Prevalence of *Plasmodium falciparum* sulfadoxine/pyrimethamine Resistant Mutations among pregnant women attending University College Hospital, Ibadan

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Certification

This is to certify that **Ibukun Ayomipo ADEYEFA** with matriculation number LCU/PG/000404 carried out this research work titled **"Prevalence of *Plasmodium* falciparum Sulfadoxine/Pyrimethamine drug resistant mutations among pregnant women attending University College Hospital Ibadan"** in the Department of Biological Sciences, Faculty of Natural and Applied Sciences, Lead City University Ibadan Nigeria for the award of Master Degree in Medical Microbiology and that this has not been previously submitted.

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Dr. O Ajibaye **Date**
(Supervisor)

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(Head of Department)

Dedication

I dedicate this Thesis to Almighty God from whom I received sufficient grace and strength for its successful completion.

Acknowledgement

All glory to Almighty God who has given me the grace to complete this research work.

My appreciation goes to the management of University College Hospital, Ibadan for giving the opportunity to collect samples among pregnant women in antenatal clinic.

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Even though the above-mentioned institutions and persons assisted in the process of this research work, I alone stand responsible for the error, if any, found in the work.

Abstract

Malaria in pregnancy is a public health problem that continues to put pregnant women and their unborn babies at risk. Therefore, this study was undertaken to investigate the prevalence Sulphadoxine-Pyrimethamine drug resistance mutations among pregnant women attending ante-natal clinic (ANC) at University College Hospital (UCH), Ibadan. A total of one hundred and five (105) eligible pregnant women attending ANC at UCH, Ibadan from February to July, 2024 were recruited for this study. Structured questionnaire was administered and about 5mls of venous blood samples were collected into EDTA bottle for malaria detection and dried blood spot samples for molecular drug resistance gene detection. Malarial parasite was detected using Microscopy and Rapid Diagnostic Test methods and antifolate wildtype genes *pfdhfr* and *pfdhps*, and their drug resistant mutant genes *S108N* and *K540E* were detected with Polymerase Chain Reaction using gene specific primers and visualized with agarose gel electrophoresis. Data analysis was done using SPSS version 25. Malaria prevalence was 17.1% with age group 25-34 years having significantly highest prevalence of 23.9% when compared to other age groups($P<0.05$).Primigravidae and first-trimester pregnancy had highest prevalence rates of 21.1% and 19.6% respectively when compared to other groups but not statistically significant ($P>0.05$).There was association between gravidity, gestational age and parasite density with primigravidae and first trimester having significantly highest parasite count of 1500 ± 100/µl and 1600±100/µl respectively when compared with other group ($P<0.05$). Moderately high levels of antifolate wildtype genes: *pfdhfr* (6.7%) and *pfdhps*(12.4%) with their corresponding alleles mutant genes: *S108N* (4.8%) and *K540E*(6.7%) were detected. There was no association between age, gestational age, gravidity and SP drug resistance mutations ($P>0.05$). The mutations linked to antifolates suggest widespread resistance to sulphadoxine-pyrimethamine used for intermittent preventive treatment during pregnancy. Further studies should be done to address the prevalence of other genetic resistance markers.

Keywords: **Malaria, Pregnancy, P.falciparum, antifolate wildtype genes, mutant genes**
Word Count: 297

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List of Acronyms

Abbreviation	Meaning
ACTs	Artemisinin-based Combination Therapies
CRP	C - reactive protein
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunosorbent assay
GPI	Glucose Phosphate Isomerase
IFA	Immunoflourescence antibody
LAMP	Loop-mediated isothermal amplification
LBW	Low Birth Weight
LDH	Lactate dehydrogenase
QBC	Quantitative Buffy Coat
RBM	Roll Back Malaria
RDTs	Rapid Diagnostic Tests
SP	Sulfadoxinepyrimethamine
WBC	White Blood Cells
WHO	World Health Organization

Chapter One

Introduction

1.1 Background of the Study

Household responses to illness are influenced by socio-economic and cultural factors and ease of access to treatment sources[1]. In sub-Saharan Africa, rural and urban populations differ demographically, in socio-economic and cultural composition, and in proximity to formal and informal treatment sources[2]. Malaria is one of humanity's worst diseases and the suffering it causes is now a global crisis. One fifth of the world population is at risk of malaria and there are more than 300 million cases each year. It is responsible for over a million deaths each year and most of the deaths occur in pregnant women and children under five years[3].

Malaria is a serious and sometimes fatal disease which makes it the leading cause of morbidity and mortality worldwide with higher burden in sub-Saharan Africa even though it is both preventable and treatable, and effective preventive and curative tools have been developed[4]. Despite several efforts by international communities to provide funds in billions of dollars to fight malaria in Africa, the problem has continued unabated leading to loss of lives daily[5]. Many of the malaria patients do not have access to health facilities on time while others who do may not access modern facilities. Some healthcare professionals still depend on the conventional method such as rapid diagnostic test for malaria parasites and which may not accurately detect the parasite on time[6].

Despite global efforts to eradicate the disease it remained a great challenge to health system for countries in the developing world. It is also a public-health problem and the principal cause of childhood mortality[7]. It is a vector-borne infectious disease caused by *plasmodium spp*. Malaria is widespread in tropical and subtropical regions in a broad band around the equator. It is prevalent in tropical and subtropical regions because rainfall, warm temperatures, and stagnant waters provide habitats suitable for mosquito to breed[8]. Five species of *plasmodium* can infect and be transmitted by humans, however, majority of deaths

from malaria are caused by *plasmodium falciparum* while *plasmodium vivax, plasmodium ovale*, and *plasmodium malarie* causes a generally milder form of malaria that is rarely fatal[9]. The zoonotic species, *Plasmodium knowlesi* is a parasite that causes malaria in humans and other primates, it is found throughout Southeast Asia; it causes malaria in macaques, but can also cause severe infections in humans[10]. The disease causes symptoms that typically begin 8-25 days following the infection, symptoms may occur later in those who have taken anti malaria medications as prevention. The classic symptom of malaria is paroxysm cyclical occurrences of sudden coldness followed by rigor and then fever. Severe malaria has varying patterns and the relative contributions to individual symptoms to mortality differ with endemicity, geographic locations and access to health services[11].

Pregnant women infected with malaria represent a significant obstetric problem that predisposes them and their unborn baby to adverse consequences[12]. Findings from clinical studies revealed that 25% women with placental malaria had evidence of malaria during parturition. In most endemic areas, malaria during pregnancy is associated with maternal anemia. It has been estimated that about 26% of maternal anemia is due to malaria[12]. In Africa, it was reported that severe anemia as a result of falciparum malaria is responsible for approximately 10,000 maternal deaths per annum[13]. Infants with congenital malaria also experience devastating effects from the infection. It has been estimated that 1 in every 5 low birth weight (≤2500 g) deliveries are a consequence of maternal malaria. This invariably results in poor infant development and survival and has led to approximately 100,000 infant deaths every year[14].

Malaria during pregnancy is a major cause of maternal morbidity worldwide and leads to poor birth outcomes. Pregnant women are more prone to complications of malaria infection than nongravid women[15]. Prevention involves chemoprophylaxis and mosquito avoidance. Treatment involves antimalarial drugs and supportive measures. One hundred twenty-five

million pregnant women are at risk for contracting malaria, a preventable cause of maternal and infant morbidity and death[16]. Malaria parasites contribute to adverse pregnancy and birth outcomes due to their preferential accumulation in placental intervillous spaces. Pregnant women are particularly vulnerable to malaria infections, and malaria infections during pregnancy put their fetuses at risk[17]. Malaria in pregnancy is associated with anemia, stillbirth, low birth weight and maternal and fetal death. A study was reviewed to diagnosing the challenges of malaria in pregnancy, as well as strategies to prevent and treat malaria in pregnancy[18].

The introduction of a new antimalarial treatment has been soon followed by the emergence of resistance to that treatment; perhaps most notably, parasites resistant to the antimalarial atovaquone were discovered the same year the drug was introduced[19]. After *P. falciparum* parasites became resistant to chloroquine, pyrimethamine/sulfadoxine, mefloquine and then atovaquone, *P. falciparum* malaria became very difficult to cure. Thus, the world enthusiastically welcomed the appearance of a new class of medicines based on extracts from the sweet wormwood plant, *Artemisia annua*[20].

The antimalarial activity of *A. annua* had been rediscovered in a screen of traditional medicines for those able to cure mice and monkeys that had rodent and simian malaria, respectively. Artemisinin derivatives are typically combined with a partner drug, typically from a chemical family such as the aryl alcohols or 4-aminoquinolones, to comprise artemisinin-based combination therapies (ACTs)[21]. Indeed, the World Health Organization only supports the use of artemisinins in combinations, reasoning that this will delay the appearance of drug resistance because a parasite will need to acquire resistance to two drugs as opposed to just one. Although not recommended for use everywhere, ACTs are currently considered the most effective treatments for *P. falciparum* malaria in areas where drug resistance to other therapies has been a problem[22]. Nevertheless, clinical trials from Southeast

Asia indicate parasites have now acquired resistance to artemisinin-based monotherapies and some ACTs appear to be losing effectiveness. Although no deaths can be directly attributed to resistance, further reductions in ACT efficacy could result in malaria again becoming a possibly incurable and often fatal disease[23].

Later-stage clinical trials on novel classes of antimalarial compound to replace artemisinin are currently underway, but no new drugs are expected to be licensed within the next few years[24]. In the absence of a forthcoming replacement medicine, concerned physicians, scientists, and government officials have been working diligently to try to find parasite genetic markers that predict artemisinin resistance[25]. Such markers will facilitate tracking of the spread of resistance and hopefully will allow resistance to be contained before early-stage treatment failures and possible deaths result. Importantly, having a genetic marker could also prevent deaths: if surveillance identifies the widespread presence of resistance-associated alleles in a given geographic region, patients in those regions might be admitted to a hospital for closer observation during treatment or given alternative therapies. In this study, the genes involved in artemisinin resistance will be elucidated[26].

The emergence of resistance to former first-line antimalarial drugs has been an unmitigated disaster. In recent years, artemisinin class drugs have become standard and they are considered an essential tool for helping to eradicate the disease[27]. However, their ability to reduce morbidity and mortality and to slow transmission requires the maintenance of effectiveness. Recently, an artemisinin delayed-clearance phenotype was described. This is believed to be the precursor to resistance and threatens local elimination and global eradication plans. Understanding how resistance emerges and spreads is important for developing strategies to contain its spread. Resistance is the result of two processes: (i) drug selection of resistant parasites; and (ii) the spread of resistance[28].

The cornerstone of antimalarial treatment, artemisinin, has reduced malaria associated morbidity and mortality worldwide[29]. However, *Plasmodium falciparum* parasites with reduced sensitivity to artemisinin have emerged, and this threatens malaria control and elimination efforts. In this minireview, they describe the initial development of artemisinin as an antimalarial drug, its use both historically and currently, and our current understanding of its mode of action and the mechanisms by which malaria parasites achieve resistance[30].

Resistance appears to occur through spontaneous mutations that confer reduced sensitivity to a given drug or class of drugs. For some drugs, only a single point mutation is required to confer resistance, while for other drugs, multiple mutations appear to be required[31]. Provided the mutations are not deleterious to the survival or reproduction of the parasite, drug pressure will remove susceptible parasites while resistant parasites survive. Single malaria isolates have been found to be made up of heterogeneous populations of parasites that can have widely varying drug response characteristics, from highly resistant to completely sensitive[32]. Similarly, within a geographical area, malaria infections demonstrate a range of drug susceptibility. Over time, resistance becomes established in the population and can be very stable, persisting long after specific drug pressure is removed[33].

Over 90% of malaria cases occur in Sub-Saharan Africa, where a child dies from this illness every 30 seconds[34]. In Nigeria, the burden of malaria is well documented and has been shown to be a big contributor to the economic burden of disease in communities where it is endemic and is responsible for annual economic loss to the tune of 132 billion Naira. It is estimated that out of 300,000 deaths occurring each year, 60% of outpatient visits and 30% hospitalizations are all caused by malaria[35]. No doubt Nigeria is a malaria-endemic area and malaria is the principal cause of childhood mortality. Global malaria deaths peaked at 1.82 million in 2004 and have fallen steadily since then, dropping by 32% to 1.24 million in 2010

(714,000 children aged less than 5 years, 524,000 individuals aged less than or equal to 5years)[36].

However, the number of deaths in individuals aged less than or equal to 5 years in 2017 exceeded WHO estimates by 433,000. The situation has remained consistent ever since, this is why investigation on how to mitigate malaria spread among under-five is pertinent and of policy relevance[37]. Malaria is common among pregnant women and children under 5 years of age due to their low levels of immunity. This is why these categories of the population were the primary focus of the Roll Back Malaria (RBM) program[38]. RBM aims at maintaining an overall vision of a malaria-freeworld. The goals and targets for malaria control was to reduce global malaria deaths to near zero by 2020, reducing global malaria cases by 75% from what it was in 2000 by end of 2018 and eliminate malaria by end 2020 in 10 new countries, this goal is yet to be achieved[39].

The increase in the prevalence of malaria in Nigeria is due to both behavioural and non-behavioural factors. The behavioural factors relates to some cultural practices, which promote mosquito breeding and mosquitoes' access to the people as well as the failure of 'at risk' population to use technologies proven to be effective for the treatment, control and prevention of malaria promptly and adequately[40]. The non-behavioural factors include geographical or ecological peculiarities, the availability of mosquitoes and the presence of plasmodia. Thorough understanding of both behavioural and non-behavioural factors is very important for the design of appropriate interventions for tackling malaria. Creating awareness and improving the understanding of the transmission of malaria has been found to greatly contribute to ameliorating malaria menace and facilitate the sustainability of malaria elimination programmes[41].

Malaria during pregnancy is not only a major public health problem in Nigeria but in other sub-Saharan African countries, especially in malaria-endemic areas[42]. Malaria in pregnancy is

said to be one of the leading contributors to the unacceptably high maternal mortality ratio in the developing countries. It is believed to be responsible for approximately 50 million cases of malaria among pregnant women worldwide annually with more than 30 million of them live in the African region[43]. It is more frequent and severe in primigravidae, both during pregnancy and at the time of delivery. "The serious consequences of malaria in pregnancy are attributed to the sequestration of malaria parasites in the placenta, leading to impeded trans-placental nutrient transport. This, combined with malaria induced anemia, compromises foetal growth and result in low birth weight (LBW) and a subsequent increase in infant and childhood mortality[44]. In addition, malaria in pregnancy is responsible for as much as 50% of low birth weight (LBW) among primigravidae especially in endemic areas. In short, malaria has many untoward effects on both the mother and the fetus, thus making this study of great importance[45].

In all the endemic areas, the frequency of malaria is higher in pregnant women than the same women before pregnancy. It causes severe maternal anemia, spontaneous abortion, stillbirth, premature delivery (gestation of less than 37 weeks), intrauterine growth retardation and low birth weight increasing rise of infant death[46]. Low birth weight is a well-documented risk factor; along with poor neuro-sensory, cognitive and behavioral performance and achievement. The interaction between HIV and malaria during pregnancy are complex studies. In Kenya and Malawi it was shown that the prevalence and density of malaria parasites are also found in HIV positive victims[47]. The risk of infants dying during the post neonatal period has also been identified to rise higher in children born with HIV positive mothers and with placental malaria than in those born to HIV positive mothers without placental malaria[48].

1.2 Statement of the Problem

Malaria is a major public health concern among pregnant women in sub-Saharan Africa. Within the region, Nigeria has the highest malaria cases. Malaria during pregnancy leads to

serious adverse effects on mothers and foetus[49]. Malaria complicates up to 58.1% of pregnancies in Nigeria. Pregnant women in their first and second pregnancies are particularly susceptible to Malaria. Placental parasitaemia limits transfer of nutrients and oxygen to the foetus, leading to intrauterine growth restriction, low birth weight or intrauterine death[50]. The prevalence of *Plasmodium falciparum* sulfadoxine/pyrimethamine resistant mutations among pregnant women represents a significant public health concern, particularly in regions where malaria is endemic. Pregnant women are at an increased risk of severe malaria due to physiological changes that occur during pregnancy, including altered immune responses and increased susceptibility to infections[51]. This vulnerability is compounded by the potential adverse effects of malaria on both maternal and fetal health, including anemia, low birth weight, and increased risk of maternal mortality. Sulfadoxine/pyrimethamine (SP) has been widely used as a first-line treatment for uncomplicated malaria and as part of intermittent preventive treatment in pregnancy (IPTp). However, the emergence and spread of drug-resistant strains of *P. falciparum* have raised concerns about the effectiveness of SP in treating and preventing malaria in this vulnerable population. Resistance mutations in the P. falciparum dihydropteroate synthase (dhps) and dihydrofolate reductase (dhfr) genes have been identified as key markers of reduced sensitivity to SP. The prevalence of these mutations varies geographically, influenced by factors such as drug usage patterns, local malaria transmission dynamics, and the socio-economic context. Several factors such as educational level, family income, and occupation are known to increase mothers' knowledge about malaria and its management[51]. Pregnant mothers who are knowledgeable about the disease are likely to use proper preventive measures and take sick children to a health facility in time.

1.3 Justification of the Study

Despite the high risk of malaria among pregnant women in this area, there is limited evidence about the burden and anti-malarial drug resistance genes.

Therefore, the data from this study can be used to formulate policy which can be used to reduce the maternal and child mortality due to the disease.

1.4 Aim and Objectives of the Study

1.4.1 Aim

The study is set out to assess the prevalence of *Plasmodium* falciparum malaria dhps, dhfr and K540 sulfadoxine/pyrimethamine resistant mutation among pregnant women attending ante-natal clinic at University College Hospital, Ibadan.

1.4.2 Objectives of the Study were to:

i. Determine the prevalence of malaria in pregnancy among pregnant women attending UCH
ii. Determine the prevalence of *pfdhps* k540E mutations among pregnant women attending UCH
iii. Determine the prevalence of *dhfr S108N* mutations among pregnant women attending UCH
iv. Investigate the relationship between *pfdhps k540E*, *dhfr S108N* SP resistance mutations and participants' age, parasiteamia, gravidity and trimester among pregnant women attending antenatal clinic at UCH.

1.5 Research Questions

1. What is the prevalence of malaria in pregnancy among pregnant women attending UCH
2. What is the prevalence of *pfdhps* k540E mutations among pregnant women attending UCH?
3. What is the prevalence of *dhfr S108N* mutations among pregnant women attending UCH?
4. What is the relationship between *pfdhps k540E*, *dhfr S108N* SP resistance mutations and participants' age, parasiteamia, gravidity and trimester among pregnant women attending antenatal clinic at UCH?

1.6 Hypotheses

This study is Based on Null Hypothesis (H_o) that:

- Prevalence of *plasmodium falciparium* malaria dhps, dhfr and K540 sulfadoxine/pyrimethamine resistant genes are significantly low among pregnant women attending ante-natal clinic at UCH, Ibadan

Alternate Hypothesis

- Prevalence of *plasmodium falciparium* malaria dhps, dhfr and K540 sulfadoxine/pyrimethamine resistant genes are significantly high among pregnant women attending ante-natal clinic at UCH, Ibadan

1.7 Significance of the Study

Malaria, a debilitating febrile and life threatening illness, is caused by a parasite called *plasmodium*. Its route of transmission still remains as bites from infected female anopheles mosquitoes. Environmental factors and behavioral patterns of vectors and human populations combine to provide favorable conditions for malaria transmission. Proven effective options to reduce morbidity and mortality include early diagnosis, combined with prompt effective therapy and malaria prevention through reduction of human-vector contact, especially with the use of ITNs. Achieving sustainable control of the disease depend on extensive public health promotional programs which focus on current and proven methods of malaria prevention and management. Improved nutrition, identification and elimination of causes of low bed net effectiveness, and reinforced health education are promising and tangible measures to further reduce *P. falciparum*. The result of this finding will help pregnant women to have more knowledge on the prevalence of malaria. The outcomes of this study will also help government sectors and partners working in collaboration to implement an intervention program focusing on the prevalence of malaria and detection of antimalarial drug resistance genes among pregnant women. The findings of this study will have special importance for

health care providers because it will serve as base line for filling gaps of the actual prevention of malaria among pregnant women. It will also contribute to reducing maternal and child mortality, improving health systems and promoting access to effective treatments, relates to the Sustainable Development Goals (SDGs) 3. Furthermore, understanding drug resistance is essential for developing strategies to combat malaria effectively, aligning with the goal of combating epidemics and ensuring universal health coverage. It will also expose other areas of interest about prevalence of malaria and detection of antimalarial drug resistance genes among pregnant.

1.8 Scope of the Study

Current study mainly focused on assessing prevalence of malaria and detection of antimalarial drug resistance genes among pregnant women attending ante-natal clinic at University College Hospital, Ibadan

1.9 Limitation of the Study

This research work is limited to pregnant women attending ante-natal clinic at University College Hospital, Ibadan.

1.10 Operational Definition of Terms

Prevalence: The total number of cases of malaria and detection of antimalarial drug resistance genes among pregnant women attending ante-natal clinic at University College Hospital, Ibadan

Malaria: A mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans belonging to the genus Plasmodium (Phylum Apicomplexa)

Antimalarial Drug: This is the drug used for the treatment and prevention of malaria infection in pregnancy

Pregnancy: This is the physiological condition in which a woman carries a developing fetus within her uterus, typically lasting about 40 weeks from the last menstrual period to childbirth

Pregnant Women: A female human carrying a developing offspring or an expectant mother

P.*falciparum*: A species of protozoan parasite that is one of the causative agents of malaria in humans

Mutant genes: Genes that have undergone a change or alteration in their nucleotide sequence compared to the wild-type or original form

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Chapter Two

Literature Review

2.1 Overview of Malaria

Malaria is regarded as the second most common cause of infectious disease-related deaths in the world, after tuberculosis[1]. It is estimated that malaria affect between 350 to 500million people annually and accounts for 1 to 3million deaths per year. The Sub-Saharan Africa has the largest burden of malarial disease because the region's tropical climate provides ideal breeding grounds for Anopheles mosquitoes, the primary vectors of malaria. High temperatures and abundant rainfall create favorable conditions for mosquito reproduction and survival, many countries in Sub-Saharan Africa face poverty, limited access to healthcare, and inadequate infrastructure with over 90% of the world's malaria-related deaths occurring in this region. It accounts for over 10,000 maternal and 200,000 neonatal deaths per year[2]. Although malaria is known to affect all ages and sexes, its morbidity and mortality is believed to be significant in pregnant women and children less than five years of age. Malaria contributes very significantly to maternal and fetal mortality[3].

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoans belonging to the genus Plasmodium (Phylum Apicomplexa)[4]. Malaria is a vector-borne disease that is widespread in the tropical and subtropical areas of the world. "The disease is most commonly transmitted by an infected female Anopheles mosquito. Only female mosquitoes feed on blood; male mosquitoes feed on plant nectar, and do not transmit the disease[5]. The female of the Anopheles genus of mosquito prefer to feed at night. Five species of Plasmodium can infect and be spread by humans; *P.falciparum. P.vivax, P.ovale, P.malariae*, and the simian parasite *P.knowlesi*"[6].

