KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,
KUMASI, GHANA
COLLEGE OF SCIENCE
FACULTY OF BIOSCIENCES

THE DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

EPIDEMIOLOGICAL, HEMATOLOGICAL AND BIOCHEMICAL FEATURES IN COMPLICATED (SEVERE) AND UNCOMPLICATED MALARIA INFECTION IN GHANAIAN CHILDREN

THIS DISSERTATION IS PRESENTED TO THE DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF

MASTER OF PHILOSOPHY DEGREE IN BIOCHEMISTRY

BY
BERNARD BAHAAH
(BSC. BIOCHEMISTRY)

JUNE, 2018

DECLARATION

I declare that I have wholly undertaken the study reported herein under the supervision of Professor Kwabena Nsiah and that except portions where references have been duly cited, this dissertation is the outcome of my research.

Bernard Bahaah …… ……
(MPhil Candidate: PG4575315) (Signature) (Date)

Professor Kwabena Nsiah …… ……
(Supervisor) (Signature) (Date)

Dr Peter Twumasi …… ……
(Head of Department) (Signature) (Date)

DEDICATION

I dedicate this thesis to God Almighty for His grace, my dear uncle Rev. Joseph Owusu Appiah and Claudia Soro for their immense contributions and support in my life.

This thesis is also dedicated to my role model and life coach Professor Kwabena Nsiah for his guidance and support in helping me make this dream a reality.

ACKNOWLEDGEMENT

I wish to express my profound gratitude to God Almighty and my Lord Jesus Christ for sustaining me throughout this course. My sincere thanks and heartfelt gratitude go to Professor Kwabena Nsiah for his selfless enthusiasm and guidance in fashioning out the thesis topic as well as Dr. Justice Silverken, Dr. Samuel Blay Nguah and Dr. Anita Owusu Sekyere, for their encouragement, continuous interest and support throughout the period of preparation of this work. I am equally indebted to them for their direction and forbearance in the course of preparation of this thesis. I am extremely grateful to Claudia Soro for her love, care, advice and prayers throughout the period of the study.

I am also grateful to all my lecturers in the Department of Biochemistry and Biotechnology, KNUST and staff of CAn Lab-KNUST, for their diverse support in the research work. Of particular mention is Emmanuel Akowuah, who helped in almost every aspect of this work. My special thanks goes to the entire medical practitioners and laboratory staff of Maternal and Child Health Hospital, Suntreso Government Hospital, KNUST Hospital, KATH Polyclinic, KATH Child Health Directorate and Manhyia District Hospital, for their invaluable support, advice and contributions without which this work would not have materialised.

I wish to thank Mr. Emmanuel Acheampong of the Department of Molecular Medicine, KNUST, School of Medical Science for his support during data analysis. I thank Brother Simon, also of the Department of Molecular Medicine, School of Medical Science, for his contribution and technical prowess, during malondialdehyde and vitamin C determination in the serum of the subjects, using the Enzyme Linked Immunosorbent Assay method. Let me sincerely acknowledge financial support provided by Rev. Joseph Owusu Appiah, towards the payment of my tuition fees.

I could never end without acknowledging all the staff of Megalife Sciences Gh. Ltd., Ashanti Region division, for the deep concern and support they gave me. I sincerely acknowledge the diverse and useful contributions of many others whose names cannot all be listed here, due to limited space. Thank you and God richly bless you all.

ACRONYMS AND ABBREVIATION

ACT: Artemisin based combination therapy

CM: Complicated malaria

ELISA: Enzyme Linked Immunosorbent Assay

Hb: Hemoglobin

HCT: Hematocrit

HIV: Human Immunodeficiency Virus

KATH: Komfo Anokye Teaching Hospital

MCH: Mean corpuscular hemoglobin

MCHC: Mean corpuscular hemoglobin concentration

MCV: Mean Corpuscular Volume

MDA: Malondialdehyde

nmol/ml: Nanomole per litre

µmol/L: Micromole per litre

par/µl: Parasite per microliter

PC: Parasite count

PLT: Platelets

RBC: Red blood cell

RDT: Rapid Diagnostic Test

TBA: Thiobarbituric acid

UCM: Uncomplicated malaria

WBC: White blood cells

WHO: World Health Organisation

ABSTRACT

Malaria, a common parasitic infection, can progress from uncomplicated, possibly asymptomatic, to complicated/severe cases, characterised by a wide range of symptoms, depending on the pathophysiology of the disease. Due to the presence of Fe^{2+} of heme, arising from hemoglobin, oxidant stress is believed to drive the pathophysiology. Therefore, this study was carried out to investigate the levels of oxidative stress, derangements in hematological parameters and the distinctive features of pediatric complicated malaria in the study area. To provide an epidemiological background, a survey in which views of 80 healthcare personnel were sought on the various features of severe *falciparum* malaria, using a structured questionnaire, whilst vitamin C and malondialdehyde (MDA) were targeted as ideal markers for the oxidative stress investigation. Subjects were recruited from hospitals in the Kumasi Metropolis. As a cross-sectional study, 17 complicated malaria subjects, 51 uncomplicated malaria subjects and 15 non-parasitemic subjects were recruited into the oxidative stress aspect of the study. The hematological parameters of the subjects were also assessed using their full blood counts. In uncomplicated malaria, increased hemoglobin (HGB) levels were significantly associated with increased levels of mean corpuscular volume (MCV) ($r=0.404$, $p=0.009$), mean corpuscular hemoglobin (MCH) ($r=0.581$, $p<0.0001$) and mean corpuscular hemoglobin concentration (MCHC) ($p=0.413$, $p=0.007$). Red blood cell (RBC) levels were also found to be significantly associated with increased levels of hematocrit (HCT) ($r=0.897$, $p<0.0001$) and decreased levels of MCV ($r=-0.764$, $p<0.0001$) and MCHC ($r=-0.731$, $p<0.0001$). Among the complicated malaria cases, there was a positive significant correlation between HGB and RBC ($r=0.628$, $p=0.007$), HCT ($r=0.640$, $p=0.006$) and PLT ($r=0.573$, $p=0.041$). RBC positively correlated with HCT ($r=0.652$, $p=0.005$) and negative correlated with MCV ($r=-0.717$, $p=0.001$) and MCH ($r=-0.607$, $p=0.010$). Malaria disease progression was found to increase MDA levels and decrease ascorbate concentration. In conclusion, this research showed changes in the selected hematological and biochemical parameters studied with prostration and hyperpyrexia reported as the most distinctive features of complicated malaria in the study area. Severity of malaria brought extreme changes in the parameters studied.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

1.1 Background

Malaria, as a life-threatening disease affects millions of people worldwide (WHO, 2012, 2015). This deadly disease spreads through the bite of an infected female Anopheles mosquito (WHO, 2015). Studies reveal that close to half of the world's population are at risk of being infected with malaria (WHO, 2015). Those who are susceptible to the disease are infants, children under five years of age, pregnant women, HIV AIDS patients, mobile populations and migrants (Narsari *et al.*,2012 ; WHO, 2015). Of these categories of people, children below five years of age are the most susceptible due to weak immune system (English *et al.*, 1996; Narsari *et al.*, 2012 and Sakyi *et al.*, 2012). Currently, malaria is known to be the most widespread tropical disease and a chief cause of morbidity and mortality, particularly in sub-Saharan Africa (Narsari *et al.*, 2012 and WHO, 2015).

Sustained research efforts are immensely decreasing the malaria burden in many places; however, with the upsurge of antimalarial resistant organisms and difficulty in designing malaria vaccine, it has become very difficult to eliminate the disease (WHO, 2015). The World Health Organisation (WHO) has mounted a Global Technical Strategy for Malaria from 2016 to 2030 to help eliminate malaria (WHO, 2015). Three points were established as the key pillars to help in the elimination process.

- Ensure a comprehensive access to malaria prevention, diagnosis, and treatment
- Speedily increase efforts geared towards elimination and attainment of malaria free-status
- Transform malaria surveillance into a core intervention.

Beyond these three pillars are two auxiliary elements. These are “exploiting innovation and increasing research and strengthening the enabling environment”.

From the above-listed pillars and supporting elements, it has become apparent that research on malaria focuses on these as that will help in attaining the goals set out by the World Health Organisation. Malaria researchers always focus on one of the stages of the life cycle of the malaria parasite. This could be when the parasite (*Plasmodium*) is in the mosquito or when in the human host (Kayode *et al.,* 2011; Sakyi *et al.,* 2012; WHO, 2012, 2014, 2015). In humans, the focus may be when the parasite is in the liver, red blood cell or in some cases organs like the brain, kidneys, lungs, spleen, etc. Since malaria is associated with the red blood cell stage (erythrocytic stage) of the parasite, researchers mostly focus on this stage (Kayode *et al.*, 2011; Sakyi *et al.,* 2012; WHO, 2012, 2014, 2015). Malaria infection disrupts the constancy of the internal environment such that some of the hematological and biochemical parameters change. Notable amongst the biochemical parameters are catalase, glutathione, vitamin A, vitamin C, vitamin E, creatinine, urea and others (Kayode *et al.,* 2011; Sakyi *et al.,* 2012; WHO, 2012, 2014, 2015).

Amongst the hematological parameters; hemoglobin, hematocrit, mean corpuscular hemoglobin, and mean corpuscular volume have received wide attention as these changes are easily obtained from a hematological analyzer (Kayode *et al.,* 2011, Sakyi *et al.,* 2012, WHO, 2012, 2014, 2015). With regard to the biochemical parameters, the antioxidant vitamins (vitamins A, C and E) have not received much attention from researchers in Africa (Kayode *et al.*, 2011; Chickezie *et al.*, 2013). In India and other places some few studies have reported on the concentration of these vitamins in particularly in uncomplicated malaria patients (Khatib *et al.,* 2015). Generally, during

malaria infection these vitamins decrease, though in some cases adults may have the reverse (WHO, 2014).

A review by Percario *et al. (*2012) showed that antioxidant vitamins reduce in children suffering from malaria. Percario and his research team suggested possible supplementation as adjuvant therapy, during malaria treatment since the concentration of these vitamins has direct or indirect bearing on the immune system. However, it will be expedient to measure such antioxidant vitamins, first, during malaria infection, before possible supplementation. In Ghana, Sakyi and his colleagues (2012) focused on MDA, catalase and glutathione concentrations but not any of the antioxidant vitamins. In Ghana and other parts of Africa, a search in the malaria research archives reveals dearth of information on this subject matter.

1.2 Statement of Problem

Sakyi *et al.* (2012) reported that there is higher malondialdehyde (MDA) concentration, higher leukocyte count, and higher lipid peroxidation levels during complicated malaria infection, as compared to controls and uncomplicated malaria group in Ghanaian children. Ghana is known to be malaria endemic. This widespread nature of malaria in the country covers all the various districts and municipalities. Sakyi *et al.* (2012) carried out their study at the Sefwi Wiawso Municipality which contributed around 0.5% of the population in Ghana. Kumasi and Accra are known to contribute 8% and 15.4% of the Ghana population respectively, based on the 2010 census (GSS, 2012).

Due to the increasing number of deaths caused by malaria in sub-Saharan Africa, WHO in the year 2014 reviewed the criteria for determining complicated malaria infection in children. The reason for this was that several clinicians misdiagnosed complicated malaria for other febrile conditions. The features to look for during complicated malaria

infection are fever, prostration, nausea and vomiting, headache, severe anemia, convulsion and abdominal pain (WHO, 2014). The occurrence of these features varies according to personal and environmental factors. Hence it is important that studies are carried out in different localities or settings to be able to obtain data which could be a reflection of particular persons, the environment or both. In addition, studies involving several arms; epidemiological, hematological, and biochemical features in uncomplicated and complicated malaria infection in Ghanaian children are very scanty. Some authors have reported in some Ghanaian malaria studies that prostration, severe anemia and convulsions are the main manifestations of complicated malaria in Ghanaian children (Oduro *et al.,* 2007; Fordjour, 2015). These conclusions were reached with one or two health facilities in focus. Now, the question is will the findings be same should more than two health facilities be used? What about sourcing information from the healthcare personnel themselves, who handle these patients? This work addressed these important questions amongst the study population.

Derangements in hematological and biochemical parameters occur during malaria infection (WHO, 2014). In this study several hematological parameters i.e. Hb, HCT, PLT, WBC, MCHC, MCV, etc. (full blood count) were measured. Malondialdehyde (MDA) and the serum ascorbate concentration of these patients were also measured as biochemical parameters. Vitamin C was studied because of its enormous benefit to children, especially helping them build a strong immune system. Weak immune system in children has been reported by WHO (2014), as a key contributor to the many malarial-deaths seen in children. In India, some medical practitioners have started vitamin C supplementation during malaria treatment (Khatib *et al.,* 2015, WHO, 2015). A recent Nigerian study reported decreased vitamin C in the malaria-infected children studied (Chickezie *et al.,* 2013). In Ghana, no known study had focused on measuring

the vitamin C concentrations in malaria-infected children, though the MDA levels have been measured in one study (Sakyi *et al.,* 2012).

1.3 Justification

Several reports have indicated elsewhere that complicated malaria is misdiagnosed for other febrile conditions, due to similarity in features of complicated malaria to these conditions (WHO, 2012, 2014, and 2015). In medicine, the right diagnosis of a disease is key to ensuring the right treatment for the patient. Ghana is a malaria-endemic nation and as such has malaria as a major cause of death in the hospitals. However, there is no known study in Ghana that has focused on the common features to look for during complicated malaria infection in children. Again no known study in Ghana has measured the level of vitamin C in children suffering from malaria. Hence this area of research requires investigation. Findings from this study will add to knowledge, help in better understanding of features of complicated malaria and can also provide insight on the mode of treatment.

Sakyi *et al.* (2012) reported that there is higher MDA concentration, higher leukocyte count, and higher lipid peroxidation levels during complicated malaria infection, as compared to controls and uncomplicated malaria group in Ghanaian children. However, a lot remains to be done as the information on the level of oxidative stress during a typical *Plasmodium falciparum* infection among Ghanaian children is still scanty. Sakyi and his colleagues (2012) worked with children aged up to ten years, in compliance with the WHO (2010) report on tropical medicine and international health. However, this study involved age range up to twelve years, in compliance with the WHO 2014 report on tropical medicine and international health.

1.4 Objectives

The overall objective of this study was to investigate and evaluate the features of malaria infection among Ghanaian children.

The specific objectives of this study were

i. To carry out interviews of healthcare personnel and caregivers of children with both complicated and uncomplicated malaria, to gather information on the disease.
ii. To measure some hematological markers of children suffering from complicated and uncomplicated malaria.
iii. To measure the serum levels of some biochemical markers of oxidant stress, like Malondialdehyde (MDA) and Vitamin C in complicated and uncomplicated malaria cases.

The information obtained would be compared to findings from other studies like Sakyi and his colleagues (2012), Chickezie *et al.* (2013), Khatib *et al.* (2015) and Kulkani and his colleagues (2003). The study was also meant to compare the socio-demographic factors contributing to uncomplicated and complicated malaria. This could lead to the identification of factors contributing to malaria infection and its progression. The study would also reveal the prevalence of some of the complications of complicated malaria, their pathophysiologic mechanisms and possible remediation measures.

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of Malaria

Malaria is an infectious and life threatening disease that affects millions of people worldwide. In the year 2015 alone, 88% of malaria cases and 90% of malaria deaths were recorded in sub-Saharan Africa (WHO, 2015). There are 4 parasite species that cause malaria in humans, and two of these species – *P. falciparum* and *P. vivax* – pose the greatest threat. *P. falciparum* is the most prevalent malaria parasite on the African continent and the most lethal. *P. vivax* has a wider distribution than *P. falciparum*, and predominates in many countries outside of Africa (Osei-Djarbeng *et al.,* 2015).

2.2 Malaria Progression in Humans

Malaria may be uncomplicated or severe depending on the level of parasitemia and organ dysfunction (Chickezie *et al.,* 2013). A bite from an infected female anopheles mosquito introduces hundreds of sporozoites into the blood of the host (Chickezie *et al.,* 2013; Metzger *et al.,* 2001; Oliver *et al.,* 2014). They immediately migrate to the liver. These multiply and undergo structural changes to form several hundreds of merozoites within the hepatocytes (Chickezie *et al.,* 2013; Metzger *et al.,* 2001; Oliver *et al.,* 2014). With time, the liver cells burst and release the merozoites into the bloodstream (Oliver *et al*., 2014). A fast onset of asexual replication proceeds with the invasion and reinvasion of red blood cells. Sixteen to thirty two progenies can be produced within 2 days of merozoite invasion through binary nuclear fission (Schizogony) (Kayode *et al.,* 2011; Oliver *et al*., 2014). In two weeks, the human host can house several trillions of infected red blood cells (English *et al.,* 1996). Several studies have established the fact that, the release of daughter merozoites from these red

cells brings about the symptoms associated with malaria such as fever, sweats, rigor, and chills (Steketee *et al.* 1996).

When a red blood cell is infected with a malaria parasite, the cytoplasm of the parasite forms a blue signet ring with a pinkish dot inside the affected erythrocyte (Trevisan *et al.,* 2001). This stage, also known as the ring stage proceeds further to the trophozoite stage where it can be visualized microscopically (Trevisan *et al.,* 2001). Further development leads to the next important stage, schizonts stage, when the nucleus replicates (Oliver *et al*., 2014). The schizonts formed break and release merozoites to join the blood stream and infect new erythrocytes. During the infections of the red blood cells, the parasite (all four types) catabolizes about 80% of hemogloblin in the host's cell (Narsari *et al.,* 2012).

Progression of the disease leads to production of hemozoin pigment from its food vacuole. The hemozoin is abducted by the tissue marcrophages and monocytes, thereby inducing pro-inflammatory mediators like (interleukin-1β) (Oliver *et al.,* 2014). Symptoms develop afterwards and gametocytes are formed with less than 1% differentiating into either males or females, starting the cycle over again (Oliver *et al*., 2014).

2.3 Experts' Communication on Complicated malaria

Several reports claim areas of intense transmission of P. *falciparum* like sub-Saharan Africa usually record the highest number of deaths in children up to 5 years during malaria infections (English *et al.,* 1996; Black *et al.,* 2010; WHO, 2014). Complicated malaria has very infrequent occurrence amongst infants, however, babies born to mothers who had malaria during pregnancy tend to have low birthweight (LBW)

(Black *et al.,* 2010; WHO, 2014). This birth defect is caused by intrauterine growth retardation in the mother (English *et al.,* 1996; Black *et al.,* 2010; WHO, 2014). Low birthweight has strongly been linked with all forms of infant mortality especially during malaria (Steketee *et al,*. 1996). Greenwood and his colleagues in 1991 reported that about one in a hundred malaria infection progressed to complicated malaria in Ugandan infants. Several reports claim that many endemic areas tend to have malaria as one of the top three reasons for patient visit to hospitals (Roca-Feltrer *et al.,* 2008; Black *et al.,* 2010). In Ghana, it is the number one reason for patient visit to hospitals (www.ghanahealthservice.org 07th August, 2017). Cerebral malaria, severe anemia and metabolic acidosis have been reported as the commonest complications in children worldwide (WHO, 2015). These may occur separately or in co-morbid association. Studies in Kenya revealed that impaired consciousness and or respiratory distress were extremely lethal, as it predicted a whopping 84.4% of 64 deaths in 1844 children (Marsh *et al.,* 1995).

