

CHAPTER ONE

1.0 GENERAL INTRODUCTION

Malaria remains a major global public health concern, as one third of the human population is estimated to be exposed to the threat of the most virulent species; *Plasmodium falciparum* (Penna-Coutinho *et al.*, 2011). According to the latest estimates by the World Health Organisation (WHO), about 214 million cases of malaria occurred globally in 2013 and the disease led to about 438 000 deaths (WHO, 2015). The mortality levels are greatest in sub-Saharan Africa, where children under 5 years of age account for 90% of all deaths due to malaria. Malaria kills an African child every 30 seconds and 3,000 children every day (WHO, 2015). It is also of global concern because of its high economic burden on nations, high prevalence of mortality in children, pregnant women and non-immune individuals (Benjamin *et al.*, 2004). The burden is heaviest in the WHO African Region, where an estimated 90% of all malaria deaths reported worldwide occur, and in children under 5 years of age (WHO, 2015).

According to the Nigerian Medical Association (NMA, 2013), Nigeria accounts for a quarter of all malaria cases in the WHO African Region. The current malaria related maternal mortality is estimated at 11%, while malaria related annual death for children under-five years of age is estimated at around 300,000 with a loss of 132 billion to malaria annually in the form of treatment cost, prevention and loss of man hours.

Human malaria is caused by infection with intracellular parasites of the genus *Plasmodium* that are transmitted by female *Anopheles* mosquitoes. There are four species of *Plasmodium* that infect humans namely: *P. vivax*, *P. ovale*, *P. falciparum* and *P. malariae*, with *P.*

falciparum being the most lethal in sub-Saharan Africa. This is due to the fact that *P. falciparum* is able to migrate into the small blood vessels of vital internal organs. It is also not confined to peripheral blood unlike the other species. The situation is compounded by various constrictions due to the 'malaria toxin', resistance to anti-malarial drugs and insecticides, decay of public health infrastructure, population movements, and environmental changes remain major contributors to the spread of malaria (Greenwood and Mutabingwa, 2003).

A good understanding of the communities' culture- beliefs and behaviour, has been demonstrated to be crucial to the success of specific control measures (Wakgariet *al.*, 2000). Informal use of antimalarials could increase the risk of incorrect dosing (under-dosage or over-dosage), treatment failure, resistance to antimalarial drugs, occurrence of adverse drug reactions and drug interactions, all of which could compromise effective antimalarial treatment (Ekanemet *al.*, 1990).

The malaria parasite multiplies in the red blood cells, causing changes in the haematological parameters of the infected person. Haematological changes are some of the most common complications in malaria and they play a major role in malaria pathogenesis. These changes involve the major cell lines including Red Blood Cells (RBC), White Blood Cells (WBC) and thrombocytes (Ovuakporaye, 2011; Imoruet *al.*, 2013). The destroyed red blood cells can clump together and cause blockages in the blood vessels. This may give rise to brain damage or kidney damage, which are potentially lethal health conditions (Imoruet *al.*, 2013).

The pathophysiological mechanism of liver damage in *falciparum* malaria has been well studied. The malaria parasites multiply in the liver into merozoites which infect and rupture the liver cells. Thereafter, the parasite moves back into the bloodstream where infection continues. The

invasion of liver cells by the sporozoite form of the malarial parasites can cause organ congestion, sinusoidal blockage and cellular inflammation (Anstey *et al.*, 2009). These changes in hepatocytes can lead to the leakage of parenchymal (transaminases) and membraneous (alkaline phosphatase) enzymes of the liver into the circulatory system. Elevation of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP), have been observed among malaria infected patients (Burtis *et al.*, 2001).

Studies have shown that the hepatic (liver) stage of the parasite's life cycle in its human host is accompanied by significant perturbation in the hepatocyte's parenchyma and membrane, leading to leakage of the liver enzymes into the general circulation (Onyesom, 2012).

