Antimalarial Drug Resistance and Vulnerable Groups

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6.1 Introduction

Malaria is a protozoan infection in humans caused by the parasite Plasmodium which until recently was thought to be classified into four species: *Plasmodium falciparum, Plasmodium ovale, Plasmodium malariae* and *Plasmodium vivax*. However, further study (Sutherland et al. 2010) has shown that there are two non-recombining species of *ovale* (*ovale curtisi* and *ovale wallikeri*) which are non-sympatric in nature (Oguike et al. 2011). Of all the species, *Plasmodium falciparum* is the agent of the most malignant form of malaria, usually presenting with severity mostly in children in sub-Saharan Africa (Urdaneta et al. 2001). It is the most dangerous form of malaria with the highest rates of complications. It is also the commonest species in virtually all parts of Africa accounting for up to 98% of the confirmed cases in Nigeria and is associated with significant morbidity and mortality. *Plasmodium falciparum* is responsible for virtually all the features of severe malaria. *P. malariae* usually occurs as a mixed infection with *P. falciparum* (NPC, NMCP and ICP 2012). The main vector of malaria in Nigeria is *Anopheles gambiae* but *Anopheles funestus* and *Anopheles arabiensis* are also commonly encountered. *Anopheles melas* is found in the coastal areas (NPC, NMCP and ICP 2012).

Malaria remains a major and important health problem facing developing countries especially in Africa where it has been associated with great morbidity and mortality. Even though half of the world's population is at risk of malaria, it is essentially a disease of the tropics and subtropics particularly the sub-Saharan African region. Malaria cases have also been reported in temperate areas as a result of human migration from tropical countries. The African region carries a disproportionately high share of the global malaria burden, with 94% of malaria cases and deaths in 2019.

The current World Malaria Report 2020 from the World Health Organization shows an estimated 229 million cases of malaria globally in 87 endemic countries, a decline from 238 million in 2000. The report also established a 60% decrease in mortality over the period of 2000–2019, from 736,000 in 2000 to 409,000 in 2019. The African region was home to 94% of malaria cases and deaths in 2019. About 95% of global malaria deaths occurred in 31 countries and about half of these deaths occurred in Nigeria (23%), the

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Democratic Republic of Congo (11%), the United Republic of Tanzania (5%), Mozambique (4%), Niger (4%) and Burkina Faso (4%). At the beginning of 2020, malaria deaths were at the lowest point ever (World Malaria Report 2020).

Earlier reports show an estimated 863,000 malaria deaths, 767,000 (89%) of which occurred in Africa where malaria is the leading cause of mortality in children under 5 years. However, the World Malaria Report 2011 indicates that over a period of two years, malaria cases had reduced from 243 million cases in 2008 to 216 million in 2010 and the number of deaths had reduced to 655,000 (WHO 2011). Despite this reduction, the global burden of malaria has remained very high, especially in the tropics.

The burden of malaria on the endemic countries transcends beyond the health problems, as it has been associated with hampering the development with a high proportion of the wealth of the nation being drained by the disease. About 8.8 million US dollars loss has been attributed to malaria in the form of treatment costs, prevention and loss of person-hours annually (FMOH 2005). Actions taken to reduce malaria transmission as well as antimalarial drug resistance will work towards achieving the SDG3 goals.

6.2 Treatment of Malaria

SDG3 calls for good health and well-being of people. Drug treatment of malaria is the most common and possibly one of the most important measures for the control of malaria. The goals of treatment of uncomplicated malaria are: to provide a rapid and long-lasting cure, to reduce morbidity including malaria-related anaemia, to prevent the progression of uncomplicated malaria to severe and potentially fatal diseases, and to minimize the likelihood and rate of development of drug resistance in addition to reducing transmission (Sutherland et al. 2005; Sawa et al. 2013).

Recommended treatment for malaria according to the World Health Organization is the use of Artemisinin Combination Therapy (ACT), which involves the use of drugs containing an artemisinin-based drug with a partner drug with another mechanism of action so as to ensure the complete killing of the parasite in the bloodstream. Some of the partner drugs include: lumefantrine, amodiaquine, piperaquine, mefloquine, etc.