Hypoglycemia was also responsible for 31% deaths whilst jaundice also accounted for 16% deaths. Sixty eight (68) out of 147 children had cerebral malaria during complicated malaria in a Nigerian study (Elesha *et al.,* 1993). In a Gambian study reported by Waller and his colleagues in 1995, it was revealed that 43 % of the subjects admitted to the children's ward had coma in complicated malaria.

Similarly, 50 % of children enrolled in another study in Burkina Faso experienced coma in complicated malaria (Modiano *et al.,* 1995). It has also been reported in a complicated malaria study in the Papau New Guinea that majority of subjects had severe anemia (62 %), whereas another 22 % had coma (Allen, 1997). Some Zambian studies have reported 5 % cerebral malaria and 10 % severe anemia in the subjects

studied (Biemba *et al.,* 2000). This was a large study involving 6200 children. In another study conducted in Nigeria, 46.5 % of the subjects enrolled in the study had cerebral malaria (Elesha *et al.,* 1993).

Complicated malaria in children has been reported to mimic other febrile conditions like pneumonia which makes it difficult clinically to distinguish between the two (Koram and Molyneux, 2007). Gwer *et al*. (2007) reiterated this by reporting that complicated malaria manifests itself just like other severe febrile illnesses. They further reported that other febrile sicknesses like sickle cell, septicemia and others have also been shown to mimic the clinical syndromes of complicated malaria such as coma, acidosis, severe anemia and others. Hence, getting a parasitological diagnosis cannot holistically resolve the diagnostic problem, particularly in areas of high transmission like Ghana, Burkina Faso, Nigeria, Kenya, Malawi, etc., where asymptomatic parasitemia occurs frequently (Gwer *et al.,* 2007; Poschl *et al.,* 2010; Hendriksen *et al.,* 2012). In these areas, over-diagnosis of malaria has also been reported (Reyburn *et al.,* 2004).

However, this problem has somewhat been lessened with the development and proper use of Rapid Diagnostic Test (RDTs) and of microscopy during the diagnosis of malaria, in addition to clinical diagnoses (WHO, 2015). Others suggest treating these two conditions at the same time (Koram and Molyneux, 2007). In regions with high prevalence of asymptomatic parasitemia, it has been reported that other febrile conditions tend to be accompanied by the presence of malaria parasites in the blood, yet these conditions may have other cause (Koram and Molyneux, 2007). Vigilant diagnoses for comorbidities have been prescribed as the best way to tackle this mess (Koram and Molyneux, 2007). Koram and Molynaeux, (2007) further suggested that in

such an instance, evidence of high-density parasitemia and thrombocytopenia may be suggestive of malaria. However, in coma patients retinopathy has been suggested instead (WHO, 2015). Other signs and symptoms reported are dehydration, acidotic breathing, wide pulse pressure, cold, jaundice and shock (English *et al.*, 1996).

2.4 Review of Malaria Complication Studies Worldwide

According to WHO (2012), hypoglycemia, severe anemia, cerebral malaria and respiratory distress have been the most prevalent complications of P. *falciparum* malaria in children below twelve years. According to Gwer *et al.* (2007) the major complications of children with malaria include; hypoglycemia, metabolic acidosis, hyperlacticacidemia, severe anemia, seizures and raised intracranial pressure, whilst pulmonary edema and renal failure are the chief causes of death in adults. The study also revealed that the severity of anemia in children of six to 24 months was higher, compared to children of 25-60 months. Studies in Gabon (Libreville) have reported that anemia is common in children under 18 months, whilst cerebral malaria was more common in older children. Several studies in Africa on complications of complicated malaria have all produced similar but varying results. In Southern Mozambique (Manhica District) and Yemen, prostration was found to be a common indicator of complicated malaria, and this was followed by respiratory distress, then severe anemia and cerebral malaria (Gwer *et al.*, 2007). A study in some rural parts of Burkina Faso, where there is high transmission of *falciparum* malaria also found severe anemia to be more predominant, followed by prostration and neurological complications (Reyburn *et al.*, 2004). However, in Ouagadougou which is an urban area and with less *falciparum* transmission rates, coma was reported in more than half of cases of malaria, then neurological symptoms and severe anemia as the least common (Hendriksen *et al.*,

2012). Previous studies in some endemic regions of Africa have showed that the diversity of complicated malaria manifestations is contributed by factors such as sex, age, previous exposure, comorbid illnesses and genetic characteristics.

There are several complications of *P. falciparum* malaria in humans, but the most widespread and essential ones in children below twelve years are cerebral malaria, severe anemia, respiratory distress and hypoglycemia (WHO, 2012). Even though it is the same *P. falciparum* parasite, due to metabolic differences in children and adults, there exist some differences in the manifestation of malaria between these two groups. Some of these differences have been tabulated below.

Table 2:1 Differences in the manifestation of complicated malaria in children and adults

Signs or Symptoms	**Adults**	**Children**
Duration of Illness	5-7 days	Shorter (1-2 days)
Respiratory Distress/Deep Breathing(Acidosis)	Common	Common
Convulsions	Common (12%)	Very Common (30 %)
Posturing	Uncommon	Common
Prostration/Obtundation	Common	Common
Resolution of Coma	2-4 days	Faster (1-2 days)
Neurological Sequelae after Cerebral Malaria	Uncommon (1 %)	Common (5-30 %)
Jaundice	Common	Uncommon
Hypoglycemia	Less Common	Common
Metabolic Acidosis	Common	Common
Pulmonary Oedema	Uncommon	Rare
Renal Failure	Common	Rare
CSF Opening Pressure	Usually Normal	Usually Raised
Bleeding/Clotting Disturbances	Up to 10 %	Rare
Invasive Bacterial Infection(Co-Infection)	Uncommon (<5 %)	Common (10 %)

(WHO, 2012, 2014, 2015)

Complicated malaria has always been associated with high mortality. Given its history, there has mostly been the transition of asymptomatic malaria to uncomplicated malaria

through to complicated malaria and finally to lethal malaria (WHO, 2012, 2015). Previously, one out of 1000 patients (0.1%) with uncomplicated *falciparum* malaria die, despite malaria treatment, but since the introduction of Artemisinin combination treatment (ACT), the mortality has lowered below 0.1% even in patients with high ring stage parasitemia (WHO, 2015).

2.5. Review of Malaria Complication Studies Locally

Over the years, malaria has been a major cause of morbidity in Ghana and still continues to kill a lot of people, accounting for 44% of all reported cases in the hospitals for the year 2015 (Fordjour, 2015). Currently, the malaria prevalence in Ghana (second quarter of 2017) stands at 38.1 (www.ghanahealthservice.org. 07th August, 2017). The disease pattern of malaria varies according to age, geographic location, level of transmission and non-malaria comorbidities. In light of this, a study done at KNUST Hospital in Kumasi showed that anemia, prostration, convulsions and fever are the major clinical manifestation of complicated malaria (Fordjour, 2015). A study conducted in Kassena-Nankena District of Northern Ghana in 2007 on 1,921 children reported the predominant complications of complicated malaria as severe anemia (36.5%), followed by respiratory distress (24.4%), prolonged or multiple convulsions (21.6%) and cerebral malaria (5.4%) (Oduro *et al.*, 2007; Gyapong, 2009; Fordjour, 2015).

Again, a study in Tamale (northern Ghana) showed that the frequency of severe anemia in about half of the study participants decreased with age and neurological symptoms (impaired consciousness and convulsions) was the most frequent in older children (Gyapong, 2009; Fordjour, 2015).

2.5.1 Cerebral Malaria (CM)

Cerebral malaria, a neurological complication of complicated malaria, happens when parasitized red blood cells get sequestered in cerebral micro-circulation (Idro *et al.*, 2010). A collection of antigens from the parasite containing *Plasmodium falciparum* erythrocyte membrane protein-1 (PfEMP-1) mediate binding to the receptors of the host of which, intercellular adhesion molecule-1 (ICAM-1) is the most significant and whose expression is up-regulated in regions adjacent to sequestered parasites (Idro *et al.*, 2010). Children suffering from complicated malaria may develop cerebral malaria, which is mainly characterized by altered consciousness (Taylor *et al.*, 2004). However, others signs like coma, convulsions and especially retinopathy have also been very useful in the detection of cerebral malaria (Taylor *et al.*, 2004). In children and adults suffering from cerebral malaria, research has shown that retinopathy can be used as a powerful prognostic tool, in determining the severity of their condition (Beare *et al.*, 2004). Fever has been reported as the earliest symptom during typical complicated malaria infection, which leads to the development of cerebral malaria (Waller *et al.*, 1995; English *et al.*, 1996; WHO, 2015). The fever is usually followed by loss of appetite, vomiting and cough, in that order (Waller *et al.*, 1995; English *et al.*, 1996; WHO, 2015). There have been reports that in some rare cases children with cerebral malaria were not febrile (Waller *et al.*, 1995; English *et al.*, 1996; WHO, 2015). Magnetic resonance images have shown the presence of cerebral edema in a lot of children suffering from cerebral malaria (Potchen *et al.*, 2012).

In a (2010) study conducted by Dondrop and his colleagues, it was revealed that cerebral malaria has a case fatality rate of 19.7 %. Children who survive cerebral malaria may recover fully, be left with neurological sequelae, and in some few cases behavioral or cognitive difficulties or even epilepsy may be seen in these children later

in life (Bojan *et al.,* 1997). It was revealed in a Kenyan study that 9.2 % children who had suffered cerebral malaria had epilepsy, whist there was 2.2 % in the controls (Carter *et al.,* 2004). Studies from four African countries (Malawi, Gambia, Kenya and Nigeria) revealed that 1060 children suffered from neurological sequelae after recovery from cerebral malaria (Molyneux *et al.,* 1989; Brewster *et al.,* 1990; WHO, 2015). Cerebral malaria may lead to coma or abnormal posturing (Feigin and Bennet, 2006). All of these indicate severe brain injury during complicated malaria infection (Feigin and Bennet, 2006). Coma in malaria patients is usually determined by their lack of response to a local stimuli or standard pain (Maitland *et al.,* 2004). Decerebrate and decorticate posturing have also been reported in children suffering from complicated malaria (Maitland *et al.,* 2004).

2.5.2 Metabolic Acidosis

Another major complication from complicated malaria is metabolic acidosis. In this complication, the level of acidity increases, thereby disturbing homeostasis. Most diseases that end up with altering the normal pH of the human system end up with a high case fatality. Children suffering from complicated malaria may have their pH decreased beyond the normal (Stacpoole *et al.,* 1994). These acidotic patients may or may not have altered consciousness. Taylor *et al*. (1993) reported 46% children in a Malawian study to be either acidemic or to have had a compensated metabolic acidosis. At the end of the study, 72% of the children who died were acidotic. Cerebral malaria complication which developed as a result of complicated malaria recorded 42% acidemia. The level of mortality in these patients (28%) was far higher than the non-acidotic patients (3%). Acidosis has also been strongly linked with hypoglycemia which is also an important risk factor for death in complicated malaria (Taylor *et al.,*

1993). Lactic acid production has been reported as the major contributor to acidosis in complicated malaria. The lactic acid content of some malaria patients were found to be twice as high in all fatal cases, compared to those who recovered (Krishna *et al.,* 1994). A Kenyan study revealed strong link between severe acidosis and fatality in close to 300 patients with complicated malaria (Von Seidlein *et al.,* 2012). During complicated malaria an insufficient supply of oxygen to tissues may follow from severe anemia and cause a shift in metabolism within host cells to anaerobic glucose metabolism and increase lactic acid production (Pascuali *et al.,* 2006). Again, the inhibition of oxidative metabolism in the context of an on-going inflammatory response have also been reported to cause accumulation of protons (H+) and eventually lead to metabolic acidosis (Pascuali *et al.,* 2006).

2.5.3 Respiratory Distress

This feature centres on the breathing abnormality found in children suffering from complicated malaria (Sasi *et al.,* 2007). In this condition there is accumulation of lactic acid, 3-hydroxybutyric acid and other organic acids which lowers the pH of the internal environment (Sasi *et al.,* 2007). The sources of these compounds are from tissue anoxia, derivation of lactic acid from parasitized red blood cells, anaerobic glycolysis and impaired gluconeogenesis (Krishna *et al.,* 1994). It is a major risk factor for death in children infected with malaria (Lackritz *et al.,* 1992). There may be deep breathing involving overly increased amplitude of chest excursion and or indrawing of the bony structures of the lower chest wall (Lackritz *et al.,* 1992). The former may point to metabolic acidosis whereas the latter may indicate presence of a lung disease such as pneumonia or acidosis (Lackritz *et al.,* 1992). Chest indrawing or deep breathing is always seen by clinicians as key danger signs of this complication of malaria. Out of

1844 Kenyan children who were enrolled in one malaria study, 259 had respiratory distress and 37 died. Over 80% of patients who had respiratory distress were also found to have had lowered pH (Marsh *et al.,* 1995). English *et al*. (1996) also reported that out of 24 Kenyan children who were suffering from malaria and respiratory distress, 16 had anemia. The situation was corrected after they received blood transfusion. In few cases respiratory distress has been associated with neurological defect (English *et al.,* 1996).

2.5.4 Severe Anemia

Malaria is essentially the erythrocytic stage of the protozoan *Plasmodium*. Since the progression of this disease requires further breakdown of red blood cells, it is not surprising that anemia has been noted as a key complication during complicated malaria infection. The World Health Organisation has reported in their (2015) malaria report that 1.8 years (21 months) is the mean age of children suffering from severe anemia whereas three years (36 months) is the mean age of children with cerebral malaria. Anemia is common in children with *P. falciparum* infection.

If not handled promptly, anemia can progress to severe anemia. Schellenberg and his colleagues (2003) reported high prevalence of anemia in children below 5 years in Tanzania. For children between 1-2 years, 10 % experienced severe anemia (Hemoglobin < 5g/dl) in this study. Several studies have established that severe anemia is multifactorial, yet in circumstances where there is presence of malaria parasites the condition is accorded to malaria. Malaria, HIV infection, bacteremia, hookworm, Vitamin B12 and A deficiencies are all associated with severe anemia (Calis *et al.,* 2008). A study conducted in Gabon revealed that almost all 8195 febrile children were anemic (Bouyou-Akotet *et al.,* 2009). In another study in Kenya, it was reported that

out of 1116 pediatric hospital admissions, 927 were parasitemic and 235 developed into severe anemia cases (Obonyo *et al.*, 2007). A study by Bojang *et al*. (1997) claims that death rate amongst anemic patients rose when condition was associated with respiratory distress. In severe anemia cases blood transfusion has been found to reduce the case fatality (Lackritz *et al.*, 1992).

2.5.4 Hypoglycemia

Children prove difficult to feed when sick. Most of such sicknesses would come along with fever and vomiting (WHO, 2015). A child is said to be hypoglycemic when the whole blood glucose concentration is less than 2.2 mM. Hypoglycemia as a complication of malaria increases the risk of dying or sequelae from the disease (Bassat *et al.*, 2008; Camara *et al.*, 2011; Nanda *et al.*, 2011). Hypoglycemia is generally common in malaria patients below three years of age who are in a coma and with high parasitemia (Dondrop *et al.*, 2010). In addition, they may have very high concentration of circulating tumour necrosis factor and lactic acid. Gambia, Malawi and Mozambique are three African countries with in-depth studies on hypoglycemia. One of such study reported that hypoglycemia accounted for 32% of complicated malaria cases in Gambia, whereas 20% was obtained in the Malawi study (White *et al.*, 1987; Taylor *et al.*, 1988; WHO, 2014). However, the Mozambique study focused on malnutrition, malaria and pneumonia (Solomon *et al.*, 1994). It was reported that hypoglycemia accounted for 7 % of all reported cases in this study. Research has shown that the malaria parasite depend on the host's glucose for its own metabolism and energy derivation (www.malariaworld.org. 08th March, 2018). Kiely (2007) and his colleagues also reported that during malaria infection, pro-inflammatory cytokines like tumor necrosis factor α, Interleukin 1 β and interleukin 6 increase glucose in the blood. This

serves as source of nutrients for the parasite. With parasite increase and disease progression, the level of glucose decreases (serving as source of nutrients for the parasite) severely leading to hypoglycemia (www.malariaworld.org. 08th March, 2018).

2.5.5 Hyperpyrexia

This a common feature or complication of malaria but the degree of hyperpyrexia cannot be used to establish the presence of complicated malaria (WHO, 2015). Very high body temperatures have been found to contribute to coma and altered consciousness in children (Axton and Siebert, 1982; WHO, 2012, 2015). Fetal distress in pregnant mothers with malaria has been found to be associated with high body temperature (Looareesuwan *et al.,* 1987). In fetal distress, a condition where the fetus fails to receive ample oxygen supply is mainly attributed to the excessive breakdown of red blood cells during malaria infection; the amount of oxygen that can be supplied to the fetus automatically reduces (Stefferl *et al.,* 1996). Again, certain inflammatory mediators like tumor necrosis factor alpha which is pyrogenic in nature increases leading to fever which is mainly characterized by high temperature (Stefferl *et al.,* 1996). Internalization of the parasites in the placenta of these women has also been reported to contribute to this menace (Stefferl *et al.,* 1996).

2.5.6 Renal Complications

English *et al.* (1996) posit that creatinine concentration and plasma urea may be highly elevated during complicated malaria, especially where there has been excessive dehydration. Research shows that acute renal failure is rare but not totally absent in children suffering from complicated malaria (von Seidlein *et al.,* 2012). Hyponatremia (low sodium) and Hypokalemia (low potassium) have been noticed in such patients

(English *et al.,* 1996; Maitland *et al.,* 2004). Seventy three (73) out of 132 children were found to have low sodium, during complicated malaria infection in a Kenyan study (English *et al.,* 1996). Maitland *et al.,* (2004) reported that 10.5% of 38 children admitted to a Kenyan hospital were hypokalemic, but in 4-8 hours the percentage of hypokalemic patients rose to 39.5 %. These losses of potassium were reported to have happened through the urine of these patients (English *et al.,* 1996; Maitland *et al.,* 2004). This is not always the case as in other complicated malaria cases there may be hyperkalaemia (excess potassium in the blood). Sixty one (61) out of 493 complicated malaria patients were reported to be hyperkalemic in a Kenyan study (Maitland *et al.,* 2004).

2.5.7 Hepatosplenomegaly

Hepatosplenomegaly which is the enlargement of the spleen and liver has also been identified as a key complication during complicated malaria infection in children (Maitland *et al.,* 2004). Splenomegaly is attributed to clogging of red blood cells during complicated malaria infection (Buffet *et al.,* 2010). The cause of hepatomegaly as a complication of complicated malaria is multifactorial (Kochar *et al.,* 2003). Some of the factors are intravascular hemolysis due to parasitized and non-parasitized red blood cells and immune hemolysis involving the adherence of circulating antigen-antibody complexes to the surface of the erythrocytes (Kochar *et al.,* 2003). Again, change in vascular blood flow through the liver as a result of parasitized red blood cells sticking to endothelial cells block sinusoids and obstruct intrahepatic blood flow (Kochar *et al.,* 2003).

