KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICINE AND DENTISTRY

DEPARTMENT OF MOLECULAR MEDICINE

Serum angiopoietins (ANG -1) and (ANG-2) levels as prognostic biomarkers for differentiating severe malaria from uncomplicated malaria in a Ghanaian population.

BY

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KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

SERUM ANGIOPOIETINS (ANG -1) AND (ANG-2) LEVELS AS PROGNOSTIC BIOMARKERS FOR DIFFERENTIATING SEVERE MALARIA FROM UNCOMPLICATED MALARIA IN A GHANAIAN POPULATION

A thesis submitted in fulfilment of the requirement for the degree of Master Of Philosophy (Chemical Pathology)

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By

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DECLARATION

The research work described in this thesis was carried out at the Department of Molecular Medicine – KNUST, Breman SDA Hospital, and Wenchi Methodist Hospital. This work has never been submitted to any institution or for any degree.

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ABSTRACT

Malaria is classified as either uncomplicated malaria or severe malaria. The principle that underly the progression of uncomplicated malaria to severe malaria is still unclear.

This study aimed at assessing serum angiopoietins (ANG -1) and (ANG-2) levels as prognostic biomarkers for differentiating severe malaria from uncomplicated malaria in a Ghanaian population.

A descriptive cross-sectional study was employed to sample 166 study participants of which forty-two (42) were apparently healthy controls, seventy-eight (78) were uncomplicated malaria cases, and forty-six (46) were severe malaria cases. Blood samples were taken and analysed for full blood count, liver function test, renal function test, and serum angiopoietins. Statistical analyses were carried out using Graph Pad Prism 9 software. The median and interquartile ranges, Mann-Whitney U test, and Kruskal-Wallis's analysis were done to compare groups.

The Ang-1 levels in the severe malaria group were lower (3.8 ng/L, IQR: 2.2 – 12.7) in comparison to the uncomplicated malaria group (6.3 ng/L, IQR: 3.3 – 8.0) and healthy controls (9.6 ng/L, IQR: 3.5 – 15.3). Ang-2 levels were higher in the severe malaria group (19.1ng/L, IQR: 9.0 – 25.8) compared to the uncomplicated malaria group (15.7ng/L, IQR: 2.6 – 27.4). The Ang-1 levels showed a high predictive ability of 94.9% to predict severe malaria from uncomplicated malaria whiles Ang-2, and Ang-2/Ang-1 ratio levels showed no predictive ability in discriminating malaria severity. Some hematological parameters, biochemical parameters, and parasite density were also associated significantly with malaria severity.

In conclusion, the results suggest that Ang-1 and Ang-2 do not have the potential to serve as biomarkers of both severe and uncomplicated forms of malaria in a Ghanaian population.

DEDICATION

I dedicate this work to God Almighty whose grace has been sufficient for me and also to my wife, Lydia Omari, daughter Maame Akosua Birago Boadi Frimpong, and the entire family.

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Table of Contents

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

HIV	Human immunodeficiency virus
AIDS	Acquired Immunodeficiency Syndrome
USAID	United States Agency for International Development
WHO	World Health Organization
IM	Impact Malaria
NMCP	National Malaria Control Program
CEPCs	Circulating endothelial progenitor cells
ANG-1/Ang-1	Angiopoietin - 1
ANG-2 / Ang-2	Angiopoietin -2
WPB	Weibel-Palade bodies
TNF	Tumor necrotic factor
SM	Severe malaria
UM	Uncomplicated malaria
HC	Healthy controls
CM	Cerebral malaria
PM	Placental malaria
ITNs	Insecticide-treated nets
LLINs	Long-lasting insecticide nets
IRS	Indoor residual spraying
IPT	Intermittent preventive treatment
IPTi	Intermittent preventive treatment in infants

RDT	Rapid diagnostic test
PMI	President's Malaria Initiative
UNICEF	United Nations International Children's Emergency Fund
PEs	Parasitised erythrocytes
PfGPI	Plasmodium falciparum Glycosyl phosphatidyl inositol
PfEMP1	Plasmodium falciparum erythrocyte membrane protein 1
ICAM-1	Intercellular Adhesion Molecule -1
CD36	Cluster of differentiation
CSA	Chondroitin-sulfate A
PECAM-1	Platelet endothelial cell adhesion molecule 1
VCAM-1	Vascular cell adhesion molecule -1
TNF- α	Tumour necrosis factor-alpha
IL	Interleukin
TLRs	Toll-like receptors
ECs	Endothelial cells
CECs	Circulating endothelial cells
NO	Nitric oxide
EPLs	Epimedium polysaccharide-propolis flavone liposomes
SDF-1	Stromal cell-derived factor 1
MMP-9	Matrix metalloproteinase -nine
IFN-y	Interferon-gamma
PBMCs	Peripheral blood mononuclear cells
DNA	Deoxy nucleic acid

GPIs	Glycosyl phosphatidyl inositols
MAPK	Mitogen-activated protein kinases
NF-KB	Nuclear factor kappa B
HRP-2	Histidine-rich protein 2
VEGF	Vascular endothelial growth factor
Flt-1	fms-like tyrosine kinase-1
VEGFR-1	Vascular endothelial growth factor receptor -1
VWF	von Willebrand factor
k Da	Kilo Dalton
SDA	Seventh Day Adventist
Hb	Haemoglobin
CHRPE	Committee for Human Research, Publication and Ethics
KNUST	Kwame Nkrumah University of Science and Technology
°C	Degree Celsius
EDTA	Ethylenediaminetetraacetic acid
Ml	Millilitres
Rpm	Revolutions per minute
FBC	Full blood count
WMH	Wenchi Methodist Hospital
ELISA	Enzyme-Linked Immunosorbent assay
pH	potential of hydrogen
RBC	Red blood cell
WBC	White blood cell

DIFF	Differentials
RET	Reticulocytes
DC	Direct current
PLT	Platelet
TWBC	Total white blood cell
MCV	Mean corpuscular volume
MCH	Mean corpuscular haemoglobin
HCT	Haematocrit
MCHC	Mean corpuscular haemoglobin content
Ng/L	Nanograms per litre
Ul	Microlitre
HRP	horseradish peroxidase
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
AST	Aspartate Aminotransferase
GGT	Gamma – Glutamyl Transferase
BCG	bromocresol green
GLDH	glutamate dehydrogenase
ROC	Receiver operating characteristic
IQR	Inter quartile range
LYMP	Lymphocyte
NEUT	Neutrophil
MXD	Monocyte/ eosinophil /basophil

AUC	Area under the curve
NA	Not applicable
Fig	Figure
CI	Confidence Interval
AKI	Acute kidney injuries
eGFR	Estimated Glomerular Filtration Rate
CKD-EPI	Chronic Kidney Disease Epidemiology
E-selectin	Endothelial Selectin

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Amongst the main causes of death, especially in the sub-Saharan part of Africa, is malaria. It is an illness brought by a single-celled parasites of the Plasmodium genus (Antwi-Baffour *et al.*, 2016). It is very common or endemic in several tropical regions especially developing countries and even extends into regions within the sub-tropics (Jeevatharan and Wickremasinghe, 2022). Infected female Anopheles mosquitoes are the main source of malaria transmission. Plasmodium falciparum infection happens to be the cause of the worst cases of malaria and contribute to over 90% of all malaria cases globally compared to malaria caused by plasmodium ovale, vivax, and malariae. Out of these, around 86% cases of malaria disease that occurs within the Sub-Saharan part of Africa are attributed to infections of Plasmodium falciparum (Ofori *et al.*, 2021; Cassy *et al.*, 2022). Globally, it is estimated that malaria infection affects 5% of the population although the weight of the disease is presently reducing (Watson *et al.*, 2022). The reduction in malaria load, however, is not distributed equally intercontinental because only a few countries out of many countries are in Asia, and the remaining countries are in sub-Saharan Africa and these contribute to about 94% of global malaria deaths especially in children (James *et al.*, 2018; Ofori *et al.*, 2021)

Malaria affects all age groups of people, both men, and women, but children below the ages of 5 years, immunosuppressed individuals, women who are pregnant, and individuals who are non-immuned (those who have not been in an endemic area for more than six months) are the most vulnerable. Among these vulnerable groups, are under-five kids in regions of endemicity sub-Saharan Africa in which almost one-fourth of every childhood mortality is attributed to malaria especially severe forms of malaria (Oluboyo *et al.*, 2020). In regions of endemicity like Africa,

huge numbers of deaths occur because of conditions such as malnutrition, and diseases such as tuberculosis, HIV/AIDS, and others, malaria do not only contribute to the ailment stress but to economic and social hardships whereby stagnating development in numerous ways (Fowkes *et al.*, 2016; Osarfo, Ampofo and Tagbor, 2022). Globally, attempts are being made by various agencies including USAID, Bill and Melinda Gates Foundation, WHO, Impact Malaria (IM) and the National Malaria Control Program (NMCP) in Ghana to regulate malaria with the intention of controlling and possible elimination through education, research and trainings (Mutasa, 2018; Sharma, Srivastava and Mohan, 2021; Osarfo, Ampofo and Tagbor, 2022).

Some signs and symptoms range from asymptomatic infections to serious illnesses. Some clinical profiles relating to uncomplicated malaria are headaches, fever, headaches, sweating, chills, muscle pains, and vomiting. Symptoms of severe malaria include renal failure, hypoglycemia, hyperparasitemia, severe anaemia, jaundice, respiratory distress, convulsions, prostration, metabolic acidosis, and coma commonly in children (Fairhurst and Wellems, 2015; Sypniewska *et al.*, 2017; Tai, Dhaliwal and Balasubramaniam, 2022). Only Plasmodium falciparum causes cerebral malaria, a type of severe malaria. It is one of the deadliest types of malaria, causing 80% of fatalities, especially in children It results in shock, irreversible coma, and eventually, the patient may die. (Luzolo and Ngoyi, 2019; Kyei-Baafour *et al.*, 2021). Although substantial research has been done on the pathophysiology of severe malaria, yet there is still no clear explanation for why some people progress to severe malaria which may result in organ dysfunction and possible death (Luzolo and Ngoyi, 2019).

An endangered microcirculation involving one of the established factors in the pathophysiology of severe malaria is the sequestration of parasitized red blood cells. (Luzolo and Ngoyi, 2019; Hoffmeister and Valdez, 2020). Investigations found that the severity of malaria depends on the

destruction that results when parasitized red blood cells cling unto the micro vessels of the endothelial walls and leads to blockage by the formation of rosettes with non-parasitized red blood cells (Gomes *et al.*, 2016; Idris-Usman, 2016; Moxon *et al.*, 2020). The prognosis of malaria severity is primarily impacted by the parasite density and the host's reaction to the disease. The severity of the illness and the prognosis are affected by a variety of protective immune responses (Jensen, Adams and Hviid, 2020).

Some investigations have proven that the cells of the endothelium that are killed, are substituted with new cells by replication as well as through migration and integration of endothelial progenitor cells in circulation (CEPCs) produced in the marrow of bones to sites of destruction in the microvasculature (Tenreiro, Correia and Brito, 2017; Le Roux and Tchokonte-Nana, 2018; Li *et al.*, 2019). One of the ways of this succession of replication is through angiogenesis which involves these angiogenic factors, Angiopoietin - 1(ANG-1) and Angiopoietin -2 (ANG-2). Normal endothelial cell function is regulated by angiopoietin-1 and angiopoietin-2 being in equilibrium with one another. Endothelial cell activity and vascular integrity are known to be regulated by the angiopoietin-Tie-2 system. In contrast to Angiopoietin-2 (Ang-2), which counteracts these effects, Angiopoietin-1 (Ang-1) recruits endothelial receptor Tie-2, promoting endothelial cell quiescence and survival (Isidori, Venneri and Fiore, 2016). Studies conducted revealed that in severe malaria compared to uncomplicated malaria, there are low Ang-1 levels and high Ang-2 levels (De Jong *et al.*, 2016; Lekpor *et al.*, 2022). According to Sahu *et al*, (2015), a higher Ang-2 to Ang-1 ratio demonstrated a good biomarker for adverse outcomes in some diseases such as sepsis, diabetes, and malaria. Assessing the relationship between angiopoietin-1 and angiopoeitin-2 levels in both uncomplicated and severe malaria is essential given that the endothelium plays a significant role in mediating malaria pathogenesis.

1.2 Problem Statement

According to WHO, malaria infections are categorized into two groups as uncomplicated malaria and as severe malaria. The two groups of malaria infection is possible to distinguish with the aid of a meticulous clinical examination of the patient who is thought to have malaria. There is no definitive diagnosis for severe malaria because of the non-specific nature of its manifestation coupled with the presence of confounders of parasitemia in places where the infection is very endemic (Conroy, Datta and John, 2019; Li *et al.*, 2020; Oluboyo *et al.*, 2020). Due to the difficulty in identifying and treating additional life-threatening illnesses, these variables may result in misdiagnosis and negative outcomes. It is therefore crucial to carefully distinguish between uncomplicated malaria and severe malaria since doing so affects how the disease is treated, which has an impact on its prognosis and can result in additional complications (Gill *et al.*, 2013; Oluboyo *et al.*, 2020). The principle that underly the development of uncomplicated malaria to the severe forms of malaria is yet unclear (Rivera-Correa and Rodriguez, 2020).

Although only a small percentage of malaria infected individuals develop to the severe forms of the disease, they present with consequences that could be lethal, including cerebral malaria which may lead to the loss of life of the individual (WHO, 2019). Activation and dysfunction of the endothelium have been found to be associated with the development of the severe forms of the infection especially cerebral malaria (De Jong *et al.*, 2016; Lekpor *et al.*, 2022). Angiopoietin-1 (ANG-1) and angiopoietin-2 (ANG-2) are two components of angiogenesis that ensure the growth of new blood vessels, and it is now known that they are essential moderators of endothelial activity and integrity (Park *et al.*, 2019). Constitutively produced, ANG-1 maintains the vessel's dormancy by signaling via the Tie-2 receptor. In conjunction with endothelial activation, Weibel-Palade (WP) bodies produce ANG-2, which displaces ANG-1, sensitizes the endothelium to cytokine

concentrations below the threshold, making it susceptible to substances like tumour necrotic factor (TNF) (Sarkar and Chakroborty, 2018).

Per the guidelines of the national malaria control program (NMCP), no test or investigation specifically indicates the severity of malaria infection in Ghana. The identification of a definitive laboratory test that convincingly predicts patients who are, or risk of severe malaria would be beneficial in the management of malaria cases. The ability to detect and intervene in cases of severe malaria will have a greater effect on the economy, particularly in areas where resources are scarce and in underprivileged settings where the rational allotment of insufficient resources with respect to health is very important.

1.3 Justification

The capacity to distinguish between the severe forms of malaria (SM) and uncomplicated malaria (UM) in patients and the other causes of severe diseases in the Ghanaian populace is difficult, due to the unspecific nature of the clinical manifestations. Due to the possibility that individuals with uncomplicated malaria may have endothelial cell activation that may develop to the severe form of the disease, rapid detection and management are therefore likely to enhance clinical outcomes in those with malaria. Therefore, useful endothelial markers (Angiopoietin-1 and -2) required for vascular activity and integrity may be found to identify those individuals from all patients with uncomplicated malaria who would advance to severe malaria.

1.4 Hypothesis

- $H_{0:}$ Endothelial biomarkers, Angiopoietin-1 (Ang-1) and Angiopoietin -2 (Ang-2) are not linked to the malaria infection severity and may not be useful in identifying individuals with severe malaria.

- H_1: Endothelial biomarkers, Angiopoietin-1 (Ang-1) and Angiopoietin -2 (Ang-2) are linked to severity of malaria infection and may be useful in identifying individuals with severe malaria.

1.5 Aim

The primary main of the study is to evaluate blood levels of angiopoietins (ANG-1 and ANG-2) as predictive indicators for differentiating severe malaria from uncomplicated malaria in a categorized Ghanaian population.

1.6 Specific Objectives

1. To determine serum angiopoietins-1 and -2 levels in both uncomplicated and severe forms malaria and compare them to that of healthy individuals.
2. To compare angiopoietins -1 and -2 levels to the level of parasitemia (parasite density) in uncomplicated and severe malaria.
3. To assess the haematological parameters for both uncomplicated and severe malaria
4. To assess the biochemical parameters of the uncomplicated malaria group and severe malaria group and compare them to the control group.

CHAPTER TWO

LITERATURE REVIEW

2.1 Malaria

Malaria is an infection that is spread by being bitten by a female Anopheles mosquito that is infected. In some cases, it can be transmitted through blood transfusions using infected blood or through the use of infected needles and syringes (Pierrotti *et al.*, 2018). The five causative agents in humans are parasitic protozoans known as *Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, Plasmodium falciparum,* and *Plasmodium knowlesi* (Bhattacharya, Maurya and Bhattacharya, 2020; Sato, 2021). The associated symptoms of malaria are nausea, anaemia, spleen enlargement, and periodic attacks of chills and fever. In most extreme instances symptoms are respiratory discomfort, cerebral malaria, severe anaemia, and algid malaria (Kotepui, Kotepui, G. D. J. Milanez, *et al.*, 2020). Plasmodium falciparum is responsible for the most dangerous type of malaria which causes cerebral malaria which is a major constituting factor in deaths resulting from malaria in children below the age of five (Riggle, Miller and Pierce, 2020).

