

**GENE EXPRESSION SIGNATURE AND PHENOTYPIC  
VARIABLES OF *PLASMODIUM FALCIPARUM* INFECTED  
ADULTS IN SAGBAMA, BAYELSA STATE, NIGERIA**

**BY**

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### CERTIFICATION

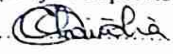
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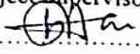
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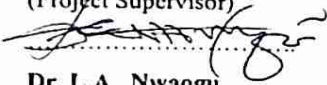
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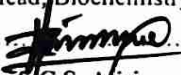
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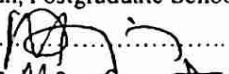
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## **DEDICATION**

To God my Ebenezer, for hitherto has He helped me and to my father, Rev S. M. E. Ogbole (of blessed memory) who was the early momentum behind my career pursuit. Daddy, although you did not wait to reap the fruit of your labour over us, your legacy still lives on.

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## ABSTRACT

Malaria is an acute inflammatory disease that requires continuous monitoring and surveillance in high risk areas. Although several molecular methods are available for the detection of submicroscopic malaria, however the most sensitive molecular method has not been established. Also, given the emergence of antimalarial drug resistance by *Plasmodium*, an alternative strategy to antimalarial drug design is needed. A knowledge of the biochemical and molecular adaptation dynamics of residents in high risk areas might provide novel targets for antimalarial drug design. The objectives of the present study were to determine the prevalence of malaria parasite infection among adults in Sagbama LGA, Bayelsa State, Nigeria using two molecular methods, evaluate the pattern of distribution of ABO/Rhesus blood groups and haemoglobin genotypes in relation to malaria, and evaluate the inflammatory role of haemoglobin genotypes and ABO/Rhesus blood group in relation to malaria in Sagbama LGA. A cross sectional community based study design was used to randomly collect 206 blood samples from the study participants. Study participants were grouped into three; uninfected, asymptomatic, mild and severe malaria group. Each group was further stratified based on ABO/Rhesus blood group and haemoglobin genotype. Nested-genomic-PCR and high resolution melting analysis (HRMA) were used to determine the prevalence of malaria parasite infection by using a set of primers to target the 18S rRNA gene of *Plasmodium falciparum* and results were compared with microscopy. Haemoglobin (Hb) genotype test, blood group test as well as real time PCR gene expression of inflammatory genes were carried out. Overall, 43.20% males and 56.80% females with mean age  $39 \pm 6.65$  years participated in the study. The prevalence of *Plasmodium falciparum* infection among adults was 92.72% (asymptomatic; 63.62%, mild; 18.93%, severe; 10.19%). HRMA was the most sensitive molecular method. Microscopy and nested-genomic PCR yielded 58.25% and 19.9% false negatives respectively. Pair-wise performance comparison of the diagnostic methods was significant ( $p < 0.001$ ). High parasitemia ( $19005 \pm 7345$  Parasites/ $\mu$ l of blood) was found among asymptomatic subjects yet they were not clinically ill. Blood group O was the most common ABO blood group (54.85%) and 96.6% of the study population were Rhesus positive, while 80.1% had haemoglobin genotype AA. Blood groups A, B, AB and O were all associated with asymptomatic malaria ( $P < 0.05$ ), blood groups A, B and AB were associated with mild malaria ( $P < 0.05$ ), while only blood group B was associated with severe malaria ( $p < 0.05$ ). Both Hb AA and AS were associated with asymptomatic malaria ( $P < 0.05$ ), while only Hb AA was associated with mild and severe malaria ( $p < 0.05$ ). A significant upregulation in the gene expression of proinflammatory  $\text{TNF}\alpha$  and  $\text{IFN}\gamma$  was found among blood group A, B and AB and Hb AA subjects compared with blood group O and Hb AS subjects ( $p < 0.001$ ). This was accompanied by a downregulation in the gene expression of anti-inflammatory IL-10 among blood group A, B and AB and Hb AA malaria subjects compared with blood group O malaria subjects ( $p < \text{value } 0.001$ ). In conclusion, the present study found a high prevalence of malaria parasite infection (92.72%) in Sagbama LGA using HRMA. It also found that the mechanism by which ABO blood groups and haemoglobin genotypes influence malaria is by differential expression of inflammatory genes. Findings may have implication for the identification of novel target for antimalarial drug development. However, further large scale study is suggested.

**Keywords:** HRM-analysis, malaria, nested-genomic-PCR, ABO/Rhesus, genotypes,  $\text{TNF}\alpha$ ,  $\text{IFN}\gamma$ , IL-10.

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background Information

Malaria is an acute febrile illness caused by *Plasmodium* parasites which are spread to people through the bites of infected female Anopheles mosquitoes (World Health Organization [WHO], 2023). Five species of *Plasmodium* parasite cause malaria in humans they are; *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi* (Li *et al.*, 2016). However, *Plasmodium falciparum* and *Plasmodium vivax* pose the greatest threat (WHO, 2023). *Plasmodium falciparum* is the deadliest and most dominant *Plasmodium* species in sub-Saharan Africa, while *Plasmodium vivax*, the second most deadly is virtually absent in sub-Saharan Africa, but is the most dominant species in Southeast Asia (WHO, 2022). *Plasmodium falciparum* infection carries poor prognosis and high mortality if untreated (WHO, 2018; Centers for Disease Control and Prevention [CDC], 2018).

Malaria is widespread in tropical and subtropical regions of the world. Globally, an estimated 619 000 malaria deaths and 247 million malaria cases were reported in 2021, with Nigeria having the highest prevalence (WHO, 2023). High prevalence of *Plasmodium falciparum* was reported among new intakes in a tertiary institution in Sagbama, a Local Government Area [LGA] in Bayelsa State, Niger Delta, Nigeria (Ebenezer and Eekpa, 2018), while a 63.3% prevalence of asymptomatic malaria was previously reported among primary school children in a community under Sagbama LGA (Abah and Temple, 2015).

The populations who are most at risk of getting very sick and dying from malaria include people who are heavily exposed to the bites of *P. falciparum* infected mosquitoes, people who have

little or no immunity to malaria, such as young children and pregnant women or travelers coming from areas with no malaria and poor people living in rural areas who lack access to health care (CDC, 2022).

Malaria is a highly inflammatory disease, among malaria subjects, cardinal signs of inflammation include cyclical fever attacks, pain, heat, redness, loss of appetite and loss of strength (Gallego-Delgado, 2014). Although the release of proinflammatory cytokines such as IL-6, IL-8, IFN- $\gamma$ , and TNF, by the host's immune system helps to eliminate *Plasmodium* parasite from the blood stream, however, excessive production of proinflammation cytokines and a dysregulation in the production of anti-inflammatory cytokines (transforming growth factor-(TGF- $\beta$  and IL-10) have been linked to the signs and symptoms experienced during malaria illness (Ty *et al.*, 2019; Popa and Popa, 2021). Also, the exact mechanism and factors that are associated with the trigger of inflammation in malaria remains to be fully understood.

Erythrocyte based antigens and proteins have been reported to either increase or decrease host susceptibility to malaria (Williams *et al.*, 2005; Williams, 2006; Timmann, Thye, and Vens, 2012; Band *et al.*, 2013; Abegaz, 2021). However, conflicting results from several studies (Herrera, Montoya, Arboleda, and Ortiz, 2009; Panda *et al.*, 2011; Afoakwah, Aubyn, Prah, Nwaefuna, and Boampong, 2016; Onanuga and Lamikanra, 2016), have made the role of erythrocyte based antigens and proteins in malaria inconclusive. Also, although malaria parasitic antigens along with various host cellular factors induce the release of cytokines from inflammatory cells (macrophages, neutrophils, dendrites) and endothelial cells (Popa, 2021), however, whether or not erythrocyte based antigens and proteins are associated with the inflammation experienced in malaria illness is yet to be established. Thus, further study is needed to establish the role of erythrocyte-associated antigens in malaria.

## **1.2 Statement of Problem**

Despite concerted efforts by organizations such as World Health Organization, Centers for Disease Control and Prevention and the National Malaria Elimination Programme in Nigeria, yet no substantial reduction in the burden of malaria has been recorded. Malaria has become a stubborn enemy to public health (WHO, 2023). The 2021 Nigeria Malaria Indicator Survey Final Report revealed that the prevalence of malaria in Nigeria was 22% in 2021, with malaria responsible for 60% of outpatient visits to health facilities in Nigeria, 30% childhood deaths, 11% of maternal death and 25% of deaths in infants (National Malaria Elimination Programme (NMEP) [Nigeria], National Population Commission (NPC) [Nigeria], and ICF. 2022). According to the 2022 World Malaria Report, Nigeria accounted for 27% of the global malaria burden in 2022 (WHO 2022).

Although malaria is transmitted through out Nigeria, however, some places in Nigeria are hotspots for malaria transmission (Onyiri, 2015). Bayelsa State is one of the States with a high prevalence of malaria in the South-South region of Nigeria (Oyiri, 2015; Oyeyemi, Davids, Edeki, Leghemo and Eguvbe, 2021; NMEP, NPC Nigeria and ICF, 2022).). Thus eradicating malaria in Nigeria requires community and state level ownership. Some communities in Bayelsa State are hard to reach rural communities that require special measures such as boats to access, as a result, routine adequate health care services are difficult to find in remote rural areas in Bayelsa State. Also, many households in Bayelsa are surrounded by standing bodies of water and mangrove swamp forest (Niger Delta Budget Monitoring Group, 2022). This might also be responsible for the high prevalence of malaria in the State.

The existing malaria control measures are challenged by insecticide resistance in the mosquito vector, drug resistance in parasite populations, and asymptomatic malaria in healthy individuals

(Prusty *et al.*, 2021). Vector management approaches involving the use of insecticides and bed nets against mosquitoes, as well as environmental modifications and pathogen management approaches involving the use of artemisinin-based combination therapy (ACT) against *Plasmodium falciparum* malaria has not yielded desired results (Prusty *et al.*, 2021; NMEP, NPC and ICF. 2022). Thus, alternative measures are needed to compliment these available malaria control measures.

Malaria is a disease that is very difficult to control. In Europe and United States of America where malaria was once eradicated, there is still a progressive increase in autochthonous or indigenous transmission of *Plasmodium* imported by travelers or immigrants from endemic regions (Piperaki, and Daikos, 2016; CDC, 2020). In Africa, malaria is majorly endemic in sub-Sahara Africa with Nigeria persistently maintaining the highest malaria prevalence globally (WHO, 2022)

Treatment of malaria using antimalarial drugs has led to a decrease in malaria morbidity and mortality. The artemisinin-based combination therapies (ACTs) recommended by World Health Organization has enhanced global malaria control, however, the emergence of *P. falciparum* resistance to antimalarial artemisinin-based combination therapies (ACTs) has been reported (WHO, 2018). Also, the other major antimalarial drug classes currently used to treat malaria which are quinoline-related compounds, antifolates, artemisinin derivatives, and antimicrobials have all showed *P. falciparum* resistance (WHO, 2018).

The undetected cases of asymptomatic malaria acting as hidden human reservoirs of malaria parasites and capable of transmitting malaria parasites to uninfected individuals has greatly hindered malaria elimination efforts (Zoghi, *et al.*, 2012; Ouma, *et al.*, 2020).

The poor outcome of the currently available malaria control measures, is that it has revealed gaps

in research that need to be filled, such as novel strategies for antimalarial drug design (Shibeshi, Kifle, Atnafie, 2020) and the utilization of a more sensitive method for early detection of malaria at asymptomatic stage (Ouma *et al.*, 2020)

### **1.3 Aim and objectives of the study**

#### **1.3.1 Aim of the study**

The aim of the present study was to determine the pattern of inflammatory cytokine gene expression and associated phenotypic variables among *Plasmodium falciparum* infected subjects in Sagbama LGA, Bayelsa State.

#### **1.3.2 Objectives of the study**

The objectives of the present study were to:

- i) comparatively determine the prevalence of malaria parasite infection in Sagbama LGA, Bayelsa State, Nigeria using molecular methods.
- ii) evaluate the association of ABO/Rhesus phenotypes with malaria.
- iii) assess the association of haemoglobin genotypes with malaria.
- iv) extract and purify total RNA from the whole blood of residents in Sagbama.
- v) identify differentially expressed pro-inflammatory genes in relation to ABO/Rhesus phenotypes and haemoglobin genotypes among malaria subjects in Sagbama.
- vi) assess differentially expressed anti-inflammatory gene in relation to ABO/Rhesus phenotypes and haemoglobin genotypes among malaria subjects in Sagbama.

## 1.4 Justification of the Study

Genetic factors are major determinants of survival to various diseases (CDC, 2021). Ethnic groups in Africa showing different levels of resistance to malaria have been previously reported (Knight, 2023). Limited knowledge and studies exist on genes conferring resistance / susceptibility to malaria in Africa and how these genes affect the malaria disease risk. This research gap is what the present study was designed to fill. These genes, if identified might provide novel molecular targets for the development of novel antimalarial drugs. The results of such studies will also help to provide a better understanding of the powerful mechanism underlying the host-parasite relationship between the humans and *Plasmodium* (Williams, 2006; Panda *et al.*, 2011; Afoakwah *et al.*, 2016).

The great genetic diversity in Africa can be harnessed to solve the menace of malaria in Africa. The time has come for Africans to start exploring their own genome to solve their endemic health problem, rather than relying on results based on the genome of continents other than Africa (CDC, 2020; Knight, 2023, WHO, 2023) This understanding can be translated into fresh approaches for the detection, prevention and treatment of malaria. This is the basis for the present study.

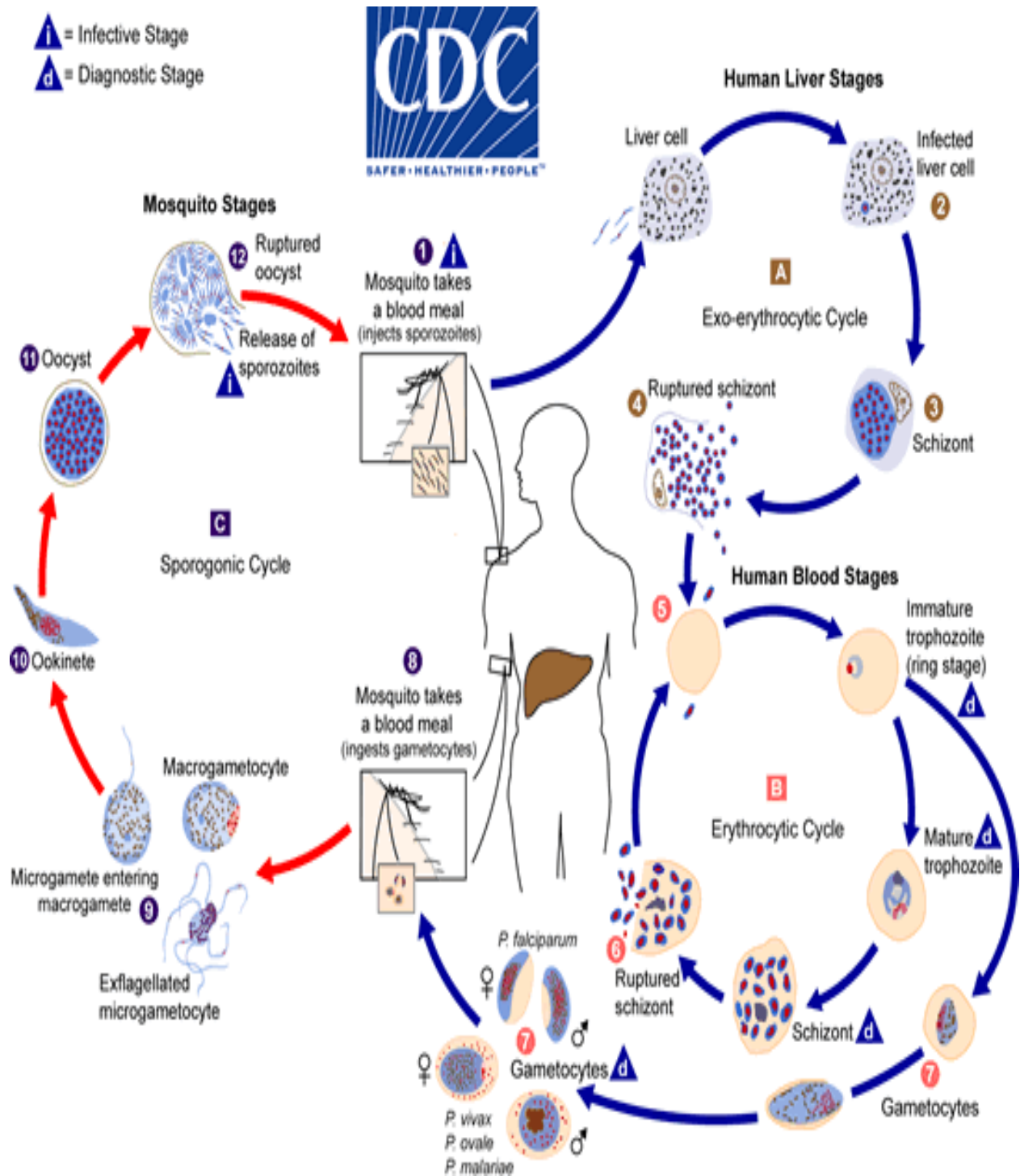
## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Malaria Parasite Life cycle

The malaria parasite lifecycle involves two host, the female *Anopheles* mosquitoes and vertebrates. The female *Anopheles* mosquitoes is the primary host, while, humans and other vertebrates are the secondary hosts (Venugopal, Hentzschel, Valkiūnas, and Marti, 2020). As shown in Figure 1, upon entering the human circulation, the parasite migrates to the liver where it grows and multiplies. Multiple copies of the parasites are released from the liver into the circulation where they enter the red blood cells. Inside the red blood cells, the *Plasmodium* parasites grow and multiplies to form schizonts. The schizonts rupture and destroy the red blood cells to release daughter *Plasmodium* parasites called merozoites. Merozoites continue the cycle by invading other red blood cells. The blood stage parasites are those that cause the symptoms of malaria (CDC, 2020).

Also, as showed in Figure 1, some blood stage parasites occur male and female gametocytes. These gametocytes are ingested by the female *Anopheles* mosquito during blood feeding. Inside the mosquitoes, the gametocytes mate in the gut of the mosquito. After 10-18 days of mating, a form of the parasite called sporozoite migrates to the mosquito's salivary glands. When the mosquito bites another human being, anticoagulant saliva is injected together with the sporozoites into the circulatory system of the human being and another cycle begins (CDC, 2020).



**Figure 2.1: Malaria parasite life cycle** Malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host 1. Sporozoites infect liver cells 2 and mature into schizonts 3, which rupture and release

merozoites ④. (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver (if untreated) and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony A), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony B). Merozoites infect red blood cells ⑤. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites ⑤. Some parasites differentiate into sexual erythrocytic stages (gametocytes) ⑦. Blood stage parasites are responsible for the clinical manifestations of the disease. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal ⑧. The parasites' multiplication in the mosquito is known as the sporogonic cycle C. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes ⑨. The zygotes in turn become motile and elongated (ookinetes) ⑩ which invade the midgut wall of the mosquito where they develop into oocysts ⑪. The oocysts grow, rupture, and release sporozoites ⑫, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites ① into a new human host perpetuates the malaria life cycle (Source: CDC, 2020).

## **2.2 Invasion and rupture of red blood cells by *Plasmodium***

The invasion of merozoites into human erythrocytes is mediated by a series of interactions from distinct classes of *P. falciparum* proteins (Gilson, Nebl, and Vukcevic, 2006; Lin *et al.*, 2016). Merozoite surface proteins (MSP) are the primary ligands responsible for low-affinity interactions with red blood cells. The most abundant MSP is the glycosylphosphatidylinositol (GPI)-anchored merozoite surface protein 1 (MSP1) (Gilson *et al.*, 2006; Lin *et al.*, 2016). MSP1 recognizes the host glycophorin A on the red blood cell surface. This mediates *Plasmodium* invasion into red blood cells, where they multiply and are released from the red blood cells leading to erythrocyte rupture and malaria illness (Baldwin, Li and Hanada, 2015). Different forms of blood stage malaria parasite can be viewed under the microscope as shown in Figure 2.2.

## **2.3 *Plasmodium* infection outcome**

### **a) Asymptomatic malaria**

Asymptomatic malaria is defined as the presence of any density of malaria parasite in the blood without the clinical symptoms of malaria (Lindblade, Steinhardt, Samuels, Kachur, Slutsker, 2013; Ouma *et al.*, 2020). As asymptomatic malaria do not get medical attention, they persist in the community and increases the risk of transmission (Zoghi *et al.*, 2012). In malaria endemic areas, people may develop partial immunity, this allows asymptomatic infections to occur. Thus, asymptomatic malaria occurs among semi- immune individuals in endemic areas with prevalence four to five times higher than clinically patent infections (Lindblade *et al.*, 2013).

Recently, researchers have been focusing on asymptomatic malaria for the elimination of malaria (Zoghi *et al.*, 2012; Ouma *et al.*, 2020). This is because total malaria elimination requires the detection of the human reservoirs of malaria parasite infection using very sensitive method other than microscopy. This is because microscopy misses the detection of *Plasmodium* when parasite

density is low (WHO, 2016; Gimenez, Marques, Regiart, and Bargieri, 2021).

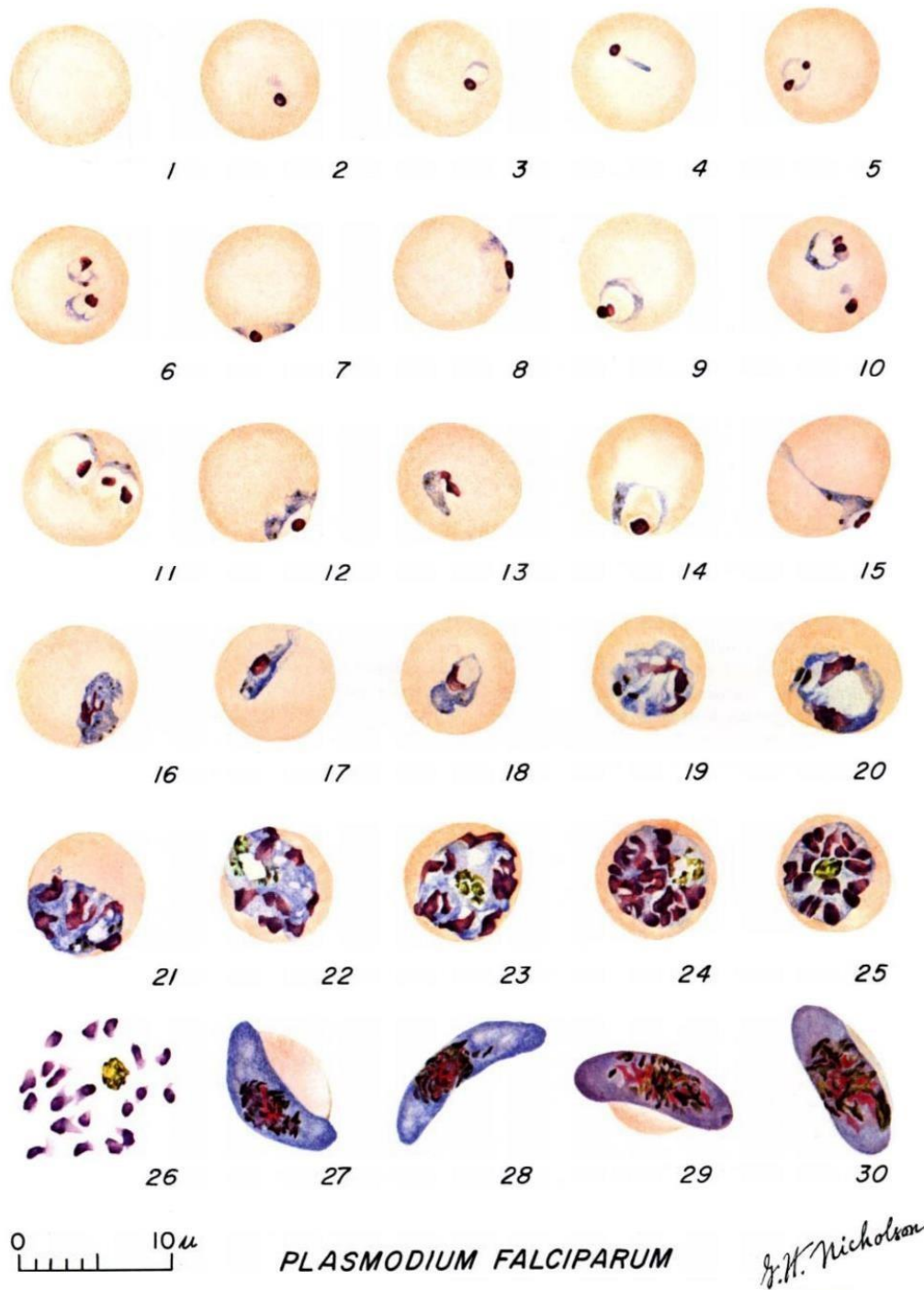
Several molecular methods for *Plasmodium falciparum* detection have been developed over the years. However, these molecular techniques differ either according to sample processing, reaction temperature, reaction to result time, ease of use, limit of detection, cost, required equipment and pre-amplification quantification or detection methods (WHO, 2016). As a result, studies using different molecular methods generate conflicting results, making the comparison of the burden of asymptomatic malaria problematic.

#### **b) Mild / uncomplicated malaria**

Individuals that are immune-compromised may show symptoms of mild malaria usually 10–15 days after infected mosquito bite for *Plasmodium falciparum*. This period may be up to 30 days for *Plasmodium malariae*. The primary clinical symptoms of mild malaria include chills, fever, headache, vomiting, sweating and tiredness. These mild symptoms may be difficult to recognize as malaria. If left untreated within 24 hours, *Plasmodium falciparum* malaria can progress to severe illness, and even death (CDC, 2022; WHO, 2023). The febrile attacks occur every second day with the “tertian” parasites (*P. falciparum*, *P. vivax*, and *P. ovale*) and every third day with the “quartan” parasite (*P. malariae*). The periodic febrile attack is due to rupture of mature schizonts. In *P. vivax* and *P. ovale* malaria, a brood of schizonts matures every 48 hours, so the periodicity of fever is tertian (“tertian malaria”), whereas in *P. malariae* malaria, fever occurs every 72 hours (“quartan malaria”). Residents in endemic countries, usually recognize these symptoms as malaria and go to get drugs to treat themselves without seeking diagnostic confirmation (“presumptive treatment”), (CDC, 2022).

### **c) Severe malaria**

Symptoms of severe malaria especially in children include, severe anaemia, respiratory distress in relation to metabolic acidosis, or cerebral malaria (abnormal behaviour, impairment of consciousness, seizures, coma, or other neurologic abnormalities), haemoglobinuria. Failure of several organs is also frequent among adults. Severe malaria requires medical attention and it has been attributed to be the main cause of preventable childhood death in tropical countries, especially in sub Saharan Africa (CDC, 2022; White, 2022; WHO, 2023).



**Fig. 1:** Normal red cell

**Figs. 2-18:** Trophozoites (among these, **Figs. 2-10** correspond to ring-stage trophozoites)

**Figs. 19-26:** Schizonts (**Fig. 26** is a ruptured schizont)

**Figs. 27, 28:** Mature macrogametocytes (female)

**Figs. 29, 30:** Mature microgametocytes (male).

Illustrations from: Coatney GR, Collins WE, Warren M, Contacos PG. The Primate Malaria. U.S. Department of Health, Education and Welfare, Bethesda, 1971.

**Figure 2.2 *Plasmodium falciparum* blood stages by thin smear microscopy (CDC, 2020)**

## 2.4 Cytokines

Cytokines are highly inducible small secreted proteins released by immune cells (innate and adaptive immune cells) to perform a specific effect such as mediating intercellular signalling, interactions and communications between cells, especially in the nervous and immune system (Rodriguez-Iturbe, Pons and Johnson, 2017). The word cytokine is a general name; other common names given to cytokines include lymphokine (that is cytokines made by lymphocytes), monokine (meaning cytokines made by monocytes), chemokine (referring to cytokines with chemotactic activities), and interleukin (used for cytokines made by one leukocyte and acting on other leukocytes). Cytokines may act on the cells that secrete them (autocrine action), on nearby cells (paracrine action), or in some instances on distant cells, endocrine action (Rodriguez-Iturbe *et al.*, 2017). Cytokines can be grouped into pro-inflammatory cytokines and anti-inflammatory cytokines. Proinflammatory cytokines help to eradicate the foreign organisms that enter the body but causes fever in the process (Krishnegowda *et al.*, 2005; Penha-Gonçalves, 2019).

Proinflammatory cytokine include, IL-6, IL-8, IFN- $\gamma$ , and TNF. Anti-inflammatory cytokines inhibit the activities of proinflammatory cytokines. Antiinflammatory cytokines include transforming growth factor (TGF- $\beta$ ) and IL-10 (Ty *et al.*, 2019; Popa and Popa, 2021). Imbalances between proinflammatory effector responses and anti-inflammatory responses determine to a large extent the severity of diseases. Inflammatory responses in the peripheral and central nervous systems play key roles in the development and persistence of malaria (Rodriguez-Iturbe, *et al.*, 2017).

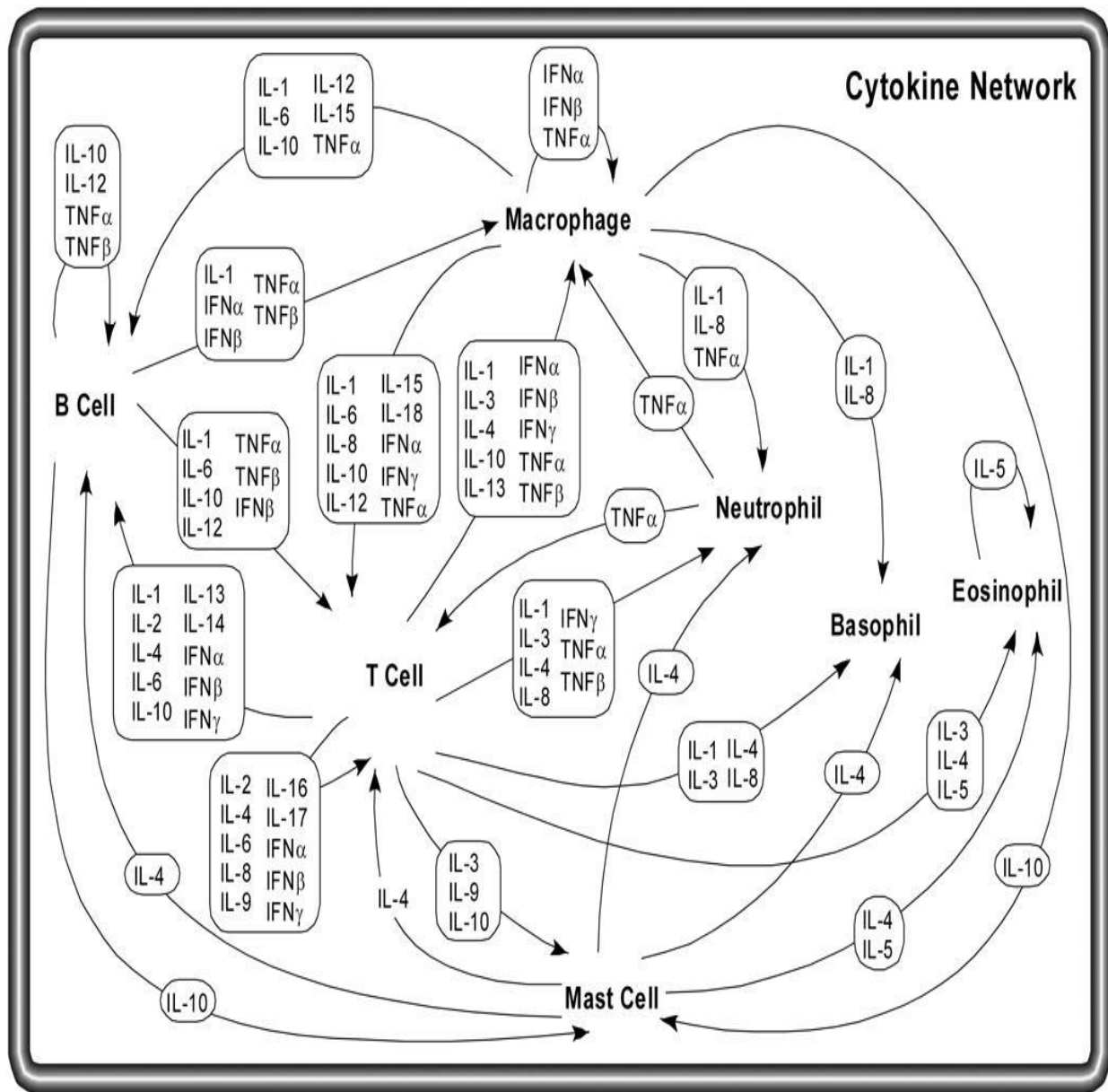
Different cell types can secrete the same cytokine or a single cytokine can act on several different cell types, pleiotropy (Figure 2.3). Cytokines are redundant in their activity, this means that similar functions can be stimulated by different cytokines and they are usually produced in a cascade, as

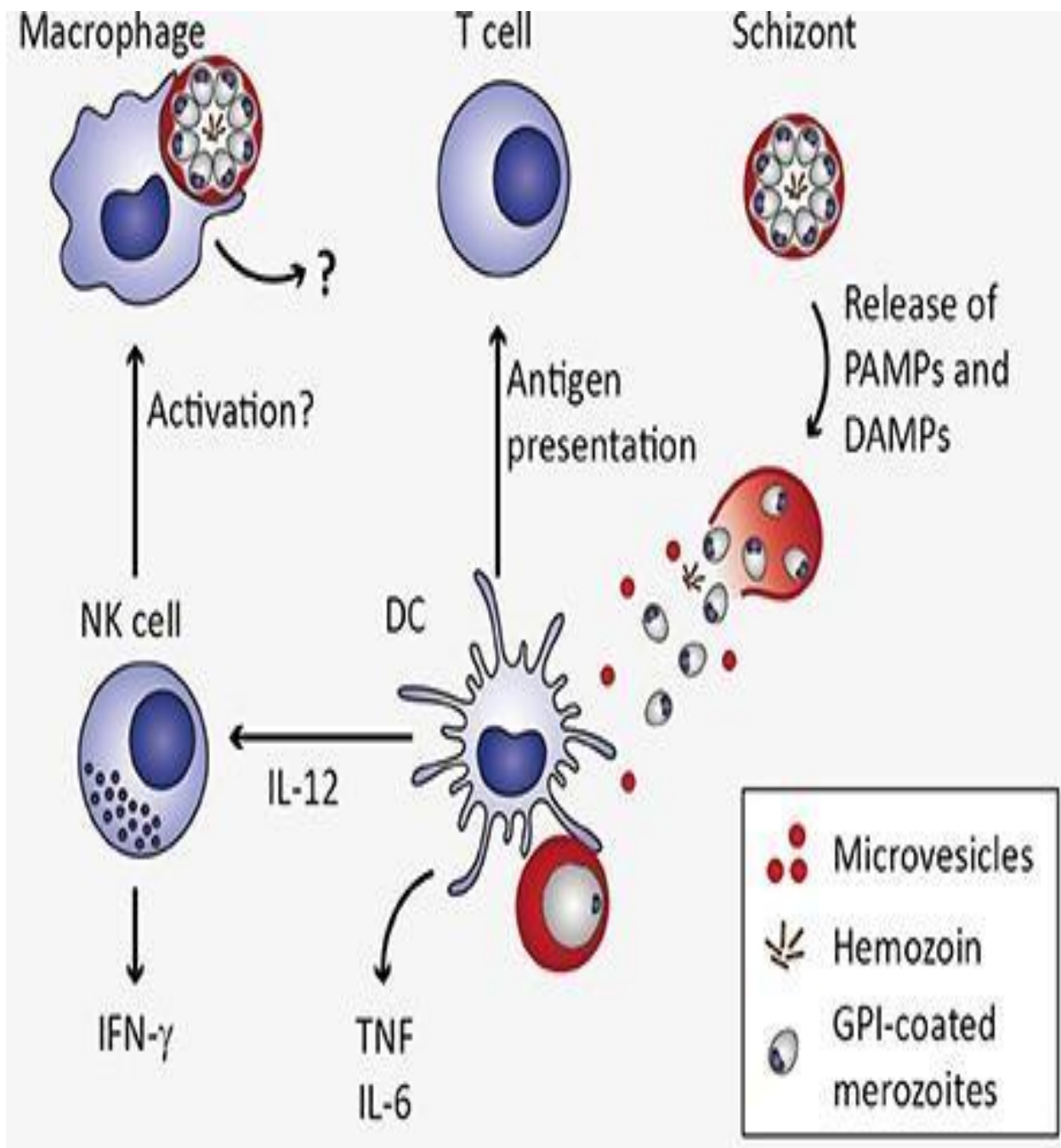
one cytokine stimulates its target cells to make additional cytokines (Figure 2.4). Cytokines can also act synergistically or antagonistically. Some cytokines and their respective functions are given in Table 2.1 (Zhang & Ann, 2007).