Plasmodium falciparum cause the largest burden of disease, followed by P. vivax. It is reported that the *P. falciparum* predominates in Africa, New Guinea, and Hispaniola (Haiti and the Dominican Republic); the P. vivax however is more common in the Americas and the

western Pacific[7]. The prevalence of these two species is approximately equal in the Indian subcontinent, eastern Asia, and Oceania. The report shows that whilst the *P. malariae* is uncommon and is found in most endemic areas, especially in Sub-Saharan Africa, the P. ovale, even less common, is relatively unusual outside of Africa and, where it is found, comprises <1% of isolates[8]. Moreso, the *P. knowlesi*, similar morphologically to *P. malariae*, has been identified by molecular methods in patients in Malaysia, the Philippines, Thailand, and Myanmar. This species has not yet been proven to be transmitted from humans to mosquitoes (i.e., a monkey reservoir may be required to infect mosquitoes)[9].

Malaria was the most important health hazard encountered by U.S. troops in the South Pacific during World War 2, where about 500,000 men were infected. According to Joseph Patrick Byrne, sixty thousand American soldiers died of malaria during the African and South Pacific campaigns[10]. Malaria is prevalent in tropical and subtropical regions because of rainfall, consistent high temperatures and high humidity, along with stagnant waters in which mosquito larvae readily mature, providing them with the environment they need for continuous breeding[11]. In drier areas, outbreaks of malaria have been predicted with reasonable accuracy by mapping rainfall. Malaria is more common in rural areas than in cities. The geographic distribution of malaria within large regions is complex, and malaria-afflicted and malaria-free areas are often found close to each other[12].

2.2 Prevalence of Malaria Fever

Malaria is an infectious disease of humans and some other animals. It is caused by parasitic protozoans called *Plasmodium* species borne by female anopheles mosquitoes [13]. Malaria fever has been ranked as one of the most important infectious diseases in tropical and sub-tropical regions of the world. It is a serious disease of childhood. It has caused an estimated 207 million cases and 627,000 deaths in 2012, mostly children in sub-Saharan Africa[14]. The clinical manifestations of malaria fever, the severity and course of a clinical attack depend on

the species and strain of the infecting *Plasmodium* parasite. It also depends on the genetic constitution, immune status and nutritional status of the infected person[15].

Malaria was the most important health hazard encountered by United State (US) troops in the South Pacific during World War 2, where about 500,000 men were infected[16]. According to Joseph Patrick Byrne, sixty thousand American soldiers died of malaria fever during the African and South Pacific campaigns[17]. Malaria fever is prevalent in tropical and subtropical regions because of rainfall, consistent high temperatures and high humidity, along with stagnant waters in which mosquito larvae readily mature. All these provide those parasites with the environment they need for continuous breeding[18]. In drier areas, outbreaks of malaria have been predicted with reasonable accuracy by mapping rainfall. It has been discovered that malaria fever is more common in rural areas than in cities and that the geographic distribution of malaria within large regions is complex. Furthermore malaria-afflicted and malaria-free areas are often found close to each other[19].

Globally, about 3.2 billion people are still at risk of contracting malaria and developing disease, and 1.2 billion are at higher risk[20]. Of the six World Health Organization (WHO) regions in the world, malaria transmission occurs in five with Europe remaining free. Climatic factors such as temperature, humidity and rainfall mainly determine where malaria could be found[21]. Malaria is prevalent in tropical and subtropical areas where malaria parasites can complete their growth cycle in the mosquitoes and where *Anopheles* mosquitoes can survive and multiply because of rainfall, high humidity and consistently high temperature [22]. It was reported that in 2017, nearly half of the world's population was at risk of malaria [23]. Most malaria cases and deaths occurred in sub-Saharan Africa. However, the WHO regions of south-east Asia, eastern Mediterranean, western pacific, and the Americas were also reported to be at risk. According to the World Health Organization reports of 2017, 90 countries and areas had ongoing malaria transmission although some population groups were

found to be at considerably higher risk of contracting malaria and developing severe disease than others[24]. These included infants, children under 5 years of age, pregnant women and patients with HIV or AIDS as well as non-immune migrants, mobile populations and travelers.

Figure 2.1:

A: Anopheles Mosquito

B: Aedes Mosquito

C: Culex Mosquito

Source: Different types of mosquitoes[25]

Anopheles mosquitoes are primarily known for their role as vectors of malaria, a disease caused by parasitic protozoa of the genus *Plasmodium*[25]. This genus includes several species, with Anopheles gambiae being one of the most efficient malaria vectors in Africa. Anopheles mosquitoes are characterized by their long legs and distinctive resting posture, where they hold their bodies at an angle to the surface[25]. They are typically active during dusk and dawn, which aligns with the feeding habits of the malaria parasite. The female Anopheles requires blood meals for egg development, and their breeding sites are often found in clean, stagnant water bodies such as ponds, marshes, and rice fields. Control measures for Anopheles mosquitoes often focus on eliminating breeding habitats and using insecticides, particularly in regions where malaria transmission is prevalent[25].

Anopheles mosquitoes have a complex life cycle, consisting of four stages: egg, larva, pupa, and adult. Females lay their eggs in clusters, often referred to as rafts, on the surface of clean, stagnant water[25]. The larvae are aquatic, feeding on organic matter and microorganisms. They undergo several molts before transitioning into the pupal stage, which is a non-feeding stage where they undergo metamorphosis into adults. The entire life cycle from egg to adult can take as little as a week under optimal conditions, although it can be longer in cooler temperatures. Behaviorally, Anopheles mosquitoes are primarily nocturnal, with many species preferring to feed during dusk and dawn[25]. This feeding pattern aligns with the life cycle of the malaria parasite, which develops within the mosquito after it has taken a blood meal from an infected host. The female Anopheles mosquito requires a blood meal to obtain the necessary proteins for egg development. After feeding, the female will typically lay her eggs within a few days, continuing the cycle[25].

The transmission of malaria occurs when an infected female Anopheles mosquito bites a human, injecting sporozoites into the bloodstream. These sporozoites migrate to the liver, where they multiply and eventually enter the bloodstream again, causing the symptoms associated with malaria. The efficiency of Anopheles mosquitoes as vectors depends on various factors, including their longevity, feeding behavior, and environmental conditions[25].

Aedes mosquitoes are recognized for transmitting several viral diseases, including dengue fever, Zika virus, chikungunya, and yellow fever. The most notable species within this genus are Aedes aegypti and Aedes albopictus[25]. Aedes mosquitoes are distinctively marked with white markings on their legs and a lyre-shaped pattern on their thorax. They are aggressive daytime feeders, although they can also bite during twilight hours. Aedes mosquitoes thrive in urban environments, often breeding in artificial containers such as discarded tires, flower pots, and water storage tanks[25]. Their adaptability to human environments makes them particularly challenging to control. Integrated pest management strategies, including community engagement and environmental management, are crucial for reducing Aedes populations and preventing the spread of the diseases they carry[25].

The life cycle of Aedes mosquitoes consists of four stages: egg, larva, pupa, and adult. Females lay their eggs in or near standing water, often in artificial containers such as discarded tires, flower pots, and water storage vessels. The eggs are capable of withstanding desiccation, allowing them to remain viable even in dry conditions until they are rehydrated by rain or flooding[25]. This adaptability helps Aedes mosquitoes thrive in urban settings where breeding sites can be abundant. The larvae of Aedes mosquitoes are aquatic and typically feed on organic matter and microorganisms in the water. They undergo several molts before transitioning into the pupal stage, which is a non-feeding phase. The pupae eventually emerge as adults, with the entire life cycle taking about a week under optimal conditions. Aedes

mosquitoes are known for their rapid reproduction, which contributes to their population density, particularly in areas with favorable environmental conditions[25].

Culex mosquitoes are another significant group, known primarily for transmitting West Nile virus, Japanese encephalitis, and filariasis. Culex pipiens, commonly known as the common house mosquito, is one of the most widespread species in temperate regions. Culex mosquitoes have a more robust body and are generally less aggressive than Anopheles and Aedes[25]. They prefer to feed during the night and are known to breed in polluted water bodies, such as storm drains, ditches, and shallow ponds. The larvae of Culex mosquitoes are often found in nutrient-rich water, which supports their growth. Control strategies for Culex mosquitoes typically involve larviciding and habitat management, focusing on reducing stagnant water where these mosquitoes breed.

The life cycle of Culex mosquitoes includes four stages: egg, larva, pupa, and adult. Females lay their eggs in rafts on the surface of stagnant or slow-moving water, often in polluted environments such as storm drains, ditches, and marshes[25]. The eggs hatch into larvae, which are aquatic and feed on organic matter and microorganisms. The larvae undergo several molts before entering the pupal stage, where they undergo metamorphosis into adults. The entire life cycle can take anywhere from a week to several weeks, depending on environmental conditions such as temperature and food availability[25]. Behaviorally, Culex mosquitoes are primarily nocturnal and are most active during the evening and night. They tend to feed on a variety of hosts, including birds, mammals, and humans. The preference for avian hosts makes them particularly important in the transmission of viruses like West Nile, as these viruses often circulate in bird populations before being transmitted to humans. Female Culex mosquitoes require blood meals for egg development, and after feeding, they typically lay their eggs within a few days[25].

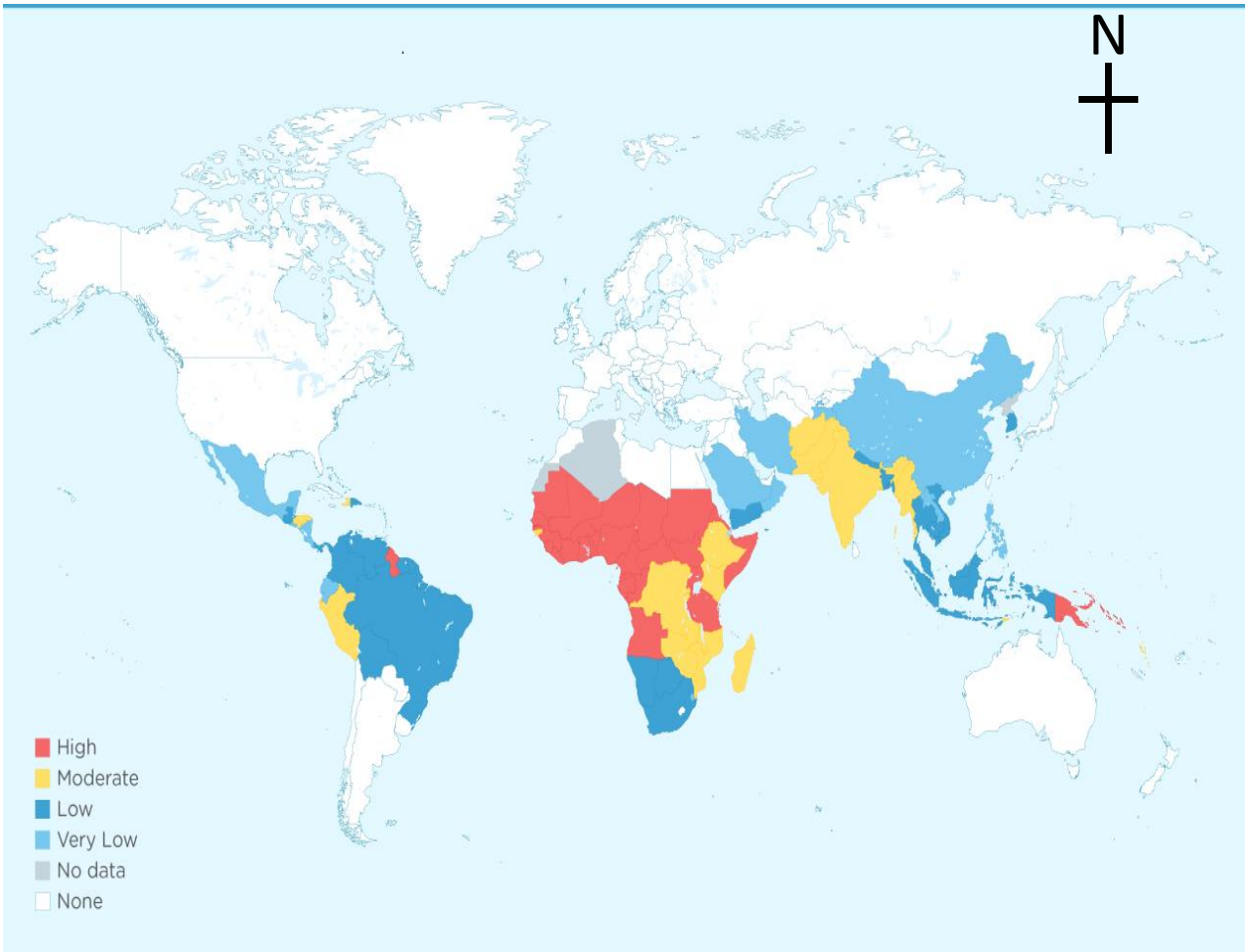

Figure 2.2: Global estimated malaria risk[26],

2.3 History of Malaria

Due to the disease's association with marshland and swamps, it was formerly called 'marsh fever' or 'ague'. However, the term malaria is reported to have originated from medieval Italian language which means "bad air" [26]. The term malaria was first used in English literature around 1829. The history of malaria stretches from its prehistoric origin as a zoonotic disease in the primates of Africa through to 21st century. The first evidence of the parasites was found in mosquitoes preserved in amber from the palaeogene period that is approximately 30 million years old[27]. However, malaria started having a major impact on human survival about 10,000 years ago at the same time with development of human settlements and advances in agriculture.

Evidences show that malaria was once common in most of Europe and North America, even though it is no longer endemic in these regions[28]. Records showed that the Roman empire was devastated by malaria and the disease so manifested throughout Rome that it was known as the "Roman fever". The presence of stagnant water in several regions in the ancient Rome provided favourable conditions for malaria vectors[29]. Throughout recorded history, beginning in 2700BC in China, there were several references describing the unique periodic fevers of the disease. While Columella associated malaria with insects from swamp, Hippocrates described the periodic fevers as tertian, quartan, subtertian and quotidian[30].

2.4 Epidemiology of Malaria

Human and mosquito vector genetics are separate and well-defined fields that influence the epidemiology of malaria but fall outside the pathogen-oriented focus of molecular epidemiology here[31]. A broader view would see them as part of the same system, with many potential points of ecological and evolutionary interaction. For example, interactive variation in human major histocompatibility complex and T-cell receptor genes can influence the type of selection operating among allelic forms of parasite antigens while hemoglobin variants can

have effects on display of variant surface antigens of the parasite on the erythrocyte membrane[32]. Vector mosquitoes such as Anopheles gambiae in Africa show genetic variation in the ability to transmit malaria parasites and have complex population structures that contain incipient species, features likely to affect the population biology of the parasite and to which it may respond adaptively. Essentially, many heritable variations in human and mosquito populations exist alongside other environmental variation that affects the molecular epidemiology of the parasite[33].

Some molecular processes lend themselves to mathematical modeling at a population level, particularly in genetics and immunology. Researchers who have modeled conceptual processes are increasingly keen to analyze the actual molecular variables that underlie these, and this helps to identify major knowledge gaps that can target research[34]. Processes that have recently received modeling attention include the relationship between immunity and the evolution of virulence, the orchestration of antigenic variation by immune responses and the emergence and spread of drug resistance[35]. The genome sequence of P. falciparum reveals the primary structure and arrangement of more than 5,000 protein coding genes and the sequences of other Plasmodium species give comparable information. This can be exploited by comparative genomic analyses that allow inferences to be drawn on molecular evolution and microarray analyses to show profiles of transcription at all stages in the life cycle (including liver stages that are accessible in rodent malaria models but not in human malaria)[36]. Data on gene evolution can lead to hypotheses about gene function, whereas studies of genome-wide patterns of gene transcription allow the potential role of otherwise nondescript genes to be highlighted. Either type of information is likely to ultimately relate to mechanistic hypotheses of clinical or epidemiological relevance[37].

Globally, about 3.2 billion people are still at risk of contracting malaria and developing disease, and 1.2 billion are at higher risk. Of the six WHO regions, malaria transmission

occurs in five with Europe remaining free. Climatic factors such as temperature, humidity, and rainfall mainly determine where malaria is found[38]. Malaria is prevalent in tropical and subtropical areas where malaria parasites can complete their growth cycle in the mosquitoes and where Anopheles mosquitoes can survive and multiply because of rainfall, high humidity and consistent high temperature[39]. It was reported that in 2017, nearly half of the world's population was at risk of malaria. Most malaria cases and deaths occur in sub-Saharan Africa. However, the WHO regions of south-east Asia, eastern Mediterranean, western pacific, and the Americas are also reported to be at risk[40]. According to the World Health Organization reports, in 2017, 90 countries and areas had ongoing malaria transmission although some population groups are at considerably higher risk of contracting malaria, and developing severe disease, than others. These include infants, children under 5 years of age, pregnant women and patients with HIV/AIDS, as well as non-immune migrants, mobile populations and travelers[41].

Malaria has been known to be widespread in the tropical and sub-tropical regions and has ravaged many communities most especially in Nigeria with considerable morbidity and death[42]. It causes the death of more than one million in Africa every year, and is responsible for fifteen percent (15%) of clinical illnesses in the tropical regions of Africa[43].Ten percent (10%) of death in children aged below three years had been estimated to be due to malaria in many parts of the tropical regions. In remote rural areas of sub-Sahara Africa, 1.5 to 2.7 million deaths of the estimated annual 300-500 million cases of clinical malaria were directly attributed to malaria and the great majority occurred in young children[44].

According to Centres for Disease Control and Prevention, 216 million people became ill of malaria in 2016 and 445,000 died of malaria disease in 2016[45]. Each year about 1,700 cases had been diagnosed in the U.S. Again nearly half of the world's populations were at risk and $12 billion was lost per year in Africa alone. In 2017, there was an estimated 219 million

cases of malaria in 90 countries and death cases caused by malaria reached 435,000. In Africa there was 92% of malaria cases and 93% of malaria deaths in 2017[46]. According to World malaria report, in 2017, five countries accounted for nearly half of all malaria cases worldwide. These include Nigeria (25%), the Democratic Republic of the Congo (11%), Mozambique (5%), India (4%) and Uganda (4%). It is worthy of note that malaria remains a major killer of children aged below five years, taking the life of a child every two minutes[47]. In general, malaria affects the rural poor, making it a leading cause of absenteeism among employees, increased health care spending, decreased productivity, and approximately fifty percent (50%) of all preventable school absenteeism in Africa. It helps to trap families in a cycle of disease and poverty. Particularly in Nigeria, malaria causes economic loss of approximately 132 billion yearly and also debilitates adults [48].

2.5 Causative Agent of Malaria

Malaria parasite is a mosquito-borne disease with incubation period of seven days longer deadliest diseases affecting people particularly in tropical and sub-tropical regions of the world. Malaria remains the most complex and overwhelming health problem facing humanity, with 300-500 million cases and 2-3 million deaths per year[49]. Malaria was once widespread in North America and other temperate regions. Today, the disease occurs mostly in tropical and subtropical regions, particularly in sub-Saharan Africa and Southeast Asia. About 90% of all malaria infections in the world today occur in Africa south of the Sahara[50].

Malaria parasite is a mosquito-borne agent of malaria disease with incubation period of seven days or longer. It is caused by the protozoan *Plasmodium*. There are five species of *Plasmodium* parasite known to infect humans. These are *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale*, *Plasmodium vivax* and *Plasmodium knowlesi*[51]. These occur in different parts of the world. Some cause a more severe type of malaria than others. *Plasmodium falciparum* is the most common species identified (75%) among those

infected, followed by *Plasmodium vivax* (20%)[52]. *Plasmodium falciparum* accounted for 99.7% of estimated malaria cases in the World Health Organization (WHO) African region. *Plasmodium falciparum* is transmitted into human during the bite of female mosquitoes of the genus *Anopheles* which injects the sporozoites, which is the invasive form of *Plasmodium* into human body[53].

2.5.1 Life Cycle of Plasmodium

Plasmodium species exhibits three life-cycle stages: gametocytes, sporozoites, and merozoites. Gametocytes within a mosquito develop into sporozoites. The sporozoites are transmitted via the saliva of a feeding mosquito to the human bloodstream. From there they enter liver parenchyma cells, where they divide and form merozoites[54]. The merozoites are then released into the bloodstream and therefore infect the red blood cells. Rapid division of these merozoites results in the destruction of the red blood cells. The newly multiplied merozoites then infect new red blood cells. Some merozoites may develop into gametocytes, which can be ingested by a feeding mosquito, starting the life cycle over again[55].

The life cycle of *Plasmodium* is a complex one that requires both a human and a mosquito host, and differentiating multiple times during its transmission and infection process[56]. A female anopheles mosquito (the definitive host) transmits a motile infective form, called the sporozoite, into a vertebrate host such as human (the secondary host), thus acting as a transmission vector. Prior to transmission, the malaria parasite resides within the salivary gland of the mosquito. The parasite is in its sporozoite stage at this point[57]. As the mosquito takes its blood meal, it injects a small amount of saliva into the skin wound. The saliva contains anti-haemostatic and anti-inflammatory enzymes that disrupt the clotting process and inhibit the pain reaction.

Typically, each infected bite contains 5-200 sporozoites which proceed to infect the human[58]. Once in the human bloodstream, the sporozoites only circulate for a matter of

minutes before infecting hepatocytes. Once in the liver, it reproduces asexually (tissue schizogony) producing thousands of merozoites. The merozoites infect the new erythrocytes and initiate a series of asexual multiplication cycles (blood schizogony) that produce 8-24 new infective merozoites, at which point the cells burst and the infective cycle begins again[59]. During the erythrocytic stage, some merozoites develop into male and female gametocytes in a process called gametogenesis[60]. The specific factors and causes underlying this sexual differentiation are largely unknown. Note that the gametocytes remain within the erythrocytes until taken up by the mosquito host. When a fertilized mosquito bites an infected person, gametocytes are taken up with the blood and mature in the mosquito gut[61]. The male and female gametocytes fuse and form an ookinete—a fertilized, motile zygote. Ookinetes develop into new sporozoites that migrate to the insect's salivary gland, ready to infect a new vertebrate host. The sporozoites are injected into the skin, through the saliva when the mosquito takes a subsequent blood meal [62].

