CHAPTER TWO

2:0 LITERATURE REVIEW

2.1 MALARIA EPIDEMIOLOGY

Malaria is the fifth leading cause of death from infectious disease worldwide, and the second leading cause of death in Africa. The World Health Organization (WHO) estimated that about 3.3 billion people in 97 countries are at risk of infection with malaria and 1.2 billion are at high risk; >1 in 1000 chance of getting malaria in a year (WHO, 2014). About 214 million cases of malaria occurred globally in 2014 and the disease led to 438,000 deaths, representing a decrease in malaria case incidence and mortality rates of 37% and 60% since 2000, respectively (WHO, 2015). Malaria kills one child every 30 seconds and it’s the third leading cause of death for children under five years worldwide, after pneumonia and diarrheal disease (CDC, 2014).

In 2010, there were an estimated 219 million malaria episodes, of which approximately 81% were recorded in Africa, and an estimated 660,000 malaria deaths, of which 91% were in Africa. The burden is heaviest in the WHO African Region, where an estimated 90% of all malaria deaths occur, and in children aged under 5 years, who account for 78% of all deaths (WHO, 2015). Thirty countries in Sub-Saharan Africa account for 90% of global malaria deaths. Nigeria, Democratic Republic of Congo (DRC), Ethiopia and Uganda account for nearly 50% of the global deaths. Malaria is the second leading cause of death from infectious diseases in Africa after HIV/AIDS. About one out of five deaths of children under 5 years in Africa is due to malaria (Breman et al., 2004).
Figure 1: Geographical distribution of malaria prevalence in children 6-59 months by microscopy in Nigeria
Malaria is a major public health problem in Nigeria where it accounts for more cases and deaths than any other country in the world. Malaria is a risk for 97% of Nigeria’s population. The remaining 3% of the population live in the malaria free highlands. There are an estimated 100 million malaria cases with over 300,000 deaths per year in Nigeria (Mangham et al., 2011). Malaria contributes to an estimated 11% of maternal mortality, 60% of outpatient visits and 30% of hospitalizations among children under five years of age in Nigeria. Malaria has the greatest prevalence, close to 50%, in children age 6-59 months in the South West, North Central, and North West regions (Fig. 1). Malaria has the least prevalence, 27.6 %, in children age 6 to 59 months in the South East region (Uzochukwu et al., 2010). Malaria impedes human development and thus having social consequences and heavy burden on economic development with Nigeria losing over 132 billion ($694.7 million) from cost of treatment and absenteeism from work, school and farm (Onwujekwe et al., 2013).

2.2 LIFE CYCLE OF HUMAN MALARIA

The life cycle of human malaria has two stages, a sexual stage and an asexual stage (Figure 2). The sexual stage occurs in female *Anopheles* mosquito (definitivehost), while the asexual stage occurs in human (intermediatehost) (CDC, 2006).

The sexual stage, also known as the sporogonic cycle, begins when a female *Anopheles* mosquito feeds on a human infected with *Plasmodium*. The red bloodcells which the mosquito ingests contain male and female gametocytes. In the mosquito, the male gametocytes or microgametocyte undergo exflagellation to form mature male gametes, while the female
Figure 2: Human malaria life cycle.
Source: CDC 2006.
gametocytes shed the red blood cell becoming female gametes. The male gametes penetrate the female gametes forming a zygote (Bloland, 2001; CDC, 2006). The zygote elongates into an ookinete, which infiltrates the stomach of the mosquito and attaches to the outer lining of the stomach. As the ookinetic enlarges it transforms into an oocyst, the oocyst rapidly divides into sporoblasts. Once the sporoblasts develop a defined nucleus and break away from the stomach lining, they become sporozoites. The sporozoites migrate and invade the salivary gland of the mosquito, which normally takes 10 to 18 days after the intake of gametocytes. This ends the sexual stage of the life cycle (Bloland, 2001; CDC, 2006).

The asexual stage begins when the sporozoites are transferred to a human host when the infected mosquito feeds. The asexual stage can be further divided into the exo-erythrocyte stage and the erythrocyte stage (Bloland, 2001). The exo-erythrocyte stage occurs first, because the sporozoites travel to the liver of the human host and invade the hepatocytes (Bloland, 2001). In the hepatocytes, the sporozoites mature into schizonts. After five to sixteen days, the schizonts rupture, they release merozoites, the haploid form of Plasmodium (CDC, 2006). Plasmodium vivax and Plasmodium ovale can remain in the hepatocyte in a dormant stage. The merozoites in the dormant phase are called hypnozoites. The merozoites exit the hepatocytes and enter the blood stream. Once the merozoites enter the blood stream, they penetrate the red blood cells of the human host. This begins the erythrocyte stage (CDC, 2006).

In the erythrocyte stage, the merozoites join together to form ringed trophozoites in the red blood cells. The parasite consumes the hemoglobin through its food vacuole. The hemoglobin is metabolized into heme, which is toxic to the parasite. The parasite uses polymerase to detoxify
heme into crystals of hemozoin pigment (Bloland, 2001). The trophozoites mature into schizonts or into gametocytes. The schizont phase will rupture into merozoites and repeat the erythrocyte stage. The gametocytes are crescent-shaped and mature very slowly (around 10 days). The gametocytes are eventually ingested by the female *Anopheles* mosquitoes starting the sexual stage (CDC, 2006).

A successful sporozoite can produce from 10,000 to more than 30,000 daughter merozoites in 6 - 8 days within the hepatocyte (White *et al.*, 2014). An asexual cycle in the blood takes roughly 48 h for *P falciparum*, *P vivax*, and *P ovale*, 72 h for *P malariae*, and 24 h only for *P knowlesi*. In a susceptible individual, the parasite population expands by between six times and 20 times per cycle (Simpson *et al.*, 2002). After 6 - 8 days of emerging from the liver, when parasite densities have reached roughly 50/µl of blood (roughly 100 million parasites in the blood), they become detectable by microscopy or rapid diagnostic tests and the symptomatic stage of infection begins. The incubation period is therefore usually 12 - 14 days from the infecting bite.

In *falciparum* malaria, the sexual cycle is delayed eventually after several asexual cycles, some merozoites invade red cells and there develop into either male or female gametocytes. Initially these resemble trophozoite forms, and they are sequestered. They mature in the small capillaries and venules and they are then released into the circulation. This sexual stage is responsible for infecting the Anopholine mosquito and thus transmission of the infection. As gamete fusion and thus meiosis takes place in the mosquito’s mid gut.
2.3 MALARIA TRANSMISSION

The malaria parasite is transmitted to human by mosquitoes belonging to the genus *Anopheles*. Malaria parasite can also be transmitted through blood transfusion, organ transplant, or the shared use of needles or syringes (CDC, 2014), and from mother to child (congenital malaria).

The nature of morbidity can also be affected by the stability of transmission. As transmission intensity decreases, the cumulative risk for experiencing a severe disease episode during childhood increases (Snow *et al*., 1998). Severe malaria becomes less likely as children grow older, but when severe malaria does occur, 8-15-year-old children (60.6%) are more likely to develop life-threatening cerebral manifestations than those who are 4-7 years old (28.2%) or younger (11.3%) (Imbert *et al*., 1997). Thus, cerebral malaria is more likely to develop from malaria infections in epidemic-prone regions, which may in part account for the high case fatality rates noted during epidemics.

Many biological and environmental factors shape the character of malaria in a given location. Nearly all the people who live in endemic areas are exposed to infection repeatedly. Those who survive malaria in childhood gradually build up some immunity. They may carry the infection, serving as reservoirs for transmission by mosquitoes without developing severe disease (Sullivan *et al*., 1999). In other areas, where the infection rate is low, people do not develop immunity because they rarely are exposed to the disease. This makes them more susceptible to the ravages of an epidemic.

Constant, frequent, year-round infection is termed stable transmission. In the sub-Saharan region from Senegal to Sudan, transmission is intense but largely confined to the 3-4 month rainy season. In areas where transmission is low, erratic, or focal (often termed unstable transmission), full protective immunity from malaria is not acquire, and symptomatic disease can occur at all
ages (Imbert et al., 1997). In such areas, changes in environmental, economic, or social conditions, for example, heavy rains after drought, large population movements, together with a breakdown in malaria control and prevention services (often because of armed conflicts) can result in epidemics, with substantial mortality in all age groups.

The probability of a mosquito being infected depends on the prevalence, duration and density of gametocyte carriage in the human host (Hogh et al., 1998; Drakeley et al., 1999; Targett et al., 2001). There are additional humoral (mainly species gametocyte antibodies) and leukocyte factors which affect transmissibility but their effects have been difficult to quantify accurately. Obviously as gametocyte densities fall much below 2/µL transmission becomes impossible. The lowest density at which transmission may occur is therefore close to the limit of detection at routine microscopy, which explains reports of mosquito infection from blood which was apparently gametocyte negative. There is a predictably lower prevalence of *P. falciparum* infection and lower intensity of oocyst infection in mosquitoes fed on blood from patients with lower gametocyte density (Drakeley et al., 1999).

### 2.3.1 HOST FACTOR

Parasites attach and enter erythrocytes via several pathways by different ligand–receptor interactions, for *P. falciparum*, these interactions can be divided into two groups according to dependency on sialic acid residues (on glycoporphins). Basigin (BSG), a transmembrane glycoprotein with two immunoglobulin-like domains, has been identified as a strain transcending receptor for PfRh5, a parasite ligand essential for blood stage growth (Crosnier et al., 2011). *Plasmodium vivax*, the predominant erythrocyte receptor is related to the Duffy blood group
antigen Fya or Fyb. Most people who live or have origins in West Africa carry the Duffy-negative FyFy phenotype and are resistant to *P. vivax* malaria.

The geographic distributions of sickle cell disease, haemoglobins C and E, ovalocytosis, thalassaemias, and glucose-6-phosphate dehydrogenase (G6PD) deficiency are suggested to confer a survival advantage in the presence of malaria. In the case of haemoglobin S [HbS], the heterozygote is protected against malaria (Williams, 2012). Malaria protective mechanisms include decreased parasite growth at low oxygen tensions (haemoglobin AS [HbAS], reduced cytoadherence (haemoglobins AC [HbAC] and CC [HbCC], HbAS), reduced invasion (ovalocytosis), reduced parasite densities (G6PD deficiency), and reduced multiplication at high densities (haemoglobin AE [HbAE]) (Cyrklaff *et al*., 2011).

The host responds to malaria by augmenting splenic immune function and filtrative clearance, accelerating removal of both parasitised and uninfected erythrocytes (Buffet *et al*., 2011). Schizont rupture releases parasite and host cellular material into the blood, which activates monocytes and macrophages and induces the release of proinflammatory cytokines, causing fever and other pathological effects (Ayimba *et al*., 2011).

The relatively low immunity of people residing in malaria epidemic prone areas exacerbates their risk of experiencing acute disease. Immunity to severe malaria generally requires only a few infections at any level of endemicity (Gupta *et al*., 1999). However, the long interval between infections and the spatial variability of transmission in areas of unstable endemicity fail to provide frequent enough challenge to sustain much disease-modulating immunity. As a result, serious clinical consequences become common during outbreaks. Where malaria is transmitted stably, the probability of dying from an untreated case of malaria is approximately 2.3% (Alles *et al*., 1998). Where unstable transmission fails to sustain immunity in the population, case
fatality rates up to 10 times greater can occur during epidemics (de Zulueta, 1988). High case fatality rates characterize epidemic malaria.