However, sometimes the desired treatment goal is not always achieved as a result of treatment failure. According to the National Guidelines for diagnosis and treatment of malaria (2015), causes of treatment failure include:

- Incorrect dosing of the drugs.
- Poor adherence to treatment.
- Poor quality of drugs.
- Interactions with other drugs.
- Poor absorption of drugs.
- Misdiagnosis of the patient.
- Drug resistance.

Among the many factors implicated in treatment failure, the present discussion is focused on drug resistance as a major cause of treatment failure.

6.3 Antimalarial Drug Resistance

The World Health Organization defines antimalarial drug resistance as 'The ability of a parasite strain to survive and multiply despite the proper administration and absorption of an antimalarial drug in the doses equal to or higher than usually recommended, provided drug exposure is adequate' (WHO 2015; WHO 2006). It is a shift to the right of the dose-response curve, thus requiring higher drug concentrations to achieve the same parasite clearance (White 2004). In many cases even the higher concentration results in treatment failure.

The implications of antimalarial drug resistance are such that there is continued transmission of drug-resistant parasites thereby limiting the efforts to control malaria. The spread of resistance through the population is facilitated through

increased gametocyte carriage that is transmitted upon new infection (Price et al. 1999). Higher rates of transmission of drug-resistant parasites are achieved through selective advantage which parasites carrying resistant genes develop as a result of the continued use of a drug with the prevalence of resistance in a locality (Handunnetti et al. 1996; Sutherland et al. 2002). Results have shown that the global burden of malaria in sub-Saharan Africa is predominantly maintained by antimalarial drug resistance (Barnes and White 2005). The resultant effect is increased difficulty in the management of malaria.

6.4 Causes of Antimalarial Drug Resistance

Widespread and indiscriminate use of antimalarial drugs exerts a strong selective drug pressure on the malaria parasites to develop high levels of resistance. This indiscriminate use includes self-medication, misdiagnosis, use of sub-optimal doses of the drugs, fake and substandard drugs, easily degradable drugs leading to poor bioavailability of the drugs, and use of monotherapy of the artemisinin drugs (Chijioke-Nwauche et al. 2021). Other factors are host factors (age, immune status, co-morbidity especially with HIV); spontaneous mutations in the parasite gene; parasite mutation rate; overall parasite load; mobile populations and migrants with resultant imported malaria; high treatment costs; and poor adherence to malaria treatment (Djimde et al. 2003).

Influencing factors to antimalarial drug resistance include parasite nature, pharmacological properties of the drug, host genetic factors particularly the immune status of the person and also age (Travassos and Laufer 2009). Individuals with lower immunity like HIV-positive patients, children and pregnant women are more vulnerable to antimalarial resistance. Reduced immunity allows the survival of a residuum of parasites thereby potentially increasing the development, intensification and spread of resistance (Byakika-Kibwika et al. 2010). Additionally, delayed cure rate and higher rate of recrudescence which occur in HIV-positive individuals accelerate the spread of resistant parasites and increase the parasite biomass in both symptomatic and asymptomatic carriers (Birku et al. 2002; Shah et al. 2006; Van Geertruyden et al. 2006). Host genetic factors have been shown to underlie some differences in resistance to malaria, and this has been observed between ethnic groups who live in the same area. It has been observed that the Fulanis have a lower incidence of classic malaria resistance genes than other sympatric ethnic populations (Quinn et al. 2017).

6.5 Vulnerable Groups

Vulnerable groups to malaria include pregnant women (especially in first pregnancies and it is worse in the second and third trimesters), children especially those under 5 years of age, immunocompromised persons [HIV-positive patients (a greater percentage are women)], transplant patients, patients that are undergoing cancer treatment, sickle cell anaemia patients, visitors from the non-endemic region and persons that have undergone splenectomy. The spleen controls the removal of infected red blood cells, and it is the first organ that generates immune response to malaria and usually gets enlarged during infection (del Portillo et al. 2012).

Malaria has been shown to have a long-term effect on cognitive function and educational attainment in children (Jukes et al. 2006; Clarke et al. 2008). Malaria is the commonest cause of fever and death especially in young children (WHO 2000). This is made worse by the situation of multi-drug resistance as a result of self-medication, sub-optimal doses of antimalarial drugs, improper diagnosis, and improper treatment due to resource limitations as is the case in Nigeria and many other sub-Saharan African countries.