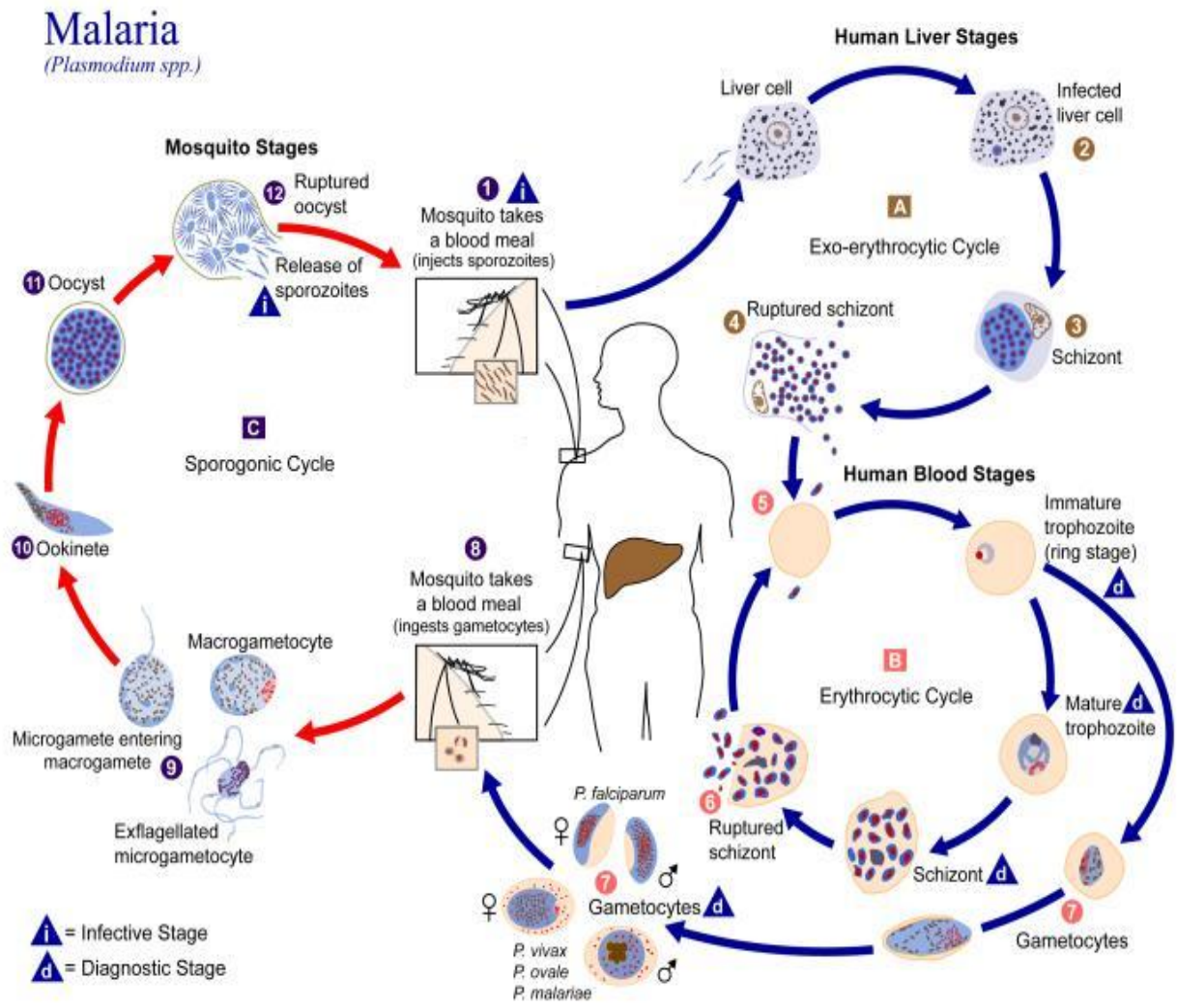

Figure 2.3: Life cycle of *Plasmodium spp.* in malaria[62].

The parasite's invasion of the liver and later the red blood cells causes a variety of symptoms like shivering, fever and sweating with enlargement of the spleen[63]. When the parasite develops in the red blood cells, a lot of known and unknown waste products such as haemozoin pigment and other toxic factors accumulate in the infected red blood cell. These waste substances are dumped into the bloodstream when the infected cells lyse to release invasive merozoites[64]. The hemozoin and other toxic factors such as glucose phosphate isomerase (GPI) stimulate macrophages and other cells to produce cytokines and other soluble factors which act to produce fever and rigors and probably influence other severe pathophysiology associated with malaria[65]. *P. vivax, P. ovale*, and *P. falciparum* repeat this chill-fever cycle every 48 hours (tertian malaria), and *P. malariae* repeats it every 72 hours (quartan malaria). *P. knowlesi* has a 24-hour life cycle and thus can cause daily spikes in fever [66].

Plasmodium-infected red blood cells, particularly those infected with mature trophozoites, adhere to the vascular endothelium of venous walls and do not circulate freely in the blood. When this occurs in the vessels of the brain it is believed to be a factor in causing cerebral malaria which is associated with high mortality[67]. Infection with malaria parasites may result in a wide range of symptoms, ranging from a mild one to severe malaria disease and even death.

Although the proportion of people exposed to malaria parasites has decreased during the last century, the absolute number of people at risk for malaria infection increased from 0.8 billion in 1900 to 3.3 billion in 2010, as a consequence of the absolute increase of the population living in malaria-endemic regions [68]. However, between 2005 and 2010 malaria cases decreased from 244 million to 216 million; moreover, malaria mortality rates showed a global reduction of 26% between 2000 and 2010.

In malaria infection, clinical manifestations of *Plasmodium* are associated with an inflammatory syndrome characterized by haematological changes. The implication of C - reactive protein (CRP) in malaria anaemia, leading to the assessment of the level of this acute phase protein during falciparum malaria in relationship to parasite density, white blood cells (WBC), age and haemoglobin was demonstrated[69].

2.5.2 Distribution of *Plasmodium* Species

Malaria in humans is caused by 5 *Plasmodium* parasites: *Plasmodium falciparum*, *P. vivax*, *P. malariae, P. ovale* and *P. knowlesi*[70]. The current distribution of human pathogenic Plasmodium species shows preponderance of *P. falciparum* in tropical Africa, while *P. vivax* prevails over *P. falciparum* in South America. Both *P. falciparum* and *P. vivax* are prevalent in south-eastern Asia and western Pacific[71]. Although *P. malariae* may occur in all malarious areas, its prevalence is generally low. In tropical Africa, *P. falciparum* and *P. malariae* co-infection is sometimes encountered. *P. ovale* is widespread principally in tropical Africa whereas *P. knowlesi* infection occurs only in certain forested areas of South-East Asia[72].

Malaria burden is hard to estimate, particularly in low income countries where data collection and reporting quality is poor. Incomplete and discontinuous reports from single health facilities may alter final global malaria prevalence[73]. Malaria cases are often under-diagnosed in hyper endemic countries, where mild symptoms of chronic malaria may possibly lead to misdiagnosis. On the contrary, over-diagnosis may also occur. In fact, not all reported malaria cases are confirmed by microscopy or others assay, such as rapid diagnostic tests (RDTs). Furthermore, in hyper endemic areas febrile illnesses from different causes might be misdiagnosed with malaria[74].

Malaria disease can be categorized as uncomplicated or severe (complicated). In general, malaria is a curable disease if diagnosed and treated promptly and correctly[75]. Early diagnosis

is the key feature for its prompt treatment and prevention of complications which may include hypoglycemia, coma, kidney failure, acidosis or pulmonary oedema. Microscopic diagnosis is needed for confirmation of the disease, but it requires technical expertise and at times may be unreliable when poorly executed [76]. However, World Health Organization (WHO) guidelines had recommended that microscopy and Rapid Diagnostic Tests (RDTs) should be used to confirm all malaria cases.

2.5.3 Discovery of Malaria Parasites

In 1880, a French army doctor, Charles Louis Alphonse Laveran, observed certain parasites inside the red blood cells of infected people for the first time and proposed that malaria fever was caused by the organism [77]. A year later, Carlos Finlay provided strong evidence that mosquitoes were transmitting the disease to and from humans. Since the discovery of these parasites, research attention was focused on their biology as well as that of the mosquitoes which transmitted them. In 1897, Sir Ronald Ross discovered the complete life-cycle of the malaria parasites in mosquitoes and that the mosquito was the vector for the malaria in humans[78].

2.5.4 Discovery and Development of Antimalarial Drugs and Vaccine

During the middle ages, treatments for malaria included blood-letting, inducing vomiting, limb amputations and trepanning [79]. For thousands of years, traditional herbal remedies have been used to treat malaria. The first effective treatment for malaria was the bark of cinchona tree [39]. In 1820, the active ingredient, quinine, was extracted from the bark, isolated and named by Pierre Joseph Pelletier and Joseph Bienaime Caventou [80]. Until the 1920s when other medications began to be developed, quinine was the main malarial medication. However, quinine was replaced with chloroquine in the 1940s until resistance to the medication superseded globally in the 1980s. In the 1970s, a Chinese scientist named Tu Youyou and her colleagues discovered artemisinins from the plant *Artemisia annua* which

became the recommended treatment for malaria when administered in combination with other antimalarials [81].

DDT was the first pesticide used for indoor residual spraying to eliminate mosquitoes, however, the large-scale agricultural use of the pesticide led to the evolution of resistant mosquitoes in many regions. Various research efforts have been made on malaria vaccines, the first being in 1967 [42]. The first vaccine was approved by European regulators in 2015 [82].

2.6 Occurrence and Prevalence of *Plasmodium*

Malaria in humans is caused by five *Plasmodium* species; *Plasmodium falciparum, P. vivax, P. malariae, P. ovale and P. knowlesi*. *Plasmodium* species are differently distributed in the world and malaria cases and deaths differs during different seasons [83].

2.6.1 *Plasmodium falciparum*

P. falciparum is widespread in nearly all malaria endemic countries. A study identified 2.37 billion people at risk of *P. falciparum* transmission worldwide, 26% of which is located in the African Region and 62% in South East Asian and Western Pacific regions [84]. In Africa, many epidemiological studies suggest that *P. falciparum* is the most prevalent malarial species. Blood samples were collected between 1998 and 2006 from nine different African countries and analyzed by PCR for the presence of each of the four human malaria parasites [85]. Out of the 2,588 samples collected, 1,737 were positive for *Plasmodium* species and 1,711 (98.5%) were positive for *P. falciparum* considering both mono and mixed infection. Another study performed in 4 villages in Mulanda sub-county, in eastern Uganda, showed a prevalence of *P. falciparum* infection of 94% during rainy season, from July to December, using thin film diagnosis[86]. A study performed in metropolitan Lagos, Nigeria, showed a microscopic prevalence of *P. falciparum* species of 88,5% in pregnant women attending antenatal care clinic, during one observation year. In Asia, *P. falciparum* and *P. vivax* were the two

prevalent species. In India, *P. falciparum* is mostly widespread in Orissa state, while in the west of the some country mixed infections were predominant [87].

2.6.2 *Plasmodium vivax*

In Central and Western Africa *P. vivax* infection was rare because of the high prevalence of the red blood cells Duffy antigen negative phenotype in the population, interfering with *P. vivax* merozoite entry into the red blood cells. Also large study carried out in some other nine African countries failed to detect *P. vivax* species[88]. Despite these studies, however, there were some evidence of *P. vivax* transmission in West and Central Africa. For instance, in Congo, specific *P. vivax* antibodies were researched in 409 samples from patients coming from a health center located on the west coast, where Duffy antigen is expected to be > 95%. Out of the 409 samples, 55 (13%) tested positive for specific *P. vivax* antibodies [89]. Another study from Kenya demonstrated the presence of *P. vivax* among mosquitoes. In addition, *P. vivax* DNA was amplified and sequenced in blood of two Duffy negative children. In eastern and southern Africa only 5% of malaria infections were attributable to *P. vivax*. However, in Asia, *P. vivax* and *P. falciparum* were the predominant species[90].

2.6.3 *Plasmodium ovale*

The real burden of *P.ovale* malaria was difficult to assess because its diagnosis was difficult. *Plasmodium ovale* might have been encountered in sub-Saharan Africa and in Asia[91]. A recent study from Mozambique tested malaria prevalence among febrile patients. Only 2 of 111 malaria positive patients presented *P. ovale* mono-infection, while *P. ovale* and *P. falciparum* mixed infections were also detected[92]. In Congo, a cross-sectional, population-based cluster household survey of adults aged 15–59 years demonstrated that *P. ovale* parasitemia was rare and its prevalence in mono-infection was only 0.1% [93].

There were many evidence of *P. ovale* infection in Asia. Samples collected during 2007/2008 in Bangladesh from 379 febrile patients who underwent microscopic, DNA extraction and

nested PCR analysis, 3 of the 189 positive samples (1.6%) were positive for *P. ovale* [94]. *Nested* PCR detected *P. ovale* parasites in 1.3% of blood samples collected from 1.356 inhabitants of eight villages of Rattanakiri Province (Cambodia) [95]. In a study performed in Myanmar, *P. ovale* was detected by PCR technique in 4.9% of malaria positive samples; most of cases were co-infections with *P. falciparum*, *P. vivax* and/or *P. malariae*. Case reports of *P. ovale* infection were recently published from Gujarat, India, Malaysia and Sri Lanka [96]. *P. ovale* infection was present in Papua, Indonesia and in Thailand, while it was very rare in Philippines where it has been reported only in the island of Palawan.

2.6.4 *Plasmodium malariae*

P. malariae was spread in sub-Saharan Africa, Southeast Asia, Indonesia, many islands in western Pacific and in areas of the Amazon Basin of South America [97]. In a recent study, blood samples were collected from the indigenous population of nine African countries and malaria parasites were searched by PCR method. *Plasmodium malariae* was found in 147 of the 1.737 positive blood samples, 14 as mono-infections, 129 as mixed infections with *P. falciparum* and 4 as triple infections with *P. ovale* and *P. falciparum*[98]. Excluding samples from Rwanda, Mozambique, Angola and Sao Tome, *P. malariae* infections represented 8.5% of all malaria infections [99]. In Nigeria, a total of 350 pregnant women attending certain antenatal clinics were randomly recruited and blood samples were collected. Of 350 blood samples, 96 (27.4%) were positive for malaria parasite and 11 (11.5%) were *P. malariae* positive as tested by microscopy[100]. During the rainy season, blood samples were collected from resident people of four village in Mulanda, Uganda. Of the 709 malaria positive samples, 6% were positive for *P. malariae*. In the state of Orissa, India, the prevalence of *P. malariae* detected by PCR method was 44.6% during the peak season of malaria incidence[101].

2.6.5 *Plasmodium knowlesi*

Plasmodium knowlesi infection was localized only in the South East Asia region and infected both monkeys (where it was first reported) and humans. Forest areas were the *reservoirs* of *P. knowlesi* that was first reported in humans in 1965 in a man who had worked in the jungle of Pahang, Peninsular Malaysia. An analysis of stored blood films identified cases of *Plasmodium knowlesi* infection occurring since 1996 in Sarawak region, Malaysian Borneo [102]. In Sabah region, Malaysian Borneo, samples were collected from patients with suspected malaria. *Nested* PCR was performed in all 243 samples collected. Of 107 samples positive for malaria parasite, 63 were positive for *P. knowlesi*, demonstrating an high incidence of *P. knowlesi* infection in the interior division of Sabah [103]. Another study performed in Thailand screened 1,874 of the samples collected from febrile patients during a period of one year. Of the 1,751 samples positive for malaria parasites, 10 were positive for *P. knowlesi* that mostly occurred in males with uncomplicated malaria coming from southern and southwestern regions of Thailand. *P. knowlesi* mono and mixed infection were also demonstrated in Myanmar in the Philippines and in Singapore [104].

2.7 Genetic Factors and Malaria

Some genetic factors present from birth that are associated with human red blood cells have been shown to influence an individual's risk of developing severe malaria[105]. For instance, individuals who have the sickle cell trait, which is heterozygous for the abnormal haemoglobin of RBC gene HbS, are relatively protected against *P. falciparum* malaria and thus enjoy a protective advantage. Birth cohort studies conducted by Center for Disease Control and Prevention in collaboration with the Kenya Medical Research Institute found that the sickle cell trait genotype (AS) provides 60% protection against overall morbidity and mortality [106]. Because *P. falciparum* malaria has been a leading cause of death in Africa since remote times, the sickle cell trait is now more frequently found in Africa and in persons of African ancestry than in other population groups. In general, the prevalence of hemoglobin-

related disorders and other blood cell dyscrasias, such as hemoglobin C, the thalassemias and G6PD deficiency, are more prevalent in malaria endemic areas and are thought to provide protection from malarial disease [107]. Also, individuals who were negative for the Duffy blood group had red blood cells that are resistant to infection by *P. vivax*. Since, most of Africans are Duffy negative, *P. vivax* is rare in sub-Saharan Africa, especially West Africa. In that area, the niche of *P. vivax* has been taken over by *P. ovale*, a very similar parasite that does infect Duffy-negative persons [108].

2.8 Trends in Malaria Cases and Deaths

Approximately 900 people were reported to have died from malaria in Europe between 1993 and 2003 [109]. An estimate for 2009 reported that countries with the highest death rate per 100,000 of population were Ivory Coast (86.15), Angola (56.93) and Burkina Faso (50.66). The World Health Organization (WHO) estimated that in 2010 there were 219 million cases of malaria resulting in 660,000 deaths. A 2010 estimate indicated the deadliest countries per population were Burkina Faso, Mozambique and Mali[110].

In 2012, there were 207 million cases of malaria with the estimated number of deaths to be between 473,000 and 789,000 people, many of whom were children in Africa[111]. World Health Organization (WHO) further orated that in 2018, there were 214 million new cases of malaria resulting in 483,000 deaths. Between 350 and 550 million cases were estimated for falciparum malaria. Most cases (65%) occurred in children under 15 years of age [112].

In 2016, an estimated 216 million cases of malaria occurred worldwide compared with 237 million cases in 2010. Five million more malaria cases were estimated to have occurred globally in 2016 compared with 2015 [113]. Most of the cases in 2016 were in the World Health Organization (WHO) African Region (90%), followed by the World Health Organization (WHO) South-East Asia Region (2%) and the World Health Organization (WHO) Eastern Mediterranean Region (2%). About 4% of estimated cases globally were caused by

Plasmodium vivax, but this proportion outside of the African continent was 36%. *Plasmodium vivax* was reported to be the predominant parasite in the Americas (64%) and above 30% in South-East Asia while 40% in the Eastern Mediterranean regions [114]. Most cases of malaria caused by *Plasmodium vivax* occurred in the World Health Organization (WHO) South-East Asia Region (58%), followed by the WHO Eastern Mediterranean Region (21%) and the WHO African Region (10%) [115].

Also, in 2016, of all malaria cases globally, fifteen countries accounted for 80%. Nigeria accounted for the highest proportion of cases globally (27%), followed by the Democratic Republic of the Congo (10%), India (6%) and Mozambique (4%) [116]. 85% of estimated vivax malaria cases in 2016 occurred in just five countries (Afghanistan, Ethiopia, India, Indonesia and Pakistan). Of the 91 countries that had an indigenous malaria case in 2016, a decrease in malaria cases of more than 20% compared with 2015 was estimated in 16 countries while an increase of a similar magnitude was estimated in 25 countries. The World Health Organization (WHO) regions of the Americas and Africa accounted for nearly 70% of the countries that had increases of more than 20% in 2016 compared with 2015 [117].

Twenty-nine high-burden countries that accounted for 85% of malaria cases in 2016 had a change of more than 50,000 cases compared with 2015. Twenty-four had estimated increases of between 50,500 (Chad) and over one million cases in Nigeria and Rwanda while five had decreases of between 151,000 (Gambia) and 856,000 (Madagascar) [118]. According to the latest *World malaria report*, released in November 2018, there were 219 million cases of malaria in 2017, up from 217 million cases in 2016. The estimated number of malaria deaths stood at 435,000 in 2017. In 2017, five countries accounted for nearly half of all malaria cases worldwide: Nigeria (25%), the Democratic Republic of the Congo (11%), Mozambique (5%), India (4%) and Uganda (4%)[119].

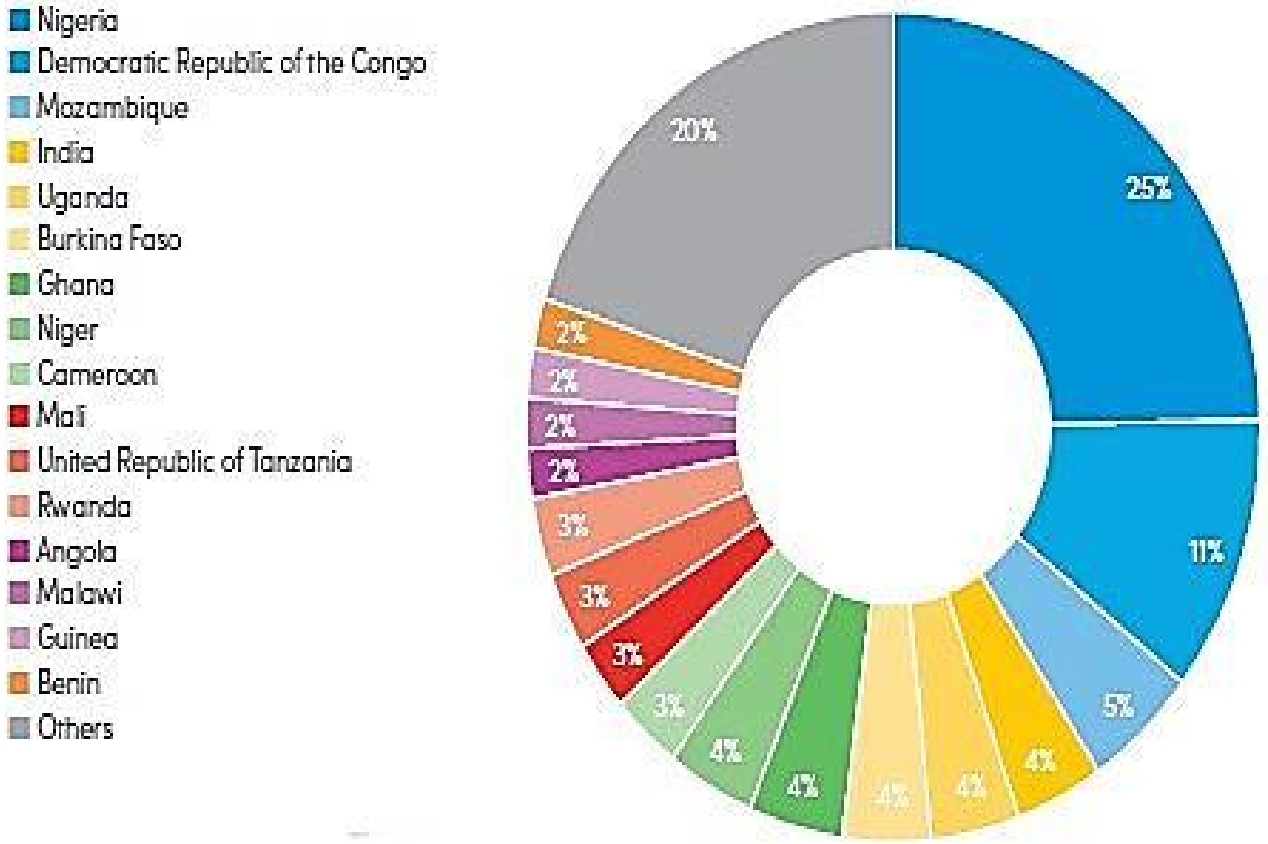

Figure 2.4: Pie chart showing global malaria[120]

Table 2.1: The global trends in the cases and deaths due to malaria between year 2017 to year 2024, World Health Organization (WHO) [120].

YEAR	CASES	DEATHS
2017	219million	435000
2018	228 million	405,000
2019	229 million	409,000
2020	229 million	409,000
2021	247 million	619,000
2022	239 million	619,000
2023	241 million	627,000
2024	245 million (projected)	635,000 (projected)

It must be noted that within the last decade, there has been a massive scale-up of malaria prevention and treatment interventions. This has saved millions of lives globally and cut malaria mortality by about 25% from 2010 to 2016. Almost 7 million lives were saved since 2001 and malaria deaths in Africa were cut by more than half leading to hopes and plans for elimination and ultimately eradication. So consequently, according to the World Health Organization (WHO) and UNICEF, deaths attributable to malaria in 2015 were reduced by 60% from year 2000 estimate of 985,000 [120].

2.9 Declining of Malaria Transmission

Estimates on malaria burden in the last 10 years shows that malaria deaths have declined and the population at risk of infection has shrunk [121]. Also, the prevalence of the infection in children between 2-10 years has declined. However, the extent of both absolute burden and the change considerably varies between reports. In many countries of the world, including the United States, malaria transmission has been eliminated, although, in many of these countries *Anopheles* mosquitoes were still present[121].