### 2.3.2 MIXED \textit{PLASMODIUM} SPECIES

One of the first questions to be considered using molecular detection methods in epidemiology is whether coinfection with one malaria parasite species (such as \textit{P. vivax} or \textit{P. malariae}) would modify the course of infection or risk of disease due to another (particularly \textit{P. falciparum}). Epidemiological evidence from microscopically detected infections in Vanuatu suggests interaction (possibly cross-immunity) between \textit{P. vivax} and \textit{P. falciparum} may be clinically important (Maitland et al., 1996), and such interactions would potentially be important in many endemic areas. An early PCR study in Cote d’Ivoire gave preliminary data showing that \textit{P. malariae} infections may reduce the risk of symptomatic \textit{P. falciparum} infection (Black et al., 1994), but little has been done to follow this up in larger studies or in other African populations. One analysis of mixed-species infections in Papua New Guinea indicated that there may be density-dependent regulation on all malaria parasites that coexist in the blood, so that a high parasitemia of any parasite clone would reduce the effective replication rate of any other parasite (of the same or a different species) (Bruce et al., 2000). This is plausible, although the presence of each species in mixed infections was determined by non-quantitative PCR, whereas the density was estimated by microscopy only (with which it is difficult to reliably estimate relative proportions of different species). Another study in Papua New Guinea has reported that the concurrence of different \textit{Plasmodium} species is apparently random, as determined by microscopy and PCR with sequence-specific probing (Mehlotra et al., 2002). The contrasting findings
indicate that it may be interesting to conduct further studies of species-specific parasitemia levels in mixed- and single-species infections using quantitative PCR estimation.

In contrast to the modest number of studies that have focused on analysis of mixed-species infections, there have been many studies that have analyzed mixed-genotype infections of *P. falciparum*. The high proportion of mixed-genotype infections in many areas of endemicity, along with the availability of simple protocols for PCR discrimination of different genotypes (Snounou et al., 1999), has encouraged many characterizations of mixed genotype infections. Experiments in a murine model of *P. chabaudi* infection have indicated that evolution of virulence may be driven by competition between coinfecting malaria parasite clones (de Roode et al., 2005). It is plausible that human malaria parasites in areas of low endemicity have evolved a lower level of virulence (due to less coinfection), whereas those in areas of high endemicity have higher virulence, although this has not been tested. However, in controlled comparisons within populations in areas of endemicity, different numbers of genotypes in *P. falciparum* infections are not generally associated with different clinical symptoms or severity of malaria. Groups of severe and mild malaria cases have been compared independently in The Gambia (Conway et al., 1991), Senegal (Robert et al., 1996) and Gabon (Kun et al., 1998) with each study showing that the numbers of genotypes per infection were similar between the clinical groups.

Clinical trials of antimalarials conducted in areas of endemicity often incorporate genotyping of highly polymorphic loci of parasites in a blood sample pretreatment (day 0) and on any occasion at which parasites have reappeared within the follow-up period and present the approach as being a robust means of distinguishing recrudescence from new infections, as applied in important recent studies (Price et al., 2006).
2.3.3 TYPES OF MALARIA

Malaria can be categorized in two categories: uncomplicated or complicated (severe).

2.3.3.1 Uncomplicated Malaria

Classical uncomplicated malaria has three stages: a cold stage, a hot stage, and a sweating stage. The cycle lasts between 6-10 hours. The cold stage consists of shivering. A patient in the hot stage suffers from fever, headaches, vomiting, and seizures (frequently in young children). The sweating stage consists of sweats and tiredness (CDC, 2014).

Tertian and quartan periodicities are associated classical attacks. In tertian attacks the symptomatic stages occur every second day. These attacks are caused by *P. falciparum*, *P. vivax*, and *P. ovale*. In quartan attacks, the symptomatic stages occur every third day. *P. malariaeis* the cause of quartan periodicity (Bloland, 2001). Uncomplicated malaria can be misdiagnosed as influenza or the common cold. This occurs in countries where malaria is not common; therefore, the patient is not expected to have malaria. In endemic areas, malaria symptoms are recognized (CDC, 2014).

2.3.3.2 Severe Malaria

Severe malaria occurs when a patient suffers from organ failure or abnormalities in the blood or metabolism. Severe malaria is associated with cerebral malaria, severe anemia, hemoglobinuria, acute respiratory distress syndrome, abnormal blood coagulation, low blood pressure, acute renal failure, hyperparasitemia, metabolic acidosis and hypoglycemia. Severe malaria requires urgent and aggressive treatment (CDC, 2014). Severe *falciparum* malaria is caused mainly by extensive
parasitised erythrocyte sequestration and consequent dysfunction of vital organs. Direct visualisation of the microcirculation and measurement of individual vessel flows in the retinal, buccal, and rectal circulations show reversible heterogeneous microvascular obstruction with patterns matching exactly those noted in tissues from fatal cases (WHO, 2000). The extent of microvascular obstruction parallels clinical severity and established prognostic measures, such as plasma lactate and base deficit (WHO, 2000). Patients with severe malaria usually have low concentrations of L-arginine, a precursor of nitric oxide, and increase in those of asymmetric dimethylarginine (Tan et al., 2011). Endothelial activation causes exocytosis of intracellular Weibel-Palade bodies, which contain bioactive molecules such as von Willebrand factor and angiopoietin 2. Ultra-long multimers of von Willebrand factor can bind activated platelets expressing CD36 (the receptor for PfEMP1), and thereby mediate cytoadherence (Wells, 2009).

2.4 PATHOGENESIS AND PATHOLOGY OF MALARIA

The majority of complications and symptoms of malaria in the human host are associated with the erythrocyte stage of Plasmodium (CDC, 2014). The Plasmodium destroys the erythrocyte, and causes waste and toxins to build up. When wastes products enter the blood stream, the resulting symptoms are fever and rigors (Newton et al., 2000). Moderate to severe shaking, chills, high fever, and profuse sweating occur in cycles. Headache, vomiting, and diarrhea may also occur (WHO, 2000).

In endemic areas malaria is often the most common cause of fever. The first symptoms of malaria are nonspecific, and include a vague absence of wellbeing, headache, fatigue, muscle aches, and abdominal discomfort, which are followed by irregular fever. Nausea, vomiting, and
orthostatic hypotension occur frequently (Feachem et al., 2010). Generalized seizures are associated specifically with *falciparum* malaria and might be followed by coma (cerebral malaria). Most patients with uncomplicated infections have few abnormal clinical presentation other than fever, mild anaemia, and, after several days, a palpable spleen. The liver can become enlarged, especially in young children, whereas mild jaundice is more likely in adults. In young children living in regions in which transmission is stable, recurrent infections cause chronic anaemia and splenomegaly (Alonso et al., 2011).

The manifestations of severe *falciparum* malaria depend on age (Yeo et al., 2010). Severe anaemia and hypoglycaemia are more common in children, whereas acute pulmonary oedema, acute kidney injury, and jaundice are more common in adults; coma (cerebral malaria) and acidosis occur in all age groups (Mohanty et al., 2011).

Malaria causes severe complications, and if left untreated usually results in death. These complications include breathing problems, organ failure, severe anemia, and low blood sugar. Breathing problems occur because of the accumulation of fluid in the lungs. Organ failure occurs in kidneys or liver. Liver dysfunction has been recognised in malaria infection. Changes in hepatocytes can lead to the leakage of parenchymal (transaminases) and membraneous (alkaline phosphatase) enzymes of the liver into the circulatory system hence the increase in liver enzymes; aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) which have been observed among malarial infected patients (Burtis et al., 2001). Pathophysiological mechanism of liver damage in *falciparum* malaria has been studied in the past by many investigators (Clark and Cowden, 2003). Factors responsible for jaundice in malaria are multiple including intravascular haemolysis of parasitized erythrocytes, liver
dysfunction microangiopathicaemolysis and associated septicaemia (Snow et al., 2005). Hepatic dysfunctions result from cytoadherence, rosetting and sequestration of erythrocytes containing mature forms of *P. falciparum* in deep vascular bed. These cases are more likely to have acute renal failure.

Severe anemia happens because the parasite destroys the red blood cells. Malaria and certain malarial drugs can cause low blood sugar (CDC, 2014). Haematological changes are some of the most common complications in malaria and they play a major role in malaria pathology (Ovuakporaye, 2011). Malaria infected patients tend to have significantly lower platelets, WBCs, lymphocytes, eosinophils, RBCs and Hb level, while monocyte and neutrophil counts were significantly higher in comparison to non-malaria infected patients (Mainaet al., 2010; Bakhubaira, 2013). A previous study found that the ratio of monocytes to lymphocytes correlated with risk of clinical malaria during follow-up (Warimweet al., 2013). The most common complication during malaria infection is thrombocytopenia (Shiraz and Mumtaz, 2012). Persons with platelet counts < 150,000/μ were 12-15 times more likely to have malaria infection than persons with platelet counts > 150,000/μ (Erhartet al., 2004). Anaemia is defined as a decrease in number of RBCs or less than the normal quantity of haemoglobin for an individual age and gender. Malaria is thought to be the primary cause of severe anaemia in at least 50% of people living in malaria endemic area (Jasbir and Kiersten, 2009).

The main manifestation of severe malaria in young children is severe anaemia especially in areas of high transmission (Caliset al., 2008), and is usually the cumulative result of repeated infections. Accelerated splenic removal of mainly the unparasitised red blood cells and erythrocyte destruction at parasite schizogony are compounded by ineffective erythropoiesis
Slight coagulation abnormalities are frequent, and thrombocytopenia is usual even in uncomplicated malaria. Substantial bleeding from disseminated intravascular coagulation in severe malaria is rare. Haematemesis from stress ulceration or acute gastric erosions can occur.

Acidosis and hypoglycaemia are an important cause of death associated with severe malaria, acidosis results from accumulation of organic acids, including lactic acid (Day et al., 2000), and is often compounded by ketoacidosis in children and acute kidney injury in adults. Of the biochemical variables in severe malaria, plasma bicarbonate, base excess, or lactate concentrations have the highest predictive value for fatal outcomes (von Seidlein et al., 2012).

Hyperinsulinaemic hypoglycaemia is an important adverse effect of quinine (a powerful stimulant of pancreatic insulin secretion), and is particularly common in pregnant women, even in those with otherwise uncomplicated malaria (White et al., 1983).

Acute respiratory distress syndrome is a complication in adults with severe falciparum malaria (particularly in pregnant women), which can also occur in P. vivax and P. knowlesi infections (Daneshvar et al., 2009). Increased pulmonary capillary permeability develops in as much as 30% of adult patients and often manifests after the start of antimalarial treatment (Taylor et al., 2012).

Pathogenesis is not fully understood, but inflammation-mediated endothelial damage might have an important role. Careful fluid management is essential; rapid infusion of large volumes of intravenous fluid can be lethal (Maitland et al., 2011). In the absence of mechanical ventilation, the mortality of acute respiratory distress syndrome exceeds 80%. With mechanical ventilation, case fatality still exceeds 50% in falciparum malaria (Daneshvar et al., 2009).