2.5.8 Bacteremia and other comorbidities

Clinically diagnosed complicated malaria patients tend to have a higher bacteremia than controls (Berkley *et al.,* 1999, 2005). It is advised that such patients should be given antibiotics in addition to antimalarials (Evans *et al.,* 2004). Children are the most affected amongst the complicated malaria patients due to low immunity (WHO, 2015). Children suffering from malnutrition and HIV also experience similar problem (WHO, 2015). The most occurring bacterial pathogen isolated from the blood cultures is non-typhi Salmonella (WHO, 2015). Some studies have reported that bacteremia in children may increase their case fatality (Berkley *et al.,* 2005), whilst others have reported otherwise (Bronzan *et al.,* 2007). Low immunity and malnutrition have been identified as factors that contribute to this (Nyirenba *et al.,* 2017).

2.6 Stressors and Malaria

Several studies have established that cells are prone to oxidative stress and they counter this with in-built antioxidative processes (Saltman, 1989). Previous research by Eaton *et al.* (1976) and Becker *et al.* (2004) revealed that red blood cells which were *Plasmodium*-infected undergo oxidative stress. It has also been reported elsewhere from a number of studies that erythrocyte membranes undergo physicochemical changes during lipid peroxidation and hemolysis in a typical malaria infection (Clark and Hunt, 1983; Clark *et al*., 1984; Das and Nanda, 1999). During malaria infection, studies have shown that neutrophils and macrophages produce reactive oxygen species and hydrogen peroxide (Egwunyega *et al.* 2004). Clark *et al.* (1984) reported that chemicals that could produce free radicals were able to cause hemolysis in *Plasmodium vinckei*-infected mice. Further studies by Golenser and Chevion (1989) established that

Plasmodium berghei-infected mice had an upsurge in malondialdehyde (MDA) concentration.

Other researchers have also reported that an increase in lipid peroxidation generates a matching effect in the concentration of enzyme antioxidants during complicated malaria infection (Egwunyenga *et al.* 2004).

Lipid peroxidation (measured by quantifying malondialdehyde levels), catalase and glutathione levels have mainly been used in the assessment of disease severity in malaria patients but not the lesser known antioxidants (Onyesom *et al.,* 2010). However, it is reported that in almost all disease conditions the antioxidant levels decrease (Kim *et al.,* 1998). A study that was conducted in Uganda on 273 children with uncomplicated malaria revealed that these children had low plasma antioxidant concentrations (WHO, 2015).

Oxidative stress has been associated with many disease conditions of which malaria is no exception; however, the role it plays in malaria infection remains unclear. Whilst some researchers claim that it has a connection with the pathophysiology of the disease, others argue that it has a protective role (WHO, 2015). In a more recent study by Kim *et al.* (1998) it became apparent that oxidative stress plays a very important role in the development of systemic complications during malaria infection (Kim *et al.,* 1998). It was reported in that particular study that hydroxyl radicals were generated in the liver after inoculation with *Plasmodium* (Kim *et al.,* 1998).

Similarly, Atamna *et al.* (1993) also reported that red cells inoculated with *P. falciparum* produced hydroxyl radicals and hydrogen peroxide about twice what is found in normal erythrocytes. Some authors have reported that a key source of these radicals is the hemoglobin molecule. This is as a result of liberation of several heme

molecules during feeding by the parasites. In the process, Fe^{2+} ions are generated, allowing for induction of intravascular oxidative stress leading to changes in endothelial cells and red cells. This causes the parasites to be internalized into tissues such as brain and the liver (Kim *et al.,* 1998; WHO, 2014). This can then generate into other complications such as cerebral malaria, respiratory distress, black water fever and others. Some authors have attributed the devastating effects of cerebral malaria to over-production of NO (nitric oxide which is a potential source of stress in the body) in the body (WHO, 2014, 2015). In another study, it was reported that oxidative stress is associated with the pathogenesis of thrombocytopenia (WHO, 2014, 2015).

Glutathione has been identified as the most important antioxidant system that can offset the effects of tumour necrosis factor-alpha (TNF-α). TNF-α and some interleukins are found in great quantities during malaria infection especially in the spleen (WHO, 2014, 2015). It has also been reported in other malaria studies that the concentration of antioxidant glutathione is reduced in these malaria patients (WHO, 2014, 2015). Some researchers reported in 1993 that malaria patients infected with *Plasmodium falciparum* parasites have reduced Vitamin C levels (WHO, 2014, 2015). Below is a figure explaining the relationships between the life cycle of *Plasmodium* and generation of stressors.

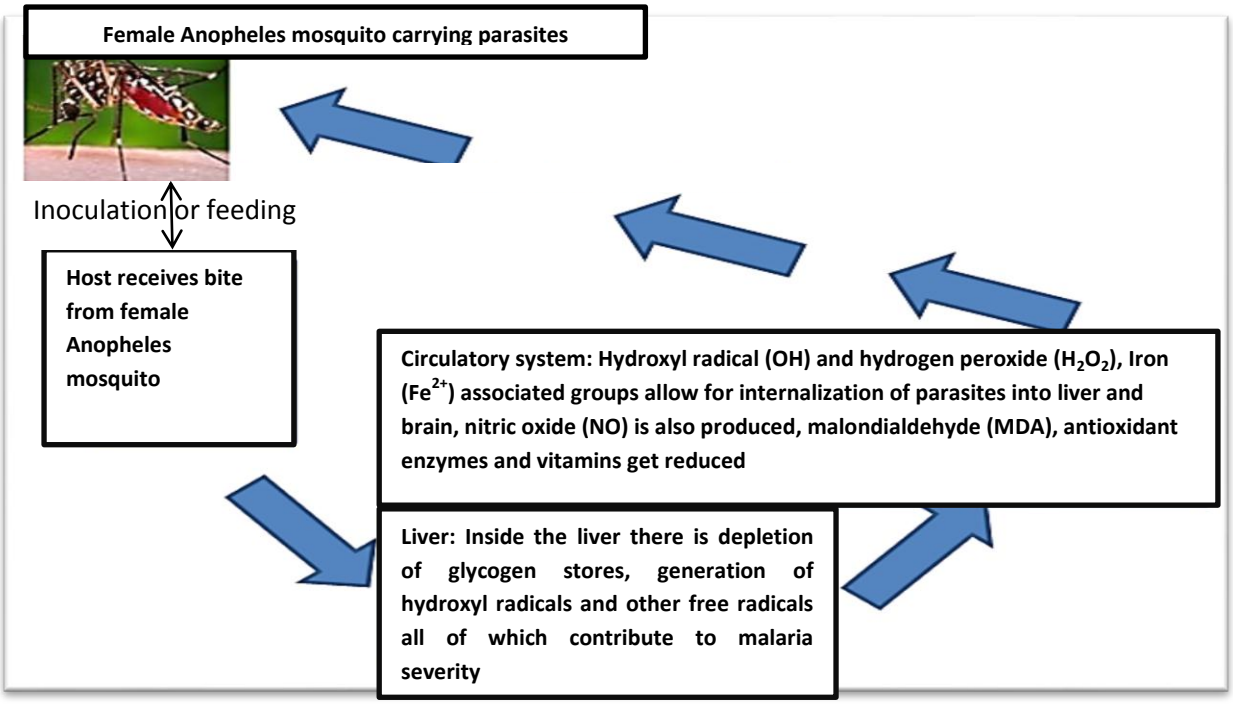

Fig. 2.1: Schematic representation of the life cycle of malaria parasite and how it produces stressors.

2.7 Measurement of Stressors in Malaria Infection

In studies involving the measurement of oxidative stress markers, malondialdehyde (MDA) has mostly been used (Sakyi *et al.*, 2012). Sakyi *et al.* (2012) demonstrated that the concentration of platelets and some antioxidant enzymes were reduced whereas the lipid peroxidation measured by MDA concentration had increased during a typical malaria infection. In such instance, the body may attempt to use other molecules like Vitamins A, C, E, beta-carotene and other small molecules to offset this imbalance (Sakyi *et al.*, 2012). This then leads to systemic reduction in these molecules (Sakyi *et al.*, 2012). Black and his team of researchers reported in 2008 that patients infected with malaria have reduced amount of Vitamin A, B1, B2, C, E and Zinc. Other notable deficiencies suffered by these patients are thiamine and folate (Sakyi *et al.*, 2012).

Pro-oxidants in the plasma of malaria patients have been quantified by the measurement of erythrocyte thiobarbituric acid-reactive (TBA) substance

concentrations. During the TBA test, one MDA molecule binds to two molecules of TBA to produce a red or pink coloured MDA-TBA complex (Sakyi *et al.,* 2012). The absorbance is then read spectrophotometrically at a wavelength of 532 nm. The intensity of the colour normally shows how high or low the readings will be. Despite its wide use, the TBA test is insensitive and non-specific to minute quantities of MDA, due to the interference from molecules like aldehydes, proteins and sugars present in the body fluid at the time of analysis (www.public.iastate.edu 06th June, 2017; Sakyi *et al.,* 2012).

Its inaccuracy to measure minute quantities has led to the use of Enzyme Linked Immunosorbent Assay (ELISA). The ELISA method in lipid peroxidation measurement is more specific, precise and requires less time than the TBA test; however its major disadvantage is the cost and life span of its reagents which is usually 6 months (www.cellbiolabs.com. 01st August, 2017). Therefore this must be considered when one wants to use this method of lipid peroxidation determination. The specificity and precision of the ELISA method relies on the interaction between the antigens and antibodies involved in this test (www.cellbiolabs.com . 01st August, 2017). The supplier of the ELISA kit may offer the researcher either antigens or antibodies, hence upon introducing a sample, there will be corresponding interaction between the antigens in the kit and the antibodies of the sample or the antibodies of the kit and the antigens in a sample.

2.8 Vitamin C and Malaria

Vitamin C which is also called ascorbic acid has been known to facilitate a host of cellular functions (Onyesom *et al.,* 2010; Neelam, 2016). This water-soluble vitamin is needed to build and maintain dentine, cartilage, collagen, bone matrix and connective

tissue (Onyesom *et al.,* 2010). Research shows that this vitamin is needed for the conversion of folate to folinate. It also play important role in regulating the respiratory cycle in mitochondria and microsomes (Onyesom *et al.,* 2010).

Vitamin C has been shown to enhance the absorption of iron through reduction of the ferric form to ferrous form which can easily be absorbed by the gastrointestinal cells thereby correcting anemia (Neelam, 2016). Vitamin C has been reported to help in the removal of iron from ferritin, which is an essential process in hemoglobin formation (Onyesom *et al.,* 2010). Research has shown that ascorbate has a lot of beneficial effects on the immune system. Some of such studies have reported that ascorbate is needed for the differentiation of lymphoid organs during the growth of cockerels and young rats (Onyesom *et al.,* 2010). Again, this vitamin has been shown to have beneficial effects on the phagocytic action of leucocytes and the migratory behaviour of neutrophils (Onyesom *et al.,* 2010; Neelam, 2016). Some studies have also shown that it can improve the regeneration and renewal of lymphoid tissues after X-irradiation (Onyesom *et al.,* 2010; Neelam, 2016). Increase in circulating interferon and virucidal effects have also been reported as some of the benefits (Onyesom *et al.,* 2010).

Infections that are febrile in nature have been shown to decrease blood level of ascorbic acid, denoting an increased need for this vitamin in such conditions (Onyesom *et al.,* 2010).

One Nigerian malaria study reported increase in this vitamin for adults and a substantial decrease in this vitamin for children especially those below 5years (Onyesom *et al.,* 2010). Onyesom *et al.,* (2010) reported the mean concentration of vitamin C in the infected children as 1.95$\pm$ 0.20 mg/dl and the healthy controls as 2.9$\pm$0.24 mg/dl.

Onyesom *et al.,* (2010) also reported a negative correlation between ascorbate and vitamins A and C but a positive correlation with vitamin E.

2.9 Review of hematological parameters

Blood as a tissue obey the law of homeostasis but it has been established that diseases that affect the hemopoetic physiology always alter hematological parameters (Maina *et al.,* 2010). Some of the parameters are red blood cells, white blood cells and platelets. Due to this the changes noticed are even used as diagnostic tools (Maina *et al.,* 2010). Such is the case of malaria. Hematological changes are amongst the most common complications of malaria and are involved in the pathology of disease (Maina *et al.,* 2010). Changes in thrombocytes, leucocytes and red cells are widely reported in malaria infection (Maina *et al.,* 2010; Kumar *et al.,* 2015).

In one Western Kenyan study, it was reported that out of the 961 subjects studied all the malaria patients (532) had severe changes in their hematological parameters (Maina *et al.,* 2010). It was reported that hematological parameters such as platelets, lymphocytes, eosinophils, red blood cell count and haemoglobin (Hb) were significantly lower in the malaria-infected children, while absolute monocyte and neutrophil counts, and mean platelet volume (MPV) were higher in the diseased children compared to the non-malarious children (Maina *et al.,* 2010).

Again it was reported that the children with platelet counts of less than150,000/uL were 13.8 times more likely to have malaria (Maina *et al.,* 2010). Thrombocytopaenia (low blood platelets count) was found in 49% of malaria-infected children and was associated with lower age, high parasitaemia levels, low Hb levels, platelet aggregation and increased mean platelet volume (MPV) (Maina *et al.,* 2010).

Kumar and his colleagues (2015) also reported similar changes in hematological parameters in the 172 malaria patients studied in India.

This devastation in the hematological parameters has also been reported in a Nigerian study involving pregnant women who were malaria-infected (Adesina *et al.,* 2009). It was revealed that the white blood cell count and mean corpuscular hemoglobin concentration were higher in these patients compared to their controls (Adesina *et al.,* 2009). Since the symptoms associated with malaria occurs at the erythrocytic stage of the disease hematological changes in the patient is highly inevitable (Adesina *et al.,* 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design/Study Site

The study was a cross sectional and partly retrospective type, carried out in some selected hospitals in Kumasi, Ashanti Region, Ghana. The study was carried out in five key hospitals in Kumasi, Ashanti Region. These were the Komfo Anokye Teaching Hospital, Maternal and Child Health Hospital, Manhyia District Hospital, Suntreso Government Hospital and KNUST Hospital. Apart from Komfo Anokye Teaching Hospital which is a quaternary health facility, the others were secondary health facilities. These secondary health facilities provide primary healthcare and hence see and treat most malaria infections. This in a way affects the number complicated malaria subjects that a researcher can obtain in a quaternary hospital like Komfo Anokye Teaching Hospital (KATH). No malaria subject was therefore selected from Komfo Anokye Teaching Hospital, but their healthcare personnel took part in a survey that involved the use of a structured questionnaire to find out the features of complicated malaria, from the perspective of providers of healthcare. This was based on reports received from the KATH Pediatric Emergency Unit.

All of the hospitals selected for the study operated under Ghana Health Service. These hospitals are in Kumasi, the capital of Ashanti Region. Kumasi is a malaria endemic zone, (www.againstmalaria.com/Distribution.aspx?proposalID=42. 26th June, 2017) has a total population of 2,069,350 (GSS, 2012), and covers a total land mass of 250 square Km (en.m.wikipedia.org/wiki/Kumasi) with a rainfall of 1484.4 mm per annum (www.kumasi.climatemps.com/precipitation.php. 26th June, 2017)

3.2 Study Subjects

Definition of children by WHO (2014) was adopted for this study. All children below twelve years who reported to the child health departments of these facilities with symptoms of malaria (malaria suspects) were screened for this study.

Following the method of Charan and Biswas (2013), a sample size of 74 to 140 malaria patients was proposed for the study. According to them, for a population more than 50,000 the formula below is usually followed in determining the sample size (Charan and Biswas, 2013).

$$Ss = \frac{Z^2 \times (P) \times (1-P)}{C^2}$$

Where Ss is the sample size, Z is a constant indicating standard normal variate (for a 95% confidence interval; it is 1.96), C is the confidence interval (95%), P is the percent of picking a choice (malaria patients below 12 years) within the population. This research targeted 5% to 10% of the population in the study area. After computing, the sample size was 74 children for 5% and 140 children for 10% of the population.

At the end of the study 83 subjects were recruited. Similarly, 80 healthcare personnel were recruited to partake in a survey for eliciting views on malaria.

3.3 Data Collection Tools

Four different data collection tools were employed in this study. Two were structured questionnaires used to obtain information from the malaria subjects and doctors, whereas the other two were for the laboratory procedures employed to obtain information on the hematological parameters, as well as biochemical parameters. The first two questionnaires were made of both closed and open ended questions. The one designed for doctors sought to extract information from these prescribers, in relation to

their practice, experience and knowledge on the features of complicated malaria. The second tool, administered to the caregivers of children with malaria was used to obtain information on demographic data, antimalarial drugs used previously, the last episode of malaria and use of insecticide-treated nets. The third and fourth data collection tools were for the capture of the laboratory tests. Hematological analyser was used to measure the differential counts. Lastly, the ELISA (Biobase Biotech, Shangai, China ELISA Kits) method was also used to determine MDA and Vitamin C concentrations in the malaria patients.

3.4 Inclusion and exclusion criteria

Patients with varying degrees of *P. falciparum* parasitemia, who have not been treated with antimalarial drugs, were used as subjects. Age-matched healthy children visiting the Out-patient Department of these hospitals were recruited as controls. The malaria subjects selected were grouped into Severe or Complicated Malaria group (CM) and Uncomplicated Malaria group (UCM).

The UCM group were those who reported with the usual symptoms of malaria, but were not admitted in the wards of these hospitals and did not have any malaria complication. The complicated or complicated malaria group were those who were admitted in the hospital wards and in addition, had one of the complications specified by WHO (2014) to be present in these patients. The hematological parameters like hemoglobin levels and hematocrit concentrations were mostly used in identifying this group.

Children who reported to these hospitals with conditions like Human Immunodeficiency Virus (HIV), enteric fever, sickle cell disease and non-malaria

infections, hepatitis B and C infections, and pyrexia of unknown origin were not included in the study.

3.4.1 Period for the study

The study lasted for a period of 6 months starting from January 2017 to June 2017. Three months was used in gathering information from healthcare givers. Another 3 months was used in recruiting patients for the hematological and biochemical study.

3.4.2 Definition of Complicated malaria

The World Health Organisation (2014) defines complicated malaria in children as in the presence of *P. falciparum* but no other confirmed cause for signs and symptoms and vital organ dysfunction with clinical features such as impaired consciousness, prostration, multiple convulsions, acidotic breathing, acute pulmonary edema and acute respiratory distress, shock, acute kidney injury, and or clinical jaundice plus one or more of laboratory findings such as hypoglycemia (less than 2.2 mmol/L), metabolic acidosis (plasma bicarbonate less than 5 g/dl), severe normocytic anemia (less than 5 g/dl and packed cell volume less than 15 %), hemoglobinuria, hyperlactatemia (greater than 5 mmol/L), pulmonary edema (radiological) and renal impairment (serum creatinine greater than 265 µmol/L . Modifications were made where necessary to suit this study. Complicated malaria was defined in this study as in the presence of *P. falciparum* asexual parasitemia and in the absence of an identified cause; Hemoglobin concentration below 5 g/dl or a hematocrit of less than 15% in children below 12 years of age (WHO, 2014).