In as much as malaria is not gender specific apart from the peculiar vulnerability of pregnant women, there are influencing factors to vulnera-

bility in women, and these include social, economic, and cultural factors and access to preventive and treatment measures. There is marginalization of women due to entrenched inequalities in areas such as education; women are less informed and have less access to treatment and preventive measures due to their low economic power and dependence on their husbands. This is particularly so in rural areas where there are more economically disadvantaged and low social status women (UNDP 2015). All these, in addition to the social pressure of providing meals for the family even when they are ill-disposed and their care-giving responsibility, predispose women more to malaria infection.

Malaria is the leading cause of morbidity and mortality in pregnant women in endemic regions such as Africa and Asia. The consequences of malaria in pregnancy include anaemia which causes about 10,000 maternal deaths, low birth weight babies due to intrauterine growth retardation, abortion, premature delivery, stillbirth and maternal death. About 11% of maternal deaths in Nigeria have been attributed to malaria. As a result of this, the WHO recommends a treatment measure to prevent malaria in pregnancy termed "intermittent preventive treatment for malaria in pregnancy" (IPTp). This is the administration of the drug Sulphadoxine-pyrimethamine (SP) to all pregnant women as part of their antenatal care usually starting in the second trimester. Each dose is given 1 month apart with the aim of taking at least three doses before delivery (WHO 2006). It is recommended that malaria be treated after a definitive or confirmed diagnosis based on tests either using a rapid diagnostic test kit (RDT) or microscopic confirmation of parasites from blood film.

Malaria is associated with many problems in pregnancy, and this includes maternal death, anaemia, abortion, poor fetal growth, low birth weight with consequences of child growth retardation and poor cognitive outcomes, stillbirth, and premature delivery which is more severe in primiparous (first time delivery) especially in partially or non-immune persons (World Malaria Report 2020).

6.6 Resistance and Immune Status of the Patient

The immune system plays a very important role in defence against the infections that attack the body, and malarial infection is not exempt from this. The human immune system is comprised of innate or natural and adaptive immunity. Adaptive/acquired immunity is antigen-specific, which develops after exposure to infection and provides long-lasting protection depending on the host, the type and the number of infections. The responses of adaptive immunity are essentially carried out by white blood cells (WBCs) which are made up of the lymphocytes, monocytes, granulocytes, basophils, neutrophils and eosinophils. In malaria infection, the T lymphocytes are the most important and are activated when a person is exposed to the antigens. The predominant T lymphocytes are the CD4 cells and are the major cells that control blood-stage malaria infection (Perlmann and Troye-Blomberg 2000). The CD4 cells are therefore seen as the bedrock of the body's immune system because upon exposure to malaria infection, they are primed for protection by initiating the recruitment and release of the other cells and chemicals that prevent the pathology of the disease. Furthermore, in HIV patients the CD4 cells are depleted leading to suppressed immunity, and this is worsened in malaria infection. HIV patients were more likely to have PCR-detectable parasitemia when compared to HIV-negative persons (Chijioke-Nwauche et al. 2013). Immunity reduces the chances of survival of resistant parasites by serving as a strong restraint on the emergence of resistance (WHO 2015).

The severity of the malaria disease is dependent on the speed of the response of the immune system. Individuals without pre-existing malaria immunity are at risk of malaria infection whereas in malaria-endemic areas where there is moderate or intense transmission, immunity that is acquired by virtue of several exposures plays a very important role in protecting the individuals from developing the clinical and severe form of

the disease (Bates et al. 2004; Cohen et al. 2005). Therefore, over time individuals infected with malaria acquire partial protection and therefore have a reduced risk of severe malaria.

Resistance to antimalarial drugs by *Plasmodium falciparum* parasite has become a major health problem since the first resistance to Chloroquine was recorded in Thailand and Cambodia in the late 1950s. This has spread to other malaria-endemic regions and against other antimalarial drugs like sulphadoxine-pyrimethamine (SP) and mefloquine. Studies (Noedl et al. 2008) reporting artesunate-resistant malaria in western Cambodia and reduced in vivo susceptibility of artesunate (Dondorp et al. 2009) and in vitro resistance to its derivative artemether (Jambou et al. 2005) indicate a great threat to the management of malaria since artemisinin and its derivatives are currently the mainstay for the treatment of uncomplicated malaria. More current reports of resistance to the latest generation partner drug piperaquine used in combination with dihydroartemisinin in Cambodia have led to increasing rates of treatment failure (Duru et al. 2016).

