on liver or mosquito transmission stages as the lower parasite numbers may decrease the potential for drug resistance (Burrows *et al.*, 2017).

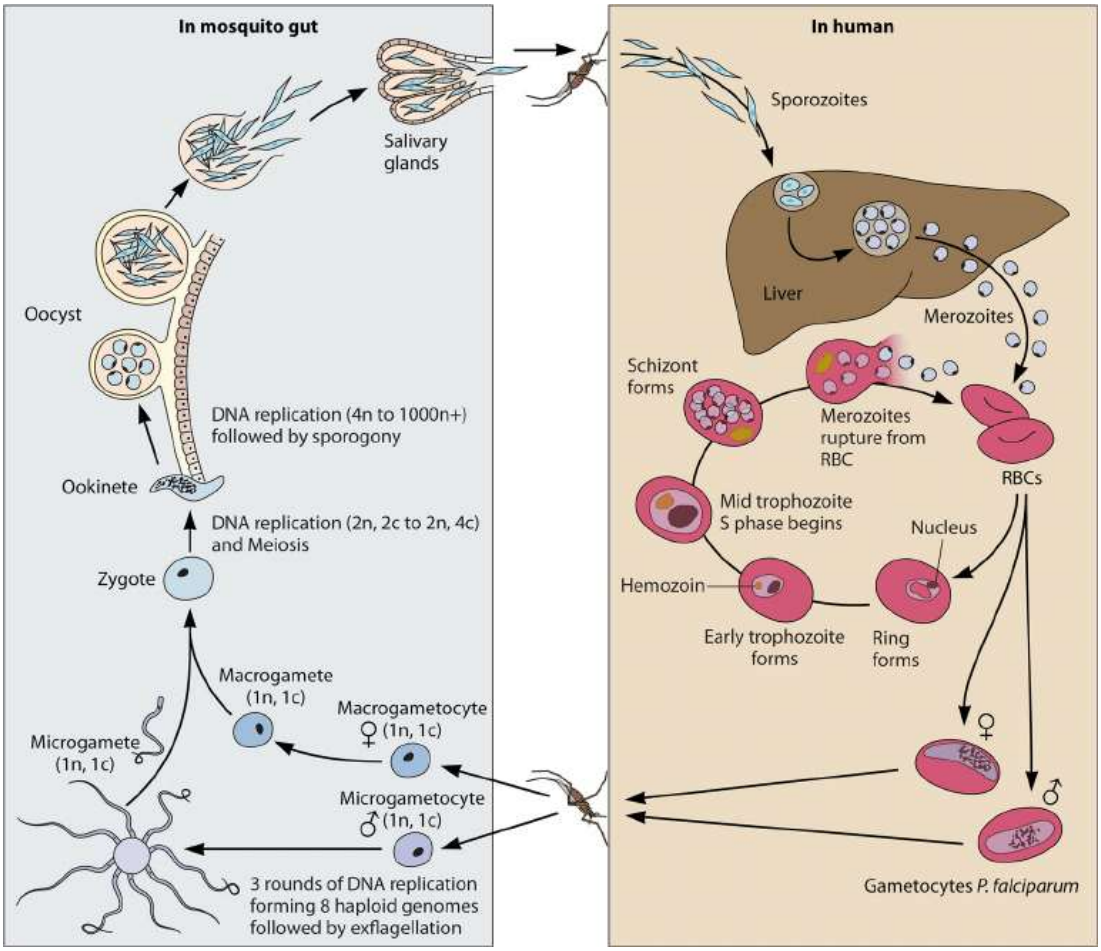

Figure 1: Plasmodium parasite sexual and Axesual life cycle (Ross and Fidock 2019).

ANTIMALARIAL RESISTANCE

Antimalarial drug resistance is a major concern for malaria control and elimination programmes. In fact,*Plasmodium falciparum* parasite has consistently developed resistance to the most widely used antimalarial,

pushing malaria control programmes to regular changes in antimalarial drug policy (Nsanzabana *et al*., 2018).

Antimalarial resistance is defined as the ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject (WHO, 2010b). This definition was later modified to include the sentence: "The form of the drug active against the parasite must be able to gain access to the parasite or the infected erythrocyte for the duration of the time necessary for its normal action" (Paloque, 2016). The development of resistance can be considered to occur in two phases. In the first phase, an initial genetic event produces a mutant (de novo mutation), the new genetic trait gives a parasite a survival advantage against the drug. In the second phase, the resistant parasite is selected and begins to multiply, eventually resulting in a parasite population that is no longer susceptible to treatment (WHO, 2010a). Resistance also develops more quickly, where a large population of parasite is exposed to drugs pressure since it will remove sensitive parasite, while resistance parasite would survive (Mebrahtu, 2015).