In as much as there is a widespread effort by public health officers in tackling malaria in terms of control and treatment, it still stands out as a very endemic disease in Africa, the Tropics and sub-tropical area contributing to 250 million infections each year as well as leaving a quarter of the global populace who face the risk of malaria (Ioannidis, Nie and Hansen, 2014; Qin *et al.*, 2020). Malaria is classified as a parasitic disease that happens when the red blood cells are attacked by the protozoan parasitic *Plasmodium species*. Malaria can also be spread by more than 30 different anopheline mosquito species. *Plasmodium falciparum* is the largest contributor of malaria in Africa being responsible for more than 95% of all cases of malaria (WHO 2020). *P. falciparum* is known to lead to various pathologies associated with different kinds of organs including the brain

known as cerebral malaria (CM) and that of the placenta referred to as placental malaria (PM) (Wassmer and Grau, 2017).

2.1.1 Malaria, the situation in Ghana

Despite several measures being implemented to reduce malaria., the prevalence of the disease is still a worry among the Ghanaian population. Studies across the country have reported different prevalence rates among different study populations. Diallo *et al.*, (2017) reported a prevalence rate of 15.1% among mobile populations whereas (Annani-Akollor *et al.*, 2020) additionally stated an 8.9% prevalence rate for expectant mothers. The plasmodium species most rampantly known for causing malaria is the *Plasmodium falciparum.* This species contributes to about 80% - 90% of all malaria infections. Plasmodium malariae contributes 20%-36% while Plasmodium ovale contributes to about 0.15% of all malaria cases in Ghana (Babayara and Addo, 2018). Each year in Ghana, about 3.5 million with a quarter of all cases lead to the death in cases of children beneath the age of 5. In most instances, malaria tends to leave extremely negative effects and majority children that survive it are frequently left with brain damages that stunt their growth (Trivedi and Chakravarty, 2022). Malaria has been proven to contribute to absenteeism in various schools amongst school-age children (Asante, Binka and Koram, 2019; Halliday *et al.*, 2020). Studies conducted proved that one bout of malaria led to negative outcomes with mathematics and languages associated tests (Bangirana, 2015; Chen *et al.*, 2016). Owusu Adjah & Panayiotou (2014) recommended that preventing malaria in the early stages of a child's development will lead to low dropout rates. With about sixty percent (60%) of non-hospitalized cases in Ghana being attributed to malaria, the weight of the disease is intense and has led to lots of pressure on medical professionals and researchers from all throughout the nation.

Strides are underway to stop the disease globally. The disease is being controlled with a significant concentration of resources. Promotion of indoor residual spraying (IRS), public education, intermittent preventive treatment (IPT) for pregnant women, long-lasting insecticide net (LLIN) use for everyone and bolstering of health services and the scientific community through the organization and hosting of workshops with regards to malaria are all the mechanisms that the state employs in curbing malaria within the country (Nyarko and Cobblah, 2014; Elmardi *et al.*, 2022; Gowelo *et al.*, 2022; Osarfo, Ampofo and Tagbor, 2022).

Small groups and communities are also part of the fight against malaria. An anti-malaria campaign, indoor residual spraying (IRS) was introduced in a mining town by Anglogold Ashanti. This program spans a vast area including private and public infrastructure and other villages within the Obuasi Municipal District. This program led to desired outcomes by limiting the cases of infection within the country and reduction of skiving in schools (Abuaku *et al.*, 2018).

2.1.2 Treatment of Malaria

The current forms of medication adopted in treating confirmed *Plasmodium falciparum* in the country are the WHO recommended artemisinin-based combination therapies (Artemether - Lumefantrine) (Okafor, Helen and Nwankwo, 2020; Kondrashin *et al.*, 2021; Vantaux *et al.*, 2021).

2.1.3 Malaria Diagnosis

Microscopy and the rapid diagnostic test (RDT) are currently being employed to diagnose malaria with microscopy being the "gold standard" (Iwuafor *et al.*, 2018). In emergencies, RDT cannot quantify the parasite, give the stage and species. Although microscopy which is the "gold standard" can quantify, gives stage and species, it requires a longer time. The RDT available in Ghana is only used to diagnose Plasmodium falciparum malaria. No surveillance mechanism has been

implemented in Ghana yet possibly due to its endemicity. The Global Fund, WHO/UNICEF, USAID/PMI and the Government all contribute money to the effort to treat, eradicate, and prevent malaria. Bill and Melinda Gates are also known contributors to the fight and research against malaria (Guitierrez, 2020).

2.1.4 The distribution of Malaria

Over 100 developing countries situated in tropical areas and sub-tropical areas with altitudes below 1,500m are currently rife with malaria (Kumar, 2016; Ferrao *et al.*, 2018; Assefa *et al.*, 2019). The rate of malaria in a particular area is highly contingent on the climate of the area. The extent of malaria in a given region is greatly influenced by socioeconomic factors as well as elements like, rainfall, temperature, immigration, and humidity, emigration. (Garamszegi, 2014; Kar *et al.*, 2014; Ferrao *et al.*, 2018). In terms of distribution, these elements have a significant impact on both the vector and the parasite. *Plasmodium falciparum* and *Plasmodium malariae* have been shown to coexist in regions with comparable topographies, while *Plasmodium vivax* and *Plasmodium ovale* have been found to coexist in habitats with similar climatic conditions (Milner, 2018; Assefa *et al.*, 2019). In tropical regions around the world, P. falciparum, which is mostly known to cause severe and extreme instances of malaria, is prevalent. Due to the absence of the Duffy antigen in Africans, P. vivax is also sporadic in Africa and extremely prevalent in Asia and the Eastern Mediterranean. Additionally, it is known that P.ovale can be found in Africa, sporadic Southeast Asia, and the Western Pacific (Gunalan *et al.*, 2019; Battle and Baird, 2021).

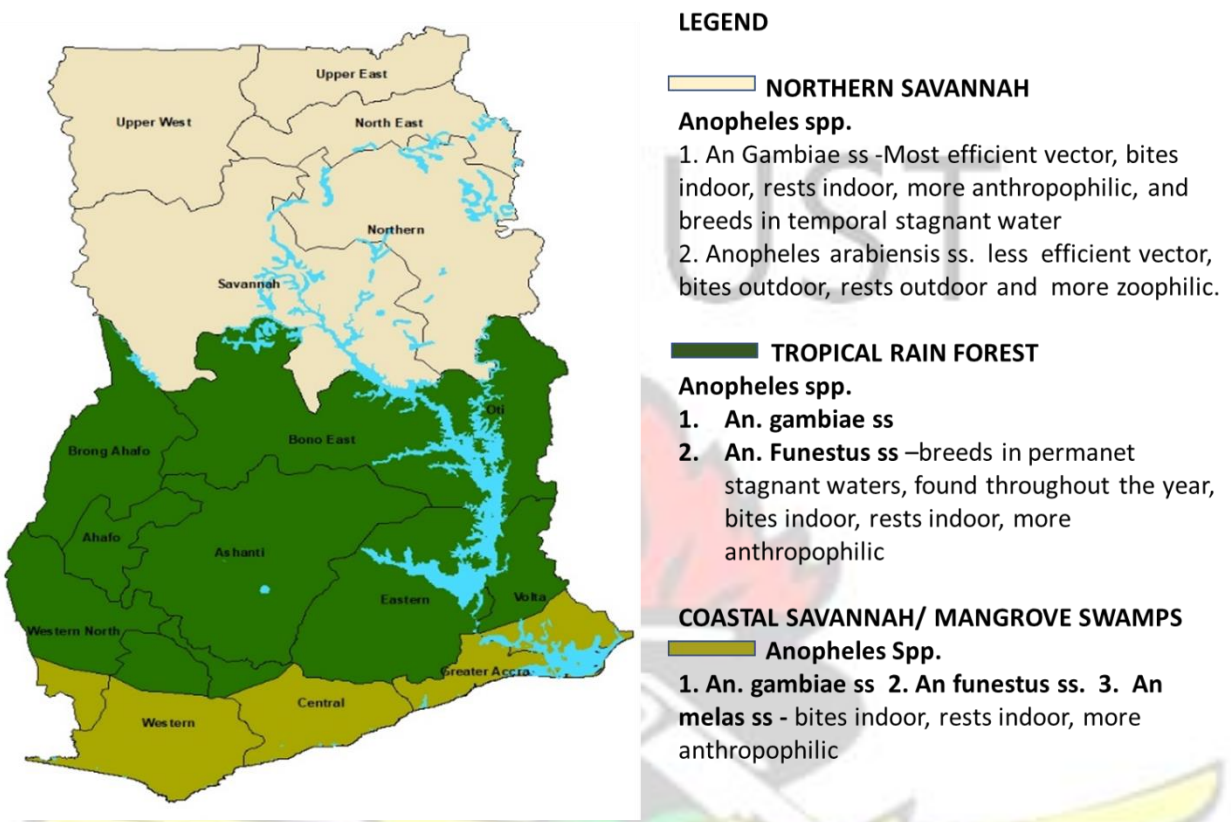

fig 2. 1 Ecological zones of Anopheles species in Ghana (https://www.researchgate.net/)

Coastal, forest, and savannah agro-ecological zones can be used to categorize Ghana's topography as seen in Figure 2.1 above. These individual zones highlight different characteristics with regard to the Plasmodium species and the mosquito distribution owing to the difference in rainfall, humidity, temperature and also the general ecology (Osei *et al.*, 2018). In coastal environments, the spread of malaria is high and perennial, but it reduces sharply during dry seasons. Coastal savannah, coastal zones, and mangrove swamps are all parts of the coastal zone. The Accra plains, the Volta area, and the lower central area are all covered by the coastal savannah. Anopheles melas, a species of mosquito, is the primary vector of malaria parasites in coastal lagoons and mangrove swamps. The spread of malaria is high, perennial, and relatively steady within rainforests (Annani-Akollor *et al.*, 2020). The conditions that characterize forest zones in Ghana are highly favourable for the spread of malaria and so these zones are the most endemic within the

country. Transmission rates are usually at the highest after rainy seasons (Kassam *et al.*, 2021). The Northern Savannah Zone is found in the country's northern region where the rainfall rate is quite uneven. Even though the transmission of malaria in these zones is very high, it reduces sharply during seasons of aridity which span from October to April. Anopheles arabiensis, the main carrier of the parasite, and Plasmodium falciparum, the main parasite, are the two main agents (Addai-Mensah *et al.*, 2018).

2.2. Taxonomy of the Plasmodium species

The following categories apply to Plasmodium falciparum: kingdom Class Sporozea, subclass Coccidia, order Eucoccidiorida, suborder Haemosporina, phylum Apicomplexa, and genus Plasmodium (Cruz-Bustos *et al.*, 2021). Plasmodium infecting vertebrates is known to span over a hundred species. Plasmodium ovale, Plasmodium vivax, Plasmodium malariae, and Plasmodium falciparum are the four of these species that can cause malaria in humans. Recent studies conducted in Southeast Asia proves that Plasmodium knowlesi is known for infecting monkeys and has the capacity to infect humans as well (Ahmad, 2016; Amir *et al.*, 2018; Vythilingam *et al.*, 2021).\

2.2.1 Plasmodium vivax

The most widespread malaria parasite is the P vivax. The majority of cases of P. vivax infection (47%) occurred in India, which is located in the South-East Asia region (53%) (Kotepui, Kotepui, G. D. J. Milanez, *et al.*, 2020). Because the Duffy gene is missing in Africans, they are naturally resistant to this parasite and so sub-Saharan Africa has very few cases of P. vivax malaria documented. (Gunalan *et al.*, 2019). According to reports, this parasite can lead to benign, uncomplicated tertian malaria in people. This also in most instances leads to enlargement of the liver. The development of hypnozoites in the human liver causes this particular strain of malaria

to frequently recur years after initial infection (Gural *et al.*, 2018; Sylvester *et al.*, 2021). (Battle and Baird, 2021) reported that the longest incubation time for the relapse of *P. vivax.* as years.

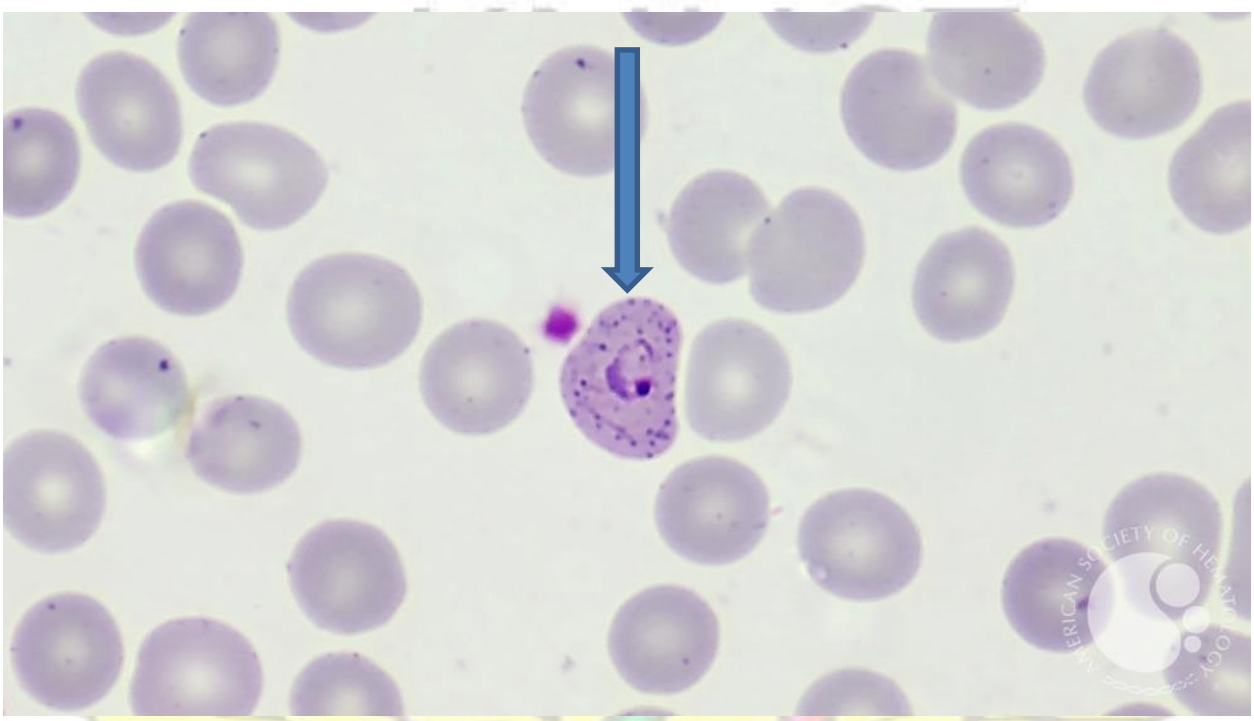

fig 2. 2 Plasmodium vivax ring form in red blood cell (https://imagebank.hematology.org/image/64015/plasmodium-vivax-ring-form?type=upload)

2.2.2 Plasmodium ovale

The least common cause of malaria infection in humans is Plasmodium ovale. It is known to exist mostly in tropical regions but has very little presence in Central and Eastern Africa. The results of infection are usually mild tertian uncomplicated malaria in humans (Groger *et al.*, 2017).

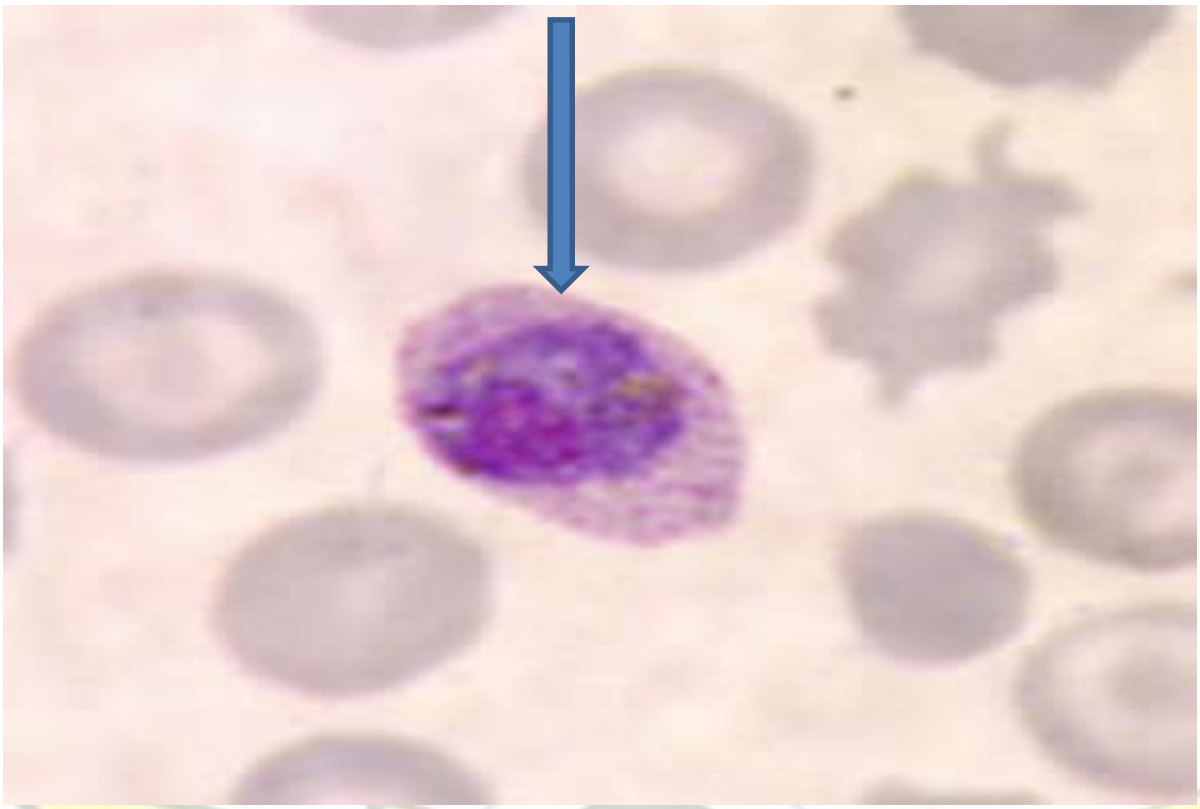

fig 2. 3 Plasmodium ovale in red blood cell (https://www.pathologyoutlines.com/topic/parasitologypovale.html)

2.2.3 Plasmodium malariae

Plasmodium malariae (P. malariae) contributes to about 3 % of the world's malaria infections and has a cosmopolitan distribution (Kotepui, Kotepui, G. D. Milanez, *et al.*, 2020). Both humans and wild animals have been reported to contract malaria because of it. Additionally, it has been linked to human quartan malaria. (Plenderleith *et al.*, 2022).