3.5 Blood Collection, Processing and Malaria Diagnosis

The Rapid Diagnostic Test (RDT) was used to determine presence or absence of *falciparum* malaria. Five millilitres of venous blood sample was collected from each participant. An aliquot of 2 ml was transferred into dipotassium ethylenediamine tetraacetic acid (K_2 EDTA) tubes for hematological analysis and the remaining 3 ml into Vacutainer plain tubes for biochemical analysis. The clotted samples in the Vacutainer tubes were centrifuged at 1500 g for 5-10 min and the serum stored at -20°C until assayed (Sakyi *et al*, 2012).

3.6 Quantification of Hematological and Biochemical Parameters

Full blood count was determined using an automated hematological analyser (Sysmex Automated Hematology Analyser, Kobe, Japan, XP-300, Model No.: AC580857). The hemoglobin level (HB), red blood cell count (RBC), hematocrit (HCT), platelets count (PLT), white blood cell count (WBC), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were recorded. The Malondialdehyde concentration (MDA) as well as vitamin C level was measured as the biochemical parameters in this study using the serum sample. ELISA kits obtained from Biobase Biotech, Shangai, China, were used. The protocol for determining Vitamin C concentration and MDA from this company (Biobase Biotech, Shangai, China) was followed. The concentrations of MDA and Vitamin C were finally measured spectrophometrically, using an ELISA microplate analyser (Inqaba Biotech, UK, Model No.: RT0400814GDM).

3.6.1 Vitamin C and MDA determination using ELISA

Materials used for this part of the study were **s**erum, MDA ELISA Kit, Vitamin C ELISA Kit, 37^{o}C incubator, standard microplate reader, precision pipettes and disposable pipettes.

The ELISA kits came with microelisa stripplate of 96 wells, 6 different standard solutions of varying concentrations (0,5,10,20,40,80 µmol/L for vitamin C and 0, 0.5, 1, 2, 4, 8 nmol/L for MDA), 6.0 mL sample diluent, 10 ml Horse radish peroxidase-conjugate reagent, 25 ml 20× wash solution, 6.0 ml chromogen solution A, 6.0 ml chromogen solution B and 6.0 ml stop solution. Two closure membranes and user manuals were made available too.

Repeated freeze-thaw cycles of the serum stored at -20^{o} C was avoided to prevent errors in the determination of these biochemical parameters, as especially vitamin C is highly volatile. The steps enumerated below were followed in determining the concentrations of vitamin C and MDA in the serum of the subjects.

1. All reagents in the kits were brought to room temperature. Standards and samples were added in duplicates to the microelisa stripplate.
2. Standard as well as sample wells were set; 50 µL standard solutions were added to standard wells.
3. A 10 µL testing sample was added to the stripplate before adding 40 µL sample diluent to the sample well. A 100 µL of horse radish peroxidase-conjugate reagent was added to the wells. At this point, an adhesive strip was used to cover the stripplates and incubated at 37^{o}C for 1 hour. At this stage antibody-antigen reaction took place.
4. Each well was aspirated and washed for a total of five times, using 400 µL wash solution in a squirt bottle. Complete removal of liquid at each step was carried out to

allow for good performance. After the last wash, the microelisa plate was inverted and blotted against a clean paper towel. During this step, the unbound molecules are what were washed.

5. Fifty microlitres each of chromogen solutions (A and B) were added to each well. Solutions, together with samples and standards were gently mixed and incubated for a period of 15 minutes at 37^{o}C. Due to the color reactions involved at this point it was essential to protect from light. Another antibody-antigen reaction took place during the incubation period.
6. Fifty microlitres of the stop solution was added to each well to bring the reaction to a stop. The color in the wells changed from blue to yellow. Some of the wells did not experience color change. These were gently tapped to allow for color change from blue to yellow.
7. The optical density was red at 450 nm using a microtitre plate reader within 15 minutes.

   Vitamin C and MDA concentrations of the various samples were read using an automated ELISA micro plate analyser (Inqaba Biotech, UK, Model No.: RT0400814GDM).

3.7 Ethical Clearance

Ethical clearance for the study was obtained from the Committee on Human Research, Publication and Ethics (CHRPE) at the KNUST School of Medical Sciences/ Komfo Anokye Teaching Hospital in Kumasi-Ghana (Reference Number: CHRPE/AP/078/17). Permission was also given by the various Medical Directors/Superintendents or Heads of Departments of the study facilities. Consent was

also obtained from healthcare personnel and guardians or parents whose wards were recruited into this study.

3.8 Statistical Analysis

Data were entered into Microsoft Excel sheet and cleaned before being subjected to analysis. Statistical analyses were performed using GraphPad Prism 5 (GraphPad© software, San Diego California USA, www.graphpad.com. 26th June, 2017).

Means were calculated for continuous variables, while percentages were calculated for categorical variables. Data presentation was done by using tables and bar graphs Analysis of variance (ANOVA), coupled with Turkey's post hoc multiple comparisons was used to compare more than two means of continuous variables. Unpaired t-test was used to compare between two means of continuous variables. A p value < 0.05 was considered statistically significant.

CHAPTER FOUR

RESULTS

4.1 Socio-demographic characteristics of study participants and guardians.

The study comprised two parts. This part of the study was conducted among the caregivers of a total of 83 subjects, comprising 15 control subjects, 17 subjects with severe or complicated malaria, and 51 subjects with uncomplicated malaria. The mean age (±SD) of the children was 4.22 (±2.65) years, with the mean age of uncomplicated malaria subjects at 4.32(±2.81) whilst that of complicated malaria stood at 4.27(±2.96). So majority of the subjects (67.5%) were within the age of 0-5 years, with 10 of such subjects from the control group, 35 from the complicated malaria group and 11 from the complicated malaria group (Table 4.1). It was parents with JHS education who were predominant, constituting 32 (38.6%), of which 23 were from the uncomplicated malaria group and 6 from the complicated malaria group; similarly, parents with tertiary education formed the minority in all the categories. Among guardians who receive daily income payment or non-regular payment, most of them receive between 10 and 25 cedis per day (38.3%) with similar data shown in the complicated (41.2%) and uncomplicated (33.3%) malaria groups.

Table 4.1 Socio-demographic characteristics of study participants and guardians

Variables	Total (n=83)	Control (n=15)	Uncomplicated (n=51)	Complicated (n=17)
Age (years) (mean ± SD)	4.22±2.65	3.80±1.66	4.32±2.81	4.27±2.96
Age range of the wards (years)				
0-5	56(67.5%)	10(66.7%)	35(68.6%)	11(64.7%)
6-10	25(30.1%)	5(33.3%)	14(27.5%)	6(35.3%)
11- 12	2(2.4%)	-	2(3.9%)	-
Gender				
Male	39(47.0%)	8(53.3%)	24(47.1%)	7(41.2%)
Female	44((53.0%)	7(46.7%)	27(52.9%)	10(58.8%)
Educational level of parents				
No formal education	18(21.7%)	3(20.0%)	12(23.5%)	3(17.6%)
Primary	21(25.3%)	6(40.0%)	9(17.6%)	6(35.3%)
JHS	32(38.6%)	3(20.0%)	23(45.1%)	6(35.3%)
SHS	7(8.4%)	2(13.3%)	3(5.9%)	2(11.8%)
Tertiary	5(6.0%)	1(6.7%)	4(7.8%)	-
Frequency of income				
Daily	54(65.1%)	11(73.3%)	32(62.7%)	11(64.6%)
Weekly	7(8.4%)	1(6.7%)	4(7.8%)	2(11.8%)
Monthly	16(19.3%)	2(13.3%)	12(23.5%)	2(11.8%)
Non-regular	6(7.2%)	1(6.7%)	3(5.9%)	2(11.8%)
Income level per day (GHS)				
Below 5	13(16.0%)	1(6.7%)	10(19.6%)	2(11.8%)
Between 5 and 10	11(13.5%)	2(13.3%)	8(15.7%)	2(11.8%)
Between 10 and 25	31(38.3%)	7(46.7%)	17(33.3%)	7(41.2%)
Between 26 and 50	11(13.6%)	4(26.7%)	4(7.8%)	3(17.6%)
Above 50	15(18.1%)	1(6.7%)	12(23.5%)	3(17.6%)
Missing data	2(0.5%)	-	-	-

SD=Standard Deviation, JHS=Junior High School, SHS=Senior High School

4.1.1 Issues related to past treatment of malaria

As shown in Table 4.2, the largest percentage of the guardians reported to have treated their ward for malaria within the past month (30.1%) (18 uncomplicated malaria subjects, 4 complicated malaria subjects and 3 control subjects), followed by within the past year (24.1% for the total and 46.7% for the control group), but 35.3% of the uncomplicated malaria group indicated that it was just a month ago that they treated their wards and another 35.3% of the complicated malaria group responded to treating

their wards for the past six months. More than half of the guardians treated their ward at the hospital or clinic (51.8%), followed by self-medication through pharmacy (47.0%) and the least frequent was self-medication, using herbal preparation (1.3%). The self-medication subject was from the complicated malaria group. The majority of the guardians (48.6%) followed the full course of treatment (23 uncomplicated malaria subjects, 11 complicated malaria subjects and 6 control subjects) and 19.3% stopped when symptoms disappeared. This 19.3% representing 16 subjects, 12 were from the uncomplicated malaria group and 2 each from the remaining groups. However, 23.0% reported that symptoms persisted even after going through the full course of treatment (Control group had 26.7%, uncomplicated malaria group had 19.6% and 17.7% for the complicated malaria group). More than half of the guardians (61.4%) reported that there was recurrence of malaria for their wards. This 61.4% was contributed by a total of 49 subjects of which 32 are from the uncomplicated malaria group, 7 from complicated malaria group and the remaining from the control group. Fifty of the guardians (60.2%) reported to be using the insecticide-treated nets method of preventing malaria, followed by mosquito coils (38.6%), repellants (9.6%), insecticide sprays (9.6%) and prophylactic drugs (2.4%). The most effective malaria preventive method reported was insecticide-treated nets (77.1%), followed by mosquito coils (16.9%) and insecticide sprays (4.8%). The percentage for the use of the insecticide-treated nets in the respective groups was also high (control group had 60.0%, uncomplicated malaria group had 62.7% and 58.8% for the complicated malaria group). The most reported reason for not using any of these methods was physical discomfort for kids (34.9%) followed by dislike of the use of cutaneous chemicals (24.1%) and difficulty of use (22.9%). In the respective groups, physical discomfort for kids was reported as the predominant reason (Table 4.2).

Table 4.2 Issues related to past treatment of malaria

Variables	Total(n=83)	Control(n=15)	Uncomplicated(n=51)	Complicated(n=17)
Last time of treating malaria				
Within the past month	25(30.1%)	3(20.0%)	18(35.3%)	4(23.5%)
Within the past six months	16(19.3%)	2(13.3%)	8(15.7%)	6(35.3%)
Within the past year	20(24.1%)	3(20.0%)	13(25.5%)	4(23.5%)
A year or more	22(26.5%)	7(46.7%)	12(23.5%	3(17.7%)
Mode of treatment				
Hospital or clinic	43(51.8%)	11(73.3%)	24(47.1%)	8(47.1%)
Self-medication through pharmacy	39(47.0%)	4(26.7%)	27(52.9%)	8(47.1%)
Self-medication using herbal preparation	1(1.2%)	-	-	1(5.8%)
No treatment provided	-	-	-	-
Course of treatment				
Full course	40(48.2%)	6(40.0%)	23(45.1%)	11(64.7%)
When symptoms disappeared	16(19.3%)	2(13.3%)	12(23.5%)	2(11.8%)
When the drugs got missing	9(10.8%)	2(13.3%)	6(11.8%)	1(5.8%)
Couldn’t afford the drugs	1(1.2%)	1(6.7%)	-	-
Symptoms persisted after full course	17(20.5%)	4(26.7%)	10(19.6%)	3(17.7%)
Recurrence of disease				
Yes	51(61.4%)	12(80.0%)	32(62.7%)	7(41.2%)
No	32(38.6%)	3(20.0%)	19(37.3%)	10(58.8%)
Type of malaria preventive methods				
Insecticide-treated nets	51(61.4%)	9(60.0%)	32(62.7%)	10(58.8%)
Mosquito coils	30(36.1%)	3(20.0%)	20(39.2%)	7(41.2%)
Repellents	7(8.4%)	3(20.0%)	3(5.6%)	1(5.8%)
Prophylactic drugs	3(3.6%)	1(6.7%)	-	2(11.8%)

Insecticide sprays	11(13.2%)	4(26.7%)	7(13.7%)	-
Others	4(4.8%)	-	4(7.8%)	-
Most effective method				
Insecticide-treated nets	66(79.5%)	12(80.0%)	42(82.3%)	12(70.7%)
Mosquito coils	15(18.1%)	1(6.7%)	10(19.6%)	4(23.5%)
Insecticide sprays	5(6.0%)	2(13.7%)	2(3.9%)	1(5.8%)
Reason for not using preventive methods				
Monetary constraints	4(4.8%)	1(6.7%)	2(3.9%)	1(5.8%)
Difficulty in use	21(25.3%)	5(29.4%)	13(25.5%)	3(17.7%)
Dislike use of cutaneous chemical	21(25.3%)	2(13.3%)	16(31.4%)	3(17.7%)
Physical discomfort for kids	37(44.6%)	9(60.0%)	23(45.1%)	5(29.4%)
Others	6(7.2%)	-	-	6(35.3%)

4.1.2 Distribution of symptoms, medications, source of information and income level

The majority of the guardians reported to have heard of malaria from the television (69.9%) followed by radio (37.3%), public health agency (15.6%), newspapers (7.2%), books (6.0%) and the least being verbal from family or friends (3.6%) (Table 4.3). Majority of the control (86.5%) and uncomplicated malaria group (68.6%) reported to have used television as their source of information on malaria but majority of the complicated malaria group (70.6%) relied on radio for their information High body temperature of the ward was the most common symptom that prompted guardians to suspect malaria (84.3%). This was followed by loss of appetite (55.4%), headaches (53.0%), general body pains and weakness (32.5%). The results obtained for the high body temperature was reflective in the control group (93.3%) and uncomplicated malaria group (80.4%) whilst a surprising 70.6% of the guardians of the complicated malaria subjects reported to have been prompted to conclude that their wards were suffering from malaria based on their loss of appetite.

Paracetamol was the most frequently used drug for treating malaria among the studied subjects (42.2%) , followed by artesunsate (18.1%), artesunate-amodiaquine (16.4%), artemether (7.2%) lonart (7.2%) and coartem (2.4%). Other drugs such as quinine. O.R.S, blood tonics and herbal preparations accounted for 24.1% of the drugs used for treating malaria.

Table 4.3: Distribution of symptoms, medications, source of information and income level

Variables	Totals(n=83)	Control(n=15)	Uncomplicated(n=51)	Complicated(n=17)
Source of information				
Newspaper	6 (7.2%)	-	5(9.8%)	1(5.9%)
Television	58(69.9%)	13(86.5%)	35(68.6%)	10(58.8%)
Radio	31(37.3%)	8(53.3%)	11(21.6%)	12(70.6%)
Public Health Agency	13(15.6%)	2(13.3%)	11(21.6%)	-
Verbal family or friend	3(3.6%)	2(13.3%)	-	1(5.8%)
Books	5 (6.0%)	1(6.7%)	1(2.0%)	3(17.6%)
Symptoms implicating malaria by guardians				
Headaches	44(53.0%)	11(73.3%)	24(47.1%)	9(52.9%)
High Body temperatures	70(84.3%)	14(93.3%)	41(80.4%)	15(29.4%)
General body pain	27(32.5%)	8(53.3%)	15(29.4%)	4(23.5%)
Shiverishness	10(12.0%)	2(13.3%)	7(13.7%)	1(5.8%)
Loss of appetite	36(55.4%)	5(33.3%)	19(37.3%)	12(70.6%)
Yellow urine	4(4.8%)	1(6.7%)	1(2.0%)	2(11.8%)
Others	21(25.3%)	4(26.7%)	14(27.4%)	3(17.6%)
Drug used for treatment				
Chloroquine	3(3.6%)	1(6.7%)	1(2.0%)	1(5.8%)
Artesunate	4(4.8%)	2(13.7%)	1(2.0%)	1(5.8%)
Artemether	20(24.1%)	4(26.7%)	14(27.4%)	2(11.8%)
Artesunate Amoadiaquine	19(22.9%)	5(33.3%)	10(19.6%)	4(23.5%)
Paracetamol	35(42.2%)	4(26.7%)	26(51.0%)	5(17.6%)
Lonart	3(3.6%)	-	2(3.9%)	1(5.8%)
Coarterm	6(7.2%)	4(26.7%)	2(3.9%)	-
Others	16(19.3%)	-	9(17.6%)	7(41.2%)

4.2 Hematological and biochemical parameters of studied subjects

Table 4.4 shows that there was no significant difference between males and females with uncomplicated malaria, in relation to WBC (p=0.423), HB (p=0.932), RBC (p=0.0.987), HCT (p=0.819), MCV (p=0.306), MCH (p=0.240), PLT (p=0.932) vitamin C (0.926) and MDA (p=0.943).

Table 4.4 Comparison of hematological and biochemical parameters according to gender among uncomplicated malaria subjects

Variables	Males (n=24)	Females (n=27)	p-value
WBC($\times 10^9$/L)	11.1±7.7	9.9±5.2	0.488
HB(g/dl)	10.3±1.7	10.7±1.6	0.494
RBC($\times 10^{12}$/L)	4.2±0.6	4.8±1.4	0.143
HCT (%)	35.0±5.1	38.3±11.3	0.268
MCV (fl)	80.2±9.5	82.4±11.2	0.956
MCH (pg)	24.4±3.0	24.3±6.0	0.940
MCHC (%)	29.8±2.8	29.6±6.9	0.883
PLT($\times 10^9$/L)	602.2±1148	1183±1595	0.219
MDA(nmol/ml)	4.8±1.4	5.1±1.9	0.943
Vitamin C (µmol/L)	40.0±11.6	36.6±12.	0.329

4.2.1 Hematological and biochemical parameters of subjects with complicated malaria

As shown in Table 4.5, female subjects with complicated malaria had significantly (p=0.046) higher levels of vitamin C (45.8±12.4), compared to the male subjects (33.0±11.1). The MCV, MCH, MCHC of the females were also higher, but not significantly different from those of the males. On the other hand, the males had higher Hb, WBC, RBC, HCT, PLT, MDA which were also not significantly different from the female values. From the haemoglobin levels, the two groups were anemic.