Acute kidney injury is common in adults with severe malaria. It behaves clinically and pathologically like acute tubular necrosis. Pathogenesis remains unclear, but reduced
microcirculatory flow probably contributes (Nguansangiam et al., 2007). Acute kidney injury is frequently associated with dysfunction of several other vital organs (leading to high mortality) or can develop more slowly as other disease manifestations resolve. Acute kidney injury is oliguric in 60–70% of cases. In survivors, urine flow resumes in a median of 4 days, and serum creatinine concentrations return to normal in a mean of 17 days (Tranget al., 1992).

Severe jaundice results from a combination of haemolysis, hepatocyte injury, and cholestasis. Jaundice is more common in adults than in children, and is often accompanied by renal impairment. Chronic carriage of hepatitis B virus might predispose to severe malaria in adults (Barcuset al., 2002).

In endemic areas, severe malaria is frequently misdiagnosed in children with severe sepsis, pneumonia, meningitis, and other diseases associated with incidental malarial parasitaemia (Reybournet al., 2004), confounding clinical studies and pathological interpretations.

2.4.1 FACTORS INFLUENCING MALARIA OUTCOME

Malaria parasites influence disease outcome through factors that include parasite density, rosetting and sequestration, toxin production, and genetic diversity including expression of virulence and immune evasion genes such as the var (variant antigen receptor) gene family (Robertet al., 1996).

2.4.1.1 Parasite density

The precise relationship between parasite density and disease severity remains unclear, as the density of sequestered parasites and circulating parasites varies greatly depending upon the stage and synchronicity of the infection (White et al., 2014). In general, high parasitaemia has been
associated with increased disease severity; however, this is not always the case as peripheral parasitaemia does not always accurately reflect the number of parasites due to sequestration. Silamut *et al.*, 1999 reported cerebral malaria as the cause of death in aparasitaemic individuals in autopsies following effective antimalarial treatment. Another study in the Solomon Islands showed that both *P. vivax* and *P. falciparum*-infected asymptomatic individuals tend to have low and submicroscopic parasite densities (Harris *et al.*, 2010). Another study suggested the association of asymptomatic parasitaemia of higher parasite density with a higher risk of symptomatic malaria (Njama-Meya *et al.*, 2004).

A study investigating the relationship that parasite density has on platelet count showed that malaria-infected children with thrombocytopenia (decreased platelet count) were younger, had higher parasitaemia, lower hemoglobin levels, an increased mean platelet volume, and exhibited platelet aggregation (Maina *et al.*, 2010). Similarly, a cross-sectional study on Nigerian children with asymptomatic malaria showed that malaria parasites cause a significant reduction in platelet counts with more pronounced reduction in children under 5 years of age (Harris *et al.*, 2010). Therefore, thrombocytopenia is not only a feature exhibited by acute malaria, but also a potentially useful indicator for monitoring asymptomatic children in high transmission areas.

### 2.4.1.2 Strain diversity

The genetic diversity of *P. falciparum* and its association with the development of clinical symptoms has been investigated in many studies. There is strong evidence that immunity to malaria is specific to the particular strain eliciting the host response, enabling an individual to
resist infection to that particular strain, but not to heterologous ones; this has been termed ‘strain-specific immunity (Giha et al., 1998). The development of strain-specific immunity might then be responsible for decreased disease severity, including asymptomatic malaria, in populations where exposure is moderate to high.

Studies have investigated some symptom-specific molecular characteristics based on polymorphisms of surface antigens, identified in isolates collected from asymptomatic, uncomplicated, and severe malaria cases across various geographic regions (Bendixen et al., 2001). Such polymorphic surface antigens include: Merozoite Surface Protein (MSP) family, Apical Membrane Antigen 1 (AMA1), Erythrocyte Binding Antigen-175 (EBA-175), and Knob-Associated Histidine Rich Protein (KAHRP).

Specific antigenic polymorphisms may allow the parasite to evade the immune response in patients with chronic infection and this may lead to their survival in the human host until transmission becomes possible.

### 2.4.1.3 Merozoite Surface Proteins (MSP)

Several studies have investigated the association of msp-1 and msp-2 with clinical severity of disease. Amoduet et al., 2005, carried out a study conducted to examine the relationship between the genetic diversity of msp-1 block 2 of *P. falciparum* and clinical severity of malaria in Nigerian children showed that the presence of K1 and MAD20 alleles were significantly associated with asymptomatic malaria and consequently reduced the risk of developing symptomatic disease (Amoduet et al., 2005). Arieyet et al., 2001, found that the association of a specific msp-1 allele (K1) with a specific var gene (var-D) was overrepresented among patients with severe malaria compared to mild disease, and this genotype combination was consistently
observed in the most severe clinical cases. However, investigations of genotype associations with disease severity need to be expanded to include all categories of disease, including asymptomatic malaria. Genetic diversity should be longitudinally monitored to ensure that such clinical associations are maintained and not biased by cross-sectional selection of isolates (Arieyet et al., 2001).

There are conflicting reports regarding the differential distribution of alleles within particular genes (such as msp-2) according to clinical status (Amoduet et al., 2008). The FC27- like genotype of msp-2 was shown to be twice as likely to be found in symptomatic cases than in asymptomatic cases, providing evidence that specific variants of msp-2 may be associated with the morbidity of malaria. A similar association was reported in Papua New Guinea, where the FC27 allele was linked to increased disease severity, however, this study did not consider asymptomatic cases (Al-Yamanet et al., 1997). Another study reported that there was no association between FC27 or 3D7 alleles of msp-2 and malaria symptoms (Cortes et al., 2004). However, further longitudinal studies in different geographical settings with standardized collection and genotyping methods will be required to clarify these findings.

### 2.4.1.4 Apical Membrane Antigen 1 (AMA 1)

There are multiple lines of evidence that indicate that polymorphisms in the \( P. falciparum \) AMA1 domain I result from selective pressures exerted by protective host immune responses (Cortes et al., 2003). A study in Papua New Guinea, showed pattern of geographical diversity and found a particular substitutions which were suggestive of strong constraints acting on the evolution of AMA1 at the population level. In addition, differences between the sequences of AMA1 domain I from symptomatic and asymptomatic infections implicate AMA1 as a possible
determinant of the morbidity associated with a particular *P. falciparum* strain (Cortes et al., 2003).

### 2.4.1.5 Erythrocyte Binding Antigen 175 (EBA-175)

Studies on the distribution of EBA-175 genotypes suggest that this gene plays a role in different clinical outcomes. Genomic studies of two *P. falciparum* strains, namely FCR-3 and CAMP, revealed two highly dimorphic segments in region III, which is located in the central part of the gene. Studies conducted by Suarez-Mutis et al., 2007, revealed that CAMP and FCR-3 genotypes of the EBA-175 were encountered in both symptomatic and asymptomatic patients, but the FCR-3 genotype predominated regardless of clinical status and the sampling period since the FCR-3(F-) genotypes were simply more prevalent in the region (Suarez-Mutis et al., 2007). However, another study by Toure et al., 2006 showed that the prevalence of mixed CAMP/FCR-3 infection was far higher in symptomatic than in asymptomatic children. This study showed that mixed CAMP/FCR-3 infection is associated with clinical malaria and may have therapeutic implications (Toure et al., 2006).

### 2.4.1.6 Virulence genes

An important aspect of *P. falciparum* virulence is the ability of infected erythrocytes to sequester and obstruct the microvasculature of different organs. Cytoadhesion to endothelial cells is mediated by electron-dense elevations of the parasite membrane referred to as knobs. Knobs consist predominantly of the Knob-Associated Histidine- Rich Protein (KAHRP), which cluster on the cytoplasmic face of the knob membrane. KAHRP is required for knob formation and has also been used as a marker because of its role in pathology. In a study, three different alleles were
found in mild cases, but only two forms were observed in severe cases (Ranjit et al., 2005). Studies on the association of these KAHRP alleles with severity need to be extended to asymptomatic malaria.

Recent studies on var gene expression and malaria severity have yielded conflicting results. These studies are complicated by extensive variation in the var gene transcription and the phenotypes displayed by circulating parasites, which may be different to those sequestered, as in severe malaria. Therefore, comparing the repertoire of genomic and expressed var genes with a particular malaria outcome will be helpful to understand the role of this gene family in asymptomatic malaria (Kyriacou et al., 2006).

Other factors that influence malaria outcomes may include, the formation of rosettes which may influence the severity of malaria by causing distinct patterns of sequestration with different pathogenic consequences (Miller et al., 2002), and parasite toxins such as glycosylphosphatidylinositol (GPI) anchors and haemozoin. Intraleucocytic malaria pigment found in neutrophils is also important and suggested to be a better indicator of disease severity than the peripheral parasite count (Miller et al., 2002).

Amoduet al. (1998) first reported an increase in the proportion of malaria-pigment containing neutrophils and monocytes in severe malaria patients, and later study by, confirmed that there was an unambiguous rise in the proportion of malaria pigment-containing neutrophils as severity increases across a range of disease outcomes, from uninfected to severe malaria cases.

2.4.2 MALARIA COMPLICATIONS

2.4.2.1 Cerebral malaria

In some cerebral malaria patients, many cerebral capillaries and venules are packed tightly with parasitized erythrocytes, whereas other adjacent vessels are not obstructed. A distinct and
specific malarial retinopathy with haemorrhages, retinal and vessel whitening occurs both in children and in adults. Corresponding microvascular pathological changes have been recorded post mortem (Beare et al., 2009).

Organ-specific and systemic blood lactate–pyruvate ratios are increased in proportion to the severity of illness (Day et al., 2000). All these findings suggest that extensive microvascular obstruction and impaired perfusion are the crucial pathophysiological processes. Also observed is little histopathological evidence of inflammation, although leucocytes are more prominent in the cerebral vessels of African children who died from cerebral malaria. A mild, generalised increase in systemic vascular permeability is noted. Cerebral malaria patients still have functionally intact blood–brain barrier, although the results of autopsies of African children suggest some increase in permeability, with disruption of endothelial intercellular tight junctions (Dorovini-Zis et al., 2011).

Cerebral oedema is most observed in adults but imaging shows cerebral oedema is often present in African children (Medana et al., 2011). Lumbar puncture opening pressures are usually normal in adults but increased in roughly 80% of children, although mean pressures (around 160 mm CSF) are similar (Newton et al., 2000). Raised intracranial pressure probably results mainly from increased intracranial blood volume, which in turn is a result of sequestration of parasitized erythrocytes (Mohanty et al., 2011).

Coma, a common pathological occurrence, can persist after the time by which cerebral parasite sequestration should have cleared. Transient disruption of axoplasmic transport (Medana et al., 2011) and persistent attachment of residual erythrocyte membranes and malaria pigment to vascular endothelium (Pongponratnet al., 2003) stimulating continued activation provide a plausible explanation. Adults rarely have neurological sequelae, 3-15% of children who survive
cerebral malaria - especially those with hypoglycaemia, severe anaemia, repeated protracted seizures, and deep coma - have residual neurological deficits, including hemiplegia, cerebral palsy, cortical blindness, deafness, and impaired cognition and learning (all of varying duration) (Dondorp et al., 2010b). Roughly 10% of children have persistent language deficits, increased incidence of epilepsy, and decreased life expectancy (Birbeck et al., 2010).