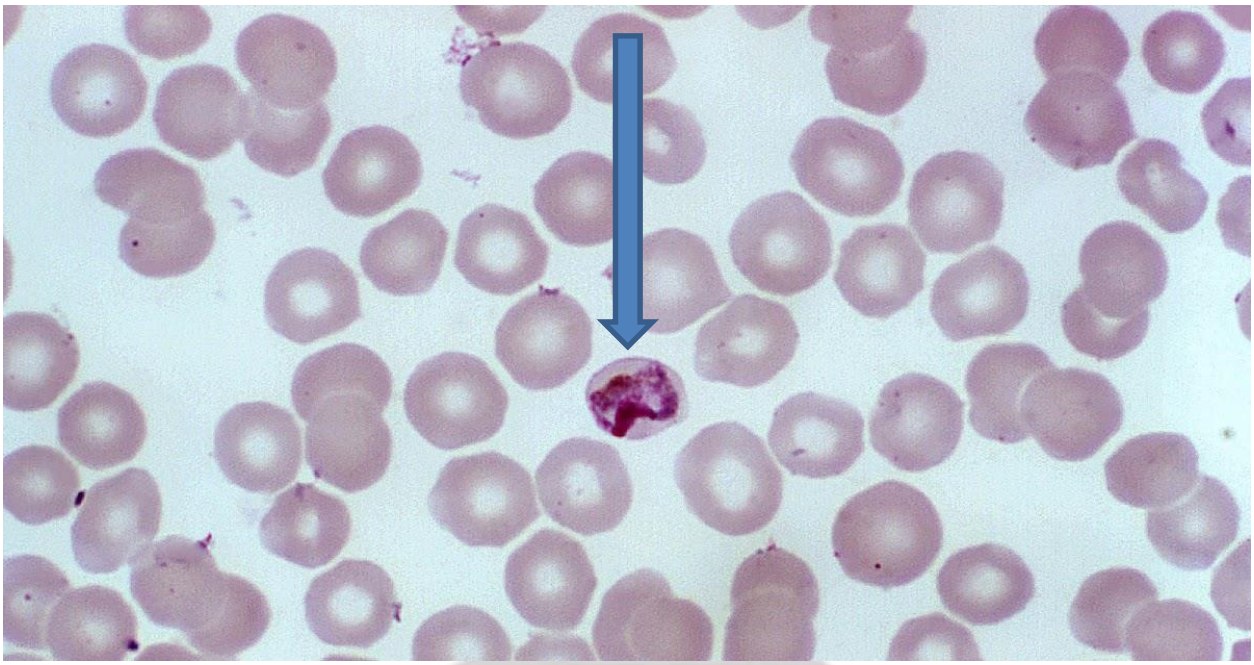

fig 2. 4Plasmodium malariae in red blood cell (https://www.medicalbiochemist.com/2020/10/mcq-malaria-plsamodium-malariae.html)

2.2.4 Plasmodium falciparum

The most deadly kind of malaria is one brought on by this parasite. Plasmodium falciparum exists mostly in Sub-Saharan Africa. Malignant tertian malaria and cerebral malaria in humans are its primary known effects (Mahmud *et al.*, 2017; Verra *et al.*, 2018). The associated symptoms are usually headaches, high fever characterized by sweats, chills, vomiting nausea, cough, and back pains and abdomen. Another extreme symptoms include jaundice, anaemia, and thrombocytopenia.

Extreme complications may arise in instances when *P. falciparum* is left without treatment. Some of these complications may include severe anaemia (1% death rate), coma (18% mortality), acidosis (15% mortality), severe failure of the liver, collapse of the circulatory system, , hypoglycemia, acute pulmonary, hyperpyrexia, oedema, and failure of the renal system (İnkaya *et al.*, 2016; Angeletti *et al.*, 2021). Red blood cell sequestration by the human placenta is another established method and so Plasmodium falciparum is a rampant contributor to abortion, maternal

death, premature delivery, stillbirth, and low birth weight in rife geographic locations (Zakama, Ozarslan and Gaw, 2020)

fig 2. 5 Plasmodium falciparum in red blood cells (https://www.pinterest.com/MLAB1231/plasmodium-falciparum/)

2.3 Life Cycle of the Plasmodium Parasite

The parasite's lifecycle is linked to its clinical manifestations. A female Anopheles mosquito that has already contracted malaria injects the host with sporozoites (Dragovic *et al.*, 2018; Roth *et al.*, 2018). The sporozoites quickly move to the liver of the host where the sporozoites locomote through several cells before infecting a hepatocyte and initiating a process of asexual and asymptotic reproduction known as exo-erythrocytic schizogony (Dundas *et al.*, 2019). At the growth and maturation phase of hepatocytes (about 7-14 days), Numerous merozoites are discharged into the bloodstream and the infection of the blood then begins. During the infection of the blood. *P. falciparum* parasites go through a process of invasion (merozoites), multiplication

(schizont), intracellular growth (trophozoites), and reinvasion of host erythrocytes over a period of about 48 hours. Some intraerythrocytic parasites develop differently and release the sexual stage of the malaria lifecycle is started by male and female gametocytes (Bancells *et al.*, 2019). Schizonts express parasite proteins that mediate the cytoadherence of PEs in the microvasculature during the latter half of the erythrocytic cycle in P. falciparum, and the schizonts then burst, releasing infectious merozoites and other parasite-derived bioactive products like PfGPI. (Duffy *et al.*, 2022).

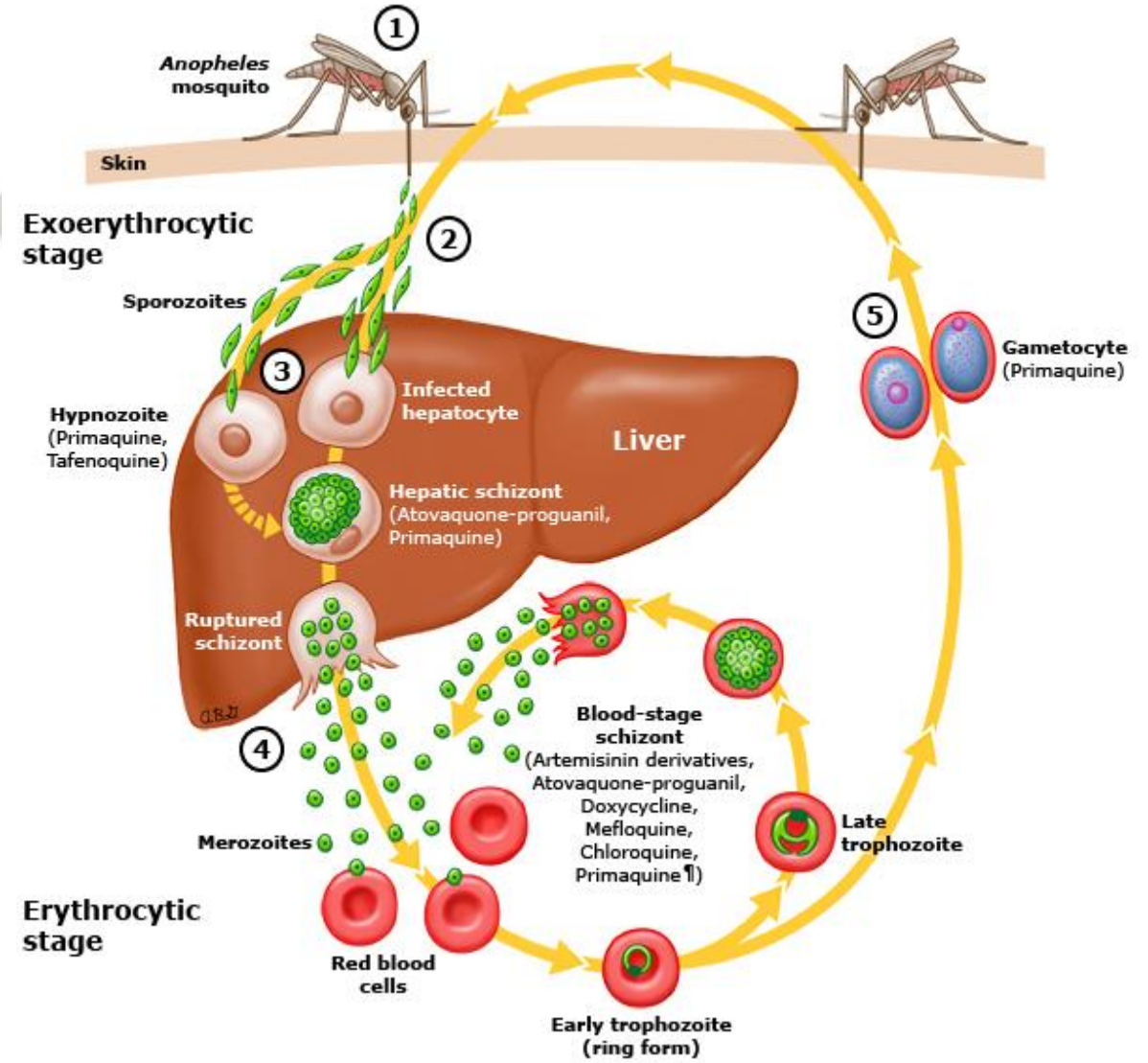

fig 2. 6 Life cycle of the plasmodium parasite (https://europepmc.org/article/PMC/5007148)

2.4 General Pathophysiology of Malaria

Malaria causes the general feeling of fever and other symptoms when parasites burst the host's red blood cells. The parasites release inflammatory products that activate the immune system's ability to release pyrogens (Sharma and Shukla, 2017; Ohiagu *et al.*, 2021). Malaria fever is usually a result of synchronous infections. Even though the timing cuts across all human infections, the maturation process of the erythrocyte plays a large role (usually between 24-72 hours). It usually takes multiple cycles for patient parasitemia to develop synchronized febrile cycles (Warrell, 2017). Individuals that are not immune to the disease usually seek medical attention when confronted with nonspecific symptoms like fever, vomiting, diarrhoea, and even before the development of malarial paroxysms. Due to this phenomenon, it is often areas difficult to tell the difference between malaria febrile illness from other forms of illness, especially in that are rife with malaria (Jairajpuri *et al.*, 2014). These pathogenic occurrences happen in both cerebral and placental malaria and will be reviewed independently below.

The parasite Plasmodium falciparum produces parasite proteins, such as PfEMP1, which is found on the surface of red blood cells and aids in the PEs' cytoadherence to the microvasculature (Kanoi *et al.*, 2018).

PfEMP1 is characterized as a transmembrane protein that spans the erythrocyte membrane and facilitates interactions between receptors and the vascular endothelium to prevent detection and clearance by the spleen. Additionally, PfEMP1 is a highly variable protein that is encoded by about 60 var genes, ensuring that the parasite is not recognised by the host's immune system (Bachmann *et al.*, 2019). Red blood cells typically express these genes on their surface in a mutually exclusive

fashion. These usually ensure individuals in areas rife with malaria take an extremely long time in developing immunity against malaria. The most comprehensive endothelium receptors for PfEMP1 are Intercellular Adhesion Molecule 1(ICAM-1) located in the brain, CD36 outside the brain, and chondroitin-sulfate A (CSA) which is found in the placenta although they still are bound to other receptors with the inclusion of thrombospondin Platelet endothelial cell adhesion molecule-1(PECAM-1), P-selectin, E-selectin, and vascular cell adhesion molecule -1 (VCAM-1) (Avril *et al.*, 2016; Tuikue Ndam *et al.*, 2017).

2.4.1 Severe Malaria

P. *Falciparum* malaria initiates anaemia at the blood stage which is usually indicative of severe malaria. Anaemia is known to be the cause of the mortality recorded as a result of malaria (White, 2018). When large paediatric populations are present in holoendemic communities, the statistic is significantly higher. Malaria mortality rates of 30% or more are typically found in these places. However, it is worthy of note that severe malaria can exist as a result of multiple factors. In these circumstances, the red blood cells lyse. Additionally, to this, erythrocytes produced in the bone marrow are less effective., inhibits the functionality of the body in replenishing the number of erythrocytes in the body. Another factor that may be responsible for anaemia during malaria could be the breakdown of erythrocytes, both infected and noninfected by the spleen (White, 2018; Oyong *et al.*, 2019). According to research, an imbalance between pro-inflammatory and anti-inflammatory cytokines, chemokines, growth factors, and effector muscles can drastically limit the body's ability to produce red blood cells, which can result in anaemia (Mavondo and Mzingwane, 2017). In some cases, the development of anaemia is attributed to tumour necrosis factor-alpha (TNF- **α**) and Interleukin-10 (IL 10). By inducing ineffectiveness in the erythropoietic

process, haemozoin has also been identified as an element that contributes to anaemia in malaria infection by increasing oxidative stress in the cells (Vasquez, Zuniga and Rodriguez, 2021).

2.4.2 Immunity

If malaria infects individuals that are not immune, it usually progresses rapidly which can lead to extreme anaemia and death in some instances (White, 2018). However severe disease only happens in 1-2% of these cases (Wassmer *et al.*, 2015; White, 2018). As much as there are effective antimalarial medications, the mortality rate of falciparum malaria is very high. Research from in vitro experiments, and murine models, on P. falciparum infections in malaria-naive people, demonstrates the importance of innate immunity for limiting the parasite's tendency to replicate. This ensures the host can have enough time to develop protective and adaptative mechanisms against the parasite (Galatas, Bassat and Mayor, 2016). The immune system provides multiple pathways and mechanisms for the recognition and clearance of parasitized erythrocytes (PEs) which include the complement system and the pattern-recognition receptors (e.g., toll-like receptors, TLRs) (Higgins *et al.*, 2019). These pathways occur in a controlled manner extremely high parasitemia levels without causing immunopathology.

2.4.3 Respiratory distress and metabolic acidosis

In more than half the cases of malaria recorded, metabolic acidosis is the major factor behind the respiratory distress experienced by patients. This happens when the concentration of lactic acid in the blood increases. This increase is usually attributed to the effects of the anaerobic respiration that hosts tissues are inadequately perfused (Sriboonvorakul *et al.*, 2018; Possemiers, Vandermosten and Van den Steen, 2021). Even though the liver is responsible for the removal of lactic acid from the body, the exponential rate at which the malaria parasites induce the production of lactate puts the liver in higher concentrations of lactic acid. In addition to these, TNF- α and IL-

10 have been found to be responsible for metabolic acidosis during malaria (Mandala *et al.*, 2017). Some of the most common symptoms that severe malaria patients suffer in respiratory distress include breathing with abdominal muscles engaged, intercostal muscle retraction, pulmonary oedema, and deep acidotic breathing (Foucher and Tubben, 2021).