The emergence of resistance to both old and new anti-malarial and its subsequent spread to non-infecting areas undoubtedly make the situation more very bad. Intervention by WHO and other malaria controlling agencies/institutions, it still exists as endemic diseases in densely populated South-East Asian and Sub Saharan African countries. In both the regions malaria became highly problematic due to eruption of multi-drug resistant *P. falciparum* mutants. Few countries, like Bangladesh, Myanmar, Philippines, Thailand, Cambodia, Eastern India, Indo-Nepal border, and Myanmar-China border become the breeding ground of multi-drug resistant *Plasmodium falciparum* (Upadhyay, 2016).

TYPES OF RESISTANCE IN *PLASMODIUM FALCIFARUM*

Drug resistance in malaria is classified as resistance type I (RI), resistance type II (RII) and resistance type III (RIII)**.** In resistance type I, there is an initial clearance of the parasite but re-appear within a month after the onset of

the treatment, while in typeII there is a reduction parastaemia after treatment but failure to clear the parasite with a sudsequent rise in parastaemia. Resistance type III is the severe form of resistance, where the parasites show no significant change with treatment (Chessbrough, 2005).

CAUSES OF RESISTANCE

There are basically two ways by which resistance occurs these are spontaneous mutation and drug abuse. These spontaneous mutations can be either minor scale modification, such as insertion, deletion or variation in a nucleotide (frame-shift mutation or single-nucleotide polymorphism), or bulky transiguration of large chromosomal regions (gene amplification/deletion/copy number variations) (Maïga *et al*., 2007). For some drugs, a single genetic event may be all that is required. A single point mutation in the parasite genome is sufficient to confer resistance (e.g. atovaquone), while for other drugs, multiple unlinked events (epistatic modulation) may be necessary (Roper *et al.,* 2004).

Resistance also arises from drug abuse such as: incorrect dosing, non-compliance with duration of dosing regimen, poor drug quality, drug interactions, poor or erratic absorption and misdiagnosis, probably all of these factors, while causing treatment failure (or apparent treatment failure) in the individual, may also contribute to the development and intensification of true drug resistance through increasing the likelihood of exposure of parasites to suboptimal drug levels (Bloland, 2001).

MECHANISMS OF ANTIMALARIAL RESISTANCE

The ability of malaria parasites to develop resistance is primarily due to the high burden of parasites in an infected person's bloodstream during the asexual blood stage of infection in conjunction with the mutability of the parasites' genomes. Mechanisms of resistance include factors such as spontaneous mutation, interaction of drug pattern, characteristics of drug itself, human host, parasite, vector and environmental factors (Abdel-Muhsin and Mackinnon *et al*. 2003).

Spontaneous mutations, in the particular genes encoding the drug target, cause the reduction in drug accumulation or efflux (chloroquine, amodiaquine, quinine, meloquine, halofantrineresistance) or reduced affinity of the drug target (pyrimethamine, cycloguanil, sulphonamide, atovaquone resistance), which finally enables the parasite to withstand the antimalarial treatment. Afterwards, the drug pressure facilitates the resistant parasites to propagate by eliminating the susceptible parasites, which are usually fit and would outcompete the resistant ones in the absence of the drug (Rossi, *et al.,* 2017).

MECHANISM OF RESISTANCE TO QUINOLINE (CHLOROQUINE)

Chloroquine resistance was first reported in both South America and South East Asia in late 1950s. By the 1980s chloroquine resistance has reached a proportion of global menace. The situation is presently worsened to such an extent that the genotype of malaria parasite so obtained from central Americas where chloroquine was earlier to be effective, has also begun to exhibit features of resistance (Dipanjan and shivaprakash, 2016). Since then chloroquine resistant strains have spread throughout the ranges where the conditions are favorable for the development of the parasite especially in the regions of sub-Saharan Africa, but some regions were more affected by these resistant parasites than others.