6.7 Mechanisms of Antimalarial Drug Resistance

Antimalarial drug resistance can result either from changes in drug accumulation or efflux or reduced affinity of the drug target resulting from point mutations in the respective genes encoding the target (White et al. 1999). Genetic polymorphisms in one or more genes that do not actually encode the drug target itself but affect drug efflux can lead to reduced drug concentrations within the parasites as in chloroquine, amodiaquine, quinine, mefloquine and halofantrine thereby resulting in drug resistance (Valderramos and Fidock 2006). The selection of parasites with genetic mutations gives rise to antimalarial drug resistance. Polymorphism involves nucleotide change of some of the base pairs in a DNA sequence possibly leading to amino acid change. When there is a change in a single base pair compared to the "common" or "wild type" sequence it is called single nucleotide polymorphism (SNP). These polymorphisms could be synonymous where the nucleotide change does not result in amino acid change, whereas a non-synonymous polymorphism involves an amino acid change. This mutation can cause a significant change in the gene which can result in resistance to the drug by the parasite. Citing an example from an unpublished study on resistance to antimalarial drugs, a nucleotide change from Cytosine to Thymine at position 290 of the gene resulted in amino acid change from Serine to Lysine. The consensus frame *Plasmodium falciparum* multidrug resistance (*pfmdr)* 3D7 is the *Plasmodium falciparum* wild type gene showing nucleotide Cytosine (C) at position 290 while on Sample 059 frame it has changed to Thymine (T). This change from Cytosine to thymine resulted in amino acid change from Serine (S) to Lysine (L) at position 97, hence it is depicted as S97L (Fig. 6.1). Similarly, in sample 207A the change at nucleotide position 218 from Thymine (T) to Cytosine (C) resulted in the amino acid change of Phenylalanine (F) to Serine (S) depicted as F73S.

Another mechanism of resistance is through altered affinity for the drug target caused by single or multiple point mutations in genes that encode the drug target as in pyrimethamine, cycloguanil, sulphonamide and atovaquone (Wang et al. 1997). Resistance can also be due to the expression of higher levels of the gene through amplification thereby resulting in increased copy number. Antimalarial drug resistance can also occur where there has been no prior resistance in the parent drug because of inadequately treated biomass infections (White and Pongtavornpinyo 2003) or reduced sensitivity to a given drug or class of drugs which occurs as a result of spontaneous mutations (Bloland 2001) and subsequent spread as a result of survival and multiplication. Where a large population of parasites are exposed to drug pressure, resistance is reported to develop more quickly (Farooq and Mahajan 2004).