**Table 2.1 Selected cytokines and their primary activities**

| Cytokines     | Principal Source                                                                             | Primary Activity                                                                                                                                                                           |
|---------------|----------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| IL-2          | Activated Th1 cells, NK cells                                                                | Proliferation of B cells and activated T cells, NK functions                                                                                                                               |
| IL-4          | Activated T cells                                                                            | B cell proliferation, eosinophil and mast cell growth and function, IgE and class II MHC expression on B cells, inhibition of monokine production                                          |
| IL-10         | Activated Th2 cells, CD8+ T and B cells, macrophages                                         | Inhibits cytokine production, promotes B cell proliferation and antibody production, suppresses cellular immunity, mast cell growth                                                        |
| IFN- $\gamma$ | Natural killer (NK) and activated Th1 cells                                                  | Induces class I MHC on all somatic cells, induces class II MHC on APCs and somatic cells, activates macrophages, neutrophils, NK cells, promotes cell-mediated immunity, antiviral effects |
| TGF- $\beta$  | Monocytes and T cells                                                                        | Chemotaxis, IL-1 synthesis, IgA synthesis, inhibit proliferation, Treg cells to differentiation from naïveCD4+ cells, maintains Treg cells homeostasis                                     |
| TNF- $\alpha$ | Nk cells, mainly by macrophages, monocyte, neutrophils, mast cells, T cells, sensory neurons | Cell death, inflammation, pain, cell proliferation and differentiation                                                                                                                     |

Source: Zhang & An (2007).





**Figure 2.3: Cytokine release** PAMP, Pathogen-associated molecular patterns and DAMPs; danger-associated molecular patterns such as microvesicles, hemozoin, and glycosylphosphatidylinositols (GPI) released during erythrocyte rupture. DC; dendritic cells (Dunst, Kamena and Matuschewski, 2017).

## **2.5 Pathogenesis of fever**

Pyrogens (protein and polysaccharide substances) released from destroyed cells are capable of raising body temperature. Pyrogens are substances that induce fever by acting on the hypothalamic thermoregulatory center to inhibit the heat-sensing neurons while stimulating the cold-sensing neurons. This makes the hypothalamus to increase the body's temperature above the normal range. The result is a condition called fever. TNF $\alpha$  can be a pyrogen by its direct action or by stimulating interleukin 1 secretion (Dinarello, 2004; El-Radhi, 2018). Certain cytokines / chemokines are involved in not only the initiation of pathologic pain but also in the persistence of pathologic pain by directly activating nociceptive sensory neurons. Certain inflammatory cytokines are also involved in nerve-injury / inflammation-induced central sensitization (Zhang & An, 2007).

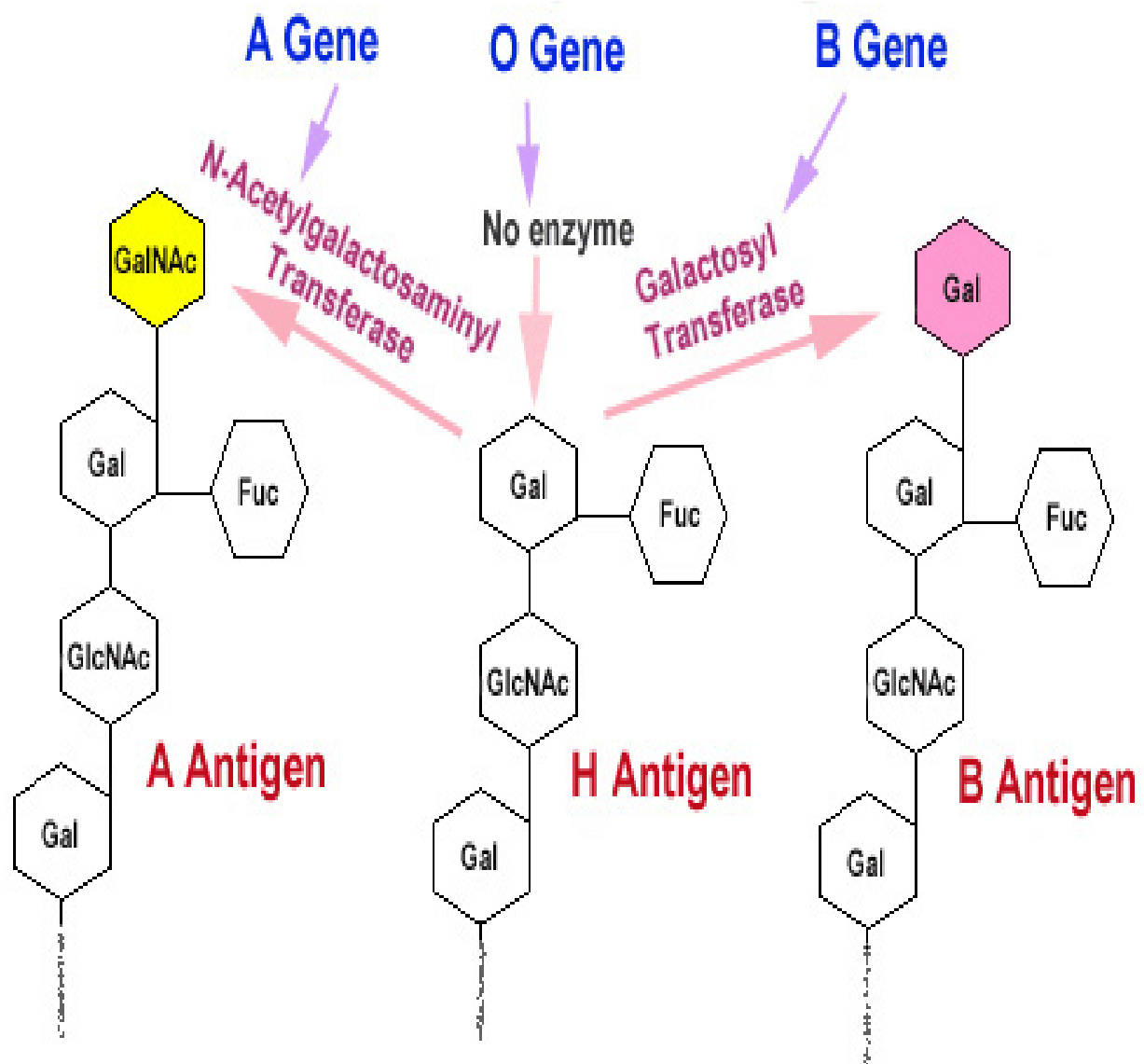
## **2.6 ABO red blood cell antigens**

The antigens of ABO blood group are sugars. They are produced by a series of reactions in which enzymes catalyze the addition of sugar units to an oligosaccharide chain (Cid, Yamamoto and Yamamoto, 2018). The red blood cell ABO antigens consist of oligosaccharide chains. These chains are attached to proteins and lipids in the bilayer of red blood cell membrane. The molecular basis of ABO antigen polymorphism is based on the three allelic forms of ABO locus: A, B and O. The A and B alleles each encode a glycosyltransferase (Figure 2.5) that catalyzes the final step in the synthesis of the A and B antigen, respectively (Dean, 2005; Jajosky *et al.*, 2023). Antigens A and B polymorphism arise from several single nucleotide polymorphisms (SNPs) in the ABO gene, which give rise to A and B transferases that differ from each other by four amino acids. The O allele encodes an inactive glycosyltransferase that leaves the ABO antigen precursor (the H antigen) unmodified. Thus, a person's DNA determines the type of enzymes they have, and,

therefore, the type of sugar antigens that end up on their red blood cells and the type of ABO blood group (Dean, 2005).

## **2.7 Rhesus antigen blood group**

The antigens of the Rhesus (RhD) blood group are proteins. The RhD gene encodes the D antigen, which is a large protein on the red blood cell membrane. Some people have a version of the gene that does not produce D antigen, and therefore the RhD protein is absent from their red blood cells and they will test negative for Rhesus D blood group test (Dean, 2005).



**Figure 2.5 Structure of terminal sugars in the A, B, and O blood antigens.** Gal = Galactose, GlcNAc = N-acetylglucosamine, Fuc = Fucose, and GalNAc = N-acetylgalactosamine (Su, Shin & Zhang, 2014).

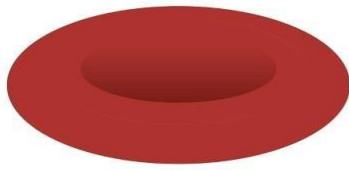
## 2.8 Genotype (Haemoglobin genotype)

Hemoglobin is a protein in the red blood cells that carries oxygen from the lungs to the rest of part of the body and carbon dioxide from body cells back to the lungs. Several different types of hemoglobin exist. Normal types of hemoglobin include: Hemoglobin (Hb) A and Hemoglobin F (HbF). Hb A is the most common type of hemoglobin in healthy adults while Hemoglobin (Hb) F is fetal hemoglobin. Fetal hemoglobin is found in unborn babies and newborns. HbF is replaced by HbA shortly after birth (Marengo-Rowe, 2006; Goncalves, Lima, and Ferreira, 2014).

Abnormal types of hemoglobin include: Hemoglobin (Hb) S, Hb C and Hb E. Hb S is the type of hemoglobin that is found in sickle cell disease. Sickle cell disease is an inherited disorder that causes the body to make stiff, sickle-shaped red blood cells. Healthy red blood cells are flexible so they can move easily through blood vessels. Sickle cells can get stuck in the blood vessels, causing severe and chronic pain, infections, and other complications. Hemoglobin (Hb) C does not carry oxygen well and can cause a mild form of anemia. Hemoglobin (Hb) E usually shows no symptoms but may show mild symptoms of anemia (Goncalves *et al.*, 2014).

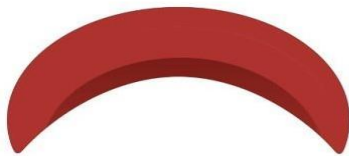
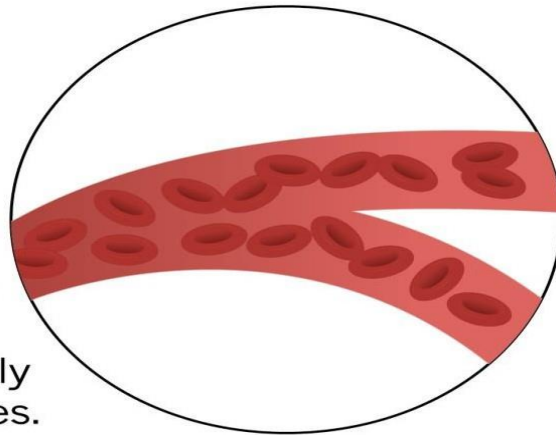
## 2.9 Sickle cell haemoglobin

Sickle haemoglobin (HbS) is the most common and clinically significant abnormal haemoglobin (Piel *et al.* 2013). It is caused by a mutation that replaces glutamic acid at residue 6 in  $\beta$ -globin with valine ( $\beta^6 \text{ Glu} \rightarrow \text{Val}$ ), as a result, linear polymers of deoxygenated HbS are formed (Goncalves *et al.*, 2014). Previous findings have demonstrated that sickle cell trait confers partial protection against lethal *Plasmodium falciparum* malaria (Williams *et al.*, 2005). Another previous study showed that the AS genotype is underrepresented among children with severe malaria (Bougouma *et al.*, 2012).



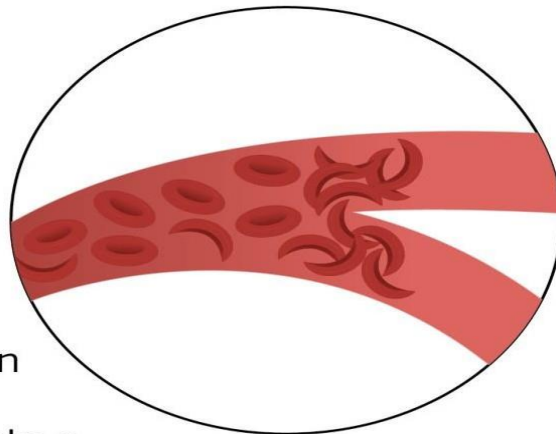
### **NORMAL CELLS**

Healthy red blood cells are flexible, biconcave discs that squeeze easily through small capillaries. They contain multiple haemoglobin molecules that carry oxygen from the lungs to tissues throughout the body. Red blood cells are made in bone marrow and have a lifespan of 120 days.



### **SICKLE CELLS**

Sickle cells contain a defective form of haemoglobin that, when not bound to oxygen, causes them to bend into a crescent shape. The sticky, rigid cells block small capillaries, which results in pain and other serious complications. Sickle cells have a lifespan of 10–20 days.

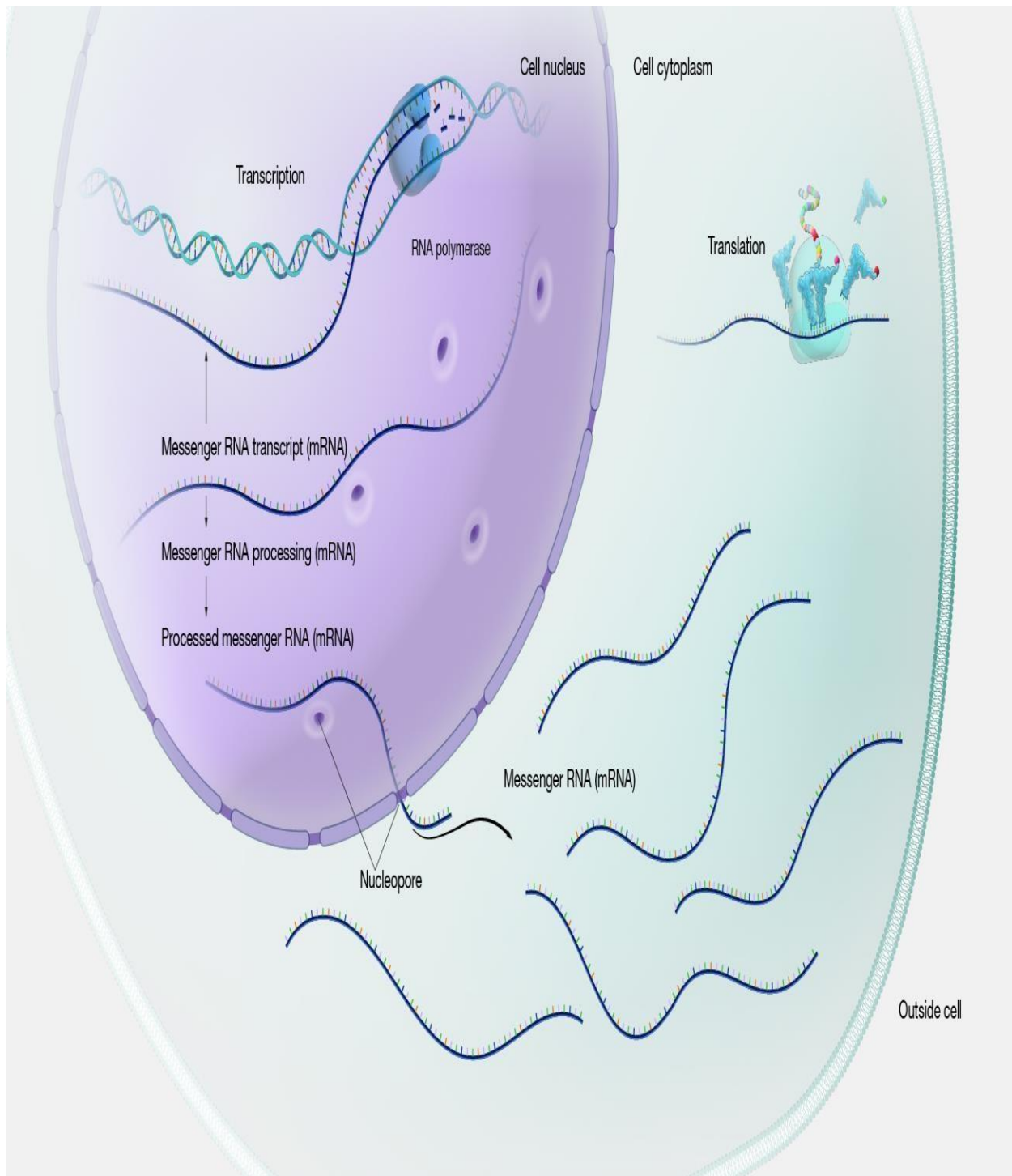


**Figure 2.6 Normal red blood cells and sickle red blood cells (Pleasant, 2014).**

The mechanism by which sickle cell trait confers partial protection against malaria is unclear. However, several mechanisms have been proposed such as aberrant cytoadherence of parasite-infected red blood cell, oxygen-dependent parasite growth inhibition and faster clearance of asymptomatic infection in children with AS. However, despite the strong epidemiologic evidence of selection for AS, the biological mechanism underlying the protection conferred by Hb AS against severe malaria is not completely understood (Archer et al., 2018).

## **2.10 Gene expression**

Gene expression is the process by which the information encoded in a gene is converted into a functional product, majorly through the transcription of RNA molecules that code for proteins or non-coding RNA molecules that serve other functions. Gene expression is synonymous to an “on/off switch” which regulates when and where RNA molecules and proteins are made. Gene expression can also be thought of as a “volume control” to determine how much of RNA and proteins are made. Gene expression is carefully regulated and changes substantially under different conditions and cell types. The RNA and protein products of many genes regulate the expression of other genes. Where, when, and how much a gene is expressed can also be assessed by measuring the functional activity of a gene product or observing a phenotype associated with a gene (Brody, 2023).

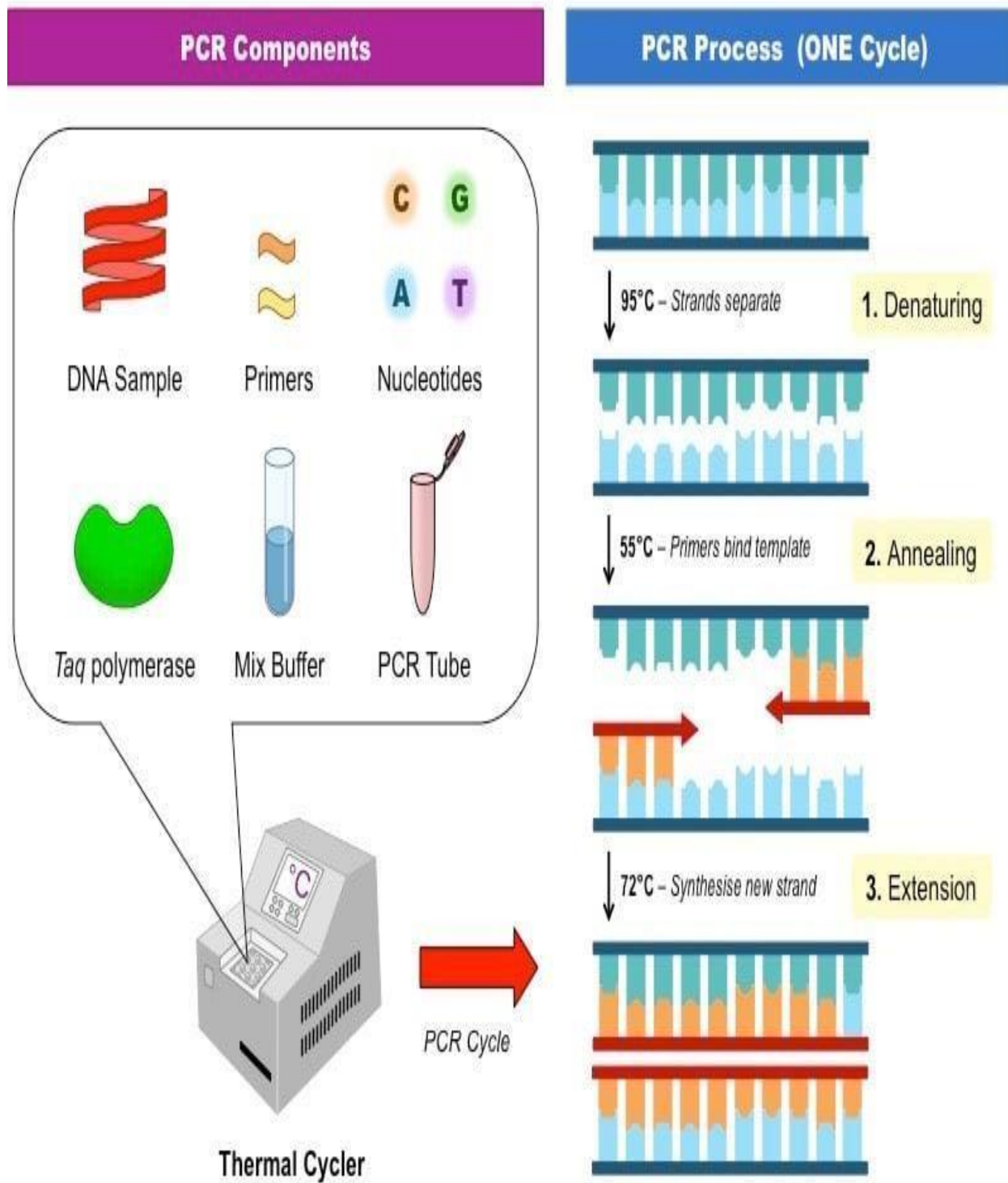


**Figure 2.7 Gene expression (Source: Brody, 2023)**

## **2.11 Gene expression signature**

A gene expression signature is the characteristic expression pattern of a single or combined group of genes in a cell which occurs in response to an altered or unaltered biological process or disease condition (Itadani, Mizuarai & Kotani, 2008). Gene expression signature investigates changes (increases [upregulation] or decreases [downregulation]) in the expression of a particular gene or a set of genes by measuring the abundance of the gene-specific transcript. With current technologies, mRNA expression of every gene in the entire genome can be measured. Real time polymerase chain reaction (qPCR) is one vital technology that is often used to quantify the expression of target genes in relation to reference genes (usually GAPDH, or  $\beta$ -actin). In carrying out real time PCR (qPCR) gene expression analysis, RNA is first extracted and purified, then it is reverse transcribed into cDNA. The cDNA is used as a template in qPCR reactions to detect and quantitate gene expression product (Taft, Pang, Mercer, Dinger, & Mattick, 2010).

Real-time PCR-based gene expression analysis measures the quantity of mRNA transcript transcribed from a specific gene of interest. Monitoring and evaluating the response of a gene of interest to an infection or treatment, or investigating the profiles or patterns of expression of a particular gene or set of genes in response to a given condition provides a molecular basis for an observed phenotypic response (Dermitzakis, 2008).



**Figure 2.8 Polymerase chain reaction (Aryal, 2022).**

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3.1 Materials**

**Materials are listed in appendix iii**

#### **3.2 Methods**

##### **3.2.1 Study location**

The study location was Sagbama LGA, Bayelsa State, Nigeria. Bayelsa is one of the 36 states in Nigeria and one of the six states in the Nigeria's South South geopolitical zone in the core Niger Delta region of Nigeria. It was created from Rivers State in 1991 and it is located on latitude 6.06990 E and longitude 4.77190 N (Oladimeji and Ohwo, 2022). Bayelsa State is one of the largest wetlands in the world and lies between the well-watered oil-palm freshwater bushy swamps and salt-water creeks and mangrove swamps. Over 40% of Nigeria's on-shore crude oil is produced in Bayelsa State. Industries are almost non-existent in the State. The State is majorly a lowland, with small uplands scattered around. The State is situated within the lower delta plain and is characterized by tidal flats, coastal beaches, beach ridge barriers and flood plains. The net features such as cliffs and lagoons are the dominant relief features of the state. The major vegetation in Bayelsa State is mangrove swamp forest with standing body of water all year round. This makes it a suitable ground for mosquito breeding and malaria transmission all year round (Bayelsa State Ministry of Health and FHI 360, 2013; Oyeyemi *et al.*, 2021). The temperature in Bayelsa State ranges from 17<sup>0</sup>C to 40<sup>0</sup>C depending on the weather condition. The climate is tropical (i.e. wet and dry season). Bayelsa State is dominated by indigenes of the Ijaw ethnic group which speak the Izon language. Other minor ethnic groups in Bayelsa are Urhobo and Isoko. The major crafts in Bayelsa include canoe building,

fish net and fish traps making, basket and mat making (Niger Delta Budget Monitoring Group, 2018; Ogbole and Oyelana, 2020). Social amenities are grossly inadequate in the State and a high level of poverty is evident in the state. The exploration and exploitation of oil and the environmental pollution that results, has had an adverse effect on communities in Bayelsa State. The high level of poverty directly results in poor health status of the state. The people of Bayelsa State are mainly rural dwellers and due to the swampy and riverine terrain, have limited access to road transportation, health services, education and other basic social infrastructure. Some communities are totally inaccessible by road. The local population engages largely in subsistence fishing and farming. There are different settlement patterns in the state: hamlets, fishing pots, seasonal farmsteads, villages; all of which are in clusters. The communities are often led by community chiefs, paramount rulers or Community Development Councils (Bayelsa Investment Promotion Agency, 2022; Bayelsa State Ministry of Health and FHI 360, 2013).

Sagbama is one of the eight Local Government Area in Bayelsa State (Figure 3.1), Nigeria. The headquarters of Sagbama LGA is Sagbama town. Part of the area of the LGA lies within the Bayelsa National Forest. It is surrounded by different bodies of Fresh water Habitats. Sagbama LGA is home to the University of Africa Toru – Orua with campuses at Boluo – Orua and Toru – Orua communities. It is also home to the College of Education located in Sagbama town and the Bayelsa Starch factory situated at Ebedebiri community. Sagbama local Government Area occupies the northern end of the State where the River Niger bifurcates into the Nun and Forcados Rivers. The major tribe in Sagbama LGA is the Ijaw (“Izon”) tribe followed by the Urhobo and Isoko tribes. The traditional occupations of the people in Sagbama LGA include farming, fishing, lumbering, hunting, and carving. Sagbama LGA has an area of 945 km<sup>2</sup> and a population of 187,146 as at the 2006 census and consist of communities like Ofoni (the home land of the

present Deputy Governor of Bayelsa State), and Toru – Orua (home land of the past Governor of Bayelsa State), (Niger Delta Budget Monitoring Group, 2022; Ogbole and Oyelana, 2020).



Figure 3.1 Map of Bayelsa State showing the study area (colour coded yellow), (Niger Delta Budget Monitoring Group, 2022).

### **3.2.2 Study design**

A community based cross sectional study design was employed using multistage random sampling. Stage one: random selection of four communities in Sagbama LGA. Stage two: random selection of households from each community. Stage three: from each household, random selection of study subjects. Probability-proportionate-to-size sampling method and stratification by sex was utilized in the selection process (Ogbole and Fatusi; 2020; Adediran, Oluleye, Ayorinde, Ugalahi, & Mpyet, 2022).

### **3.2.3 Study group**

The study population was grouped into four;

Group I: Uninfected,

Group II: Asymptomatic malaria;

Group III: Mild malaria;

Group IV: Severe malaria

Each group was further stratified based on ABO/Rhesus factor blood grouping and haemoglobin genotype variant. The standard precautions protocol developed by the US Centers for Disease Control and Prevention was followed throughout sample collection, handling, biochemical and molecular assay in order to prevent infection when working with human blood samples (Zhao *et al.*, 2017; Ouma *et al.*, 2020; CDC, 2022).

### **3.2.4 Sample size determination**

Sample size was calculated using the Cochran formula below (Charan and Biswas, 2013):

$$n_0 = \frac{Z^2 pq}{e^2}$$

Where:

- $n_0$  is the sample size
- $e$  is the desired level of precision (i.e. the margin of error), 0.05
- $p$  is the (estimated) proportion of the population having the attribute in *Plasmodium falciparum* infection. 85% (0.85) of the population in Bayelsa State was assumed to have the *Plasmodium falciparum* infection based on a previous pilot study.
- $q$  is  $1 - p$  which is 0.15
- The  $z$ -value was found in a  $Z$  table, for  $z$ -value at 95% confidence interval was 1.96  $((1.96)^2 (0.85) (0.15)) / (0.05)^2 = 195.92 = 196$ . Assuming a nonresponse rate of 5%, the sample size was increased to 206, thus a random sample of 206 individuals in the target population was selected. Selection of study participants was carried out after repeated meetings and discussions about the objectives of the research and its protocols with the heads of each selected community.

### 3.2.5 Ethical approval and informed consent

Approval for the study was sought by the Ethics Committee of Bayelsa State Primary Health Care Authority in Sagbama Local Government Area. Informed consent was obtained from each participant prior to commencement of the study.

### **3.2.6 Inclusion and exclusion criteria (indicators for each group)**

Individuals with illness other than malaria or individuals currently on antimalarial medications or have taken antimalarial medication two weeks prior to sample collection were excluded from the study. Individuals below 18 years of age were also excluded from the study, while individuals equal or above age 18 years were included in the study. Smokers and heavy alcohol consumers were also excluded from the study (CDC, 2022). Pregnant women were excluded from the study. Adults negative for *Plasmodium falciparum* infection using high resolution melting analysis were included in group 1 (uninfected). Adults positive for *Plasmodium falciparum* infection but with no febrile illness were included in group 2 (asymptomatic malaria). Adults positive for *Plasmodium falciparum* infection and with febrile illness were included in group 3 (mild malaria). Adults positive for *Plasmodium falciparum* infection and with febrile illness as well as with severe anaemia (haemoglobin concentration below 8 g/dl) were included in group 4 (severe malaria) (NMEP, NPC and ICF. 2022). In order to control for other known illnesses that can cause inflammation, participants with high blood pressure (Patrick, Van Beusecum, and Kirabo, 2021) obesity (Khanna, Khanna, Khanna, Kahar, Patel, 2022), diabetes (Tsalamandris *et al.*, 2019) and any other inflammatory disease were excluded from the study.

### **3.2.7 Evaluation of baseline biochemical and clinical characteristics**

The following baseline biochemical and clinical characteristics of the study population were evaluated: hyperglycemia (fasting blood sugar level  $\geq 100$  mg/dl; Matuschik *et al.*, 2022), overweight and obesity (BMI  $\geq 24.9$  kg/m<sup>2</sup>; Ellulu, Patimah, Khaza'ai, Rahmat, & Abed, 2017), hypertension (systolic blood pressure  $> 120$  mmHg and diastolic blood pressure  $> 80$  mmHg; Patrick *et al.*, 2021) anaemia / low haemoglobin concentration (NMEP, 2022).