The incidence rate of malaria globally declined steadily from 76 to 63 cases per 1000 population at risk from 2010 to 2016 representing an 18% decline [121]. In the World Health Organization (WHO) African Region, malaria incidence reduced from 256 to 206 cases per 1000 population at risk from 2010 to 2016, representing a 20% reduction in case incidence. Among other regions, the World Health Organization (WHO) South-East Asia Region registered the largest decline (48%), followed by the World Health Organization (WHO) Region of the Americas (22%) and the World Health Organization (WHO) Western Pacific Region (12%) [122].

Studies have predicted changes in clinical epidemiology. A recent 25-year analysis in Kenya predicted that, although the burden of malaria cases will shift to older children as transmission declines, the most severe consequences will continue to primarily affect the

children younger than 5 years [123]. In fact, the highest malaria positive result was observed to rise slightly to children older than 5 years while the median age at admission doubled significantly from approximately 2-4 years [22]. In West Africa, these similar patterns were also observed [123].

In recent studies, the view that adults are also emerging as a population that needs monitoring was supported. Due to the declining antimalarial immunity that followed decreased exposure to parasites, adults were reported to be at increased risk of clinical episodes of malaria [124]. The prevalence of parasitaemia detected via microscopy in adults with fever presenting to hospital increased from 19% to 42% in Gabon. Even as transmission declines, adults may put those surrounding them at risk by continuing to harbour chronic infections with low-density[125]. However, how clinical epidemiology is changing in older children and adults over time was largely unknown because the true extent of the malaria burden in older children and adults was less measured while household surveys for parasitaemia that contributed to estimates of the malaria burden usually comprised children only [126].

Areas where malaria transmission was high had been detected using many different malaria indices, including incidence, prevalence, mosquito density, and serological markers of exposure. These high transmission areas varied from a few households to subnational regions depending on the scale of the analysis [127]. Generally, these areas became more pronounced as transmission declined. Studies showed that malaria hotspots could be dynamic and the detection of these hotspots were not standardized. This made identification and targeting complicated. However, effectiveness of the logically attractive idea of targeted malaria control within hotspots was yet to be proven [128].

There has been a renewed focus on low-density chronic infections as the burden of malaria declines, significant burden in infants, children of school-going age, pregnant women, and non-pregnant adults[129]. Studies showed that microscopic chronic infections were

transmissible to mosquitoes and might serve as a neglected reservoir for continued transmission while the evidence was debatable for submicroscopic chronic infections. However, to better understand importance of this reservoir to the elimination agenda in different transmission settings, more research using sensitive molecular tools was needed [130]. In a study in adults in a rural area of southern Mozambique, prevalence of *Plasmodium falciparum* infection by microscopy (14%) and PCR (42%) decreased progressively during adulthood, in parallel with an increase in the prevalence of submicroscopic infections [131].

2.10 Clinical Signs and Symptoms of Malaria

The clinical manifestations of malaria, as well as the severity and course of a clinical attack, depends on the species and strain of the infecting plasmodium parasite. Other factors that affect the clinical manifestations of malaria include: immune status, malaria specific immunity, genetic constitution, nutritional status (of a child), the mode of transmission of infection and whether the individual was on prophylaxis or had earlier exposure to antimalarial drugs [132].

Initial clinical manifestations of malaria common to all plasmodium species are similar to flu-like symptoms [133]. The symptoms can resemble other conditions such as gastroenteritis, pneumonia, sepsis and viral infection. The symptoms may include headache, shivering, fever, vomiting, hemolytic anaemia, jaundice, retinal damage, haemoglobinuria, joint pain, and convulsions. Typically, the signs and symptoms of malaria begin 8-25 days following infection. These signs and symptoms may manifest later in those who had taken prophylactic antimalarial medications [134].

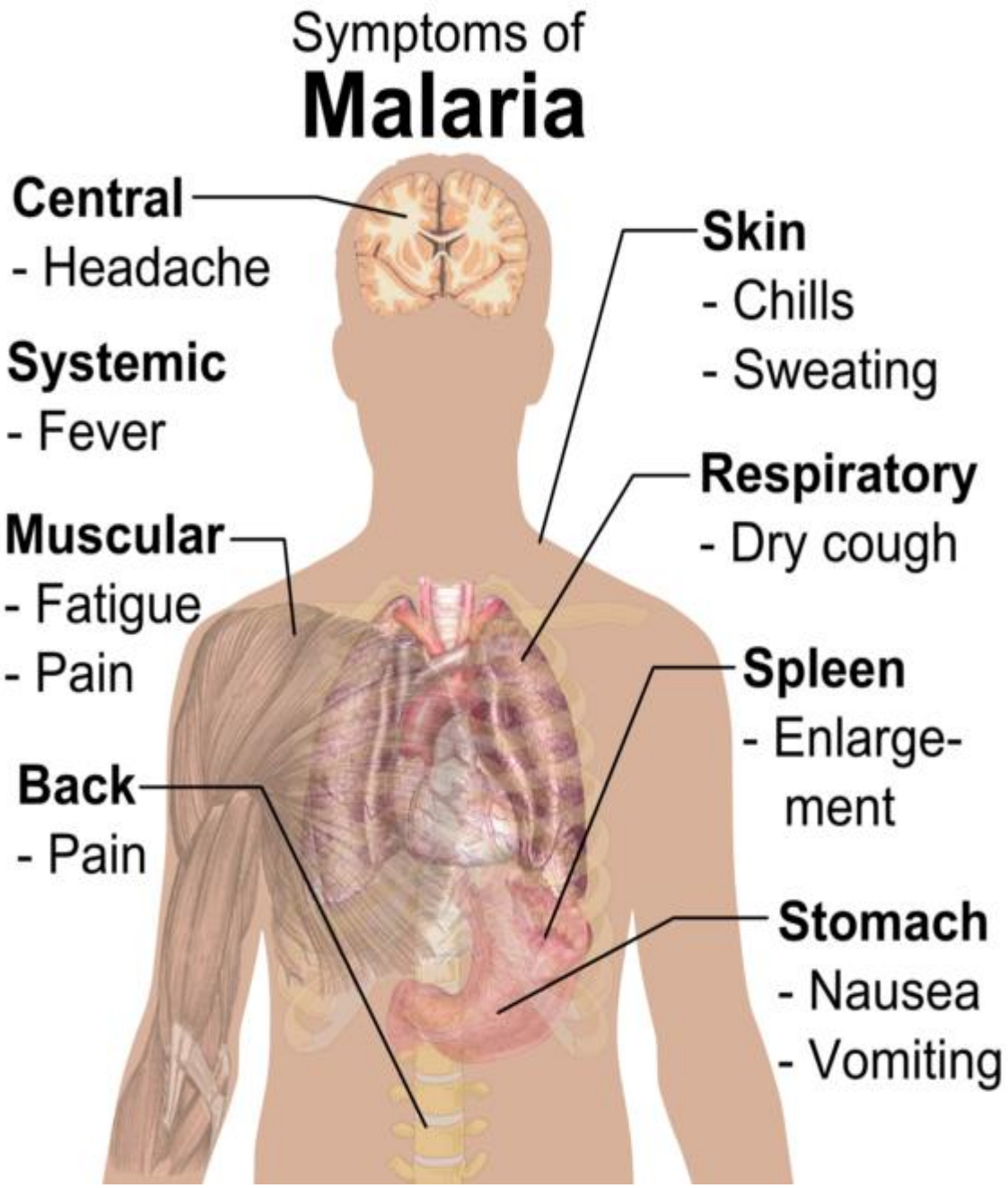

Figure 2.5: Symptoms of Malaria

Source: Main symptoms of malaria and affected organs [135].

The usual symptom of malaria is paroxysm which is a cyclical occurrence of sudden coldness followed by shivering and then fever and sweating, occurring every two days (tertian fever) in *Plasmodium vivax* and *Plasmodium ovale* infections, and every three days (quartan fever) for *Plasmodium malariae*. *Plasmodium falciparum* infection can cause recurrent fever every 36-48 hours, or a less pronounced and almost continuous fever [135]. The malaria paroxysm results from the lysis of parasitized red blood cells and release of merozoites into the circulation at the completion of the parasite's asexual reproduction.

2.11 Clinical resistance emerges

The search for markers that are associated with resistance became more urgent, and in some ways more feasible, when it became clear that parasites were developing resistance to artemisinin in the field[136]. In 2008, a letter to the editor of the *New England Journal of Medicine* publicly documented the first clinical cases of suspected artemisinin resistance, in a patient population from Western Cambodia. Clinical trials with artesunate monotherapy in 94 adults presenting with uncomplicated *P. falciparum* malaria in Battambang province was conducted[137]. This study, looking at the presence of parasites in the blood after taking a standard dose, showed that artesunate alone had failed to clear parasites in two adults. The treatment of these two individuals was prolonged, but their infections were ultimately cleared. There is an active debate as to whether this situation should be best described as artemisinin tolerance, to distinguish it from that in which drug levels in the patient cannot be safely raised high enough to kill the parasites effectively and to prevent recrudescence. For the sake of simplicity, the term 'resistant' will be used in this study[138].

In 2009, a more comprehensive study compared patient responses to artesunate monotherapy in Western Cambodia, Vietnam and north-western Thailand. Measurements of parasite clearance times for 40 patients from each site demonstrated longer parasite clearance times in

Cambodia than in Thailand. Furthermore, parasites taken from Cambodian patients into *in vitro* cultures demonstrated a significant IC_{50} increase for DHA, although not for chloroquine, mefloquine, or artesunate[139]. The authors of this study noted that artemisinin administration in 2001 in Thailand was almost exclusively in the form of ACTs, while in Cambodia, 78% of artemisinin treatment had consisted of monotherapies, which can drive parasites to acquire resistance much more quickly. Although some sought to explain the longer parasite clearance time observed in Western Cambodia by an enrichment of possible human alleles (such as the hemoglobin E (HbE) polymorphism) in this region, studies showed that parasite genotype was more predictive than human genotype[140]. Some small, but statistically insignificant, differences in parasite-clearance times were nevertheless associated with some human alleles. The human genotype theory became less likely as further studies were carried out. In 2012, resistance began to appear on the Thailand-Myanmar border where increases in parasite clearance time were quickly approaching those reported in western Cambodia. At the same time, a report of artemisinin-resistant parasites in Myanmar was also published[141].

The existence of parasites with heritable resistance spurred the design of parasite population genetic studies that could be used to map genes involved in resistance. In the absence of patient phenotype data, some groups sought simply to identify genomic regions under selection using large collections of existing parasites. It had been known for many years that there is linkage disequilibrium around genes involved with either chloroquine or pyrimethamine resistance and it was hypothesized that there might be genomic regions in disequilibrium that would correlate with artemisinin sensitivity[142].

In one study, 61 parasite lines were screened against the NIH Chemical Genomics Center Pharmaceutical Collection containing 2,816 compounds that are registered or approved for human or animal use[143]. The parasite lines were genotyped and genotypes were examined for association with differential drug sensitivity to endoperoxides. Genes associated with

responses to ART included *mal13p1.268* (a *Plasmodium* conserved protein), *pf11_0188* (a heat shock protein 90), *pfe0565w* (a conserved *Plasmodium* protein), *pf08_0130* (a ribosomal-RNA-processing WD-repeat protein), *pfa0655w* (SURFIN), and *pfi0355c* (an adenosine triphosphate-dependent heat shock protein) [144].

Subsequently, genotyped 189 culture-adapted parasites collected from diverse locations, including 146 from Asia, using a custom-built Affymetrix molecular inversion probe 3 K malaria panel array with a coverage of approximately one single nucleotide polymorphism (SNP) per 7 kb[145]. Their genome-wide scan for loci associated with responses to DHA, utilizing only Asian parasites, revealed novel loci on chromosome 1, 3 and 8. In another study with 45 cultured *P. falciparum* parasites from diverse geographical sources , some chromosomal regions (notably on chromosome 4) were found to be associated with increased sensitivity to DHA and artemisinin, but none of the associations were strong enough to be significant or worthy of follow-up[146]. It should be noted that both of these studies provided strong evidence of selection around known resistance genes, such as *pfcrt*, *pfdfhr*, and *pfmdr1,* indicating that the overall method was working. Although it is possible that artemisinin-resistance alleles might not have been suitably represented in the starting parasite populations, it is also possible that the standard IC_{50} assay that was used for phenotyping was not sufficiently sensitive. Artemisinin resistance is now considered easier to detect and quantify in cell culture using a ring-stage assay of synchronized parasites[147].

These early studies clearly lacked both clinical phenotypic data and parasite samples with demonstrated resistance[148]. To overcome this, studies were set up to recruit patients, to measure the amount of time needed to clear parasites after artemisinin monotherapy (compared with the standard ACT), and to obtain parasite material for genome analysis from areas such as Cambodia, where genetically determined resistance was present , as well as from control areas. The first major study, published in 2012, analyzed 91 parasite samples

from Cambodia, Thailand, and Laos that were phenotyped for parasite clearance time[149]. The group utilized a custom Nimblegen genotyping array scoring both SNVs at a density of 1 per 500 bp as well as CNVs, with further fine-mapping using microsatellite analysis. The authors showed that although artemisinin resistance was probably not the result of a single originating event, either geographically or temporally, a clinical slow parasite clearance rate was strongly associated with a selective sweep on chromosome 13. Hypotheses about the actual gene involved were not resolved, although a 35 kb region on chromosome 13 (bases 1,759,466 to 1,794,766, PlasmoDB 11.1) was highlighted as a probable marker of resistance[150]. Subsequent work by Ariey would eventually show that the window was slightly too narrow, potentially because genotyping markers were too sparse in the region or alternatively because a genotyping marker was in a polymorphic sequence tract, which could distort the boundaries of a selective sweep[151].

Genotyped parasites in 331 clinical infections from patients from Pailin, Cambodia, Wang Pha, Thailand and Bangladesh that had been phenotyped for parasite clearance time after artesunate monotherapy was conducted[152]. An Affymetrix SNP array was used to analyze parasite genotypes at 8,079 positions. Modeling significant association with parasite clearance half-life, the time needed for parasitaemia to be reduced by half during the log-linear phase of parasite clearance, or parasite clearance time, four SNPs were identified on chromosomes 4, 10 and 13. Of these, two SNPs were calculated to be 'located within a top-ranked signature of recent positive selection'. Both of these SNPs (MAL13-1718319 and MAL13-1719976) were found on chromosome 13, within 2,000 bp of each other; one was within *pf3d7_1343400* (formerly *mal13p1.216*, located between bases 1,714,443 to 1,719,255, PlasmoDB 11.1)[153]. This study was not designed to identify exact alleles causing resistance (as opposed to loci associated with resistance), but the authors further emphasized the

importance of the 100-kb region on chromosome 13, although narrowly missing the probable gene with causative alleles[154].

Mapping and identify possible causal SNPs in the locus under selection by genotyping 825 *P. falciparum* infections from 10 locations in West Africa and Southeast Asia was conducted[155]. Infections were phenotyped for parasite clearance time after artesunate monotherapy in Southeast Asia and genotyped using short-read high-throughput sequencing on an Illumina platform. The authors showed that one resistant subpopulation of parasites from Southeast Asia (KH2) had essentially a single haplotype extending across half of chromosome 13, from 1.4 Mb to 3.4 Mb, which is strong evidence of a recent selective sweep[156]. This group was able to postulate that the region was important, but even with genotyping at 86,158 coding SNPs, they were unable to perform further fine-scale population mapping without further sexual recombination between resistant and sensitive parasites to break up the interval[157].

2.11.1 Recrudescence of Malaria and Causes

Malaria symptoms may recur after varying period free of symptoms. Depending on the cause, the recurrence can be classified as recrudescence, relapse, or reinfection. Recrudescence is when symptoms return after a symptom-free period. It is caused by parasites surviving in the blood as a result of inadequate or ineffective treatment [158]. Relapse is when symptoms reappear after the parasites have been eliminated from blood but persist as dormant forms in liver cells. Relapse commonly occurs between 8–24 weeks and is often seen in *Plasmodium vivax* and *Plasmodium ovale* infections [159]. However, relapse-like *Plasmodium vivax* recurrences are probably being over-attributed to hypnozoite activation. Some of them might have an extra-vascular merozoite origin, making these recurrences recrudescences, not relapses [160]. One newly recognized, non-hypnozoite, possible contributing source to recurrent peripheral *Plasmodium vivax* parasitemia is erythrocytic forms in bone marrow. Reinfection means the parasite that caused the past infection was eliminated from the body, but a new

parasite was introduced. Reinfection cannot readily be distinguished from recrudescence, although recurrence of infection within two weeks of treatment for the initial infection is typically attributed to treatment failure. People may develop some immunity when exposed to frequent infections [161].

2.11.2 *Falciparum* Malaria Symptoms

Plasmodium falciparum usually causes severe malaria which is often referred to as falciparum malaria. A symptom of falciparum malaria manifests 9-30 days after infection [162]. Neurological symptoms including abnormal posturing, coma, rapid involuntary eye movement (nystagmus), spasm in which body is bent backwards and stiffen (opisthotonus), failure of the eyes to turn together in the same direction (conjugate gaze palsy), or seizures are frequently exhibited by individuals with cerebral malaria [163]. In children, symptoms are varied and often mimic other common childhood illness particularly gastroenteritis, meningitis/encephalitis, or pneumonia. Fever and headache may be the sole symptoms, or gastrointestinal symptoms may predominate. Fever is the key symptom, but the characteristic regular tertian and quartan patterns are seen in < 25% of children. However, children are more likely to have high fever (≥40°C), which may also lead to febrile convulsions. Nausea and vomiting are also common (especially for *Plasmodium falciparum*) and may hamper treatment with oral anti-malaria drugs [164]. Pneumonia and acute diarrhoea are the most common conditions associated with malaria and are both strong predictors of mortality. The diagnosis of pneumonia in a child with malaria might be a coexisting bacterial or viral respiratory illness, but the diagnosis might also be given to a child with malaria-related respiratory distress. Likewise, acute diarrhoea might be a feature of clinical malaria, or the result of concurrent diarrheal disease from an enteric pathogen. Even if rigors frequently accompany infections with *Plasmodium vivax*, compared with adults, children are less likely

to complain chills, arthralgia/myalgia or headache but are more likely to have hepatomegaly, splenomegaly and jaundice [167].

2.11.3 Categories of Malaria Disease with Symptoms

Malaria disease can be categorized as uncomplicated and severe (complicated) malaria. Symptoms of uncomplicated malaria typically lasts for 6-10 hours. It consists of a cold stage (sensation of cold and shivering), a hot stage (fever, headaches, vomiting and seizures in young children), and finally a sweating stage (sweats, return to normal temperature with tiredness) [168]. In severe malaria, clinical or laboratory evidence shows signs of vital organ dysfunction. It occurs when infections are complicated by serious organ failures or abnormalities in the patient's blood or metabolism. Symptoms of severe malaria include: fever and chills, impaired consciousness, prostration, or adopting a prone position, multiple convulsions, deep breathing and respiratory distress, abnormal bleeding and signs of anemia, clinical jaundice and evidence of vital organ dysfunction and severe malaria can be fatal without treatment [169].

2.11.4 Parasitaemia

Plasmodium falciparum caused malaria is the most severe form of malaria, with fatality rates up to 15% in non-immune children with anaemia and severe respiratory distress if appropriate therapy is not promptly instituted [170]. Since *Plasmodium falciparum* is the only *Plasmodium* species that infects all ages of erythrocytes, it can lead to intense parasitaemia that can reach 60% or more. In general, increasing parasitaemia increases severity of symptoms and risk of death. Malaria caused by the other species of *Plasmodium* usually results in parasitaemia of less than 2% [171]. *Plasmodium vivax* and *Plasmodium ovale* preferentially infect reticulocytes, and Plasmodium malariae infects mostly senescent red cells. Thus, severe complications of malaria are more often encountered in Plasmodium falciparum infection. However, only a small proportion of the large number of people infected with *Plasmodium falciparum* develop severe malaria [172].

2.12 Complications of Malaria

Several serious complications are reported in malaria. Development of respiratory distress is one of these complications. It occurs in up to 25% of adults and 40% of children with severe Plasmodium falciparum malaria [173]. The possible causes of the respiratory distress include respiratory compensation of metabolic acidosis, concomitant pneumonia, noncardiogenic pulmonary oedema and severe anaemia. Although uncommon in young children with severe malaria, acute respiratory distress syndrome is known to occur in 5-25% of adults and up to 29% of pregnant women and mortality is increased in individuals with HIV [174].

Cerebral malaria, which is a severe form of malaria that involves encephalopathy, may develop from *Plasmodium falciparum* infection. It is associated with retinal whitening, which may be a useful clinical sign in distinguishing malaria from other causes of fever [175]. Hepatomegaly, splenomegaly or both of these, hypoglycaemia and haemoglobinuria with renal failure may occur. Spontaneous bleeding, coagulopathy, and shock are other likely complications. In pregnant women, malaria is an important cause of stillbirths, abortion, infant mortality and low birth weight, particularly in *Plasmodium falciparum* infection but also with *Plasmodium viva* [176].

2.13 Aetiology of Malaria

Malaria parasites belong to the genus *Plasmodium* and phylum Apicomplexa. Among those infected, *Plasmodium falciparum* is the most common species identified (approximately 75%) followed by *Plasmodium vivax* (20%) [177]. Although *P. falciparum* has been known to cause the majority of deaths. Recent evidence suggests that *P. vivax* malaria is associated with potentially life-threatening conditions about as often as with a diagnosis of *P. falciparum* infection. *P. vivax* is known to be more common outside of Africa. Evidence suggests that *P. falciparum* may have originated in gorillas while close relatives of the human parasites remain common in chimpanzees [178]. *P. vivax*, another malarial *Plasmodium* species among

the six that infect humans, also likely originated in Afrcan Gorillas and Chimpanzees. Another malarial species recently discovered to be transmissible to humans, *P. knowlesi* was known to have originated in Asian macaque monkeys. *P. knowlesi* is a zoonotic species that is known to cause malaria in macaques [15]. While *P. malariae* is highly host specific to humans, there is spotty evidence that low level non-symptomatic infection persists among wild chimpanzees [179].

2.14 Pathogenesis of Malaria

Malaria infection develops through two phases: exoerythrocytic phase (in the liver) and erythrocytic phase. When an infected mosquito pierces the skin to take a blood meal, sporozoites in the saliva of the mosquito enter the bloodstream and carried into the liver where they infect liver cells and multiply asexually. They remain asymptomatically for a period of 8-30 days [180]. Some *Plasmodium vivax* sporozoites do not immediately develop into exoerythrocytic phase merozoites, instead, they produce hypnozoites that remain dormant for periods ranging from several months, typically 7-10 months- to several years [181]. After a period of dormancy, they reactivate and produce merozoites. Hypnozoites are responsible for long incubation and late relapses in *P. vivax* infections, although their existence in *P. ovale* is uncertain. *P. falciparum,* especially, causes severe malaria via sequestration, a distinctive property not shared by any other human malaria parasites. It is haploid during nearly all stages of its life-cycle, except for a brief period after fertilization when it is diploid from the ookinete to sporogenic stages within the mosquito gut [182]. Within the 48-hour asexual blood stage cycle, the mature forms change the surface properties of infected red blood cells, causing them to stick to blood vessels a process called cytoadherence. This leads to obstruction of the microcirculation and results in dysfunction of multiple organs, typically the brain in cerebral malaria.