2.4.2.2 Malaria in pregnancy

In high transmission area, women with placental malaria have double the risk of low birthweight, with the effect being greatest during first pregnancies. This lower birth weight is associated with increased infant mortality. Maternal anaemia is exacerbated, but most mothers remain asymptomatic despite intense accumulation of infected erythrocytes in the placental microcirculation. Congenital malaria occurs in roughly 5% of neonates but clears spontaneously in 62% of cases (Falade et al., 2007). Maternal HIV infection predisposes pregnant women to malaria, congenital malaria, and increases chances of reductions in birthweight. In areas with unstable malaria transmission, pregnant women are at increased risk of developing severe *falciparum* malaria with a very high mortality rate of 50%. High parasitaemias, severe anaemia, hypoglycaemia, and acute pulmonary oedema are all more frequent in pregnant women having malaria, than in non-pregnant women with malaria. In severe disease, fetal distress, premature labour, and stillbirth often occur. The risk of infant death is particularly high if maternal malaria occurs during late (near-term) pregnancy (Bardajiet al., 2011). Maternal death from haemorrhage at childbirth is correlated with malaria-induced anaemia (McGready et al., 2012).
In Nigeria, intermittent preventive treatment with sulfadoxine - pyrimethamine is usually given to pregnant women as prophylaxis. A full course of sulfadoxine - pyrimethamine twice during later pregnancy provided partial protection (WHO, 2012). A minimum of three doses of sulfadoxine–pyrimethamine are now recommended to provide continuous preventive effects. Resistance to sulfadoxine–pyrimethamine is increasing in Africa, and thus alternative drugs are being investigated for use in intermittent preventive treatment in pregnancy (WHO, 2012). Mefloquine is the only drug recommended for chemoprophylaxis in pregnant women travelling to areas with drug-resistant malaria, and is thought to be safe in the second and third trimesters of pregnancy (Schlagenhaufet al., 2012). The safety of other prophylactic antimalarials in pregnancy has not been established, although no harmful effects have been associated with atovaquone–proguanil (WHO, 2012).

The intermittent preventive treatment approach has been extended to infants, where it can be delivered to all at-risk infants via the expanded programme on immunisation. However, much malaria-related illness and death in Africa occurs in children aged 3 - 59 months in the Sahel sub-region during 4 months of the rainy season. WHO has recommended administration of monthly amodiaquine and sulfadoxine–pyrimethamine (maximum four doses) to all children aged 3 - 59 months in this region from the start of the yearly transmission season (WHO, 2012). This seasonal malaria prophylaxis has superceded intermittent preventive treatment in infants. Intermittent preventive therapy is effective when delivered through schools or to adults at high risk of malaria (WHO, 2012).

2.4.2.3 Placental Malaria
Placental *P. falciparum* infection during pregnancy causes a substantial risk of miscarriage or poor birth outcome. The enhanced infection of the placenta is largely due to a variant ligand on the *P. falciparum*-infected erythrocyte that binds to chondroitin sulfate A on the placental capillary endothelia (Haywood *et al*., 1999), with some evidence that a secondary ligand may bind to hyaluronic acid. Substantial evidence indicates that the product of a particular *P. falciparum var* gene (*var2csa*) that encodes a variant of PfEMP1 is responsible and is expressed at high levels in most infections of pregnant women but only occasionally in infections of others (TuikueNdam *et al*., 2005).

Infected pregnant women can make antibodies, pregnancy associated malaria (PAM)-specific parasite molecules, of which the response to the *var2csa*-encoded PfEMP1 appears to be the most important, as it can block adhesion of these parasites to chondroitin sulfate A. The presence of such antibodies is associated with better pregnancy outcome, such as higher birth weight. A recent study has suggested the existence of serological polymorphism in the chondroitin sulfate A-binding pregnancy-associated malaria antigen at a population level, indicating that the adaptive and immunological significance of polymorphisms in *var2csa* should be investigated.

An initial analysis of divergent allelic sequences from laboratory isolates, and a partial sequence from the homologous *var* gene in the chimpanzee parasite *Plasmodium reichenowi*, indicates that *var2csa* is under diversifying selection in *P. falciparum* (Trimnell *et al*., 2006).

2.5 DIAGNOSIS OF MALARIA

Effective management of malaria relies on prompt and accurate diagnosis. The global epidemiological data of malaria has spurred interest in developing effective diagnostic strategies not only for resource-limited areas where malaria is a substantial burden on society, but also in
developed countries, where malaria diagnostic expertise is often lacking. Malaria diagnosis involves identifying malaria parasites or antigens/products in patient blood. The leading causes of death in many countries can be attributed to delays in diagnosis and treatment (CDC, 2014).

### 2.5.1 CLINICAL DIAGNOSIS OF MALARIA

Clinical diagnosis of malaria is traditional among medical doctors. It is most widely practiced method and is least expensive. Clinical diagnosis is based on the patients’ signs and symptoms, and on physical findings at examination. The earliest symptoms of malaria are very nonspecific and variable, and include fever, headache, weakness, myalgia, chills, dizziness, abdominal pain, diarrhea, nausea, vomiting, anorexia, and pruritus (CDC, 2014). The non-specific nature of the signs and symptoms of malaria has made clinical diagnosis of malaria very challenging. These signs and symptoms overlap considerably with other common, as well as potentially life-threatening diseases, e.g. common viral or bacterial infections, and other febrile illnesses. The overlapping of malaria symptoms with other tropical diseases impairs diagnostic specificity, which can promote the indiscriminate use of antimalarials and compromise the quality of care for patients with non-malarial fevers in endemic areas. Therefore, the accuracy of malaria diagnosis can be greatly enhanced by combining clinical-and parasite-based findings (Kyabayanze et al., 2008).

### 2.5.2 LABORATORY DIAGNOSIS OF MALARIA

In the laboratory, malaria diagnoses can be done using different techniques, which include microscopic diagnosis by staining thin and thick peripheral blood smears, rapid diagnostic tests and molecular diagnostic methods such as PCR.
2.5.2.1 Microscopic diagnosis

Malaria is conventionally diagnosed by microscopic examination of stained blood films using mostly Giemsa stains (Ngasala et al., 2008). Microscopic detection and identification of *Plasmodium* species in Giemsa-stained thick blood films (for screening the presenting malaria parasite), and thin blood films (for species’ confirmation) remains the gold standard for laboratory diagnosis. Malaria is diagnosed microscopically by staining thick and thin blood films on a glass slide, to visualize malaria parasites.

The patient’s finger is cleaned with 70% ethyl alcohol, allowed to dry and then the side of fingertip is picked with a sharp sterile lancet and two drops of blood are placed on a glass slide. To prepare a thick blood film, a bloodspot is stirred in a circular motion with the corner of the slide, taking care not make the preparation too thick, and allowed to dry without fixative. After drying, the spot is stained with diluted Giemsa (1:20, vol/vol) for 20 min, and washed by placing the film in buffered water for 3 min. The slide is allowed to air dry in a vertical position and examined using a light microscope (Chotivanich et al., 2006). As they are unfixed, the red cells lyse when a water-based stain is applied.

A thin blood film is prepared by immediately placing the smooth edge of a spreader slide in a drop of blood, adjusting the angle between slide and spreader to 45° and then smearing the blood with a swift and steady sweep along the surface. The film is then allowed to air-dry and is fixed with absolute methanol. After drying, the sample is stained with diluted Giemsa (1:20, vol/vol) for 20 min and washed by briefly dipping the slide in and out of a jar of buffered water (excessive washing will decolorize the film). The slide is then allowed to air-dry in a vertical position and examined under a light microscope (Chotivanich et al., 2006).
This technique is widely accepted by laboratories all around the world and this is due to its simplicity, low cost, its ability to identify the presence of parasites, the infecting species, and assess parasite density—all parameters useful for the management of malaria. This method has its own shortcoming, the staining and interpretation processes are labor intensive, time consuming, and require considerable expertise and trained healthcare workers, particularly for identifying species accurately at low parasitemia or in mixed malarial infections. The most important shortcoming of microscopic examination is its relatively low sensitivity, particularly at low parasite levels.

2.5.2.2 Rapid Diagnostic Tests (RDTs)

A rapid diagnostic test (RDT) is an alternate way of quickly establishing the diagnosis of malaria infection by detecting specific malaria antigens in a person's blood (CDC, 2014). Rapid diagnostic tests are fast and easy to perform, and do not require electricity or specific equipment. Unlike conventional microscopic diagnosis by staining thin and thick peripheral blood smears, RDTs are all based on the same principle and detect malaria antigen in blood flowing along a membrane containing specific anti-malaria antibodies; they do not require laboratory equipment. Most products target a *P. falciparum*-specific protein, examples is histidine-rich protein II (HRP-II) or lactate dehydrogenase (LDH). Some tests detect *P. falciparum* specific and pan-specific antigens (aldolase or panmalarialpLDH), and distinguish non-*P. falciparum* infections from mixed malaria infections. Although most RDT products are suitable for *P. falciparum* malaria diagnosis, some also claim that they can effectively and rapidly diagnose *P. vivax* malaria (Park *et al.*, 2006).
Recently, a new RDT method has been developed for detecting *P. knowlesi* (McCutchan *et al.*, 2008). RDTs provide an opportunity to extend the benefits of parasite-based diagnosis of malaria beyond the confines of light microscopy, with potentially significant advantages in the management of febrile illnesses in remote malaria-endemic areas. One of the major shortcomings of RDT is that it cannot quantify parasitemia in patients but microscopy can be used to quantify the proportion of red blood cells that are infected, which is an important prognostic indicator (CDC, 2014).

### 2.5.3 SEROLOGICAL TESTS

Diagnosis of malaria using serological methods is usually based on the detection of antibodies against asexual blood stage malaria parasites. Immunofluorescence Antibody Testing (IFA) has been a reliable serologic test for malaria in recent decades. Although IFA is time-consuming and subjective, it is highly sensitive and specific. Literatures clearly showed the reliability of IFA, therefore it was usually regarded as the gold standard for malarial serology testing (Doderer *et al.*, 2007).

Immunofluorescence Antibody Testing is useful in epidemiological surveys, for screening potential blood donors, and occasionally for providing evidence of recent infection in non-immunes. It was a validated method for detecting *Plasmodium* specific antibodies in various blood bank units, which was useful for screening prospective blood donors (Reesing, 2005). The principles of IFA is that, following infection with any *Plasmodium* species, specific antibodies are produced within 2 week of initial infection, and persist for 3-6 months after parasite clearance.
The IFA uses specific antigen or crude antigen prepared on a slide, coated and kept at -30°C until used, and quantifies both IgG and IgM antibodies in patient serum samples. Titers >1 : 20 are usually deemed positive, and < 1 : 20 unconfirmed. Titers >1 : 200 can be classified as recent infections (Chotivanich et al., 2006). A major shortcoming of IFA is that it is time-consuming and cannot be automated, which limits the number of sera that can be studied daily.