Almost 80% of malaria parasite are chloroquine resistant and spread all over the world at present (Sandeep and Shailja, 2014), for that, chloroquine is no longer recommended to treat malaria in sub-saharan Africa (Linda *et.al.,* 2009).The emergence and spread of drug resistant malaria parasites in endemic regions has posed a great threat to usefulness of chloroquine (CQ) (Folarin,et.al.,2008), thus, limiting the effective use of this low cost antimalarial drug(Muheet, *et.al.,*2013). The available epidemiological data suggest that, chloroquine resistance in Africa may have been imported from southeast Asia in the late 1970s (Donald, *et.al.,*1988**).**At the moment, all sub-Saharan African countries have reported the presence of chloroquine resistant strains.

Although, the exact mechanism of action and resistance to quinolones (chloroquine) have not been fully elucidated (Jelagat, et.al.,2014), however, its believed that the malaria parasite, through some uncertain mechanism effluxes chloroquine from vacuole to survive the drugs pressure (Diganta, *et.al.,* 2014). Studies which demonstrate that, susceptible and resistant parasite initially accumulate chloroquine at the same rate (28 to 29fmol/10^6 parasitized erythrocyte per min), suggest that, the chloroquine-concentrating mechanism is the same in susceptible and resistant parasite (Donald, *et.al*.,1988**),** but resistant Plasmodium falcifarum parasites have a mechanism for releasing chloroquine (an efflux process). This efflux is either absent or greatly reduced in the susceptible parasite.

The fact that, the initial rate of Chloroqine accumulation is the same in resistant and susceptible parasite suggest that, efflux causes the observed difference in steady-state Chloroqine accumulation and that, it may be the only significant difference between resistant and susceptible parasites. The resistant Plasmodium falcifarum parasite releases chloroquine 40-50 folds more rapidly than the susceptible parasite.

In addition, various genetic alterations have been shown to be associated with Chloroqine resistance. Mainly, two genes known as *P. falciparum* multi drug resistance-1 gene (*Pfmdr1)*,(which is found on chromosome 5) which codes for Pgh1, a P-glycoprotein homologue, and the Chloroqine resistance transporter gene (*Pfcrt)*, (which is found on chromosome 7) which codes for CQ resistance transporter protein have been identified as potential candidates of Chloroqine resistance (Wahib,et.al.,2012). PfCRT is a 48 kDa protein containing 424 aminoacids, 10 predicted transmembrane-spanning domains and is localized to the DV membrane in erythrocytic stage parasites. Fifteen polymorphic amino acid residues in PfCRT are associated with CQR in field isolates (Ferreira, 2010).*Plasmodium falciparum* multidrug resistance 1(PfMDR1) contains five amino acid polymorphisms that are suggested to be involved in altered drug transport from the parasite's cytosol in to the digestive vacuole (DV) (Friedrich *et al.*, 2014).

Resistance to chloroquine is associated with a Lysine/Threonine amino acid substitution at position 76(K76T,) in the Pfcrt gene (Makoah and

Gabriele,2013) other mutations include C72S/R,73,74,N75E/D/K/I , Q271E,N326S,I356T and R371I. Several point mutations in *Pfmdr1* gene at positions 754, 1049, 3598, 3622 and 4234 result in amino acid changes at codons N86Y, Y184F, 1034, N1042D & D1246Y, respectively. These amino acid changes have been shown to be associated with CQ resistance. Out of the several mutations described, the mutation in codon 86 (from asparagine to tyrosine, N86Y), involved in the substrate specificity of the gene product (P-glycoprotein), appears to be the most important as this may alter the transport activity of the protein (Sandeep, *et.al.,* 2014).

MECHANISM OF RESISTANCE TO ANTIFOLATE

Drugs that target the folate synthesis pathway have a long history of effectiveness against a variety of pathogens (Sarmah *et al.,* 2017; McCollum *et al.,* 2006). As antimalarials, the antifolates are safe and well tolerated, but resistance emerged quickly and has persisted even with decreased drug pressure. The primary determinants of antifolate resistance in *Plasmodium falciparum* are well-described point mutations in the enzymes dihydropteroate synthase (*dhps*) and dihydrofolate reductase (*dhfr*) targeted by the combination sulfadoxine–pyrimethamine (SP) (Adina and Laura,2015).

The discovery of changes in codons of *pfdhfr*-gene, strongly indicated that single amino acid changes lead to observe resistance (Ole, et.al., 2003). It has been well established that, mutations at codon 108 of plasmodium falcifarum from serine to asparagine (S108N) reduces the sensitivity of the drug. Indeed, almost all the parasite isolate showing pyramethamine resistance were found to contain this mutation (Sharma, 2005). In addition, mutations at amino acid position 51, 59 and 164 have been linked with resistance of *Plasmodium falcifarum* to antifolate antimalarials, absolute resistance is conferred by addition of I164L mutation in quadruple mutant form N108/I51/R59/L164 (Ole, et.al.,2003).