During the erythrocytic stage of the parasite's life cycle, it uses intracellular haemoglobin as a food source. The organism avidly ingests host hemoglobin and the protein is broken down into peptides, and the heme group is released and detoxified by biocrystallization in the form of hemozoin [183]. During these stages, the parasite produces its energy mainly through anaerobic glycolysis, with pyruvate being converted into lactate. Merozoites contained high levels of cell recognition and invasion proteins. Trophozoites contained proteins implicated in erythrocytes remodeling and haemoglobin digestion. Gametocytes contained high amounts of gametocyte-specific transcription factors and cell cycle/DNA processing proteins. The gametocytes had low levels of polymorphic surface antigens. Sporozoites contained large amounts of proteins related to invasion [184]. The parasite has different subsets of its proteome expressed during various stages of its developmental cycle. In a study, 2,415 proteins were identified in four stages (sporozoite, merozoite, trophozoite, gametocyte), representing 46% of the theoretical number of proteins [68]. Only 6% of the proteins were found in all of the four stages. Of the proteins found, 51% were annotated as hypothetical proteins. It has been hypothesized that the parasite obtains all, or nearly all, of its amino acids by salvaging from the host or through the degradation of haemoglobin. This is supported by the fact that genomic analysis has found no enzymes necessary for amino acid biosynthesis, except for glycine-serine, cysteine-alanine, aspartate-asparagine, proline-ornithine, and glutamine-glutamate interconversions [185]. *Plasmodium spp.* is unable to biosynthesize purines. Instead, the parasite is able to transport and interconvert host purines. Conversely, the parasite can produce pyrimidines *de novo* using glutamine, bicarbonate, and aspartate.

2.15 Diagnosis of Malaria

Prompt and accurate diagnosis is the key to effective disease management and this is one of the main interventions of the Global Malaria Control Strategy [186]. Therefore poor diagnosis continues to hinder effective malaria control. This is due to a combination of factors, including non-specific clinical presentation of the disease, high prevalence of asymptomatic

infection in some areas, lack of resources and insufficient access to trained health care providers and health facilities and widespread practice of self-treatment for clinically suspected malaria [187]. One major contributing factor, however, is that the laboratory diagnosis of malaria has up to now relied nearly exclusively on microscopy, a valuable technique when performed correctly but unreliable and wasteful when poorly executed. A better utilization of microscopy and the development of alternative diagnostic techniques could substantially improve malaria control. Such objectives prove particularly relevant to the Roll Back Malaria initiative, a global movement that emphasizes better application of existing tools and the development of new ones[189].

Malaria diagnosis involves identifying malaria parasites or antigens/products in patient blood. Prompt and accurate diagnosis is critical to the effective management of malaria. Delays in diagnosis and treatment had been identified as the leading causes of patient's death in many countries. There is therefore a need for 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 in lacking [190].

Clinical diagnosis is the most widely used approach. It has been the only feasible one in many situations, particularly in rural areas and at the periphery of the health care system where laboratory support to clinical diagnosis does not exist [191]. Among the many clinical signs and symptoms associated with malaria, the most prominent is fever, which is often accompanied by chills, perspiration, anorexia, headaches, vomiting and malaise. Residents of endemic areas are often familiar with this combination of symptoms, and frequently self-diagnose malaria based on symptoms alone. In addition to these symptoms of uncomplicated malaria, other manifestations may develop that signal severe malaria which is almost always due to *Plasmodium falciparum*. These include confusion or drowsiness with prostration together with severe manifestations such as cerebral malaria, severe anaemia and others [192].

Clinical diagnosis is inexpensive to perform, and requires no special equipment or supplies. However, the symptoms of malaria are very non-specific and overlap with those of other febrile illnesses. A diagnosis of malaria based on clinical grounds alone is therefore unreliable, and when possible should be confirmed by laboratory tests. In spite of this lack of specificity, in some settings, disease management based on clinical diagnosis alone is justifiable [193].

2.15.1 Microscopic Diagnosis

Conventional light microscopy is the established method for the laboratory confirmation of malaria [194]. The careful examination by an expert microscopist of a well prepared and well stained blood film remains currently the "gold standard" for detecting and identifying malaria parasites. In most settings, the procedure consists of: collecting a finger-prick blood sample, preparing a thick blood smear (in some settings a thin smear is also prepared), staining the smear (most frequently with Giemsa stain) and examining the smear through a microscope (preferably with a 100X oilimmersion objective) for the presence of malaria parasites [195].

2.15.2 Microscopy and many Advantages

When used by skilled and careful technicians. Microscopy can detect densities as low as 5–10 parasites per µl of blood. Under general field conditions, however, the detection capabilities of a typical microscopist might be more realistically placed at 100 parasites per µl of blood.

It is informative. When parasites are found, they can be characterized in terms of their species (*P. falciparum, P. vivax, P. ovale, and/or P. malariae*) and of the circulating stage (e.g. trophozoites, schizonts, gametocytes) [196]. Occasionally, expert microscopists can detect morphological alterations induced by recent drug treatment. In addition, the parasite densities can be quantified from ratio of parasites per number of leukocytes or erythrocytes. Such quantifications are needed to demonstrate hyperparasitaemia (which may be associated with severe malaria) or to assess parasitological response to chemotherapy. This is relatively

inexpensive. Cost estimates for endemic countries range from about US$ 0.12 to US$ 0.40 per slide examined.

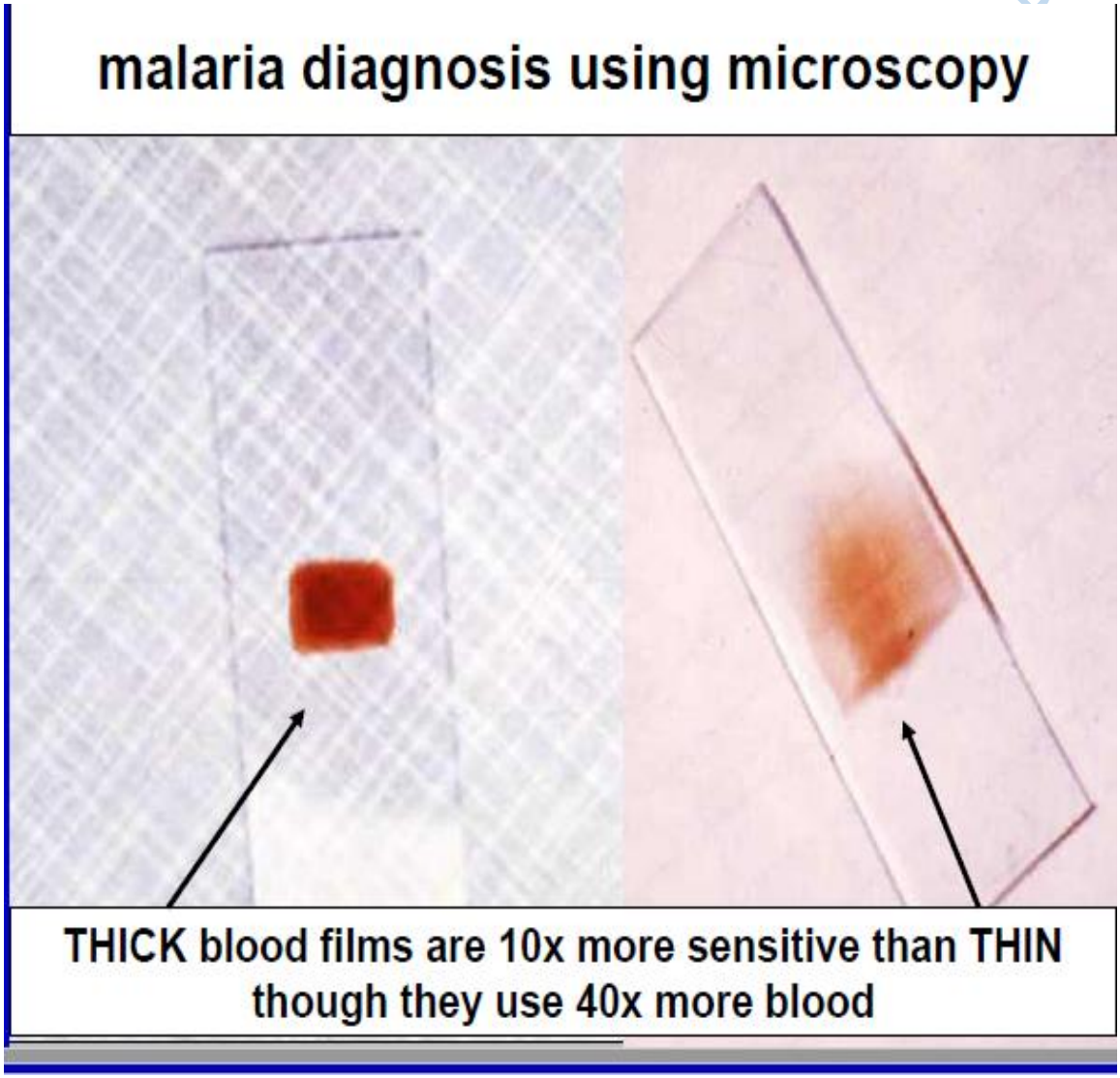

Figure 2.6: Immunochromatographic antigen detection "CARD" Rapid Diagnosis TEST (RDT)

The conventional diagnostic method for malaria in the laboratory is microscopic examination of stained blood films using Geimsa, Wright's or Field's stains [197]. For years, microscopic detection and identification of *Plasmodium spp.* in Geimsa-stained thick blood films (for screening), and thin blood films (for species' confirmation) remains the gold standard for diagnosis in the laboratory. The wide acceptance of the technique can be attributed to its low cost, its ability to identify the presence of parasites, and assess parasite densities, which are all useful for management of malaria. Recently, a study showed that conventional malaria microscopic diagnosis at primary healthcare facilities in Tanzania could reduce the prescription of antimalarial drugs, and also appeared to improve the appropriate management of non-malarial fevers [198]. However, the technique is time consuming, labor intensive, and requires considerable technical expertise, particularly for identifying species accurately at low parasitaemia or in mixed malarial infection. The most important shortcoming of the microscopic examination is its relatively low sensitivity, particularly at low parasite density which has resulted in underestimating malaria infection rates [199].

2.15.3 Rapid Diagnostic Test (RDT)

Deficiencies of light microscopy as a diagnostic method for malaria has led to an increase in the use of RDTs for malaria diagnosis. It is fast and easy to perform, and do not require electricity or any laboratory equipment [200]. Rapid diagnostic test (RDT) detects malaria antigen in blood flowing along a membrane containing specific anti-malaria antibodies. Most RDTs detects histidine-rich protein 2 (HRP-2) or lactate dehydrogenase (LDH), which are *Plasmodium falciparum* specific proteins. Others distinguish non-*P. falciparum* infections from mixed malaria infections by detecting *P. falciparum* specific and pan-specific antigens (aldolase or pan-malaria LDH) [201]. RDTs provide an opportunity to extend the benefits of parasite-based diagnosis of malaria beyond the limitations of light microscopy. The techniques' performance for diagnosis of malaria has been reported as excellent. Although,

there are some reports on the wide variations in sensitivity, however, RDTs has been highly valuable and a rapid malaria-diagnostic method.

2.15.4 Quantitative Buffy Coat (QBC)

This technique is another method for malarial diagnosis. This technique involves staining parasite DNA in micro-haematocrit tubes with fluorescent dyes like acridine orange, and subsequently detected by epi-flourescent microscopy. The QBC technique was developed to enhance microscopic detection of parasites and simplify malaria diagnosis [202]. Recently, it has been shown that acridine orange is the preferred diagnostic method over light microscopy and immunochromatographic tests in the context of epidemiological studies in asymptomatic populations in endemic areas, probably because of increased sensitivity at low parasitaemia. Although, this technique is simple, user friendly, reliable, it requires specialized instrumentation, is more costly than conventional light microscopy, and is poor at determining species and numbers of parasites [203].

2.15.5 Serological Tests

These tests are also employed in the diagnosis of malaria. The tests are usually based on the detection of antibodies against malaria parasites in asexual blood stage. In recent decades, Immunoflourescence antibody testing (IFA) has been a reliable serologic test for malaria. Although it is time-consuming and subjective, it is highly sensitive and specific.

2.15.6 Polymerase Chain Reaction (PCR)

Recent advancements in molecular biology has helped in the development of molecular diagnostic methods for malaria diagnosis which permits extensive characterization of the malaria parasite. Polymerase Chain Reaction (PCR) based techniques are some of the new strategies in molecular diagnosis generated for malaria diagnosis. The PCR technique continues to be used extensively to confirm malaria infection, follow-up treatment response, and identify drug resistance [204].

2.15.7 Loop-Mediated Isothermal Amplification (LAMP)

This is another molecular based technique for malaria diagnosis. It is claimed to be simple and inexpensive test that detects the conserved 18S ribosome RNA gene of *P. falciparum*. Studies have shown high sensitivity and specificity of this test, not only for *P. falciparum*, but also *P. vivax, P. ovale* and *P. malariae* [205]. Flow cytometry has reportedly been used for malaria diagnosis has been suggested to provide a sensitivity of 49-98%, and a specificity of 82-97%, for malarial diagnosis. It disadvantages include, labor intensiveness, the need for trained technicians, costly diagnostic equipment, and likely occurrence of false-positive results with bacterial or viral infections [206].

There are three reported techniques based on automated blood cell counters for malaria diagnosis[207]. None of these methods are routinely available in clinical laboratory. Further studies are required to improve and validate the method. However, the accuracy these methods promise for detecting malaria parasites means that the methods could become a valuable and routine malaria-diagnostic method. Mass spectrophotometry is another promising diagnostic technique in malaria diagnosis. It involves in vitro detection of malaria parasites, with a recently reported sensitivity of 10 parasites/ µl of blood. It identifies a heme from hemozoin, which is a parasite-specific biomarker, in clinical samples. This high-tech mass spectrometers cannot be used in the remote rural areas without electricity, therefore, future improvements in equipment and techniques should make this method more practicable.

A pan-microbial oligonucleotide microarray has been developed for infectious diseases diagnosis and has identified *P. falciparum* accurately in clinical specimens. However, this diagnostic technique is still in the early stages of development [208]. Other reliable diagnostic tests for malaria has been developed and introduced in recent years. Some are commercially available, which include; enzyme linked immunosorbent assay (ELISA) or enzyme immunoassay (EIA), latex agglutination assay, and cultivation of live malaria parasites [16].

Investigating malaria parasites in tissue autopsy (post-mortem organ diagnoses) has been described e.g. liver, spleen, kidney, and brain [209].

Although, parasite culture, serological techniques, and molecular techniques are valuable in research laboratories, they are not practical or appropriate for the routine clinical diagnosis of malaria [210]. Field studies of malaria in endemic areas frequently use the presence or levels of parasitaemia, together with the measurement of fever, as the primary criteria with which to identify cases. However, since malaria cases do not always present with measurable fever, and since asymptomatic parasitaemia occurs, additional episode markers may be useful epidemiological tools.

2.16 Treatment of Malaria during Pregnancy

Pregnant women, particularly in the second and third trimesters of pregnancy are more likely to develop severe malaria than other adults, often complicated by pulmonary oedema and hypoglycaemia [211]. Maternal mortality is approximately 50%, which is higher than in non-pregnant adults. Fetal death and premature labour are common. The role of early Caesarean section for the viable live fetus is unproven, but is recommended by many authorities. Obstetric advice should be sought at an early stage, the paediatricians alerted, and blood glucose checked frequently. Hypoglycaemia should be expected and is often recurrent if the patient is receiving quinine. Severe malaria may also present immediately following delivery [212]. Postpartum bacterial infection is a common complication in these cases. Falciparum malaria has also been associated with severe mid-trimester haemolytic anaemia in Nigeria. This often requires transfusion, in addition to antimalarial treatment and folate supplementation.

Parenteral antimalarials should be given to pregnant women with severe malaria in full doses without delay [213]. Artesunate or artemether are preferred over quinine in the second and third trimesters because quinine is associated with recurrent hypoglycaemia. Recent evidence

shows that in non pregnant adults with severe malaria in areas of low transmission, artesunate was superior to quinine, reducing mortality by 35% compared to quinine, which makes artesunate the preferred option in the second and third trimesters [214]. In the first trimester, the risk of hypoglycaemia associated with quinine is lower, and the uncertainties over the safety of the artemisinin derivatives are greater. However, weighing these risks against the above evidence in favour of the efficacy of artesunate, and until more evidence becomes available, both artesunate and quinine may be considered as options. Treatment must not be delayed, so if only one of the drugs artesunate, artemether or quinine is available, it should be started immediately. The National vector Borne Disease Programme in India recommends quinine for the treatment of severe malaria in pregnancy [215].

2.17 Antimalarial Drug Resistance Genes among Pregnant Women

Resistance to antimalarial drugs has been described for two of the four species of malaria parasite that naturally infect humans, *P. falciparum* and *P. vivax. P. falciparum* has developed resistance to nearly all antimalarials in current use, although the geographical distribution of resistance to any single antimalarial drug varies greatly. *P. vivax* infection acquired in some areas has been shown to be resistant to chloroquine and/or primaquine[216]. Chloroquine-resistant P. falciparum malaria has been described everywhere that P. falciparum malaria is transmitted except for malarious areas of Central America (north-west of the Panama Canal), the island of Hispaniola, and limited areas of the Middle East and Central Asia. Sulfadoxinepyrimethamine (SP) resistance occurs frequently in South-East Asia and South America. SP resistance is becoming more prevalent in Africa as that drug is increasingly being relied upon as a replacement for chloroquine. Mefloquine resistance is frequent in some areas of South-East Asia and has been reported in the Amazon region of South America and sporadically in Africa. Cross-resistance between halofantrine and

mefloquine is suggested by reduced response to halofantrine when used to treat mefloquine failures[217].

Each year between 75,000 and 200,000 infant deaths are attributed to malaria infection in pregnancy. Globally, between 200,000 and 500,000 pregnant women developed severe anaemia as a result of malaria in Sub-Saharan Africa and this is because the majority of infections in Africa are caused by Plasmodium falciparum. In a study in Uyo, the result showed that majority of their respondents (71%) had knowledge about malaria prevention in pregnancy and were aware that malaria had adverse effects in pregnancy[218]. Also in another study, the result revealed that 89% of respondents attributed malaria to the bites of mosquito and were aware of the prevention strategies while 11% still attributed the aetiology of malaria to other factors, including excessive ingestion of oil, exposure to sunlight, bite of cockroaches, witches and poisoned foods. They added that knowledge of the cause of malaria was directly proportional to the educational attainment of the women and that fever was the most acknowledged symptom of malaria[220].

Despite the public health priority given to malaria, in recent times, the parasites have developed resistance to most of the commonly available prophylactic and therapeutic antimalarial agents. In view of this, there is a crucial need for periodic antimalarial sensitivity testing. The main objective of antimalarial sensitivity testing is to evaluate parasite(s) sensitivity to increasing antimalarial dosages[221]. This approach allows for the complete exclusion of interfering host immunity and metabolism of the compound, thus offering an accurate evaluation of drug impact on parasites. Over the past five decades, antimalarial resistance by *Plasmodium falciparum* has become an issue of utmost concern. There are several methods for the assessment of parasite growth inhibition by antimalarial drug testing, using either in vivo or in vitro testing. The in vivo approach uses a standard dose regimen of chloroquine and daily parasite checks for 7 or 28 days[222]. The results permit classification

into sensitive response, recurrence, overt resistance, and high resistance. This type of test can also be run with other antimalarial drugs, provided the period of the post treatment follow-up is adjusted to the half-life of the medication employed.

In vivo antimalarial tests have various drawbacks, especially the need for regular checks over a relatively long period, the strong influence of host immunity on test result outcomes, the purely qualitative nature of the results, and eventual clinical deterioration in cases of high resistance responses[223]. *In vitro* assays have become indispensable tools for surveillance of drug resistance and planning therapeutic guidelines. These include the schizont maturation assay, radiolabeled hypoxanthine isotopic assay, malaria histidine-rich protein 2 and lactate dehydrogenase quantification by enzyme-linked immunosorbent assay and nucleic acid amplification tests (such as polymerase chain reaction [PCR])

Due to the high burden of malaria among children under the age of five years and pregnant women, targeting women in the reproductive age group to deal with the disease has recently been widely recognized[224]. It has been acknowledged that the success of malaria control in children and pregnant women depends on the understanding of the local socio-cultural factors affecting women's perceptions of causes and modes of transmission of the disease, health seeking behaviour and practices of malaria prevention measures. However, research focusing on malaria issues among women in the reproductive age group with children under the age of five years in areas of seasonal transmission has particularly been scarce.

The coverage and proper utilization of the most promising malaria preventive measure, ITNs, in the country is also limited by lack of sustainable distribution and issues related to replacement of free nets, seasonality of malaria, poor knowledge of the community with regard to the link between mosquitoes and malaria[225]. It is also well recognized that accessibility to anti-malaria interventions alone will not bring about the desired change. Several studies have demonstrated compliance to anti-malaria interventions depends

substantially on social, behavioural and cultural factors that affect understanding of the causes, the relationship between mosquitoes and the disease, diagnosis, treatment and practices about prevention. In addition, factors such as vulnerability, economic constraints, inadequacy or unavailability of appropriate health services, and other related factors play an important role in explaining health seeking behaviour of the people [226].

Studies in the past were mainly carried out in areas of stable malaria transmission due to the overwhelming burden of the disease among young children and pregnant women in those areas. Recently, however, there has been an increased need to understand perceptions and practices of the community about the disease, particularly health and treatment seeking behaviours in areas of seasonal transmission frequently exposed to malaria epidemics in SSA. Understanding the local perceptions and practices of women is of utmost relevance to enhance community's potential to deal with malaria interventions[227].

Pregnant women infected with malaria represent a significant obstetric problem that predisposes them and their unborn baby to adverse consequences[228]. Findings from clinical studies revealed that 25% women with placental malaria had evidence of malaria during parturition. In most endemic areas, malaria during pregnancy is associated with maternal anemia. It has been estimated that about 26% of maternal anemia is due to malaria. In Africa, it was reported that severe anemia as a result of falciparum malaria is responsible for approximately 10,000 maternal deaths per annum[229]. Infants with congenital malaria also experience devastating effects from the infection. It has been estimated that 1 in every 5 low birth weight (≤2500 g) deliveries are a consequence of maternal malaria. This invariably results in poor infant development and survival and has led to approximately 100,000 infant deaths every year.

Despite the public health priority given to malaria, in recent times, the parasites have developed resistance to most of the commonly available prophylactic and therapeutic

antimalarial agents. In view of this, there is a crucial need for periodic antimalarial sensitivity testing. The main objective of antimalarial sensitivity testing is to evaluate parasite(s) sensitivity to increasing antimalarial dosages[230]. This approach allows for the complete exclusion of interfering host immunity and metabolism of the compound, thus offering an accurate evaluation of drug impact on parasites. Over the past five decades, antimalarial resistance by Plasmodium falciparum has become an issue of utmost concern. There are several methods for the assessment of parasite growth inhibition by antimalarial drug testing, using either in vivo or in vitro testing. The in vivo approach uses a standard dose regimen of chloroquine and daily parasite checks for 7 or 28 days[231]. The results permit classification into sensitive response, recurrence, overt resistance, and high resistance. This type of test can also be run with other antimalarial drugs, provided the period of the post treatment follow-up is adjusted to the half-life of the medication employed.