2.5.4 MOLECULAR DIAGNOSTIC METHODS

2.5.4.1 Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR)-based techniques are recent development in the molecular diagnosis of malaria, and have proven to be one of the most specific and sensitive diagnostic methods, particularly for malaria cases with low parasitemia or mixed infection. This technique is used extensively to confirm malaria infection, follow-up therapeutic response, and identify drug resistance (Chotivanich et al., 2006).

Some modified PCR methods are proving reliable, e.g., nested PCR, real-time PCR, and reverse transcription PCR, and appear to be useful second-line techniques when the results of traditional diagnostic methods are unclear for patients presenting with signs and symptoms of malaria; they also allow accurate species determination (Mlambo et al., 2008).

The PCR method has become widely accepted for identifying *P. knowlesi* infections (Ng et al., 2008). Although PCR appears to have overcome the two major problems of malaria diagnosis- sensitivity and specificity- the utility of PCR is limited by complex methodologies, high cost, and the need for specially trained technicians. PCR, therefore, is not routinely implemented in developing countries because of the complexity of the testing and the lack of resources to perform these tests adequately and routinely (Mlambo et al., 2008).
2.5.4.2 Loop-Mediated Isothermal Amplification (LAMP) technique

The loop-mediated isothermal amplification (LAMP) technique is a simple and inexpensive molecular malaria-diagnostic test that detects the conserved 18S ribosome RNA gene of *P. falciparum* (Han *et al.*, 2007). Studies have shown high sensitivity and specificity, not only for *P. falciparum*, but also *P. vivax, P. ovale* and *P. malariae*. These observations suggest that LAMP is more reliable and useful for routine screening for malaria parasites in regions where vector-borne diseases, such as malaria, are endemic. LAMP appears to be easy, sensitive, quick and lower in cost than conventional PCR. However, reagents require cold storage, and further clinical trials are needed to validate the feasibility and clinical utility of LAMP (Erdman and Kain, 2008).

2.5.4.3 Microarrays

Publication of the *Plasmodium* genome offers many malariadiagnostic opportunities (Cramerier *et al.*, 2007). Microarrays may play an important role in the future diagnosis of infectious diseases. The principle of the microarrays technique parallels Southern hybridization. Hybridization of labeled targets divided from nucleic acids in the test sample to probes on the array enables the probing of multiple gene targets in a single experiment. A pan-microbial oligonucleotide microarray has been developed for infectious disease diagnosis and has identified *P. falciparum* accurately in clinical specimens (Patarakul, 2008).

2.6 MALARIA CHEMOTHERAPY

Chemotherapy has traditionally played an important role in the treatment and control of malaria. Quinoline containing antimalarial components are the most effective drugs for malaria
chemotherapy. This group of compounds has evolved from the structural modification of quinine and includes 4-aminoquinoline compounds such as chloroquine and mefloquine of which the former is more effective, cheap, safe and commonly available drug (Foley and Tilley, 1997). The dihydrofolatereductase inhibitors include proguanil, chloroprophuanil, pyrimethamine and trimethoprim and sulfa drugs like dapsone, sulfalene, sulfamethoxazole and sulfadoxine. These drugs are used in combinations. A classic example of such combination is sulphadoxine and pyrimethamine (SP) used as first line drug in Thailand and other parts of the world. Tetracycline and its derivatives such as doxycycline are very potent antimalarials and are used for both treatment and prophylaxis. In areas where response to quinine has deteriorated, tetracyclines are often used in combination with quinine to improve cure rates (Landgraf et al., 1994).

The other useful antimalrials are Artemisinin compounds synthesised from the plant Artemisia annua. These compounds (artesunate, artemether, arteether) are most effective antimalarials and seem to have effect on protein synthesized by the malaria parasite. These are used for the treatment of severe malaria and have shown very rapid parasite clearance in comparison to quinine compounds (Dondorp et al., 2010).

Plasmodium parasite has extremely complex genome and they can switch with ease between the micro environments in different hosts and the metabolic changes they require illustrates the difficulty in studying the exact modes of action of the antimalarial drugs on parasite metabolism (WHO, 1987).

2.6.1 Chloroquine

Chloroquine resistant P. falciparum malaria has been reported from wherever falciparum malaria is endemic except in Central America, CarribeanHisparivala Island and some parts of middle-
east and central Asia (WHO, 2001). Resistance to chloroquine in *P. falciparum* first appeared virtually simultaneously in Southeast Asia (Thai-Cambodian border) and South America (Colombia) in late 1950s (Wernsdorfer and Payne, 1991). Since then chloroquine resistance has spread far beyond the first focus and is now found in all parts of the world where malaria is endemic. Chloroquine resistant *falciparum* strains had spread in all endemic areas of South America by 1970 and almost all in Asia and Oceania by 1989. Chloroquine resistance in Africa was first reported in the eastern part in 1978, which then spread to the central and southern parts before arriving in West Africa in 1983 (Peters, 1987). By 1989 chloroquine resistance was widespread in sub-Saharan Africa. The severity of resistance in West and Central Africa was less than in East Africa, but even in West Africa, its intensity varies from an advanced stage with severe effects on morbidity and mortality in focal areas of Senegal to a moderate degree in Ghana (Landgraf *et al.*, 1994), Cameroon (Ringwald *et al.*, 2000) and at a low level in Mali (Djimde *et al.*, 2001).

Chloroquine resistance in *P. vivax* was noted for the first time in Papua New Guinea (Rieckman *et al.*, 1989) and from there it has spread to other parts of the world. Resistance in *P. vivax* is more serious as hypnozoites will cause relapse of resistant parasites and *P. vivax* is a mixture of various strains with respect to incubation period, relapsing pattern and response to primaquinesince sulpha drugs are not effective in its treatment.

It is believed that resistance of *P. falciparum* to chloroquine is due to increased capacity for the parasite to expel chloroquine at a rate that does not allow chloroquine to reach levels required for inhibition of heamepolymerization (Foley and Tilley, 1997). This chloroquine efflux occurs at a rate 40 to 50 fold faster among resistant parasites than that in sensitive ones. Further, evidence supporting this mechanism is provided by the fact that chloroquine resistance would be reversed
by drugs which interfere with this efflux system (Martin et al., 1987) but the biochemical basis of this efflux is a matter of debate. The efflux of chloroquine and in fact the entire chloroquine resistant phenotype can be reversed with Ca\(^+\) channel blocker, such as verapamil and dilitazem (Martin et al., 1987).

2.6.2 Sulphadoxine-Pyrimethamine (SP)

The increasing chloroquine resistance in early 1960s led to a significant increase in mortality. The sulphadoxine-pyrimethamine combination was used as a drug of choice to treat chloroquine resistant malaria. Resistance to SP was first described from the Thai-Cambodian border in 1960s(Bjorkman and Phillips-Howard, 1990). SP resistance has been reported from large parts of Southeast Asia, southern China and Amazon basin (Aramburuet al., 1999). Low degree of resistance is found in Pacific Coast of South America, southern Asia, east of Iran and western Oceania (Bloland, 2001). In Africa, SP resistance was detected in the late 1980s, which has since spread more in the east than in the west.

The intrinsic frequency of the genetic events that confer antimalarial resistance (while retaining parasite viability) varies between antimalarials, but this is generally a rare event. The most readily occurring mechanisms of resistance to current antimalarials are the single point mutations in Cytochrome b which confer high level atovaquone resistance (Bloland, 2001). Viable mutations in the gene encoding dihydrofolatereductase (\textit{dhfr}), which confer pyrimethamineresistance, were also estimated to develop with a similar frequency. An adult with approximately 2\% parasitaemia carries a burden of 10 parasites so these arise readily.
The antifolate compounds like sulphadoxine-pyrimethamine inhibit the action of dehydrofolatereductase ($dhfr$) while sulphones and sulphonamide compounds inhibit the action of dihydropteroate synthase ($dhps$). The dehydrofolatereductase enzymes of resistant strains bind to pyrimethamine 400–800 fold less readily than to the enzymes of drug sensitive strains (Fidock *et al*., 2000).

### 2.6.1.3 Mefloquine

Mefloquine resistance was first observed in late 1980s near the Thai-Cambodian border (Shanks, 1994). It is frequent in some parts of Southeast Asia and has been reported in the Amazon region of South America and sporadically in Africa (Muckenaupt, 1995). Mefloquine acts on the erythrocyte stage *Plasmodium*; however, the medication is not effective against the exo-erythrocytes stages of *Plasmodium*. Thus, it is not effective against the dormant phase of *P. ovale* and *P. vivax*. The exact mechanism is unknown. In *P. falciparum*, melfloquine may cause swelling of the food vacuole. Mefloquine is suspected to bind with heme to generate toxic complexes, which then damage parasitic membranes and disrupt other cellular components. Mefloquine has also shown cross-resistance with halofantrine, which is an antimalarial drug used against erythrocyte stages (Bjorkman and Phillips-Howard, 1990).

### 2.6.1.4 Artemisinin
Artemisinin and its derivatives are the newest and most effective antimalarial drugs. These drugs affect the protein synthesis of the parasite. One of the most important benefits of artemisinin based combination therapy is the potential to delay the spread of antimalarial resistance.

Artemisinin based combination therapy decreases the transmission advantage of the resistant parasites over sensitive parasites, from a gametocyte carriage ratio of 4:1 (monotherapy resistant: sensitive) to a ratio of 1:1 (ACT resistant: sensitive) (Price *et al.*, 1996). In patients with no detectable gametocytaemia at baseline included in a meta-analysis of randomized controlled trials comparing artemisinin-based combination therapy with monotherapy, the addition of 3 days artesunate dramatically reduced gametocyte carriage on day 7, with larger effects at days 14 and 28 (International Artemisinin Study Group, 2004). Decreased gametocyte carriage following artemether-lumefantrine treatment has been shown to limit post-treatment transmission of *P. falciparum* to Anopheles mosquitoes. Although artesunate consistently reduces post-treatment infectivity to mosquitoes, primarily by decreasing gametocytes in peripheral blood and preventing recrudescence, it does not abolish infectivity completely.

A previous study with *P. falciparum* suggested that a sarcoplasmic and endoplasmic reticulum Ca\(^{2+}\) ATPase (SERCA)-type protein encoded by a gene denoted *pfatp6* might be the major chemotherapeutic target of these drugs (Eckstein-Ludwig *et al.*, 2003). SERCA is responsible for the maintenance of calcium ion concentrations, which is important for the generation of calcium-mediated signaling and the correct folding and post-translational processing of proteins. *pfatp6*, the only SERCA-type Ca\(^{2+}\) ATPase in *P. falciparum*, was expressed in *Xenopus laevis* oocysts and the effect of antimalarial drugs was examined. Artemisinin completely inhibited *PfATP6* activity, with a half-maximal inhibition constant (Ki) of 150 nM. This inhibition was highly
specific, such that even at 50 nMartemisinin, no other transporters, including the non-SERCA Ca$^{2+}$-ATPase PfATP4, were affected (Eckstein-Ludwig et al., 2003).