2.4.4 Host endothelial cell

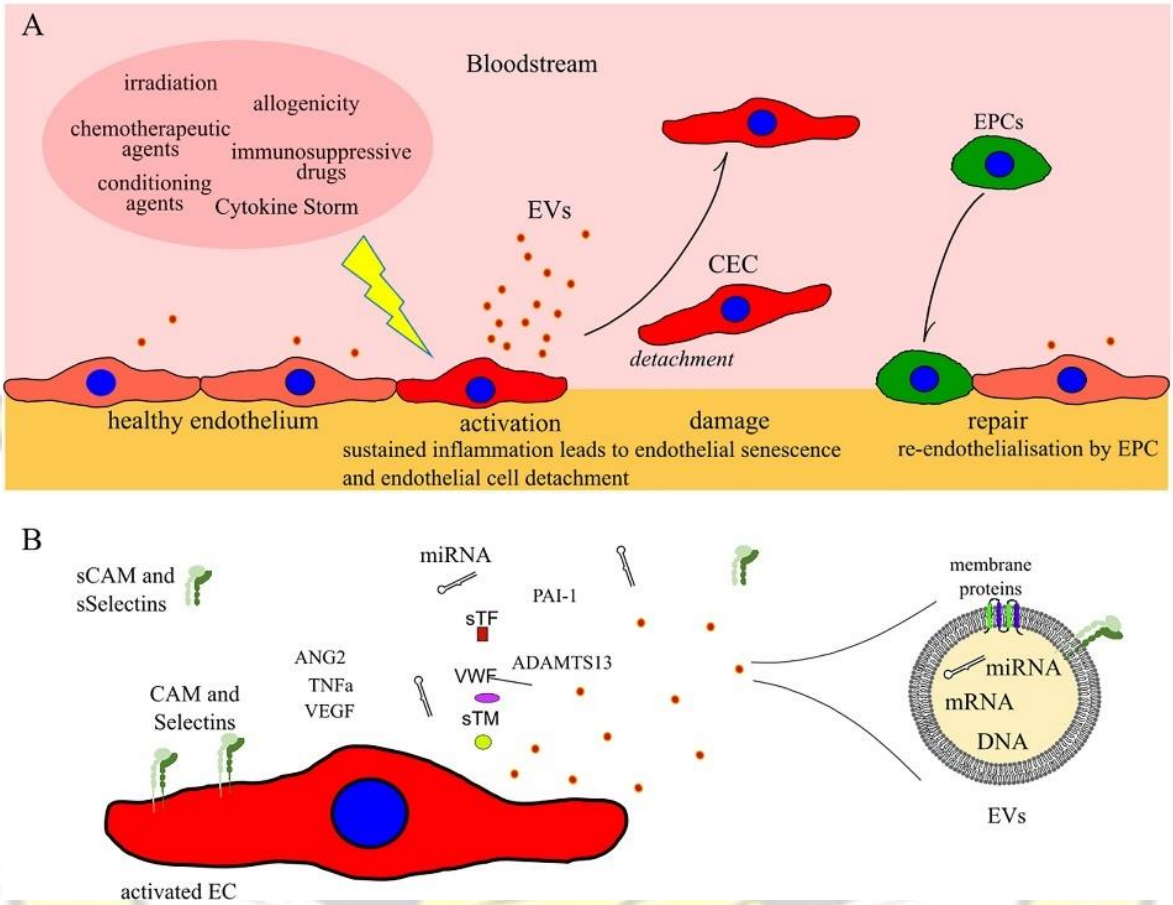

fig 2. 7 Host endothelial cell (https://www.frontiersin.org/articles)

The vascular endothelium is a complex structure that is capable of responding to the environment and interacts with it by switching from a dormant to an active state and back to resting state. It is available in all human organs and is in charge of all oxygen regulation, nutrient transportation, blood pressure regulation, coagulation, inflammatory processes, and all other physiological functions that the organs carry out (Hennigs *et al.*, 2021). A monolayer of endothelial cells makes

up the vascular endothelium (ECs), which divide to separate blood circulation from the surrounding tissues (Krüger-Genge *et al.,* 2019). They could be generated through the hematopoietic lineage or endothelial lineage if they both function in similar ways. Both endothelial progenitor cells (EPCs) and circulating endothelial cells (CECs) are categorized as non-hemopoietic cells. The endothelium produces the CECs while the bone marrow, through a mediator by the enos-derived nitric oxide (NO), produces the EPCs. Nonetheless, studies show that through differentiation, immature EPCs can produce CECS (Chopra *et al.*, 2018; Morrone *et al.*, 2021). Researchers have postulated that certain signals from the regeneration sites of the bones, assemble EPCs produced by the bone marrow and guide them into peripheral circulation (Zigdon-Giladi *et al.*, 2015; de Araújo Leitão, 2020). In addition to this, it is explained that, peripheral blood contains a specific number of EPCs found in bone marrow were drawn there in response to some injuries (Tetteh, 2014; Emontzpohl *et al.*, 2017). These function in ways that support the development and production of new vessels that are used in the regeneration of vascular endothelium in times of injury (Wakabayashi *et al.*, 2018). The process involves their migration and spread into injury sites and spreads different kinds of cells that make up the bone marrow. These include multipotent adult progenitor cells, which are responsible for regenerating several layers of tissue, mesenchymal stem cells, and haemangioblasts which are known as the most common precursors of haemotopoietic and endothelial lineages (Corliz et al, 2013). With about zero to one per cent daily renewal rate in persons with good health, the rate at which endothelial layers regenerate is classified as very low.

Epimedium polysaccharide-propolis flavone liposomes (EPLs) are broadly classified as a group of circulating cells with the capacity to show off numerous and varying cell surface indicators that are identical to that the vascular cells of the endothelium expresses. They also have the capacity to

all areas of hypoxia/ischemia, and to adhere to endothelium while still holding on to their capacity to form new vessels (Ma *et al.*, 2015; Hasan *et al.*, 2016).

Three distinct markers usually characterize the classification of EPCs - the CD133, the CD34, and the vascular endothelium growth factor receptor -2. However, in times of differentiation, the CD33 is lost and an expression of CD31 begins. These are likely to spread and differentiate to grown versions of CECs When the endothelium sustains any damage or injury, it is immediately triggered to act as the surface's pro-adhesive and pro-coagulant as a result of it losing all of its optimal roles (Peng *et al.*, 2019). The reason behind the replication of CECs is to replace or repair endothelial cells that have experienced damage. However, due to their limited ability to be mobile and spread quickly to sites where such damages do not occur, they are not as efficient as they are expected to be (Mavondo *et al.*, 2019; Fosse *et al.*, 2021)

Experts have explained EPCs that malfunction and their insufficient distribution which affect their mobilization together with their performance like that of the chemokines. The stromal cell-derived factor one (SDF-1 and the matrix metallo-proteinase -nine (MMP-9), do exude ineffective outcomes in numerous diseases that are linked to microvascular damage. This explanation suggests that there is a circulation of endothelial progenitor cells by incorporating itself into a site that has been damaged (Rana, Kumar and Sharma, 2018). In the consideration of some acute malaria, microvascular damage is one of the major outcomes to be experienced (Erice and Kain, 2019). Damage to endothelial cells is common for malaria infections (Angchaisuksiri, 2014). This together with a significant spike in the number of endothelial microparticles circulating sometimes serve as very strong markers of activating the endothelium in people with malaria in situations complicated by coma (Angchaisuksiri, 2014).

2.4.5 Inflammation

The coordination of the production of pro-inflammatory cytokines like IFN-y and IL-12, which are in charge of controlling the early parasitemia in murine models of malaria, is a highly vital and important part of the immune response to the disease (Punsawad, 2013). Once the parasite emerges, a coordinated attempt is made to get the liver to start producing IFN- γ and IL-12. These are quickly produced in vitro by peripheral blood mononuclear cells (PBMCs) exposed to Plasmodium falciparum (Gleeson and Bosch, 2013; Yakubu, Weiss and Silmon de Monerri, 2018). Even though in the control of parasitemia, inflammation is deemed a necessity, excessive inflammation and dysregulation through the production of inflammatory mediators could result in immunopathology (Deroost *et al.*, 2016; Dobbs *et al.*, 2017; Drewry and Harty, 2020). A vital part of the innate immune response generated by the body in response to immune *plasmodium falciparum* malaria infection is the part that recognizes pathogen ligands and ensures the initiation of the right and corresponding effector responses. These are found among the family of pattern recognition receptors, the family within which Toll-like receptors (TLRs) are found.

Three kinds of TLRs have been noted to participate in the malaria infection process. These are the TLR2, TLR5, and the TLR9. TLR2 is able to recognize Plasmodium falciparum Glycosyl phosphatidyl inositol (PfGPI) while TLR9 is able to recognize the parasite DNA that is bound to the home polymer hemozoin (Lamb, Schenk and Todryk, 2010; Tannous and Ghanem, 2018; Mukherjee, Huda and Sinha Babu, 2019). Despite the ubiquitous expression of GPIs by eukaryotes to serve as membrane anchors, the GPIs that are expressed by parasitic protozoa wield significant and staggering differences from the ones expressed in humans and show immunostimulatory activity (Ghazanfari, Mueller and Heath, 2018; Liu and Fujita, 2020).

When an engagement between TLRs and components of the malaria parasite happens, inflammation is initiated in response through Mitogen-activated protein kinases (MAPK) and Nuclear factor kappa B (NF-κB) which produces cytokines (TNF, IL-1), chemokines, and type 1 interferon (Kanmani and Kim, 2018; Shi and Sun, 2018; Efferth and Oesch, 2021). It is known that these cytokines can cause immunopathology in mouse models of severe malaria as well as in human disorders when they are produced in excess. (Ghazanfari, Mueller and Heath, 2018; Burrack, Hart and Hamilton, 2019). TLRs have been discovered to contribute to the development of human murine malaria (Zamboni and Lima-Junior, 2015; Kobia *et al.*, 2022). Even though inflammation caused by TLRs could be damaging to the host in instances of poor regulation, TLRs are also able to collaborate with other innate scavenger receptors to improve the clearance of PEs (Di Gioia and Zanoni, 2015).

2.5 Biomarkers

Biomarkers are biological molecules that can be detected in blood, other bodily fluids, or tissues and are indicators of a condition, stage of an illness, or a physiological or an unhealthy process. A biomarker may be used to assess the body's response to a condition or treatment. (Mullington *et al.*, 2016; Venkatasubramanian and Keshavan, 2016). Biomarkers serve mainly as tools of biological quantitative evaluation that give information about the state of diseases. They may perform the function of identifying individuals who exhibit a high risk of contracting a disease. For instance, the intracellular cell adhesion molecule-1 (ICAM-1) and cerebral malaria are closely related, and the histidine-rich protein 2 (HRP-2) antigen is helpful in detecting P. falciparum in human blood.(Jain *et al.*, 2014; Sahu *et al.*, 2015). Biomarkers can in some cases, be used to predict the poor outcome of a disease such as in the case of kids with malaria affecting the cerebral portion of the brain (Jain *et al.*, 2014; Foko *et al.*, 2022).

They can also be used in the identification of early stages of very invasive diseases that require expensive tests that are inaccessible by patients or unavailable. With the advancement recorded in the field of molecular diagnosis, the development of very rapid tests with the capacity to provide multiple quantitative assessments is becoming acceptable. By incorporating the idea of biomarkers into ongoing scientific trials, it may be possible to study malaria's disease process in greater detail and to identify important side effects before they develop into clinical issues (Blennow *et al.*, 2015; Sharif *et al.*, 2016)

2.5.1 Angiogenesis and Endothelial Activation

By lining the inner surface of blood cells, endothelial cells create a systemic boundary between the blood and the other parts of the body (Rönnbäck and Hansson, 2019; Yazdani *et al.*, 2019). The migration and apposition of endothelial cells to one another and their supporting cells during vasculogenesis (the formation of new blood vessels) and angiogenesis (the growth and remodeling of existing blood vessels) depends on the proteins produced by endothelial cells and their underlying mural cells. (Xia *et al.*, 2018; Coelho-Santos and Shih, 2020; Jones *et al.*, 2021).

Numerous studies have been conducted on vascular endothelial growth factor (VEGF) and its receptors Flt-1 (fms-like tyrosine kinase-1, VEGFR-1) and Flk-1 (VEGFR-2) for their function in normal and pathologic angiogenesis, as have angiopoietin-1 and angiopoietin-2 and their corresponding receptor, Tie-2 (Margadant, 2021). In situations of endothelial activation and dysfunction, aberrations in these two families of proteins have been the topic of extensive research.

Angiopoietins are a significant class of angiogenic proteins that regulate angiogenesis in a context-dependent manner in conjunction with VEGF. Pericytes and vascular smooth muscle cells, sometimes known as vascular mural cells express Ang-1 on a constant basis, and it communicates

with its corresponding receptor, Tie-2, to promote vascular stability and quiescence (Amita, 2019; Barretto, 2020)

Ang-2 mostly inhibits Ang-1 signaling, although it can also cause Tie-2 phosphorylation in the absence of Ang-1 (Fagiani and Christofori, 2013). Ang-2, like other Weibel–Palade bodies (WPB) products like von Willebrand factor (VWF) and its propeptide (VWFpp), is found in the endothelium's Weibel-Palade bodies (WPB) and can be rapidly mobilized and released when the endothelium is activated. At sub-saturating TNF-α concentrations, ICAM-1 and VCAM-1 expression has been discovered to be promoted by Ang-2, indicating that it plays a crucial part in sensitizing the endothelium to inflammatory stimuli (Norooznezhad and Norooznezhad, 2017; Chen *et al.*, 2018).

2.5.2 The Role of Angiopoietin– 1 in Malaria.

As a 70-k Da glycoprotein, Angiopoietin – 1(Ang – 1) primarily originates from smooth muscle cells that are found around the monolayer of the endothelial cell, the vasculature support cells, and the specialized pericytes found in the kidney and the liver's T cells like podocytes (Chittiboina *et al.*, 2013). These are the reasons why Ang-1 functions in a paracrine form (Liang *et al.*, 2018). Ang-1 functions as a ligand for the Tie-2 receptor discovered to belong to a collection of vascular tyrokinase receptors that are fundamentally expressed in endothelial cells (Pirouzpanah *et al.*, 2019).

To become an effective mediator, there is an activation of Tie-2 which is done by Ang-1. The mediation role is played during the angiogenetic process and also functions after the development process is complete to ensure that vascular leakage is prevented. It also functions to enhance the extent of endothelial vascular quiescence through the strengthening of the junctions of endothelial cells and is also engaged in the down-regulation of molecules that adhere to surfaces like VCAM-

1 and endothelial selectin (E-Selectin)**Error! Bookmark not defined.**. Ang-1 is found as a soluble substance in the plasma or serum. Known as the antagonist of Angiopoeitin-1, (Fagiani and Christofori, 2013) Angiopoeitin-2 levels are always lower than that of Ang-1(Fang *et al.*, 2015). In instances where there is deficiency of Ang-1 in the presence of microvascular damage results in damage to organs, accelerated angiogenesis, and fibrosis (Elpek, 2015). Research done by (Costa-Fraga *et al.,* 2018) explains that Angiopoeitin-1 have strong capacity to regulate the vascular reactions that occurs.

Endothelial versions of Ang-1 are ones that are broadly found in tissues of normal adults and are also expressed as a constituent of the endothelium (Melincovici *et al.*, 2018). Several studies are being conducted by researchers studying conditions like stroke, arteriosclerosis, sepsis, acute kidney damage and lung damage with the goal of discovering how the protective properties of Ang-1 can be used and weaponized to generate a cure (Reilly *et al.*, 2018; Hayashi, Rakugi and Morishita, 2020). When the endothelial cells are activated, several disease complications can arise because the activation induces a dysregulation of the equilibrium between Angiopoeitin-1 and Angiopoeitin-2. These dysregulations have been found to be, in some cases, responsible for tumour-associated angiogenesis, cancer and hypertension.

In considering the *Plasmodium falciparum* malaria for instance, Weibel-Palade body products including VWF, VWFpp, and Ang-2 are induced into the systemic circulation because of acute endothelial activation and the presence of mature parasitized erythrocytes that cling to the microvasculature of critical organs. The severity of malaria is therefore indicated by these biomarkers (Mahittikorn *et al.*, 2022). One of the factors that have generated investigative attention in the pursuit of more understanding of cerebral malaria is angiopoietins. Angiopoietin-1 was found to be significantly low in patients with cerebral malaria while the accompanying

Angiopoietin-2 levels were significantly high, according to research conducted by Lovegrove et al., (2009) to evaluate the relevance of angiopoietins in the diagnosis of cerebral malaria found in patients based in Thailand and Uganda. Angiopoeitin-1 and Angiopoeitin-2 levels have been found to be significantly different between patients with cerebral malaria and those with moderate malaria.

2.5.3 The Role of Angiopoietin-2 in Malaria.

Due to the need for it to be rapidly released during various incidents of inflammation and similar stimuli, the Angiopoietin-2 is generated by cells of the endothelium at low levels which are preserved in Weibel-Palade bodies (WPB) (Cossutta *et al.*, 2019; Schillemans *et al.*, 2019). The autocrine regulator angiopoietin-2 controls the actions of endothelial cells. When Angiopoeitin-2 is expressed in ECs, a destabilization of the quiescent autocrine loop happens and results in the detachment of the EC and the regression of the vessel(Korhonen, 2020). Ang-2 functionality is predicated on VEGF. This is because the presence of VEGF during Ang-2 activity results in active promotion of capillary diameter expansion, basal lamina remodeling, blood vessel stimulation, endothelial cell migration and proliferation (Fagiani and Christofori, 2013). If VEGF is not present, endothelial cell death and vessel regression are facilitated by Angiopoeitin-2's functional effects. (Melincovici *et al.*, 2018). Angiopoeitin-2 must be either an agonist or an antagonist for Tie-2 to function is dependent on circumstances and the composition of its environment. Studies have found that when Ang 2 combines with Ang-1, it may have a role in the formation of endothelial cells from the circulation of blood CD34 + progenitors(Higgins *et al.*, 2016; Shi *et al.*, 2021). While Ang 2 is in charge of the CD34+ progenitor cells' circulation and proliferation, Ang-1 is typically engaged in controlling the early stages of endothelial cell commitment for these cells. The synergy experienced between the activities of Ang-1 and Ang-2 directly initiates the activation

of the neutrophils and the ECs to generate and advance a pro-inflammatory response through signaling from the Tie-2 and B2 integrin (Gacche and Assaraf, 2018).

The level of Ang 2 recorded in septic proteins was found to positively correspond to the severity of malaria and was used as a substantive basis to project substantive development of factors such as shock or death (Storm and Craig, 2014). In mice, high levels of Ang 2 recorded were correlated to the increase in survivability of the mice who had been induced with the pseudomonal aeruginosa experimented sepsis. This was observed after recombinant Ang-2 was induced in the mice a few hours before the commencement of the experiment (Amison *et al.*, 2018). In some instances, the functionality of Ang-1 is antagonized by Ang-2 which leads to rise in vascular permeability and activation of the endothelium (Fagiani and Christofori, 2013). Additionally, Ang-2 has been labelled and described as an indicator of secondary injury and a modulator of the breakdown of the blood-brain barrier (BBB) together with apoptosis of the endothelium (Chittiboina *et al.*, 2013).