Table 4.5 Comparison of hematological and biochemical parameters according to gender among complicated malaria

Variables	Males (n=7)	Females (n=10)	p-value
WBC($\times 10^9$/L)	9.6±4.5	13.1±5.5	0.195
HB(g/dl)	7.6±1.5	7.0±2.5	0.557
RBC($\times 10^{12}$/L)	3.2±0.6	2.6±1.1	0.222
HCT (%)	14.5±0.5	13.9±1.6	0.353
MCV (fl)	76.3 ±9.37	84.9±24.6	0.396
MCH (pg)	23.8±3.6	31.0±19.9	0.363
MCHC (%)	31.6±5.9	34.7±9.0	0.430
PLT($\times 10^9$/L)	815.8±1360.1	504.6±492.4	0.582
MDA(nmol/L)	5.6±1.3	5.0±2.1	0.534
Vitamin C (µmol/L)	33.0±11.1	45.8±12.4	0.046

Table 4.6 shows the comparison of hematological and biochemical parameters amongst control subjects, uncomplicated malaria and complicated malaria. There were non-significant increasing mean levels of WBC in the order: control <uncomplicated malaria< complicated malaria (p=0.081). Mean levels of HB decreased significantly in the order; control subjects>uncomplicated malaria subjects> complicated malaria subjects (p<0.0001). There was no statistical significance amongst control subjects, uncomplicated and complicated malaria subjects in relation to the mean levels of MCV (p=0.281). There was a statistically significant reduced mean levels of HCT (p<0.0001), MCHC (p=0.004) and PLT (p=0.012) in the order: control subjects > uncomplicated malaria and complicated malaria. Mean levels of MDA was significantly lower in control subjects compared to complicated malaria subjects (4.62±1.85 vs.6.68±0.70, p=0.0008) and also lowered in uncomplicated malaria compared to complicated malaria (4.50±1.58 vs. 6.68±0.70, p<0.0001). There was a statistically significant reduced mean level of vitamin C (p=0.036) in the order: control subjects > uncomplicated malaria > complicated malaria subjects.

Table 4.6 Comparison of hematological and biochemical parameters between control subjects, uncomplicated malaria and complicated malaria

Variables	Control (n=15)	Uncomplicated Malaria (n=51)	Complicated Malaria(n=17)	p-value	Significant pairs
WBC($\times 10^9$/L)	7.25±3.04	10.44±6.11	11.64±10.44	0.081	ns
HB(g/dl)	10.70±1.65	10.50±1.64	7.25±1.91	**<0.0001**	A vs. C(**<0.0001**)
RBC($\times 10^{12}$/L)	5.90±7.78	4.53±1.17	2.87±0.93	0.062	A vs. C (**0.037**)
HCT (%)	32.69±14.62	36.97±9.33	14.15±1.25	**<0.0001**	A vs. C **(<0.0001**)
MCV (fl)	75.26±16.49	82.39±19.77	81.39±19.77	0.281	ns
MCH (pg)	31.86±14.51	28.07±15.55	24.35±4.97	0.064	ns
MCHC (%)	35.17±3.02	33.42±7.84	29.68±5.52	**0.005**	A vs. C (**0.003**)
PLT($x10^9$/L)	2155±1921	916.2±1419	648.2±958.4	**0.026**	A vs. B(0.0251), A vs. C(**0.028**)
MDA(nmol/ml)	4.62±1.85	4.50±1.58	6.68±0.70	**<0.0001**	B vs. C (**<0.0001**), A vs. C (**0.008**)
Vitamin C (µmol/L)	49.57±21.80	40.53±13.22	38.17±12.33	**0.036**	A vs. C (**0.019**)

A: control; B: uncomplicated malaria; C: complicated or complicated malaria; ns: not significant

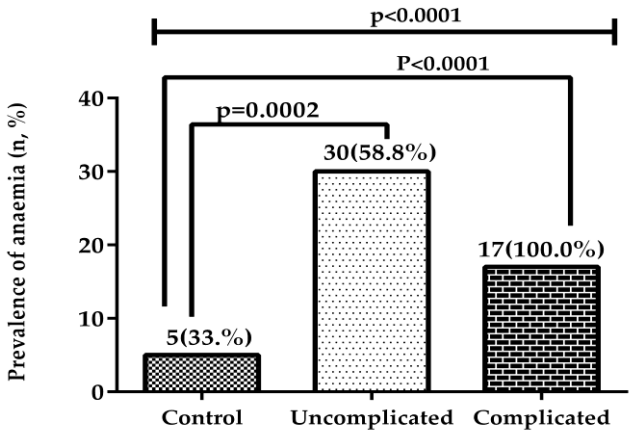

Figure 4.1 Prevalence of anemia among uncomplicated and complicated malaria cases

For the patients with uncomplicated malaria, 58.8% were anemic, while all the patients with complicated malaria were anemic, as shown in Figure 4.1

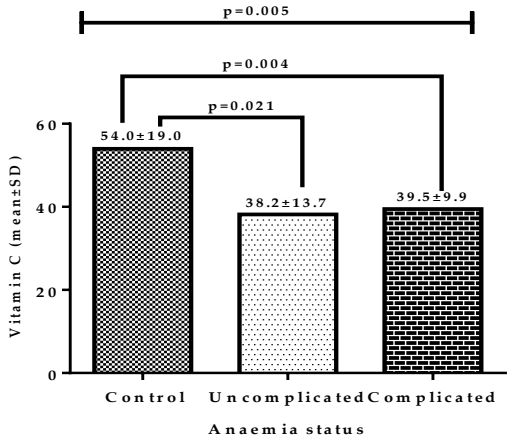

Figure 4.2: Comparison of vitamin C between the anaemic control subjects, anaemic uncomplicated malaria and complicated malaria

The mean level of vitamin C was higher among the anaemic control subjects, anemic uncomplicated malaria and complicated malaria. The mean levels of vitamin C was higher in anemic control subjects compared to anemic uncomplicated (54.0±19.0 vs. 38.2±13.7, p=0.004) and anemic complicated malaria subjects (54.0±19.0 vs. 39.5±9.9,

p=0.021). However, no significant difference of mean levels vitamin C was observed between anemic uncomplicated and complicated (severe) malaria subjects (38.2±13.7 vs. 39.5± 9.9, p=0.942).

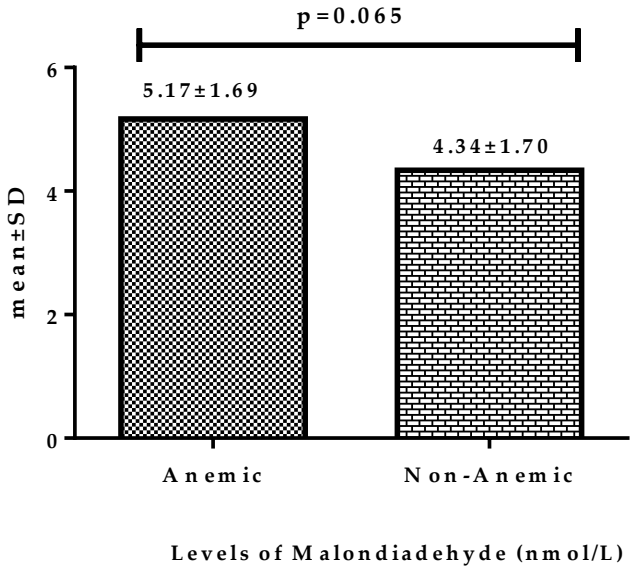

Figure 4.3 Comparison of malondialdehyde levels between anemic and non-anemic subjects with uncomplicated malaria

Higher mean level of malondialdehyde was observed in the anemic subjects, compared to the non-anemic subjects with uncomplicated malaria but this is not statistically significant (5.17±1.69 vs. 4.34±1.70, p=0.065).

4.2.2 Clinical manifestations of complicated malaria

Table 4.7 shows the various clinical manifestations seen in the complicated malaria subjects studied. Prostration (69.2%) was the most prevalent complication of complicated malaria in the subjects, followed by hyperpyrexia (38.5 %), acute respiratory distress (30.2%) and then convulsion (23.1%). All complicated malaria subjects had a parasite count > 10,000 par/µl and hematocrit ≤15%. Four out of the 17 complicated malaria subjects had hemoglobin values ≤ 5g/dl.

Table 4.7 Clinical manifestations of complicated malaria subjects

Clinical Manifestation	MCHH (n=13)	MDH (n=2)	SGH (n=2)
Hyperpyrexia (Temp≥ 40^{o}C)	5(38.5%)	0(0)	0(0)
Prostration	9(69.2%)	0(0)	0(0)
Acute Respiratory Distress	4(30.2%)	0(0)	0(0)
Coma	1(7.7%)	0(0)	0(0)
Convulsion	3(23.1%)	0(0)	0(0)
Unconsciousness	2(15.4%)	0(0)	0(0)
Hemoglobin (Hb ≤ 5g/dl)	2(15.4%)	2(100%)	0(0)
Hematocrit (HCT≤15%)	13(100 %)	2(100%)	2(100)
Parasite count (PC >10000 par/µl)	13(100%)	2 (100%)	0(0)

MCHH: Maternal and Child Health Hospital; MDH: Manhyia District Hospital; SGH: Suntreso Government Hospital.

4.3 Correlation between biochemical and hematological parameters in uncomplicated malaria subjects

As shown in the Table 4.8, increasing hemoglobin (HGB) level is significantly associated with increasing levels of MCV (r=0.404, p=0.009), MCH (r=0.581, p<0.0001) and MCHC (p=0.413, p=0.007). RBC levels was found to be significantly associated with increased levels HCT (r=0.897, p<0.0001) and inversely related with MCV (r=-0.764, p<0.0001) and MCHC (r=-0.731, p<0.0001).

Table 4.8 Correlation between biochemical and haematological parameter among uncomplicated malaria subjects

Variables		HGB	RBC	HCT	MCV	MCH	MCHC	PLT	MDA	VIT C
WBC	r	-0.086	-0.038	-0.190	-0.446	-0.037	0.288	-0.001	0.062	0.012
	p-value	0.550	0.814	0.234	**0.003**	0.821	0.068	0.995	0.668	0.933
HGB	r		0.007	0.172	0.404	0.581	0.413	0.217	-0.189	0.078
	p-value		0.966	0.282	**0.009**	**<0.0001**	**0.007**	0.197	0.185	0.585
RBC	r			0.897	-0.203	-0.764	-0.731	-0.043	-0.046	-0.085
	p-value			**<0.0001**	0.203	**<0.0001**	**<0.0001**	0.807	0.776	0.599
HCT	r				0.186	-0.547	-0.740	-0.317	-0.027	-0.048
	p-value				0.245	**<0.0001**	**<0.0001**	0.067	0.868	0.765
MCV	r					0.525	-0.088	-0.351	-0.040	0.022
	p-value					**<0.0001**	0.586	**0.042**	0.806	0.893
MCH	r						0.798	0.303	-0.049	0.080
	p-value						**<0.0001**	0.081	0.759	0.621
MCHC	r							0.775	-0.044	0.072
	p-value							**<0.0001**	0.783	0.655
PLT	r								-0.236	0.044
	p-value								0.159	0.798
MDA	r									0.030
	p-value									0.835

r= Correlation Coefficient, p<0.05: statistically significant

4.3.1 Correlation between biochemical and hematological parameters in complicated malaria subjects

Among the complicated malaria cases, there was a positive significant correlation between HGB and RBC (r=0.628, p=0.007), HCT (r=0.640, p=0.006) and PLT (r=0.573, p=0.041). RBC positively correlated with HCT (r=0.652, p=0.005) and negatively correlated with MCV (r=-0.717, p=0.001) and MCH (r=-0.607, p=0.010) (Table 4.9).

Table 4.9 Correlation between biochemical and hematological parameter among complicated malaria subjects

		HGB	RBC	HCT	MCV	MCH	MCHC	PLT	MDA	VIT C
WBC	r	-0.330	-0.159	-0.500	-0.217	-0.127	-0.010	-0.133	0.269	0.207
	p-value	0.195	0.542	0.041	0.403	0.627	0.970	0.664	0.297	0.424
HGB	r		0.628	0.640	-0.067	0.134	0.360	0.573	-0.119	0.201
	p-value		**0.007**	**0.006**	0.797	0.609	0.156	**0.041**	0.650	0.439
RBC	r			0.652	-0.717	-0.607	-0.403	0.194	0.210	-0.027
	p-value			**0.005**	**0.001**	**0.010**	0.109	0.525	0.419	0.918
HCT	r				-0.191	-0.194	-0.165	0.014	0.233	0.048
	p-value				0.463	0.457	0.528	0.964	0.368	0.854
MCV	r					0.909	0.639	-0.140	-0.179	0.188
	p-value					**<0.0001**	**0.006**	0.649	0.491	0.470
MCH	r						0.890	0.115	-0.166	0.293
	p-value						**<0.0001**	0.709	0.525	0.254
MCHC	r							0.436	-0.220	0.336
	p-value							0.136	0.396	0.188
PLT	r								-0.038	0.104
	p-value								0.902	0.735
MDA	r									0.047
	p-value									0.858

r= Correlation Coefficient, p<0.05: statistically significant

The results for the survey involving 80 healthcare personnel are first presented and interpreted after which the results of the 83 subjects follow.

4.4. Gender and designations of healthcare professionals

About half (~ 50%) of the healthcare personnel was males (52.5%). The majority of the participants were medical officers (32.5%) followed by physician assistants (27.5%).

Table 4.11: Frequency distribution of respondents by gender and designations

Variables	Frequency (n=80)	Percentages (%)	
Gender			
Male	42	52.5%	~ 50%
Female	38	47.5%	~ 50 %
Designations			
Consultant	1	1.25%	
House Officer	2	2.5%	
Intern	5	6.25%	
Medical Assistant (MA)	1	1.26%	
Medical Officer	26	32.5%	~ 30 %
Medical Sup	1	1.25%	
Nurse	3	3.75%	
Physician Assistant (PA)	22	27.5%	~ 30 %
Pediatrician	1	1.25%	
Resident	7	8.75%	
SMO	5	6.25%	
Senior Pharmacist	1	1.25%	
SPA	1	1.25%	
Specialist	4	5.0%	

SMO=Senior Medical Officer, SPA= Superintendent Physician Assistants

4.5 Distribution of malaria symptoms in children according to healthcare personnel

Fever recorded the highest frequency of the symptoms, represented by 98.8%, followed by general malaise (97.4%), nausea and vomiting (93.8%), chills (78.8%) and headaches (72.5%).

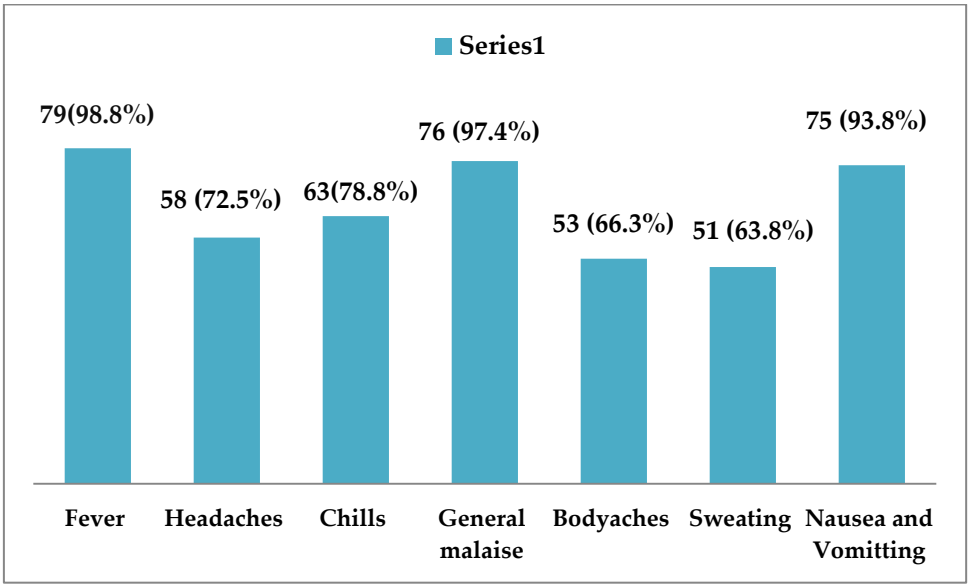

Figure 4.4: Frequency of symptoms of malaria in children.

4.6 Clinical manifestations of complicated malaria infections in children

Table 4.12 shows the frequency distribution of clinical manifestations of complicated malaria in children. Prostration (45.0%), hyperpyrexia (38.75%) and hypoglycemia (13.75%) were the most frequently reported complications. Complications which were distinctive feature of complicated malaria infection in children reported by healthcare personnel were hyperpyrexia (17.5%), prostration (12.5%), impaired consciousness (8.75%) and cerebral malaria (7.5%). The majority of the participants reported cerebral malaria (30.0%), severe anemia (23.75%), acute respiratory distress (17.5%), repeated generalized convulsions (16.25%) to be the most lethal feature of complicated malaria infection in children. The overwhelming majority of the respondents use the WHO 2014 standard guide for determining complicated malaria.

Table 4.12 Frequency distribution of complications of complicated malaria infections

Variables	Frequency	Percentages (%)
Complications which are almost always present		
Prostration	36	45.0%
Severe Anemia	8	10.0%
Hypoglycemia	11	13.75%
Impaired consciousness	2	2.5%
Repeated Generalized Convulsions	3	3.75%
Hyperpyrexia	31	38.75%
Thrombocytopenia	0	0
Complications which are distinctive feature		
Thrombocytopenia	4	5.0%
Prostration	10	12.5%
Severe Anemia	1	1.25%
Cerebral Malaria	6	7.5%
Impaired consciousness	7	8.75%
Repeated Generalized Convulsions	4	5.0%
Hyperpyrexia	14	17.5%
Missing	46	42.5%
Complications which are most lethal		
Thrombocytopenia	5	6.25%
Acute respiratory distress	14	17.5%
Severe Anemia	19	23.75%
Cerebral Malaria	24	30.0%
Hypoglycemia	16	20.0%
Impaired consciousness	6	7.5%
Repeated Generalized Convulsions	13	16.25%
Hyperpyrexia	1	1.25%
Complications which leads to misdiagnosis		
Thrombocytopenia	13	16.25%
Acute respiratory distress	19	23.75%
Prostration	12	15.0%
Severe Anemia	4	5.0%
Cerebral Malaria	8	10.0%
Hypoglycemia	2	2.5%
Impaired consciousness	13	16.25%
Repeated Generalized Convulsions	17	21.25%
Hyperpyrexia	15	18.75%
Standards for determining complicated malaria		
WHO 2014 Standard	72	90.0%
Standard Treatment Guideline	8	10.0%

The data collection tool employed also required the healthcare personnel to report other malaria complications their patients have experienced which were not part of the list of complications on the questionnaires. Table 4.13 provides a list of such malaria complications.

Table 4.13: Frequency of other complications of malaria

Variables	Frequency (n=47)	Percentages (%)
Acute hemoglobinuria	1	1.25%
Acute renal failure	3	3.75%
Arthalgia	1	1.25%
Bitter taste	1	1.25%
Black water fever	10	12.5%
Excessive vomiting	1	1.25%
Splenomegaly	3	3.75%
Cerebral palsy	2	2.5%
Coma	1	1.25%
Shock	5	6.25%
Jaundice	8	10.0%
Polydipsia	1	1.25%
Intravascular hemolysis	2	2.5%
Metabolic acidosis	4	5.0%
Oliguria	2	2.5%
Neurological effect	1	1.26%
Opisthotonus	1	1.25%

CHAPTER FIVE

DISCUSSION

The study revealed that the spectrum of healthcare professionals that come into contact with malaria patients are medical officers, physician assistants, residents, senior medical officers, medical interns, consultants, nurses, pharmacists and specialists (Table 4.11). All healthcare professionals involved in the study followed WHO standards or the accepted Standard Treatment Guidelines, adopted by the Ghana Health Service in treating both complicated malaria and uncomplicated malaria conditions. Their knowledge base regarding the features of complicated malaria is very high.