2.6.5 DRUG PREFERENCE AND UTILIZATION

Inappropriate behaviour can interfere with the effectiveness of a control measure, such as vector control or chemotherapy. These issues are particularly important in tropical areas where malaria control options are limited because of the parasite and vector resistance to antimalarial drugs and insecticides, respectively. In such cases, an understanding of the communities' beliefs and behaviour may be crucial to the success of specific control measures (Wakgari et al., 2000). Informal use of antimalarials could increase the risk of under-dosage, over-dosage or incorrect dosing, treatment failure, the resistance to antimalarial drugs, occurrence of adverse drug reactions and drug interactions which could compromise effective antimalarial treatment (Ekanem et al, 1990).

Studies carried out by the WHO, evidence shows that 80% of malaria cases are inadequately managed at community level by the home-based caregivers and 96% of caregivers initiated actions within 24 hours but only 15% of their actions are appropriate due to inadequate dosages (WHO and UNICEF, 2005). Pattern of drug-use in cases of malaria infection either on prescription basis or self-medication can result in high incidence of resistance strain. The knowledge and attitude of patient can influence drug-therapy.

Community perceptions relating to causation, transmission, prevention and treatment are the main socio-cultural factors that can influence malaria control. Most malaria cases are managed at the household level. Treatment is usually sought from the community pharmacist or licensed chemical seller after the initial therapy has failed, and most patients reporting at clinics and
hospitals facilities would have gone through homebased treatment or community drug shops/pharmacies initially (Pagnoniet al., 1997). However, severe, or recurrent episodes are reported at clinics and hospitals.

Self-treatment may be attributed to previous experience with a particular drug, such as having used the same drug for similar symptoms, or neighbour, friend, or relative previously taking the same drug for similar symptoms (Watsierahet al., 2011). Self-medication, which is mostly based on presumptive diagnosis, will similarly be faced with consequences of misdiagnosis, overtreatment of malaria, masking of underlying, potentially fatal conditions and unnecessary side-effects. The knowledge about the symptoms of malaria is usually high in endemic areas where people are aware of the clinical manifestations of the disease. However, in a holoendemic area of western Kenya only approximately 30% of the respondents were aware of malaria symptoms (Ongoreet al., 1989). Place of purchase of drugs could also interfere with behavioural pattern of respondents to antimalarial drug-use. Drug purchased from hawkers or market places could be detrimental to health.

In Nigeria, ACT has been adopted for first-line treatment of uncomplicated malaria since 2004; evidence abounds on the improper use of anti-malarial drugs, such as the use of monotherapy and other less effective anti-malarial drugs, as well as inappropriate use of ACT(Okekeet al., 2006). This is especially so in the retail sector where studies have reported significant inappropriate use of anti-malarial drugs. Providers in the retail sector are often influenced by their knowledge, financial incentives, competition, perceptions of patients’ attitudes, and regulatory sanctions (Hanson et al., 2004).

Okekeet al., 2006 further suggested that prescribing patterns are more likely to follow patient demands and expectations as well as profit motive rather than professional principles.
Appropriate attention to this sector is therefore critical to achieve the goals of malaria case management. Regular monitoring of drug utilization, as recommended by Federal Government policy (FGN, 2005) becomes important when identifying opportunities for enhancing effective implementation of the ACT policy.

The study by Uzochukwuet al., (2010), about five years after policy change in the same area suggested utilization rate of 24.2% for ACT in medicine outlets using patients’ exit questionnaires. Findings corroborate the results of previous, related studies in the area in terms of availability and utilization of anti-malarial drugs (Uzochukwuet al., 2010). Prescriptions were mostly adults, while children cases were limited, comprising prescriptions from hospitals. Predominance of adult prescriptions agrees with previous findings that adults make more use of retail outlets than children (Hetzelet al., 2008). The study shows that ArthemetherLumenfrantrine (AL), as the policy first-line drug in Nigeria, is the most commonly used ACT, corroborating the study Hetzelet al., (2008) and Uzochukwuet al., (2010), which documented similar findings for both public and private health facilities. Studies in Uganda and Zambia also suggested preference for AL regimen over other ACT in health facilities (Sears et al., 2013).

Prescriptions from hospitals which consists almost entirely of ACT is consistent with findings that the public sector conforms more to policy on the use of ACT and that prescribing pattern is not influenced by patient demand, compared to the private retail sector (Uzochukwuet al., 2010). Fewer cases of monotherapy prescription through this mode may be explained by the use of SP for prophylaxis in pregnancy. This may have contributed to making SP the highest used monotherapy in their study. Adequate knowledge which will influence behavioural attitude to
antimalarial in terms of correct dosing, compliance factors, appropriate use of available antimalarials is crucial in averting antimalarial drug use pattern and drug resistance. Lack of knowledge about rational use of antimalarial drugs among patients is a serious problem, especially in areas of intense transmission, where antimalarial drugs are given repeatedly to treat fevers (even in the absence of malaria), thus increasing the risk of resistance and adverse drug reactions (Whitty et al., 2002). Thus, the main aim of treatment for malaria is to reduce morbidity and mortality and also delay the development of antimalarial drug resistance. The attitude of the community and correct use of these drugs are very important to treatment and prevention of emergence of resistant parasite to treatment.

2.6.3 METHODOLOGY FOR DETERMINATION OF ANTIMALARIAL DRUG RESISTANCE

2.6.3.1 MOLECULAR BASIS OF DRUG RESISTANCE IN MALARIA

Drug resistant malaria has become a major problem in malaria control. Drug resistance by malaria parasites has been defined as the ability of a parasite strain to survive or multiply despite the administration and absorption of a drug when given in doses equal to or higher than those normally recommended and within the limits of tolerance of the subject (White, 2004). This definition may be applied to the response of the parasite to antimalarial drugs used as schizontocides, gametocytocides or sporontocides. The drug resistance in the parasite can be determined either in vivo or by in vitro drug susceptibility tests (WHO, 2005).

In vivo tests: Resistance in vivo has been reported against almost all antimalarial drugs. Resistance to antimalarials has been reported in both *P. falciparum* and *P. vivax*. In vivo tests are based on the observation of parasite response in the patients to a fixed dose of a drug within the limits of tolerability (Wernsdorfer and Payne, 1991), one of the key characteristics of in vivo test
is the interplay between host and parasite. Decreased therapeutic efficacy of a drug can be marked by immune clearance of parasite in patients with a high degree of acquired immunity (White, 1999).

The assessment of *in vivo* drug response of *P.falciparum* to antimalarials require prolonged periods of follow-up (28 days) and seclusion of patients in screened rooms to prevent the possibility of reinfection. In 1990, WHO introduced a modified protocol, involving shorter period of follow-up (7–14 days) without seclusion, under the assumption that reappearance of parasites in peripheral blood within 14 days of treatment is more likely due to recrudescence than reinfection. Traditionally, response to treatment was categorised according to the WHO criteria purely on parasitological ground as sensitive, R-I, R-II and R-III (Bruce–Chwatt, 1986) level of resistance. Later modifications are based on adequate clinical response, early and late treatment failure. The test procedure is based on a 14-day follow-up with clinical, parasitological, haematocrit and fever assessment on Day 0, 3, 7 and 14 (WHO, 2005).

*In vitro* tests: The problem related with the assessment of antimalarial drug resistance *in vivo* has led to the introduction of a number of *in vitro* tests for the measurement of antimalarial drug susceptibility in the late 1970s. Traditionally, two types of *in vitro* assays are commonly used, WHO schizont maturation assay and the isotopic micro test. These tests are based on the estimation of the parasite metabolic process in short- or long-term culture. The data derived from *in vitro* tests have to be interpreted in relation to the *in vivo* and pharmacological tests to determine individual susceptibility levels for the drug tested. *In vitro* tests avoid many of the confounding factors, which influence the *in vivo* test, by removing parasites from the host and placing them in a controlled experimental environment. These tests more accurately reflect the intrinsic antimalarial drug resistance (Bloland, 2001). The *in vitro* assays not only yield
quantitative results, but also determine the phenotype of the parasite independently of the immune and physiopathological status of the host.

In general, resistance appears to occur through spontaneous mutations that confer reduced sensitivity to a given drug or class of drugs (White, 1999). Resistance also develops more quickly where a large population of parasites is exposed to drug pressure since it will remove sensitive parasites, while resistant parasite would survive.

Drug resistance by malaria parasites has been defined as the ability of a parasite strain to survive or multiply despite the administration and absorption of a drug when given in doses equal to or higher than those normally recommended and within the limits of tolerance of the subject (WHO, 2005).

_P. falciparum_ has developed clinically significant resistance to almost all classes of antimalarial drugs. Resistance is a shift to the right of the dose–response curve, thus requiring higher drug concentrations to achieve the same parasite clearance (White, 2004). Resistance emerges de novo through spontaneous mutations or gene duplications, which are thought to be independent of drug selection pressure, but these mutants are then selected for and spread as a result of the drug pressure which provides a selective advantage to resistant parasites. Resistance arises mainly during asexual reproduction, and may require only a single genetic event (e.g. antifol or atovaquone resistance) or multiple events (epistasis).

Genotyping of molecular markers; Single Nucleotide Polymorphisms (SNPs) and microsatellites, situated very close to drug resistance genes provides a powerful approach to determining the numbers of origins of spread of resistance and compliments epidemiological and clinical data describing the emergence of resistance.
2.6.3.1.1 Chloroquine Resistance

Current molecular studies of *P. falciparum* isolates suggest that few gene loci are associated with chloroquine resistance to *P. falciparum*. These genes have been named as *pfmdr-1* and 2, *pfcrt*. *Pfmdr-1* gene located on chromosome-5 and coding for P-glycoprotein homologue-1 (*Pgh-1*) has generated interest in resistance to chloroquine and other antimalarials. Studies conducted in different geographical areas of the world suggest that the point mutation of aspartic acid to tyrosine in codon 86 (A86T) is associated with chloroquine resistance (Babiker *et al*., 2001). Several other *pfmdr-1* polymorphisms—Phe 184, Cys 1034, Asp1042 and Tyr 1246 have been implicated to varying degrees in chloroquine resistance. Another locus governing chloroquine resistance has been identified on chromosome 7 and encodes a transmembrane protein in a digestive vacuole of malaria parasites. Sets of point mutations in *pfcrt* gene have been found to be associated with *in vitro* chloroquine resistance in *P. falciparum* from Africa, South America and Southeast Asia (Durand *et al*., 2001).

Djimdeet *et al*.,(2001)found that the substitution of thyroxine (T76) for lysine (K76) at codon 76 was present in all chloroquine resistant isolates and absent in all sensitive isolates. Seven other point mutations have been associated with chloroquine resistant strains of *Plasmodium*: M74I, N75E, A220S, Q271E, N326S, I356T, and R371I (Djimdeet *et al*.,2001). This mutation does not allow chloroquine to accumulate in the parasites food vacuole, thus, blocks the inhibition of the heme polymerase mechanism of the drug. The rate of expulsion of chloroquine in resistant strains is 30 to 40 times greater than strains that are sensitive to chloroquine (Peter, 1987).
2.6.3.1.2 Sulphadoxine-Pyrimethamine Resistance

The molecular basis of resistance to SP is the best characterised one. Specific gene mutations encoding for resistance to \(dhfr\) and \(dhps\) have been identified. Point mutations in the five codons of \(dhps\) gene known to date are implicated in conferring resistance by decreasing binding affinity of the enzyme. Serine to alanine at codon 436 or phenylalanine; alanine to glycine at codon 437; lysine to glutamic acid at codon 540; alanine to glycine at codon 581; alanine to serine or threonin at codon 613. Gly437 and Gly540 have been reported to occur together or single in various parts of the world including Indonesia, Malawi, Bolivia, Kenya (Plowe et al., 1997) and Gabon while Gly581 has been observed in South America alone. Specific point mutation in \(dhfr\) gene is known to be associated with pyrimethamine resistance by reduction in drug-binding affinity of \(dhfr\). Alanine to valine at codon 16, aspargine to isoleucine at codon 51, cysteine to arginine at codon 59, serine to asparginine at codon 108, threonine and isoleucine to leucine at codon 164, this combination of mutations has been observed in Thailand, where high level of SP resistance is well recognised. The point mutation from serine to asparginine at codon 108 is a key mutation for pyrimethamine resistance. Additional point mutations in three other codons ILe51, Arg59 and Leu164 are known to increase progressively the degree of resistance (Fidock et al., 2000).