A change in the Ang-1: Ang-2 ratio which happens to be usually low results in dysregulation of angiopoietin. This happens when Ang-1 levels drop, Ang-2 levels rise or when both situations occur. During serum analysis, Ang-2: Ang-1 ratios (Ratio, 0.857, $p < 0.001$) (Conroy *et al.*, 2014) serve as a substantial indication of the severity of malaria and are hence identified as being implicated in the pathogenesis of various diseases (Storm and Craig, 2014). During the very early stages of an injury in the endothelium, a reduction in Ang-1 levels is recorded accompanied by an upscaling of the peaks of Ang-2 present. The extent of Angiopoeitin-2 upscaling experienced or recorded is useful in determining the severity of the damage done, failure of an organ, sudden physiology and long term health (Chittiboina *et al.*, 2013).

In cases of malaria induced by Plasmodium falciparum, Ang-2 has been determined to be involved in the sensitization of the endothelium. This sensitization leads to a surge in the intensity with

which adhesion molecules express themselves. These adhesion molecules, including ICAM-1, attach to infected red blood cells (Kucukal *et al.*, 2020). Storm and Craig, (2014), postulated that, the rise in plasma Ang-2 levels is a result of a rise in venous lactate concentrations which can affect the concentration of plasma ICAM-1, the parasite biomass and chance of death in people with severe malaria. In short, it was discovered by Oluboyo *et al.*, 2020, that the levels of Ang-2 concentration served as a good basis for predicting the death of patients.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site and Study Design

The cross-sectional nature of this study allowed for individuals to be accessed at one point in time at the Outpatients Department and Laboratory Department of the Breman Seventh Day Adventist Hospital (Adventist Hospital, Breman). The facility, which serves as the municipal hospital, is situated in the Suame Municipality and the only Quasi-government facility within the Municipality hence a referral site for most facilities around the catchment area. Almost in the middle of the Ashanti Region, between Latitude 6.35°N and 6.40°S and Longitude 1.30°W and 1.35°E, is the Suame Municipal, founded in 2017. The Municipality's topography rises from 250 to 300 metres above sea level. The municipality's northern, eastern, and southern borders are shared with the Afigya Kwabre South District, Old Tafo Municipality, and Kumasi Metropolis

The Municipality has an estimated population of 250,365 people. The Municipality is cosmopolitan with almost all ethnic groups. The municipality is home to West Africa's largest enclave of artisanal engineers which is popularly known as the “Suame Magazine’’ .It provides the majority of individuals in the municipality and elsewhere with a significant source of income.

3.2 Sampling and Sample Size

A total of one hundred and sixty-six (166) participants were used for the study. Out of this number, forty-two (42) were apparent healthy individuals serving as controls, seventy-eight (78) were patients presenting with uncomplicated malaria, and forty-six (46) were subjects presenting with severe malaria. The World Health Organization's (WHO) categorization guidelines for malaria served as the foundation for classifying the severity of the disease. Participants recruited for the study were all Ghanaians. It included both children (between 6 months and 13yrs) and adults

(above 14 years) both males and females. According to WHO guidelines, the cases were diagnosed as uncomplicated or severe malaria in outpatients after malaria was diagnosis with microscopy. Controls were healthy individuals who did not have any signs or symptoms of any ailment and tested negative for malaria. Non-Ghanaians were exempted from the study. Individuals with conditions such as diabetes, hypertension, inflammation, and other chronic infections as well as children less than 6 months were excluded. Also, people who had taken antimalarial drugs for less than one month were excluded from this research.

Individuals in the first group (uncomplicated malaria) and the second group (severe malaria) were made up of children and adults between the ages of six months and eighty years who reported to the Outpatient Department of Breman SDA Hospital for treatment. The individuals who met the inclusion criteria, which are described in the following two sections (case definition), were chosen after clinical examination of these participants. Children and adults without malaria who agreed to participate in the study made up the third group, or the control group, and they ranged in age from 1 year to 60. An informed consent was signed by the grownups. while the parents and guardians signed on behalf of their children after making them understand the purpose of the study.

3.2.1 Sample size Justification

The appropriate sample size needed for the investigation was determined using Cochran's method.

$$N = \frac{P(1-P)Z^2}{e^2}$$

Where;

N = Sample size required, **Z** = Confidence interval at 95% (standard value of 1.96), **P** = prevalence rate (8.9%), **e** = error margin (standard value of 0.05). Hence **N= 124** (minimum). 166 people made up the study's sample size.

3.3 Case Definition

According to the WHO, the case definition for the categories of malaria are;

a. **Uncomplicated malaria** – the patient must present with fever or previous history of fever, a positive malaria test either with microscopy or Rapid Diagnostic Test (RDT) with no sign of any vital organ dysfunction.
b. **Severe malaria** – the patient must present with fever or previous history of fever, a positive malaria test either with microscopy or Rapid Diagnostic Test (RDT) and with a sign of any vital organ dysfunction (a condition where an organ does not work as it should).

Participants who were recruited as controls had to meet the following inclusion criteria: normal body temperature, no parasitaemia, sickling negative, no chronic disease, and no recent history of receiving treatment for malaria.

The enrolled participants were divided into three (3) groups: healthy controls (HC), severe malaria (SM), and uncomplicated malaria (UM). Patients who presented with malaria but did not meet any of the World Health Organization's (WHO) severe malaria criteria fell into the category of uncomplicated malaria, whereas those in the severe malaria category did meet any of the WHO's severe malaria criteria (Wilairatana *et al.*, 2021). The healthy controls group included individuals with haemoglobin (Hb) levels greater than 11.0g/dL who did not have malaria or any other form of the disease at the time of enrollment,

3.4 Ethical clearance

The researcher received ethical approval from the Kwame Nkrumah University of Science and Technology's Committee for Human Research, Publication, and Ethics (CHRPE / KNUST) with Reference Number CHRPE/AP/129/21. This study was also subject to the approval of the Management of Breman SDA Hospital. The researcher observes and prioritizes cardinal ethical principles of voluntary participation, anonymity, and confidentiality for all participants whose direct and retractable consents were sought for this study.

3.5 Data collection

A systematic questionnaire was used to gather information from participants and controls about sociodemographic characteristics and patient profiles, including sex, age, ethnicity, religion, place of residence, level of education, and occupation. Also, the history of taking anti-malaria drugs in the previous month, symptoms and signs of uncomplicated malaria and severe malaria were captured from the study subjects. The questionnaire was administered to participants or their guardians who voluntarily consented to the study. Those who could read and write were given the forms to be filled by themselves and the questionnaire was read and explained to individuals who couldn't read or write immediately before their samples were obtained.

3.6 Anthropometric Measurement

Measurements such as body weight in kilograms (kg) temperature in degrees Celsius (°C) and for both cases of uncomplicated and severe malaria, blood pressure was measured in mmHg and pulse rate in beats per minute (bpm).

3.7 Blood Sample Collection and Processing

After obtaining the participants' consent, a skilled phlebotomist took a sample of venous blood measuring 5ml from the arm. Three millilitres (3 ml) of the whole blood were dispensed into a gel-separated yellow corked test tube and allowed to clot and centrifugated at 5000 rpm for 5 minutes

to harvest the serum. Two millilitres (2 ml) were dispensed to EDTA test tubes for Blood films for malaria parasite detection and quantification and Full Blood Count. The serum harvested was put into Eppendorf tubes and stored at -20 ºC for measurements of serum angiopoietins 1 and 2 levels and biochemical assay (LFT and RFT).

3.8 Laboratory Investigations

Laboratory tests were run at Wenchi Methodist Hospital and Breman SDA Hospital's Laboratory Department. Detection and quantification of malaria parasites on blood films, full blood count (FBC) analysis and biochemical assays were carried out at Breman SDA Hospital laboratory while ELISA for angiopoietins 1 and 2 was done at the Wenchi Methodist Hospital (WMH) Laboratory.

3.9 Assay Methods

3.9.1 Parasitological examination of Blood Films for malaria parasites detection and quantification

Using the technique described by Haggaz et al., (2014), thick and thin films of peripheral blood were prepared for the examination of plasmodium parasites. On a fresh frosted end microscope slide, thin and thick blood films were prepared using a slide preparation template. A glass microscope slide has 6ul of blood deposited at one end of it using an automatic micropipette and evenly spread on the slide to the size of about 12mm in diameter for the thick film. With a smooth-edge spreader held at a 45° angle to the microscope glass slide, 2ul of blood was uniformly spread out to cover nearly the full length of the slide for the thin blood films. The blood films made were labelled properly and allowed to air- dry completely. By gently immersing for 2 seconds, the thin blood films were fixed with 100% methanol. After 10 minutes of staining with newly made 10% Giemsa solution (in phosphate buffer), the blood films were carefully washed with buffered water that had a pH of 7.2. The slides were air-dried and analyzed for the presence of plasmodium parasites and species identification using immersion oil under a light microscope (X100 objective)

by a nationally certified grade "A" malaria microscopist using a Carl Zeiss Primo Star microscope (Carl Zeiss Suzhou Co. Ltd China, 2016) supplied by the national malaria control program (NMCP). On 200 white blood cells (WBCs), the plasmodium parasites were enumerated in the thick film or against 2000 Red Blood Cells (RBCs) depending on the parasite density. Parasite densities were estimated using the standard formulae below.

$$\text{Parasites}/\mu l = \frac{\text{Parasite count X 8,000}}{\text{\# of WBC counted}} \quad \text{or}$$

$$\text{Parasites}/\ \mu l = \frac{\text{Count of parasitized RBCs X 5,000,000}}{\text{Total Red Cell Counted (parasitized RBCs + Non- parasitized RBCs)}}$$

3.9.2. Haematological measurements (Full Blood Count analysis)

The haematological parameters were measured using a five (5) parts automated haematology analyzer, Sysmex XN – 350 (Sysmex Corporation, Kobe, Japan 2019) as indicated by Akatse - Tsesu, 2022. The automated haematology analyzer functions with the principles of DC Impedance method with hydrodynamic focusing (RBC/PLT), Flow Cytometry (WBC), Fluorescence Flow Cytometry (WBC DIFF/RET), and Cyanide-free SLS method (HB).

In Fluorescence flow cytometry, examination of cells and particles occurs whiles the cells traversed through a very small flow cell. A blood sample is first aspirated, proportioned, and then diluted according to a predetermined ratio and marked with a proprietary fluorescent marker that binds only to nucleic acids. The side fluorescence represents the quantity of nucleic acids in the cell. In a graph known as a "scattergram," cells with comparable physical and chemical characteristics group together.

When performing flow cytometry (WBC), a sample comprising cells or other particles is dissolved in fluid and injected into the flow cytometer device. The sample is ideally focused such that it flows through the laser beam one cell at a time, where the light scattered by the scattered light is indicative of the cells and their components. With the DC Impedance method with hydrodynamic focusing (RBC/PLT), a fluid sheath surrounds diluted red blood cells, causing them to line up in a single line as they pass through the detecting aperture. To prevent cell recirculation after they have passed through the aperture, the cells are then guided away from the back of the aperture. The device then finds the cells' volume, size, and number.

In the Cyanide-free SLS method (HGB), red and white blood cells in the sample are lysed by the reagent. The oxidation of the haeme group comes after the globin is altered in the chemical process. The haeme group can now bind to the hydrophilic groups of the SLS to create a stable, coloured complex (SLS-HGB), which is then measured using a photometric technique.

Haemoglobin (HGB) level, total white blood cell (TWBC), total red blood cell (RBC), mean corpuscular volume (MCV), total red blood cell (RBC), platelets (PLT) count, mean corpuscular haemoglobin (MCH), haematocrit (HCT), and mean corpuscular haemoglobin content (MCHC), as well as differential white blood cell count - lymphocyte, neutrophil, and monocyte - are among the parameters measured. Eosinophil and basophil (percentage and absolute count).

3.9.3 Measurement of Human Angiopoietin-1 and Angiopoietin-2.

The enzyme-linked immunosorbent assay (ELISA) method was used to quantify the levels of human angiopoietins-1 and angiopoietin-2. The double sandwich ELISA was used in accordance with the manufacturer's instructions to measure the amounts of human angiopoietins in the samples (serum) of research participants. (Melson Shanghai Chemical Ltd, 2020, ANG-1 KIT10062 ANG 2 -KIT10691) (Le Cras *et al.*, 2017) which works on the principle that any antigen present binds to the capture antibody once the sample is put to wells coated with a

capture antibody. Before an enzyme-linked secondary antibody is administered and binds to the detecting antibody, an antigen-binding detecting antibody is first added. Following the addition of a substrate, this is changed by an enzyme to a form that can be detected.

3.9.3.1 Assay Procedure

Dilution of the original standard density was done as follows:

Table 3. 1 Dilution of Original Standard Density

60ng/L	Standard 5	150ul original standard density +150ul standard diluent
30ng/L	Standard 4	150ul standard 5 + 150ul standard diluent
15ng/L	Standard 3	150ul standard 4 + 150ul standard diluent
7.5ng/L	Standard 2	150ul standard 3+ 150ul standard diluent
3.75ng/L	Standard 1	150ul standard 2 + 150ul standard diluent

For sample addition, blank wells were set separately from test wells. For test wells, 50ul of the standard to micro-Elisa strip plate was added, 40ul samples dilution fluid and the 10ul of test samples were subsequently added and gently mixed well. This was incubated at 37ºC for 30 minutes after closing the microplate with a plate closure membrane.

The plate closure membrane was uncovered, and the microplates were washed repeatedly 5 times (with an automatic microplate washer, Bio-Rad PW 40) and allowed to dry.

50ul the enzyme, horseradish peroxidase (HRP) conjugate was put into every well, except the blank well and were kept in an incubator at 37ºC for half an hour after closing the microplate with plate closure membrane.

The plate closure membrane was uncovered, and the microplates were washed repeatedly 5 times (with an automatic microplate washer, Bio-Rad PW 40 manufactured by Bio-Rad Co. Ltd, 2014)

and allowed to dry. 50ul of chromogen solutions A and B to the wells and put in an incubator for 10 minutes at 37ºC.

By adding 50ul of stop solution to each well, the reaction was stopped. From blue to yellow, the colour changed. This was measured by zeroing with the blank, read absorbance at 450nm using the microplate reader (Mindray, MR -96A manufactured by Mindray Medical International Limited, 2013) within 15 minutes after adding the stop solution.

3.10 Biochemical Analysis

The samples' biochemical analysis was performed using the LE – Scientific Fully Automated Chemistry Analyzer LE Max – 2000 (LE Scientific Medfuture, Hamburg, Germany, 2021). The analyzer employs different principles depending on the analyte to be measured (Al-Salahy *et al.*, 2016).

Alanine Aminotransferase (ALT)

Glutamate and pyruvate are produced as a result of the reversible amino group transfer from alanine to -ketoglutarate, which is catalyzed by the enzyme alanine aminotransferase (ALT). By the action of LDH and NADH, the pyruvate generated is converted to lactate.

$$\text{L – Alanine + α- ketoglutarate} \xrightarrow{\text{ALT}} \text{Glutamate and Pyruvate}$$

$$\text{Pyruvate + NADH + } H^{+} \xrightarrow{\text{LDH}} \text{Lactate +} NAD^{+}$$

The catalytic concentration of ALT present in the sample determines how quickly the concentration of NADH decreases when evaluated photometrically.

Alkaline Phosphatase (ALP)

At a pH of 10.4, alkaline phosphatase catalyzes the hydrolysis of p-nitrophenyl phosphate, releasing both p-nitrophenyl and phosphate, as per the process.

$$\text{p- Nitrophenylphosphate +} H_2O \xrightarrow{\text{ALP}} \text{p-Nitrophenol + Phosphate}$$

The catalytic concentration of alkaline phosphatase present in the sample correlates with the rate of p-nitrophenol production as evaluated photometrically.

Aspartate Aminotransferase (AST)

To create glutamate and oxalacetate, Aspartate Aminotransferase (AST) catalyzes the reverse transfer of an amino group from aspartate to -ketoglutarate. Malate-by-malate dehydrogenase (MDH) and NADH are formed from the reduced oxalacetate

$$\text{L -Aspartate + } \alpha\text{ - Ketoglutarate} \xrightarrow{\text{AST}} \text{Glutamate + Oxalacetate}$$

$$\text{Oxalacetate + NADH + } H^{+} \xrightarrow{\text{MDH}} \text{Malate + } NAD^{+}$$

The amount of AST present in the sample determines how quickly the concentration of NADH decreases when measured photoelectrically.

Gamma – Glutamyl Transferase (GGT)

The transfer of the gamma-glutamyl group from gamma-glutamyl-p-nitroanilide to acceptor glycylglycine is catalyzed by the enzyme gamma-glutamyl transferase (GGT)..

$$\text{Gamma-L-Glutamyl-3-carboxy-4-nitroanilide + Glycylglycine} \xrightarrow{\text{GGT}} \text{Gamma- L- Glutamyl-glycylglycine + 2-Nitro- 5- aminobenzoic acid}$$

The amount of GGT present in the sample affects how quickly 2-nitro-5 aminobenzoic acid forms.

Total Protein

In an alkaline medium, proteins produce a strong violet-blue complex with copper salts. An antioxidant used is iodide.

$$\text{Protein + } Cu^{2+} \xrightarrow{\text{alkali}} \text{Coloured complex}$$

The amount of total protein present in the sample directly relates to how intense the colour that results is.

Albumin

When albumin is present with bromocresol green at a pH that is somewhat acidic, the indicator's colour changes from yellow-green to green-blue.