Two thirds of healthcare professionals involved in the study were medical officers (32.5%) and physician assistants (27.5%). This means that there is 60% chance for a child suffering from malaria to come into contact with this cadre of healthcare professionals whenever they visit clinics or hospitals for treatment. It is therefore very necessary for this group of healthcare professionals to be always updated on all policies of malaria, in order to attain the malaria-free world that WHO has targeted to achieve by 2030 (WHO, 2015).

Fever, general malaise, nausea and vomiting are the most common symptoms of malaria in Ghanaian children below 12 years, according to the healthcare givers. This agrees with a report of WHO (2014) but not entirely with reports from other African studies. For example, fever has been reported by Fordjour (2015) but not general malaise and vomiting. Headaches were also common in these children. Body aches and sweating were the least occurring symptoms, based on the experiences of the healthcare professionals interviewed.

Prostration (45.0%), hyperpyrexia (38.75%), hypoglycemia (13.75%) were reported as the three most prevalent manifestations of complicated malaria in the children (Table

4.12). This does not agree entirely with WHO report on tropical medicine and international health (WHO, 2014), as prostration was the only one highlighted in that report. This makes it clear that the manifestation of complicated malaria is varied, depending on the patients and their environments. Similarly, hypoglycemia was mentioned as one of the most prevalent complications of complicated malaria (WHO, 2012) but not prostration.

The current results agree in part with a complicated malaria study conducted at the KNUST Hospital, Ghana, and others in Mozambique (Gwer *et al.*,2007), Yemen (Gwer *et al.,* 2007) and Burkina Faso (Reyburn, 2004). It was revealed in the KNUST study that anemia, prostration, convulsions and fever are the major clinical manifestation of complicated malaria (Fordjour, 2015); whilst in the other studies Reyburn (2004) and Gwer *et al.* (2007) prostration was found to be a common indicator of complicated malaria. Prostration was found in the current Ghanaian study and the previous Ghanaian (KNUST) study (Fordjour, 2015), all in the same region and city. Hence prostration should be given premium attention by healthcare givers in Ghana when treating malaria patients below 12 years. The presence of hyperpyrexia is not surprising as malaria is febrile. All febrile conditions elevate the body temperature, except in very rare cases.

It has been reported elsewhere (WHO, 2012) that respiratory distress is the most lethal complicated malaria complication. This study shows this to occupy the third position. Studies in Kenya revealed that impaired consciousness, together with respiratory distress were extremely lethal as it predicted as high as 84.4% of 64 deaths in 1844 children (Marsh *et al.,* 1995). Respiratory distress is common to two reports on the lethality of complicated malaria (Marsh *et al.,* 1995 and WHO, 2012).

The finding of this current study on the lethality of the various malaria complications agree with two previous Ghanaian complicated malaria studies. Oduro *et al.* (2007) and Gyapong (2009) have reported the predominant complications of complicated malaria as severe anemia (36.5%), followed by respiratory distress (24.4%), prolonged or multiple convulsions (21.6%) and cerebral malaria (5.4%). Extra care should be taken by healthcare professionals in Ghana especially when any of the above four complications affect a malaria patient who is below 12 years as this could be fatal.

Another revelation from this current study is that the 23.75% prevalence of acute respiratory distress is a major malaria complication which may lead to misdiagnosis in Ghanaian children (Table 4.12). This is primarily due to its resemblance in manifestation with other febrile conditions, especially Pneumonia (WHO, 2012). In addition to acute respiratory distress repeated generalized convulsions (21.25%), hyperpyrexia (18.75%), thrombocytopenia (16.25%) and impaired consciousness can all be misleading (Table 4.12) especially in a high transmission area like Ghana. Hence, extra care and vigilance should be used in managing these patients.

Several reports claim areas of intense transmission of *P. falciparum* like sub-Saharan Africa usually record the highest number of deaths in children up to 5 years during malaria infections (English *et al.,* 1996; Black *et al.,* 2010; WHO, 2014). Though no death was recorded in the subjects during the period of this study, a higher number of study participants were within the age of 0-5 years (Table 4.1). This observation agrees with several reports (English *et al.,* 1996; Black *et al.,* 2010; WHO, 2014) that malaria usually affects children below 5 years especially in areas of intense transmission like Kumasi. The mean ages for both uncomplicated malaria subjects (4.32±2.81) and complicated malaria subjects (4.27±2.96) were extremely. This is not surprising as complicated malaria is a result of uncomplicated malaria that has not been dealt with so

such uncomplicated malaria patients develop complications with time as the disease persists. It has been reported elsewhere that most malaria infection and death occur within this age group (English *et al.,* 1996; Black *et al.,* 2010; WHO, 2014). Underdeveloped immunity has been the major explanation for this experience (WHO, 2014). The ages of the two groups being the same is not surprising, as the complicated malaria is sequelae to the uncomplicated state which has been inappropriately diagnosed and attended to.

Coincidentally, most of the inhabitants of high malaria transmission areas are poor (WHO, 2014). There is also a strong link between poverty and illiteracy in such instances. Ghana is no exception to this fact. The current study substantiated this observation as majority of the guardians or parents receive between 10 and 25 Ghana cedis per day (Table 4.1). For the guardians whose wards suffered from severe or complicated malaria infection, 64.8% receive below 25 Ghana cedis per day (Table 4.1). Poverty therefore has hidden hands in malaria progression. Similarly, little or no education has also been reported in such parents or guardians (WHO, 2014).The study again revealed that none of the guardians whose wards suffered from complicated malaria had attained education up to the tertiary level. In order for the WHO global malaria strategy for eradicating malaria and making the world a malaria-free place to materialize, improvement in education should be made to go along with these strategies; otherwise, it will be very difficult to achieve these targeted goals.

More than half of both complicated and uncomplicated malaria subjects responded to have had malaria in the past 6 months (Table 4.2). This is very worrisome, as it is likely to contribute to absenteeism in schools. It is gratifying to note however, that previous malaria programs and education in the study area are yielding encouraging results, as more than 92% of both complicated and uncomplicated malaria subjects seek

healthcare assistance from hospitals, clinics or pharmacies. In order to help the others who do not seek immediate healthcare assistance, malaria programs and education must be intensified in the study area.

The study also revealed that 54.9% of uncomplicated malaria subjects and 35.3% of complicated malaria subjects had previously not taken their full course of medications given to them to treat malaria infections (Table 4.2). This is very alarming, especially due to the recurrence problem associated with the disease (WHO, 2012, 2014, 2015). This can contribute to genetic modification in the *Plasmodium* species (WHO, 2015) and if care is not taken it could hinder the achievement of the malaria-free world that has been targeted by WHO. In fact, 62.7% of the uncomplicated malaria subjects had experienced recurrence of malaria infection in the study area. Similarly, 41.2% of the complicated malaria subjects had also experienced this menace before. Majority of the subjects in the study asserted that the use of insecticide-treated bed nets is the most effective method of malaria prevention; however, many would not use it mainly due to physical discomfort for children. Education must be intensified to help in the use of these insecticide-treated bed nets.

Over 85% (Table 4.3) of guardians to these subjects agreed that they get most of their information on malaria from either radio or television. Hence, in the battle to eradicate malaria, the media are very powerful tools that must be rightly used to win the war against malaria. High body temperature made most of the guardians conclude their wards were suffering from malaria. Elevation of body temperature is a common feature of most febrile illnesses (WHO, 2012). However, other features like loss of appetite, nausea and vomiting, headaches and general malaise must all be monitored as they are all essential indicators or red flags.

On drugs use, Paracetamol seems to be the most preferred than the real medications for treating malaria. This however, cannot be true, as when all the drugs used for treating malaria are put together, they surpass the response obtained for Paracetamol alone. Paracetamol most likely received such response due to its antipyretic effect. It is the most common antipyretic known, whereas the anti-malaria drugs are varied. Majority of subject used ACTs (artemisinin-based combination therapies); Coartem, Lonart, Artesunate, Artemether, Artesunate Amodiaquine. One subject each from both complicated and uncomplicated malaria groups had used Chloroquine for treating malaria. Currently, the standard treatment guidelines of Ghana states that ACTs should be used in treating uncomplicated malaria; however, when complication arises then Quinine could be used intravenously (Standard Treatment Guidelines, 2004). Prompt and right use of malaria medications can be very helpful, hence a lot of education needs to be done since some people still use Chloroquine for treating pediatric malaria.

Other important aspects of this study are the hematological and biochemical features. Studies by Narsari *et al.* (2012) revealed significant increase in MDA, but decrease in ascorbic acid and other hematological and biochemical parameters during complicated malaria infection. In the same study, severe anemia (54%) was the most common presentation of complicated malaria. In the current study, there was no significant difference between males and females with uncomplicated malaria, in relation to WBC ($p=0.423$), HB ($p=0.932$), RBC ($p=0.0.987$), HCT ($p=0.819$), MCV ($p=0.306$), MCH ($p=0.240$), PLT ($p=0.932$) vitamin C (0.926) and MDA ($p=0.943$) (Table 4.4). This implies that the level of disease severity was similar in both sexes. However, with disease progression, depending on an individual patient and the patient's circumstances, there could be varied alterations occurring in some of the hematological and biochemical parameters (Narsari *et al.*, 2012). This is exemplified by the female subjects with complicated malaria having significantly ($p=0.046$) higher levels of

vitamin C (45.8±12.4), compared to the male subjects (33.0±11.1) (Table 4.5). Again, the MCV, MCH, MCHC of the females were also higher, but not significantly different from those of the males. The opposite occurred in some of the parameters. This is seen in the males having higher Hb, WBC, RBC, HCT, PLT, MDA which were not significantly different from the female value. The study also revealed that high concentration of vitamin C results in less production of MDA. Ascorbate is known to act as antioxidant, so until the antioxidant system is overpowered by free-radicals, high concentrations of antioxidant molecules are likely to be present in the body.

Malondialdehyde is a lipid peroxidation product which can be used to monitor disease progression in many febrile illnesses, including malaria (Narsari *et al.,* 2012). However, a strong antioxidant system will prevent excessive formation of this molecule. Therefore, it is not surprising that patients with higher vitamin C (antioxidant vitamin) had lower MDA values (Table 4.5). In the studied participants, females with complicated malaria recorded 5.0±2.1nmol/ml of MDA and 45.8±12.4µmol/L of ascorbate. Similarly, the males had 5.6±1.3 nmol/ml and 33.0±11.1 µmol/L of ascorbate. It appears higher vitamin C may lead to lower MDA formation. This can be very good for parasite clearance and prevention of the progression of malaria.

In malaria infection, the white blood cell count is expected to increase (Kayode *et. al,* 2011). The findings of this study agree with this assertion as increasing mean levels of WBC were observed in the order; control <uncomplicated malaria< complicated malaria (Table 4.6). On the other hand, a contrasting observation was made with regards to the hemoglobin levels, as there was decreasing trend as follows; control subjects>uncomplicated malaria subjects> complicated malaria subjects ($p<0.0001$). It was not surprising to find the same trend in some parameters like RBC ($p=0.062$), HCT ($p<0.0001$), MCHC ($p=0.004$) and PLT ($p=0.012$) in the order: control subjects >

uncomplicated malaria and complicated malaria as most of these parameters are calculated in relation to red blood cell and for that matter hemoglobin levels.

Mean levels of MDA was significantly lower in control subjects, compared to complicated malaria subjects (4.62±1.85 vs.6.68±0.70, p=0.0008) (Table 4.6) as reported elsewhere in literature (Sakyi *et al.,* 2012). Oxidative stress plays a key role in the pathogenesis of malarial anemia, and *Plasmodium falciparum*-infected red blood cells are known to produce more reactive oxygen species, thus making anemia a dependable predictor of the progression and severity of disease (Kayode *et al.,* 2011 and Narsari *et al.,* 2012). The increased plasma MDA level shows lipid peroxidation due to free radical injury.

There was a statistically significant reduced mean level of vitamin C (p=0.036) in the order: control subjects > uncomplicated malaria > complicated malaria subjects (Table 4.6). This observation agrees with other findings (Narsari *et al.,* 2012) as the more severe the disease gets, the lesser the concentration of ascorbate in the serum of the patient. Research has shown that ascorbate has a lot of beneficial effects on the immune system. Some of such studies have reported that ascorbate is needed for the differentiation of lymphoid organs during the growth of cockerels and young rats (Onyesom *et al.,* 2010). Again, this vitamin has been shown to have beneficial effects on the phagocytic action of leucocytes and the migratory behaviour of neutrophils (Onyesom *et al.,* 2010; Neelam, 2016). Increase in circulating interferon and virucidal effects have also been reported as some of the benefits of vitamin C (Onyesom *et al.,* 2010).

Infections that are febrile in nature have been shown to decrease blood level of ascorbic acid, denoting an increased need for this vitamin in such conditions (Onyesom *et al.,*

2010). Results obtained in this study agree with studies by Kim *et al.* (1998) and Onyesom *et al.* (2010).

The prevalence of anemia among uncomplicated malaria patients stood at 58.8%, whereas that of the complicated or complicated malaria cases stood at 100% (Fig. 4.2). During the erythrocytic stage of the malaria parasite, important amino acids from hemoglobin are needed for the development and continuation of the life cycle of the parasite. In many studies complicated malaria have been defined as Hb<5g/dl or Hct <15 % (WHO, 2012). This was observed in all complicated malaria patients for the hematocrit component but not hemoglobin; though other researchers like Narsari *et al.,* (2012) have reported 54% severe anemia and English *et al.,* (1996) 66.7% anemia in studied subjects. Schellenberg and his colleagues (2003) reported high prevalence of anemia in children below 5 years in Tanzania.

Several studies have established that severe anemia is multifactorial, yet in circumstances where there is presence of malaria parasites the condition is attributed to malaria. Hence all forms of anemia in the parasitic subjects are attributed to malaria. Again, a study in Tamale (northern Ghana) proved that the frequency of severe anemia was very high among children below five years (Gyapong, 2009; Fordjour, 2015).

The malaria parasite feeds on hemoglobin so it is always expected that there will be decrease in the level of hemoglobin. In some cases, the hemoglobin concentration alone may not be able to tell or be used to monitor disease progression. The World Health Organisation (2014) has therefore advised that the hematocrit which is the ratio of the volume of red blood cells in relation to the total blood volume can also be used. When the hematocrit is found to be less than 15%, such a patient can be said to be suffering from complicated malaria (WHO, 2014). Such was the case of all the complicated malaria subjects in this study (Table 4.6). Among uncomplicated malaria subjects,

increasing hemoglobin (HGB) level is significantly associated with increasing levels of MCV ($r=0.404$, $p=0.009$), MCH ($r=0.581$, $p<0.0001$) and MCHC ($p=0.413$, $p=0.007$). RBC levels was found to be significantly associated with increased levels of HCT ($r=0.897$, $p<0.0001$). This correlation explains the fact that the hematocrit concentration is as good as hemoglobin when monitoring disease progression in malaria patients (WHO, 2014).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The study revealed the following;

1. Prostration with hyperpyrexia is the most distinctive feature of complicated malaria in the study area.
2. Radio and television is the best means to disseminate information on malaria to people in the study area.
3. Use of insecticide-treated bed nets is the most effective way of preventing malaria.
4. Poverty and lack of formal education are key barriers to malaria prevention.
5. Malaria disease progression increases MDA levels and decreases the ascorbate concentration.
6. The decrease in vitamin C is highest amongst the severely ill malaria patients.
7. Malaria severity in children reflects in decrease in vitamin C levels.
8. Malaria progression reduces the hemoglobin levels, hematocrit concentration, red blood cell count and platelet count while there is increase in the white blood cell concentration.

6.2 Novelty in the current study

1. The first research work in the study area to interview healthcare givers on the various complications of complicated malaria.
2. The first Ghanaian study to report the level of vitamin C in malaria patients.
3. The use of ELISA in determining the level of MDA in malaria patients below 12 years.
4. The first study in which the redefined age of children of 12 years has been used.

6.3 Limitations of study

Some of the limitations of the current study within the 3 months for recruiting the patients are;

1. Difficulty in getting complicated malaria patients as education in the study area is yielding results.
2. High cost of ELISA kits restricting the study to work on antioxidant vitamins to vitamin C only.

6.4 Recommendations

Based on this study, it is recommended that

- Future studies with more robust designs should investigate derangements of other antioxidant vitamins, like vitamins A and E.
- Higher number of subjects should be recruited into similar study to validate some of the findings in this study.
- Studies on possible supplementation with antioxidant vitamins before, during and after intake of ACTs.

REFERENCES

Adesina, K.T., Balogun, O.R., Babatunde, A.S., Sanni, M.A., Fadeyi, A., Aderibigbe, S. (2009). Impact of malaria parasitemia on hematologic parameters in pregnant women at Booking in Florin, Nigeria. *Trends in medical research.* **4**:84-90.

Allen, S.J., Donnell, A., Alexander, N.D., Bush, N. and Bill, H. (1997). Alpha thalassemia protects children against disease caused by other infections as well as malaria. *Proceedings of the National Academy of Sciences of the United States of America.* **94:**4736–4741.

Angulo, I., and Fresno, M. (2002). Cytokines in the pathogenesis and protection against Malaria *Clin. Diagn. Lab. Immunol.* **9(6)**: 1145-1156.

Atamna, H., Ginsburg, H. and Seaman, S. (1993). Origin of reactive oxygen species in erythrocytes infected with *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* **61:**231–234.

Axton, J.H. and Siebert, S.L. (1982). Aetiology of convulsions in Zimbabwe children three months to eight years old. *The Central African Journal of Medicine.* **28**, 246–249.

Bassat, Q., Guinovart, C., Sigauque, B., Siquel, V. Willet, J. and Iquel, H. (2008). Malaria in rural Mozambique. Part II: children admitted to hospital. *Malaria Journal* **7:** 37-38.

Beare, N.A., Southern, C., Chalira, C., Taylor, T.E., Molyneux, M.E. and Harding, S.P. (2004). Prognostic significance and course of retinopathy in children with complicated malaria. *Archives of Ophthalmology* **122:**1141–1147.

Becker, K.L., Tilley, J.L., Vennerstrom, D., Roberts, S., Rogersonand, H. and Ginsburg, C. (2004). Oxidative stress in malaria parasite infected erythrocytes: host-parasite interactions. *Int. J. Parasitol*. **34**: 163-189.

Berkley, J.A., Lowe, B.S., Mwang,i I., Lowe, K. and Makuso, H. (2005). Bacteremia among children admitted to a rural hospital in Kenya. *New England Journal of Medicine* **352**:39–47.

Berkley, J.A., Mwarumba, S., Bramham, K., Lowe, B. and Marsh, K. (1999). Bacteremia complicating complicated malaria in children. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **93:**283–286.

Biemba, G., Dolmans, D., Thuma, P.E., Weiss, G. and Gordeuk, V.R. (2000). Severe anemia in Zambian children with *Plasmodium falciparum* malaria. *Tropical Medicine and International Health.* **5**:9–16.