### **3.2.8 Sociodemographic variables**

Sociodemographic variables were measured using a semi-structured questionnaire (Ogbole and Fatusi, 2020).

### **3.2.9 Temperature measurement**

Axillary method (under the armpit) was used for temperature measurement following the manufacturer's protocol for Royal care diagnostics digital thermometer (Royal Care Diagnostic, Indiral colony, Chennai, India). Briefly, the tip of the thermometer was placed in the centre of the armpit. The arm of the subject was tucked snugly (closely) against their body and the thermometer was left in that position for about 1 minute, until a “beep” was heard. The thermometer was removed and the temperature was read. Readings between 35 – 37 °Celsius were regarded as normal, while readings above 37.4 °C were regarded as febrile malaria. An armpit temperature is generally 1 degree lower than an oral temperature (Marui, Misawa, Tanaka, and Nagashima, 2017).

### **3.2.10 Blood sample collection**

A total of 206 blood samples was randomly collected from adults living in Bayelsa State, Nigeria. Five millilitre of whole blood was drawn by venipuncture from each participant and 3 ml was transferred into a Vacutainer® EDTA tube for microscopy, ABO blood grouping and haemoglobin genotyping (WHO, 2016), while the remaining 2 ml was transferred into another Vacutainer® EDTA tube for PCR analysis. Blood samples for PCR analysis were transported at 4°C to the laboratory (CDC, 2016), while blood samples for malaria microscopy, ABO blood grouping and haemoglobin genotyping were transported immediately (within 1 h), without cooling or refrigeration, to the laboratory and were not kept for more than 4 h before preparing thick and thin blood films (WHO, 2016).

### 3.2.11 Haemoglobin genotype test

Hemoglobin electrophoresis on cellulose acetate paper following standard methods was used for haemoglobin genotype test (Khosa, Usman, Moinuddin, Mehmood, & Qamar, 2015).

### 3.2.12 Erythrocyte ABO/Rhesus antigen phenotyping

The principle of erythrocyte ABO/Rhesus antigen phenotyping was based on antigen-antibody agglutination test using the tile method and commercially available ABO and Rhesus factor blood grouping test kit (Alemu and Mama 2016).

### 3.2.13 Detection of *Plasmodium falciparum* by microscopy method

Thick and thin blood films were made on labelled clean glass slide as recommended by World Health Organization (WHO, 2016). The thin films were fixed with methanol. Both thick and thin films were stained with 3% Giemsa stain (pH 7.0) and left for 30 minutes. Thick and thin blood films stained with Giemsa stain were read by two experienced microscopists using an oil immersion lens (100×) and following the World Health Organization malaria microscopy standard operating procedures. Malaria parasites were counted against 200 white blood cells. Thin films for positive slides were further examined for parasite species identification. The Thick films were used to determine parasite density. The parasite density of each *Plasmodium falciparum* positive thick film was determined with an assumed white blood cells count of 8000 white blood cells/μL using the formula below (WHO, 2016).

Parasite density (Parasites/μL blood) =

$$\frac{\text{Number of asexual parasites counted} \times 8000 \text{ white blood cells}/\mu\text{L}}{\text{Number of white blood cells counted}}$$

Parasitemia was classified as low (< 1000 parasites/ $\mu$ l blood = +), moderate (1000 – 4999 parasites/ $\mu$ l blood = ++), high (5000 – 99,999 parasites/ $\mu$ l blood = +++), and hyperparasitaemia ( $\geq$  100,000 parasites/ $\mu$ l blood = ++++) (Chipwaza and Sumaye, 2020).

### **3.2.14 DNA extraction and purification**

gSYNC<sup>TM</sup> extraction kit (Geneaid, Biotech. Ltd, New Taipei, Taiwan) was used for genomic DNA extraction following the manufacturer's protocol. Briefly, whole blood (200  $\mu$ l) was mixed with proteinase K (20  $\mu$ l), and incubated at 60°C for 5 min. GSB buffer (200  $\mu$ l) was added, followed by incubation at 60°C for 5 minutes and vigorous mixing of absolute ethanol (200  $\mu$ l) with the sample lysate. Centrifugation at 14,000 $\times g$  was carried out to separate / extract the precipitated DNA from the mixture. Extracted DNA was purified in a two-step process. Firstly, W1 buffer (400  $\mu$ l) was added to the extracted DNA followed by centrifugation and decantation. Secondly, a mixture of wash buffer and absolute ethanol (600  $\mu$ l) was added to the extracted DNA followed by centrifugation and decantation. The column matrix was dried by centrifugation and the purified DNA was eluted with a 100  $\mu$ l of pre-heated elution buffer.

### **3.2.15 Agarose gel electrophoresis of extracted and purified DNA**

Given that a degraded DNA might result in amplification failure, agarose gel electrophoresis was carried out to determine if the extracted genomic DNA was degraded. Exactly 0.8 g of agarose powder was dissolved in a 100 ml of 1X Tris Acetate EDTA (TAE) buffer (pH 8.0), heated up on a microwave for few minutes until bubbles were seen in the boiling gel and the gel turned from a milky appearance to a crystal clear colourless solution. The gel was made to cool under running water. A gel casting tray previously sealed at all ends by pipetting a little quantity of the gel around

all the edges was positioned and 5  $\mu$ l of 10 mg/ml Ethidium Bromide was stirred into the cooled gel (care was taken to avoid bubbles while stirring). The agarose solution was poured into the gel casting tray and combs were inserted into the gel. The gel was allowed to solidify / set on the tray and the combs were removed to create a well in the gel where the samples will be loaded. TAE buffer was poured over the gel up to 3 mm depth. Ten microliter from 100  $\mu$ l eluates of purified genomic DNA was mixed with 6x blue DNA loading dye (ratio 5:1) and loaded into the wells along with a 10 kilo base molecular marker (DNA ladder). Electrophoresis was carried out at 50 volts for one hour, after which the buffer was poured out and the gel was transferred into a UV transilluminator for visualization of DNA integrity (Zhao *et al.*, 2017).

Nanodrop spectrophotometer was used to measure absorbance. The concentration of genomic DNA was extrapolated from absorbance at 260 nm, on the basis that a 50  $\mu$ g/ml solution of pure double stranded genomic DNA has an absorbance of 1 at 260 nm (Gong & Li, 2014).

Purified genomic DNA Concentration ( $\mu$ g/ml) = Absorbance at 260 nm – Absorbance at 320 nm (turbidity absorbance)  $\times$  dilution factor  $\times$  50  $\mu$ g/ml  $\times$  path length of cuvette.

Dilution factor = 1

Path length = 1 cm.

The ratio absorbance at 260 nm / absorbance at 280 nm was used to determine genomic DNA purity (Gong & Li, 2014).

### **3.2.16 Detection of *Plasmodium falciparum* infection by Nested-genomic PCR method**

**First nested PCR:** A pair of primers (forward 5'TCAAAGATTAAGCCATGCAAGTGA3' and reverse 5'CCTGTTGTTGCCTTAAACTTC3') previously used pair by Zhao *et al.*, 2017 was

employed to amplify a target sequence located on the 18S small subunit ribosomal RNA (SSU rRNA) gene of *Plasmodium* genus. 5 x HOT FIREPol® EvaGreen® qPCR Mix Plus (Solis BioDyne, Estonia) was used for nested-genomic PCR following the manufacturer's protocol. Briefly, the 20  $\mu$ l reaction mix contain 4  $\mu$ l of DNA template, 4  $\mu$ l 5 x HOT FIREPol® EvaGreen® qPCR Mix Plus, 0.1  $\mu$ l primer mix and 11  $\mu$ l nuclease-free water. PCR was carried out in a ProFlex™ 96-well PCR Thermal Cycler Systems (Applied Biosystems, Foster City, California, USA) under the following conditions: an initial activation at 95°C for 15 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 65°C for 20 s, and elongation at 72°C for 20 s. Water replaced DNA template for negative controls and PCR was carried out in duplicates (Zhao *et al.*, 2017; Zhou *et al.*, 2014).

**Second nested PCR:** Species specific nested genomic PCR was performed under the same conditions and reaction mixture as the first PCR, except that the DNA templates was the product of the first PCR and a species-specific pair of primers for *Plasmodium falciparum* previously used by Zhao *et al.*, (2017) was used.

Forward primer: 5`TTAAACTGGTTTGGGAAAACCAAATATATT3` and reverse primer: 5`ACACAATGAACTCAATCATGACTACCCGTC3` were employed to target the 18S small subunit (SSU) rRNA gene of *Plasmodium falciparum*. Positive controls (previously established *P. falciparum* positive samples using microscopy) was included for confirmatory results and negative controls (water instead of DNA template) was also included in order to certify that the reaction mixture have not become contamination while setting up the PCR run. The reaction was carried out in duplicates (Zhao *et al.*, 2017; Zhou *et al.*, 2014).

### 3.2.17 Agarose gel electrophoresis of nested genomic PCR products

The nested PCR products was run on 2.0% agarose gel alongside a 100 base pair molecular marker and visualized under a UV transilluminator. The expected molecular size of *Plasmodium falciparum* PCR fragment was 205 base pairs. Matching positions between sample bands and positive control band was used to indicate a positive malaria parasite case (Zhao *et al.*, 2017; Hassanpour *et al.*, 2016; Zhou *et al.*, 2014).

### 3.2.18 Detection of *P. falciparum* infection using high resolution melting analysis

HOT FIREPol® EvaGreen® HRM Mix-ROX (SolisBiodyne, Tartu Estonia) was used for high resolution melting analysis (HRM-analysis) following the manufacturer's protocol. Briefly, the reaction mixture contained 4  $\mu$ l genomic DNA template, 4  $\mu$ l HOT FIREPol® EvaGreen® HRM Mix-ROX, 11  $\mu$ l nuclease-free water and 1  $\mu$ l primer mix. A pair of primer (forward: 5'TTAAACTGGTTTGGGAAAACCAAATATATT3' and reverse 5'ACACAATGAACTCAATCATGACTCCCGTC 3') previously used by Zhao *et al.*, 2017 was employed to amplify the 18S SSU rRNA gene of *Plasmodium falciparum*. PCR was carried out in a StepOnePlus™ Real-Time PCR Thermal Cycler Systems (Applied Biosystem, Foster City, USA) under the following conditions: an initial activation at 95 °C for 15 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 65 °C for 20 s, extension at 72 °C for 20 s and a post-amplification high resolution melting curve was prepared from 65 °C to 90 °C for 10 mins using a default setting. Positive controls and negative controls were included in the assay and the reaction was carried out in duplicates. Samples with melting temperatures ( $T_m$ ) aligning with the  $T_m$  of positive control (with a  $T_m$  shift interval of  $\pm 0.4$  °C) and with fluorescent signal within 30 cycles ( $C_t$ ) were considered positive for malaria parasite. HRM curves were generated using high resolution melt

software v 3.0.1 (Applied Biosystems, step one plus), (Chua, Lim, Ng, Lee, Lim, Lau, 2015; Ouma *et al.*, 2020).

### 3.2.19 Calculation of sensitivity and specificity (Van Honacker *et al.*, 2022).

#### Definitions:

Positive cases: *Plasmodium falciparum* present in the blood (asymptomatic, mild and severe malaria).

Negative cases: *Plasmodium falciparum* absent in the blood

True positive (TP) = the number of cases correctly identified as patient

False positive (FP) = the number of cases incorrectly identified as patient

True negative (TN) = the number of cases correctly identified as healthy

False negative (FN) = the number of cases incorrectly identified as healthy

Sensitivity: Sensitivity refers to a test's ability to designate an individual with disease as positive.

Mathematically, sensitivity can be stated as:

$$\text{Sensitivity (\%)} = \frac{\text{TP}}{\text{TP} + \text{FN}}$$

Specificity: The specificity of a test is its ability to designate an individual who does not have a disease as negative.

Mathematically, this can be stated as:

$$\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}}$$

### **3.2.20 Extraction of total RNA from human peripheral blood mononuclear cells**

Total RNA was extracted from whole blood samples within 24 hours of collection following the manufacturer's instruction for total RNA minikit (Geneaid, New Taipei city, Taiwan). Briefly, to a sterile 1.5 micro-centrifuge tube, RBC lyses buffer (1 ml) and whole human blood (300  $\mu$ l) were added and mixed by inversion. The tube was incubated on ice for 10 minutes (vortexed twice during incubation) and was centrifuged for 5 minutes at 3000xg and the supernatant was removed completely. RB buffer and  $\beta$ -mecarptoethanol (400  $\mu$ l) was added to the pellet and the pellet was re-suspended by pipetting, and then incubated at room temperature for 5 minutes. The reaction mixture was shaken vigorously. A mixture of de-ionized distilled water (ddH<sub>2</sub>O RNase and DNasefree) and 70% ethanol (400  $\mu$ l) was prepared and kept aside. RB column was placed in a 2 ml collection tube and 500  $\mu$ l of mixture was transferred into the RB column and was centrifuged at 16000xg for 1 minute then the flow through was discarded. The remaining mixture was transferred to the same RB column and then centrifuged for another 1 minute at 16000xg. The flow-through was discarded and the RB column was placed in a new 2 ml collection tube. W1 buffer (400  $\mu$ l) was added into the RB column and was centrifuged at 16000xg for 30 seconds. The flow through was discarded then the RB column was placed back into the 2 ml collection tube. Into the RB column, wash buffer (600  $\mu$ l) was added (with ethanol) and was then centrifuged at 16000xg for 30 seconds; then the flow-through was discarded (this step was repeated). RB column was placed back into the 2 ml collection tube and was centrifuged at 16,000xg for 3 minutes to dry the column matrix. The dried RB column was placed in a clean 1.5 micro-centrifuge tube and RNase-free water (50  $\mu$ l) was added into the center of the column matrix. The column matrix was left to stand for 1 minute to ensure that the RNase water was well absorbed. It was then centrifuged at 16,000xg for a minute in order to elute the purified RNA.

### **3.2.21 Agarose gel electrophoresis of extracted total RNA**

A total of 100 ml of 1X Tris Acetate EDTA buffer (TAE) was measured into a conical flask and 0.7% of agarose powder was added and mixed by swirling. The mixture was boiled using a microwave and was allowed to clear while boiling. The gel was allowed to cool by leaving at room temperature for some few minutes until it was no longer hot to touch (the gel was not allowed to solidify while cooling). Ethidium Bromide (10 mg/ml, 5 µl) was added to the gel before pouring. The base of the gel tray was sealed with little quantity of gel dispensed from a pipette. The gel was poured into the tray and combs were inserted. The gel was allowed for approximately twenty minutes to solidify. The RNA sample was be mixed with 6x blue RNA loading dye (in a ratio of 5:1 that is 10 µl of RNA sample to 2 µl of loading dye). The mixture of sample and loading dye (10 µl) was loaded into the gel wells created by the comb (one sample per well). The electrophoresis gel was run at 100 volts for 1 hour. After which the gel was transferred unto a UV transilluminator, visualized and documented (Zhao *et al.*, 2017).

### **3.2.22 cDNA synthesis**

The extracted RNA was reverse transcribed into complementary DNA (cDNA) using Fire Script cDNA synthesis kit (Solis BioDyne, Tartu Estonia) following the manufacturer`s instruction. Briefly, 5 µl of Solisbiodyne Firescript cDNA synthesis kit (containing 0.5 µl of Oligo(dT) primer (G), 0.5 µl random hexamer primer (H), 0.5 µl dNTP mix (I), 1 µl reverse transcriptase (RT), 2 µl of 10x reaction buffer (J), and 0.5 µl riboGrip RNase inhibitor) was mixed with 15 µl of RNA template in a 0.2 ml PCR tube. Spin, vortex, spin of all reagents and RNA template was carried out before dispensing. After dispensing, spin, vortex, spin of the reaction mixture was carried out

before loading unto the thermal cycler. The conventional cDNA PCR run; primer annealing at 25°C for 10, reverse transcription at 48°C for 15 min, inactivation at 85°C for 5 min and hold at 4°C was run for cDNA synthesis.

### **3.2.23 Real time PCR quantification of pro- and anti-inflammatory gene expression**

The gene expression level of pro-inflammatory and anti-inflammatory cytokines was measured using real time PCR method. Each target and reference gene were amplified using the pair of primers given below. 5x HOT FIREPol Evagreen qPCR Supermix (Solis BioDyne, Tartu, Estonia) was used for gene expression analysis following the manufacturer's protocol. The reaction was carried out in an Applied Biosystem StepOne Plus real-time polymerase chain reaction system (Thermo Fisher Scientific, California, USA). Custom designed primers synthesized by Genewiz (Genewiz, South Plainfield, New Jersey), were used to amplify mRNA expression of the genes of interest. Amplification cycle (Ct) was normalized by the mRNA expression of endogenous control ( $\beta$ -actin). The cycling protocol were: Initial activation at 95°C for 12 mins, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 53°C for 30 s, elongation at 72°C for 30 s. The reaction mixture contained 4  $\mu$ l of PCR supermix, 1  $\mu$ l primer mix, 4  $\mu$ l of cDNA template, and 11  $\mu$ l of water. The assay was run in duplicates using water as negative control (negative control was used to ascertain that the setup was free from contamination). Fold change in gene expression was calculated and all data was reported as normalized fold change in gene expression relative to control (uninfected group). Gene expression was quantified using StepOne™ Software v2.3 (Applied Biosystem StepOnePlus™ Real-Time PCR, Foster City, USA). Relative quantification of gene expression was calculated using  $2^{-\Delta\Delta CT}$  method (Livak and Schmittgen, 2008).

### **Primer sequences for the selected genes of interest for the present study**

TNF- $\alpha$  Forward: 5`-CTGAACTTCGGGGTGATCG-3`

Reverse: 5`-GCT TGG TGG TTT GCT ACG AC-3

IFN- $\gamma$  F: 5'-ATTCGGTAACTGACTTGAATGTCC-3'

R: 5'-CTCTTCGACCTCGAAACAGC-3'

IL-10 F: 5'-5'-GGCCGTGGAGCAGGT-3'

R: 5'-CACTCATGGCTTTGTAGATGCC-3'

$\beta$ -actin F: 5`-ACA ACC TTC TTG CAG CTC CTC-3`

R: 5-TGACCCATACCCACCATCAC-3

#### **3.2.24 Statistical analysis**

SPSS software version 24 (SPSS Inc., Chicago, IL, USA) was used for data analysis and results were presented as percentages and as mean  $\pm$  standard deviation. Chi square test and multinomial logistic regression were used to test for significant association between categorical variables. Independent t-tests and one way-ANOVA were used to test for significant difference between means. McNemar's exact test was used for pair-wise comparison among the proportions of positive detections for the different detection methods and significant level was set at  $p < 0.05$  (Witt *et al.*, 2019).

## **CHAPTER FOUR**

### **RESULTS AND DISCUSSION**

#### **4.1 Results**

##### **4.1.1 Sociodemographic, biochemical and clinical phenotypes of the study population in Sagbama LGA, Bayelsa State, Nigeria**

The sociodemographic phenotypes of the study population in Sagbama, Bayelsa State, Nigeria are presented in Table 4.1, while the biochemical and clinical phenotypes are presented in Table 4.2. As presented in Table 4.1, more males than females participated in the study. Age group 26 – 35 years had the highest number of study participants. More than half of the study participants were married. Fishing / farming was the major occupation of the study population. Majority of the study population had below tertiary education. As shown in Table 4.2, individuals with high blood pressure, obesity and diabetes (major inflammation inducing confounding variables other than malaria) were excluded from the present study. The randomly selected study participants had the normal range of these variables as shown in Table 4.2.

**Table 4.1: Sociodemographic phenotypes of adults in Sagbama LGA, Bayelsa State, Nigeria**

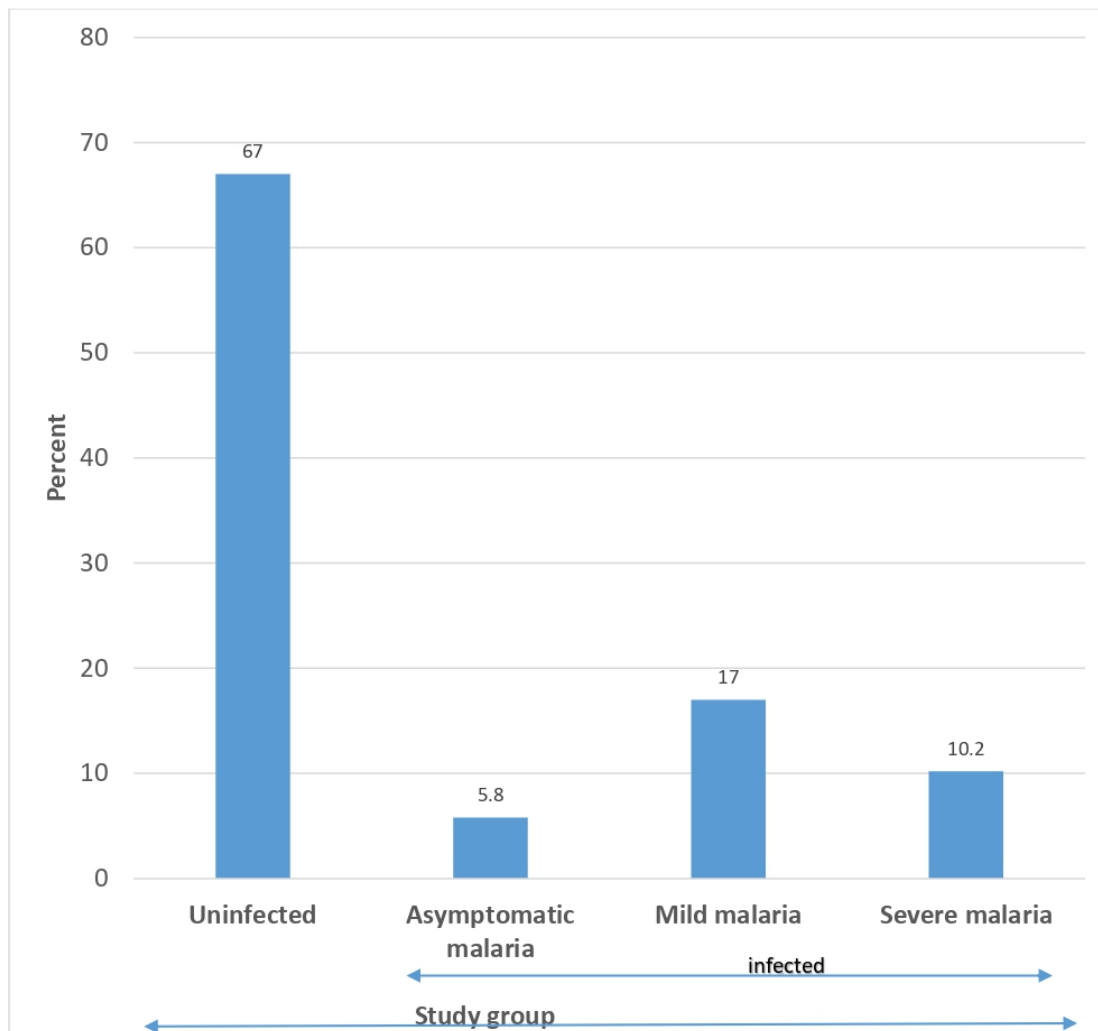
| <b>Sociodemographic</b>   | <b>Male</b>        | <b>Females</b>     | <b>Total</b>        |
|---------------------------|--------------------|--------------------|---------------------|
| <b>Phenotypes</b>         | <b>N (%)</b>       | <b>N (%)</b>       | <b>N (%)</b>        |
|                           | <b>91 (44.17%)</b> | <b>115 (55.83)</b> | <b>206 (100.00)</b> |
| <b>Age (years)</b>        |                    |                    |                     |
| 18 – 25                   | 22 (10.68)         | 27 (13.11)         | 49 (23.79)          |
| 26 – 35                   | 27 (13.11)         | 32 (15.53)         | 59 (28.64)          |
| 36 – 45                   | 21 (10.19)         | 28 (13.60)         | 49 (23.79)          |
| 46 – 55                   | 9 (4.37)           | 16 (7.77)          | 25 (12.14)          |
| ≥56                       | 12 (5.83)          | 12 (5.83)          | 24 (11.65)          |
| <b>Marital status</b>     |                    |                    |                     |
| Single                    | 34 (16.50)         | 51 (24.76)         | 85 (41.26)          |
| Married                   | 57 (27.67)         | 64 (31.07)         | 121 (58.74)         |
| <b>Occupation</b>         |                    |                    |                     |
| Farming / fishing         | 33 (16.02)         | 81 (39.32)         | 114 (55.34)         |
| Petty trading             | 30 (14.56)         | 18 (8.74)          | 48 (23.30)          |
| Civil servants            | 17 (8.25)          | 10 (4.85)          | 27 (13.11)          |
| Craftsmanship             | 11 (5.34)          | 6 (2.91)           | 17 (8.25)           |
| <b>Level of education</b> |                    |                    |                     |
| None                      | 22 (10.68)         | 36 (17.48)         | 58 (28.16)          |
| Primary                   | 27 (13.11)         | 15 (7.28)          | 42 (20.39)          |
| Secondary                 | 11 (5.34)          | 54 (26.21)         | 65 (31.55)          |
| Tertiary                  | 31 (15.05)         | 10 (4.85)          | 41 (19.90)          |

**Table 4.2: Biochemical and clinical phenotypes of the selected study population in Sagbama LGA**

| <b>Characteristics</b>                      | <b>Mean <math>\pm</math> Standard error of mean</b> |
|---------------------------------------------|-----------------------------------------------------|
| Body mass index (kg/m <sup>2</sup> )        | 22.06 $\pm$ 2.08                                    |
| Fasting blood glucose concentration (mg/dl) | 89.00 $\pm$ 9.98                                    |
| Systolic blood pressure (mmHg)              | 101.00 $\pm$ 18.00                                  |
| Diastolic blood pressure (mmHg)             | 69.00 $\pm$ 8                                       |

#### **4.1.2. Malaria infection prevalence among adults in Sagbama LGA, Bayelsa State, Nigeria based on microscopy**

The prevalence of malaria parasite infection and density by microscopy in Sagbama LGA, Bayelsa State, Nigeria are presented in Figure 4.1 and Table 4.3 respectively. As shown in Figure 4.1, the prevalence of malaria parasite infection by microscopy was 30% (asymptomatic; 5.8%; mild; 17% and severe; 10.2%). As presented in Table 4.3 a very high malaria parasite density was found in the blood of adults in Sagbama LGA. A large proportion of individuals with high parasite density were asymptomatic. Significant difference ( $P < 0.05$ ) in parasitemia level was found between asymptomatic, mild and severe malaria groups ( $p < 0.05$ ). However, no significant difference ( $P > 0.05$ ) in malaria parasite density was found between males and females as shown in Table 4.3.



**Figure 4.1: Malaria infection prevalence among adults in Sagbama LGA, Bayelsa State, Nigeria by microscopy**

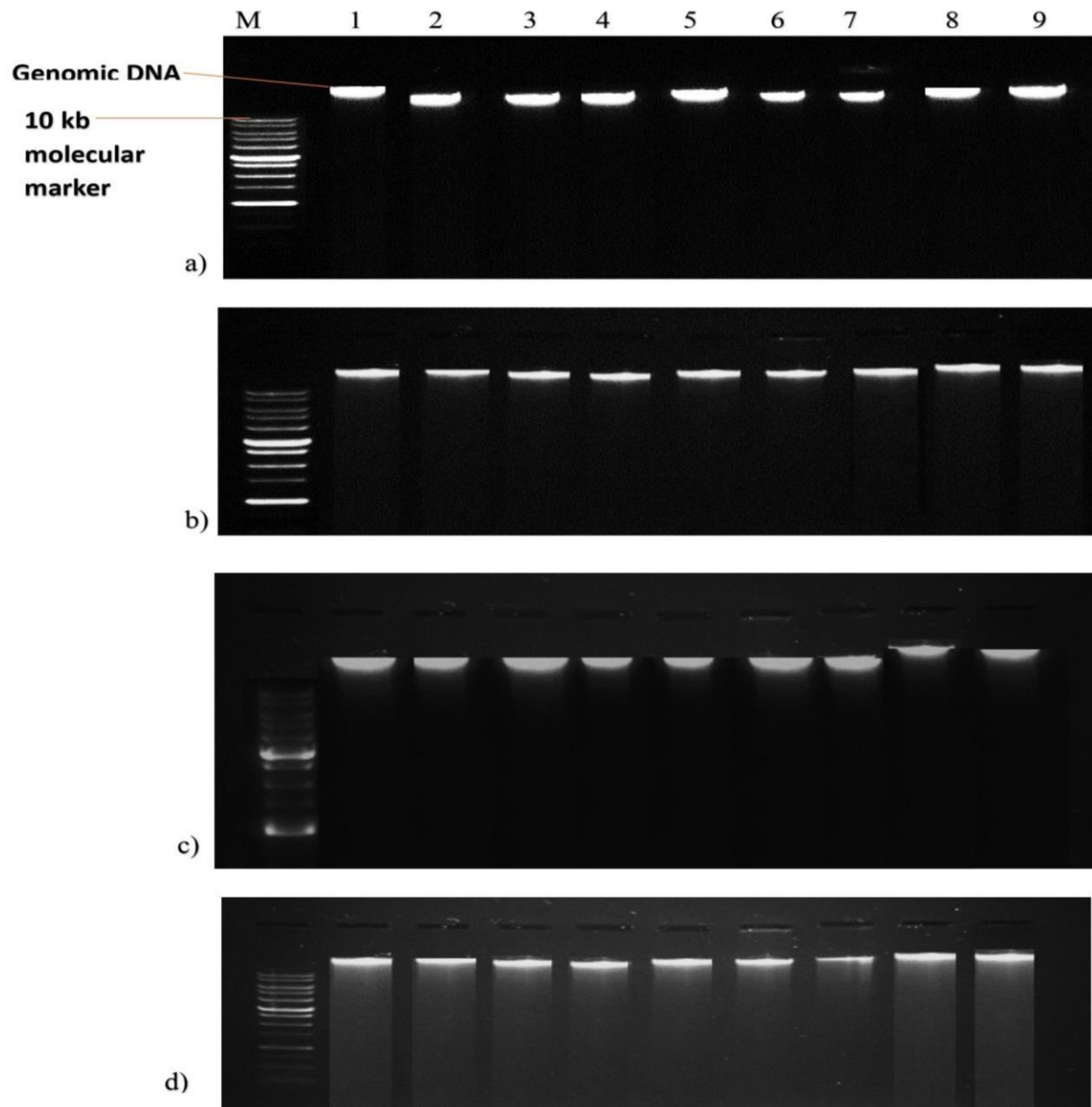
**Table 4.3: Malaria parasite density among adults in Sagbama LGA, Bayelsa State, Nigeria**

| Study group    | Malaria parasite density (parasites/ $\mu$ l of blood) |                                      |                                      |
|----------------|--------------------------------------------------------|--------------------------------------|--------------------------------------|
|                | Male                                                   | Female                               | Total                                |
| Uninfected     | 0                                                      | 0                                    | 0                                    |
| Asymptomatic   | 20065.29 $\pm$ 164.73 <sup>a</sup>                     | 19733.40 $\pm$ 485.24 <sup>a</sup>   | 19927.00 $\pm$ 748.95 <sup>a</sup>   |
| Mild malaria   | 62240.67 $\pm$ 4896.62 <sup>b</sup>                    | 72406.35 $\pm$ 5045.62 <sup>b</sup>  | 68049.63 $\pm$ 3620.10 <sup>b</sup>  |
| Severe malaria | 104406.00 $\pm$ 2065.63 <sup>c</sup>                   | 102995.45 $\pm$ 1164.87 <sup>c</sup> | 103667.14 $\pm$ 5213.93 <sup>c</sup> |

Results presented as mean  $\pm$  SEM; Values with different superscript on a column are significantly different ( $P < 0.05$ ).

#### **4.1.3. Integrity and yield of extracted human genomic DNA of adults in Sagbama LGA**

Figure 4.2 shows a section of agarose gel electrophoretogram of human genomic DNA extracted and purified from 200  $\mu$ l of the whole blood from adults in Sagbama LGA, Bayelsa State. Overall; two hundred and six DNA samples were extracted and purified. The DNA bands in Figure 4.2 a), b), and c) are sharp, clear and above the 10 kilo base pair molecular marker, indicating that the extracted and purified DNA were non degraded. The yield of the extracted and purified human genomic DNA was  $4.5 \pm 0.02 \mu\text{g}$ , while the purity was  $1.81 \pm 0.02$ .