BCG + Albumin $\xrightarrow{\text{controlled pH}}$ Green BCG albumin complex

The amount of albumin present in the sample directly correlates with the intensity of the colour produced.

Bilirubin (Total and Direct)

A result of azobilirubin is created when bilirubin combines with diazo salt in an acidic environment.

Bilirubin + diazo salt $\xrightarrow{\text{H+, surfactant}}$ Azobilirubin

The amount of bilirubin present in the sample directly correlates with the increase in absorbency.

Creatinine

The assay is based on Jaffe's description of the interaction between creatinine and sodium picrate. Alkaline picrate and creatinine interact to generate a red complex. The measuring window used prevents interference from other serum components.

Creatinine + sodium picrate $\xrightarrow{\text{alkali}}$ creatinine – picrate complex

The sample concentration directly relates to the intensity of the colour generated..

Urea

Enzymatic hydrolysis of the urea in the sample produces ammonia (NH4+) and carbon dioxide (CO2). The resulting ammonia ions react with ketoglutarate. glutamate dehydrogenase (GLDH) catalyzes a step in which NADH is simultaneously oxidized to NAD+

$$\text{Urea} + H_2O + 2H^+ \xrightarrow{\text{Urease}} (NH_4^+)_2 + CO_2$$

$$NH_4^+ + \alpha\text{- Ketoglutarate} + NADH \xrightarrow{\text{GLDH}} H_2O + NAD^+ + \text{L-Glutamate}$$

The ratio of the concentration of urea to the reduction in NADH concentration is linear.

3.11 Estimation of Glomerular Filtration Rate

For healthy controls (HC), the uncomplicated malaria (UM) group, and the severe malaria (SM) group, the estimated glomerular filtration rate (eGFR) was determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI).

3.12 Data analysis

The Kolmogorov-Smirnov test was used to examine the distribution of the continuous variables, none of the variables passed the normality test, hence were analysed using non-parametric tests. The median and interquartile ranges (1^{st} and 3^{rd} quartiles) were used to summarize the data. In order to compare two groups Mann-Whitney U test was used, while Using Kruskal-Wallis analysis, three or more groups were compared followed by Dunn's Multiple Comparison tests. Correlations between two continuous variables of interest were tested using the nonparametric Spearman's rank correlation (rho). To find the most accurate indicators of severe malaria, multiple logistic regression was used. The prognostic accuracy of angiopoietin levels (Ang-1 and Ang-2) was determined by the use of receiver operating characteristic (ROC) curves. Using the GraphPad Prism 9 software (GraphPad Software, San Diego, CA, USA), all statistical analyses were performed. A p-value of 0.05 was used to define statistically significant differences.

CHAPTER FOUR

RESULTS

4.1 Description of Study Population

This research recruited a total of 166 participants between the ages of 1 – 70 years comprising 46 (27.7%) severe malaria cases, 78 (47.0%) uncomplicated malaria cases and 42 (25.3%) healthy subjects as control. The median age of the healthy control was 24 (IQR: 5 - 38) and 26 were female while 16 were male.

Table 4.1 summarises the parasite count differences between uncomplicated malaria and severe malaria based on their demographic characteristics. Within the uncomplicated malaria patients, children below the ages of 5 years recorded the highest parasitaemia (7346, IQR: 1994 – 26693); however, this was not statistically different from the other age groups (5-17 and ≥ 18 years) (p=0.075). Sex, marital status, education, and occupation were all not related to parasitaemia within the uncomplicated malaria subjects. Conversely, within the severe malaria population, those with basic education significantly recorded higher parasitaemia than their counterpart with secondary education. The rest of the demographic characteristics for the severe malaria population did not affect parasitaemia levels.

Table 4. 1: Comparison of parasitaemia levels of uncomplicated and severe malaria within the various demographic characteristics

[a] only participants ≥ 18 years were included; [b]only participants ≥6 years were included; n = sample size; IQR = 1st–3rd quartile

Demographic characteristics	Uncomplicated malaria			Severe Malaria		
	n	Parasitemia (/µL) Median (IQR)	P value	n	Parasitemia (/µL) Median (IQR)	P value
Age range						
<5	6	7346 (1994 – 26693)		16	29589 (17150 – 102905)	
5-17	14	2808 (1397 – 7568)		14	29793 (14542 – 88230)	
≥18	58	2670 (1109 – 6716)	0.649	16	5461 (1871 – 32617)	0.075
Sex						
Female	42	2670 (680 – 6716)		22	26823 (2595 – 179603)	
Male	36	3623 (1208 – 8470)	0.190	24	23542 (5856 – 57785)	0.744
Marital status						
Single	26	2670 (1208 – 8470)		10	26823 (6542 – 50000)	
Marriage	30	1275 (787 – 6112)	0.546	4	3079 (1777 – 4380)	0.120
Education[b]						
No education						
Basic	36	2413 (840 – 4400)		14	29793 (9439 – 88705)	
Secondary	18	4208 (2670 – 8676)		14	6542 (2282 – 40296)	0.037
Tertiary	8	1641 (947 – 4531)	0.071		NA	
Occupation[a]						
Student	18	2670 (1208 – 8470)		8	28271 (5250 – 53455)	
Informal sector	32	3623 (1103 – 6586)		8	3141 (1871 – 9991)	0.253
Formal sector	6	2041 (1441 – 9510)	0.937		NA	-

4.2 Evaluation of clinical parameters and biomarkers in malaria patients (UM and SM) and healthy controls (HC)

Table 4.2 shows the comparison of biomarkers and clinical indicators in malaria patients (UM and SM) and healthy controls (HC). The parasite counts of severe malaria (24615, IQR: 3943 – 60541)) population were significantly higher than uncomplicated malaria (2808, IQR: 1090 – 8189).

Patients with severe malaria had significantly lower haemoglobin and platelet counts than those with uncomplicated malaria ($p<0.05$). However, the WBC of severe malaria patients (7.4, IQR: 5.4 – 10.6) was significantly higher than the uncomplicated malaria population (5.7, IQR: 5.0 – 6.5) ($p < 0.001$).

Serum levels of bilirubin (total and direct), aspartate transaminase (AST), alkaline phosphatase (ALP), alanine transaminase (ALT), gamma-glutamyl transferase (GGT) and total proteins in severe malaria were significantly higher than uncomplicated malaria group ($p < 0.001$).

The median Angiopoietin-1 levels in SM were lower (3.8 ng/L, IQR: 2.2 – 12.7) in comparison to UM (6.3 ng/L, IQR: 3.3 – 8.0) and healthy controls (9.6 ng/L, IQR: 3.5 – 15.3). Ang-2 levels were higher in SM (19.1ng/L, IQR: 9.0 – 25.8) compared to UM (15.7ng/L, IQR: 2.6 – 27.4), however, this did not reach statistical significance ($p=0.152$).

Table 4. 2: Comparison of clinical parameters and biomarkers in malaria patients and healthy controls

Variable	Healthy controls (HC), n= 42	Uncomplicated malaria (UM), n=78	Severe malaria (SM), n = 46	P value for UM vs. SM
Haemoglobin	11.8 (11.3 – 12.4)	11.8 (10.8 – 13.1)	10.9 (9.2 – 12.5)	0.003
WBC	6.22 (5.0 – 8.1)	5.7 (5.0 – 6.5)	7.4 (5.4 – 10.6)	<0.001
Platelet count	270 (229 – 342)	149 (117 – 196)	108 (46 – 130)	<0.001
NEUT	41.6 (37.8 – 60.7)	69.8 (55.2 – 78.1)	71.5 (61.9 – 82.7)	0.124
LYMP	46.5 (30.3 – 53.3)	23.5 (14.9 – 33.5)	19.0 (12.1 – 29.9)	0.249
MXD	8.8 (7.2 – 10.2)	7.7 (6.1 -12.1)	7.9 (4.7 – 8.9)	0.012
Total Bilirubin	5.2 (3.8 – 6.2)	8.1 (5.4 – 10.7)	11.2 (7.4 – 24.3)	<0.001
Direct Bilirubin	2.1 (1.2 – 3.2)	3.4 (1.9 – 4.3)	6.6 (4.1 – 16.1)	<0.001
ALT	7 (6 – 8)	9 (5.3 – 11.8)	12 (10 – 18.7)	<0.001
AST	21 (13 – 26)	17 (12 – 23.3)	26 (16.3 – 50)	<0.001
GGT	18 (11 – 22)	20 (13.3 – 28.8)	40 (18.3 – 49.5)	<0.001
ALP	80 (66 – 97)	66 (49.3 – 107.5)	92 (67.8 – 126.8)	0.054
Total protein	69.2 (64.9 – 70.7)	64.1 (60 – 69.4)	68.3 (66.2 – 72.7)	0.002
Albumin	38.6 (37.1 – 41.6)	37.6 (35.9 – 39.7)	35.9 (31.7 -37.9)	0.002
Parasite count	NA	2808 (1090 – 8189)	24615 (3943 – 60541)	<0.001
Ang-1	9.6 (3.5 – 15.3)	6.3 (3.3 – 8.0)	3.8 (2.2 – 12.7)	0.129
Ang-2	18.7 (12.4 – 22.1)	15.7 (2.6 – 27.4)	19.1 (9.0 – 25.8)	0.429
Ang-2/Ang-1	1.9 (1.2 – 3.5)	2.0 (0.6 – 3.2)	3.1 (1.4 – 5.3)	0.152

Wbc = White blood cell(×109/L); Neut = Neutrophil (%); Lymph = Lymphocyte (%);Mxd = Mixed Cell Count (%): ALT = alanine transaminase : AST = aspartate transaminase; GGT = gamma-glutamyl transferase ; ALP = alkaline phosphatase; Ang-1 = Angiopoietin 1; Ang-2 = Angiopoietin 2

The serum creatinine of severe malaria patients (95, IQR: 84.3 – 127.0)) was significantly higher than uncomplicated malaria (72.4, IQR: 59.8 – 90) and healthy control group (58, IQR: 43 – 74) (Figure 4.1) (p <0.0001). Similarly, the serum urea levels of SM patients (7.6, IQR: 5.3 – 9.3) were significantly higher than uncomplicated malaria (5.6, IQR: 4.8 – 7.2)) and the control group (5.4, IQR: 4 – 6.5)). However, the urea levels of UM and HC were comparable (Figure 4.1) (p =0.001)

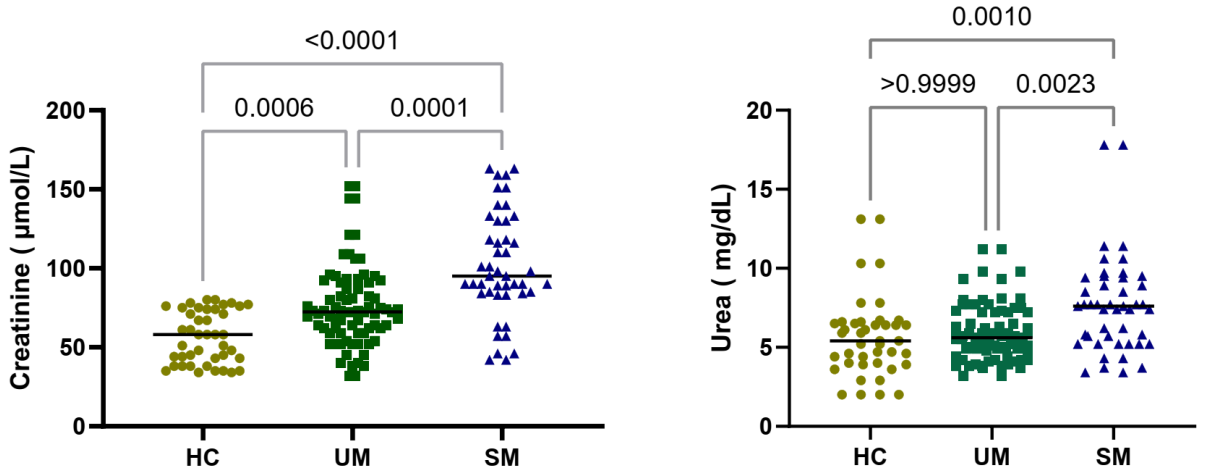

Figure 4. 1 Comparison of serum creatinine and urea levels in malaria patients (UM and SM) and healthy controls (HC)

4.3 Correlation analysis of Angiopoietin-1 and Angiopoietin-2 with platelet count and malaria parasite count

A correlation between angiopoietin-1 and angiopoietin-2 levels and platelet count of HC, UM and SM is shown in Figure 4.2. In healthy controls, Ang-1 recorded a stronger but not statistically significant association with platelet count (rho = 0.29, p =0.190) than Ang-2 (rho = 0.19, p = 0.21). However, there was a slight non-significant inverse connection between Ang-1 and platelet count in the population with uncomplicated malaria (-0.15, p = 0. 20) similar results were observed for Ang-2 and platelet count (rho = -0.06, p = 0.612). While Ang-1 inversely correlated with platelet count (rho = 0.22, p = 0.139), Ang-2 correlated positively with the platelet count (rho = 0.12, p = 0.43) in the severe malaria population; however, both were not significant.

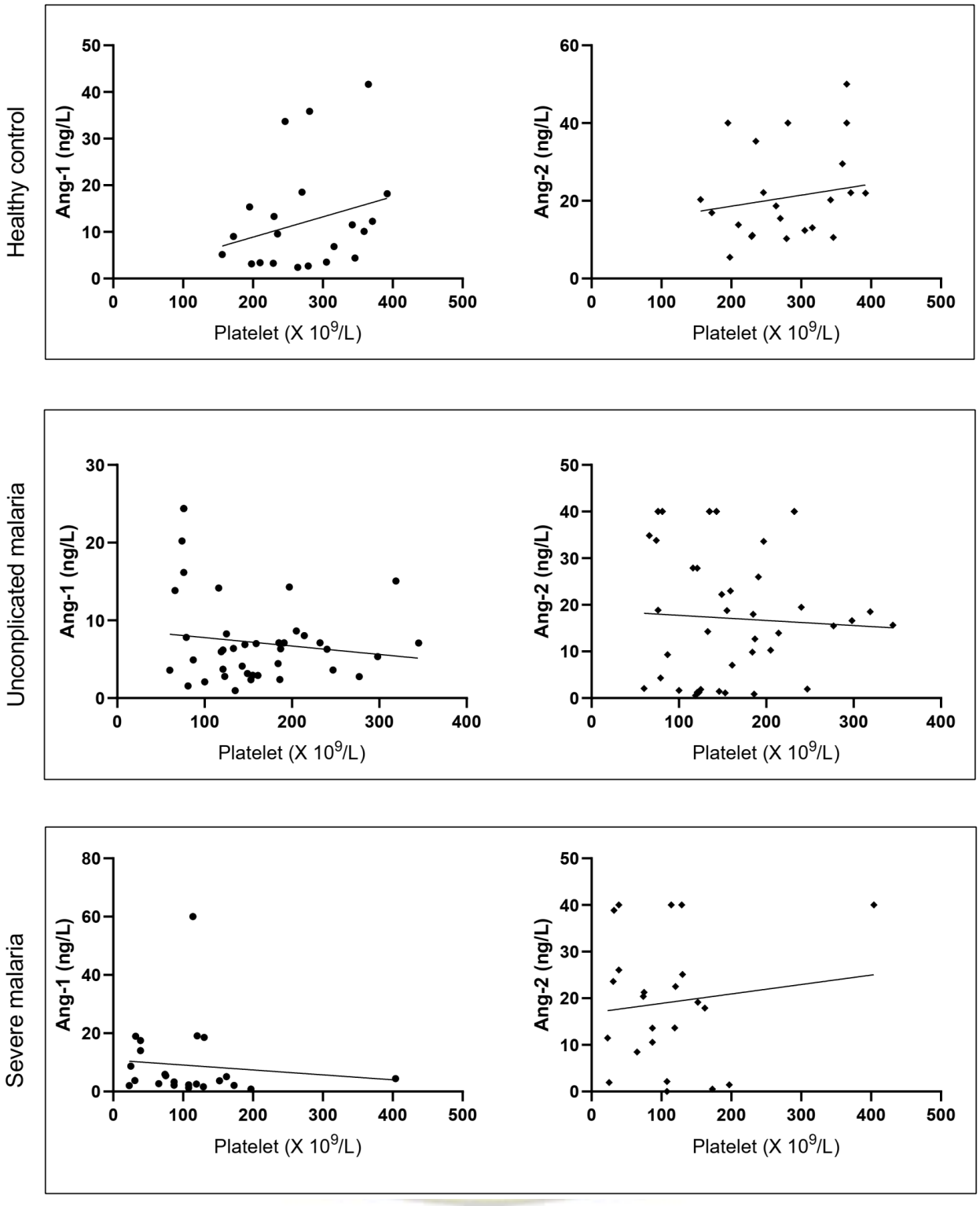

Figure 4. 2: Correlation analysis of angiopoietin levels (Ang-1 and Ang-2) and platelet count

There was no correlation (rho = 0.0, p = 0.89) between Ang-2 and parasite count in people with uncomplicated malaria but Ang-1 correlated weakly with parasite count (rho = 0.10) but this was not significant (p = 0.382). However, Ang-1 and parasite count had a significant positive weak correlation (rho = 0.15, p = 0.003), though it was similarly not significant, Ang-2 had a positive correlation with parasite count (rho = 0.15) (p = 0.332).