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The ideal approach for detecting and monitoring antimalarial resistance is the use of a PCR. This method detects the genes responsible for resistance, and results are usually available within 8–12 h with high sensitivity and specificity. However, the need for technical training of laboratory personnel (especially in developing countries) and the relatively high cost of procurement and maintenance limits PCR applicability in resource-limited settings[233].

In Nigeria and other endemic areas, malaria is a major etiology of morbidity and mortality, particularly among pregnant women. In 2015 alone, there were an estimated 214 million new cases of malaria and 438,000 deaths worldwide. Of these, Nigeria accounted for up to 25% of global cases and deaths with 500,000 cases in pregnant women. The physiological attributes of pregnant women and the pathology due to malaria exert synergistic effects on each other, thus making both the mother and the child vulnerable to adverse consequences[234].

The main strategies for the prevention of malaria during pregnancy in Nigeria are by chemoprophylaxis using sulphadoxine-pyrimethamine (SP) and use of long-lasting insecticide treated nets. In spite of the recommendation of two treatment doses of SP for intermittent preventive treatment of malaria during pregnancy, other antimalarial medicines such as CQ, pyrimethamine and proguanil are still used in many antenatal clinics[235]. A wide range of un-recommended antimalarial medicines are available and sold over the counter in medicine stores in Nigeria and are accessible to multigravid pregnant women who continued with medications used during their previous pregnancies. Healthcare providers also prescribed un-recommended antimalarials due to poor knowledge on best practices, low confidence on the safety of SP, and challenges in the institution of on-the-job supervision by stakeholders, such as the Ministry of Health, that ensures compliance with recommend malaria in pregnancy guidelines[236].

Surveillance of antimalarial drug resistance markers in Plasmodium falciparum populations is an important tool in attempts aimed at predicting the level of resistance to antimalarial drugs. Mutations in specific genes of Plasmodium falciparum that confer resistance to antimalarial drugs are selected by sustained drug pressure. The emergence and spread of drug resistance depends, in part, on the number of mutations required to encode resistance and their effects on parasite fitness. Specific multiple point mutations in a gene make up a resistance marker for an antimalarial drug[237].

The level of susceptibility of P. falciparum strains to quinoline antimalarial drugs such as CQ, amodiaquine, lumefantrine and mefloquine has been attributed to mutations in Pfcrt and Pfmdr 1 genes. Resistance to type 1 antifolates (pyrimethamine, chlorproguanil, trimethoprim) has been associated with amino acid substitutions in the Pfdhfr gene while resistance to type II antifolates (sulfonamides: sulfadoxine and dapsone) are associated with mutations in P. falciparum dihydropteroate synthase (Pfdhps) gene[238].

2.18 Challenges to Working with Malaria Parasites: A Complex Lifecycle and Logistical Barriers

Malaria parasites have a complex lifecycle. Although the parasite replicates asexually as a haploid organism in human and mosquito tissues, it has a sexual cycle with meiosis and a brief diploid phase, which occurs in the mosquito[239]. The sexual re-assortment that occurs within mosquitoes is the basis for the genome-wide association studies of parasites in humans. Sexual crosses between resistant and sensitive parasites can be performed for *P. falciparum* and have been used to map drug-resistance genes in the past, but the method is not particularly accessible. Few researchers have access to all stages of the complex lifecycle, which is needed to complete genetic crosses. Although there are rodent models of malaria, which in some cases (such as *Plasmodium chabaudi*, *Plasmodium berghei*, and *Plasmodium yoelii*) can be more readily used in forward and reverse genetics, other human malaria parasites, such as *Plasmodium vivax*, cannot even be cultured long-term[240].

P. falciparum has an approximately 24 megabase haploid genome, notable for its extreme AT-richness. Although malaria has been and continues to be a strong selective force on the human genome, the function of many of the predicted approximately 5,300 proteins encoded by the parasite genome can only be inferred from studies of orthologs in model organisms[241]. A notable feature is that the genome bears long tracts of repetitive, recombinogenic sequence that may assist with immune evasion, but which makes genome manipulation and cloning challenging. Some of these recombinogenic tracts are within multigene families, some are

intergenic, and some are within genes. For example, the amino terminus of PfKelch13 is predicted to have the low complexity protein coding sequence 'NNNINHNNNNNNLTANNITNNLINNNMN' within its first 200 amino acids[242]. *In vitro* evolution studies have shown that repetitive sequences are more prone to mitotic gene conversion than sequences that do not contain repetitive sequences, but they are also more difficult to sequence and study. Outside of repetitive regions, the rate of mutation is probably similar to those found in other organisms.

Although the blood stages of *P. falciparum* can be maintained in cell culture using human erythrocytes obtained from donors, parasites cannot be as readily taken into cell culture for drug sensitivity testing[243]. Furthermore, given that the disease can rapidly turn fatal, treatment recommendations may be made on the basis of the number of parasites that are PCR positive for a resistance marker in a region. The patient parasite clearance studies in which parasite numbers are counted by simple light microscopy involve consented clinical trials in which patients agreed to be treated with a monotherapy (versus an ACT) initially but are closely monitored and then treated with a second drug or ACT[244]. Although simple in design, these studies are relatively costly and are influenced by host factors, including a person's immunity or whether the person has alleles that protect against malaria, such as the sickle cell allele, HbS. Individuals with this allele could theoretically clear parasites more quickly than those without. *In vitro* drug-sensitivity assays, in which parasites are incubated in the presence of increasing drug concentrations (to obtain an EC_{50}), are more quantifiable but may require more specialized laboratory equipment, such as incubators and tissue culture facilities. For artemisinin-resistance studies, a modified RSA in which parasites are first synchronized is typically used. Genotyping parasites that have been phenotyped by both types of tests may be complicated by multiclone infections[245].

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Chapter Three

3.0 Materials and Methods

3.1 Study Area and Period

The study was conducted (from February, 2024 to July, 2024) at the ante-natal unit in the department of Obstertrics and Gyneacology, University College Hospital, Ibadan. Detection and quantification of malaria parasite in blood smear and detection of anti-malarial drugs (Sulphadoxine-pyrimethamine) resistance genes was done at the department of Medical Microbiology and Parasitology, UCH and Institute of Postgraduate Research Centre, UCH Ibadan

3.2 Study Design

A facility-based study was conducted. The source population for this study included pregnant women with sign and symptoms (Fever, malaise, nausea, body pain, etc) at any gestational age attending the ante-natal clinic at UCH, Ibadan.

3.3 Study population

The study population was pregnant women with signs and symptoms of malaria who reported for booking at University College Hospital, Ibadan, Southwestern Nigeria and provided informed consent were enrolled into the study.

3.3.1 Inclusion criteria:

To be eligible for inclusion in the study:

1. Clients must be confirmed pregnant (Abdominal ultrasound in early pregnancy or positive pregnancy test).
2. Must have signs and symptoms of malaria.
3. Must not be on any antimararial drug.
4. Pregnant women who meet the inclusion criteria and gave their consent will be enrolled at the booking clinic which runs on Mondays, Tuesdays and Thursdays. At

the booking, information on the age of the women, length of pregnancy, or gestational age at booking, gravidity, parity, level of education and other demographic details will be obtained.

5. Information on drug use before booking e.g antibiotics, antimalarial drug and hematinics will be obtained
6. Medical examination was carried out by trained midwives who work with the hospital. Temperature was measured to the nearest 0.1^0C using an electronic thermometer. Fever was defined as axillary temperature >37.5^0C.

3.3.2 Exclusion criteria:

1. Non-pregnant women
2. Pregnant women with mental illness
3. Pregnant women with severely debilitating diseases

Sample and Sampling Technique:

Sample size was determined using a single proportion formula (below) using 50% prevalence of malaria among pregnant women, 95% confidence level, 5% margin of error, and design effect of 2. To compensate for non-response rate, 10% of the determined sample size was added. Finally, finite population correction was done to adjust the final sample size which gives a total sample size of 325

- The sample size of was used using the Cochrane formula[1]
- $N= Z^2 X P (1-P) / d^2$
- Where
- N=minimum sample size
- Z= Standard score corresponding to a given confidence level. At a 95% confidence level. At a 95% confidence level or 5% significance level (0.05), Z =1.96

- P= prevalence of malaria parasitemia; and prevalence of 8.70% in a previous study in UCH, Nigeria will be used[2]
- d= precision limit or proportion of sampling error at 6% (0.06)
- N= 325.281
- N= 325.

3.4 Description of Research Instrument

The research instrument used in this study was a combination of molecular biology techniques and a structured questionnaire. Blood samples were collected from pregnant women attending antenatal clinics in Universty College Hospital, Ibadan, and DNA was extracted and amplified using polymerase chain reaction (PCR) to detect the presence of *Plasmodium falciparum*. The amplified DNA was sequenced to identify the presence of sulfadoxine/pyrimethamine resistant mutations. A structured questionnaire was also used to collect demographic information towards malaria and its treatment. The research instrument was validated through a pilot study and administered by trained research assistants.

3.5 Validity of the Research Instrument

The validity of the research instrument is a critical aspect of any research study, as it ensures that the data collected is accurate, reliable, and relevant to the research question. In this study, the research instrument consisted of molecular biology techniques, including polymerase chain reaction (PCR) and microscopy method, as well as a structured questionnaire. To establish the validity of the research instrument, a pilot study was conducted among pregnant women attending antenatal clinics in Adeoyo Maternity Teaching Hospital. The pilot study aimed to assess the feasibility of the instrument, refine the molecular biology techniques, and evaluate the validity and reliability of the structured questionnaire. The molecular biology techniques used in this study, including PCR and microscopy method are widely accepted and validated methods for detecting and identifying *Plasmodium falciparum*.

3.6 Reliability of the Research Instrument

The reliability of the research instrument is a critical aspect of any research study, as it ensures that the data collected is consistent, dependable, and accurate. In this study, the research instrument consisted of molecular biology techniques, including polymerase chain reaction (PCR) and and microscopy method, as well as a structured questionnaire. To establish the reliability of the research instrument, several measures were taken. First, the molecular biology techniques used in this study, including PCR and microscopy method are widely accepted and standardized methods for detecting and identifying *Plasmodium falciparum*. The questionnaire was designed to collect demographic towards malaria and its treatment. The questionnaire was pre-tested among pregnant women to assess its clarity, relevance, and acceptability.The results of the pre-test showed that the questionnaire was well understood and accepted by the participants. The questionnaire was also found to be reliable, with high internal consistency and test-retest reliability. Internal consistency refers to the extent to which the items on the questionnaire measure the same concept. Test-retest reliability refers to the extent to which the questionnaire produces consistent results when administered on two separate occasions. To further establish the reliability of the questionnaire, Cronbach's alpha coefficient was calculated. Cronbach's alpha coefficient is a statistical measure of internal consistency. A high Cronbach's alpha coefficient indicates that the items on the questionnaire are highly correlated and measure the same concept. In this study, the Cronbach's alpha coefficient was found to be 0.8, indicating high internal consistency.

3.7. Data Collection

3.7.1 Collection of venous blood sample:

About 5ml venous blood samples were collected from the eligible pregnant women by trained medical laboratory scientist. The pucture site was clean by making a smooth circular pass

over the site with 70% alcohol pad, moving in and outward spiral from the zone of penetration. The skin was allowed to dry before proceeding to venepuncture. Appropriate needle was attached to the syringe hub by removing the plastic cap over the small end of the needle and inserting into the hub, twiting it tight. The plastic cap over the needle was removed and the bevel held up. Using the index finger, the skin was pulled thightly just below the puncture site. By holding the needle in line with the vein, use a quick, small thrust to penerate the skin and enter in one smooth motion. After blood starts to flow, the tourniquet was released and patient asked to open her hand. After the required volume of blood was collected, the needle was removed from the vein and the blood gently dispensed into K2 EDTA bottled previously labelled with patient's identifiers.

3.7.2 Dried Blood Spot (DBS) Collection

DBS was collected from venous whole blood collected in K2 EDTA bottle. Capillary tube was used to collect blood from a well- mixed (at least 7 times) EDTA bottle and spotted blood each delineated 12-millimeter circle of the whatman 903 filter paper card. It was ensured that the entire circle is covered with blood (approximately 60 -70 µL). The card was air-dried at room temperature for four hours. Each card was packed in individual resealable bags with a desicacant sachet in each bag.

3.7.3 Detection of Parasiteamia:

3.7.3.1 Preparation of Thick and Thin blood smears

The thick blood film was used to detect parasiteamia, while the thin blood film was used to determine the parasite density. Thick and thin blood smears were prepared on the same slide. Using an automatic pipette, 6µL and 2µL of blood were placed separately on a clean greese-free slide. The 6µL was made to prepare thick film by using another slide to spread the blood in circular motion, while 2µL was used to make thin film by using another slide as spreader, held at about 45^0 to the blood and spread gently to form a thin film. The smears were allowed

to air-dry at room temperature for 1 hour. Thereafter, the thin film was fixed using abosulte methanol, while the thick film was left unstained. The smears were flooded with 10% freshly prepared Giemsa stain at pH 7.2, and allowed to stain for 10 minutes. The smears were rinsed gently with clean water and allowed to air-dry. The thick blood film was examined microscopically using 100x objective lens of the CX22 Olympus microscope. Smears were screened by a trained microscopist following standard, quality control procedure. Parasitaemia is expressed, as the number of asexual forms of *Plasmodium falciparum* per microlitre of blood and blood smear is considered negative only after reading 100 high power fields without detecting a parasite under a light microscope at X 1000 magnification[3].

3.7.4 Determination of Parasite Density

CX 22 Olympus microscope was used for parasite count in thin blood film. Parasite density was determined by counting the number of asexual malaria parasite relative to 1000 RBC and assuming a mean leucocyte count of 8000/µl of blood[2.] The following formular was used to calculate the parasite density:

Parasite density: Number of parasite counted in 1000 RBC/1000 X 8000 parasite/µl

3.7.5 Molecular Detection of Antimalarial Drugs Resistance Genes

P.falciparum wildtype and Sulphadoxine-pyrimethamine drug resistance genes including *Pfdhps*, *S108N*, *Pfdfr* and *K540E* were evaluated among the study pregnant women[4].

3.7.6 DNA Extraction: Extraction of parasite from dried blood spots(DBS) using Qiagen Qiaamp DNA extraction mini-kit.

Two pieces of 3mm disk from the filter paper blood spots were punched out using a sterile hole punch and placed into appropriatly labelled 1.5 micro-centrifuge tube> The punch was cleaned and sterilized wach time in sequence with 5% bleach followed by distilled water and then 70% ethanol. Animal tissue lysis buffer, 180µl, was added ensuring the filter paper were soaked before incubation at 85^{o} C for 10 minutes followed by addition of 20µl of proteinase

K stock solution. The mixture was vortexed and incubated at 56ºC for 1 hour after which buffer AL was added to the sample, thoroughly mixed by vortexing and incubated at 70^0 c for 10 minutes. Absolute ethanol (200 µl was added and mixed thoroughtly by vorttexing). The mixture was then added to QIAamp mini kit spin column placed in a 2ml collection tube and centrifuged at 6000 x g (8000 rpm) for 1 minute. The collection tube containing the filtrate was discarded. Buffer aw2 (500 µl) was added to the spin column and then centrifuged at full speed (20,000 x g or 14,000 rpm) for 3 minutes. Again, the filtrate was discarded and the spin column was placed in a 1.5ml microcentrifuge tube. DNA was eluted with 150µl elution buffer AE, incubated at room temperature for 1 minute and centrifuged at 6,000 x g (8000 rpm) for 1 minute. The extracted DNA was -20^0 C until it was used for subsequent molecular studies [5]

3.7.7: Amplification

Table 3.1; Primer Sequences and PCR Amplification Conditions for Pfdhfr [6]

PCR Conditions	**Primary Reaction** DHFR
Initial Denaturation	94^0C x 3 minutes
Denaturation	94^0C x 1 minute
Annealing	52^0C x 2 minutes
Extension	72^0C x 1 minutes
Final Elongation	72^0C x 10 minutes
Total Number of Cycles	**40 Cycles**

Table 3.2: Primer Sequences and PCR Amplification Conditions for Pfdfr [6]

PCR Conditions	**Primary Reaction** DHFR
Initial Denaturation	94^0C x 15 minutes
Denaturation	94^0C x 30 seconds
Annealing	57^0C x 90 seconds
Extension	90^0C x 1 minutes
Final Elongation	72^0C x 10 minutes
Total Number of Cycles	**55 Cycles**

Table 3.3: Primer Sequences and PCR Amplification Conditions for PfK450E [6]

PCR Conditions	**Primary Reaction**
Initial Denaturation	94^0C x 1 minutes
Denaturation	91^0C x 30 seconds
Annealing	62^0C x 30 seconds
Extension	72^0C x 20 seconds
Final Elongation	4^0C x 10 minutes
Total Number of Cycles	**40 Cycles**

Table 3.4: Primer Sequences and PCR Amplification Conditions for Pf S108N [6]

PCR Conditions	**Primary Reaction** DHFR
Initial Denaturation	94^0C x 1 minutes
Denaturation	91^0C x 30 seconds
Annealing	62^0C x 30 seconds
Extension	72^0C x 20 seconds
Final Elongation	20 ^{0}C x 10 minutes
Total Number of Cycles	**45 Cycles**

3.7.7.1: PCR amplification of *Pfdhfr*

The PCR assays for *dhfr* mutation detection involved reactions to enhance specificity, followed by sequencing to identify the mutations. PCR primer sequences and reaction conditions are indicated in Table 3. In the initial reaction of total volume of 25 µl containing; [2.5 µl of reaction buffer, 12.5 µl Master mix (containing 2mM $MgCl_2$, 250 µM each deoxynucleoside triphosphate (dNTPs), 1x Bioline Taq DNA polymerase), and 0.5 µl of forward and reverse primers]. A 650-basepair (bp) segment of the *dhfr* gene was amplified using the primers DHF forward and DHFR reverse as described in Table 3. In the second reaction, 3 µl of the amplified product from the first PCR was added to the second PCR mixture, using primers DHFR Nested M3b (5'TGATGGAACAAGTCTGCGACGTT3') and DHFR Nested M9 (5'CTGGAAAAAATACATCACATTCATATG3') to amplify a 594-bp fragment containing polymorphic codons 51, 59, 108, and 164. The PCR products were analyzed via electrophoresis on 1.0% agarose gels and visualized by transillumination. [7]

3.7.7.2: PCR amplification of *Pfdhps*

For Pfdhps amplification, 25µl reaction volume containing 12.5µl ready Master mix (Roche), 1.0µl of each primer, 5.5µl of nuclease free water and 5µl of *P. falciparum* DNA. Amplification was done using a Thermocycler following conditions as described in Table 4. PCR products were examined using 1% agarose gel electrophoresis stained with ethidium bromide.[8]

3.7.7.3: PCR amplification of *K450e*

For K450E, 25µl reaction volume containing 12.5µl ready Master mix (Roche), 0.5µl of each primer, 6.5µl of nuclease free water and 5µl of *P. falciparum* DNA. Amplification was done using a Thermocycler following conditions as described in Table 3. PCR products were examined using 1% agarose gel electrophoresis stained with ethidium bromide. [9]

3.7.7.4: PCR amplification of *S108N*

For S108N 25µl reaction volume containing 12.5µl ready Master mix (Roche), 1.0 µl of each primer, 6.5µl of nuclease free water and 5µl of *P. falciparum* DNA. Amplification was done using a Thermocycler following conditions as described in Table 4. PCR products were examined using 1% agarose gel electrophoresis stained with ethidium bromide.

3.8 Data Analysis

SPSS Version 21.0 was used to analyse the data set, while ChromasPro Software v.1.5 was used to assemble the forward and reverse sequences of the genes. MEGA 7 (Moecular Evolutionary Genetics Analysis, https:www.megasoftware.net/show eua) software was used to identify the mutations by comparing with their reference genomes.

3.9: Ethical Approval

Ethical approval was obtained from the Joint University of Ibadan/University College Hospital Institution Review Committee UI/UCH Ethical Committee assigned number was: UI/EC/23/0523.

Endnotes

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Chapter Four

4.0 Results and Discussion of Findings

Characteristics of the study subjects are shown in table 4.1. Age group 25-34 years had the highest percentage of 43.8%, followed by 30.5% from age group <25 years, 24.8% from age group 35-44 years, while the least percentage of 3.8% was found among the age group >45 years. Married pregnant women had higher percentage of 89.5% when compared with 10.5% single pregnant women. Primigravida had the highest percentage of 36.2%, followed by 33.3% and 30.5% from secundigravida and multigravida respectively. First trimester gestational age had highest percentage of 43.8%, 2nd trimester had 32.4% while 3rd trimester had the least value of 23.8%.

Educational status of the respondents shows 71.4% had tertiary education, 22.9% had secondary education and 5.7% had primary education. HIV status: 98.1% were HIV negative, and 1.9% were HIV negative. Awareness about Intermittent preventive therapy (IPT) use shows that 78.1 of the responded used IPT, while 21.9% did not use IPT. Only 20% of the respondents used insecticide treated net (ITN), while 80% did not use it. A good number of the respondents (89.5%) used insecticide, while 10.5% did not use it.

Table 4.2 shows the prevalence of malaria among the various age groups. Age group 25-34 years had significantly highest malaria prevalence of 23.9% ($P<0.05$), followed by 12.5% rate obtained from <25 years, 11.5% from 35-44 years while >45 years recorded no prevalence.

Prevalence of malaria among the various gravidity is shown in table 4.3. Primigravida had the highest prevalence rate of 21.1%, followed by 17.1% rate from secundigravida, and the least rate of 12.5% from multigravida.

Table 4.1: Socio-demographic characteristics

Parameters	Frequency	Percentage (%)
Age range (Years)		
<25	32	30.5
25 – 34	46	43.8
35 – 44	26	24.8
>45	4	3.8
Total	**105**	**100.0**
Marital Status		
Single	11	10.5
Married	94	89.5
Total	105	100.0
Gravidity		
Primigravida	38	36.2
Secundigravida	35	33.3
Multigravida	32	30.5
Total	105	100.0
Gestational age		
1st Trimester	46	43.8
2nd Trimester	34	32.4
3rd Trimester	25	23.8
Total	105	100.0
Educational status		
Primary	6	5.7
Secondary	24	22.9
Tertiary	75	71.4
Total	105	100.0
HIV Status		
Negative	103	98.1
Positive	2	1.9
Total	105	100.0
IPT use		
Yes	82	78.1
No	23	21.9
Total	105	100.0
ITN Use		
Yes	21	20.0
No	84	80.0
Total	105	100.0
Commercial Insecticide use		
No	11	10.5
Yes	94	89.5
Total	**105**	**100.0**

Source: Author's Lab Work, 2024.

Table 4.2: Prevalence of Malaria among the various age groups

Age range	N	Positive n_1(%)	Negative n_2(%)
<25 Years	32	4 (12.5)	28 (87.5)
25 - 34	46	11 (23.9)	35 (76.1)
35 - 44	26	3 (11.5)	23 (88.5)
>45	4	0 (0.0)	4 (100.0)
Total	105	18(17.1)	87(82.9)

Key: N= Total number examined, n_1 = number of positive, n_2 = number of negative

(P<0.05)

Source: Author's Lab Work, 2024.