The mutations associated with resistance to sulfadoxine (in \(dhps\)) and pyrimethamine (\(dhfr\)) are widespread, and it was thought that these arose de-novo very frequently, and that this accounted for the rapid development of sulfadoxine-pyrimethamine resistance wherever it has been deployed. There are two properties of sulfadoxine-pyrimethamine that may have contributed to this remarkable spread of resistance; the apparent stimulation of gametocytogenesis associated with poor therapeutic responses to sulfadoxine-pyrimethamine and the widespread exposure of
*P. falciparum* parasites in these regions to sub-therapeutic sulfadoxine-pyrimethamine concentrations either during its long elimination phase from the body or following sub-therapeutic dosing (Terlouw *et al.*, 2003).

### 2.6.3.1.3 Mefloquine Resistance

Molecular studies have suggested that the copy number and polymorphism of *pfmdr-1* gene is associated with mefloquine resistance. A study from Thailand has suggested that a higher copy number confers mefloquine resistance (Price *et al.*, 2004) but other studies did not confirm that finding from Brazil and Africa. Some studies have shown increased sensitivity to mefloquine with *pfmdr-1* Tyr86 mutation (Price *et al.*, 2004) suggesting a possible inverse relationship between sensitivity to mefloquine and chloroquine, while Ser1034, Asn1042 and Asp1246 mutations were cause of resistance to mefloquine (Reed *et al.*, 2000). These findings strengthen the role of *pfmdr-1* as the key modulator of mefloquine resistance.

### 2.6.3.1.4 Artemisinin Resistance

To deal with the threat of resistance, Artemisinin-based Combination Therapy (ACT) is being promoted as a strategy to counteract the increasing resistance of the parasite as well as to prevent disease transmission (WHO, 2011). Despite the precautionary measures however, artemisininresistant *P. falciparum* malaria has emerged in western Cambodia and the bordering regions with Thailand, the hotspot of multidrug resistance parasites (Dondorp *et al.*, 2010; Noedl *et al.*, 2010), and appears to be emerging in the western border of Thailand (Na-Bangchang *et al.*, 2010; Phyo *et al.*, 2012).
SERCA-type PfATPase6, a sarcoendoplasmic reticulum calcium-dependent ATPase6, has been implicated as genetic markers that potentially confer resistance to artemisinins (Flegg et al., 2011). The polymorphism of ATPase6 is being monitored by several scientific research teams. Lionel et al., 2009 reported the genotyping results of ATPase6 from 98 P. falciparum field isolates collected in 2006 to 2007 in South Vietnam. They found a total of eight mutations: four nonsynonymous (I89T, N463S, N465S, and N683K), three synonymous (N460N, I898I, and C1031C), and one double deletion leading to the loss of two asparagines (_463–464). Five of these have not been described previously (N460N, N463S, _463–464, N465S, and C1031C). All of the mutations were detected on different isolates, except for I898I, which was found alone or associated with others. Studies conducted in Tanzania (Wongsrichanalai and Meshnick, 2008) and China (Flegg et al., 2011), did not find either the S769N mutation or the A623E, E431K double mutation, associated with reduced susceptibility to artemether, similar to the report of Lionel et al., 2009 (Noedl et al., 2010). Previously, the N683K mutation was only found in Cambodia, suggesting that it may be specific to P. falciparum from South-East Asia. However, they did not detect this mutation in the South-East Asiatic strains W2 and Dd2 (both from Indochina, Malaria Research and Reference Reagent Resource Center), IMT-A4 (Vietnam), and IMT-K2. Interestingly, the N460N, N463S, N465S, and N683K mutations and the _463–464 double deletion are in a stretch of nine asparagines located in the interspecies variable region of PfATP6, a domain specific for Plasmodium species (Noedl et al., 2010). Consequently, these modifications could be adaptive changes that might alter susceptibility to artemisinins.

In 2014, the WHO reported a new molecular marker of artemisinin resistance (WHO, 2014). Mutations in the Kelch 13 (K13)-propeller domain were shown to be associated with delayed
parasite clearance in vitro and in vivo. The function of the K13-propeller of *P. falciparum* is largely unknown, and direct predictions of function are precluded by the diverse functions of kelch-containing proteins in other species (Taylor *et al.*, 2014).

Strong evidence of genetic linkage to a region of *P. falciparum* chromosome 13 has now been translated (Ariey *et al.*, 2014) into the discovery of a molecular marker: Single-Nucleotide Polymorphisms (SNPs) in the propeller region of a kelch protein encoded by *kelch13* (Ashley *et al.*, 2014). In Cambodia, where this delayed parasite clearance was first reported, 3 polymorphisms: C580Y, R539T, and Y493H are prevalent and associated with prolonged parasite half-life after treatment. An additional polymorphism, M476I, was detected in a Tanzanian parasite by cyclic in vitro artemisinin pressure (Taylor *et al.*, 2014).

### 2.6.4 FITNESS OF DRUG RESISTANT MALARIA PARASITES

As a general rule, mutant forms of an organism are likely to be less fit than their wild-type strains in the absence of selection. Mutations that render pathogens resistant to drug treatment are likely to result in a loss of fitness, and such mutants could therefore be outgrown by sensitive forms if the drug pressure were removed. However, a complication which needs to be borne in mind is that resistant mutants may themselves develop ‘compensatory’ mutations, which could then allow them to grow and survive in competition with wild-type sensitive forms (Levin *et al.*, 2000).

Fitness differences between resistant and sensitive clones of malaria parasites may be assessed simply by comparing their phenotypes for characteristics such as growth rates, gametocyte production, variations in mosquito transmission. The ideal parasites for such work are sensitive and resistant clones which are isogenic apart from the mutation(s) underlying the resistance
phenotype, that is, a sensitive wild-type clone and a resistant mutant derived from it. Mixed infections or cultures of each clone can then be established to examine whether either has a competitive advantage over the other (David et al., 2005).

Monitoring the growth of individual clones in a mixture can be done by the rather laborious procedure of re-cloning parasites at various stages after initiation of the mixed culture and then testing each new clone for drug response. Some experimental work of this type has been done using rodent malaria models. The monitoring of such mixed infections has been greatly simplified in recent years with the identification of mutations in genes determining drug-resistance, i.e. mutations in *dhfr* (dihydrofolatereductase) (Nair et al., 2003) determining pyrimethamine resistance, in *dhps* (dihydropteroate synthase) determining sulfadoxine resistance, and in *pfcrt* (*P. falciparum* chloroquine resistance transporter) and *pfmdr1* (*P. falciparum* multi-drug-resistance) determining chloroquine resistance. PCR-based methods have been developed to determine the proportion of resistant and sensitive alleles of each gene in a mixture of resistant and sensitive parasites (David et al., 2005).

Evaluation of fitness as reproductive success requires consideration of the capacity of resistant mutants to develop gametocytes and hence to be transmitted through mosquitoes. Very few studies have been done on this subject. Shinondo et al., 1994 using *P. berghei*, showed that the growth of a pyrimethamine-resistant mutant in mice was similar to that of its sensitive parent; however, when in mosquitoes, the mutant grew more slowly, producing sporozoites in the salivary glands several days later than the sensitive form.

Mutations underlying drug resistance are likely to have a negative impact upon reproductive fitness. This expectation arises from understanding of evolutionary and population genetics and selection. However, it is instructive to attempt to understand the mechanisms by which mutations
may lead to fitness costs. For example, where enzymes are drug targets (e.g. \textit{dhfr} in pyrimethamine resistance) (Nair \textit{et al}., 2003), mutations may alter the kinetics of the enzyme. This may result in a change in the flux of the pathway or a change in the concentration of pathway metabolites. Both of these may change the fitness of the parasite (David \textit{et al}., 2005). The elegant studies of selective sweeps in \textit{P. falciparum} by chloroquine and pyrimethamine (Nair \textit{et al}., 2003) showed that alleles at loci closely linked to \textit{pfcrt} and \textit{dhfr}, respectively, had also been selected by drug treatment, due to limited recombination in the chromosome regions containing these genes in the time since drug selection was imposed. The probability that compensatory mutations would be needed to restore the fitness of resistant parasites was discussed by Nair \textit{et al}., 2003, who pointed out that such mutations would ideally need to be closely linked to resistance genes, since recombination would break down any linkage disequilibrium of unlinked genes in the absence of selection. Nair \textit{et al}., 2003 also made an interesting point that if a gene conferring drug resistance is linked to a polymorphic antigen locus, purifying selection by the drug on the resistance gene could lead to a reduction in the diversity of alleles at the antigen locus in the parasite population, thereby reducing its mean fitness (Nair \textit{et al}., 2003).

\textbf{2.6.5 TRANSMISSION OF RESISTANT MALARIA PARASITE}

Although eliminating the asexual stages of \textit{P. falciparum} is the focus of treatment of individual symptomatic patients, at a population level, reducing carriage of the gametocytes is necessary to limit the transmission of malaria parasites, and in particular, the transmission of resistant parasites. Antimalarial drugs are important in controlling the passage of \textit{falciparum} parasites from humans to the mosquito vector, although this relationship is complex as different
antimalarials have different effects on the various stages of gametocytogenesis, on gametocyte infectivity to the mosquito, and on the development of the parasites in the mosquito vector. Antimalarial drugs which kill the asexual stages of Plasmodium vivax, P. malariae and P. ovale also act against the sexual stages of these parasites (terKuile et al., 1993). Sulfadoxine-pyrimethamine is associated with the highest post-treatment prevalence and density of gametocyte carriage of all antimalarial drugs. This may reflect a drug induced release or redistribution of gametocytes as this increase is seen as early as 4 days after treatment (Targett et al., 2001). After adjusting for gametocyte density, there is a lower probability of infecting mosquitoes in gametocyte carrying children treated with sulfadoxine-pyrimethamine compared with chloroquine (Hoghet al., 1998). This relatively lower infectivity per gametocyte may be due to a sulfadoxine-pyrimethamine-triggered release of immature forms that are not yet infectious (Targett et al., 2001). However, the higher prevalence and density of gametocytes following sulfadoxine-pyrimethamine treatment more than offsets this apparent lower per patient gametocyte transmissibility.