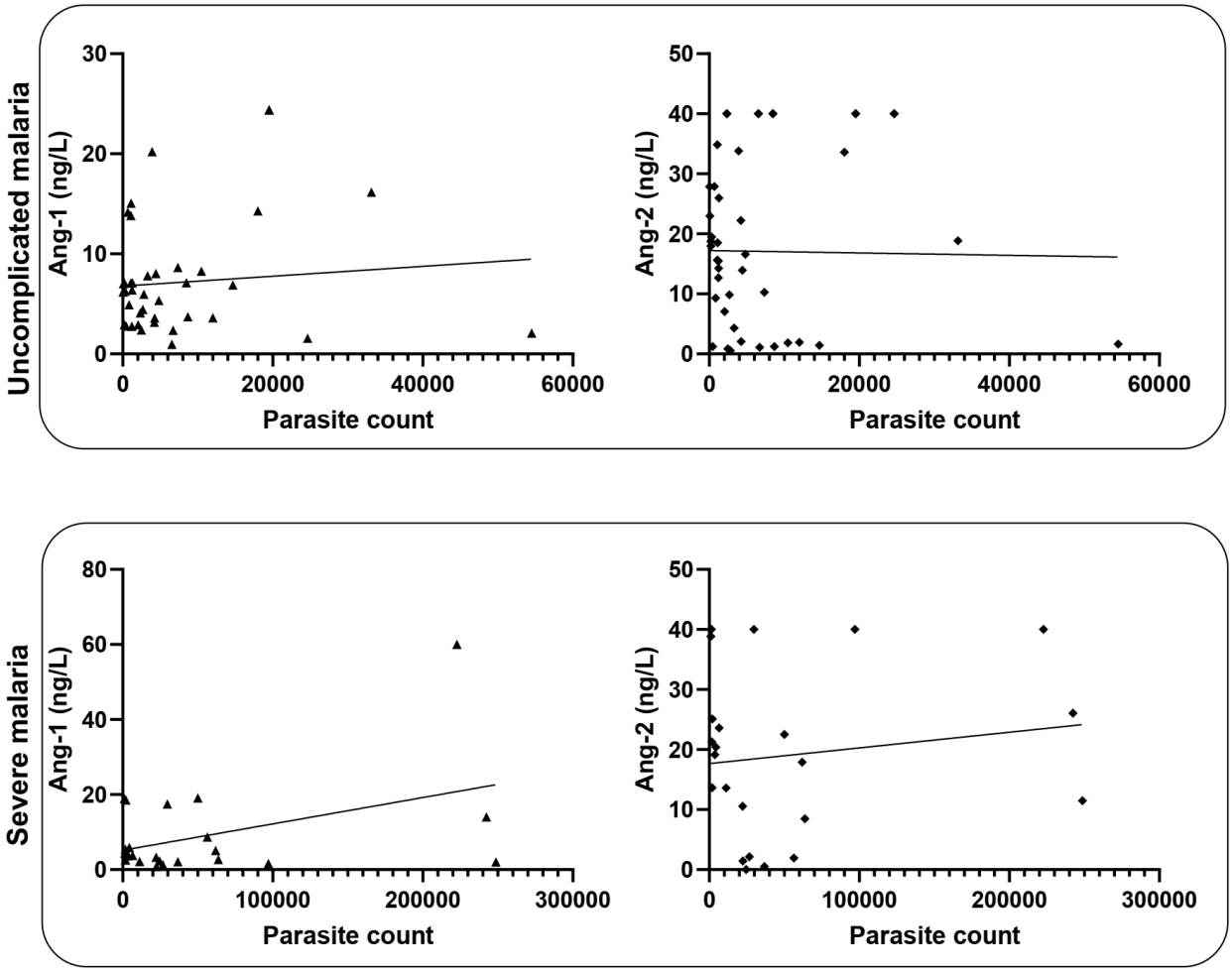

Figure 4. 3: Correlation analysis of angiopoietin levels (Ang-1 and Ang-2) and malaria parasite count

4.4 Multivariable logistic regression model

Because 46 (37.1%) of the total malaria patients (124) were categorised as having severe malaria (SM), the categorization cut-off value was set at 0.37, and the model without predictors can classify 37.1% of the severe malaria patients accurately by chance. The relationship between clinical variables and malaria severity was further explored using a multivariable logistic regression model (Table 4.3). Among the 10 variables in the model, only platelets and creatinine were substantially related to severe malaria The odds ratios of platelet was 0.9909 and creatinine was 1.032. This means that as platelet increases by 1 unit, the odds of someone with uncomplicated malaria getting severe malaria also decrease by 1%, while a 1 unit increase in creatinine will increase the odds of getting severe malaria by 3%.

Table 4. 3: Multivariable logistic regression model to predict severe malaria using clinical parameters and biomarkers.

Variable	β	OR	95% CI for OR	P value
AGE	-0.01336	0.9867	0.9521 to 1.020	0.4401
HB	-0.2853	0.7518	0.5334 to 1.037	0.0897
PLT	-0.009133	0.9909	0.9823 to 0.9982	0.0249
WBC	0.1090	1.115	1.025 to 1.335	0.1451
ANG-1	0.01862	1.019	0.9599 to 1.083	0.5252
ANG-2	0.03785	1.039	0.9909 to 1.091	0.1198
ALT	-0.04155	0.9593	0.9086 to 1.007	0.1091
AST	0.02238	1.023	0.9893 to 1.058	0.1905
UREA	0.1545	1.167	0.8101 to 1.680	0.4063
CREATININE	0.03165	1.032	1.008 to 1.061	0.0152
Constant	2.339	0.2869	0.002346 to 23.65	0.5934

HB = Haemoglobin; PLT = platelet count; WBC = white blood cell; ANG-1 = Angiopoietin 1; ANG-2 = Angiopoietin 2; ALT= alanine transaminase; AST= aspartate transaminase

The classification percentage accuracy for classifying severe malaria increased from 37.1 for the model without predictors to 83.1% for the final model with predictors (Figure 4.4). The model's sensitivity for accurately classifying patients with severe malaria is 70.0%; its specificity or capacity to correctly classify patients with uncomplicated malaria is 90.0%. The high positive and negative predictive values of the logistic regression model are 82.1% and 83.5%, respectively.

The Receiver Operating Characteristics (ROC) curve has an area under it of 0.86% (95% CI: 0.79 - 0.94, p=0.01) (Figure 4.4). Because of this, the model in Table 4.3 has strong predictive performance and has an area under the curve (AUC) value >0.5.

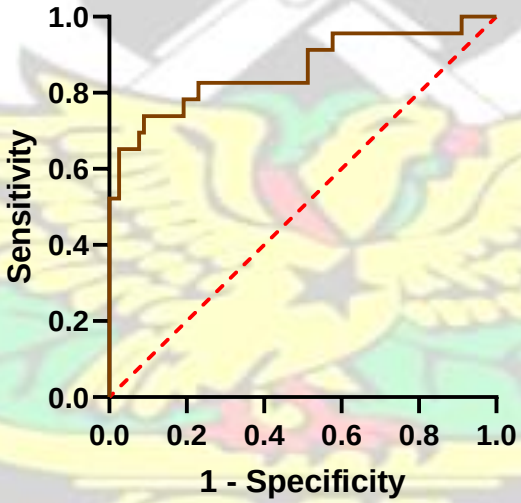

Figure 4. 4: Receiver Operating Characteristics (ROC) curve of the multivariable logistic model

4.5 Receiver Operating Characteristic (ROC) Curves to determine differences in Angiopoietin Levels of HC, UM, and SM

In order to test the ability of Ang-1, Ang-2, and Ang-2/Ang-1 biomarkers to distinguish between Healthy Controls (HC), Uncomplicated Malaria (UM), and Severe Malaria (SM), Receiver

Operating Characteristics (ROC) curves were used. For each ROC curve, the AUC was determined, and at a classification cut-off value of 0.5, the sensitivity, specificity, and positive and negative predictive values were obtained.

Comparing the angiopoietin levels of healthy controls (HC) vs. UM individuals, Ang-1 had the highest area under the curve (AUC = 0.63, 95% CI: 0.52 – 0.75, p = 0.015) differs considerably from that of a chance outcome (AUC = 0.5) (Figure 4.5). The Ang-1 model showed a high predictive ability of 94.9% and a failure rate of 23.8% as shown in table 4.4 below. Furthermore, this model identified that patients with UM are 3 times more likely to have higher levels of Ang-1 than the control group (p= 0.0023). For Ang-2 and Ang-2/Ang-1, the area under the curve was 0.59 (95% CI: 0.49 – 0.69, p>0.05) and 0.55 (95% CI: 0.45 – 0.65, p>0.05) respectively, hence their models have no class separation capacity or ability to discriminate between HC and UM (Figure 4.5).

Table 4. 4: Receiver Operating Characteristics table indicating Area Under Curve, Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of Angiopoietins

Indicators	**Area under the ROC**	**Sensitivity**	**Specificity**	**PPV (%)**	**NPV (%)**
HC vs. UM					
Ang-1	0.63	23.8	94.8	69.8	71.4
Ang-2	0.59	2.38	100	65.5	100
Ang-2/Ang-1	0.54	100	0.00	-	35.0
HC vs. SM					
Ang-1	0.66	78.2	28.5	54.5	54.5
Ang-2	0.52	18.2	23.8	50.0	52.9
Ang-2/Ang-1	0.56	52.1	57.4	52.1	57.1

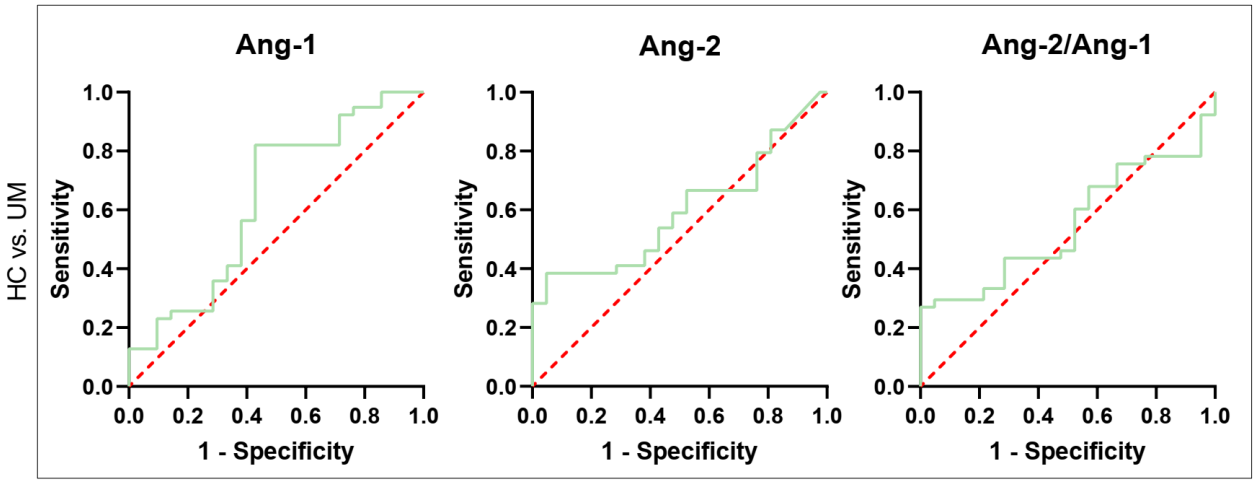

Figure 4. 5: Assessment of angiopoietin levels in discriminating between uncomplicated malaria (UM) and healthy controls (HC).

Figure 4.6 shows the angiopoietin levels of healthy controls (HC) vs. SM Patients, the ROC curve with the ability to discriminate between HC and SM is Ang-1 (AUC = 0.66, 95% CI: 0.55 – 0.77, p=0.01). This model's sensitivity to SM prediction is 78.3%, and its misclassification rate is 28.6%. The models with Ang-2 (AUC = 0.53, 95% CI: 0.41 – 0.65) and Ang-2/Ang-1 (AUC= 0.57, 95% CI: 0.45 – 0.69) showed no ability to discriminate between HC and SM (p>0.05).

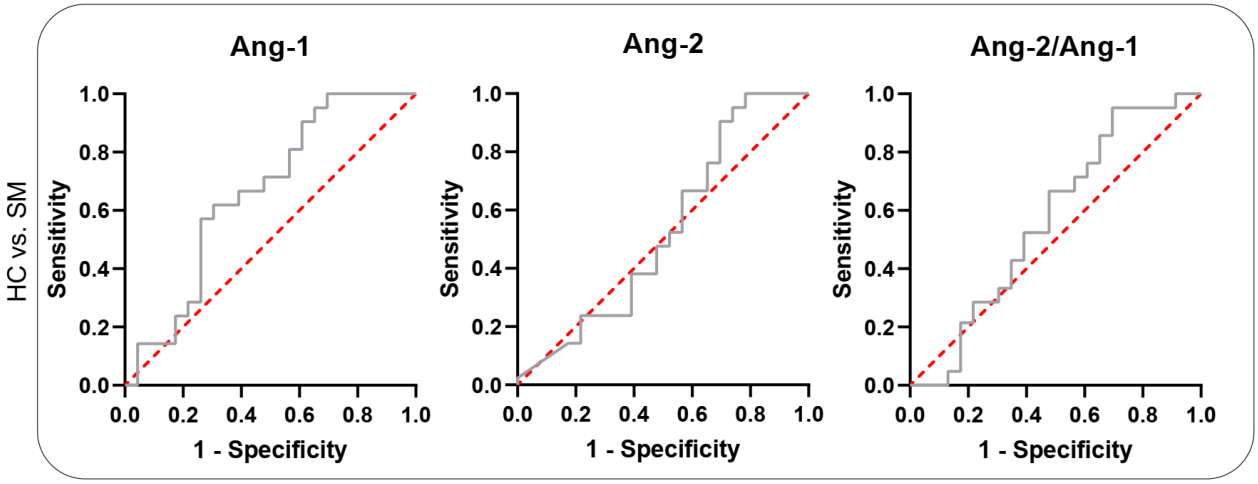

Figure 4. 6: Assessment of angiopoietin levels in discriminating between severe malaria (SM) and healthy controls (HC)

Figure 4.7 displays a ROC curve that illustrates the predictive performance of the biomarkers Ang-1, Ang-2, and Ang-2/Ang-1 in distinguishing UM and SM. For all the models in Figure 4.7, the curves come closer to the 45-degree diagonal of the ROC space. ROC curve analysis of the biomarkers Ang-1, Ang-2, and Ang-2/1 ratio yielded an AUC score of 0.58 (95% CI: 0.47 – 0.70, p=0.13), 0.54 (95% CI: 0.43 – 0.64, p=0.43), 0.57 (95% CI: 0.47 – 0.68, p=0.15) respectively. Hence all three models are less accurate in discriminating between UM and SM.

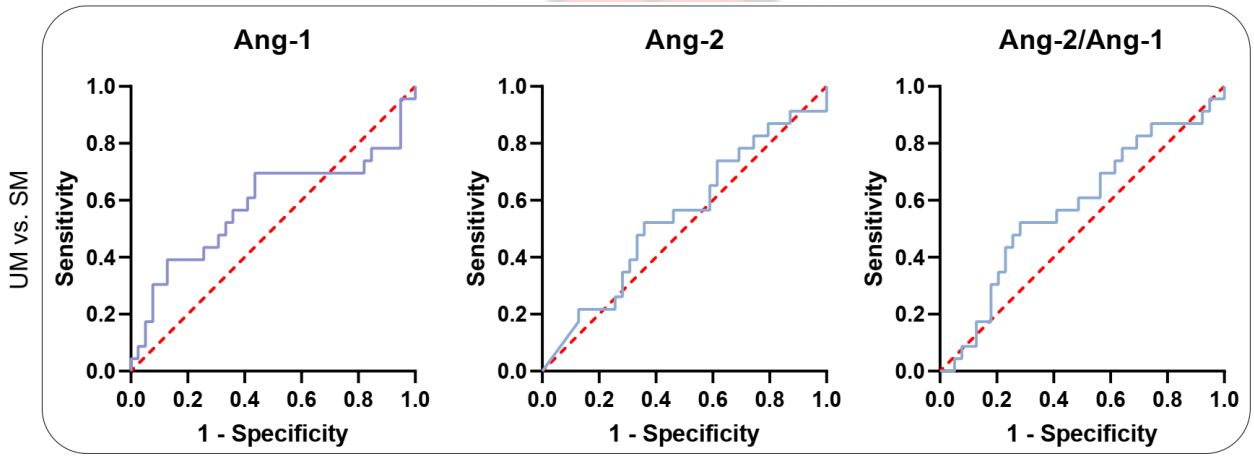

Figure 4. 7: Assessment of angiopoietin levels in discriminating between severe malaria (SM) and uncomplicated malaria (UM)

Comparison of estimated Glomerular Filtration Rate between healthy controls (HC), uncomplicated malaria (UM) and severe malaria (SM)

The eGFR of severe malaria patients (99, IQR: 64 – 138) was significantly lower than uncomplicated malaria (129, IQR: 95 – 153) and healthy control group (136, IQR: 115 – 176) ($p<0.05$). However, eGFR values in the control group and those with uncomplicated malaria were comparable ($p = 0.18$) (Figure 4.8)

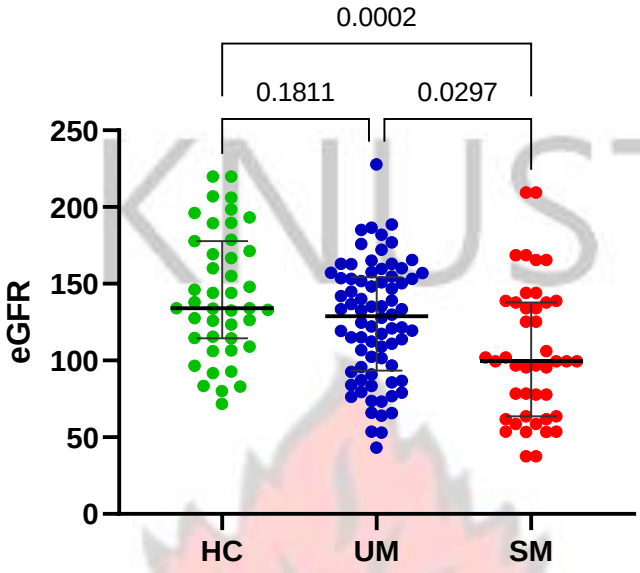

Figure 4. 8 Comparison of eGFR levels in malaria patients (UM and SM) and healthy controls (HC)

CHAPTER FIVE

DISCUSSION

Socio demographics of uncomplicated malaria and severe malaria patients

From this study, a total of 166 participants were recruited which comprised 78 (47%) uncomplicated malaria cases, 46 (27.7%) severe malaria cases and 42 (25.3%) control groups between 1 to 70 years for both males and females. The percentage of uncomplicated cases, severe cases and the control group conform to a study by Oluboyo *et al, 2020* whose study recruited very similar subjects and control groups in Nigeria but in contrast with the study population since Oluboyo *et al, 2020* recruited only children. This is also in agreement with a work by De Jong et al, 2016 whose reviewed data employ both adults and children. Again, the study population of this work (all group of ages) is in contrast with research conducted by Tadesse, Fogarty and Deressa, 2018 which also recruited only older adults.