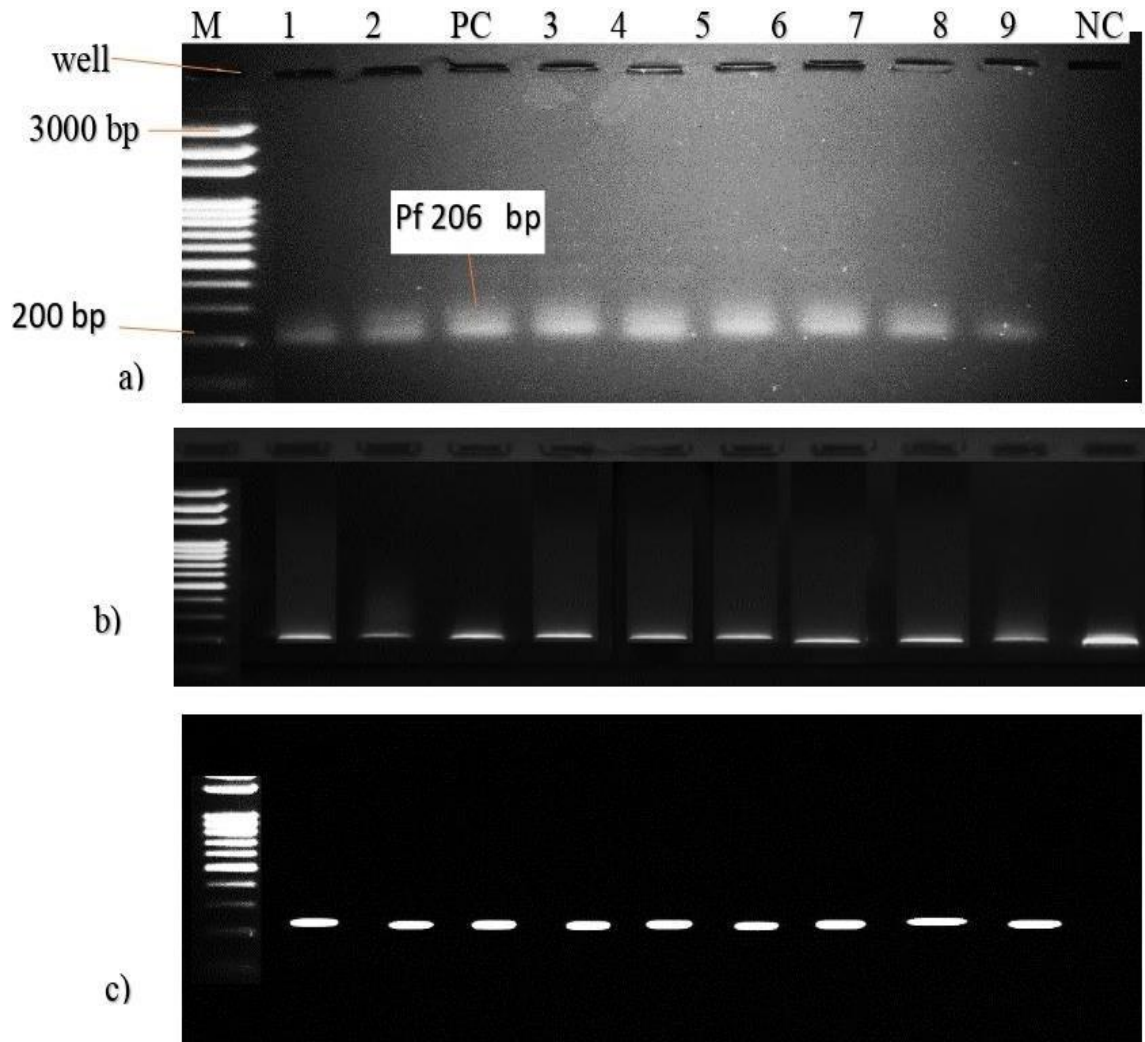


**Figure 4.2: A section of agarose gel electrophoretogram of extracted human genomic DNA.**

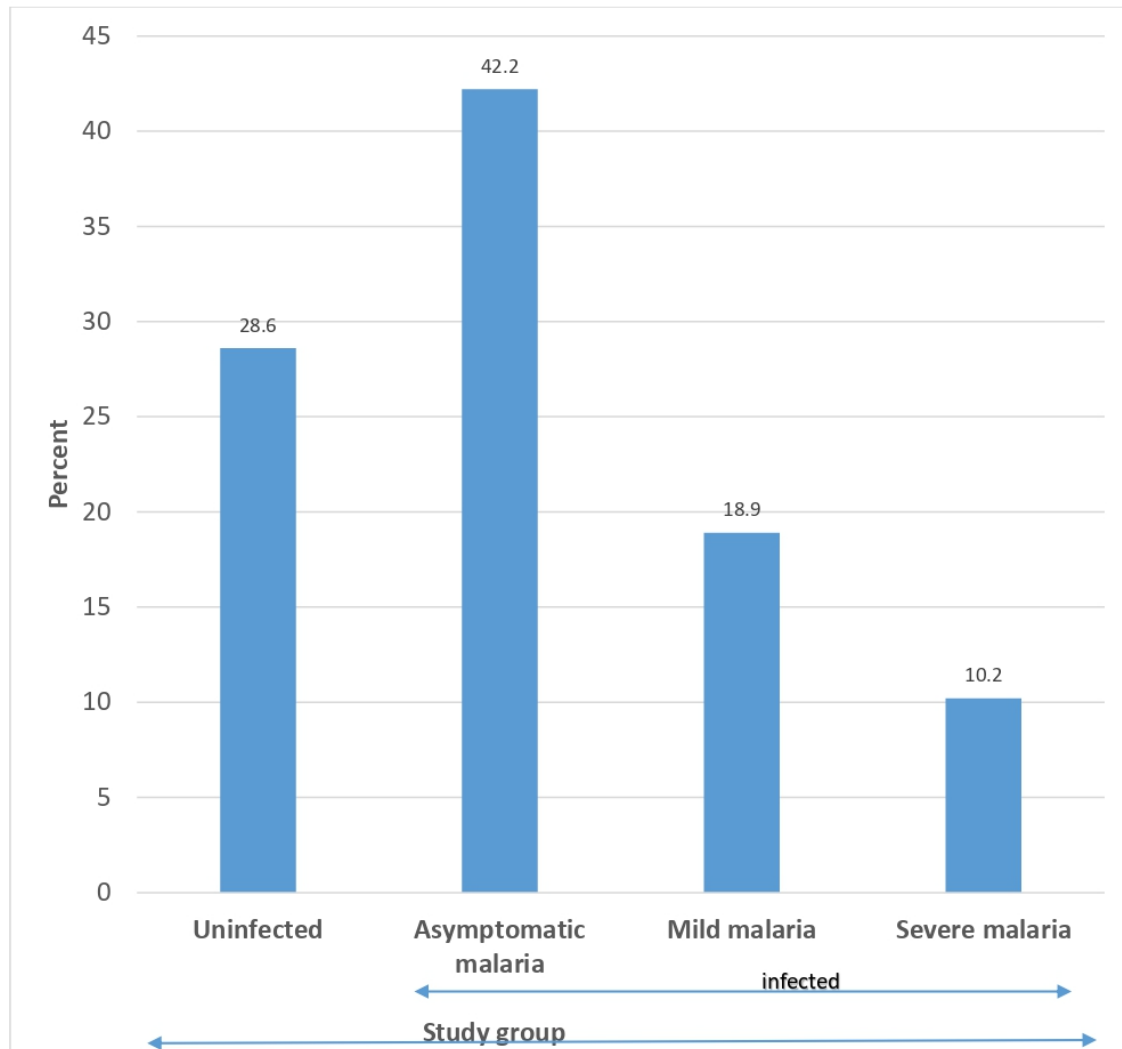
(a) Uninfected (b) asymptomatic malaria (c) mild malaria (d) severe malaria. DNA was extracted and purified from 200  $\mu$ l of the whole blood from adults in Sagbama LGA, Bayelsa State. M: 1 kilo base pair molecular marker (DNA ladder). Values represent the positions of the white DNA sample bands and wells.

#### **4.1.4. Malaria infection prevalence among adults in Sagbama LGA, Bayelsa State, Nigeria by nested genomic PCR**

Figure 4.3 shows a section of nested genomic PCR result displayed on agarose gel electrophoretogram, while, Figure 4.4 shows the overall prevalence of malaria parasite infection by nested genomic PCR among adults in Sagbama LGA, Bayelsa State, Nigeria. Based on the total count of the white DNA sample bands were visible on the agarose gel electrophoretogram (Figure 4.3) and that aligned with the positive control at the expected 206 base pair region of molecular marker, the prevalence of malaria parasite infection by nested genomic PCR as showed in Figure 4.4 was 71.30% (asymptomatic malaria; 42.2%, mild malaria; 18.9% and severe malaria; 10.2%).



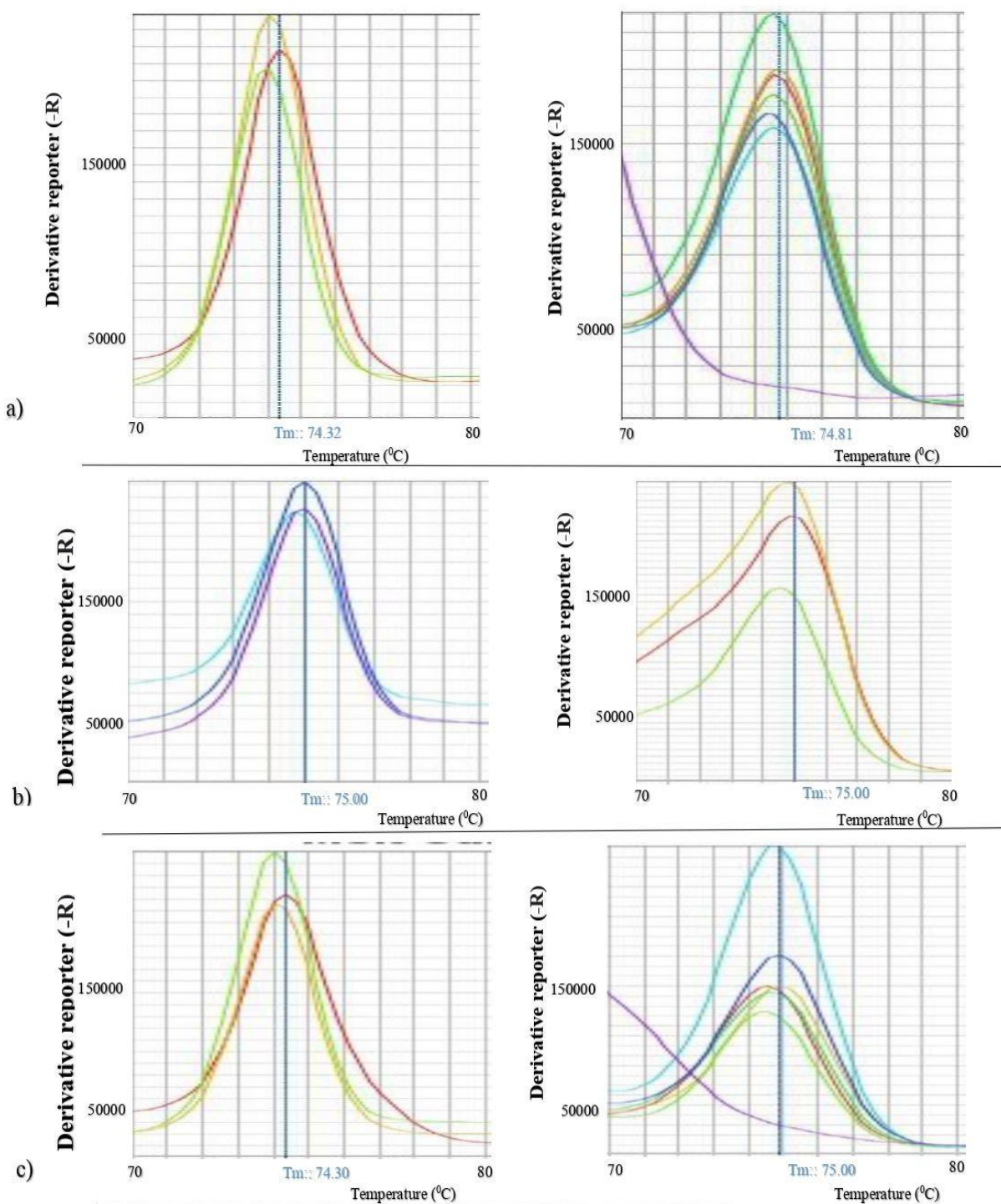
**Figure 4.3: A representative section of nested genomic PCR-agarose gel electrophoretogram of *Plasmodium falciparum* positive samples among adults in Sagbama LGA. a) Asymptomatic malaria, b) Mild malaria, and c) Severe malaria. PC; positive control, NC; negative control, M; 100 base pair (bp) molecular marker (DNA ladder), Pf; 206 base pair long *Plasmodium falciparum* 18S rRNA gene fragment of positive control.**



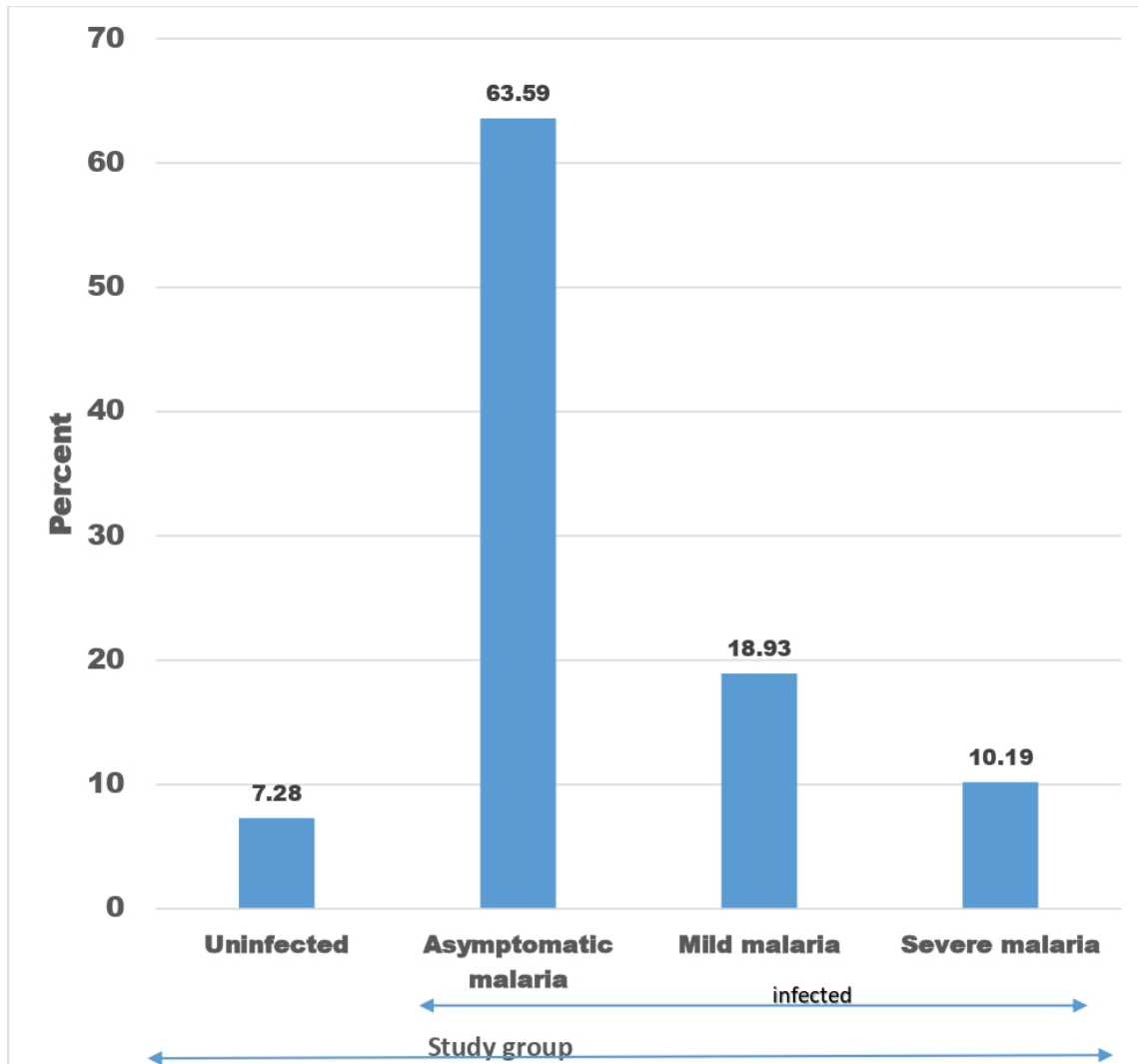
**Figure 4.4: Prevalence of malaria parasite infection by nested genomic PCR among adults in Sagbama LGA, Bayelsa State, Nigeria**

#### **4.1.5. Prevalence of malaria parasite infection by high resolution melting analysis in Sagbama LGA, Bayelsa State, Nigeria**

Figure 4.5 shows a section of the melt curves of *Plasmodium falciparum* positive samples among adults in Sagbama LGA, Bayelsa State, while Figure 4.6 shows the prevalence of malaria parasite infection by high resolution melting analysis in Sagbama LGA, Bayelsa State, Nigeria. Based on the total count of the sample melt curves that aligned with positive control melt curve within an average melting temperature ( $T_m$ ) of  $74.74 \pm 0.34$  °C, the prevalence of malaria parasite infection by high resolution melting analysis as shown in Figure 4.6 was 92.71% (asymptomatic malaria, 63.59%; mild malaria 18.93% and severe malaria 10.19%).



**Figure 4.5: A section of melt curves of *Plasmodium falciparum* positive samples among adults in Sagbama LGA. (a) Asymptomatic malaria, b) mild malaria, and c) severe malaria**



**Figure 4.6: Prevalence of malaria parasite infection by high resolution melting analysis among adults in Sagbama LGA, Bayelsa State, Nigeria**

#### **4.1.6 Comparative performance of microscopy, nested genomic PCR and high resolution melting analysis for *Plasmodium falciparum* infection detection**

Table 4.4 shows the result for Mc Nemar's pair-wise comparison of the positive (infected) cases detected by microscopy, nested genomic PCR and high resolution melting analysis methods. Mc Nemar's pair-wise comparison between the proportions of positive (infected) cases for the different methods was significant at  $P < 0.001$ . Table 4.4 showed that microscopy yielded 59.71% false negatives while nested genomic PCR yielded 21.36% false negatives (high resolution melting analysis was used as the reference method). Table 4.4 also showed that high resolution melting analysis was the most sensitive method.

**Table 4.4: Comparative performance of microscopy, nested genomic PCR and high resolution melting analysis for *Plasmodium falciparum* infection detection**

|                      | Microscopy              | Nested-G-PCR             | HRMA (reference method)  |
|----------------------|-------------------------|--------------------------|--------------------------|
|                      | N (%)                   | N (%)                    | N (%)                    |
| Uninfected           | 138 (67.00)             | 59 (28.64)               | 15 (7.28)                |
| *Infected            | 68 (33.00) <sup>a</sup> | 147 (71.36) <sup>b</sup> | 191 (92.72) <sup>c</sup> |
| Asymptomatic cases   | 12 (5.8)                | 87 (42.26)               | 131 (63.59)              |
| Malaria cases        | 56 (27.20)              | 60 (29.10)               | 60 (29.1)                |
| True positives (TP)  | 68 (33.00)              | 147 (71.36)              | 191 (92.72)              |
| False negatives (FN) | 123 (59.71)             | 44 (21.36)               | 0 (0.00)                 |
| True negatives (TN)  | 15 (7.28)               | 15 (7.28)                | 15 (7.28)                |
| False positive (FP)  | 0 (0.00)                | 0 (0.00)                 | 0 (0.00)                 |
| Sensitivity (%)      | 35.60                   | 76.96                    | 100                      |
| Specificity (%)      | 100                     | 100                      | 100                      |

\*Pair-wise comparison of positive cases for the different methods was significant at  $P < 0.001$ . Values with different superscript are significantly different. G; Genomic, HRMA; High resolution melting analysis, N; number of participants. Sensitivity (%) =  $(TP / TP + FN) \times 100$ ; Specificity (%) =  $(TN / TN + FP) \times 100$ . HRMA was the reference method employed to estimate sensitivity and specificity.

#### 4.1.7 Sociodemographic determinants of malaria in Sagbama LGA, Bayelsa State

Table 4.5 shows the sociodemographic variables associated with susceptibility or protection against malaria among adults in Sagbama LGA, Bayelsa State, Nigeria. As showed in Table 4.5, compared with the uninfected group, sociodemographic variables with  $P$  values less than 0.05 were associated with malaria, implying that malaria had no respect for such variables. However, variables with  $P$  values greater than 0.05 were not associated with malaria, implying that malaria had respect for such variables. As shown in Table 4.5, down the asymptomatic column, all selected sociodemographic variables were associated ( $P < 0.05$ ) with asymptomatic malaria ( $P < 0.05$ ), implying that asymptomatic malaria had no respect for age, sex, marital status nor level of education. Thus possessing any of these sociodemographic variables did not guarantee protection against *Plasmodium* infection in the present study. This suggests that once an individual is exposed to the *Plasmodium*, by way of finding oneself in a locality of high transmission, the likelihood of acquiring asymptomatic malaria infection is very high. On the contrary, down the mild malaria column, the present study found that some of the socio-demographic variables were associated with mild malaria ( $P < .05$ ), while only sex was associated ( $P < 0.05$ ) with severe malaria. This suggests that certain sociodemographic variables are implicated in the transition from asymptomatic malaria to mild and severe malaria. Farmers and fishermen were most prone to infection by *Plasmodium* ( $P = 0.000$ ).

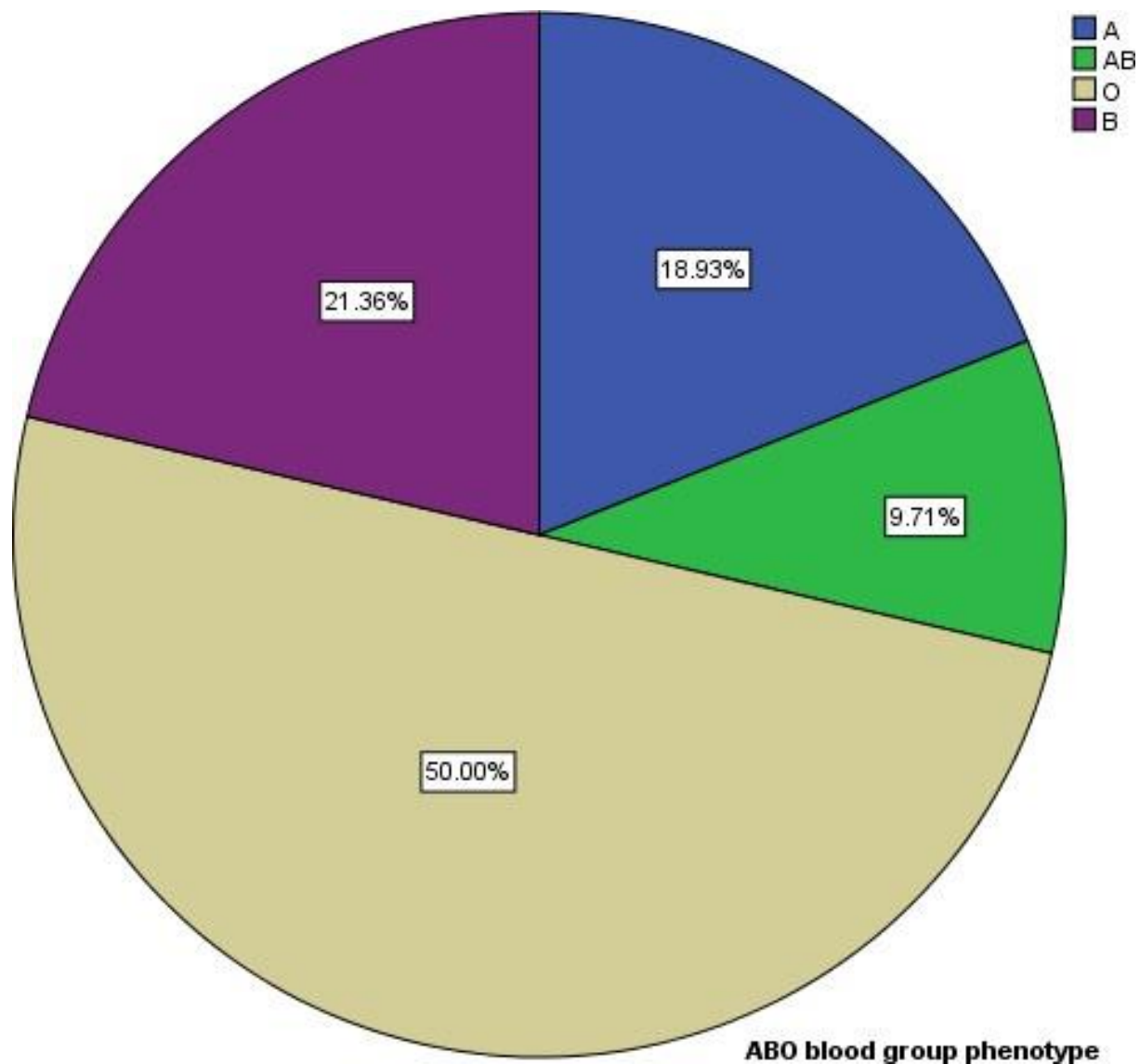
**Table 4.5 Sociodemographic determinants of malaria in Sagbama LGA, Bayelsa State**

| <b>Sociodemographic Determinant</b> | <b>Uninfected<br/>N (%)</b> | <b>Asymptomatic<br/>N (%; <i>P</i>-value)</b> | <b>Mild<br/>N (%; <i>P</i>-value)</b> | <b>Severe<br/>N (%; <i>P</i>-value)</b> |
|-------------------------------------|-----------------------------|-----------------------------------------------|---------------------------------------|-----------------------------------------|
| <b>Sex</b>                          |                             |                                               |                                       |                                         |
| Male                                | 7 (47%)                     | 55 (42%; .00)                                 | 19 (49%; .02)                         | 10 (48%; .05)                           |
| Female                              | 8 (53%)                     | 76 (58%; .00)                                 | 20 (51%; .03)                         | 11 (48%; .05)                           |
| <b>Age (years)</b>                  |                             |                                               |                                       |                                         |
| 18 – 25                             | 3 (20%)                     | 28 (21%; .00)                                 | 9 (23%; .10)                          | 9 (43%; .10)                            |
| 26 – 35                             | 3 (20%)                     | 43 (33%; .00)                                 | 10 (26%; .07)                         | 3 (14%; 1.00)                           |
| 36 – 45                             | 3 (20%)                     | 30 (23%; .00)                                 | 10 (26%; .07)                         | 6 (29%; .33)                            |
| 46 – 55                             | 2 (13%)                     | 16 (12%; .01)                                 | 4 (10%; .42)                          | 3 (14%; .66)                            |
| ≥56                                 | 4 (27%)                     | 14 (11%; .03)                                 | 6 (15%; .53)                          | 0                                       |
| <b>Marital status</b>               |                             |                                               |                                       |                                         |
| Single                              | 5 (33%)                     | 50 (38%; .00)                                 | 17 (44%; .02)                         | 13 (62%; .07)                           |
| Married                             | 10 (67%)                    | 81 (62%; .00)                                 | 22 (56%; .04)                         | 8 (38%; .64)                            |
| <b>Occupation</b>                   |                             |                                               |                                       |                                         |
| Farming / fishing                   | 7 (47%)                     | 71 (54%; .00)                                 | 22 (56%; .01)                         | 14 (67%; .13)                           |
| Petty trading                       | 3 (20%)                     | 28 (21%; .00)                                 | 13 (33%; .03)                         | 5 (24%; .48)                            |
| Civil servants                      | 3 (20%)                     | 17 (13%; .01)                                 | 4 (10%; .48)                          | 2 (10%; .66)                            |
| Craftsmanship                       | 2 (13%)                     | 15 (11%; .01)                                 | 0                                     | 0                                       |
| <b>Formal education</b>             |                             |                                               |                                       |                                         |
| None                                | 4 (27%)                     | 29 (22%; .00)                                 | 17 (44%; .01)                         | 8 (38%; .26)                            |
| Primary                             | 3 (20%)                     | 20 (15%; .00)                                 | 14 (36%; .02)                         | 5 (24%; .48)                            |
| Secondary                           | 5 (33%)                     | 52 (40%; .00)                                 | 4 (10%; .74)                          | 4 (19%; .74)                            |
| Tertiary                            | 3 (20%)                     | 30 (23%; .00)                                 | 4 (10%; .71)                          | 4 (19%; .71)                            |

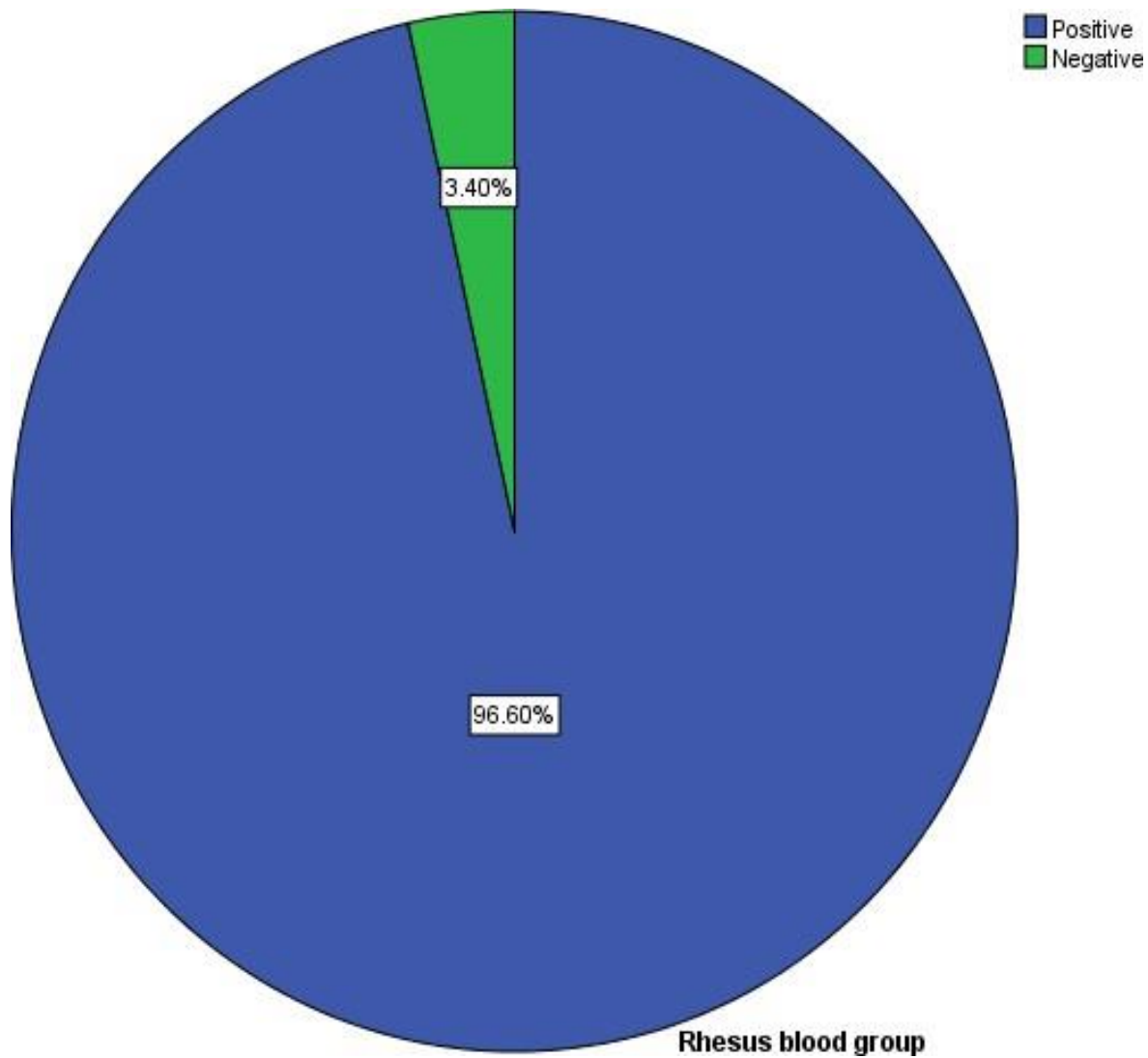
N = number of participants; Using logistic regression, each study group was compared with the uninfected group (reference category).