Black, R.E., Cousens, S., Johnson. H.L., Luuk. V. and Mahamud, C. (2010). Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet.* **375**:1969–1987.

Bojang, K.A., van Hensbroek, M.B., Palmer, A., Banya, W.A., Jaffar, S. and Greenwood, B.M. (1997). Predictors of mortality in Gambian children with complicated malaria anemia. *Annals of Tropical Paediatrics.* **17:** 355–359.

Bouyou-Akotet, M.K., Dzeing-Ella. A., Kendjo, E., Palmer, A., Banya, W.A. and Jaffar, M. (2009). Impact of *Plasmodium falciparum* infection on the frequency of moderate to severe anemia in children below 10 years of age in Gabon. *Malaria Journal.* **8**:166.

Brewster, D.R., Kwiatkowski, D. and White, N.J. (1990). Neurological sequelae of cerebral malaria in children. *Lancet*. **336:**1039–1043.

Bronzan, R.N., Taylor, T.E., Mwenechanya, J, Kuntam, V. and Erisa, M. (2007). Bacteremia in Malawian children with complicated malaria: prevalence, etiology, HIV coinfection, and outcome. *Journal of Infectious Diseases.* **195**:895–904.

Buffet, P.A., Safekui, I., Deplaine, G., Brousse, V.P., Thellier, M., Turner, G.D., Puijalon O.M. (2010). The pathogenesis of plasmodium falciparum malaria in humans: insights from splenic physiology. *Blood*. **117 (2)**:381-392.

Calis, J.C., Phiri, K.S., Faragher, .EB.,Das B. and Nanda, M. (2008). Severe anemia in Malawian children. *New England Journal of Medicine.* **358**:888–899.

Camara, B., Diagne-Gueye, N.R., Faye, P.M. and Silic, B. (2011). Malaria severity criteria and prognostic factors among children in Dakar. *Med. Inf. Dis*. **41:**63–67.

Carter, J.A., Neville, B.G., White, S., Dase B. and Namda, M. (2004). Increased prevalence of epilepsy associated with severe *falciparum* malaria in children. *Epilepsia* **45:**978–981.

Charan, J. and Biswas, T. (2013). How to calculate sample size for different study designs in medical research. *Indian J. Psychol Med.* **35(2):** 121-126.

Chikezie, P., Chidoka, P. and Okpara, R. (2013). Hematologic and biochemical indices of *Plasmodium falciparum* infected inhabitants of Oweri, Imo state, Nigeria. *Joun. Med. Lab.Diag.* **4(3):** 38-44

Clark, I.A. and Hunt, N. H. (1983). Evidence for reactive oxygen intermediates causing hemolysis and parasite death in malaria Infect. *Immun*. **39**: 1-6.

Clark, I.A., and Hunt, N.H. (1983). Evidence for reactive oxygen intermediates causing hemolysis and parasite death in malaria. *Infect. Immun.* **39:** 1–6.

Clark, I.A., Hunt, N.H., Cowden W.B., Maxwell, L.E. and Mackie, E.J., (1984). Radical-mediated damage to parasites and erythrocytes in *Plasmodium* vinckei infected mice after injection of t-butyl hydroperoxide. *Clin. Exp.Immunol.* **56:**524-530.

Clark, T.A., and Hunt, N.H., (1983). Evidence for reactive oxygen intermediates causing hemolysis and parasite death in malaria. *Infect. Immun.* **39:**1-6.

Das, B.S. and Nanda, N.K. (1999). Evidence for erythrocyte lipid peroxidation in acute *falciparum*- malaria. *Trans. Royal Soc. Trop. Med. Hyg.* **93**: 58-62.

Das, B.S. and Nanda, N.K. (1999). Evidence for erythrocyte lipid peroxidation in acute *falciparum* malaria*. Trans. R. Soc. Trop. Med. Hyg*. **93**: 8–62.

Das, B.S., and Nanda, N.K. (1999). Evidence for erythrocyte lipid peroxidation in acute *falciparum* malaria. *Trans. Royal Soc. Trop. Med. Hyg.* **93:** 58-62

Das, B.S., Satpathy, S.K., Mohanty, D., Maluba, J.M. and Kaluwa, C.V. (1988). Hypoglycemia in severe *falciparum* malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* **82**:197–201.

Das, S.N., Mohapatra, B., Mohanty, R., Dash, P.C., Kar, K. and Dash, P.K. (2007). Malarial hepatitis as a component of multi-organ failure: a bad prognostic sign. *J. Indian Med.Ass.*105, 247–250.

Dondrop, A.M., Fanello, C.I., Hendriksen, I.C., Ellui, C., Kumpa, D.. Malu, V. (2010). Artesunate versus quinine in the treatment of severe *falciparum* malaria in African children (AQUAMAT): an open-label, randomised trial. *Lancet* **376**:1647–1657.

Eaton, J.W., Eckman, J.R., Berger, E. and Jacob, H.S. (1976). Suppresion of malaria infection by oxidant sensitive erythrocytes. *Nature*.**264:** 758-760

Egwunyenga, A.O., Isamah, G. and Nmorsi, O.P. (2004).Lipid peroxidation and ascorbic acid levels in Nigeria Children with acute *falciparum* malaria. *Afri. J. Biotech.***3:**560-563.

Elesha, S.O., Adepoju, F.B., and Banjo, A.A. (1993). Rising incidence of cerebral malaria in Lagos, Nigeria: a post-mortem study. *East Afr. Med. J.* **70**:302–306.

en.m.wikipedia.org/wiki/Kumasi. 26th July, 2016

English, M., Punt, J., Mwangi, I., McHugh, K. and Marsh, K. (1996). Clinical overlap between malaria and severe pneumonia in Africa children in hospital. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* **90**:658–662.

Evans, J.A., Adusei, A., Timmann, C. Namaba, C. and Kinsa, M. (2004). High mortality of infant bacteremia clinically indistinguishable from complicated malaria. *Monthly Journal of the Association of Physicians.* **97:**591–597.

Fordjour, F. (2015). Antimalarial usage in pregnancy: a cross-sectional study in selected health facilities in the Brong-Ahafo Region of Ghana : a thesis submitted to the Department of Social and Clinical Pharmacy of KNUST for the award of MSc. Clinical Pharmacy.

Ghana Statistical Service. Summary Report on 2010 Housing and Population Census. (2012). Accra: Sakoa Press Limited. pp. 16-17.

Golenser, J. and Chevion, M. (1989a). Oxidant stress and malaria: host-parasite interrelationships in normal and abnormal erythrocytes. *Semin Hematol*. **26**: 313-325.

Golenser, J., and Chevion, M. (1989b). Oxidant stress and malaria:host-parasite interrelationships in normal and abnormal erythrocyte. *Semin. Hematol.* **26:** 313-325.

GraphPad© software, San Diego California USA, www.graphpad.com. 26th June, 2017

Gwer, S., Newton, C.R. and Berkley, J.A. (2007). Over-diagnosis and co-morbidity of complicated malaria in African children: a guide for clinicians. *American Journal of Tropical Medicine and Hygiene.* **77**:6–13.

Gyapong, R. (2009). Characterization of complicated malaria and treatment-related adverse drug reactions among hospitalised children at the KNUST hospital, Kumasi,Ghana : A thesis submitted to the Department of Social and Clinical Pharmacy of KNUST for the award of MSc. Clinical Pharmacy

Hendriksen, I.C., Ferr. J. and Montoya, P. (2012). Diagnosis, clinical presentation, and in hospital mortality of complicated malaria in HIV-coinfected children and adults in Mozambique. *Clinical Infectious Diseases* **55**:1144–1153.

Hendriksen, I.C., Mwanga-Amumpaire, J., von Seidlein, L., Muutu, C. and Evale, P. (2012). Diagnosing severe *falciparum* malaria in parasitaemic African children: a prospective evaluation of plasma PfHRP2 measurement. *PLoS*

http://www.public.iastate.edu/~duahn/teaching/Lipid%20oxidation/Measurement%20of%20Lipid%20Oxidation.pdf. Accessed 1st August, 2017.

https://malariaworld.org/blog/glucose-and-malaria. 08th March, 2018

https://www.cellbiolabs.com/lipid-peroxidation. Accessed 1st August,2017.

https://www.public.iastate,edu/duahn/teaching/lipidoxidation/measurementoflipid-peroxidation.pdf 02. 06th June, 2017

Idro, R., Marsh, K., John, C.C., Newton, C.R., (2010). Cerebral malaria:mechanisms of brain injury and strategies for improved neurocognitive outcome. *Pediatri Res.* **63:**267-274.

Kayode, O.T., Kayode, A.A.A. and Awonuga, O.O. (2011). Status of selected hematological and biochemical parameters in malaria and malaria-typhoid co-infection. *J. Biol. Sci.* **11(5):** 367-373.

Khatib, Y., Patel, R., Sequeira, K., Agrawal, G., and Chikhale, N. (2015). Hematological and biochemical alterations in malaria and their correlation with parasitic index. *Iosr Jr. Pharmacy.* **5(9):** 53-56.

Kim, M., Oldham, R.D. and Bowen, P.E. (1998). Oxidative stress in critical care: Is antioxidant supplementation beneficial?. *Journal of the American Dietetic Association*. **98(8):** 1002-1007.

Kochar, D.K., Singh, P., Agarwal P., Kochar, S.K., Pokharna, R. Sareen, P.K. (2003). Malaria/Hepatitis. *JAPI.* **(51)**:1069-1072.

Koram, K.A. and Molyneux, M.E. (2007). When is malaria? The different burdens of malaria infection, malaria disease, and malaria-like illnesses. *American Journal of Tropical Medicine and Hygiene* **77:**1–5.

Krishna, S., Waller, D.W., Ter Kuile, F., Campbell, V. and Lackritz, E.M. (1994). Lactic acidosis and hypoglycemia in children with complicated malaria: pathophysiological and prognostic significance. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88:**67–73.

Kulkani, A.G., Suryakar, A.N., Sardeshmukh, A.S., and Rathi, D.B., (2003). Studies on biochemical changes with special reference to oxidant and antioxidant in malaria patients. *Indian J. Clin. Biochem.***18**:136-149.

Kumar, S.S.S., Bhargavi, M.V. and Palaniandaran, S. (2015), Significance of haematological parameters as a diagnostic test for malaria patients with acute febrite illness. *Int. J. Pharm. Bio.* **6(4):** B 868 – B874.

Lackritz, E.M., Campbell, C.C., Ruebush, T.K., Aminu, C., Peray, T. (1992). Effect of blood transfusion on survival among children in a Kenyan hospital. *Lancet* **340:**524–528.

Looareesuwan, S., Merry, A.H., Phillips, R.E. (1987). Reduced erythrocyte survival following clearance of malarial parasitemia in Thai patients. *British Journal of Haematology* **67**:473–478.

Maina, R., Walsh, S.D., Gaddy C., Hongo, G., Waitumbi, J., Otieno, L., Jones, D., Ogutu, B.R. (2009). Impact of Plasmodium falaparum infection on haematological parameters in children living in Western Kenya. *Malaria Journal*. **9 (suppl 3):**54.

Maitland, K., Pamba, A., Newton, C.R., Lowe, B. and Levin, M. (2004). Hypokalemia in children with severe *falciparum* malaria. *Pediatric Critical Care Medicine* **5:**81–85.

Marsh, K., Forster, D., Waruiru, C., Melia, T. and Kruus, Y. (1995). Indicators of life threatening malaria in African children. *New England Journal of Medicine.* **332**:1399–1404.

Metzger, A., Mukasa, G., Shankar, A.H., Ndeezi, G., Melikan, G. and Semba, R. (2001). Antioxidant status and acute malaria in children in Kampala, Uganda. *Am. J. Trop. Med.* **65(2):** 115-119.

Modiano, D., Sawadogo, A. and Pagnoni, F. (1995). Indicators of life threatening malaria. *New England Journal of Medicine.* **333:**1011.

Molyneux, M.E., Looareesuwan, S., Menzies, I.S., Borgstein, A and Brenha, B. (1989). Reduced hepatic blood flow and intestinal mal-absorption in severe *falciparum* malaria. *American Journal of Tropical Medicine and Hygiene.* **40**:470–476.

Molyneux, M.E., Taylor, T.E., Wirima, J.J. and Borgstein, A. (1989). Clinical features and prognostic indicators in paediatric cerebral malaria: a study of 131 comatose Malawian children. *Quarterly Journal of Medicine.* **71**:441–459.

Nanda, N.C., Rath, P., Acharya, J., Mishra, P. and Mishra, S.K. (2011). *Falciparum* malaria in children-a brief report of 305 patients from Rourkela, eastern India. *Indian Journal of Pediatrics.* **78:**475–477.

Narsari, N., Mohanty, C., Das, B.K., Mishra, S.P. and Prasad, R. (2012). Oxidative stress in childgnhren with complicated malaria. *J. Trop. Ped.* **58(2):** 1-4.

Neelam. W., (2016). Ascorbic acid co-administration with artesiminin based combination therapies in *falciparum* malaria. *Indian J. Med. Res.*, **143:** 539-541.

Nyirenba, S.T., Mandala, W.L., Gordon, A.M, Mastroeni, P. (2017). Immunological bases of increase susceptibility to invasive nontyphoidal Salmonella infection in children with malaria and anemia. *Microbes and infection.* **2017**:1-10.

Obonyo, C.O., Vulule, J., Akhwale, W.S. and Grobbee, D.E. (2007). In hospital morbidity and mortality due to complicated malarial anemia in western Kenya. *American Journal of Tropical Medicine and Hygiene*.**77:**23–28.

Oduro, A.R., Koram, K.A., Rogers, W., Delofou, M. and May, H. (2007). Severe *falciparum* malaria in young children of the Kassena-Nankana district of northern Ghana. *Malaria Journal.* **6**:96.

Oliver, M., Ham, K.V.D., Shio, M.T., Kassa, F.A. and Fougeray, S. (2014). Malarial pigment hemozoin and the innate inflammatory response. *Front. Immunol.* **5:**25.

Onyesom, I., Ekeanyanwu, R.C., and Achuka, N., (2010). Correlation between moderate *Plasmodium falciparum* malarial parasitemia and antioxidant vitamins in serum of infected children in South Eastern Nigeria. *Afri. J. Biochem. Res.* **4(12):** 261-264.

Osei-Djarbeng, S.N., Agyekum-Attobra, E., Nkansah, R., Saloga, D., Osei-Asante, S. and Owusu-Dapaah, G. (2015). Medicinal plants constituting antimalaria herbal preparations in the Ghana Market. *BJPR.* **5(3)**:153-162.

Pascuali, C.C., Kai, O., Lowe, B., English, M., Williams, I.N., Maitland, K., Newton, C.R.C.J, Peshu, N., Roberts, D.J. (2006). Lactate levels in complicated malaria anemia are associated with hemozoin containing neutrophils and low levels of IL-2. *Malaria Journal*. **5**:101.

Percario, S., Moreira, D.R., Gomes, B.A.Q., Ferreira, M.E.S., Conclaves, A.C.M., Laurindo, P.S.O, Vilhena, T.C., Dolabela, M.F., and Green, M.D. (2012). Oxidative stress in malaria. *Int. J. Mol. Sci.* **13:** 16346-16372.

Poschl, B., Waneesorn, J., Thekisoe, O., Chutipongvivate, S. and Karanis, P. (2010). Comparative diagnosis of malaria infections by microscopy, nested PCR, and LAMP in northern Thailand. *American Journal of Tropical Medicine and Hygiene* **83**:56–60.

Potchen, M.J., Kampondeni, S.D., Seydel, K.B., Karma, J. and Able, T. (2012). Acute brain MRI findings in 120 Malawian children with cerebral malaria: new insights into an ancient disease. *American Journal of Neuroradiology* **33**:1740–1746.

Reyburn, H., Mbatia, R., Drakeley, C., Muanda, W., Kalumba, C. and Malumba, P. (2004). Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. *British Medical Journal.* **329**:1212.

Roca-Feltrer, A., Carneiro, I. and Armstrong, J.R. (2008). Estimates of the burden of malaria morbidity in Africa in children under the age of 5 years. *Tropical Medicine and International Health.* **13**:771–783.

Sakyi, S.A., Ephraim, K.D.R., Antoh, E.O., Obirikorang, C. and Berchie, G.O. (2012). Lipid peroxidation and catalase levels among children presenting with severe *falciparum* malaria in the Sefwi Wiawso municipality, Ghana. *J.Med. Sci.* **12(5)**:141-147.

Saltman, P. (1989). Oxidative stress: a radical review. *Seminars Haematol*. **26**: 249-256.

Sasi, P., Burns, S.P., Waruiru, C., Pue., A and Axel., H.M. (2007). Metabolic acidosis and other determinants of hemoglobin-oxygen dissociation in severe childhood *Plasmodium falciparum* malaria. *American Journal of Tropical Medicine and Hygiene* .**77**:256–260.

Schellenberg. D., Schellenberg. J., Schellenberg, T., Mushi, A. and Xui, M. (2003). The silent burden of anemia in Tanzanian children: a community-based study. *Bulletin of the World Health Organization* **81**:581–590.

Solomon, T., Felix, J.M., Samuel, M., Kylian, M. and Riya, S. (1994). Hypoglycemia in paediatric admissions in Mozambique. *Lancet.* **343**:149–150.

Stacpoole, P.W., Wright, E.C., Baumgartner, T.G., Bersin, R.M., Buchalter, S., Curry, S.H.. Jackinson, S. (1994). Natural history and course of acquired lactic acidosis in adults. DCA-Lactic Acidosis Study Group. *Am. J. Med*. **97**:47–54.

Standard Treatment Guidelines (2004), National Drugs Programme, Malaria Section,Yamen Press Ltd., Accra Ghana, West Africa. Retrieved from https://collections.infocollections.org/whocountry/en/d/Js6861e/15.5.html

Stefferl, A., Hopkins, S.J., Rothwell, N.J., and Luheshi, N.G., (1996). The role of TNF-α in fever opposing actions of human an dmurine TNF- α and interactions with IL-β in the rat. *B.J. Pharm*. **118(8)**:1919-1924.

Steketee, R.W., Wirima, J.J., Slutsker, L., Khoromana, C.O., Heyman, P.. Bay. C. (1996). Malaria parasite infection during pregnancy and at delivery in mother,

placenta, and newborn: efficacy of chloroquine and mefloquine in rural Malawi. *American Journal of Tropical Medicine and Hygiene.* **55:**24–32.

Taylor, T.E., Borgstein, A. and Molyneux, M.E. (1993). Acid-base status in paediatric *Plasmodium falciparum* malaria. *Quar. Jour. Med* .**86**:99–109.

Taylor, T.E., Fu, W.J., Carr, R.A., Whitten, R.O., Muellar, J.S.. Molyneaux, M.E. (2004). Differentiating the pathologies of cerebral malaria by post-mortem parasite counts. *Nature Medicine.* **10**:143–145.

Taylor, T.E., Molyneux, M.E., Wirima, J.J., Fletcher, K.A. and Morris, K. (1988). Blood glucose levels in Malawian children before and during the administration of intravenous quinine for severe *falciparum* malaria. *New England Journal of Medicine.* **319**:1040–1047.