This study, conducted in the Suame Municipality's urban environment, is consistent with earlier research published by other authors (Higgins *et al.*, 2016; Oluboyo *et al.*, 2020). Their studies were also carried out in the urban settings of Mahidol in Thailand, Kampala in Uganda and Edo Ekiti state of Nigeria.

In this current study, students and people within the non-formal sector occupation were much more infected with malaria as compared with those within the formal sector. The result is in agreement with studies conducted in Kenya (Wumba *et al.*, 2015) and in Malaysia (Ramdzan, Ismail and Zanib, 2020) who reported similar findings. This result can be explained by the fact that schools and the informal sector of employment are mostly overcrowded which paves the

way for enough mosquito bleeding, resting sites and smooth malaria transmission which contributes to malaria infection (Joseph *et al.*, 2015).

Parasitaemia levels of uncomplicated malaria and severe malaria patients

In this study, only *Plasmodium falciparum* specie was identified to be causing all malaria which is consistent with other published studies who also identified only *P. falciparum* (Anabire *et al.*, 2019; Annani-Akollor *et al.*, 2020). This may buttress the point that *P. falciparum* is the predominant specie of plasmodium in Ghana as this parasite is carried by the vector *Anopheles gambiae* and *funestus* which are mostly found over Ghana's central belt (Dadzie *et al.*, 2014; Mattah *et al.*, 2017). The parasitaemia levels ranged from 680 to 6690 parasites per microliter of blood for uncomplicated malaria cases and 1871 to 179603 parasites per microliter of blood for severe malaria cases. Children less than 5 years from this study recorded the highest number of parasites as in relation to other age groupings and this is consistent with other studies (Awosolu, Yahaya and Farah Haziqah, 2021; Uhomoibhi *et al.*, 2022).

In the current study, those with basic education significantly recorded higher parasitaemia than their counterparts with secondary education or higher education. This result, therefore, supports a study conducted by (Awosolu, Yahaya and Farah Haziqah, 2021). This higher level of parasitaemia seen in people with low educational levels may be due to the fact that people who have a higher education have acquired knowledge of malaria transmission, prevention and control (Awosolu, Yahaya and Farah Haziqah, 2021).

Haematological parameters of severe malaria and uncomplicated malaria

White blood cell (WBC) counts significantly increased in those with severe malaria infection compared to those with uncomplicated malaria or control patients ($p < 0.001$). On the other hand,

in both severe and uncomplicated malaria, there was no discernible variation between the WBC based on age categories (Munyenyembe *et al.*, 2018). The activation of WBCs as a response to an infection may be the cause of the observed rise in the proportion of white blood cells in the blood.

Results of this study showed that there was a statistically significant difference in haemoglobin levels between patients with severe malaria and those with uncomplicated malaria (p=0.003). The sequestration of red blood cells that occurs during and even after treatment for Plasmodium-induced malaria may be the cause of the lower haemoglobin levels in the severe malaria group. Additionally, these outcomes are in line with past research findings, which found that severe cases of malaria were associated with lower platelet counts (Devineni, Suneetha and Harshavardhan, 2015; Bhattacharya, 2016; Kumar, 2016).

Biochemical parameters of severe malaria and uncomplicated malaria

Results emanating from this study indicate that the biochemical derangements are clinically evident in the severe cases of malaria in comparison to those with uncomplicated malaria and those with healthy controls.

Serum levels of total and direct bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) were all significantly higher in the group with severe malaria than in the group with uncomplicated malaria ($p< 0.05$).

The above findings support a study by Al-Salahy et al. (2016) that found that patients with falciparum malaria had significantly higher serum levels of ALP, AST, ALT, total bilirubin, and direct bilirubin ($p < 0.0001$). The elevated levels of serum liver enzymes, transaminases (AST and ALT), and ALP represents the markers of hepatic injuries. The indicators of liver injury are

elevated serum levels of hepatic enzymes, transaminases (AST and ALT), and alkaline phosphatase. It has been previously reported that the elevation in enzymes of the hepatocytes is due to leakage, likewise the elevated levels of bilirubin (total and direct) are also because of haemolysed red blood cells by increased density of plasmodium parasites (Asare, 2019).

Renal Function of severe malaria and uncomplicated malaria patients

Renal injuries are usually seen as one of the complications of severe malaria mostly in the older age group (Koopmans *et al.*, 2015). Some studies have reported some percentages of acute kidney injuries (AKI) in severe malaria patients (Koopmans *et al.*, 2015; Anghan *et al.*, 2018). Markers such as urea and creatinine usually peak during severe malaria episodes. From this study, results indicate that serum creatinine of severe malaria patients (95, IQR: 84.3 – 127.0) were significantly higher than in uncomplicated malaria (72.4, IQR: 59.8 – 90) and healthy control group (58, IQR: 43 – 74). Similarly, the serum urea levels of SM patients (7.6, IQR: 5.3 – 9.3) were significantly higher than uncomplicated malaria (5.6, IQR: 4.8 – 7.2) and the control group (5.4, IQR: 4 – 6.5). This presentation is very consistent with studies conducted by Saravu, Rishikesh and Parikh, (2014) who found that severe malaria cases had higher serum creatinine and urea levels than groups who had uncomplicated malaria and healthy people. The results again are consistent with a study by Vinhaes *et al.*, (2021) who stated higher creatinine and urea levels in women with severe plasmodium malaria. Das, Rajkumari and Chinnakali, (2019) also reported that patients with severe malaria and a high parasitic index had elevated levels of serum creatinine and urea and therefore presented with acute renal injury. Furthermore, the above results also compares with several other studies (Fiona E. Lovegrove *et al.*, 2009: Gai *et al*, 2018: Wilairatana *et al.*, 2021) conducted worldwide which concluded high levels of serum creatinine and urea in leading to acute kidney injuries in severe malaria episodes.

The high creatinine and urea levels observed in this, and several other studies are the main features of acute kidney injuries (AKI) associated with severe malaria. According to Silva *et al.*, (2017), severe malaria causes damage to the glomerulus, interstitial areas and tubules. Diseases of the kidney in severe malaria are usually caused by red blood cell (erythrocytes) anomalies. Infected erythrocytes attach themselves to non-infected red blood cells, platelets, and vascular endothelium, which lead to forming rosettes and clumps. This impairs microcirculation in the vessels and possibly contributes to kidney injuries. Again, hemodynamic variability, such as hypovolemia and shock due to diarrhoea, excessive sweating and vomiting also cause acute kidney injury. Activation of the endothelium helps in the issuance of cytokines, including catecholamines, , thromboxane, endothelin and other inflammatory mediators that are also concerned with the pathophysiology of malaria-related kidney injury (Silva *et al.*, 2017).

This study's results for the estimated Glomerular Filtration Rate (eGFR) indicated that the severe malaria group had significantly lower eGFR as in comparison with their uncomplicated malaria counterparts and healthy controls ($p< 0.05$). This findings are therefore in agreement with studies conducted by Rivera-Correa *et al.*, (2019); Bhardwaj *et al.*, (2020) who reported similar lover eGFR in patients having severe malaria infection. This alteration in the function of the kidney in terms of fluid output resulting in lower eGFR in severe malaria may be a result of rosette formation or sequestration in blood vessels which reduces blood supply to the kidneys. This then results in high levels of creatinine accumulation leading to acute kidney injury (Bhardwaj *et al.*, 2020).

Angiopoietin 1 and 2 levels in Uncomplicated malaria, Severe Malaria and Healthy Controls

In this investigation, a difference in Ang-1 levels was detected between individuals with severe malaria and healthy control individuals, between individuals with uncomplicated malaria and

healthy control individuals, or between individuals with severe malaria and uncomplicated malaria.

The results of this study, which are in line with those of Storm and Craig (2014), revealed that people in the healthy control group had much greater levels of Ang-1 than subjects with severe malaria and subjects with uncomplicated form of the disease, in contrast to individuals in the control group. Patients with severe malaria and those with uncomplicated malaria had levels of Ang-2 that could be distinguished from those in the control group. In comparison to patients with simple malaria and healthy controls of all ages and age groups, patients with severe malaria had considerably greater Ang-2 levels This result is consistent with Storm and Craig, (2014), who found that, the levels Ang-2 were greater in adults who were suffering from severe malaria. It is also consistent with the findings of another study that was conducted on children in Malawi (Gomes, Alves-, *et al.*, 2014) According to that study, children with severe malaria had greater Ang-2 levels than children with uncomplicated malaria. Therefore, the data, when compared with those obtained from prior publications (Gomes, Alves-, *et al.*, 2014; Jain *et al.*, 2022), suggest that Ang-2 is a measurably accurate indicator of malaria severity.

The ability to distinguish between uncomplicated malaria and severe malaria is enabled by decreased levels of Ang-1 and higher levels of Ang-2. Although the results of this study did not indicate whether Ang-1 and Ang-2 could be used to predict which of the subjects with uncomplicated malaria is slowly progressing to severe malaria, the evidence provided by the results nonetheless showed that decreased Ang-1 and increased Ang-2 levels are indicators of severe malaria disease (Storm & Craig, 2014). As a result, in terms of endothelium activation and the severity of the sickness, the ratio between Ang-2 and Ang-1 may be the most advantageous. (Fang *et al.*, 2018). Furthermore, the results of this research show remarkable consistency with the

results of Gomes et al, (2014) who postulated that the ratio of Ang-2 : Ang-1 was greater in severe cases of malaria.

An analysis of variance (ANOVA) revealed a modest decrease in the Ang-2: Ang-1 ratio between the groups with severe malaria and uncomplicated malaria, as well as with the control group, although this was not noticeable between the group with severe malaria and the healthy control group (p=0.362). This suggests that angiopoietin dysregulation, which is known to ultimately lead to endothelial dysfunction and increased disease severity, is not a contributing factor in malaria infections. This provides more evidence that the perception of equilibrium between Ang-1 and Ang-2 might not be especially helpful in providing details about the condition of the endothelium and the severity of the sickness. Also, research with findings quite similar to this one reported that the ratio of Ang-2 to Ang-1 was larger in patients who had severe Plasmodium falciparum malaria as compared to individuals who did not have severe malaria, which better reflected the severity of the condition (Prapansilp *et al.*, 2013; Gomes, Alves-, *et al.*, 2014; De Jong *et al.*, 2016).

Despite the fact that an increased risk of developing severe malaria is frequently associated with a high level of parasitemia, persons with a relatively low parasitemia can get severe malaria as well. A ROC analysis also proved the value of Ang-2 and Ang-2: Ang-1 as highly effective markers of malaria and the severity of the illness. The analysis of Ang-1 is necessary in order to determine the ratio of Ang-2 to Ang-1, despite the fact that it did not exhibit a high level of discriminating power (Gomes, Alves-Junior, *et al.*, 2014; Jain *et al.*, 2022).

Angiopoietin 1 and 2 levels in discriminating between severe malaria (SM) and uncomplicated malaria (UM)

These findings show that serum Ang-1 and Ang-2 levels are reliable predictors of both severe and straightforward forms of malaria. The ratio of Ang-2 to Ang-1 between both UM and SM and Ang-1 levels further distinguished people with severe malaria from those with uncomplicated malaria, and there was a difference in Ang-2 and Ang-1 levels, which is particularly interesting (Figure 4.7). The ROC curve analysis demonstrated that these biomarkers had a high diagnostic accuracy in distinguishing between uncomplicated malaria and severe malarial disorders (Figure 4.4). When considered in conjunction with more recent research, this study suggests that angiopoietin dysregulation may contribute to the pathophysiology of complicated malaria and that these proteins may serve as clinically relevant indicators of the severity of the illness (Gomes, Alves-, *et al.*, 2014; Jain *et al.*, 2022).

It's been demonstrated that angiopoietins are able to differentiate between uncomplicated and severe forms of malaria in three different populations and geographical areas (Ugandan children, Papuan adults, and Thai adults). In the past, these proteins (Ang-1 and Ang-2) were tested in serum, which may now be measured directly in whole blood samples obtained from a separate population of people who have malaria (Gomes, Alves-, *et al.*, 2014; Jain *et al.*, 2022).

Severe malaria, which can be fatal, only rarely develops in a small percentage of people. (Wassmer *et al.*, 2015). Because of this, a quick point-of-care test that can determine whether someone infected with malaria has severe malaria or is at risk of getting severe malaria may be useful in clinical settings. Ang-1 and Ang-2 are excellent candidates for inclusion into rapid lateral flow immunochromatographic tests that are combined with the detection of malaria antigens because of their ability to detect in whole blood and distinguish between patients with and without severe disease. However, in order to establish and verify the utility of biomarkers such as Ang-1 and Ang-2 in a variety of populations before they can be included in quick malaria testing and clinical

practice, more prospective studies will be required (Foko *et al.*, 2022). Standardized procedures for the collecting of samples, their processing, and the use of laboratory protocols for biomarker testing need to be used in order to find the optimal cut-offs. The stable proteins Ang-1 and Ang-2 are both capable enduring several cycles of freezing and thawing without experiencing major changes in their protein levels (Chittiboina *et al.*, 2013; Fourier *et al.*, 2015; Pöss *et al.*, 2015). Furthermore, as this work reveals, Ang-1 and Ang-2 are easily accessible in serum. Angiopoietins, which are associated with angiogenesis, inflammation, as well as endothelial function and integrity, also play a significant role in many of the disease processes that contribute to the development of severe malarial syndromes. Ang-2 is pre-stored inside Weibel Palade bodies; following endothelial activation, it is promptly released and may help endothelial cells to become more activated (Gurnik *et al.*, 2016). As a consequence of this, variations in the levels of Ang-2 which signify advance alterations in the endothelium beds; hence, further research needs to investigate its potential use as a biomarker for prediction. In addition, various endothelial beds could have varying degrees of sensitivity to the effects of exogenous stimuli, which offers a potential mechanism for explaining why there is a higher prevalence of specific organ failure symptoms in severe malaria cases. These include cerebral malaria, acute renal failure, and respiratory disorders. (Hawkes *et al.*, 2013; Karnad *et al.*, 2018).

According to this study, Ang-1 was capable of distinguishing severe malaria from uncomplicated as its levels were low in uncomplicated malaria cases compared to severe malaria cases. This may be especially useful with regard to cerebral malaria. In the present investigation, higher Ang-2 levels were seen in patients with severe malarial syndromes in comparison to individuals with uncomplicated malaria. This lends credence to previous research that suggested that in diseases associated with endothelial activation, Ang-2 serves as a marker of disease severity (Hanson *et al.*,

2015). On the other hand, this research demonstrates that Ang-1 can distinguish between mild and severe forms of malaria. In order to find answers to such problems, it will be necessary to verify these discoveries in a larger group of people who have clinical characteristics that have been well defined. The fact that biomarkers are not always illness-specific places a natural restriction on how they may be used to study disease. There are a variety of severe viral diseases that have been linked to endothelial activation and dysfunction, which may be connected with the dysregulation of angiopoietins. If these proteins are going to be utilized as markers for malaria, then the consequences of co-illnesses must be taken into consideration. Co-infections may include bacterial and viral infections (such as HIV); thus, it will be necessary to take this into account.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

In conclusion the findings of this study indicate that Ang-1 and Ang-2 are less accurate in discriminating severe malaria from the uncomplicated malaria in a Ghanaian population. However both haematological and biochemical parameters can predict the severity of severe malaria.

6.2 Recommendation

Due to the way the research was designed, there is a need for prospective research to explore the dynamics of angiopoietin levels, as well as their link to the start of symptoms, the patient's responsiveness to treatment and the patient's clinical history. Angiopoietins may still be helpful biomarkers that support clinical decision-making (for instance, patient triage, referral, or admission) and the best distribution of medical resources for the treatment of severe infections that are linked to endothelial dysfunction even if they are unable to distinguish between malaria and other infectious diseases.

6.3 LIMITATIONS

The study design which did not allow for the cases to be followed from onset of signs and symptoms, and for resampling to measure their haematological, biochemical and Angiopoietins levels after treatment, served as a limitation to the study.

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