#### **4.1.8. ABO/Rhesus (D) blood group distribution among adults in Sagbama LGA, Bayelsa State, Nigeria.**

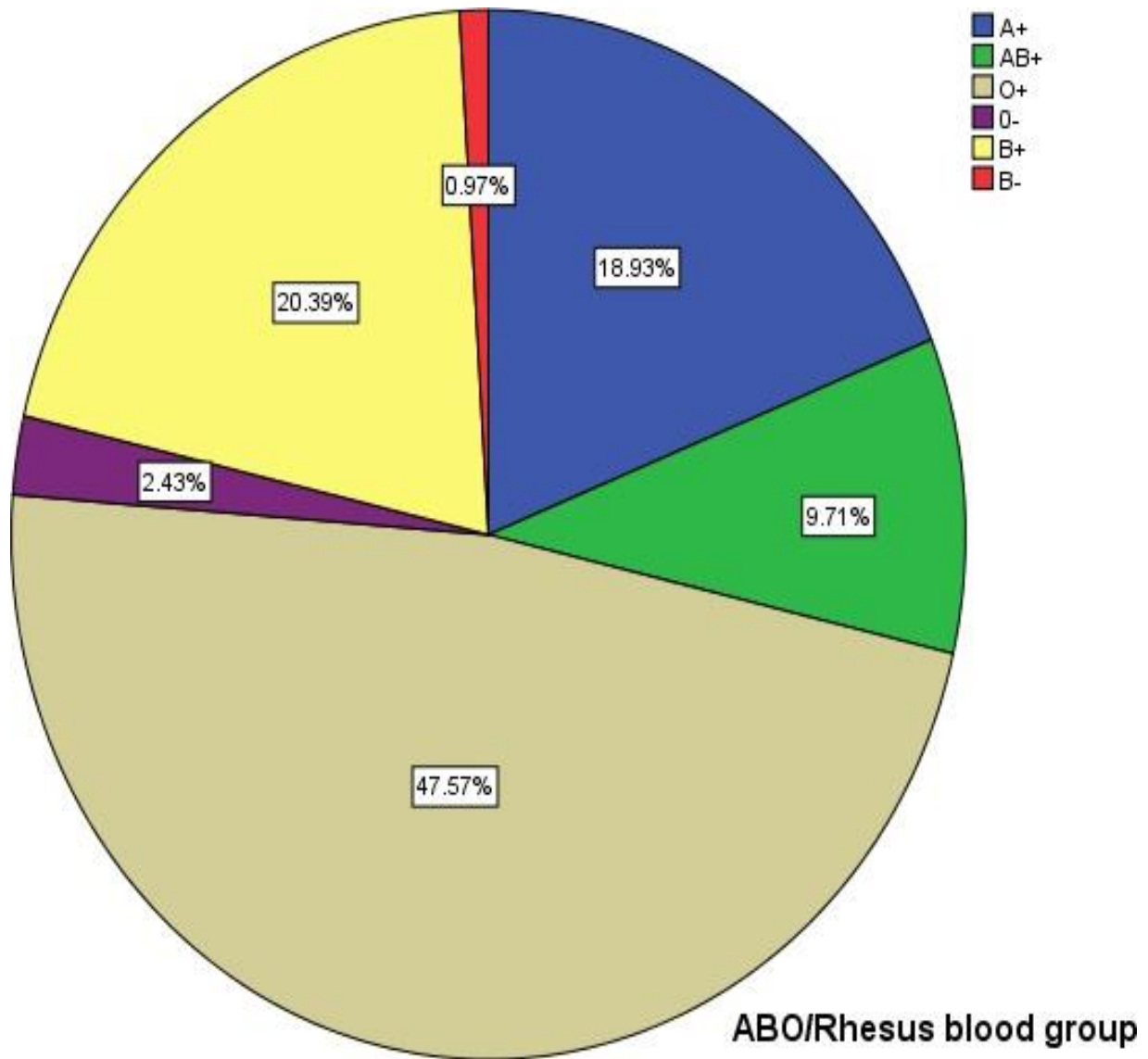
Presented in Figure 4.7 is the ABO/Rhesus (D) blood group distribution among adults in Sagbama LGA, Bayelsa State, Nigeria. As shown in Figure 4.7 (a), blood group O was the most prevalent ABO blood group (50%), followed by blood group B and A, while the least frequent was blood group AB (9.71%), while as showed in Figure 4.7 (b), almost all the study participants were Rhesus (D) negative. As presented in Figure 4.7 (c), the pooled ABO/Rhesus group distribution showed that blood group O<sup>+</sup> was the most prevalent (47.57%), while the least prevalent was B<sup>-</sup>. Blood group and A<sup>-</sup> and AB<sup>-</sup> were not found among the study population.



**Figure 4.7 (a): Pie chart showing the distribution of ABO blood group polymorphism in Sagbama LGA, Bayelsa State, Nigeria**



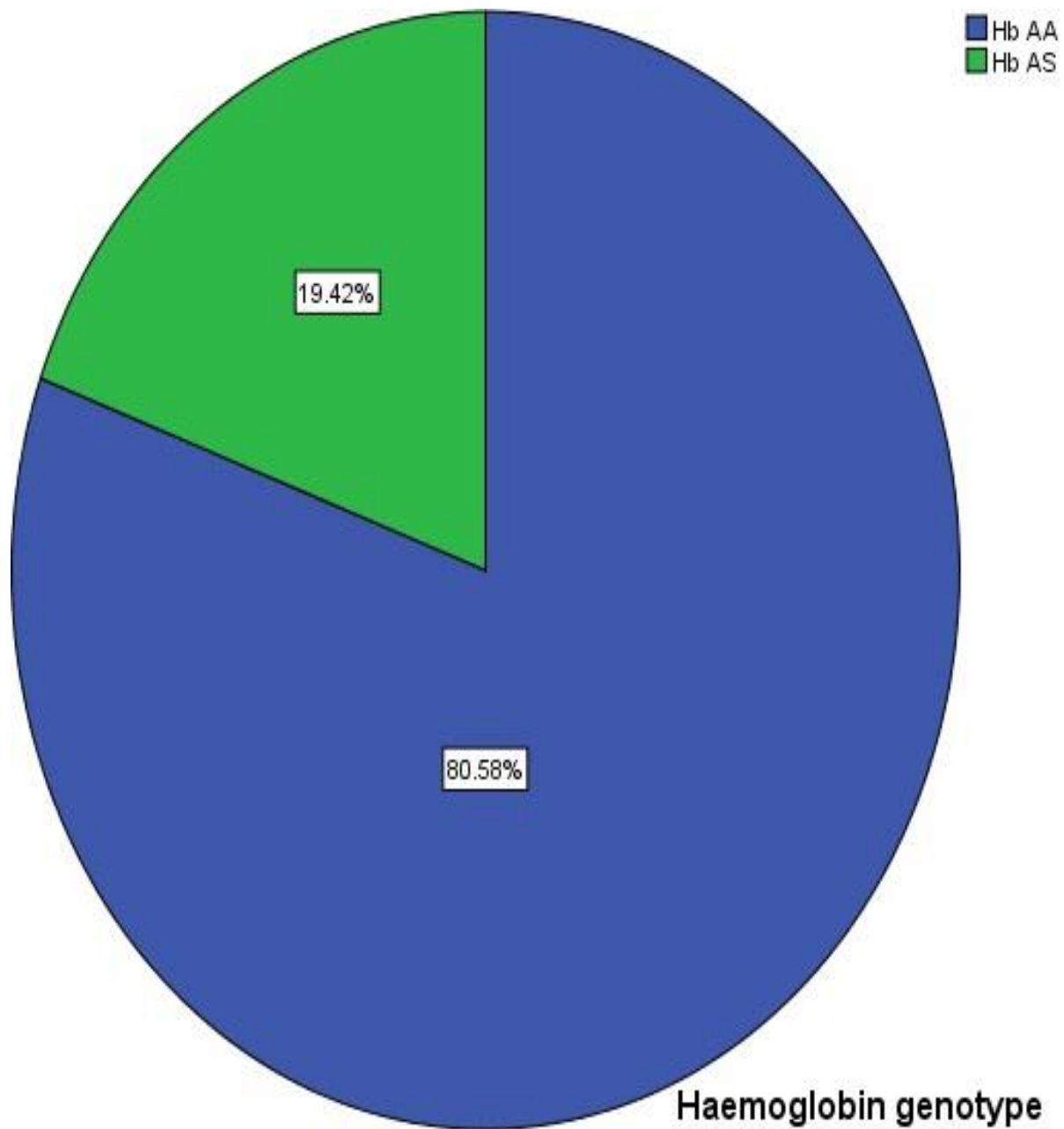
**Figure 4.7 (b): Pie chart showing the distribution of Rhesus (D) blood group polymorphism in Sagbama LGA, Bayelsa State, Nigeria.**



**Figure 4.7 (c): Pie chart showing the distribution of the pooled ABO/Rhesus (D) blood group polymorphism in Sagbama LGA, Bayelsa State, Nigeria.**

#### **4.1.9. Haemoglobin genotype distribution among adults in Sagbama LGA, Bayelsa State, Nigeria.**

Figure 4.8 shows the haemoglobin genotype distribution among adults in Sagbama LGA, Bayelsa State, Nigeria. As shown in Figure 4.8, haemoglobin genotype (Hb) AA was the most prevalent in Sagbama LGA, Bayelsa State while Hb AS was the least prevalent. Hb SC, Hb AC, Hb CC were not found among the study population.



**Figure 4.8: Haemoglobin genotype distribution among adults in Sagbama LGA, Bayelsa State, Nigeria.**

#### **4.1.10 ABO blood group polymorphism and malaria susceptibility**

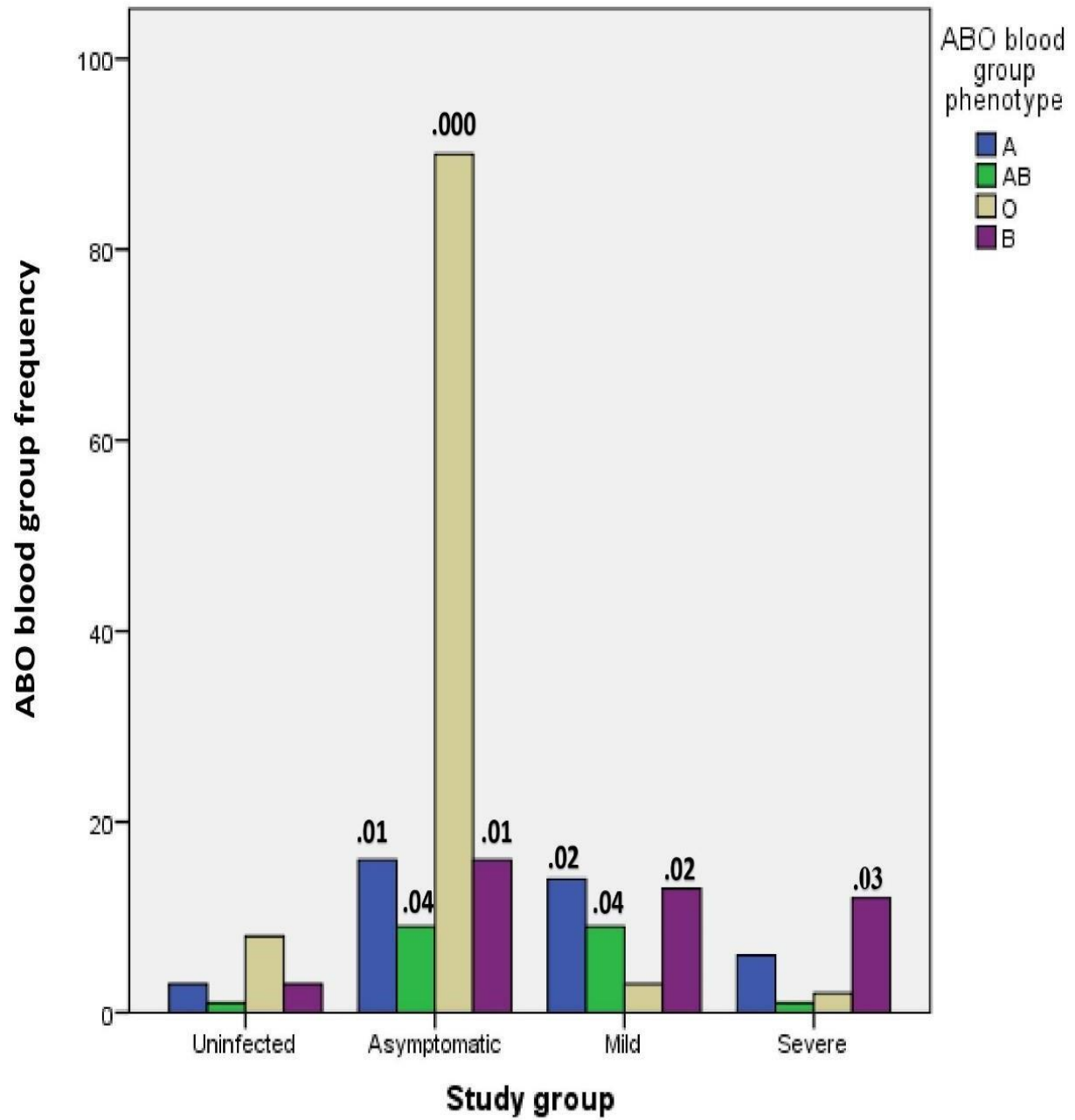
Table 4.6 and Figure 4.9 shows the association of ABO blood group polymorphism with malaria susceptibility among adults in Sagbama LGA, Bayelsa State using multivariate logistics regression analysis. As shown in Table 4.6 and Figure 4.9, although blood group O was significantly associated with the likelihood of acquiring asymptomatic malaria infection ( $P$  value = .000), it was however not significantly associated with the risk of mild ( $P$  value = .147) or severe malaria ( $P$  value: .484). This suggests that blood group O is protective against mild and severe malaria. On the other hand, blood groups A, B and AB were associated with the likelihood of acquiring asymptomatic malaria infection [( $P$  values: .008 (A), .008 (B), .037 (AB))] as well as with the risk of mild malaria [( $P$  values = 0.015 (A), 0.022 (B) and .037 (AB). Only blood group B was associated with the risk of severe malaria ( $P$  value: .032).

A  $P$  value of .000 for blood group O under the asymptomatic malaria study group (Table 4.6 and Figure 4.9) indicate that blood group O subject are at a higher risk of getting infected with malaria parasite compared with other blood groups. Although some blood group O subjects had mild malaria but there was no association compared with blood group A, B and AB subjects. As shown in Table 4.6 among the severe malaria subjects, 57% had blood group B.

**Table 4.6: Multivariate logistics regression showing the likelihood of association between ABO antigen polymorphisms and malaria among adults in Sagbama LGA, Bayelsa State**

| Study group <sup>a</sup> | Blood group | P value | OR     | 95%confidence interval |
|--------------------------|-------------|---------|--------|------------------------|
| Asymptomatic             | A (12%)     | .008    | 5.333  | 1.554 – 18.304         |
|                          | B (12%)     | .008    | 5.333  | 1.554 – 18.304         |
|                          | AB (7%)     | .037    | 9.000  | 1.140 – 71.038         |
|                          | O (69%)     | .000    | 11.250 | 5.459 – 23.184         |
| Mild                     | A (36%)     | .015    | 4.667  | 1.341 – 16.239         |
|                          | B (33%)     | .022    | 4.333  | 1.235 – 15.206         |
|                          | AB (23%)    | .037    | 9.000  | 1.140 – 71.038         |
|                          | O (8%)      | .147    | .375   | .099 – 1.414           |
| Severe                   | A (29%)     | .147    | .375   | .099 – 1.414           |
|                          | B (57%)     | .032    | 4.000  | 1.129 – 14.175         |
|                          | AB (5%)     | 1.000   | 1.000  | .063 – 15.988          |
|                          | O (10%)     | .484    | 1.667  | .398 – 6.974           |

<sup>a</sup>. the reference category is: uninfected. OR = odd ratio; association significant at  $p < 0.05$ .



**Figure 4.9: Association of erythrocyte ABO antigens polymorphisms with malaria infection outcome. Bars with *P-values* are significantly associated with the respective study group.**

#### **4.1.11. Erythrocyte Rhesus (D) polymorphism and malaria susceptibility in Sagbama**

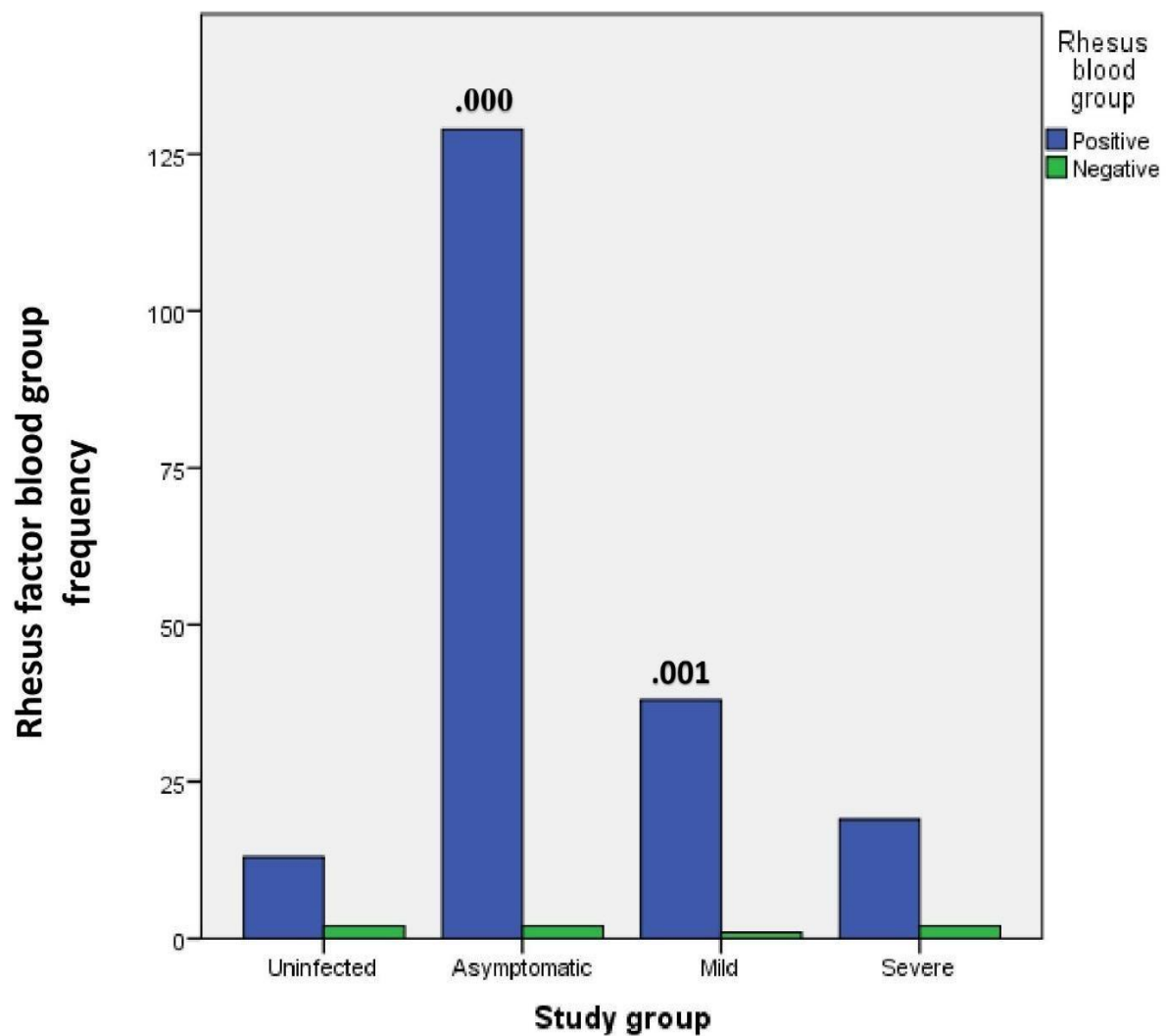
##### **LGA, Bayelsa State, Nigeria**

Presented in Table 4.7 and Figure 4.10 is the association of Rhesus factor blood group polymorphism with malaria. As showed in Table 4.7 Rhesus factor D was associated with the risk of mild malaria (95% confidence interval; OR: 2.923; *P*-value: .001), thus it is susceptible to malaria and not protective against the clinical manifestation of mild malaria. As clearly shown in Figure 4.10, Rhesus factor D was also significantly associated with the risk of asymptomatic malaria (*P* value: .000) and thus, it is not protective against the risk of malaria infection. Rhesus factor D was however not associated with severe malaria as showed in Figure 4.10 and Table 4.7.

**Table 4.7: Multivariate logistics regression showing the likelihood of association between Rhesus antigen polymorphisms and malaria among adults in Sagbama LGA**

| <b>Study group <sup>a</sup></b> | <b>Rhesus D</b> | <b>P value</b> | <b>AOR</b> | <b>95% confidence interval</b> |
|---------------------------------|-----------------|----------------|------------|--------------------------------|
| Asymptomatic                    | +               | .000           | 9.923      | 5.610 – 17.552                 |
|                                 | -               | 1.000          | 1.000      | .141 – 7.099                   |
| Mild                            | +               | .001           | 2.923      | 1.557 – 5.487                  |
|                                 | -               | .571           | .500       | .045 – 5.514                   |
| Severe                          | +               | .292           | 1.462      | .722 – 2.959                   |
|                                 | –               | 1.000          | 1.000      | .141 – 7.099                   |

a. the reference category is: uninfected.



**Figure 4.10: Association between erythrocyte Rhesus D antigens polymorphisms and malaria infection outcome. Bars with *P*-values are significantly associated with respective study group.**

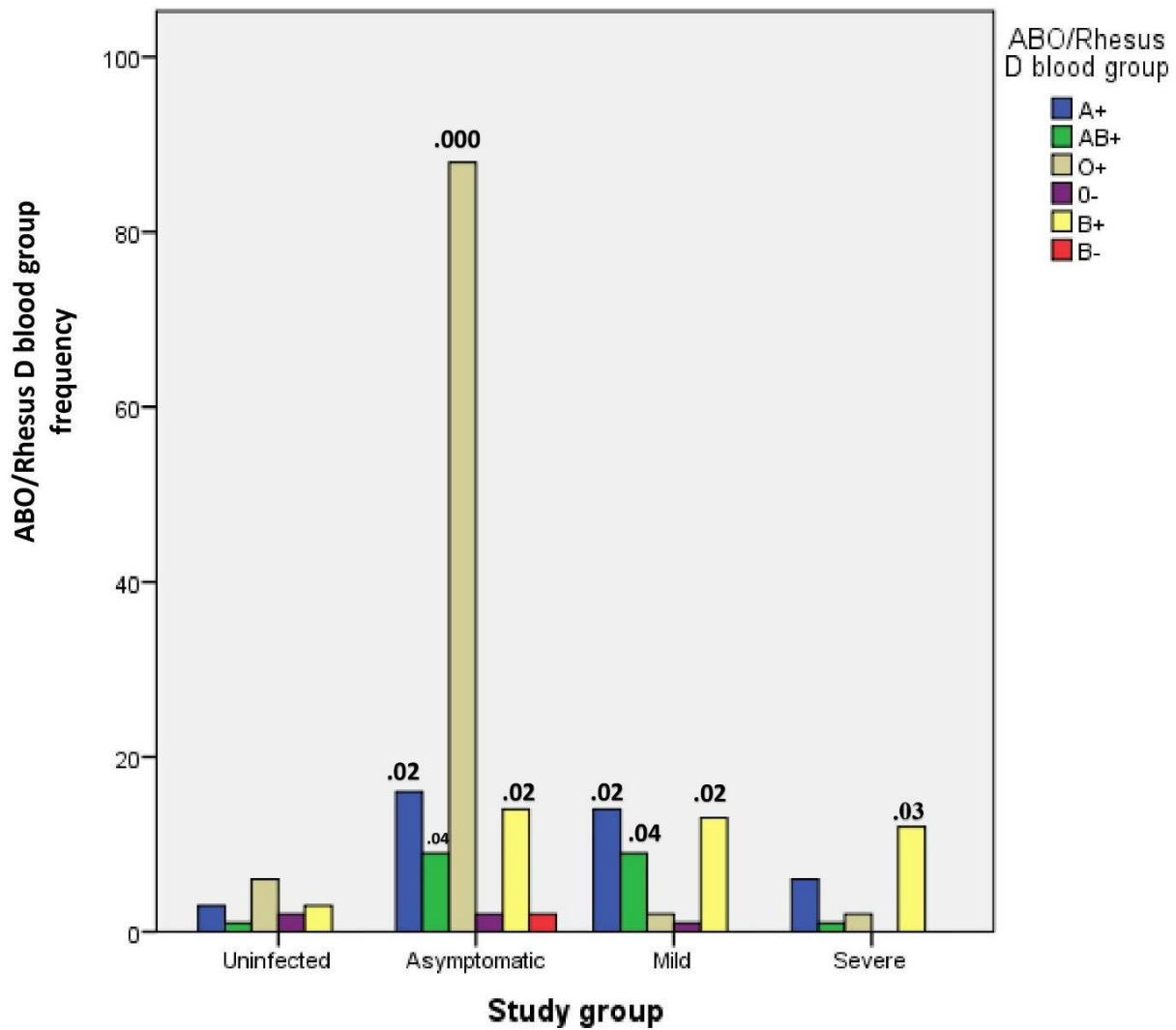
#### **4.1.12. Pooled ABO/Rhesus factor polymorphism and malaria susceptibility in Sagbama LGA, Bayelsa State, Nigeria**

As shown in Table 4.8 and Figure 4.11, only ABO blood groups with rhesus positive were associated with malaria ( $P < 0.05$ ). ABO blood groups with Rhesus negative were not associated with malaria.

**Table 4.8: Association of ABO/Rhesus antigen polymorphism with malaria in Sagbama LG**

| <b>Study group <sup>a</sup></b> | <b>ABO/Rhesus D</b> | <b>P value</b> | <b>AOR</b> | <b>95% CI</b>  |
|---------------------------------|---------------------|----------------|------------|----------------|
| Asymptomatic                    | A+                  | .008           | 5.333      | 1.554 – 18.304 |
|                                 | A-                  | -              | -          | -              |
|                                 | B+                  | .015           | 4.667      | 1.341 – 16.239 |
|                                 | B-                  | -              | -          | -              |
|                                 | AB+                 | .037           | 9.000      | 1.140 – 71.038 |
|                                 | AB-                 | -              | -          | -              |
|                                 | O+                  | .000           | 14.667     | 6.415 – 33.534 |
|                                 | O-                  | 1.000          | 1.000      | .141 – 7.099   |
| Mild                            | A+                  | .015           | 4.667      | 1.341 – 16.239 |
|                                 | A-                  | -              | -          | -              |
|                                 | B+                  | .022           | 4.333      | 1.235 – 15.20  |
|                                 | B-                  | -              | -          | -              |
|                                 | AB+                 | .037           | 9.000      | 1.140 – 71.04  |
|                                 | AB-                 | -              | -          | -              |
|                                 | O+                  | .178           | .333       | .067 – 1.652   |
|                                 | O-                  | .571           | .500       | .045 – 5.514   |
| Severe                          | A+                  | .327           | 2.000      | .500 – 7.997   |
|                                 | A-                  | -              | -          | -              |
|                                 | B+                  | .032           | 4.000      | 1.129 – 14.175 |
|                                 | B-                  | -              | -          | -              |
|                                 | AB+                 | 1.000          | 1.000      | .063 – 15.988  |
|                                 | AB-                 | -              | -          | -              |
|                                 | O+                  | .178           | .333       | .067 – 1.652   |
|                                 | O-                  | -              | -          | -              |

<sup>a</sup>. the reference category is uninfected. – indicates ABO/Rhesus D blood group without comparable values among the study population.



**Figure 4.11 Association of pooled ABO/Rhesus antigen polymorphism with malaria infection outcome. Bars with *P*-values are significantly associated with respective study group.**

#### **4.1.13 Association of ABO and Rhesus blood groups with malaria parasite density in Sagbama LGA**

The association of ABO and Rhesus factor with malaria parasite density in Sagbama LGA is presented in Table 4.9 and Table 4.10 respectively. As showed in Table 4.9, no association of ABO blood groups with malaria parasite density was found among the adults in the present study. As presented in Table 4.10, the association between Rhesus D factor and parasite density among asymptomatic malaria subjects could not be computed because none of the asymptomatic malaria subjects were Rhesus D negative. Also, as showed in Table 4.10, no association between Rhesus D blood group and mild or severe malaria parasite density was found.

**Table 4.9: Association between malaria parasite density and ABO antigens in Sagbama**

| <b>Blood group</b> |                   | <b>Malaria parasite density (parasites/<math>\mu</math>l of blood)</b> |                                |                                |
|--------------------|-------------------|------------------------------------------------------------------------|--------------------------------|--------------------------------|
| <b>Phenotype</b>   | <b>Uninfected</b> | <b>Asymptomatic</b>                                                    | <b>Mild</b>                    | <b>Severe</b>                  |
| A                  | 0                 | 19672 $\pm$ 337 <sup>a</sup>                                           | 83330 $\pm$ 12476 <sup>b</sup> | 101622 $\pm$ 1108 <sup>c</sup> |
| B                  | 0                 | 20217 $\pm$ 267 <sup>a</sup>                                           | 79587 $\pm$ 8898 <sup>b</sup>  | 103515 $\pm$ 1558 <sup>c</sup> |
| AB                 | 0                 | 20083 $\pm$ 726 <sup>a</sup>                                           | 82467 $\pm$ 1651 <sup>b</sup>  | 112000 $\pm$ 0000 <sup>c</sup> |
| O                  | 0                 | 19495 $\pm$ 495 <sup>a</sup>                                           | 77340 $\pm$ 8229 <sup>b</sup>  | 105600 $\pm$ 4600 <sup>c</sup> |

Results presented as mean  $\pm$  SEM; Values with different superscript on a column are significantly different.

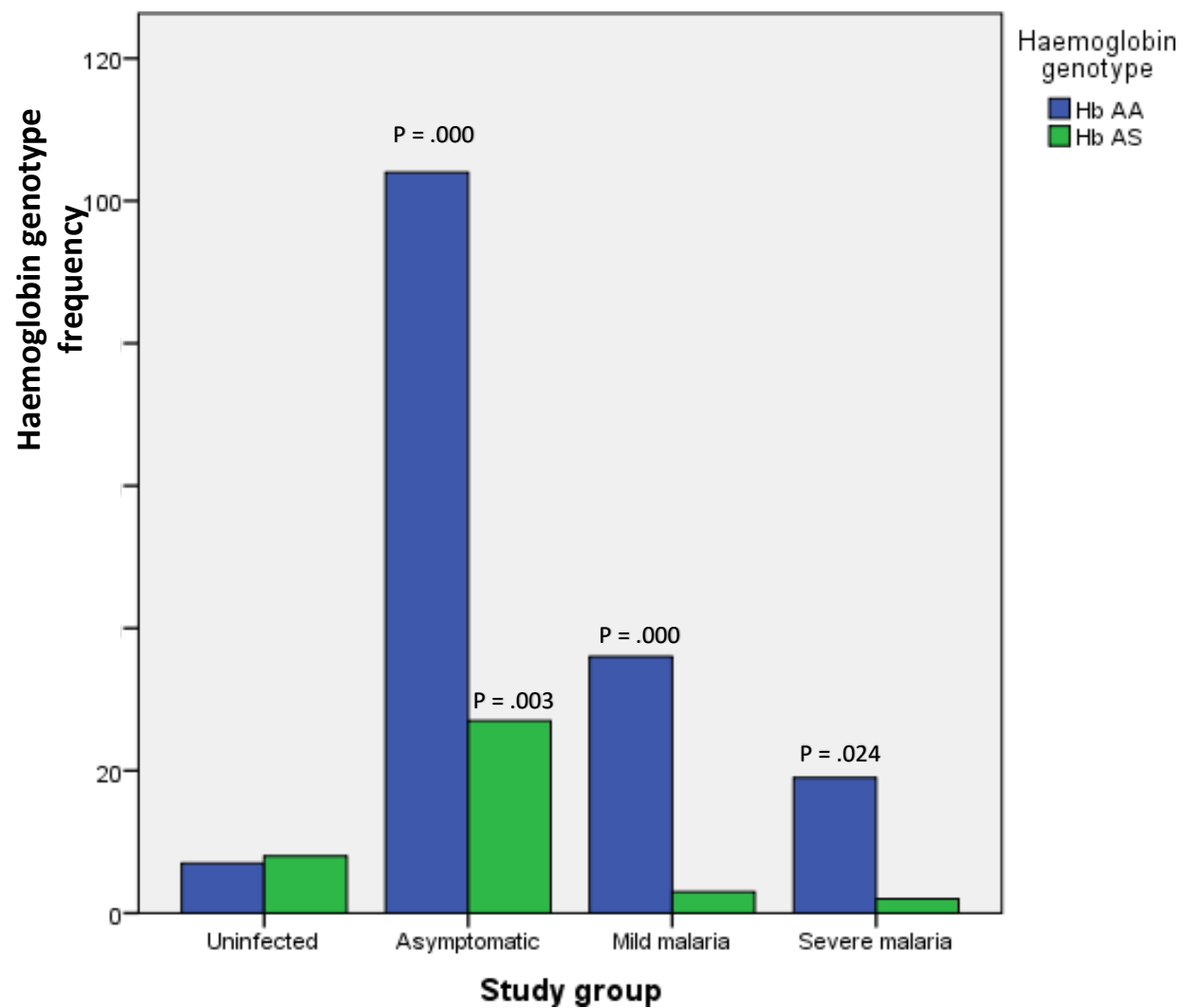
**Table 4.10: Association between malaria parasite density and Rhesus (D) antigens in Sagbama**

| <b>Rhesus D</b> | <b>Malaria parasite density (parasites/<math>\mu</math>l of blood)</b> |                     |                               |                                |
|-----------------|------------------------------------------------------------------------|---------------------|-------------------------------|--------------------------------|
|                 | <b>Uninfected</b>                                                      | <b>Asymptomatic</b> | <b>Mild</b>                   | <b>Severe</b>                  |
| Positive        | 0                                                                      | 19927 $\pm$ 0000    | 74185 $\pm$ 5985 <sup>a</sup> | 103779 $\pm$ 1244 <sup>b</sup> |
| Negative        | 0                                                                      | 0                   | 80000 $\pm$ 0000 <sup>a</sup> | 102600 $\pm$ 2400 <sup>b</sup> |

Results presented as mean  $\pm$  SEM; Values with different superscript on a column are significantly different

#### **4.1.14. Haemoglobin genotypes and malaria susceptibility in Sagbama LGA, Bayelsa State, Nigeria**

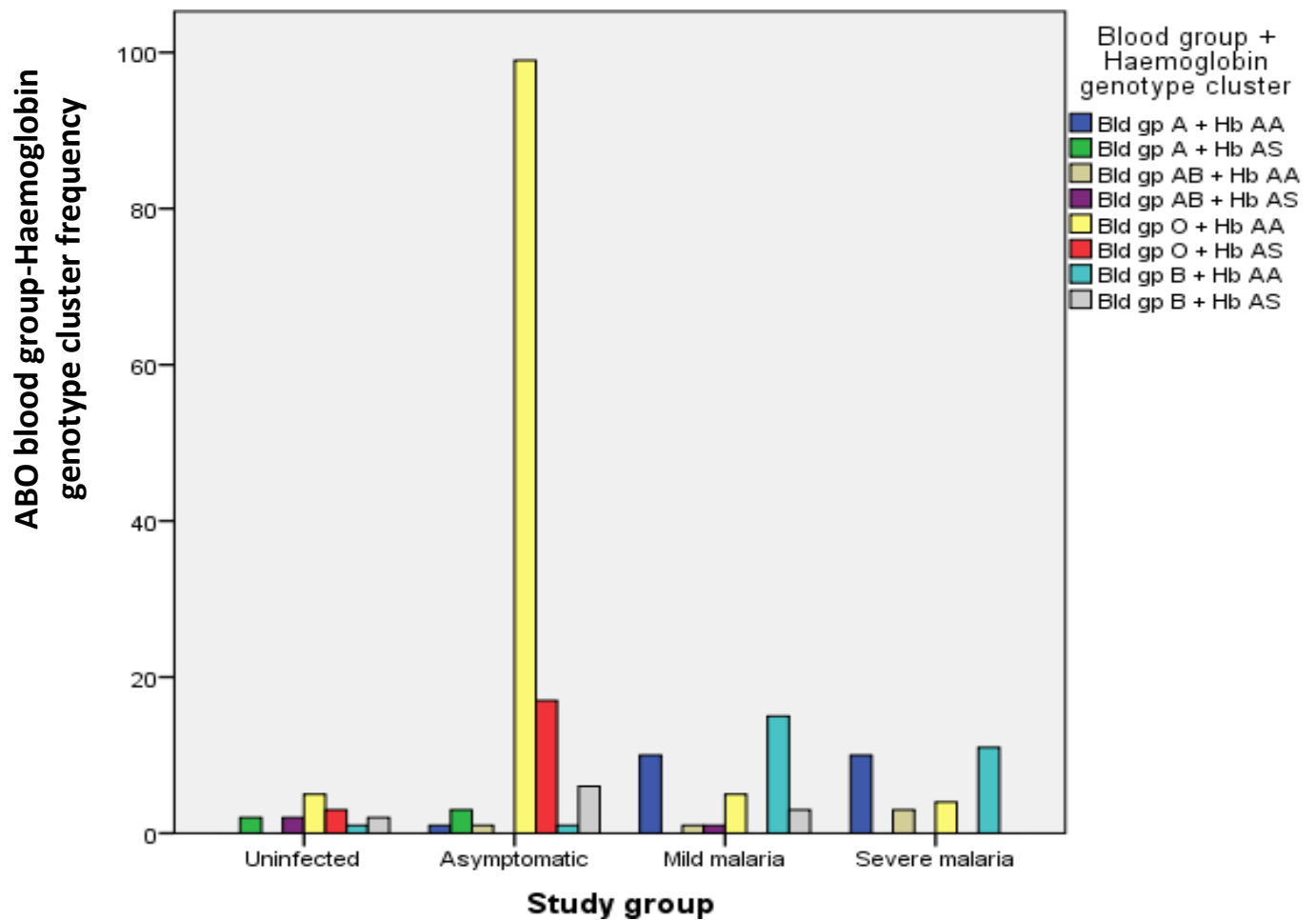
Figure 4.12 shows the relationship between haemoglobin genotype and malaria in Sagbama LGA, Bayelsa State, Nigeria. As showed in Figure 4.12, compared with the control group, Hb AA was associated with the likelihood of having asymptomatic ( $P = .000$ ), mild ( $P = .000$ ) and severe malaria ( $P = .024$ ). Thus Hb AA is susceptible to malaria. Hb AS was associated with the likelihood of having asymptomatic malaria ( $P = .003$ ), indicating susceptibility and not protection against malaria.