Trevisan, M., Browne, R., Ram, M., Muti, P., Freudenheim, J., Carosella, A.M., and Armstrong, D. (2001). Correlates of markers of oxidative status in general population. *American J. Epid.* **154(4):**348-355.

Von Seidlein, L., Olaosebikan. R., Hendriksen, I.C.,Lee, S.J., Adedoyin, O.T.. White, N.J. (2012). Predicting the clinical outcome of severe *falciparum* malaria in African children: findings from a large randomized trial. *Clinical Infectious Diseases.* **54**:1080–1090.

Waller, D., Krishna, S., Crawley, J., Miller, K., Nosten, F.. Peace. P. (1995). Clinical features and outcome of complicated malaria in Gambian children. *Clinical Infectious Diseases.* **21**:577–587.

White, N.J., Miller, K.D., Brown, J., Marsh, K. and Greenwood, B. (1987) .Prognostic value of CSF lactate in cerebral malaria. *Lancet.* **1**:1261.

White, N.J., Miller, K.D., Marsh, K., Warrel, D.A, Seepi, K.. Moan. J. (1987). Hypoglycemia in African children with complicated malaria. *Lancet.* **1**:708–711.

World Health Organisation. (2012). Annual Report on Malaria: Tropical Medicine and International Health.

World Health Organisation. (2014). Tropical Medicine and International Health. July 2014. Wiley and Sons. **19**:2.

World Health Organisation. (2015). Cooperated Strategy. Pg. 29.

World Health Organisation. (2015). Malaria fact sheet for Ghana

World Health Organisation. (2015). Malaria Key Facts. Pp. 2-8.

World Health Organization. (2000). Communicable diseases cluster. *Trans. R. Soc. Trop. Med. Hyg*. **94**: 1-90.

www.againstmalaria.com/Distribution.aspx?proposalID=42. 26th June, 2016.
www.ghanahealthservice.org/ghanamalariabulletin. 07th August, 2017.
www.kumasi.climatemps.com/precipitation.php. 26th June, 2016.

APPENDIX I

ETHICAL CLEARANCE APPROVAL

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES / KOMFO ANOKYE TEACHING HOSPITAL
COMMITTEE ON HUMAN RESEARCH, PUBLICATION AND ETHICS

Ref: CHRPE/AP/078/17

24th January, 2017

Mr. Bahaah Bernard
Department of Biochemistry
and Biotechnology
KNUST-KUMASI.

Dear Sir,

LETTER OF APPROVAL

Protocol Title: ***"Epidemiological, Haematological and Biochemical Features Complicated and Uncomplicated Malaria Infection in Ghanaian Children."***

Proposed sites: ***Paediatric Emergency Unit, -Komfo Anokye Teaching Hospital, MCHH, Manhyia District Hospital and KNUST Hospital.***

Sponsor: ***Principal Investigator.***

Your submission to the Committee on Human Research, Publications and Ethics on the above named protocol refers.

The Committee reviewed the following documents:

- Notification letters of 16th December, 2016 and 13th December, 2016 from the Komfo Anokye Teaching Hospital and Maternal and Child Health Hospital respectfully (study sites) indicating approval for the conduct of the study in the Hospitals.
- Notification letters from the Department of Biochemistry and Biotechnology seeking permission from Manhyia District Hospital and KNUST Hospital (study sites) and were duly approved.
- A Completed CHRPE Application Form.
- Participant Information Leaflet and Consent Form.
- Research Protocol.
- Questionnaire.

The Committee has considered the ethical merit of your submission and approved the protocol. The approval is for a fixed period of one year, beginning 24th January 2017 to 23rd January, 2018 renewable thereafter. The Committee may however, suspend or withdraw ethical approval at any time if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the Committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at the close of the project, whichever one comes first. It should also be informed of any publication arising from the study.

Yours faithfully,

Osomfo Prof. Sir J. W. Acheampong MD, FWACP
Chairman

Room 7 Block J, School of Medical Sciences, KNUST, University Post Office, Kumasi, Ghana
Phone: +233 3220 63248 Mobile: +233 20 5453785 Email: chrpe.knust.kath@gmail.com / chrpe@knust.edu.gh

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

Private Mail Bag
University Post Office
Kumasi, Ghana

Loc: Aboagye Menyeh Complex, Room No. GF4

Our Ref: BC/R/3/Vol.2

Date: 7th November, 2016

The Head
Manhyia District Hospital
Manhyia – Kumasi

LETTER OF INTRODUCTION: Mr. Bahaah Bernard

This is to affirm that Mr. Bahaah Bernard is a second year MPhil Biochemistry student of this Department.

Mr. Bahaah Bernard wants to involve the subjects in your facility to undertake his research project titled: **"Epidemiological, Haematological and Biochemical Features in complicated and uncomplicated malaria infection in Ghanaian children."**

The study will not be harmful to the subjects. Any courtesies extended to him would be appreciated.

Thank you.

H.D. Zakpaa (PhD)
Head of Department

GHANA HEALTH SERVICE
RECEIVED
DATE 14/11/16
MANHYIA DISTRICT HOSPITAL

1479

meet Supt and permission
15/11/16

Adm
permission granted!
16/11/16

Tel: 233 3220 60298 / Email: biochem@knust.edu.gh / bbt.sci@gmail.com / Fax: 233 3220 60298/60137 / Website: biochemistry.knust.edu.g

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

Loc: Aboagye Menyeh Complex, Room No. GF4

Private Mail Bag
University Post Office
Kumasi, Ghana

BC/R/3/Vol.2

Our Ref:.....

7th November, 2016

Date:.....

RECEIVED 14/11/16
DEPARTMENT OF CHILD HEALTH
SMS-KATH 3:41 pm

The Head
Child Health Department
Komfo Anokye Teaching Hospital
Bantama – Kumasi

LETTER OF INTRODUCTION: Mr. Bahaah Bernard

This is to affirm that Mr. Bahaah Bernard is a second year MPhil Biochemistry student of this Department.

Mr. Bahaah Bernard wants to involve the subjects in your facility to undertake his research project titled: **"Epidemiological, Haematological and Biochemical Features in complicated and uncomplicated malaria infection in Ghanaian children."**

The study will not be harmful to the subjects. Any courtesies extended to him would be appreciated.

Thank you.

H.D. Zakpaa (PhD)
Head of Department

(3) Contact Student to talk to Dr Sylvia HOD-PEL.

(2) Seen 17/11/16 Can the Researcher first of all clarify where he intends to carry out his work so it can be forwarded to appropriate place. There exist no such unit as "the Child Health Clinic of the Okomfo Anokye Teaching Hospital" There is the General Specialist CR and several Special clinics. There is also the Polyclinic which is under Family Health (OS Hod)

I have had a discussion with Researcher & clear that he needs primary care cases (Polyclinic → Family Medicine) and complicated malaria cases (CPED - Prof Awuku) kindly advise!

(1) Seen and golden to contribute. Please communicate promptly at PEL

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF SCIENCE
DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY
Private Mail Bag
University Post Office
Kumasi, Ghana
Loc: Aboagye Menyeh Complex, Room No. GF4

Our Ref: BC/R/3/Vol.2 Date: 13th March, 2017

SUNTRE HOSPITAL RECEIVED 15 MAR 2017 GHANA HEALTH SERVICE-KUMASI

The Medical Superintendent
Suntreso Government Hospital
Suntreso

LETTER OF INTRODUCTION: MR. BAHAAH BERNARD

This is to introduce Mr. Bahaah Bernard, a second year MPhil Biochemistry student of this Department.

Mr. Bahaah Bernard wants to involve the subjects in your facility to undertake his research project titled: **"Epidemiological, haematological and biochemical features in complicated and uncomplicated malaria infection in Ghanaian children."**

The study will not be harmful to the subjects. Any courtesies extended to him would be appreciated.

Thank you.

H.D. Zakpaa (PhD)
Head of Department

16/03/17
attn. Admin
pls Consent letter
& Ethics.

ccc
for your assistance
pb
15/3/17

Tel: +233 3220 60298 / Email: biochem@knust.edu.gh / bbt.sci@gmail.com / Fax: 233 3220 60298/60137 / Website: biochemistry.knust.edu.gh

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF SCIENCE
DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

Private Mail Bag
University Post Office
Kumasi, Ghana

Loc: Aboagye Menyeh Complex, Room No. GF4

Our Ref:..... Date:.....

BC/R/3/Vol.2 11th November, 2016

The Medical Director
KNUST Hospital
Kumasi

LETTER OF INTRODUCTION: Mr. Bahaah Bernard

This is to affirm that Mr. Bahaah Bernard is a second year MPhil Biochemistry student of this Department.

Mr. Bahaah Bernard wants to involve the subjects in your facility to undertake his research project titled: **"Epidemiological, Haematological and Biochemical Features in complicated and uncomplicated malaria infection in Ghanaian children."**

The study will not be harmful to the subjects. Any courtesies extended to him would be appreciated.

Thank you.

H.D. Zakpaa (PhD)
Head of Department

Lab. Technologist on Duty
Pls assist the bearer of this note.
Thanks
Lab. II C
Approved
14/11/16

Fax: 233 3220 60298/60137 / Website: biochemistry.knust.edu.g

In case of the reply the number
And the date of this letter
should be quoted

My Ref. No: MCHH/ADM-13
Your Ref. No:
E-mail:

GHANA HEALTH SERVICE
MATERNAL & CHILD HEALTH HOSPITAL
P. O. BOX 16
KUMASI

Your Health · Our Concern

13th December, 2016

THE HEAD OF DEPARTMENT
BIOCHEMISTRY AND BIOTECHNOLOGY
KNUST.

RE: INTRODUCTORY LETTER

With reference to your letter BC/R/3/ VOl. 2 dated 7th November, 2016. I wish to inform you that management of Maternal and Child Health Hospital has granted approval for Mr. Bahaah Bernard to conduct research on the project: (**Epidemiological, Hematological** and **Biochemical Features** in **Complicated** and **Uncomplicated Malaria Infection** in **Ghanaian Children**) in our facility.

This letter is issued to assist in the acquisition of ethical clearance for the research.

Hoping for a fruitful collaboration.

Thank you.
Yours faithfully,

DR. KOFI BUAH BOSQUE-HAMILTON
MEDICAL SUPERINTENDENT

APPENDIX 2

DATA COLLECTION TOOLS

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

I am a student of KNUST, pursuing an MPhil in Biochemistry. I am conducting research on the topic **"Epidemiological, Heamatological and Biochemical Features in Complicated and Uncomplicated Malaria in Ghanaian Children"**. This is to enable me partially fulfill the academic requirement for the award of MPhil Degree. **Participation is voluntary and all responses will be treated with confidentiality.** Thank you.

INSTRUCTION: Please circle or tick (√) in the right bracket provided and write where applicable.

DEMOGRAPHIC DATA

AGE:

SEX:

COMMUNITY:

1. EDUCATIONAL LEVEL OF PARENT/GUARDIAN:

A. NONE
B. BASIC
C. JUNIOR HIGH
D. SENIOR HIGH
E. TERTIARY
F. OTHERS *PLEASE SPECIFY*……

2. FREQUENCY OF INCOME PAYMENT

A. DAILY

B. WEEKLY

C. MONTHLY

D. NON-REGULAR

E. OTHER

3. IF DAILY OR NON-REGULAR, INCOME LEVEL PER DAY (GHc)

A. BELOW 5

B. BETWEEN 5 AND 10

C. BETWEEN 10 AND 25

D. BETWEEN 26 AND 50

E. ABOVE 50

4. WHEN WAS THE LAST TIME YOU TREATED YOUR WARD FOR MALARIA?

A. WITHIN THE PAST MONTH

B. WITHIN THE PAST SIX MONTHS

C. WITHIN THE PAST YEAR

D. A YEAR OR MORE AGO

5. WHAT SYMPTOMS PROMPTED YOU TO CONCLUDE THAT IT WAS MALARIA?

A. HEADACHE

B. HIGH BODY TEMPERATURE

C. GENERAL BODY PAINS AND WEAKNESS

D. SHIVERISHNESS

E. LOSS OF APPETITE

F. YELLOWISH URINE

G. OTHERS ***PLEASE SPECIFY*** …….

6. WHAT WAS THE OPTION OF TREATMENT USED

A. HOSPITAL /CLINIC

B. SELF-MEDICATION THROUGH A PHARMACY

C. SELF-MEDICATION USING A HERBAL PREPARATION

D. NO TREATMENT PROVIDED, RESOLVED ON ITS OWN

7. WHAT DRUG(S) WAS /WERE USED FOR THE TREATMENT? DOSAGE?

A. CHLOROQUINE

B. ALAXIN

C. ARTESUNATE

D. ARTEMETHER

E. ARTENSUATE/AMODOAQUINE

F. PARACETAMOL

G. LONART

H. COARTEM

I. OTHERS (*PLEASE SPECIFY)*……..

8. WHEN DID YOU STOP THE TREATMENT?

A. WHEN THE DRUGS GOT FINISHED

B.WHEN SYMPTOMS DISAPPEARED

C. WHEN THE DRUGS GOT MISSING

D. WHEN I COULD NO LONGER AFFORD THE DRUGS

9. WAS THERE A RECURRENCE OF THE DISEASE SHORTLY AFTERWARDS?

YES ☐ NO ☐

10. WHAT IS/ ARE YOUR SOURCE(S) OF INFORMATION ON MALARIA?

A. NEWSPAPERS

B. TELEVISION

C. RADIO

D. PUBLIC HEALTH AGENCY

E. VERBAL FROM FAMILY OR FRIENDS

F. OTHERS *PLEASE*

SPECIFY……

11. WHAT MALARIA PREVENTION METHOD(S) DO YOU PRACTICE?

A. INSECTICIDE -TREATED NETS

B. MOSQUITO COILS

C. REPELLANTS

D. PROPHYLATIC DRUGS

E. INSECTICIDE SPRAYS

F. OTHERS (*PLEASE SPECIFY*)…….

12. WHAT METHODS DO YOU THINK ARE THE MOST EFFECTIVE?

A. INSECTICIDE -TREATED NETS

B. MOSQUITO COILS

C. REPELLANTS

D. PROPHYLATIC DRUGS

E. INSECTICIDE SPRAYS

F. OTHERS (*PLEASE SPECIFY*)…….

13. WHY DON'T YOU USE THIS METHOD

A. MONETARY CONSTRAINTS

B. DIFFICULTY IN USE

C. DISLIKE OF THE USE OF CUTANEOUS CHEMICALS

D. PHYSICAL DISCOMFORT FOR KIDS

14. **WHAT IS THE AGE RANGE OF YOUR WARD**

A. 0-5 YRS ☐

B. 6-10 YRS ☐

C. 11-12 YRS ☐

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

I am a student of KNUST, pursuing an MPhil in Biochemistry. I am conducting research on the topic **"Epidemiological, Heamatological and Biochemical Features in Complicated and Uncomplicated Malaria in Ghanaian Children"**. This is to enable me partially fulfill the academic requirement for the award of MPhil Degree. **Participation is voluntary and all responses will be treated with confidentiality.** Thank you.

INSTRUCTION: Please circle or tick (√) the right bracket provided and write where applicable.

THIS SECTION IS TO BE FILLED BY HEALTHCARE PROFESSIONALS. KINDLY COMPLETE, TICK OR CIRCLE WHERE NECESSARY.

SEX:

NAME OF HOSPITAL:

DESIGNATION:

1. RATE THE FOLLOWING SYMPTOMS IN ORDER OF FREQUENCY TO YOU WHEN DIAGNOSING MALARIA IN CHILDREN.

FEVER, CHILLS, SWEATS, HEADACHES, NAUSEA AND VOMITTING, BODY ACHES, GENERAL MALAISE.

……

RATE THE FOLLOWING IN ORDER OF FREQUENCY DURING COMPLICATED MALARIA INFECTION INDICATING WHETHER IT IS PRESENT ALL THE TIME, RARELY PRESENT OR ABSENT DURING COMPLICATED MALARIA INFECTION IN GHANAIAN CHILDREN.

ACUTE RESPIRATORY DISTRESS, PROSTRATION, SEVERE ANEMIA, CEREBRAL MALARIA, HYPOGLYCEMIA, IMPAIRED CONSCIOUSNESS, REPEATED GENERALIZED CONVULSIONS, HYPERPYREXIA, AND THROMBOCYTOPENIA

2. ACUTE RESPIRATORY DISTRESS

A. PRESENT ALL THE TIME
B. SOMETIMES PRESENT
C. RARELY PRESENT
D. ABSENT

3. PROSTRATION

A. PRESENT ALL THE TIME
B. SOMETIMES PRESENT
C. RARELY PRESENT
D. ABSENT

4. SEVERE ANEMIA

A. PRESENT ALL THE TIME
B. SOMETIMES PRESENT
C. RARELY PRESENT
D. ABSENT

5. CEREBRAL MALARIA

A. PRESENT ALL THE TIME

B. SOMETIMES PRESENT

C. RARELY PRESENT

D. ABSENT

6. HYPOGLYCEMIA

A. PRESENT ALL THE TIME

B. SOMETIMES PRESENT

C. RARELY PRESENT

D. ABSENT

7. IMPAIRED CONSCIOUSNESS

A. PRESENT ALL THE TIME

B. SOMETIMES PRESENT

C. RARELY PRESENT

D. ABSENT

8. REPEATED GENERALIZED CONVULSIONS

A. PRESENT ALL THE TIME

B. SOMETIMES PRESENT

C. RARELY PRESENT

D. ABSENT

9. HYPERPYREXIA

A. PRESENT ALL THE TIME

B. SOMETIMES PRESENT

C. RARELY PRESENT

D. ABSENT

10. THROMBOCYTOPENIA

A. PRESENT ALL THE TIME

B. SOMETIMES PRESENT

C. RARELY PRESENT

D. ABSENT

11. WHICH OF THE ABOVE COMPLICATIONS IS ALMOST ALWAYS PRESENT DURING COMPLICATED MALARIA INFECTION IN CHILDREN?

……

12. WHICH OF THE ABOVE COMPLICATIONS IS A DISTINCTIVE FEATURE OF COMPLICATED MALARIA IN CHILDREN?

……

13. WHICH OF THE ABOVE COMPLICATIONS IS THE MOST LETHAL DURING COMPLICATED MALARIA INFECTION IN CHILDREN?

……

14. ARE THERE SOME FEATURES OR COMPLICATIONS OF COMPLICATED MALARIA THAT ARE NOT LISTED IN THE ABOVE COMPLICATIONS? KINDLY WRITE THEM IN THE SPACE PROVIDED.

……

15. WHICH OF THE ABOVE COMPLICATIONS OR FEATURES MOSTLY LEADS TO MISDIAGNOSES OF COMPLICATED MALARIA IN CHILDREN?

……

16. ANY OTHER SUGGESTIONS, OBSERVATIONS, FEATURES OR COMPLICATIONS OF COMPLICATED MALARIA CAN BE WRITTEN BELOW.

……

……

17. WHAT STANDARDS DO YOU FOLLOW IN DETERMINING IF A CHILD HAS COMPLICATED MALARIA INFECTION?

A. WHO 2014 STANDARD

B. OTHERS (*PLEASE SPECIFY)*…….