The control of malaria relies principally on the use of drugs for prophylaxis or treatment. The thrust is to reduce burden of malaria due to the spread of resistance of the major human malaria pathogen, *P. falciparum*, to currently available drugs. Lack of knowledge about rational use of antimalarial drugs among patients is a serious problem, especially in areas of intense transmission. In areas where antimalarial drugs are given repeatedly to treat fevers (even in the absence of malaria), there are increased risks of resistance and adverse drug reactions (Whitty *et al.*, 2002). The main goal of treatment for malaria is to reduce morbidity and mortality and also delay the development of antimalarial drug resistance, therefore the attitude of the community and correct use of drugs are very important to treatment and prevention of emergence of resistant parasite.

The resistance of *Plasmodium falciparum* to Chloroquine (CQ) and Pyrimethamine-Sulfadoxine (SP) has become a serious obstacle to the control of malaria. Genetic mutation associated with CQ and SP resistance includes *pfmdr1* and *pfcr1*. *Pfmdr1* encodes membrane transporter proteins,

which are localized in the membrane of the parasite's food vacuole while *pfcr* code for an integral membrane protein and it is a member of the drug/metabolite transport superfamily (Fidock *et al.*, 2000). One particular mutation in the latter (*pfcr* K76T) has been strongly associated with chloroquine resistance in a genetic cross, allelic replacement transfections (Fidock *et al.*, 2000), and natural populations of *P. falciparum* (Djimde *et al.*, 2001). *Plasmodium falciparum* allele replacement studies and the analysis of progeny from a genetic cross have shown that point polymorphisms in the *pfmdr1* gene were correlated with small changes in artemisinin sensitivity (Durainghet *et al.*, 2000). Additionally, increased copy number of *pfmdr1* rather than single nucleotide polymorphisms has been shown to be associated with reduced artesunate sensitivity *in vitro* in two previous studies (Price *et al.*, 2004).

Other genetic mutations have been associated with resistance to other antimalarials and have been demonstrated to affect *in vitro* sensitivity to artemisinin derivatives. Mutations in *pfmdr1* which decrease sensitivity to mefloquine, tend to decrease sensitivity to artemisinin derivatives (Durainghet *et al.*, 2000).

The WHO in 2001 recommended Artemisinin-based Combination Therapy (ACTs) as the first-line drug for the treatment of uncomplicated *falciparum* malaria in countries experiencing resistance to antimalarial monotherapy (WHO, 2001), and the national antimalarial policy in Nigeria adopted ACTs as treatment choice in 2004 (FMOH, 2004). All artemisinin compounds induce a very rapid reduction of parasitemia, starting almost immediately after administration, killing all stages of the malaria parasite, including young gametocytes (Akomponget *et al.*, 2000). Artemisinin and its derivatives contain a stable endoperoxide bridge, which is cleaved by intraparasitic heme. The cleaved endoperoxide becomes a carbon-centered free radical which

then functions as an alkylating agent, reacting with both heme and parasite proteins (Akomponget *al.*, 2000).

The use of molecular tools in the last two decades has enormously enhanced the knowledge of the biology of *P. falciparum* and its vectors. Parasite genes have been characterised and used to examine the basic genetics of the parasite life cycle among laboratory parasite lines. These studies have been extended to examine the genetic structure of natural parasite populations. At the same time, work on the genetic basis of drug resistance in *P. falciparum* has been revolutionised by genome sequence data. This has accelerated the identification of genes controlling response of the parasite to some antimalarials.

1.2 STATEMENT OF PROBLEM

Presently, ACT is the drug of choice in the treatment of uncomplicated malaria in Nigeria. There are confirmed reports of *Plasmodium* resistance to ACTs in Southeast Asia (Dondorpet *al.*, 2010; Noedlet *al.*, 2010). ACT resistance has not been reported or documented in developing countries such as Nigeria. There is therefore need to urgently map and provide potential evidence of susceptibility or resistance of *P. falciparum* to ACT in Nigeria.