In The Gambia, where sulfadoxine-pyrimethamine resistance is uncommon, 25.6% of children treated with sulfadoxine-pyrimethamine compared with 12.2% of children treated with chloroquine were found to be infectious 7 days after treatment (Targett et al., 2001). Treatment with artemisinins result in the lowest levels of gametocyte carriage, even when administered together with sulfadoxine-pyrimethamine (Targett et al., 2001; International Artemisinin Study Group, 2004). This is as a result of both the lower production of gametocytes, due to the rapid reduction in asexual parasite biomass, and their direct gametocytocidal effect against immature stages (terKuile et al., 1993; White, 2004).
Gametocyte carriage is significantly higher in patients infected with resistant parasites than sensitive ones, fuelling the spread of antimalarial resistance. This is expected as the slower clearance and prolonged presence of asexual parasites associated with resistance increases gametocytogenesis. Furthermore, the apparent half-life of gametocytes was longer and their apparent clearance slower in children with chloroquine-resistant infections than those with chloroquine-sensitive infections (Drakeley et al., 2004). It should be noted that these kinetic terms are in fact hybrids of formation in and removal from the circulation. The ratios of post treatment gametocyte prevalence (i.e. the proportion of patients carrying any gametocytes) of treatment failures to sensitive *falciparum* infections was 4:0 for me quine (Price et al., 1996), 4:1 for sulfadoxine-pyrimethamine (Terlouw et al., 2003) and for chloroquine ranged between 2:9 and 1:2 (Hogh et al., 1998; Drakeley et al., 2004; von Seidlein et al., 2012). However, these studies differ in their baseline prevalence of gametocytaemia, age distribution, levels and definitions of antimalarial drug resistance and duration of follow up (7–28 days). Variations in the intensity of malaria transmission will result in differences in the effects of acquired immunity on gametocyte carriage (Drakeley et al., 2004).

### 2.7 STRATEGIES FOR INTEGRATED MALARIA CONTROL

Malaria control is too complex to be addressed by a single approach, and any attempt to do so is fraught with danger. It is therefore important to tailor the strategy to the prevailing ecological and epidemiological conditions (Mouchet and Carnavale, 1998). The immune status of the population and the patterns of malaria will affect control strategies. The following strategies can be adopted for the control of malaria: (i) mortality control, (ii) transmission control, and (iii) eradication.
2.7.1 Mortality Control.

The major impact of malaria in any community is that of the death of individuals. The strategy of mortality control involves detecting presumptive cases, determining which cases are parasite positive, and administering effective treatment. Such a strategy has little impact on morbidity due to malaria and has little or no effect on the overall transmission of the disease. In areas of holoendemic infection, this morbidity results in a major burden on the population.

Mortality control is the main thrust of the current Global Malaria Control Strategy (WHO, 1993). It relies on chemotherapy, no particular program is required, nor is there any need for nationwide strategies and the development of local priorities. All that is required is some means for record keeping and a system for distribution of the drugs of choice to the peripheral clinics. Training is minimal and could also involve local commercial outlets for antimalarial drugs. It is an effective stopgap strategy to cope with epidemics of malaria when emergency situations arise (WHO, 2000). In crisis situations such as severe flooding or epidemic outbreaks, it is the strategy of choice because the tools can be mustered simply and quickly.

The main problem is that chemotherapy alone is not a means of controlling malaria and is not sustainable in the long term. There are good data to indicate that treatments obtained from unskilled sources are frequently inappropriate and often ineffective and may promote drug resistance in the parasite population (Dunyo et al., 2000).
2.7.2 Transmission Control.

The transmission control strategy recognizes that malaria is an important cause of morbidity as well as mortality. Appropriate treatment is one aspect of the transmission control strategy, vector control is also a major player, and, properly applied, these aspects together have an impact on both the mortality and morbidity of malaria. This approach is effective in most epidemiological conditions and is an effective control strategy for a sustained attack on the malaria problem (Shiff et al., 1996). It is adaptable to the use of insecticide-treated mosquito nets as well as indoor spraying of insecticide. It can be implemented in specific circumstances where malaria is a local priority or on a wide scale as part of a major program of intervention. Transmission control requires coordination and the development of strategic plans to intervene against malaria (Kouznetsov, 1977). A high level of expertise is needed with personnel trained in epidemiology and vector control as well as in planning, mapping, and communications to coordinate and supervise the operations.

2.7.3 Malaria Eradication

Eradication can be considered only in certain areas, for example, in places where malaria has been eradicated and where it has been reintroduced and in areas of hypoendemic malaria where there are sufficient resources to undertake the process and where there is little likelihood of future introduction. The advantage of an eradication program is that it is time limited and, once it has achieved its objective, can be terminated with little further oversight (WHO, 1957). Eradication programs have been extremely successful, but eradication could not be achieved in many places and the technique must be considered not appropriate in most areas of endemic infection.
2.7.4 Malaria Vaccines

In the backdrop of malaria treatment, drug resistance and insecticides, appropriate means of disease control are currently lacking; the development of a vaccine remains the only tool for disease eradication. The purpose of vaccination strategies is to induce protective memory immune responses in advance of infection, to provide protection in the case of encountering the disease-causing agent again. Malaria vaccine development is an active research area with enormous challenges. As the parasite proceeds from a sporozoite through the liver stage to the replicating cycle of the blood stage, it undergoes morphological changes and displays antigenic variations. This allows the parasite to evade the protective immune responses of the host.

An ideal malaria vaccine requires three essential features: (i) multiple components that will induce an effective immune response to the different stages of the malaria infection (sporozoites, infected hepatocytes and asexual and sexual stages); (ii) multiple epitopes that are restricted to presentation by different major histocompatibility complex (MHC) molecules to overcome genetic diversity and antigenic variation; and (iii) multi-immunogenicity inducing more than one type of immune response, including cell-mediated and humoral components. Such a multicomponent vaccine should increase the probability of a sustainable and effective host response (Shi et al., 1999).

Three types of vaccine candidate targeting different stages in the life cycle of the malaria parasite have been intensively investigated: (i) transmission-blocking vaccines (TBVs); (ii) pre-erythrocytic vaccines; and (iii) blood-stage vaccines.
Transmission-blocking vaccines (TBVs)

Transmission-blocking vaccines target antigens on gametes, zygotes and ookinetes to prevent parasitedevelopment in the mosquito midgut. The aim of these vaccines is to induce antibodies against thesexual-stage antigens to block ookinete-to-oocyst transition to stop the subsequent generation of infectious sporozoites (Carter et al., 2000). TBVs do not protect the recipient from contracting malaria, but could be helpful in preventing the spread of the disease. The leading vaccine candidates in this group include the *P. falciparum* ookinete surface antigens Pfs25 and Pfs28 and their *P. vivax* homologues Pvs25 and Pvs28 (Hisaeda et al., 2000).

Pre-erythrocytic vaccines

Liver-stage vaccines are designed to prevent malaria in the human host. However, because of the high rate of replication of sporozoites, a single parasite may be sufficient for the infection to proceed to the blood stage. The liver stage of *P. falciparum* is an attractive therapeutic target for the development of both antimalarial drugs and vaccines, as it provides an opportunity to interrupt the life cycle of the parasite at a critical early stage (El Sahly et al., 2010). The most advanced pre-erythrocytic vaccine candidate is RTS,S, which consists of a truncated circumsporozoite protein (CSP) of *P. falciparum* directly fused to the hepatitis B surface (S) antigen.

Blood-stage vaccines

Blood-stage vaccines are designed to elicit antiinvasion and antidisease responses (Moorthy et al., 2004). The underlying principle of these strategies is that if a vaccine could block the invasion of erythrocytes by merozoites, it would prevent malarial disease. Several blood-stage antigens are in
clinicaltrials: apical membrane antigen 1 (AMA1), erythrocyte-binding antigen-175 (EBA-175) (El Sahly et al., 2010), glutamate-rich protein (GLURP), merozoitesurface protein (MSP) 1, MSP2 and MSP3 (Jepsen et al., 2013) and serine repeat antigen 5 (SERA5) (Palacpac et al., 2013).

**Adjuvants and delivery systems for malaria vaccines**

A range of adjuvant formulations and viral/bacterial vectors is available for use with different malaria vaccine candidates. An ideal adjuvant is a component that enhances the potency, longevity and quality of specific immune responses to antigens, with minimal toxicity to the recipient. Adjuvants have been divided into two main groups according to their component sources, physiochemical properties or mechanisms of action: (i) immunostimulants such as Toll-like receptor (TLR) ligands, cytokines, saponins and bacterial exotoxins that act directly on the immune system to increase the response to antigens; and (ii) vehicles such as mineral salts, emulsions, liposomes, virosomes and biodegradable polymer microspheres that present vaccine antigens (Bruder et al., 2010).

Trials of the efficacy of candidate malaria vaccines against natural infections present an opportunity to study whether vaccine-induced immune responses are selectively effective against the vaccine-type allele. If this is so, deployment of such a vaccine might cause the non-vaccine-type alleles in a population to replace the vaccine-type allele, and the overall efficacy of the vaccine would progressively decline. Research has brought several candidate vaccines through phase 1 and in some cases phase 2 of vaccine development.

The only vaccine to reach phase 3 was a multicomponent synthetic peptide, SPf66, which contains a short sequence from near the N terminus of the *P. falciparum* merozoite surface protein 1 (MSP1), which was claimed to have protective effects in trials in Columbia. The
vaccine was tested in a series of trials in holoendemic situations in Africa (D’Alessandro et al., 1995) and showed marginal, if any, protection. Finally, no protection was observed in a trial when it was incorporated in an Extended Program of Immunization program and administered to children as part of the initial immunization program. The recombinant protein-based RTS,S/AS02A that contains a large portion of the *P. falciparum* circumsporozoite protein was tested in The Gambia (D’Alessandro et al., 1995) and Mozambique (Nardin, 1999). The RTS,S/AS02A vaccine had a significantly protective effect but did not affect the allele frequencies of the antigen in the infections that occurred in the vaccine group compared to the control group (Anders and Saul, 2000).

Vaccination with RTS,S/AS01 significantly reduced overall hospital admissions, admissions because of malaria, severe anaemia, and the need for blood transfusion in children, with these protective effects being more marked in those who received a booster dose (*RTS,S Clinical Trials Partnership*, 2015). Administration of a booster dose of RTS,S/AS01 led to an increase in anti-circumsporozoite geometric mean titres in both young infants and children, as noted in adults immunised with an earlier formulation of the vaccine (RTS,S/AS02), but the anti-circumsporozoite geometric mean titres after the booster remained lower than concentrations after the primary course and the booster effect was only transitory. Changes in anti-circumsporozoite concentration over time paralleled changes in efficacy against clinical malaria. However, there was a significant imbalance in cases of meningitis in children vaccinated at the age of 5–17 months between the RTS,S/AS01 and control groups (*RTS,S Clinical Trials Partnership*, 2015).

Development of a successful malaria vaccine is an important component of the global effort for malaria eradication as well as a major challenge to the scientific community and funding.
agencies. Recent reports of limited protection from severe malaria in young African children vaccinated with RTS,S, a recombinant subunit of malaria vaccine and renewed interest in the production and testing of live attenuated sporozoite bases candidate vaccines have sparked the prospects of an effective malaria vaccine in the near future (Nardin, 1999).