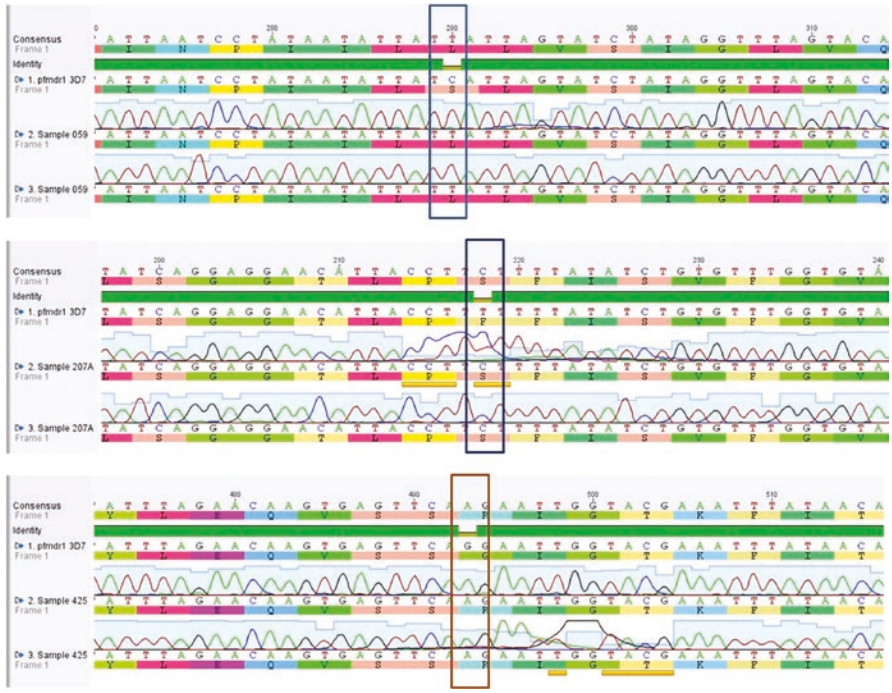

Fig. 6.1 Chromatograms showing examples of non-synonymous mutations from nucleotide changes in a genome of *Plasmodium falciparum* [Source: Unpublished data from PhD thesis of Ifeyinwa Chijioke-Nwauche: Use of Artemether-Lumefantrine in the treatment of asymptomatic malaria in HIV-positive and HIV-negative Nigerian adults in London School of Hygiene and Tropical Medicine, 2014].

6.8 Detecting Resistance

Detection of drug resistance to antimalarial drugs can be achieved through different ways. These include animal model studies, in vitro studies which involve use of drug assays, in vivo studies usually regarded as the gold standard and molecular characterization (Hiasindh and Subhash 2016). Molecular markers of drug-resistant malaria are based on genetic changes that confer parasite resistance to drugs used to treat and prevent malaria (Plowe et al. 2007). They have been proven to be tools for surveillance of resistance and provide additional data that complement clinical observations of the in vivo efficacy of a drug and have been instrumental to policy making with regards to the control of the malaria epidemic (Djimde et al. 2001; Mugittu et al. 2004). They have also served as monitoring tools in parasite drug susceptibility following a change in treatment policy (Laufer et al. 2006).

6.9 Implications of Antimalarial Drug Resistance to Public Health

- Increase in malaria transmission.
- Frequency in severe illness especially in vulnerable groups.
- Increased treatment costs.
- Higher parasite burden which leads to a higher likelihood of resistant parasites.
- Frequency of intake of antimalarials may increase the risk of adverse drug reactions, drug pressure on the parasites and subsequent spread of drug resistance.

Antimalarial drug resistance is a major public health challenge in endemic countries, and the impact is not easily quantifiable. High drug pressure on the parasites which comes as a result of treatment of acute manifestations of the disease rapidly propagates resistance in low transmission

areas. However, in areas of high transmission, the manifestation of resistance usually presents clinically, and there is increasing risk of severe anaemia as a result of prolonged or chronic infections. Reports have shown that when resistance develops, there is an associated 2–11-fold increase in malaria mortality in African children (Björkman and Bhattarai 2005). Other clinical consequences of antimalarial drug resistance are increased treatment failure, reduced treatment efficacy, delayed initial therapeutic response, complications such as anaemia and complications during pregnancy.

The World Health Organization advocates that to stop the spread of resistant parasites, in areas with no known resistance, control measures should be used to reduce transmission. Also, preventive measures to reduce transmission such as vector control methods including nets and indoor residual spraying should be used (WHO 2006). However, in areas with existing artemisinin resistance, a combination of control and elimination measures should be used to stop the survival and spread of resistance (WHO 2011).

6.10 Approaches to Fighting Resistance

To prevent or slow down the onset of resistance, the following should be done (World Health Organisation WHO. Global plan for artemisinin resistance containment (GPARC) 2011):

- Use of artemisinin combination drugs with different mechanisms of action because combination therapy slows resistance as recommended by WHO.
- Strict adherence to the dosage regimens as recommended by the manufacturers of the drugs.
- Adhering to the T3 (test, treat and track) principle of WHO. This implies testing to confirm parasite presence, treating with the right drug and tracking the patient for review.
- Definitive diagnosis of malaria before treatment, no presumptive or empirical treatment.
- Improved access to diagnostics to ensure definite diagnosis.
- Improved access to affordable, quality-assured artemisinin-based combination therapy.
- Avoidance of artemisinin monotherapy or partner drugs.
- Improve on documentation and reports of resistance.
- Increased monitoring and surveillance to evaluate resistance.

6.11 Conclusion

Resistance against antimalarial drugs has been a great public health change to the control of malaria. Transmission and spread of resistant parasites are major factors that have sustained the global burden of malaria, and this is particularly so in vulnerable groups. There is a need for continuous active surveillance and detection of molecular markers that will help in monitoring of resistance to the existing antimalarial drugs. The establishment of sentinel surveillance sites for monitoring molecular markers as well as in vivo and in vitro studies to arrest the trend of resistance will be beneficial in reducing the spread of resistant parasites.

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