According to Alyson *et.al*.(2010), resistance to antifolate results from the accumulation of mutation in the *Plasmodium falcifarum dhfr,* principally, A16V, N51I, C59R, S108N or S108T and I164L. In addition mutations at codons

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436, 437, 540, 581 and 613 of *pfdhps* are also associated with resistace to antifolate (Triglia, *et.al.*,1997).

MECHANISM OF RESISTANCE TO ARTEMISININ

Artemisinins are found to be active against multi-resistant strains of *P. falciparum* and have broad stage specificity against the *Plasmodium* life cycle including activity throughout the asexual blood stages and also the sexual gametocyte stages which may reduce the spread of the disease in areas of low transmission (Neill *et al.*,2010). PfATP6 is the only SERCA-type Ca^{2+}ATPase present in the malaria parasite and artemisinin exerts its action via PfATP6. Inhibition of this enzyme subsequently inhibits the action of artemisinin (Bhattacharjee and Shivaprakash 2016). Artemisinin resistant of *P. falciparum* was originated from Thai-Cambodian border and dispersed to many other malaria endemic countries including Africa (Mishra *et al.*,2016). Artemisinin resistant is define as the delayed parasite clearance following treatment with an artesunate monotherapy or with artemisinin- based combination therapy (WHO,2016). Artemisinin-resistant *Plasmodium falciparum* malaria has emerged in western Cambodia and has been detected in western Thailand. The situation is ominously reminiscent of the emergence of resistance to chloroquine and to sulfadoxine–pyrimethamine several decades ago. Artemisinin-based combination treatments are the mainstay of treatment for *Plasmodium falciparum* malaria globally, but artemisinin resistance, evidenced by delayed parasite clearance after artemisinin treatment, is now prevalent across an expanding area of southeast Asia and is characterised by reduced susceptibility of the ring stage of parasite development. It is clearly associated with increasing rates of failure of artemisinin-based combination treatments (Kyaw,*et.al.*,2015). Artemisinin resistance is a major threat to global public health, with the most severe potential effects in sub-Saharan Africa, where the disease burden is highest and systems for monitoring and containment of resistance are inadequate (Ambrose, *et.al.*, 2012).Combined therapy with artemisinin derivatives plus amodiaquine or lumefantrine is now implemented in Africa (Halima,*et.al.*,2006)

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A genome-wide association approach pinpointed a specific gene associated with artemisinin resistance, the K13 gene on chromosome 13 of *Plasmodium falcifarum* (Zaw and Myo, 2015). Mutations in the Kelch13 (K13) - propeller domain were shown to be associated with delayed parasite clearance in vivo and in vitro. Analysis of the of the recently identified molecular marker for artemisinin resistant showed that C580Y mutation was the most prevalent in Greater Mekong Sub region, but many other mutations in and near the K13 region were also found to be associated with artemisinin resistance, for example N458Y,Y493H,R539T and I543T (WHO,2014; Daily, 2016). Similarly, amino acid mutation at position 263 of *PfATP6* enzyme tremendously affects the sensitivity of the enzyme to artemisinin (Nagasundaram *et al.*,2016)

However, since the list of mutations associated artemisinin resistance is still evolving, the definition of artemisinin resistance will continue to evolve based on the new findings. The current definition of artemisinin resistance is divided into suspected artemisin resistance, which is defined as the high prevalence of delayed parasite clearance or highest prevalence of *K13* mutant and Confirmed resistance which is regarded as the of delayed parasite clearance and *K13* resistance- validated mutations for the same patient (WHO, 2016).

CONCLUSSION AND RECOMMENDATIONS

Plasmodium falcifarum has developed resistance to almost all the classes of antimalarial drugs in use, and one of the major factors associated with this resistance is genetic mutations on some selected ADR-genes at some specific position on the genes. Other factors like drug pressure, the use sub-standard and sub-curative dose contribute immensely towards antimalarial resistance in *Plasmoduium falcifarum.*

Use of sub-standard and sub-curative doses of antimalarial drugs should be avoided and monitoring of genetic mutations should be carried out regularly in order to halt the spread of resistant gene in a population.

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