Table 4.3: Prevalence of Malaria among the various gravidity

Gravidity	N	Positive n_1(%)	Negative n_2(%)
Primigravida	38	8 (21.1)	30 (78.9)
Secundigravida	35	6 (17.1)	29 (82.9)
Multigravida	32	4 (12.5)	28 (87.5)
Total	105	18(17.1)	87(82.9)

Key: N= Total number examined, n_1 = number of positive, n_2 = number of negative (P>0.05)

Source: Author's Lab Work, 2024.

Table 4.4 shows the prevalence of malaria among the various gestational age. First trimester had the highest prevalence rate of 19.6%, followed by 17.6% and 12.0% obtained from 2nd and 3rd semester respectively (P>0.05).

The prevalence of malaria among the various educational status is shown in figure 4.1. Pregnant women with secondary education had significantly highest prevalence rate of 25.0%, when compared with 14.7%, and 0.9% rates recorded by Tertiary and primary education respectively.

Table 4.5 shows malaria prevalence Vs. HIV Status of the pregnant women. HIV seropositive pregnant women had significantly higher prevalence rate of 50.0% when compared with 16.5% recorded by HIV seronegative pregnant women (P<0.05).

Table 4.4: Prevalence of Malaria among the various gestational age

Gestational age	N	Positive n_1(%)	Negative n_2(%)
1st Trimester	46	9(19.6)	37 (80.4)
2nd Trimester	34	6(17.6)	28 (82.4)
3rd Trimester	25	3(12.0)	22 (88.0)
Total	105	18(17.1)	87(82.9)

Key: N= Total number examined, n_1 = number of positive, n_2 = number of negative (P>0.05)

Source: Author's Lab Work, 2024.

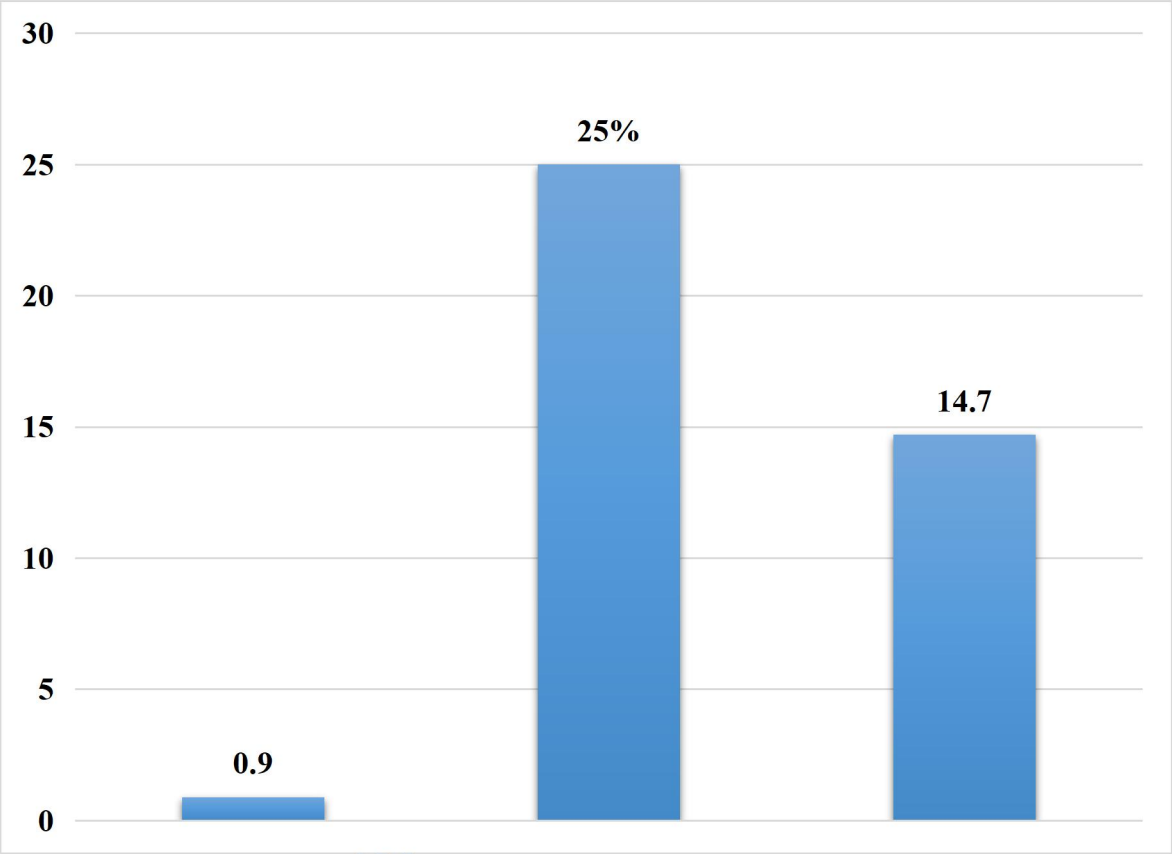

Figure 4.1; Prevalence of Malaria among the various educational status

Table 4.5: Malaria Vs. HIV status

HIV Status	N	Positive n_1(%)	Negative n_2(%)
Positive	2	1(50.0)	1 (50.0)
Negative	103	17(16.5)	86(83.5)
Total	105	18(17.1)	87(82.9)

Key: N= Total number examined, n_1 = number of positive, n_2 = number of negative

($P<0.05$)

Source: Author's Lab Work, 2024.

Table 4.6 shows malaria prevalence and IPT use. Pregnant women that didn't use IPT had significantly higher prevalence rate of 30.4% when compared with 13.4% rate recorded by pregnant women that used IPT ($P<0.05$).

Table 4.7 shows malaria prevalence and ITN use by the respondents. Pregnant women that did not use ITN had significantly higher prevalence rate of 28.6% when compared with 14.3% obtained from pregnant women that used ITN ($P<0.05$)

Table 4.8 shows malaria prevalence and use of commercial insecticide. Pregnant women who didn't use insecticide had significantly higher prevalence rate of 45.6% when compared to 13.7% rate recorded by pregnant women who used insecticide ($P<0.05$).

Table 4.6: Malaria Vs. IPT use

IPT Use	N	Positive n_1(%)	Negative n_2(%)
Yes	82	11(13.4)	71 (67.6)
No	23	7(30.4)	16(69.6)
Total	105	18(17.1)	87(82.9)

Key: N= Total number examined, n_1 = number of positive, n_2 = number of negative

($P<0.05$)

Source: Author's Lab Work, 2024.

Table 4.7: Malaria Vs. Insecticide Treated Net use

ITN Use	N	Positive n_1(%)	Negative n_2(%)
Yes	84	12(14.3)	72 (85.7)
No	21	6 (28.6)	15(71.4)
Total	105	18(17.1)	87(82.9)

Key: N= Total number examined, n_1 = number of positive, n_2 = number of negative

Source: Author's Lab Work, 2024.

Table 4.8: Malaria Vs. Commercial Insecticide use

Insecticide Use	N	Positive n_1(%)	Negative n_2(%)
Yes	94	13(13.7)	81 (67.6)
No	11	5 (45.6)	6(15.2)
Total	105	18(17.1)	87(82.9)

Key: N= Total number examined, n_1 = number of positive, n_2 = number of negative ($P<0.05$).

Source: Author's Lab Work, 2024.

Table 4.9 shows the age and parasite counts. Age group 35-44 years had significantly highest mean parasite count of 1742±300 µL when compared to 1040±200 count/ µL, 110±10 count/ µL obtained from age group 25 -34 years, and age group <25 years respectively, while age group >45 years had zero count.

Table 4.10 shows gravidity of pregnant women and parasite count. Primigravida had significant highest parasite count of 1500 ± 100 count/ µL when compared to 1010 ± 200 parasite/ µL and 900 ± 20 parasite/ µL obtained from secundigravida and multigravida respectively.

Gestational age of pregnant women and parasite count is shown in table 4.11. Significantly highest prevalence rate of 1600 ± 100 parasite/ µL, followed by 1000 ±200 parasite/ µL from 2nd trimester, while the least rate of 600 ±10 parasite/ µL.

Table 4.9: Age Vs. Parasite count

Age range (Years)	N	Mean Parasite Count/µL
<25	32	110 ± 10
25 – 34	46	1040 ± 200
35 – 44	26	1742 ±300
>45	4	0 ± 0

Key: N= Total number examined, (P<0.05)

Source: Author's Lab Work, 2024.

Table 4.10: Gravidity Vs. Parasite count

Gravidity	N	Mean Parasite Count/µL
Primigravida	38	1500 ± 100
Secundigravida	35	1010 ± 200
Multigravida	32	900 ±20

Key: N= Total number examined, (P<0.05)

Source: Author's Lab Work, 2024.

Table 4.11: Gestational age Vs. Parasite count

Gravidity	N	Mean Parasite Count/µL
1st Trimester	46	1600 ± 100
2nd Trimester	34	1000 ± 200
3rd Trimester	25	600 ±10

Key: N= Total number examined, (P<0.05)

Source: Author's Lab Work, 2024.

Table 4.12 shows the age group and parasite species. *P.falciparum* had the highest prevalence rate of 19.6% among the age group 25 -34 years, 12.5% among age group < 25 years, 11.5% among age group 35-44 years, while age group > 45 years recorded no rate of *P.falciparum*. P.ovale had 2.2% rate among age group 25 -34 years, and Gametocyte prevalence rate of 2.2% among age group 25 -34 years. The overall prevalence rate of P.falciparum was found to be 15.2%, P.ovale 0.9%, and gametocyte 0.9%.

Table 4.13 shows gravidity of pregnant women and parasite species. *P.falciparum* had the highest prevalence rate of 15.7% among secundigravida, 15.7% among primigravida, while multigravida recorded rate of 9.4%. *P.ovale* had 2.6% rate among primigravida, and Gametocyte prevalence rate of 2.6% among primigravida. The overall prevalence rate of *P.falciparum* was found to be 15.2%, *P.ovale* 0.9%, and gametocyte 0.9%.

Figure 4.2 shows age group and RDT positivity. Significantly highest RDT positivity rate of 15.2% was found among age group 25-34 years, followed by 12.5% obtained from age group < 25 years, while the least rate of 11.5% was recorded by age group 35 – 44 years as age group > 45 years recorded no positivity rate.

Table 4.12: Age Vs. Parasite species

Age range	N	Parasite species/ forms		
		P.falciparum **n_1 (%)**	***P.ovale*** **n_2 (%)**	**Gametocyte n_3 (%)**
<25 Years	32	4 (12.5)	0 (0.0)	0 (0.0)
25 – 34	46	9 (19.6)	1 (2.2)	1 (2.2)
35 – 44	26	3 (11.5)	0 (0.0)	0 (0.0)
>45	4	0 (0.0)	0 (0.0)	0 (0.0)
Total	105	16 (15.2)	1 (0.9)	1 (0.9)

Key: N= Total number examined, (P<0.05)

Source: Author's Lab Work, 2024.

Table 4.13: Gravidity Vs. Parasite species

Gravidity	N	Parasite species/ forms		
		P.falciparum **n_1 (%)**	***P.ovale*** **n_2 (%)**	**Gamectocyte n_3 (%)**
Primigravida	38	6 (15.7)	1 (2.6)	1 (2.6)
Secundigravida	35	6 (17.1)	0 (0.0)	0(0.0)
Multigravida	32	3 (9.4)	0 (0.0)	0 (0.0)
Total	105	16 (15.2)	1 (0.9)	1 (0.9)

Key: N= Total number examined, ($P<0.05$)

Source: Author's Lab Work, 2024.

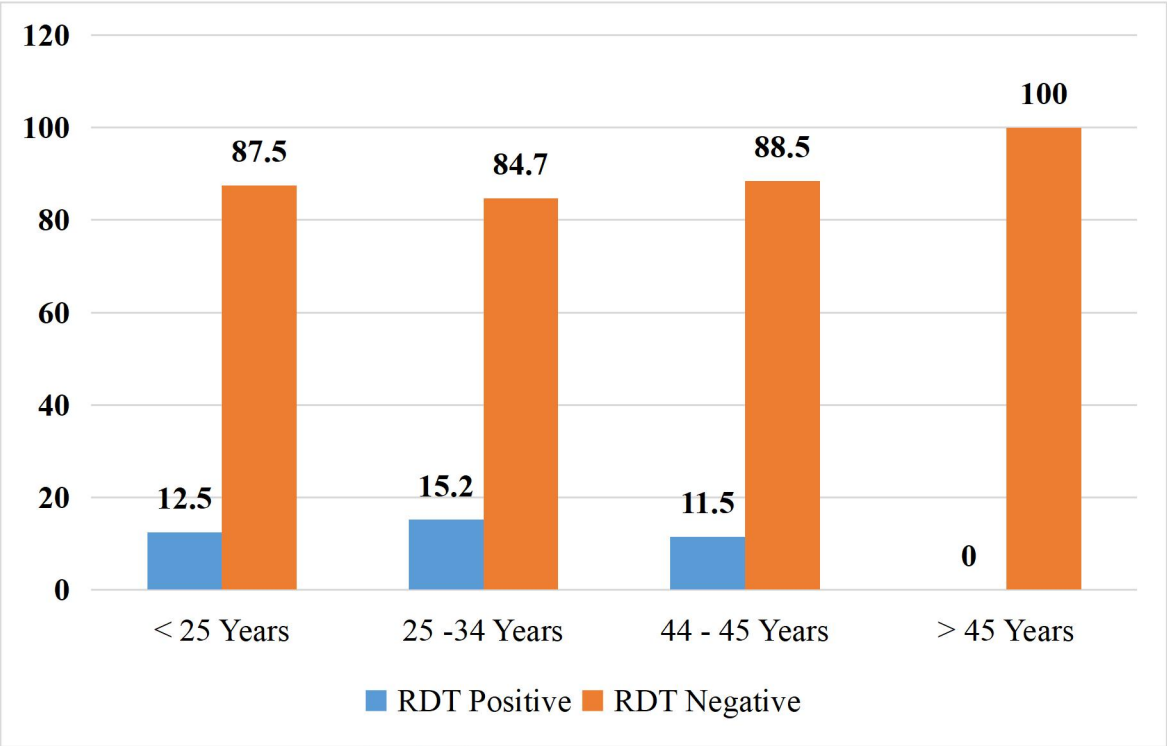

Figure 4.2: Age group Vs RDT

Figure 4.3 shows gravidity and RDT positivity. Significantly highest RDT positivity rate of 21.1% was found among primigravida, when compared to 8.6% obtained from secundigravida, and 3.1% rate from multigravida.

Figure 4.4 shows gestational age and RDT positivity. Significantly highest RDT positivity rate of 21.1% was found in first trimester, when compared to 17.1% obtained from second trimester, and 12.5% rate from third trimester.

Table 4.14 shows the diagnostic performance of Microscopy and RDT. The sensitivity, Specificity, PPV and NPV of RDT were found to be 77.8%, 100%, 100% and 95.6% respectively.

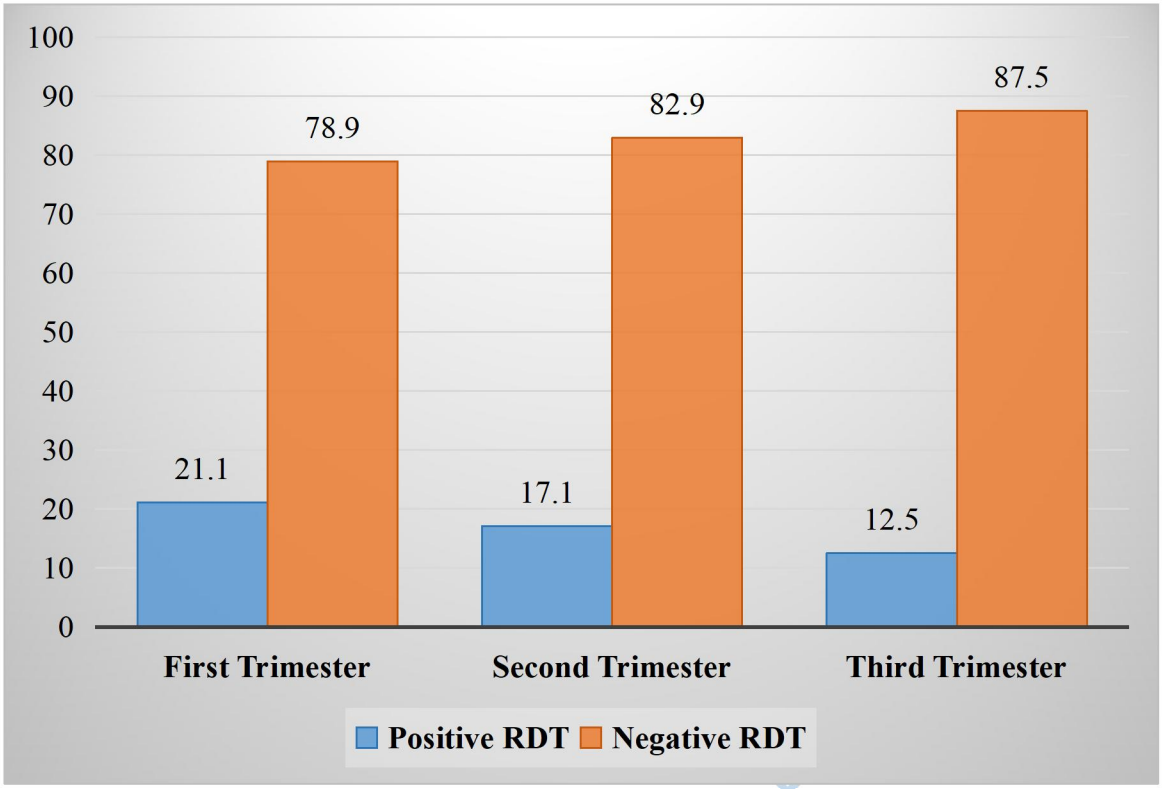

Figure 4.3: Gravidity Vs RDT Positivity

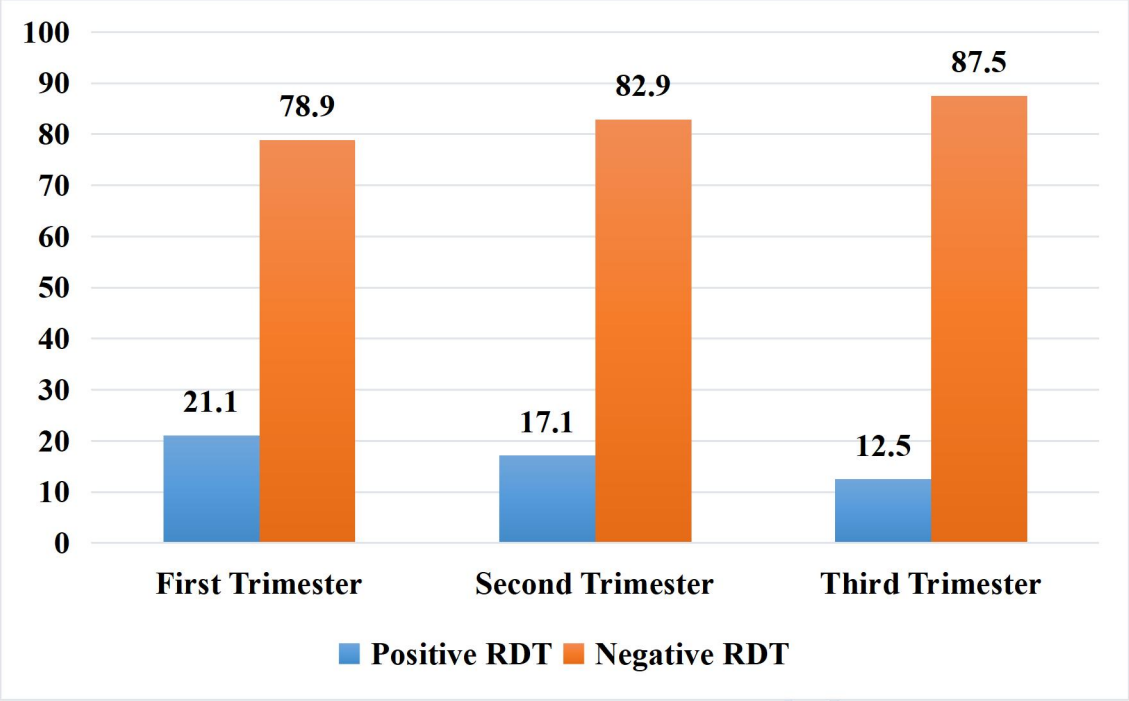

Figure 4.4: Gestational age and RDT positivity

Table 4.14: Diagnostic performance of Microscopy Vs RDT

RDT	Microscopy		Total
	Positive	Negative	
Positive	14	0	14
Negative	4	87	91
Total	18	87	105

RDT Sensitivity = True positives/ Total positives x 100%

RDT Sensitivity= 14/18 x 100 = 77.8%

RDT Specificity = True negatives / Total negative x 100%

RDT specificity = 87/987 x 100 = 100.0 %

Positive Predictive Value (PPV) = True positives/ Tested positive x 100

PPV = 14/14 x 100 = 100%

Negative Predictive Value (NPV) = True negatives/ Tested negatives x 100

NPV = 87/91 x 100 = 95.6%.

Source: Author's Lab Work, 2024.

Table 4.15 shows the prevalence of *Pfdhps* and *S108N* among the various age group. Age group 35-44 years had highest prevalence of *Pfdhps* and mutant gene S108N when compared with other age groups but not statistically significant having values of 8.7% and 6.5% respectively (P>0.05), while age group >45 years recorded no prevalence of the two genes. Similarly, there was no significant difference between wildtype *Pfdhps* and its mutant gene *S108N* among the various age group.

Table 4.16 shows the prevalence of *Pfdfr* and *K540E* among the various age group. Age group 35-44 years had highest prevalence of *Pfdfr* and mutant gene *K540E* when compared with other age groups but not statistically significant having values of 15.2% and 8.7% respectively (P>0.05), while age group >45 years recorded no prevalence of the two genes. Similarly, there was no significant difference between wildtype *Pfdfr* and its mutant gene *K540E* among the various age group.

Table 4.17 shows the Prevalence of *pfdhps* and *pfdhfr* Wildtype and Mutant genes. *Pfdhps* wildtype gene had higher prevalence rate of 6.7% when compared to 4.8% rate obtained from S108N mutant gene but not statistically significant (P>0.05). Similarly, *pfdhfr* wildtype gene had higher prevalence rate of 12.4% when compared to 6.7% rate obtained from k540E mutant gene.

Figure 4.5 shows detection of *pfdhps* genes among the *P. falciparum* positive pregnant women expressed on agarose gel. Lanes 1 and 10 are DNA ladder; Lanes 3,4,6,9,11, 14 and 15 are *pfdhps* genes.

Figure 4.6 shows detection of *pfdhfr* gene among Plasmodium falciparum positive pregnant women. Lane 1 and 8 are DNA ladders, Lane 3-16 (except 8) are the wildtype *pfdhfr* genes.

Figure 4.7: shows gel electrophoresis plate of S108N mutant gene. Lane M is the DNA ladder, lane 1,2,6, 11 and 15 are S108N genes each with a size of 23bp

Table 4.15: Prevalence of *Pfdhps* and S108N among the various age group

Age	N	*Pfdhps* n(%)	Mutant gene(S108N) n(%)	X^2	P-value
<25 Years	32	2 (6.3)	1 (3.1)	2.653	0.120
25-34	46	4 (8.7)	3 (6.5)	1.568	0.921
35-44	26	1 (3.8)	1 (3.8)	0.561	0.102
>45	4	0 (0.0)	0(0.0)	0.873	0.589
Total	**105**	**7 (6.7)**	**5 (4.8)**	**1.450**	**0.430**

Source: Author's Lab Work, 2024.