**Figure 4.12: Association between haemoglobin genotypes and malaria in Sagbama LGA, Bayelsa State, Nigeria**

#### **4.1.15. Association of pooled ABO blood group-haemoglobin genotype cluster with malaria in Sagbama LGA, Bayelsa State, Nigeria**

Figure 4.13 shows the relationship between blood group-haemoglobin genotype cluster and malaria in Sagbama LGA, Bayelsa State, Nigeria. Compared with the control group, the cluster of blood group O and Hb AA was the most prevalent in the study population and was found in the asymptomatic malaria group. The predominant clusters in severe the malaria group were blood group A and HbAA as well as blood group B + HbAA clusters.

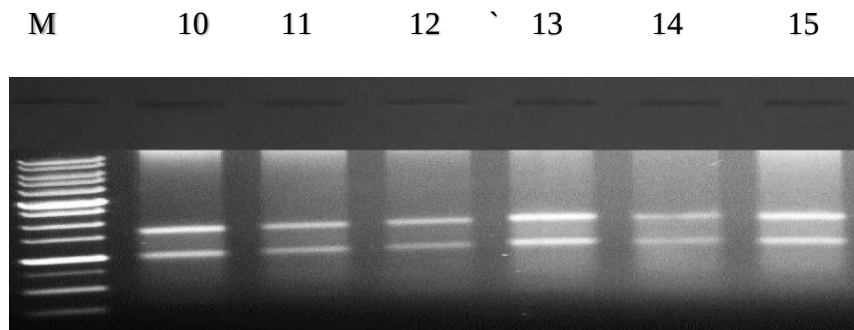
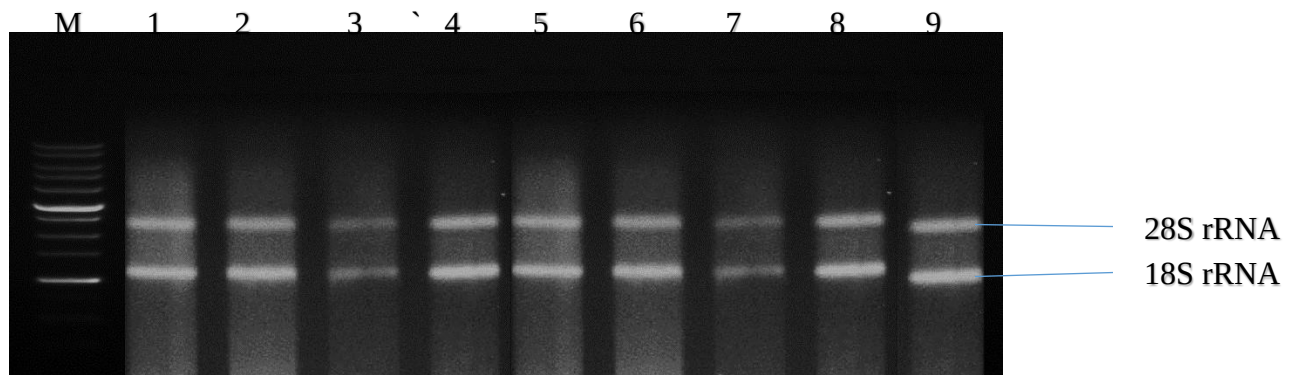


**Figure 4.13: Association of ABO blood group-haemoglobin genotype cluster with malaria in Sagbama LGA, Bayelsa State, Nigeria**

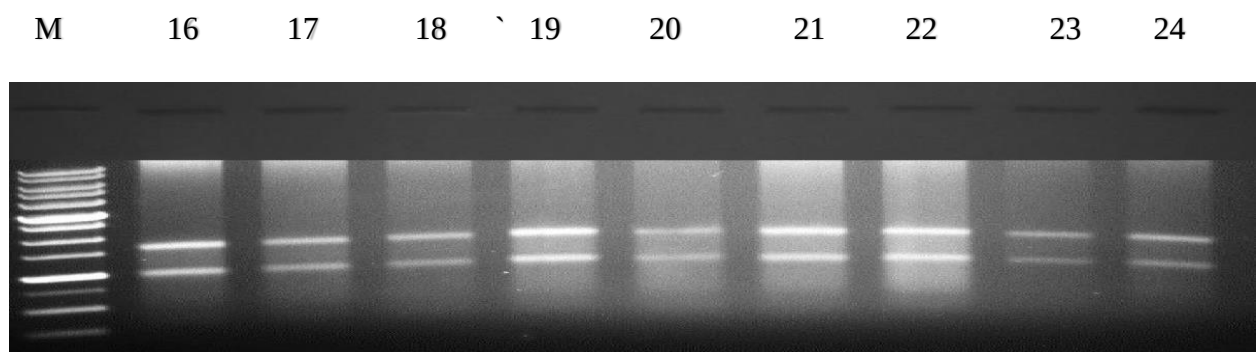
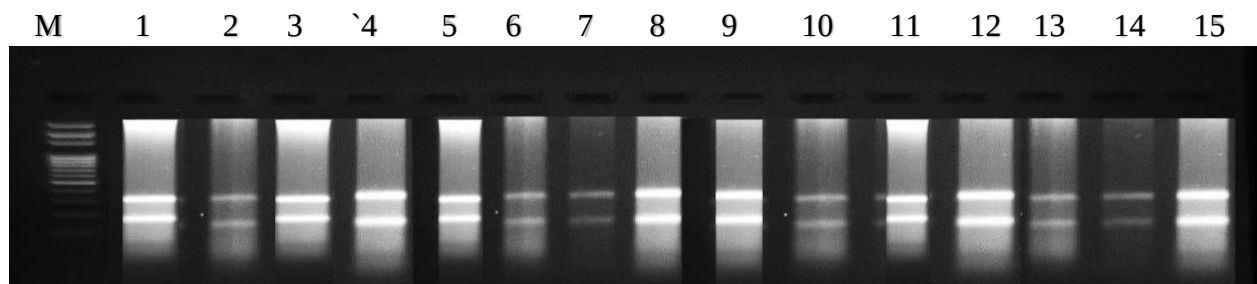
#### **4.1.16. Agarose gel electrophoresis of extracted RNA**

Presented in Figure 4.14 is the agarose gel electrophoretogram of extracted total RNA. Sharp and clear visible bands for 28S and 18 rRNA were observed on each lane, indicating the extraction of non-degraded and high quality total RNA. A total of 206 RNA samples were extracted.

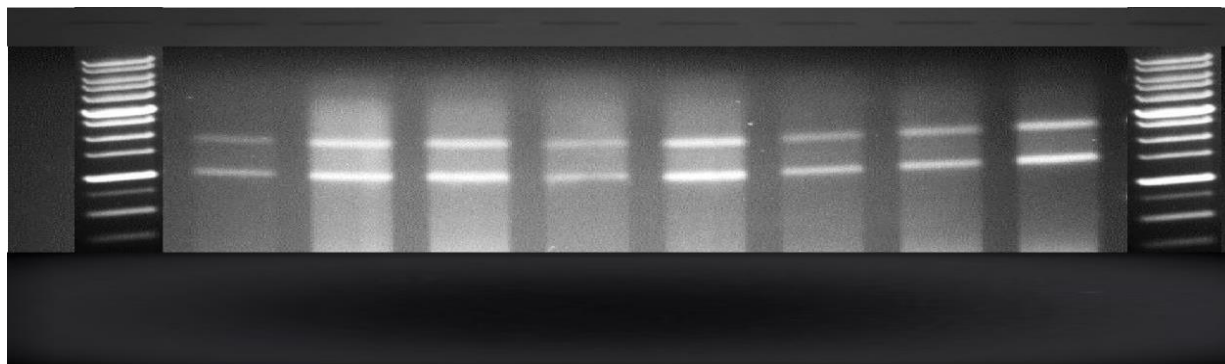
**a) Total RNA for group 1 (uninfected group): 15 samples**



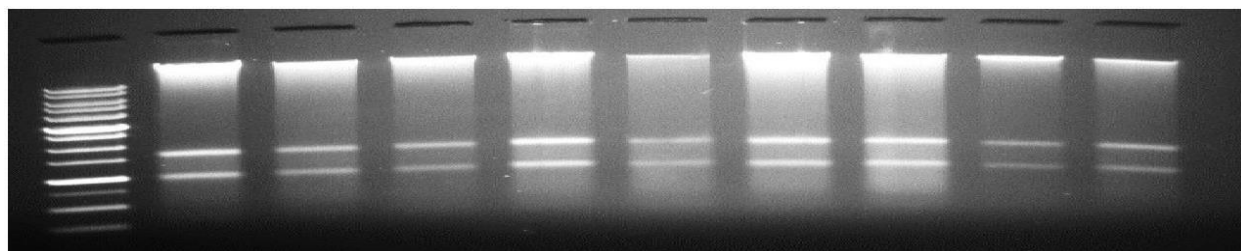
**b) Total RNA for group 2 (Asymptomatic group): 131 samples**



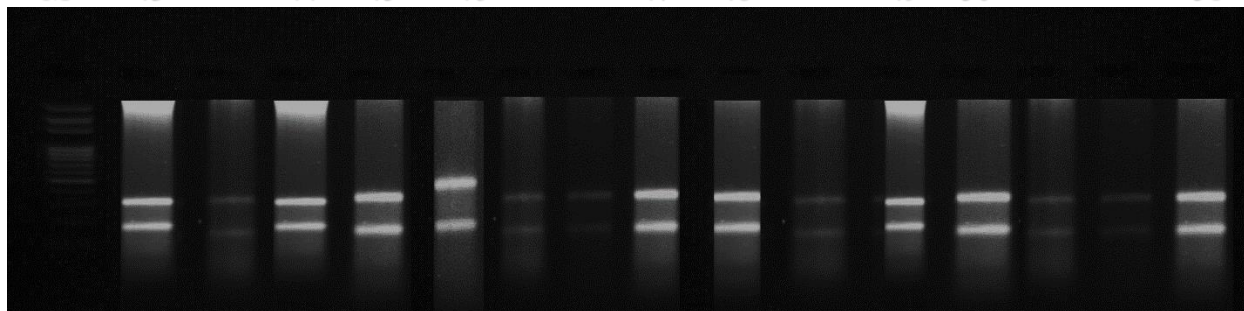
M 25 26 27 ` 28 29 30 32 33 M



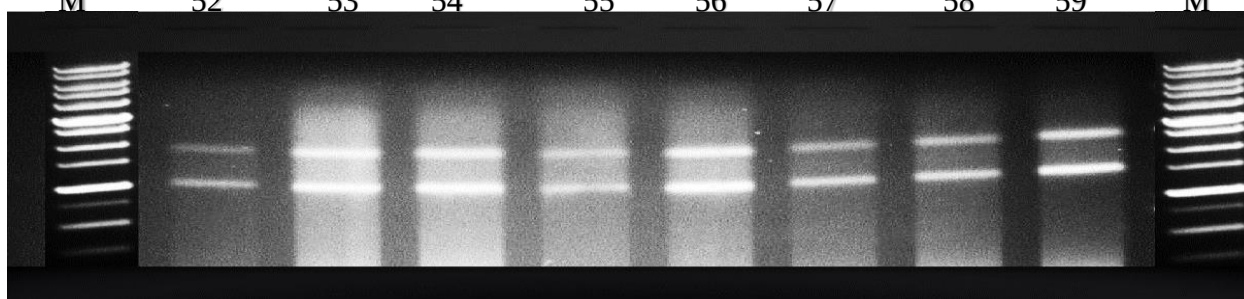
M 34 35 36 ` 37 38 39 40 41 42

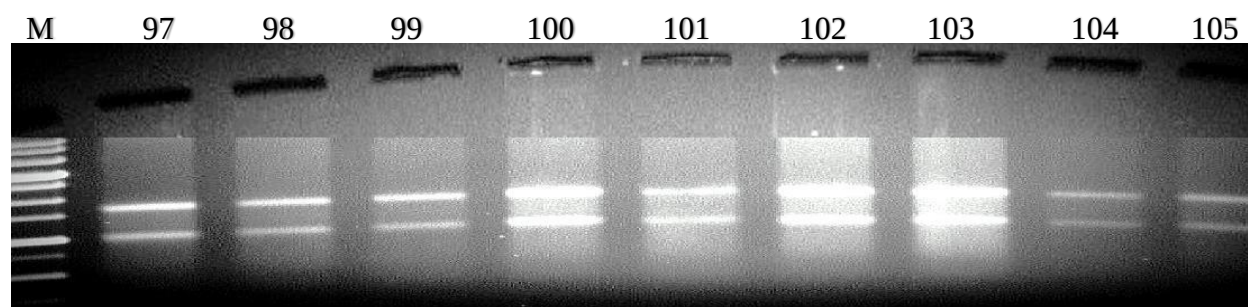
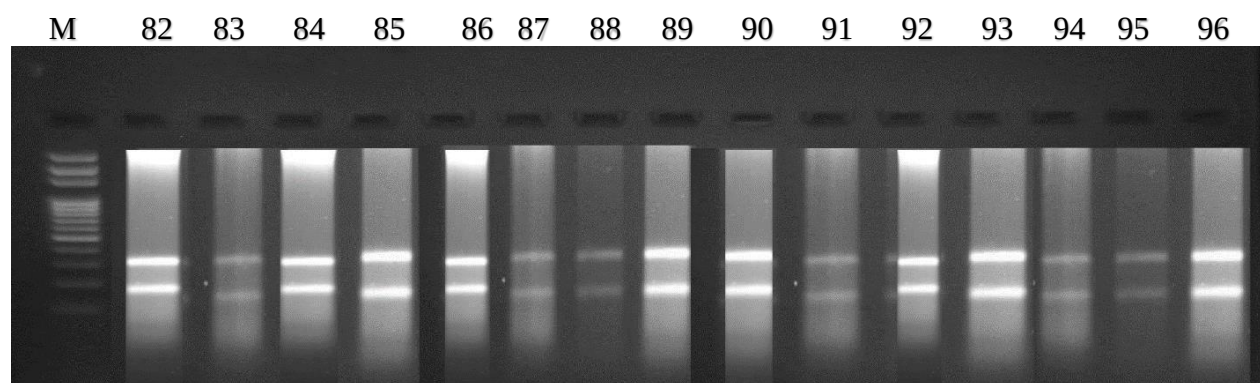
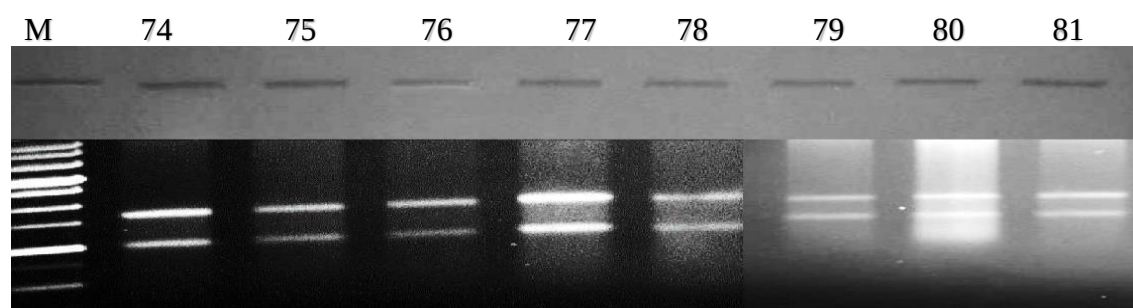
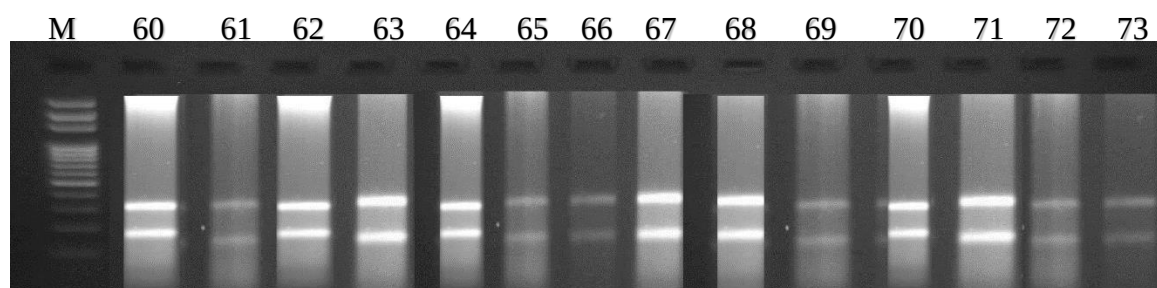


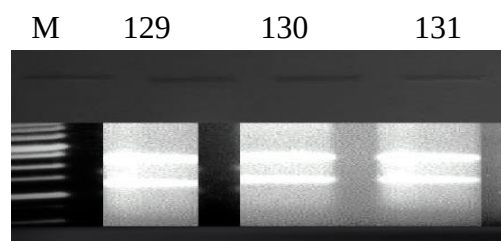
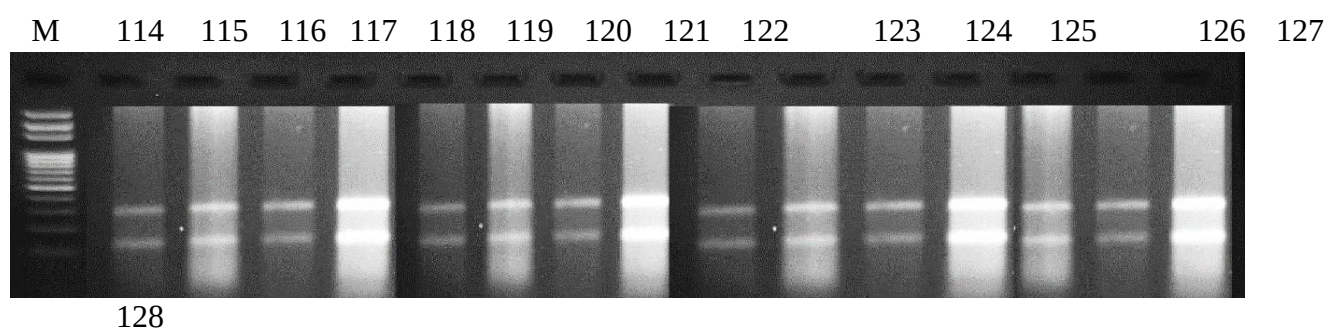
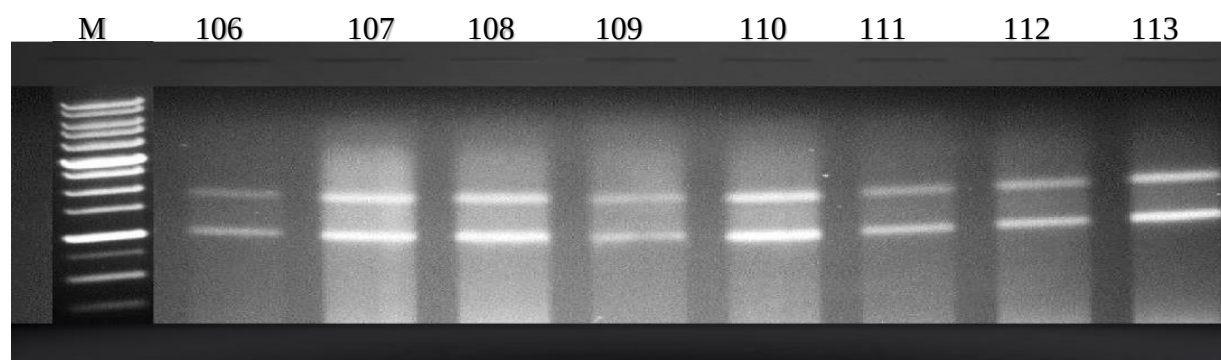
M 43 44 45 ` 46 47 48 49 50 51



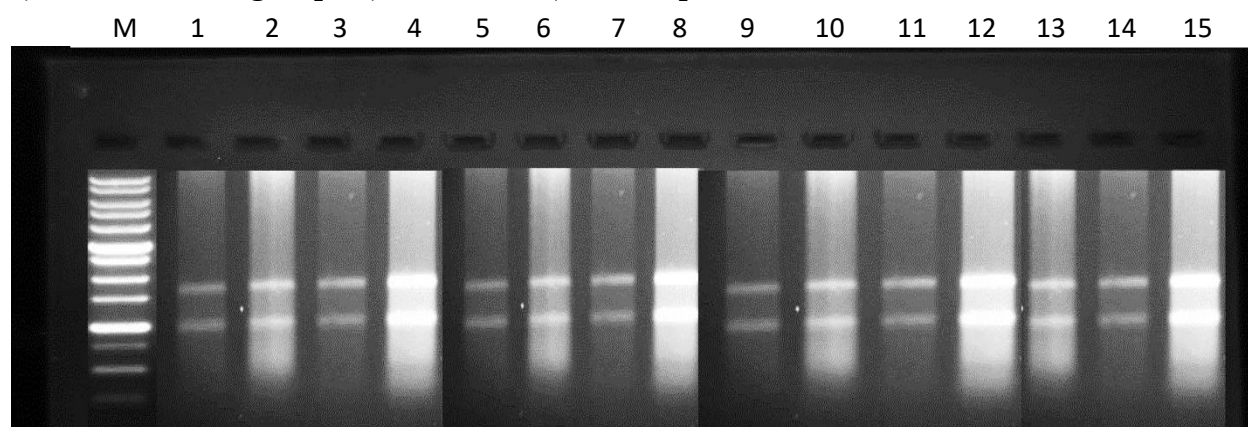
M 52 53 54 55 56 57 58 59 M

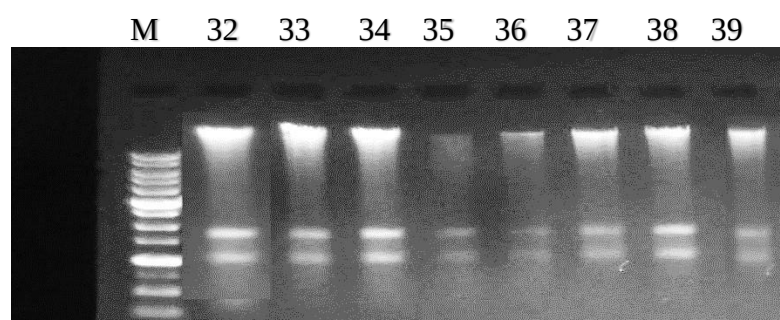
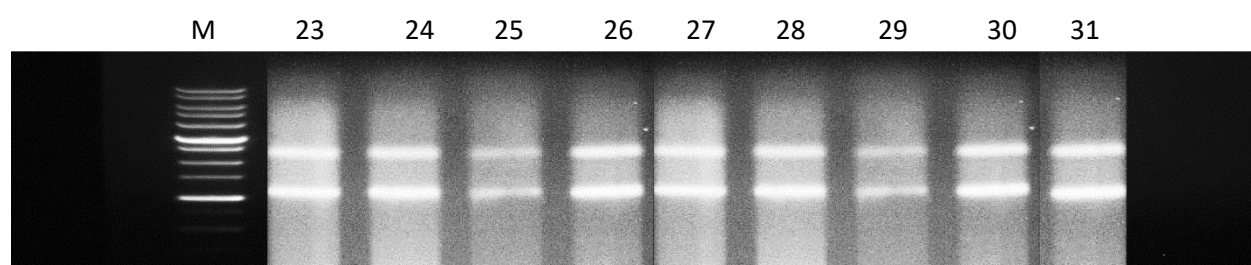
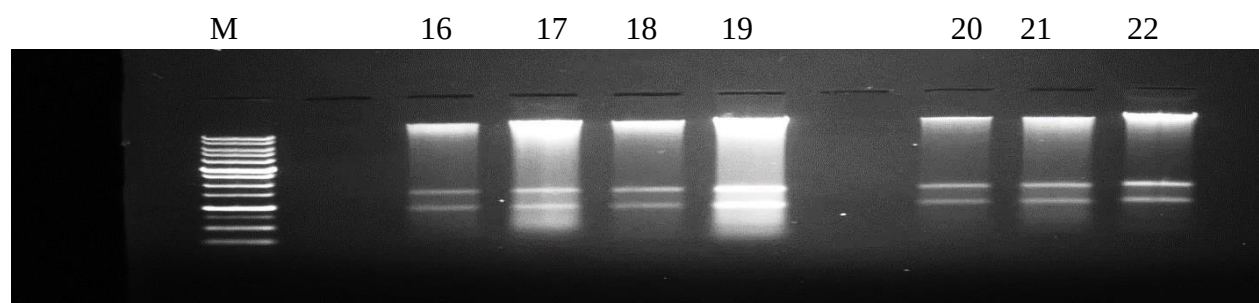




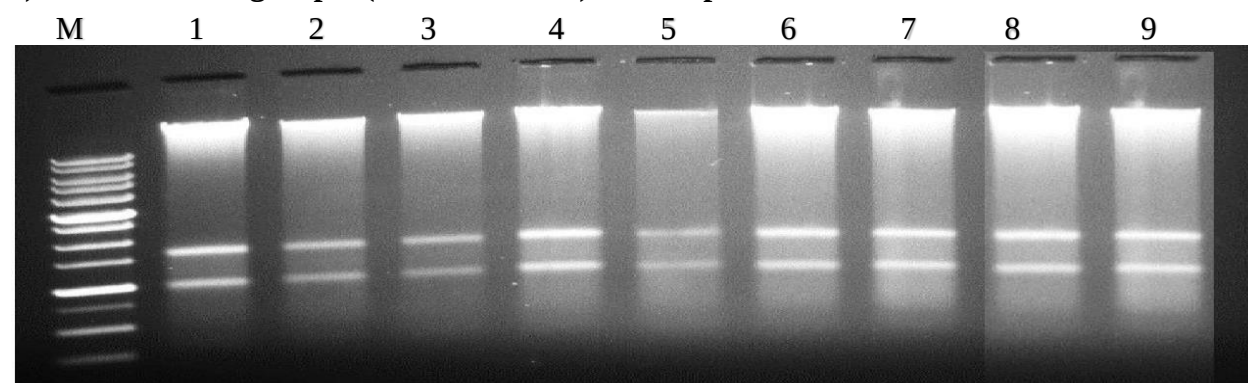


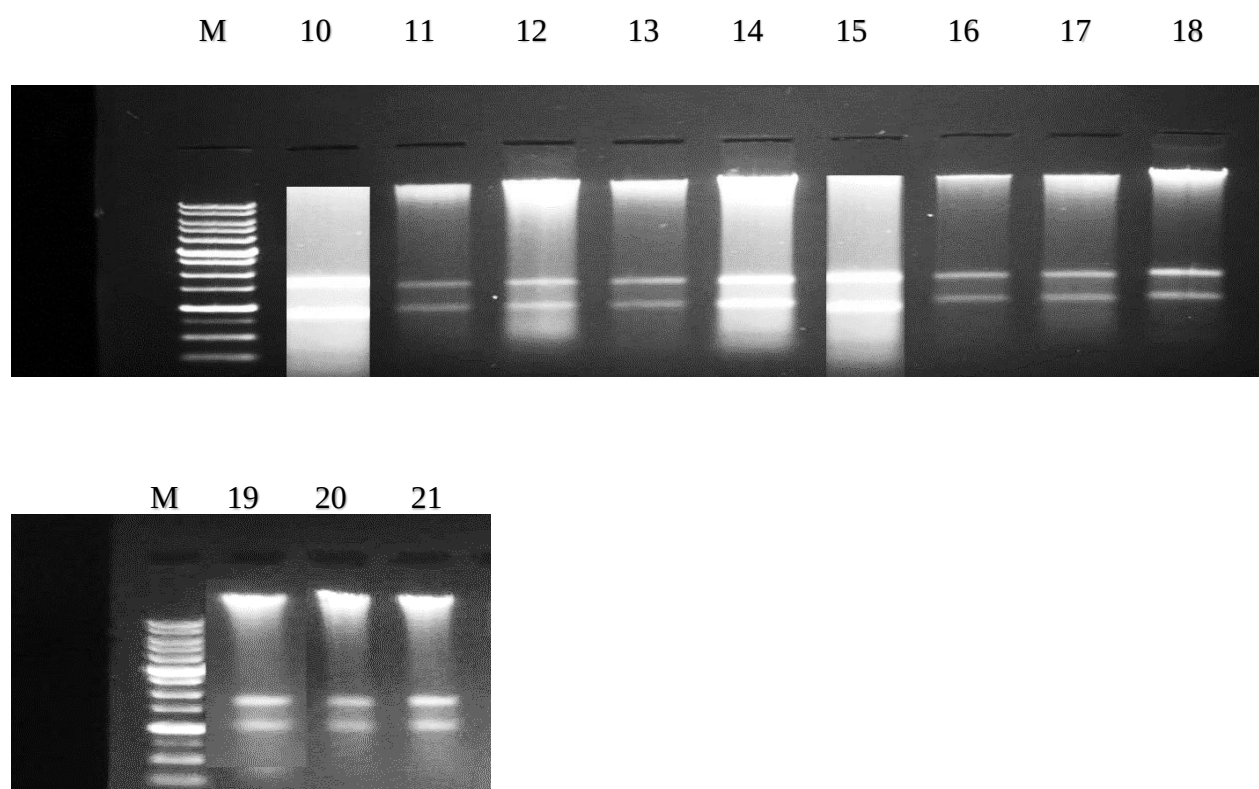
**c) Total RNA for group 3 (Mild malaria): 39 samples**





**c) Total RNA for group 4 (Severe malaria): 21 samples**





**Figure 4.14: Agarose gel electrophoretogram of total RNA extracted from whole blood of the study population in Sagbama LGA, Bayelsa State. M = molecular marker (1 kilo base pairs).**

#### **4.1.17. Immunomodulatory role of ABO blood group on TNF $\alpha$ gene expression in relation to malaria in Sagbama LGA**

Table 4.11 shows the immunomodulatory role of ABO blood group on TNF $\alpha$  gene expression in relation to malaria in Sagbama LGA. A significantly higher expression of TNF alpha gene was found among non-blood group O malaria subjects (blood group A, B and AB malaria subjects) compared with blood group O malaria subjects ( $p < \text{value } 0.001$ ).