Nigeria accounts for a quarter of all malaria cases in the WHO African Region with over 95% of the country been endemic with year-round malaria transmission. A number of factors have contributed to the worsening malaria situation in the country, which include: weak health systems, poor access to health care facilities, increasing resistance to anti-malarial drugs, poor access to knowledge about appropriate health behavior in the population which leads to delay in treatment, climate changes leading to epidemics, inadequate knowledge on the disease and costly preventive interventions.

The identification of genes that control important parasite phenotypes such as drug resistance, growth rate, and strain-specific immunity, is of immense importance in the development of vaccine against malaria. Furthermore, identification of these genes involved in resistance to a drug can provide the molecular basis of drug resistance and aid in the design of novel drugs that evade mutations which are usually the cause of parasite drug resistance.

1.3 AIM AND OBJECTIVES

The aim of this study is to carry out biochemical and genetic studies to determine potential resistance of *Plasmodium falciparum* to Artemisinin-based Combination Therapy (ACT) in Lagos and Osun States, Southwestern Nigeria.

The specific objectives of this study are to:

1. determine Artemisinin-based Combination Therapy (ACT) usage and antimalarial preference in the study population.
2. determine the impact of *Plasmodium falciparum* on the expression profile of liver function enzymes and some haematological parameters in individuals treated with artemisinin.
3. Genotype loci (K-13, *pfmdr*, *pfATPase 6*, *pfert* polymorphism) associated with ACT resistance in the study communities.
4. Sequence and quantification of genes associated with ACT resistance.

1.4 SIGNIFICANCE OF STUDY

With the establish resistance of *P. falciparum* to ACT in Southeast Asia, and no data in Nigeria to track susceptibility or otherwise. This study will help to map and provide evidence of the status of *P. falciparum* to ACT in Nigeria. It will also activate the surveillance and monitoring of how antimalarial drug preference, usage and health-seeking behavior influence circulating malaria parasite populations. The origin and spread of antimalarial drug resistance (especially for ACT) are currently being monitored using genetic markers like K13 and *pfATPase* 6.

1.5 OPERATIONAL DEFINITION OF TERMS

CODON: It is a sequence of three DNA or RNA nucleotides that corresponds with a specific amino acid or stops signal during protein synthesis.

GENOME: A genome is an organism's complete set of DNA, including all its genes. Each genome contains all of the information needed to build and maintain an organism.

MUTATION: It is a permanent change of the nucleotide sequences of the genome of an organism, virus, or extrachromosomal DNA or other genetic elements.

POLYMORPHISM: This is when two or more clearly different phenotypes (form) exist in the same population of species. It functions to retain variety of forms in a population living in a varied environment.

RESISTANCE: An increase in parasite clearance time, as evidenced by $\geq 10\%$ of cases with parasites detectable on day 3 after treatment with an ACT.

UNCOMPLICATED MALARIA: This is when malaria signs and symptoms are present but there are no clinical or laboratory signs to indicate severity or vital organ dysfunction.

1.6 LIST OF ABBREVIATIONS/ACRONYMS

ACT: Artemisinin-based Combination Therapy

ALP: Alkaline phosphatase

ALT: Alanine aminotransferase

AST: Aspartate aminotransferase

cDNA: Complimentary Deoxyribonucleic Acid

DNA: Deoxyribonucleic Acid

EXO-SAP: Exonuclease S-Alkaline Phosphatase

FBC: Full Blood Count

FMOH: Federal Ministry of Health

K-13: Kelch-13 propeller domain gene

LFT: Liver Function Enzymes

PCR: Polymerase Chain Reaction

PCV: Packed Cell Volume

pfATPase: Sarcoplasmic and endoplasmic reticulum Ca^{2+} ATPase (SARCA)-type gene

pfert: *Plasmodium falciparum* chloroquine transporter gene

pfmdr: *Plasmodium falciparum* multidrug resistance gene

RDT: Rapid Diagnostic Test

RNA: Ribonucleic Acid

SNP: Single Nucleotide Polymorphism

SP: Sulphadoxine-Pyrimethamine

WHO: World Health Organisation