Table 4.16: Prevalence of *Pfdfr* and K540E among the various age group

Age	N	*Pfdfr* n(%)	Mutant gene(S108N) n(%)	X^2	P-value
<25 Years	32	4 (12.5)	2 (6.25)	1.621	0.954
25-34	46	7 (15.2)	4 (8.7)	0.568	0.872
35-44	26	2 (7.7)	1 (3.8)	0.765	0.518
>45	4	0 (0.0)	0 (0.0)	0.873	0.589
Total	**105**	**13 (12.4)**	**7 (6.8)**	**0.965**	**0.545**

Source: Author's Lab Work, 2024.

Table 4.17: Prevalence of *pfdhps* and *pfdhfr* Wildtype and Mutant genes

Gene/Haplotype	**N= 105**			
Pfdhps				
	Wildtype	**Mutant**	**X^2**	**P-value**
	n (%)	**n (%)**		
S108N	7 (6.7)	5 (4.8)	1.397	0.226
Pfdhfr				
k540E	13 (12.4)	7 (6.7)	0.416	0.921

Source: Author's Lab Work, 2024.

Figure 4.5 shows detection of *pfdhps* genes among the *P. falciparum* positive pregnant women expressed on agarose gel. Lanes 1 and 10 are DNA ladder; Lanes 3,4,6,9,11, 14 and 15 are *pfdhps* genes.

Figure 4.6 shows detection of *pfdhfr* gene among Plasmodium falciparum positive pregnant women. Lane 1 and 8 are DNA ladders, Lane 3-16 (except 8) are the wildtype *pfdhfr* genes.

Figure 4.7: shows gel electrophoresis plate of S108N mutant gene. Lane M is the DNA ladder, lane 1,2,6, 11 and 15 are S108N genes each with a size of 23bp

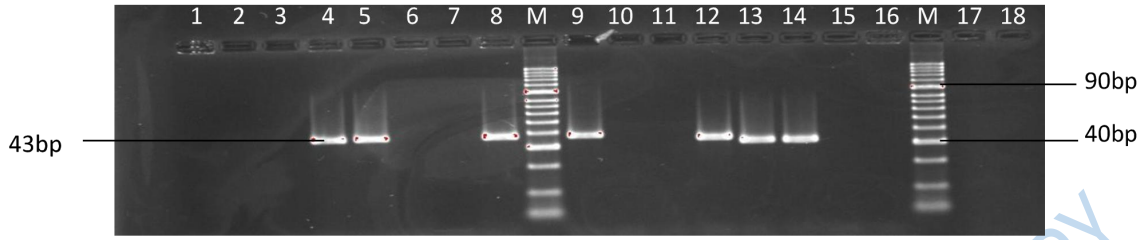

Figure 4.5: Detection of *pfdhps* genes among the *P. falciparum* positive pregnant women expressed on agarose gel

Source: Author's Lab Work, 2024.

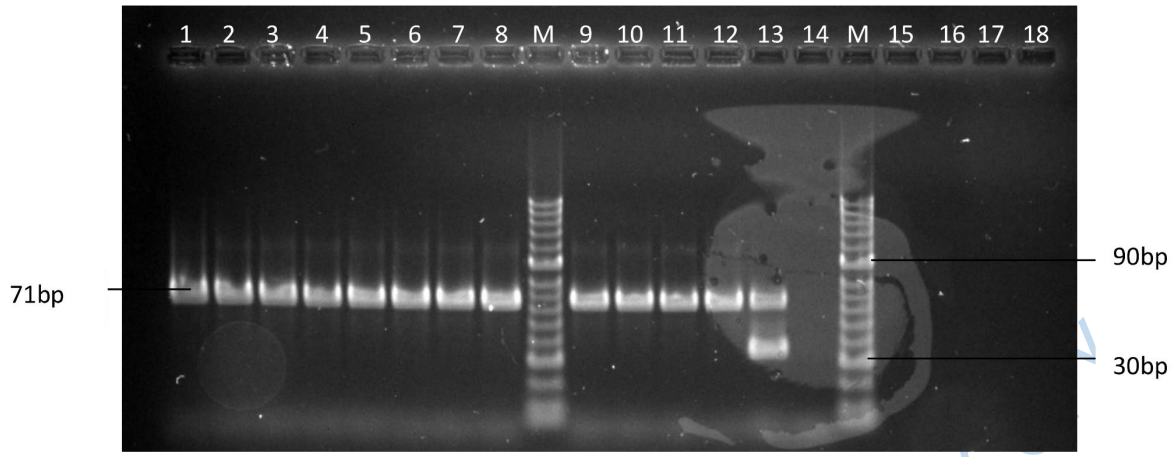

Figure 4.6: Detection of *pfdhfr* gene among Plasmodium falciparum positive pregnant women

Source: Author's Lab Work, 2024.

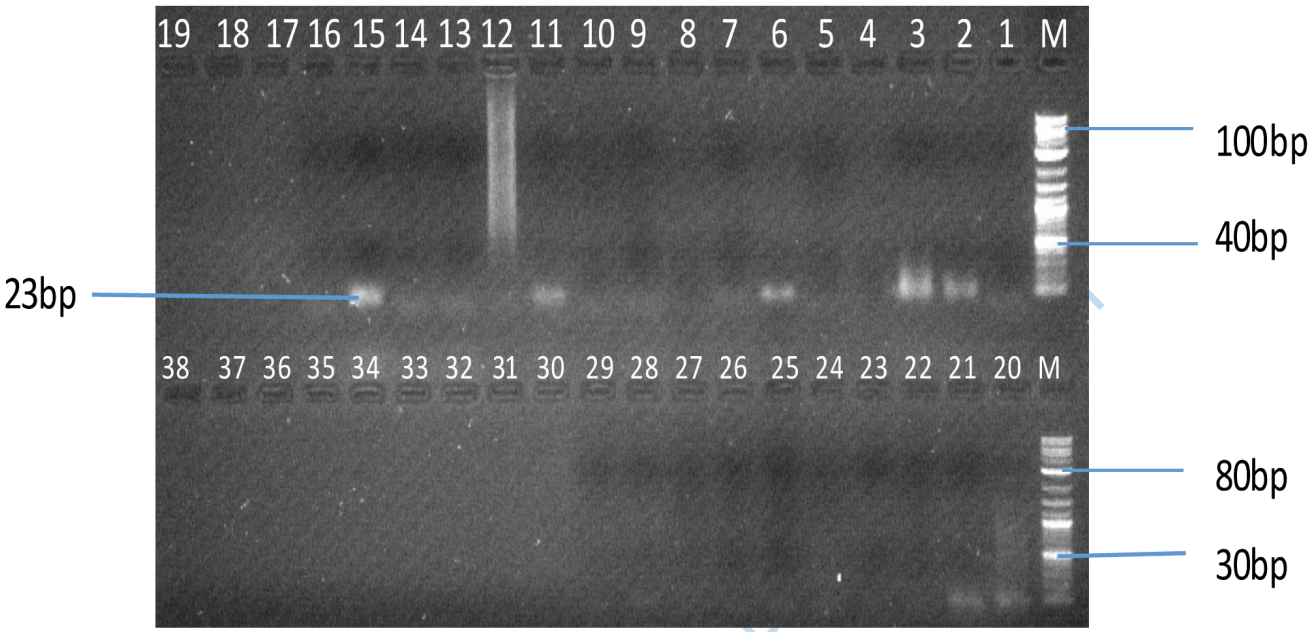

Figure 4.7: Gel electrophoresis plate of S108N mutant gene.

Source: Author's Lab Work, 2024.

4.1 Discussion of Findings

Malaria in pregnancy is a serious public health problem that continually put pregnant women and their unborn babies at risk[1]. Nigeria has made considerable progress in the fight against malaria in pregnancy and remains one of the leaders in the testing and large scale application of new recommendations and some innovative strategies. However, malaria remains a public health problem in Nigeria.

This study investigates the prevalence of malaria and anti-malaria drug resistances genes among pregnant women attending ante-natal clinic at University College Hospital, Ibadan. In this study, the prevalence of malaria was 17.1%. This is comparable to the rate found in Ghana, Burkina Faso, and Malawi which reported 20.4%, 18.1%, and 19% respectively[2]. However, this is higher than 8.7% reported but much lower than other national sub-regions: 42% in the north-east, 58% in the South-South and 92-99% in the north-east but lower than 4.3% prevalence rate[3,4,5,6,7]. These findings significantly affect malaria control programmes, especially those focused on pregnant women. Based on the local epidemiological situation, the study highlights the need for targeted interventions and strategies to combat malaria in pregnancy. Targeted strategies and interventions could lead to better health outcomes for pregnant women and their newborns. These interventions can reduce the burden of malaria in affected communities and contribute to achieving global health targets such as the sustainable development targets such as the Sustainable Development Goals. Pregnancy increases the risk of severe disease for women infected with malaria due to immune system suppression[8]. Variations in malaria prevalence among pregnant women in the country could be attributed to difference in diagnosis methods, the impact of geographical location, and seasonal variations in malaria endemicity.

Malaria causes about 11% of maternal deaths in Nigeria[9]. Malaria complicates 8.4% to 58.1% of pregnancies in Nigeria[10]. In this study, pregnant women in the age bracket 25-34 years had

significantly highest malaria prevalence rate of 23.9% (P<0.05). This is slightly lower than 32.8% rate reported in Ibadan[11].

Women of low parity have the highest risk of malaria in pregnancy, as was also corroborated in this study with primigravidae having prevalence rate of 21.1%. This is in line with 21.3% rate reported, but higher than 8.4% and lower than 41.8%. Multigravidae were significantly less likely to have malaria parasites, while primigravidae were more likely to (p=0.012)[12,13,14,15,16].

Most participates were recruited in their first trimester with high utilization of Sulphadoxine pyrimethamine (SP). This early booking is in contrast with late gestational age at booking[17]. Early booking enhances the benefit that can be derived from preventive measures. Participants in first trimester of pregnancy had highest prevalence rate of 19.6% when compared to other gestational ages (p>0.05). This is slightly higher than 17.6% rate obtained in a study done in Ibadan[19]. However, despite the fact that most participants were recruited in their early gestational age, the prevalence of malaria is the highest in this group. This is probably due to other factors like suppressed immunity that is associated with pregnancy. Pregnant women in their first and second pregnancies are particularly susceptible to malaria[19]. Placental parasitaemia limits transfer of nutrients and oxygen to the foetus, leading to intrauterine growth restriction, low birth weight or intrauterine death[20].

In this study, participants with only secondary education had highest prevalence of malaria. This is similar with a report of high prevalence (73.13) of malaria in pregnancy[21]. Pregnant women with lower levels of education are more likely to be affected by malaria than those with higher levels of education. Malaria is still a public health issue and risk factors like educational class less than tertiary education may be contributing to increase in prevalence of malaria among pregnant women[22].

Malaria disease is still a known health threat in tropical and subtropical areas in pregnancy. Pregnant women with HIV had significantly higher prevalence of malaria when compared to non-HIV pregnant women ($p<0.05$). Risk factors for malaria disease play a significant role in evaluating any program to prevent malaria in pregnant women. There is great to malaria disease and morbidity in women who have been infected with HIV[23]. It was suggested that death of pregnant mothers where malaria is endemic may be greater than 25%[24].

During pregnancy, malaria prevention is essential and it can lead to considerable reductions in neonatal mortality and low birth weight. Timely diagnosis and effective case management alongside the use of insecticide treated nets (ITNs), plus Sulfadoxine-pyrimethamine (SP) as intermittent preventive treatment (IPTp) were recommended for malaria control in pregnancy by World Health Organization. In this study, those who used ITN, IPT and insecticide had lower prevalence of malaria when compared to those who didn't use ($P<0.05$) and this is similar to a report[25].

Age group 35-44 had significantly highest parasite count when compared to other age groups. This is at variant with a report which shows that maternal age of <25 years was found to be significantly associated with an increased with the risk of malaria parasitaemia in pregnancy (OR=2.054, C.I.=1.118-3.772, p=0.019)[26].

Majority of malaria infection in Nigeria are caused by *P.falciparum*[27]. In this study, *P.falciparum* significantly highest prevalence of 15.2%, when compared to 0.9% rate recorded by P.ovale and gametocyte ($p<0.05$). This is also similar to same report of highest prevalence of *P.falciparum* in their study titled "A cross-sectional study of risk factors associated with malaria diseases in pregnant women attending a State hospital Iwo, Osun State, Southwest Nigeria". Infection with *P.falciparum* during pregnancy may lead to several severe complications, including abortion, fever, placenta malaria, maternal anaemia and foetal exposure to the parasite[28].

Recurrent infections with placenta strain-specific P.*falciparum* is common among women in areas of high endemicity during their first and second pregnancies[29]. Prevention and control strategies are used to combat the negative consequences of malaria for women and their fetuses.

A Rapid Diagnostic Test (RDT) is a quick way to determine if a person has malaria by detecting the malaria parasites in their blood. Malaria RDTs detect specific antigens (proteins) produced by malaria parasites that are present in the blood of infected individuals. Histidine-rich proteins (HRP2) malaria RDT test was used in this study. The HRP2 malaria test is a rapid test that detects the presence of the P. falciparum HRPs antigen in blood samples. In this study, RDT positivity was found to be 13.3%, with sensitivity of 77.8% and specificity of 100% when compared with blood smear microscopy (reference standard). This finding corroborate the results who reported RDT sensitivity 73.0%[30,31].

The progress in the development and administration of chemotherapeutic agents is threatened by evolved resistance to most of the antimalarials currently in use, including SP. Mutations in the *pfdhps* and *pfdhfr* genes are associated with antifolate resistance[32]. In this study, the prevalence of *pfdhps* mutant gene (S108N) was found to be 4.8%, *pfdhfr* mutant gene (K540E), 6.7% as against the wild type genes of 6.7% and 12.4% for wildtype *pfdhfr* and *pfdhfr* respectively and the findings are in line with the report[33]. The detection of *pfdhps* and *pfdhfr* mutant genes suggesting resistance to the SP drug combination currently used for intermittent preventive treatment during pregnancy. Resistance to SP is associated with polymorphism in the *P.faciparum* dihydrofolate reductase (pfdhfr) and dihydropteroate synthase (pfdhps) genes.

Endnotes

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Chapter Five

Conclusion

5.1 Summary of Findings

A total of one hundred and five (105) eligible pregnant women attending ANC at UCH, Ibadan from February to July, 2024 were recruited for this study. Structured questionnaire was administered and about 5mls of venous blood samples were collected into EDTA bottle for malaria detection and dried blood spot samples for molecular drug resistance gene detection. Malarial parasite was detected using Microscopy and Rapid Diagnostic Test methods and antifolate wildtype genes *pfdhfr* and *pfdhps*, and their drug resistant mutant genes *S108N* and *K540E* were detected with Polymerase Chain Reaction using gene specific primers and visualized with agarose gel electrophoresis. Data analysis was done using SPSS version 25. Malaria prevalence was 17.1% with age group 25-34 years having significantly highest prevalence of 23.9% when compared to other age groups($P<0.05$).Primigravidae and first-trimester pregnancy had highest prevalence rates of 21.1% and 19.6% respectively when compared to other groups but not statistically significant ($P>0.05$).There was association between gravidity, gestational age and parasite density with primigravidae and first trimester having significantly highest parasite count of 1500 ± 100/µl and 1600±100/µl respectively when compared with other group ($P<0.05$). Moderately high levels of antifolate wildtype genes: *pfdhfr* (6.7%) and *pfdhps*(12.4%) with their corresponding alleles mutant genes: *S108N* (4.8%) and *K540E*(6.7%) were detected. There was no association between age, gestational age, gravidity and SP drug resistance mutations ($P>0.05$).

5.2. Conclusion

The study revealed a high prevalence of malaria among pregnant women attending the University College Hospital, Ibadan. The detection of antimalarial drug resistance genes is a major public health concern, as it may compromise the effectiveness of currently used

antimalarial drugs. The use of insecticide-treated bed nets (ITN), intermittent preventive treatment (IPT), and insecticides was associated with a lower prevalence of malaria. The study also found that the age group pregnant women in the age bracket 25-34 years had the highest parasite count, and *Plasmodium falciparum* was the predominant species causing malaria infection in Nigeria.

RDT as a rapid diagnosis test was found to have lesser sensitivity compared to blood smear microsocopy. However, it has comparable specificity, positive predictive value (PPV) and negative predictive value (NPV) with blood smear microscopy.

Moderately high levels of antifolate resistance associated mutations were found in the *pfdhfr* (S108N) and *pfdhps* (K540E) genes suggesting high prevalence of Sulphadoxine-pyrimethamine drug combination currently used for intermittent preventive treatment during pregnancy.

5.3. Recommendation

Based on the findings, the following recommendations are made:

- Regular malaria screening should be conducted among pregnant women attending antenatal clinics.
- Effective antimalarial treatment should be provided promptly to prevent complications.
- Monitoring of antimalarial drug resistance should be continued to inform treatment policies.
- Pregnant women should be educated on the use of insecticide-treated bed nets and other preventive measures to reduce the risk of malaria infection.
- Scale up the use of ITN, IPT, and insecticides: These interventions have been shown to be effective in reducing the prevalence of malaria among pregnant women.

- Targeted interventions for high-risk groups: Women in the age group 35-44 should be targeted for intensified malaria prevention and control measures due to their high parasite count.
- Improved diagnosis and treatment: Healthcare providers should be trained to promptly diagnose and treat malaria infections, especially among pregnant women.
- Continued surveillance and monitoring: Regular monitoring of malaria prevalence and antimalarial drug resistance should be conducted to inform treatment policies and interventions
- Education and awareness: Pregnant women should be educated on the importance of malaria prevention and control measures, such as the use of ITN, IPT, and insecticides. All negatve RDT results should be confirmed with blood smear microscopy (gold standard method) to exclude false negative results.
- Antifolate resistance associated genes were found in this study, further studies should be done to address the prevalence of other genetic resistance markers.

5.4. Contribution to Knowledge

- The prevalence of malaria in pregnancy is high in our environment, with age group 25-34 years having the highest prevalence, there is an association between malaria in pregnancy and educational level.
- Malaria is significantly associated with primigravidae and 1st trimester pregnancy.
- Malaria is significantly associated with non-use of insecticide treated net (ITN), intermittent preventive therapy and commercial insecticide.
- The prevalence of Sulphadoxine-Pyrimethamine (SP) resistance gene mutations is high in our environment.
- There is no association between SP drug resistance gene mutations and gravidity, and gestational age.

5.5. Suggested Area for Further Research

Further studies should be done to address other genetic resistance markers especially quintuple mutant genotype consisting of triple mutations of *pfdhfr*(*N511*/*S59R*/*S108N*) and double mutations of pfdhps (*A437G+K540E+ K540E*). Further research also needed to improve our understanding of the burden of antimalarial drug resistance and to develop effective strategies for controlling and eliminating malaria.

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Appendix I

Questionnaire on Malaria in Pregnancy study

Date: ……

Instruction: Please tick appropriate response

1. Age : < 25 years ( ) 25 – 34 Years ( ) 35 – 44 Years ( ) above 45 Years

2. Marital status? Single ( ) Married ( )

3. Gravidity? Primigravidae and Secundigravidae ( ) Multigravidae (three or more) ( )

4. Gestational age: 1st Trimester ( ) 2nd Trimester ( ) 3rd Trimester ( )

5. Level of education: Primary only ( ) Secondary ( ) Higher education ( )

6. HIV Status : Positive ( ) Negative ( ) Unknown ( )

7. Have heard about how to avoid getting malaria in the last 12 months: Yes ( ) No ( )

8. IPT : No ( ) Yes (any dose , One dose, two dose, three doses)

9. ITN Use : No ( ) Yes : (regularly (four or more times per week), Occasionally (twice or three times per week) Seldom (once or twice per week)

10. Commercial insecticide use: No ( ) Yes : (regularly (four or more times per week), Occasionally (twice or three times per week) Seldom (once or twice per week)

11. Repellants use: No ( ) Yes: (regularly (four or more times per week), Occasionally (twice or three times per week) Seldom (once or twice per week)

Thank you for your response

Author's Lab Work, 2024.

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NOTICE OF EXPEDITED REVIEW AND APPROVAL

Re: Prevalence of Malaria and Detection of Antimalarial Drug Resistance Genes among Pregnant Women Attending Ante-natal Clinic at UCH, Ibadan

UI/UCH Ethics Committee assigned number: UI/EC/23/0523
Name of Principal Investigator: **Adeyefa Ibukun Ayomipo**
Address of Principal Investigator: **Department of Medical Microbiology, Faculty of Natural Sciences, Lead City University, Ibadan**

Date of receipt of valid application: **06/09/2023**

This is to inform you that the research described in the submitted protocol, the consent forms, and other participant information materials have been reviewed and *given expedited approval by the UI/UCH Ethics Committee.*

This approval dates from **06/09/2023 to 05/09/2024.** If there is delay in starting the research, please inform the UI/UCH Ethics Committee so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the* UI/UCH EC *assigned number and duration of* UI/UCH EC *approval of the study.* It is expected that you submit your annual report as well as an annual request for the project renewal to the UI/UCH EC at least four weeks before the expiration of this approval in order to avoid disruption of your research.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the UI/UCH EC. *No changes are permitted in the research without prior approval by the* UI/UCH EC *except in circumstances outlined in the Code. The* UI/UCH EC *reserves the right to conduct compliance visit to your research site without previous notification.*

Dr O. S. Michael
For: Chairperson, UI/UCH Research Ethics Committee
E-mail: uiuchec@gmail.com

Bio-data

A Personal Data

1. Full name:	Ibukun Ayomipo ADEYEFA
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E-mail Address:	ibukunadeyefa@gmail.com
	Phone number: 08035983134
2. Date of Birth:	01/03/1982, Place of Birth: Ilesha
3. Nationality:	Nigerian
4. Name and Address of Next of Kin:	Adeyefa Adebayo O.
	20 Amao Street, Ojota, Lagos

B. Educational Background

i.	School of Science Ile-Ife	1999 SSCE
ii.	Babcock University Ilishan Remo	2004 Bsc Microbiology
iii.	School of Medical Laboratory Science OAUTHC Ile Ife	2008 AMLSCN
iv.	School of Medical Laboratory Science OAUTHC Ile Ife	2010 FMLSCN

Nigeria Youth Service Corps — NYSC Certificate 2006

Post graduate Diploma, Lead City University, Ibadan — 2021

C. Working Experience with Dates

Medical Laboratory Scientist University College Hospital — May 2009 till date

D. Award and Fellowship (if any) — FMLSCN

E. Membership of Academic Professional Bodies

Fellow of the Medical Laboratory Science Council of Nigeria	FMLSCN 2010
Associate of the Medical Laboratory Science Council of Nigeria	AMLSCN 2008

F. Publication (if any) Nil

1. Thesis/Desertation Nil
2. Book/Monographs Nil
3. Scholarly articles Nil
4. Notable scholar or professional Accomplishment Nil

G. Major conferences/workshop attended Nil

H. **Referees**

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_____ _____

Signature **Date**

The University Compliance Certification

This is to certify that the thesis was written by Ibukun Ayomipo ADEYEFA with matriculation number LCU/PG/000404 in the Department of Biological Science, Faculty of Natural and Applied Sciences, Lead City University, Ibadan, Nigeria is in full compliance with the approval format and style.

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Signature **Date**