**Table 4.11: Immunomodulatory role of ABO blood group on TNF $\alpha$  gene expression in relation to malaria**

| Relative gene expression of TNF $\alpha$ (fold change) |                                 |                                 |                                 |            |
|--------------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|------------|
| Phenotype                                              | Severe malaria                  | Mild malaria                    | Asymptomatic                    | Uninfected |
| A                                                      | 231.44 $\pm$ 24.34 <sup>a</sup> | 224.54 $\pm$ 31.12 <sup>a</sup> | 136.74 $\pm$ 60.39 <sup>a</sup> | 1          |
| B                                                      | 248.29 $\pm$ 21.21 <sup>a</sup> | 207.90 $\pm$ 35.39 <sup>a</sup> | 153.83 $\pm$ 64.40 <sup>a</sup> | 1          |
| AB                                                     | 226.68 $\pm$ 14.38 <sup>a</sup> | 208.11 $\pm$ 27.68 <sup>a</sup> | 126.52 $\pm$ 40.79 <sup>a</sup> | 1          |
| O                                                      | 180.92 $\pm$ 17.06 <sup>b</sup> | 116.17 $\pm$ 1.82 <sup>b</sup>  | 53.59 $\pm$ 11.45 <sup>b</sup>  | 1          |

Each value represents mean fold change  $\pm$  standard deviation. Values with different superscript on the same column are significantly different ( $p < 0.05$ ).

#### **4.1.18. Immunomodulatory role of ABO blood group on IL-10 gene expression in relation to malaria in Sagbama LGA**

Table 4.12 shows the immunomodulatory role of ABO blood group on IL-10 gene expression in relation to malaria in Sagbama LGA. A significantly higher expression of IL-10 gene was found among non-blood group O malaria subjects (blood group A, B and AB malaria subjects) compared with blood group O malaria subjects ( $p < \text{value } 0.001$ ).

**Table 4.12: Immunomodulatory role of ABO blood group on IL-10 gene expression in relation to malaria in Sagbama LGA**

| Relative gene expression of IL-10 (fold change) |                            |                            |                             |            |
|-------------------------------------------------|----------------------------|----------------------------|-----------------------------|------------|
| Phenotype                                       | Severe malaria             | Mild malaria               | Asymptomatic                | Uninfected |
| A                                               | 20.56 ± 2.58 <sup>b</sup>  | 42.54 ± 13.49 <sup>a</sup> | 126.28 ± 66.36 <sup>a</sup> | 1          |
| B                                               | 17.69 ± 4.76 <sup>a</sup>  | 42.12 ± 9.79 <sup>a</sup>  | 104.99 ± 62.37 <sup>a</sup> | 1          |
| AB                                              | 14.58 ± 2.00 <sup>a</sup>  | 83.30 ± 14.73 <sup>a</sup> | 122.28 ± 71.99 <sup>a</sup> | 1          |
| O                                               | 52.40 ± 11.31 <sup>c</sup> | 47.54 ± 13.98 <sup>b</sup> | 269.28 ± 19.61 <sup>b</sup> | 1          |

Each value represents mean fold change ± standard deviation. Values with different superscript on the same column are significantly different ( $p < 0.05$ ).

#### **4.1.19. Immunomodulatory role of ABO blood group on IFN $\gamma$ gene expression in relation to malaria in Sagbama LGA**

Table 4.13 shows the immunomodulatory role of ABO blood group on IFN $\gamma$  gene expression in relation to malaria in Sagbama LGA. A significantly higher expression of IFN $\gamma$  gene was found among non-bloodgroup O malaria subjects (blood group A, B and AB malaria subjects) compared with blood group O malaria subjects ( $p < \text{value } 0.001$ ).

**Table 4.13: Immunomodulatory role of ABO blood group on IFN $\gamma$  gene expression in relation to malaria in Sagbama LGA**

| Relative gene expression of IFN $\gamma$ (fold change) |                                 |                                 |                                 |            |
|--------------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|------------|
| Phenotype                                              | Severe malaria                  | Mild malaria                    | Asymptomatic                    | Uninfected |
| A                                                      | 231.62 $\pm$ 28.10 <sup>a</sup> | 207.90 $\pm$ 35.39 <sup>a</sup> | 171.71 $\pm$ 39.70 <sup>a</sup> | 1          |
| B                                                      | 252.93 $\pm$ 39.34 <sup>a</sup> | 201.56 $\pm$ 28.16 <sup>a</sup> | 164.17 $\pm$ 21.36 <sup>a</sup> | 1          |
| AB                                                     | 297.73 $\pm$ 20.88 <sup>a</sup> | 229.14 $\pm$ 30.57 <sup>a</sup> | 174.40 $\pm$ 11.67 <sup>a</sup> | 1          |
| O                                                      | 150.24 $\pm$ 28.10 <sup>b</sup> | 112.29 $\pm$ 6.87 <sup>b</sup>  | 122.60 $\pm$ 21.52 <sup>b</sup> | 1          |

Each value represents mean fold change  $\pm$  standard deviation. Values with different superscript on the same column are significantly different ( $p < 0.05$ ).

#### **4.1.20. Immunomodulatory role of haemoglobin genotype on TNF $\alpha$ gene expression in relation to malaria in Sagbama LGA**

Table 4.14 shows the immunomodulatory role of haemoglobin genotype on TNF $\alpha$  gene expression in relation to malaria in Sagbama LGA. A significantly higher expression of TNF $\alpha$  gene was found among Hb AA malaria subjects compared with Hb AS malaria subjects ( $p < \text{value } 0.001$ ).

**Table 4.14: Immunomodulatory role of haemoglobin genotype on TNF $\alpha$  gene expression in relation to malaria in Sagbama LGA**

| Haemoglobin |                                 | Relative gene expression of TNF $\alpha$ (fold change) |                                 |            |
|-------------|---------------------------------|--------------------------------------------------------|---------------------------------|------------|
| Phenotype   | Severe malaria                  | Mild malaria                                           | Asymptomatic                    | Uninfected |
| AA          | 240.21 $\pm$ 38.49 <sup>a</sup> | 207.24 $\pm$ 43.24 <sup>a</sup>                        | 97.43 $\pm$ 73.71 <sup>a</sup>  | 1          |
| AS          | 184.63 $\pm$ 17.63 <sup>b</sup> | 145.92 $\pm$ 64.73 <sup>b</sup>                        | 109.64 $\pm$ 60.19 <sup>a</sup> | 1          |

Each value represents mean fold change  $\pm$  standard deviation. Values with different superscript on the same column are significantly different ( $p < 0.05$ ).

#### **4.1.21. Immunomodulatory role of haemoglobin genotype on IFN $\gamma$ gene expression in relation to malaria in Sagbama LGA**

Table 4.15 shows the immunomodulatory role of haemoglobin genotype on IFN $\gamma$  gene expression in relation to malaria in Sagbama LGA. A significantly higher expression of IFN $\gamma$  gene was found among Hb AA malaria subjects compared with Hb AS malaria subjects ( $p < \text{value } 0.001$ ).

**Table 4.15: Immunomodulatory role of haemoglobin genotype on IFN $\gamma$  gene expression in relation to malaria in Sagbama LGA**

| Phenotype | Relative gene expression of IFN $\gamma$ (fold change) |                                 |                                 |            |
|-----------|--------------------------------------------------------|---------------------------------|---------------------------------|------------|
|           | Severe malaria                                         | Mild malaria                    | Asymptomatic                    | Uninfected |
| AA        | 237.58 $\pm$ 23.68 <sup>a</sup>                        | 205.85 $\pm$ 37.27 <sup>a</sup> | 118.64 $\pm$ 63.58 <sup>a</sup> | 1          |
| AS        | 179.01 $\pm$ 14.39 <sup>b</sup>                        | 168.03 $\pm$ 59.49 <sup>b</sup> | 61.45 $\pm$ 2.94 <sup>a</sup>   | 1          |

Each value represents mean fold change  $\pm$  standard deviation. Values with different superscript on the same column are significantly different ( $p < 0.05$ ).

#### **4.1.22. Immunomodulatory role of haemoglobin genotype on IL-10 gene expression in relation to malaria in Sagbama LGA**

Table 4.16 shows the immunomodulatory role of haemoglobin genotype on IL-10 gene expression in relation to malaria in Sagbama LGA. A significantly higher expression of IL-10 gene was found among haemoglobin AS subjects compared with haemoglobin AA malaria subjects ( $p < \text{value } 0.001$ ).

**Table 4.16: Immunomodulatory role of haemoglobin genotype on IL-10 gene expression in relation to malaria in Sagbama LGA**

| <b>Relative gene expression of IL-10 (fold change)</b> |                           |                            |                             |                   |
|--------------------------------------------------------|---------------------------|----------------------------|-----------------------------|-------------------|
| <b>Phenotype</b>                                       | <b>Severe malaria</b>     | <b>Mild malaria</b>        | <b>Asymptomatic</b>         | <b>Uninfected</b> |
| AA                                                     | 10.56 ± 2.48 <sup>a</sup> | 42.54 ± 13.49 <sup>a</sup> | 226.28 ± 66.36 <sup>a</sup> | 1                 |
| AS                                                     | 65.40 ± 9.31 <sup>b</sup> | 57.54 ± 13.98 <sup>b</sup> | 239.28 ± 19.61 <sup>a</sup> | 1                 |

Each value represents mean fold change ± standard deviation. Values with different superscript on the same column are significantly different ( $p < 0.05$ ).

#### **4.1.23 Immunomodulatory role of Rhesus blood group on TNF $\alpha$ gene expression in relation to malaria in Sagbama LGA**

Table 4.17 shows the immunomodulatory role of Rhesus blood group on TNF $\alpha$  gene expression in relation to malaria in Sagbama LGA. No significant difference in the expression of TNF $\alpha$  gene was found between Rhesus positive and negative malaria subjects.

**Table 4.17: Immunomodulatory role of Rhesus blood group on TNF $\alpha$  gene expression in relation to malaria in Sagbama LGA**

| Relative gene expression of TNF $\alpha$ (fold change) |                                 |                                 |                                |            |
|--------------------------------------------------------|---------------------------------|---------------------------------|--------------------------------|------------|
| Phenotype                                              | Severe malaria                  | Mild malaria                    | Asymptomatic                   | Uninfected |
| Rhesus+                                                | 210.21 $\pm$ 48.40 <sup>a</sup> | 150.24 $\pm$ 43.24 <sup>a</sup> | 67.43 $\pm$ 73.71 <sup>a</sup> | 1          |
| Rhesus-                                                | 204.63 $\pm$ 57.44 <sup>a</sup> | 145.92 $\pm$ 34.03 <sup>a</sup> | 64 $\pm$ 60.19 <sup>a</sup>    | 1          |

Each value represents mean fold change  $\pm$  standard deviation. Values with the same superscript on the same column are not significantly different at  $p < 0.05$ .

#### **4.1.24 Immunomodulatory role of Rhesus blood group on IFN $\gamma$ gene expression in relation to malaria in Sagbama LGA**

Table 4.18 shows the immunomodulatory role of Rhesus blood group on IFN $\gamma$  gene expression in relation to malaria in Sagbama LGA. No significant difference in the expression of IFN $\gamma$  gene was found between Rhesus positive and negative malaria subjects.

**Table 4.18: Immunomodulatory role of Rhesus blood group on IFN $\gamma$  gene expression in relation to malaria in Sagbama LGA**

| Phenotype | Relative gene expression of IFN $\gamma$ (fold change) |                                 |                                 |            |
|-----------|--------------------------------------------------------|---------------------------------|---------------------------------|------------|
|           | Severe malaria                                         | Mild malaria                    | Asymptomatic                    | Uninfected |
| Rhesus+   | 201.38 $\pm$ 22.68 <sup>a</sup>                        | 120.00 $\pm$ 37.27 <sup>a</sup> | 110.44 $\pm$ 63.58 <sup>a</sup> | 1          |
| Rhesus-   | 200.01 $\pm$ 33.32 <sup>a</sup>                        | 138.03 $\pm$ 59.49 <sup>a</sup> | 91.45 $\pm$ 42.84 <sup>a</sup>  | 1          |

Each value represents mean fold change  $\pm$  standard deviation. Values with the same superscript on the same column are not significantly different at  $p < 0.05$ .

#### **4.1.25. Immunomodulatory role of Rhesus blood group on IL-10 gene expression in relation to malaria in Sagbama LGA**

Table 4.19 shows the immunomodulatory role of Rhesus blood group on IL-10 gene expression in relation to malaria in Sagbama LGA. No significant difference in the expression of IL-10 gene was found between Rhesus positive and negative malaria subjects.

**Table 4.19: Immunomodulatory role of Rhesus factor blood group on IL-10 gene expression in relation to malaria in Sagbama LGA**

| Relative gene expression of IL-10 (fold change) |                           |                            |                             |            |
|-------------------------------------------------|---------------------------|----------------------------|-----------------------------|------------|
| Phenotype                                       | Severe malaria            | Mild malaria               | Asymptomatic                | Uninfected |
| Rhesus+                                         | 45.06 ± 5.28 <sup>a</sup> | 65.14 ± 10.49 <sup>a</sup> | 126.28 ± 66.36 <sup>a</sup> | 1          |
| Rhesus-                                         | 46.40 ± 9.31 <sup>a</sup> | 57.54 ± 13.98 <sup>a</sup> | 139.28 ± 19.61 <sup>a</sup> | 1          |

Each value represents mean fold change ± standard deviation. Values with the same superscript on the same column are not significantly different at  $p < 0.05$ .

## 4.2 Discussion

A comprehensive community based active case detection for malaria was carried out in the present study using two comparative molecular methods. This was done in order to determine which molecular method was most sensitive in determining *Plasmodium falciparum* infection active cases in Sagbama LGA, Bayelsa State, Nigeria. Over the years, the enormous case detection gap for malaria has resulted in late presentation at hospitals and poor disease outcome (Abiodun and Ilori, 2022). Active Case detection gap has also been reported as a major factor sustaining malaria transmission cycle (Ouma *et al.*, 2020). The call to eliminate malaria globally especially in Nigeria requires improvement in the current case detection method. Currently, the gold standard for malaria detection is microscopy (WHO, 2016), however as shown in the present study (Table 4.4), the use of microscopy for active malaria case detection gave false negatives who may never seek medical attention and even if they do might not be given malaria treatment (Lindblade *et al.*, 2013). Nested genomic PCR and the post real time PCR amplification high resolution melting analysis were the two molecular methods compared in the present study for malaria parasite detection. The present study found that high resolution melting analysis (HRMA) was more sensitive compared with nested genomic PCR. A lot of *Plasmodium falciparum* positive cases would have been missed if only microscopy was employed as the detection method in the present study.

The high proportion of asymptomatic malaria cases found in the present study using HRMA suggest the need to embark on treatment for asymptomatic malaria (Ogutu, Tiono, Makanga, 2010). Asymptomatic carriers do not seek medical help because they do not show any clinical

sign. Previous studies have also reported a high prevalence of asymptomatic malaria in other parts of the world (Ouma *et al.*, 2020, Chua *et al.*, 2015), however, a higher prevalence of asymptomatic malaria was found in the present study. This might probably be due to the fact that these previous studies were carried out outside sub-Saharan Africa where malaria is not as prevalent as in tropical sub-Saharan Africa (WHO, 2022).

Mapping and monitoring malaria reservoirs in tropical wetlands may facilitate malaria elimination interventions because tropical wetlands, are important hydrological features that serve as mosquito breeding habitats (Midekisa, Senay, & Wimberly, 2014). Several molecular PCR methods have been developed for the determination of *Plasmodium falciparum* infection (WHO, 2016), however, studies on the standard and most sensitive molecular method for *Plasmodium falciparum* determination has yielded conflicting result (Zhao *et al.*, 2017). HRM-analysis gave the highest prevalence of *Plasmodium falciparum* in the present study compared with nested genomic PCR and microscopy ( $P < 0.05$ ; Table 4.4). Therefore, HRM-analysis is recommended as a standard molecular method for future malaria parasite detection. In addition, the high prevalence of asymptomatic malaria in Bayelsa State calls for the need to include asymptomatic malaria detection and elimination in malaria control programs.

Although nested-genomic PCR was comparable with microscopy in the present study as reported in previous studies (Zhou *et al.*, 2014; Zhao *et al.*, 2017) however, it was less sensitive compared with HRM-analysis in the present study. Compared with nested-genomic PCR, HRM analysis was more sensitive, less laborious and less time consuming in the present study. Nested-genomic-PCR involves two separate PCR reactions, agarose gel electrophoresis and visualization of agarose gel under a transilluminator, making it a laborious and time process (Zhao *et al.*, 2017).

The present study provided the rare opportunity to detect asymptomatic malaria parasite infection in one of the most at risk populations around the globe. The contribution of asymptomatic malaria to the persistence of disease transmission was revealed in the present study. The asymptomatic subjects in the present study were apparently healthy with no malaria symptoms but some had high parasitemia (Table 4.3). This makes asymptomatic malaria a hidden reservoir for malaria transmission and an impediment to malaria elimination efforts.

The high parasitemia found among some apparently healthy asymptomatic malaria subjects in the present study is not in agreement with the low parasitemia usually reported among asymptomatic malaria subjects (Zhao *et al.*, 2017; Ouma *et al.*, 2020). This variation underscores the relevance of this study and call for malaria interventions to be directed to Sagbama LGA. The harmful effect of asymptomatic malaria to human health is that although they look healthy, however, the parasite can still be transmitted from them to other people around them especially to vulnerable groups like children, through mosquito bites (Chen *et al.*, 2016).

The association of all sociodemographic phenotypes in the present study with susceptibility to asymptomatic malaria ( $P < .05$ ), points to the high endemicity of malaria in Sagbama. This shows that once exposed, by way of finding oneself in a locality of high transmission, the malaria *Plasmodium* infection has no respect for age, sex, occupation, marital status nor level of education and possessing either of them will not guarantee the likelihood of protection against the risk of infection ( $P < .05$ ).

Variations in ABO/Rhesus blood group distribution was found in the present study. Blood group O was the most frequent in the present study, followed by blood group B, blood group A and the least frequent was blood group AB (Figure 4.7a). This distribution is consistent with previous studies carried out in Chongjing, China and South Ethiopia respectively (Alemu and mama, 2016; Liao and Li, 2020), but it is inconsistent with a previous study carried out in India where the

frequencies of blood group O and B were almost the same (Patidar, and Dhiman, 2021). In yet another study among the Mexican population, blood group A was the second most prevalent blood group after blood group O (Canizalez-Román, *et al.*, 2018). Also, in other regions such as Istanbul, Turkey, blood group A was the most common (Kayiran, Oktem, Kayiran, Paloglu, Gurakan, 2012) while blood group B was found to be the most common in District Swat, Pakistan (Khattak *et al.*, 2005). These results from various studies as well as results of the present study show that around the world, ABO blood group distribution varies. This variation in ABO blood group distribution around the world underscores the need to assess the pattern of blood group distribution among populations in different geographical regions. The present study reaffirms the heterogeneity in the distribution of ABO blood groups in different regions and races. The distribution of Rhesus factor D blood group was consistent with findings of a previous study, where Rh D positive blood group was 95.58% prevalent, and Rhesus D negative was 4.42% prevalent (Canizalez-Román *et al.*, 2018). This shows less geographical variation in Rhesus D distribution.

Furthermore, the pooled ABO/Rhesus blood group distribution found in the present study, is similar to findings of a previous study carried out by Canizalez-Roman *et al.*, (2018) where O+ blood group was the most frequent. However in contrast to the present study, blood group A- and AB- which were absent among the study population in the present study were present among the study population in the Canizalez-Román *et al.*, (2018) study. Studies on the geographical variations in blood group polymorphism distribution is fundamental to health and clinical practice because ABO/Rhesus blood groups have been associated with several diseases (Canizalez-Roman *et al.*, 2018; Abegaz, 2021). Unlike previous studies in Bayelsa State, to our knowledge, this is the first community based study on ABO/Rhesus blood group distribution and malaria in Bayelsa State, Nigeria in which a wider population distribution in terms of age, sex, occupation, tribe, hard to reach and educational level have been analysed (Egesie, Egesie, Usar, Johnbull, 2008; Onuoha,

Eledo, Young-Dede, & Agoro, 2015).

The distribution of haemoglobin genotype found in the present study (Figure 4.8) is consistent with reports from previous studies (Umoh, Abah, Ekanem, Essien, 2010; Ngwengi, Fon, Mbanya, 2020), however, a higher prevalence of Hb AS was found in the present study compared with findings of Ngwengi et al., (2020). Nearly 80% of individuals born with sickle cell anemia live in sub-Saharan Africa, where most *Plasmodium falciparum* malaria cases and deaths occur. This might be due to the fact that AS confers partial protection to lethal malaria (Piel *et al.*, 2013). However, genetic counselling has reduced the number of neonates born with haemoglobin S variant. This has led to an increase in the number of children born with genotype AA (Piel *et al.*, 2013). Haemoglobin genotype AA was predominantly distributed among symptomatic subjects, while haemoglobin genotype AS was skewed towards the asymptomatic malaria group. Association of haemoglobin genotype with malaria has been previously reported (Bougouma *et al.*, 2012) with individuals having genotype AA more susceptible to malaria parasite than AS and SS individuals. This is in agreement with the present findings. Compelling genetic evidence collected in recent years has showed that malaria contributed to shape the human genome (Penha-Goncalves, 2019).

The association of ABO blood group with malaria which was found in the present study (Table 4.6 and Figure 4.9), corroborates results of previous studies. A previous study carried out by Panda *et al.*, (2011) found an association between ABO blood group and malaria. Another study carried out by Afoakwah *et al.* (2016), also found similar result. However contrary results have been found in other studies. A study carried out by Onanuga and Lamikanra (2016) found no association between malaria infection and ABO blood groups. Also, another study carried out by Herrera, Montoya, Arboleda, & Ortiz (2009), found no statistically significant association of malaria with ABO bloodgroup. In the Herrera *et al.* (2009) study, however, severe malaria was more frequent among patients classified with blood group O but the association was not significant.

A previous study reported that blood group A was associated with about four times the risk of having malaria compared with blood group O (Tazebew, Munshea, & Nibret, 2021). Many previous studies on malaria and ABO blood group only reported the blood group with the highest proportion of malaria without stating whether or not there was an association (Panda *et al.*, 2011). Significant association helps to predict the risk a given blood group has to malaria disease. Blood group O was strongly associated with the risk of harbouring the parasite ( $P = .000$ ) under asymptomatic malaria study group in the present study (Figure 4.9). However blood group O was not associated with the transition from infection to the clinical manifestation of malaria. A previous study reported that blood group O had the highest infection (36%) and AB group the least (Abegaz, 2012). Although several previous studies have showed the association between ABO blood types and malaria, other studies refute the claim. This makes the exact conclusion on the association of ABO blood groups with malaria to fall into uncertainty due to inconsistent results (Abegaz, 2012). However the present study has helped to clear this uncertainty by demonstrating the association of blood group A, B and AB with malaria susceptibility and the non-association / protective role of blood group O to malaria.

Despite the importance of inflammation in the pathology and mortality induced by malaria, factors heightening inflammation in malaria is not fully understood. Before now, erythrocyte factors contributing to inflammation in malaria were yet to be fully characterized (Gallego-Delgado, Ty, Orengo, van de Hoef, & Rodriguez, 2014; Ademolue, Aniweh and Kusi, 2017). The present study has thus helped to fill this gap in research. However, the present study showed a significantly high gene expression of the proinflammatory  $TNF\alpha$  and  $IFN\gamma$  cytokines among malaria subjects with blood groups A, B and AB and haemoglobin AA compared with blood group O and Hb AS subjects (Tables 4.11, 4.13, 4.14 and 4.15). On the contrary, a significantly high gene expression of the anti-inflammatory IL-10 was found among blood group O and Hb genotype AS subjects compared with

blood group A, B and AB (Tables 4.12, and 4.16). This suggests that the mechanism by which ABO blood group and haemoglobin genotype is associated with malaria might probably be through the modulation of inflammation. The influence of blood groups and haemoglobin genotypes on inflammation among malaria subjects is hardly available. This makes the present study novel especially in Nigeria. Thus, the present study is of immense importance to health care

## CHAPTER FIVE

### CONCLUSION, RECOMMENDATION AND EXPECTED CONTRIBUTION TO KNOWLEDGE

#### 5.1 Conclusion

The present study determined the prevalence of malaria using comparative molecular methods and found that high resolution melting analysis was the most sensitive molecular method for malaria infection determination compared with nested genomic PCR. Also, a very high prevalence of malaria parasite infection was found among adults in Sagbama LGA.

Also, from the determination of the association of ABO/Rhesus blood groups and haemoglobin genotypes with malaria, the present study found the possible role of ABO antigen polymorphism in malaria with blood group O conferring protection to malaria while blood group A, B and AB conferred susceptibility to malaria. The pattern of distribution of ABO antigen polymorphism found in the present study in decreasing order was blood group O > B > A > AB. The present study also found haemoglobin genotype AA was the most prevalent genotype compared with haemoglobin genotype AS. Aside from being the most prevalent, the present study found that haemoglobin genotype AA conferred susceptibility to malaria compared with HbAS which conferred protection against malaria but not against acquiring the *Plasmodium falciparum* infection.

Furthermore, the present study found that the possible mechanism by which erythrocyte factors (haemoglobin genotypes and ABO/Rhesus blood groups) influenced malaria outcome was through differential expression of TNF alpha, interferon gamma (pro-inflammatory genes) and interleukin-10 (anti-inflammatory genes). Heterogeneity in inflammatory response in variants of haemoglobin genotypes and ABO/Rhesus factor was demonstrated in the present study. Further

malaria related molecular studies in Africa is highly recommended, this may lead to the development of diagnostic kits using genetic variants found in Africa rather than using European population variants. In the present study, adults who inherited blood group O and haemoglobin AS combination had high expression of anti-inflammatory IL-10 gene and a decreased risk of malaria compared with adults who had blood group A and haemoglobin genotype AA combination. Thus partial immunity to malaria in endemic areas might be based on inflammatory gene expression modulation by ABO/Rhesus blood group antigens and haemoglobin genotypes. Findings from the present study suggest that efforts aimed at targeting the ABO/Rhesus blood and haemoglobin genotypes may offer important treatment alternatives for malaria.

## **5.0 Recommendation**

Given the high prevalence of malaria in the present study, malaria interventions to Sagbama LGA is highly recommended. Also, given the high prevalence of asymptomatic malaria in the present study active asymptomatic malaria detection in wetlands using the very sensitive high resolution melting (HRM) analysis and treatment is recommended, since they have the potential of transmitting the parasite to others and at present treatment for asymptomatic malaria is been neglected. Also, further large scale study on the use of HRM analysis for malaria parasite detection is recommended. Government has a role to play in the early detection of asymptomatic malaria by providing real time PCR machine in rural health care centers for high resolution melting analysis based asymptomatic malaria detection. Furthermore, given the heterogeneity in inflammatory response to malaria due to differences in haemoglobin genotypes and ABO/Rhesus blood groups as reported in the present study, development of ABO/Rhesus factor and haemoglobin genotype based malaria intervention is recommended.

### 5.3 Contribution to knowledge

The present study showed that high resolution melting analysis is the most sensitive molecular method for *P. falciparum* determination. Thus, it can be used for future malaria determination, especially asymptomatic malaria. The present study was community based, this helped to capture individuals that may not seek medical help. To our knowledge, this study is the first of such study in Nigeria wetland. To date, diagnosis of malaria and asymptomatic malaria in Nigeria wetlands using the very sensitive HRM-analysis, has not been published. This study has thus filled the gap in research.

The present study has also provided information on the influence of ABO blood groups and haemoglobin genotypes on malaria outcome as well as the possible molecular mechanism for the observed influence of ABO blood groups and haemoglobin genotypes on malaria. Human evidence-based data that can be used as a blue print to plan for a scale up of malaria interventions and early detection using high resolution melting analysis has been provided in the present study. Stratification and better management of malaria subjects based on their blood groups and haemoglobin genotypes is implicated in the present study.

The present study also showed that the mRNA transcripts of some cytokines in the immune system are differentially expressed in relation to blood groups and genotypes during malaria. Thus, information on potential transcriptomic pathways that could be targeted for immunotherapy based antimalarial drug design has been revealed. Also, information on African based gene expression and phenotypic signatures which could be translated into fresh approaches for clinical treatment of malaria in Africa has also been revealed in the present study. It is hoped that findings of the present study will foster the development of future therapeutics to reduce the burden of malaria morbidity and mortality.

## References

- Abah, A. and Temple B. (2016). Prevalence of Malaria Parasite among Asymptomatic Primary School Children in Angiama Community, Bayelsa State, Nigeria. *Tropical Medicine and Surgery*, 4(203), 1-3.
- Abegaz, SB. (2021). Human ABO blood groups and their associations with different diseases. *Journal of Biomed Research International*, 6629060. doi: 10.1155/2021/6629060.
- Abiodun, M.T. and Ilori, O.R. (2022). Caregivers' perception and determinants of delayed presentation of children with severe malaria in an emergency room in Benin City, Nigeria *Nigerian Postgraduate Medical Journal*, 29(3), 198-205.
- Adediran, O.A., Oluleye, T.S., Ayorinde, O.O., Ugalahi, M.O. and Mpyet, C.D. (2022). Survey of blindness in Saki East, Oyo State, Nigeria. *Journal of West African College Surgeons*, 12(1), 41-47.
- Afoakwah, R., Aubyn, E., Prah, J., Nwaefuna, E.K., and Boampong, J. N. (2016). Relative Susceptibilities of ABO Blood Groups to *Plasmodium falciparum* Malaria in Ghana. *Journal of Advances in Hematology*, 5368793, 1-4, doi.org/10.1155/2016/5368793.
- Ademolue, T.W., Aniweh, Y. and Kusi, K.A. (2017). Patterns of inflammatory responses and parasite tolerance vary with malaria transmission intensity. *Malaria Journal*, 16(145), doi.org/10.1186/s12936-017-1796-x.
- Alemu, G. and Mama, M. (2016). Assessing ABO/Rh Blood group frequency and association with asymptomatic malaria among blood donors attending Arba Minch Blood Bank, South Ethiopia. *Journal of Malaria Research and Treatment*, 8043768. doi: 10.1155/2016/8043768.

- Aryal, S. (2022). Polymerase chain reaction (PCR) – Principle, steps, application. Available at:  
Polymerase Chain Reaction (PCR)-Principle, Steps, Applications.  
<https://microbenotes.com/polymerase-chain-reaction-pcr-principle-steps-applications/>
- Archer, N.M., Petersen, N., Clark, M.A., Buckee, C.O., Childs, L.M. and Duraisingh, M.T. (2018). Resistance to *Plasmodium falciparum* in sickle cell trait erythrocytes is driven by oxygendependent growth inhibition. *Proceedings of the National Academy of Sciences of the United States of America*, 115(28), 7350-7355.
- Baldwin, M.R., Li, X. and Hanada, T. (2015). Merozoite surface protein 1 recognition of host glycophorin A mediates malaria parasite invasion of red blood cells. *Journal of Blood*, 125, 2704–2711.
- Band, G., Le, Q.S., Jostins, L., Pirinen, M., Kivinen, K., Jallow, M., ... & Spencer, C.C.A. (2013). Imputation-based meta-analysis of severe malaria in three African populations. *PLoS Genetics*, 9(5), doi: 10.1371/journal.pgen.1003509
- Bayelsa Investment Promotion Agency (2022). Discover Bayelsa.  
<https://explore.investbayelsa.by.gov.ng/sagbama-lga/>
- Bayelsa State Ministry of Health and FHI 360 (2013). Bayelsa State-wide Rapid Health Facility Assessment, Nigeria: Bayelsa State Ministry of Health and FHI 360.  
<https://www.fhi360.org/sites/default/files/media/documents/Bayelsa%20RHFA%20report.pdf>.
- Bougouma, E.C., Tiono, A.B., Ouédraogo, A., Soulama, I., Diarra, A., Yaro, J.B... & Sirima, S.B. (2012). Haemoglobin variants and *Plasmodium falciparum* malaria in children under five years of age living in a high and seasonal malaria transmission area of Burkina Faso. *Malaria Journal*, 11(154), <https://doi.org/10.1186/1475-2875-11-154>.

Brody, L. (2023). Gene expression. Division of Genomics and Society, National Human Genome Research Institute. <https://www.genome.gov/genetics-glossary/Gene-Expression>.

Canizalez-Román, A., Campos-Romero, A., Castro-Sánchez, J.A., López-Martínez, M.A., Andrade-Muñoz, F.J., Cruz-Zamudio, C.K., ... & Alcántar-Fernández J. (2018). Blood Groups Distribution and Gene Diversity of the ABO and Rh (D) Loci in the Mexican population. *Biomed Research International*. 1925619. doi: 10.1155/2018/1925619.

Centers for Disease Control and Prevention (2016). Blood Specimens - Molecular Diagnosis. Global Health, Division of Parasitic Diseases and Malaria, 1600 Clifton Rd. Atlanta, GA 30329, United States.

Centers for Disease Control and Prevention (2018). Drug resistance in the malaria endemic world. Global Health, Division of parasitic diseases and malaria, 1600 Clifton Rd. Atlanta, GA 30329, United States.

Centers for Disease Control and Prevention (2020). Malaria – About malaria – Biology. Global Health, Division of parasitic diseases and malaria, 1600 Clifton Rd. Atlanta, GA 30329, United States.

Centers for Disease Control and Prevention (2022). Malaria disease. Global Health, Division of parasitic diseases and malaria, 1600 Clifton Rd. Atlanta, GA 30329, United States.

Charan, J. and Biswas, T. (2013). How to calculate sample size for different study designs in medical research. *Indian Journal of. Psychological Medicine*, 35(21), 121-126.

- Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X. and Zhao, L. (2017). Inflammatory responses and inflammation-associated diseases in organs. *Journal of Oncotarget*, 9(6), 7204-7218.
- Chen, I., Sian, E.C., Gosling, R., Hamainza, B., Killeen, G. and Magill A. (2016). Asymptomatic malaria a chronic and debilitating infection that should be treated. *Plos Medicine*, 13(1), e1001942. doi: 10.1371/journal.pmed.1001942.
- Chipwaza, B. and Sumaye, R.D. (2020). High malaria parasitemia among outpatient febrile children in low endemic area, East-Central Tanzania in 2013. *BMC Research Notes*, 13(251), doi: 10.1186/s13104-020-05092-4.
- Chua, H.K., Lim, S.C., Ng, C.C., Lee, P.C., Lim, Y.L. and Lau, T.P. (2015). Development of high resolution melting analysis for the diagnosis of human malaria. *Scientific Reports*, 28(5),15671. doi: 10.1038/srep15671.
- Cid E, Yamamoto M, Yamamoto F. (2018). Blood group ABO gene-encoded A transferase catalyzes the biosynthesis of FORS1 antigen of FORS system upon Met69Thr/Ser substitution. *Blood Advances*, 2(12):1371-1381.
- Dean, L. (2005). Blood Groups and Red Cell Antigens [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); Chapter 5, The ABO blood group. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK2267/>
- Dermitzakis, E. (2008). From gene expression to disease risk. *Nature Genetics*, 40, 492–493.
- Deroost, K., Pham, T.T., Opdenakker, G. and Van den Steen PE. (2016). The immunological balance between host and parasite in malaria. *FEMS Microbiology Reviews*, 40(2), 208-257.
- Dinareello, C.A. (2004). Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed. *Journal of Endotoxin Research*, 10(4), 201-222.

- Dugue, A.G. and Descoteaux, A. (2014). Macrophage cytokines: involvement in immunity and infectious diseases. *Frontiers in Immunology*, 7(5). 491. doi: 10.3389/fimmu.2014.00491
- Dunst, J., Kamena F., and Matuschewski, K. (2017). Cytokines in cerebral malaria pathogenesis. *Journals of Frontiers in Cellular and Infection Microbiology*, 7(324), 1-16. doi: 10.3389/fcimb.2017.00324.
- Dwivedi, S., Purohit, P., Misra, R., Pareek, P., Goel, A., Khattri, S., Pant, K.K., Misra, S. and Sharma, P. (2017). Diseases and Molecular Diagnostics: A Step Closer to Precision Medicine. *Indian Journal of Clinical Biochemistry*, 32(4), 374-398.
- Ebenezer, A., and Eekpa, P. (2018). Prevalence of *Plasmodium falciparum* Among New Intakes in the Isaac Jasper Boro College of Education, Sagbama, Bayelsa State, Nigeria. *American Journal of Biomedical and Life Sciences*, 6(2):33-36.
- Ellulu, M.S., Patimah, I., Khaza'ai, H., Rahmat, A., and Abed Y. (2017). Obesity and inflammation: the linking mechanism and the complications. *Archives of Medical Science*, 13(4), 851-863.
- El-Radhi, A.S. (2018). Pathogenesis of Fever. *Clinical Manual of Fever in Children*. 2009, 47–61. doi: 10.1007/978-3-540-78598-9\_3.
- Cid, E., Yamamoto, M., Yamamoto F. (2018). Blood group ABO gene–encoded A transferase catalyzes the biosynthesis of FORS1 antigen of FORS system upon Met69Thr/Ser substitution. *Blood Advances*, 2 (12), 1371–1381.
- Egesie, U.G., Egesie, O.J., Usar, I. and Johnbull, T.O. (2008). Distribution of ABO, Rhesus blood groups and haemoglobin electrophoresis among the undergraduate students of Niger delta niversity Nigeria. *Nigerian Journal of Physiological Sciences*, 23(1-2), 5-8.

- Futagbi, G., Out, P.S., Abdul-Rahman, M., Aidoo, E.K., Lo, A.C., Gyan, B.A., Afrane, A.Y. and Amoah, I.E. (2022). Association of TNF-Alpha, MBL2, NOS2, and G6PD with malaria outcomes in people in Southern Ghana. *Journal of Genetics Research*, 6686406. doi: 10.1155/2022/6686406.
- Gallego-Delgado, J., Ty, M., Orenge, J.M., van de Hoef, D. and Rodriguez A. (2014). A surprising role for uric acid: the inflammatory malaria response. *Current Rheumatology Reports*, 16(2), 401. doi: 10.1007/s11926-013-0401-8.
- Gimenez, A.M., Marques, R.F., Regiart, M. and Bargieri, D.Y. (2021). Diagnostic methods for non-*falciparum* malaria. *Frontiers in Cellular and Infection Microbiology*, 11:681063.
- Gilson, P.R., Nebl, T. and Vukcevic, D. (2006). Identification and stoichiometry of glycosylphosphatidylinositol-anchored membrane proteins of the human malaria parasite *Plasmodium falciparum*. *Molecular and Cellular Proteomics*, 5(7), 1286–1299.
- Glennon, E.K.K., Dankwa, S., Smith, J.D. and Kaushansky, A. (2018). Opportunities for Hosttargeted Therapies for Malaria. *Trends in Parasitology*, 34(10), 843-860.
- Goncalves, M.R., Lima, N.F. and Ferreira, M.U. (2014). Parasite virulence, co-infections and cytokine balance in malaria. *Journal of Pathogens and Global Health*, 108(4), 173-178.
- Gong, R., and Li, S. (2014). Extraction of human genomic DNA from whole blood using a magnetic microsphere method. *International Journal of Nanomedicine*, 9, 3781-3789.
- Itadani, H, Mizuarai, S, and Kotani, H. (2008). “Can systems biology understand pathway activation? Gene expression signatures as surrogate markers for understanding the complexity of pathway activation” *Current Genomics*, 9(5): 349 – 360.

- Hassanpour, G., Mirhendi, H., Mohebbali, M., Raeisi, A., Zeraati, H. and Keshavarz H. (2016). Simplified Pan-species Real-time PCR-based detection of *Plasmodium Spp.* in blood smear. *Iranian Journal of Parasitology*, 11(4), 463–470.
- Herrera, A.M., Montoya, L.P., Arboleda, M., and Ortiz, L.F. (2009). Association of severe malaria with ABO-blood group types in an endemic zone of Colombia. *Ces Medicina*, 23(2), 7-14.
- Hodgson, S.H., Muller, J. and Lockstone, H.E. (2019). Use of gene expression studies to investigate the human immunological response to malaria infection. *Malaria Journal*, 18(418), <https://doi.org/10.1186/s12936-019-3035-0>.
- Jajosky, R. P., Wu, S. C., Zheng, L., Jajosky, A.N., Jajosky, P. G., Josephson, C.D., Hollenhorst, M. A., Sackstein, R., Cummings, R. D., Arthur, C. M., Stowell, S. R. (2022). ABO blood group antigens and differential glycan expression: Perspective on the evolution of common human enzyme deficiencies. *Journal of iScience*, 26(1):105798. doi: 10.1016/j.isci.2022.105798
- Jefferies, C.A. and Fitzgerald, K.A. (2005). Interferon gene regulation: not all roads lead to Tolls. *Trends in Molecular Medicine*, 11(9), 403 – 411.
- Jude, P.J., Dharshini, S. and Vinobaba, M. (2010). *Anopheles culicifacies* breeding in brackish waters in Sri Lanka and implications for malaria control. *Malaria Journal*, 9(106), [doi.org/10.1186/1475-2875-9-106](https://doi.org/10.1186/1475-2875-9-106).
- Kayiran, S.M., Oktem, O., Kayiran, P.G., Paloglu, E. and Gurakan, B. (2012). Frequency of ABO and rhesus blood groups among neonates born at a private hospital in Istanbul. *Southeast Asian Journal of Tropical Medicine and Public Health*. 43(2), 467-470.

- Khanna, D., Khanna, S., Khanna, P., Kahar, P. and Patel, B.M. (2022). Obesity: A Chronic Low-Grade Inflammation and Its Markers. *Cureus, Journal of Medical Science*, 14(2), e22711. doi: 10.7759/cureus.22711.
- Khattak, I.D., Khan, T.M., Khan, P., Shah, S.M., Khattak, S.T. and Ali, A. (2008). Frequency of ABO and Rhesus blood groups in District Swat, Pakistan. *Journal of Ayub Medical College Abbottabad*, 20(4), 127-129.
- Khosa, S.M., Usman, M., Moinuddin, M., Mehmood, H. and Qamar, K. (2015). Comparative analysis of cellulose acetate hemoglobin electrophoresis and high performance liquid chromatography for quantitative determination of hemoglobin. *British Journal of Haematology*, 50(1), 46-50.
- Knight, J.C. (2023). Parasite wars: Human Genetic Diversity: Functional Consequences for Health and Disease. Oxford, Online edn, Oxford, Oxford Academic, 341-364. doi.org/10.1093/acprof:oso/9780199227693.003.0013.
- Krishnegowda, G., Hjjar, A.M. Zhu, J., Douglass, E.J., Uematsu, S. and Akira, S. (2005). Induction of proinflammatory responses in macrophages by the glycosylphosphatidylinositols of *Plasmodium falciparum*: cell signaling receptors, glycosylphosphatidylinositols (GPI) structural requirement, and regulation of GPI activity. *Journal of Biological Chemistry*, 280, 8606-8616.
- Kwok, A.J., Mentzer, A. and Knight, J.C. (2021). Host genetics and infectious disease: new tools, insights and translational opportunities. *Nature Reviews Genetics*, 22, 137–153.
- Leão, L., Puty, B., Dolabela, M.F., Povia, M.M., Né, Y.G.S., Eiró, L.G., ... Lima, R.R. (2020). Association of cerebral malaria and TNF- $\alpha$  levels: a systematic review. *BMC Infectious Diseases*, 20(1), 442.

- Li, P., Zhao, Z., Xing, H., Li, W., Zhu, X., Cao, Y. ... Cui, L. (2016). Plasmodium malariae and Plasmodium ovale infections in the China-Myanmar border area. *Malaria Journal*, 15(557), doi.org/10.1186/s12936-016-1605-y.
- Liao, H. B. and Li, J. (2020). Distribution characteristics of ABO and RhD blood groups among the voluntary blood donors in Chongqing: A retrospective study. *Journal of Medicine* 99(42), e22689. doi: 10.1097/MD.00000000000022689.
- Lin, C.S., Uboldi, A.D., Epp, C., Bujard, H., Tsuboi, T., Czabotar, P.E., & Cowman, A.F. (2016). Multiple *Plasmodium falciparum* Merozoite Surface Protein 1 Complexes Mediate Merozoite Binding to Human Erythrocytes. *Journal of Biological Chemistry*, 291(14):7703-7715.
- Lindblade, K.A., Steinhardt, L., Samuels, A., Kachur, S.P. and Slutsker, L. (2013). The silent threat: asymptomatic parasitemia and malaria transmission. *Expert Review of Anti-Infective Therapy*, 11(6), 623-639.
- Livak, K.J. and Schmittgen, T.D. (2008). Analyzing real-time PCR data by the comparative CT method. *Nature Protocols*, 3, 1101-1108.
- Luckheeram, R. V., Zhou, R., Verma, A. D. and Xia, B. (2012). CD4<sup>+</sup>T cells: differentiation and functions. *Clinical and Developmental Immunology*, 925135, doi: 10.1155/2012/925135.
- Mahittikorn, A., Mala, W., Masangkay, F.R., Kotepui, K.U., Wilairatana, P. and Kotepui M. (2022). Increased interferon- $\gamma$  levels and risk of severe malaria: a meta-analysis. *Scientific Reports*, 12(1):1891.
- Marengo-Rowe, A.J. (2006). Structure-function relations of human hemoglobins. *Baylor University Medical Center Proceedings*, 19(3), 239-245.

- Marshall, J.S., Warrington, R., Watson, W. and Kim, H.L. (2018). An introduction to immunology and immunopathology. *Allergy Asthma and Clinical Immunology*, 14(2), 49.
- Matuschik, L., Riabov, V., Schmuttermair, C., Sevastyanova, T., Weiss, C., Klüter, H. and Kzhyshkowska, J. (2022). Hyperglycemia Induces Inflammatory Response of Human Macrophages to CD163-Mediated Scavenging of Hemoglobin-Haptoglobin Complexes. *International Journal of Molecular Sciences*. 23(3), 1385, doi: 10.3390/ijms23031385.
- Marui, S., Misawa, A., Tanaka, Y. and Nagashima, K. (2017). Assessment of axillary temperature for the evaluation of normal body temperature of healthy young adults at rest in a thermoneutral environment. *Journal of Physiological Anthropology*, 36(1), 18, 925135. doi: 10.1155/2012/925135.
- Midekisa, A., Senay, G.B., and Wimberly, M.C. (2014). Multisensor earth observations to characterize wetlands and malaria epidemiology in Ethiopia, *Water Resources Research*, 50, 8791–8806.
- Mohamedahmed, K.A., Abakar, A.D., Ahmed, M.O., Mukhtar, M.M., Yousif, B. and Nour, M. (2019). The Role of TNF- $\alpha$  Levels as Predictive Diagnostic Biomarker among Children with Severe Falciparum Malaria in Endemic Area in Sudan. *International Journal of Academic Health and Medical Research*, 3(7), 1-6.
- Naing, C., Htet, N.H., Aye, S.N., Aung, H.H., Tanner, M. and Whittaker, M.A. (2022). Detection of asymptomatic malaria in Asian countries: a meta-analysis of diagnostic accuracy. *Malaria Journal*, 21(1), 50. doi.org/10.1186/s12936-022-04082-0

- Nasr, A., Allam, G. and Hamid, O. (2014). IFN-gamma and TNF associated with severe falciparum malaria infection in Saudi pregnant women. *Malaria Journal*, 13(314), doi.org/10.1186/1475-2875-13-314.
- National Malaria Elimination Programme (NMEP) [Nigeria], National Population Commission (NPC) [Nigeria], and ICF. 2016. Nigeria Malaria Indicator Survey 2015 Final Report. Abuja, Nigeria, and Rockville, Maryland, USA.
- National Malaria Elimination Programme (NMEP), (2019). Malaria Programme Review 2019 Department of Public Health, Ministry of Health Abuja, Nigeria.
- National Malaria Elimination Programme (NMEP) [Nigeria], National Population Commission (NPC) [Nigeria], and ICF. 2022. Nigeria Malaria Indicator Survey 2021 Final Report. Abuja, Nigeria, and Rockville, Maryland, USA: NMEP, NPC, and ICF.
- Ngwengi, N.Y., Fon, P.N. and Mbanya, D. (2020). Distribution of haemoglobin genotypes, knowledge, attitude and practices towards sickle cell disease among unmarried youths in the Buea Health District, Cameroon. *Pan African Medical Journal*, 37(109), doi: 10.11604/pamj.2020.37.109.17864. 3120256063
- Niger Delta Budget Monitoring Group (2022). Overview of Bayelsa State. Retrieved from <https://www.nigerdeltabudget.org/overview-of-bayelsa/>
- Nwaefuna, E.K., Ibalafake, B.I., Gbogbo, F., and Osae, M. (2019). Oviposition and Development of *Anopheles coluzzii* coetzee and Wilkerson in Salt Water. *Malaria Research and Treatment*. 9523962. doi: 10.1155/2019/9523962.
- Ogbole, F.A. and Fatusi, A.O. (2020). Co-occurrence of health risk behaviours for communicable and non-communicable diseases in a tertiary institution in South West Nigeria. *International Journal of Research and Scientific Innovation*, 7(1), 10-14.

- Ogbole, F.A. and Oyelana, O. (2020). Health risk assessment of the drinking water from Sagbama River, Bayelsa State, Niger Delta, Nigeria. *International Journal of Scientific & Engineering Research*, 11(9):1455-1460.
- Ogbole, F.A. (2021). Urinalysis for dehydration, kidney injury and urinary tract infection assessment in rural riverside, Bayelsa State, Nigeria. *International Journal of Innovative Science and Research Technology*, 6(12), 806–810.
- Ogutu, B., Tiono, A.B. and Makanga, M. (2010). Treatment of asymptomatic carriers with artemetherlumefantrine: an opportunity to reduce the burden of malaria? *Malaria Journal* 9(30), doi.org/10.1186/1475-2875-9-30.
- Oladimeji, O. E. and Ohwo, O. (2022). Assessment of Flood Risk and Mapping of Flood Risk Zones in Yenagoa, Bayelsa State, Nigeria. *Journal of Applied Sciences and Environmental Management*, 26(2):219-226.
- Omoweh, D.A. (2009). The Paradox of Water Crisis and Rural Poverty in the Niger Delta of Nigeria: The Case of Bayelsa State. *OpenEdition Books*, 201 – 220.
- Onanuga, A. and Lamikanra, A. (2016) Association of ABO blood group and *Plasmodium falciparum* malaria among children in the Federal Capital Territory. *African Journal of Biomedical Research*, 19(1), 1-6.
- Onuoha, E.C., Eledo, B.O., Young Dede, E.U., and Agoro, E.S. (2015). Distribution of ABO, Rhesus Blood Groups and Haemoglobin Variants among Residents of Yenagoa and Environs, Bayelsa State, Nigeria. *Advances in Life Science and Technology*, 34, 26-31.
- Ouma, F.F., Nateghpour, M., Haghi, A.M., Mohebal, M., Farivar, L. and Hosseini-Safa A. (2020). Application of high-resolution melting (HRM) technique towards the detection of asymptomatic malaria in a malaria endemic area of Southeastern Iran under elimination program. *Journal of Arthropod-Borne Diseases*, 14(4), 353-362.

- Onyiri N. (2015). Estimating malaria burden in Nigeria: a geostatistical modelling approach. *Geospatial Health*, 10(2), 306. doi: 10.4081/gh.2015.306.
- Oyeyemi, A.S, Davids, K.I., Edeki, S.O., Leghemo, K.T. and Eguvbe, A.O. (2021). Galvanizing Communities towards Zero Malaria in Nigeria: An Account of 2019 World Malaria Day in Bayelsa State. *Journal of Biomedical Research and Clinical Practice*, 4, 105-108.
- Panda, A.K., Panda, S.K., Sahu, A.N. Tripathy, R., Ravindran, B. and Das, K. B. (2011). Association of ABO blood group with severe falciparum malaria in adults: case control study and meta-analysis. *Malaria Journal*, 10(309). doi.org/10.1186/1475-2875-10-309
- Patidar, G.K. and Dhiman, Y. (2021), Distribution of ABO and Rh (D) Blood groups in India: A systematic review. *International Society of Blood Transfusion*, 16, 37-48.
- Patrick, D.M, Van Beusecum, J.P. and Kirabo, A. (2021). The role of inflammation in hypertension: novel concepts. *Current Opinion Physiology*, 19, 92-98.
- Penha-Gonçalves, C. (2019). Genetics of Malaria Inflammatory Responses: A Pathogenesis Perspective. *Frontiers in Immunology*, 10(1771), doi: 10.3389/fimmu.2019.01771.
- Piel, F.B., Patil, A.P., Howes, R.E., Nyangiri, O.A., Gething, P.W., Dewi, M, Temperley, W.H., Williams, T.N., Weatherall, D.J. and Hay, S.I. (2013). Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. *Lancet*, 381(9861):142-151.
- Piperaki, E.T. and Daikos, G.L. (2016). Malaria in Europe: emerging threat or minor nuisance? *Clinical Microbiology and Infection*, 22(6), 487-493.
- Pleasants, S. (2014). Epidemiology: A moving target. *Nature* 515, S2. doi.org/10.1038/515S2a.

- Popa, G.L. and Popa, M.I. (2021). Recent Advances in Understanding the Inflammatory Response in Malaria A Review of the Dual Role of Cytokines. *Journal of Immunology Research*, 7785180. doi: 10.1155/2021/7785180.
- Prusty, D., Gupta, N., Upadhyay, A., Dar, A., Naik, B., Kumar, N., and Prajapati, V.K. (2021). Asymptomatic malaria infection prevailing risks for human health and malaria elimination. *Infection Genetics and Evolution*, 93, 104987. doi: 10.1016/j.meegid.2021.104987.
- Rattanapan, Y., Duangchan, T., Wangdi, K., Mahittikorn, A., and Kotepui, M. (2023). Association between Rhesus Blood Groups and Malaria Infection: A Systematic Review and Meta-Analysis. *Tropical Medicine and Infectious*, 8(4), 190 doi.org/10.3390/tropicalmed8040190.
- Rodriguez-Iturbe, B., Pons, H. and Johnson, R.J. (2017). Role of the Immune System in Hypertension. *Physiological Reviews*, 97(3), 1127-1164.
- Scholzen, A., Mittag, D., Rogerson, S.J., Cooke, B.M. and Plebanski, M. (2009). *Plasmodium falciparum* mediated induction of human CD25<sup>hi</sup>Foxp3<sup>hi</sup> CD4 T cells is independent of direct TCR stimulation and requires IL-2, IL-10, and TGFβ. *PLoS Pathogens*. 5(8), e1000543. doi: 10.1371/journal.ppat.1000543.
- Scholzen, A., Cooke, B.M. and Plebanski, M. (2014). *Plasmodium falciparum* induces Foxp3<sup>hi</sup> CD4 T cells independent of surface PfEMP1 expression via small soluble parasite components. *Frontiers in Microbiology*, 5(200), doi: 10.3389/fmicb.2014.00200.
- Shibeshi, M.A., Kifle, Z.D., Atnafie, S.A. (2020). Antimalarial Drug Resistance and Novel Targets for Antimalarial Drug Discovery. *Infection and Drug Resistance*, 10(13), 4047-4060.

- Sinha, S., Medhi, B. and Radotra, B.D. (2022). Antimalarial and immunomodulatory potential of chalcone derivatives in experimental model of malaria. *Complementary Medicine and Therapies*, 22(330), doi.org/10.1186/s12906-022-03777-w.
- Su, D. K., Shin, K and Zhang, B. (2003). Molecular immunocomputing with application to alphabetical pattern recognition mimics the characterization of ABO blood type. *The 2003 Congress on Evolutionary Computation*, 4, 2549-2556.
- Su, W.M., Gu, X.J., Duan, Q.Q., Jiang, Z., Gao, X., Shang, H., and Chen, Y. (2022). Genetic factors for survival in amyotrophic lateral sclerosis: an integrated approach combining a systematic review, pairwise and network meta-analysis. *BMC Medicine*, 20(209), doi.org/10.1186/s12916-022-02411-3.
- Sumbele, I.U.N., Kimbi, H.K., Ndamukong-Nyanga, J.L., Nweboh, M., Anchang-Kimbi, J.K., and Lum, E., (2015). Malarial Anaemia and Anaemia Severity in Apparently Healthy Primary School Children in Urban and Rural Settings in the Mount Cameroon Area: Cross Sectional Survey. *PLoS ONE*, 10(4): e0123549. doi: 10.1371/journal.pone.0123549.
- Sun, Q., Li, J, and Gao, F. (2014). New insights into insulin: The anti-inflammatory effect and its clinical relevance. *World Journal of Diabetes*, 5(2), 89-96.
- Sund-Levander, M., Forsberg, C. and Wahren, L.K. (2002). Normal oral, rectal, tympanic and axillary body temperature in adult men and women: a systematic literature review. *Scandinavian Journal of Caring Sciences*, 16, 122-128.
- Taft, R. Pang, K.C., Mercer, T.R., Dinger, M. and Mattick, J.S. (2010). Non-coding RNAs: regulators of disease. *Journal of Pathology*, 220, 126-139.

- Tazebew, B., Munshea, A and Nibret, E. (2021). Prevalence and association of malaria with ABO blood group and haemoglobin level in individuals visiting Mekaneeyesus Primary Hospital, Estie District, northwest Ethiopia: a cross-sectional study. *Parasitology Research*, 120, 1821–1835.
- Timmann, C., Thy, T. and Vens, M. (2012). Genome-wide association study indicates two novel resistance loci for severe malaria. *Nature Journal*, 489, 443–446.
- Titilayo, O.A., Samuel, A.O., David, O.O. and Adewunmi, T.I. (2018). Genetic variations among three major ethnic groups in Nigeria using RAPD. *Molecular Biology Research Communications*, 7(2), 51-58.
- Tsalamandris S, Antonopoulos AS, Oikonomou E, Papamikroulis GA, Vogiatzi G, Papaioannou S, Devereux S, and Tousoulis D. (2019). The Role of Inflammation in Diabetes: Current Concepts and Future Perspectives. *European Cardiology Review*, 14(1), 50-59.
- Ty, M.C., Zuniga, M., Götz, A., Kayal, S., Sahu, P. K., Mohanty, A., Mohanty, S., Wassmer, S. C. and Rodriguez, A. (2019). Malaria inflammation by xanthine oxidase-produced reactive oxygen species. *Journal of EMBO Molecular Medicine*, 11(8), 1757-4676.
- Umoh, A.V., Abah, G.M., Ekanem, T.I. and Essien, E. M. (2010). Haemoglobin genotypes: a prevalence study and implications for reproductive health in Uyo, Nigeria. *Nigerian Journal of Medicine*, 19(1), 36-41.
- Van Honacker, E., Coorevits, L., Boelens, J., Verhasselt, B., Van Braeckel, E., Bauters, F., Padalko E. (2022). Sensitivity and specificity of 14 SARS-CoV-2 serological assays and their diagnostic potential in RT-PCR negative COVID-19 infections. *Acta Clinica Belgica*, 77(2), 315-320.

Varo, R. (2018). Adjunctive therapy for severe malaria: a review and critical appraisal. *Malaria Journal*, 17(1), 47. doi: 10.1186/s12936-018-2195-7.

Venugopal, K., Hentzschel, F., Valkiūnas, G., Marti, M. G. (2020). *Plasmodium* asexual growth and sexual development in the haematopoietic niche of the host. *Nature Reviews Microbiology*, 18, 177–189.

Wetlands International (2018). Conserving and restoring wetlands in Nigeria`s Niger River Delta. Retrieved from <https://www.wetlands.org/case study/conserving-and-restoring-wetlands-in-nigerias-niger-river-delta/>

White, N.J. (2022). Severe malaria. *Malaria Journal*, 21(284). doi.org/10.1186/s12936-022-04301-8

Williams, T.N., Mwangi, T.W., Wambua, S., Alexander, N.D., Kortok, M., Snow, R.W. and Marsh, K. (2005). Sickle cell trait and the risk of *Plasmodium falciparum* malaria and other childhood diseases. *Journal of Infectious Diseases*, 192(1), 178-186.

Williams, T. N. (2006). Human red blood cell polymorphisms and malaria, *Current Opinion in Microbiology*, (9)4, 388-394.

Witt, E., Hammer, E., Dorr, M., Weitmann, K., Beug, D. and Lehnert, K. (2019). Correlation of gene expression and clinical parameters identifies a set of genes reflecting LV systolic dysfunction and morphological alterations. *Journal of Physiological Genomics*, 51(8), 356-367.

World Health Organization (2016). Global Malaria Programme: Microscopy, Rapid diagnostics test and Nucleic acid amplification-based diagnostics. Geneva, Switzerland.

World Health Organization (2018). Artemisinin resistance and artemisinin-based combination therapy efficacy. Global Malaria Programme. Geneva, Switzerland.

- World Health Organization (2018). Malaria: responding to antimalarial drug resistance. Geneva, Switzerland.
- World Health Organization (2020). Malaria Factsheets. Geneva, Switzerland.
- World Health Organization (2022). World malaria report. Geneva, Switzerland.
- World Health Organization (2023). Malaria. Geneva, Switzerland.
- Zerihun, T., Degarege, A. and Erko, B. (2011). Association of ABO blood group and *Plasmodium falciparum* malaria in Dore Bafeno Area, Southern Ethiopia. *Asian Pacific Journal of Tropical Biomedicine*, 1(4), 289-294.
- Zhao, Y., Zhao, Y., Lv, Y., Liu, F., Wang, Q., and Li, P. (2017). Comparison of methods for detecting asymptomatic malaria infections in the China–Myanmar border area. *Malaria Journal*, 16(159), doi.org/10.1186/s12936-017-1813-0
- Zhang, J.M. and An, J. (2007). Cytokines, inflammation, and pain. *Journal of International Anesthesiology Clinics*, 45(2), 27-37. DOI: 10.1097/AIA.0b013e318034194e
- Zhou, X., Huang, J.L., Njuabe, M.T., Li, S.G., Chen, J.H. and Zhou, X.N. (2014). A molecular survey of febrile cases in malaria-endemic areas along China-Myanmar border in Yunnan province, People`s Republic of China. *Journal of Parasite*, 21(27). doi: 10.1051/parasite/2014030
- Zhu, J. and Paul, W.E. (2008). CD4 T cells: fates, functions, and faults. *Journal of Blood*. 112(5), 1557-1569. doi: 10.1182/blood-2008-05-078154.
- Zoghi, S., Mehrizi, A.A., Raeisi, A., Haghdoost, A.A., Turki, H., Safari, R., Kahanali A.A., and Zakeri, S. (2012). Survey for asymptomatic malaria cases in low transmission settings of Iran under elimination programme. *Malaria Journal*, 11(126), 1-10. doi.org/10.1186/1475-2875-11-126.

## Appendix i



## Appendix ii

### QUESTIONNAIRE

#### INTRODUCTION AND CONSENT

Dear Respondent,

Greetings, I am a Ph.D. student in the Department of Biochemistry, Federal University of Technology, Owerri. I am conducting a research on “**GENE EXPRESSION SIGNATURE AND PHENOTYPIC VARIABLES OF *PLASMODIUM FALCIPARUM* INFECTED SUBJECTS IN SAGBAMA LGA, BAYELSA STATE**” It involves the collection of blood samples and answering some questions, which is purely for academic purpose. The information we collect will help the government to plan health services and produce better drugs. Your household was selected for the survey. The questions and test will take about 30 minutes. It would be appreciated if you supply honest and sincere responses to the questions. All information will be strictly confidential. Your identity is not required on this questionnaire. I therefore solicit your **voluntary consent** in participating in this study.

Thanks for your co-operation.

| NO                                                                                                                         | QUESTIONS AND FILTERS | RESPONSES / CODING CATEGORIES | SKIP |
|----------------------------------------------------------------------------------------------------------------------------|-----------------------|-------------------------------|------|
| <b>SECTION 1: SOCIO-DEMOGRAPHIC CHARACTERISTICS</b>                                                                        |                       |                               |      |
| <b>I would like to start by asking you few questions about yourself.</b> <input type="checkbox"/> <input type="checkbox"/> |                       |                               |      |
| Q001                                                                                                                       | How old are you?      |                               |      |

|       |                                                                                                                            |                         |  |
|-------|----------------------------------------------------------------------------------------------------------------------------|-------------------------|--|
| Q002  | What is your sex?                                                                                                          | a) Male<br>b) Female    |  |
| Q003  | What is your current marital status?                                                                                       | a) Single<br>b) Married |  |
| Q004  | What is your occupation / where are you working currently?                                                                 |                         |  |
| Q005  | What is your religion?                                                                                                     |                         |  |
| Q006  | What was your highest level of education with certificate                                                                  |                         |  |
| Q007  | Are you a temporary or permanent resident in Sagbama LGA                                                                   |                         |  |
| Q008  | What language do you speak / what is your tribe?                                                                           |                         |  |
| Q009  | Have you been ill with a fever at any time in the last 2 weeks?                                                            |                         |  |
| Q0010 | In the past two weeks have you taken any antimalarial medicine?<br><br>If yes what is the name of the medicine?            |                         |  |
| Q0011 | Do you suffer from any health issues / do you have any of the following illnesses or symptoms:<br><br>a) Extreme weakness? |                         |  |

|                                                                                                                                                                                                                                            |                                                                                                                                                                                                                                                                |  |  |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|--|
|                                                                                                                                                                                                                                            | b) Heart problems?<br>c) Loss of consciousness?<br>d) Rapid or difficult breathing?<br>e) Seizures?<br>f) Abnormal bleeding?<br>g) Jaundice or yellow skin?<br>h) Dark urine?<br>i) Vomiting?<br>j) Pallor?<br>k) Refusal to eat?<br>Very cold hands and feet? |  |  |
| <b>Biochemical and clinical characteristics: Please we would like to carry out the following test, some of which might require collecting your blood samples and taking it to the laboratory. Will you allow us to collect your blood?</b> |                                                                                                                                                                                                                                                                |  |  |
| Q0012                                                                                                                                                                                                                                      | Can I take your Blood pressure?                                                                                                                                                                                                                                |  |  |
| Q0013                                                                                                                                                                                                                                      | Can I take your Height?                                                                                                                                                                                                                                        |  |  |
| Q0014                                                                                                                                                                                                                                      | Can I take your Weight?                                                                                                                                                                                                                                        |  |  |
| <b>It`s time to take your blood for laboratory analysis. Would you like me to collect your blood?</b>                                                                                                                                      |                                                                                                                                                                                                                                                                |  |  |

This is the end of the survey thank you very much. Doo! Mbana!!

## **Appendix iii**

### **Reagents and equipment**

#### **Reagents**

Methylated spirit, ethanol, primers, TAE buffer, agarose powder, ethidium bromide, agarose gel powder,  $\beta$ -mecarptoethanol, de-ionized distilled water (ddH<sub>2</sub>O). DNA, RNA, HRM analysis and cDNA kits, forward and reverse primers, DNA loading dye, malaria giemsa stain, and immersion oil

#### **Equipment**

Microscope, glucometer, weighing scale, measuring tape, ice making machine, microwave, vortex machine, dry bath, Vacutainer needle and holder, micro-centrifuge tubes, thermal cycler (PCR machine), cellulose acetate and gel electrophoresis machine, micropipettes, beaker, conical flask, pH meter, incubator, EDTA anticoagulant tubes (for blood sample collection for microscopy), total RNA minikit, gel documentation system, centrifuge, cotton wool, safety gloves, face mask, ice packs, micropipettes (1  $\mu$ l - 1000  $\mu$ l), pasteur pipettes, measuring cylinder (1,000 ml), tourniquet, and Andon automatic blood pressure monitor.