ENSIGN COLLEGE OF PUBLIC HEALTH, KPONG EASTERN REGION, GHANA

CLINICAL AND LABORATORY FINDINGS IN CHILDREN PRESENTING WITH CEREBRAL MALARIA TO FIVE REFERRAL HOSPITALS IN GREATER ACCRA, GHANA 2012-2016

BY

PATIENCE OKANTEY

A Thesis submitted to the Department of Community Health in the Faculty of Public Health in partial fulfillment of the requirements for the degree

MASTER OF PUBLIC HEALTH

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DECLARATION

I declare that this submission is my own work towards the MPH and that to the best of my knowledge it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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DEDICATION

This thesis is dedicated to my wonderful children Nelly and Ivan. You have been a great inspiration to me.

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ABBREVIATIONS

ACT……Arteminsinin-based Combination Therapy

ADP……Adenosine Diphosphate

BCS…….Blantyre Coma Scale

CBC…….Complete Blood Count

CM……...Cerebral Malaria

CNS…….Central Nervous System

EDTA……..Ethylenediaminetetraacetic acid

ELISA…….Enzyme-Linked Immunosorbent Assay

GDP……Gross Domestic Product

GHS……Ghana Health Service

HCT……Haematocrit

iRBC……infected Red Blood Cells

ITN……..Insecticide Treated Net

LEKMA……...Ledzokuku Krowor Municipal Assembly

LGH…….La General Hospital

MCH……Mean Corpuscular Haemoglobin

MCHC……..Mean Corpuscular Haemoglobin content

NMIMR……Noguchi Memorial Institute for Medical Research

NTS……..Non Typhoidal Salmonella

OPD……..Out Patient Department

PCR……...Polymerase Chain Reaction

PML……..Princess Marie Louis

RBC……..Red Blood Cell

RBM…….Roll Back Malaria

SMA……..Severe Malaria Anaemia

TGH……..Tema General Hospital

WBC…….White Blood Cell

WCMC……..Weill Cornell Medical College

WHO…….World Health Organization

ABSTRACT

Introduction

Cerebral malaria is the severest form of malaria. About a fifth (20%) of children who get malaria develop cerebral malaria. An improved understanding of the clinical manifestation and mediators of cerebral malaria can form the basis of improved therapeutic options. This study aimed to describe the demographic and clinical profile of children presenting with cerebral malaria to five referral facilities in the Greater Accra Region of Ghana from 2012-2016.

Methods

Data was obtained from children presenting with cerebral malaria at five referral hospitals in the Greater Accra Region. Cerebral malaria was diagnosed on the basis of changes in mental status and or coma accompanied by malaria parasitaemia. The analysis aimed to describe the clinical presentations and explored how degree of parasitaemia was related to clinical presentation. The study was nested within a large study that is investigating the immunological markers of severe malaria.

Results

Eight-three (65% males) children with cerebral malaria were enrolled. Their mean age was 5.5 (standard deviation-SD=2.8) years. While most (62.7%) children presented with temperatures above 37.5^0C, all (100%) presented with a Blantyre Coma Score of less than 3. Convulsion was present in 53% of cases. About half (49%) of patients who presented unconscious only regained consciousness 24 to 48 hours after arrival. The mean levels of parasitaemia and haemoglobin level were 36113.3(SD=77747.4)/uL and 8.9 (SD=1.8) g/dl respectively. Thrombocytopenia (54.2%) and moderate anaemia (32.5%) were the major laboratory findings. Bacterial coinfection was

present in only 2.4% of cases. The case fatality rate was 7.23%. Of all the explored clinical parameters at time of presentation, only duration of fever was significantly associated with degree of parasitaemia (P-value=0.02).

Conclusion

There is a male preponderance among cases presenting with cerebral malaria. The majority of cases present acute conditions and require emergency care. The lack of association between degree of parasitaemia and features of clinical presentation make a case for the continued investigation of other mediators of cerebral malaria.

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CHAPTER 1

INTRODUCTION

1.1 Background

Malaria has been known since millennia and yet it still remains a major public health problem in most tropical countries. An estimated 300 to 500 million individuals are affected every year with nearly half of these people dying of the infection annually (English et al., 2004). Out of about 500 million clinical malaria cases that are reported each year, one percent of these cases may become complicated and develop into severe malaria (Idro et al., 2010). In some cases, however, the disease becomes so severe and may lead to death. It has also been shown that malaria is the cause of nearly a quarter of all childhood deaths (Miller et al., 2013). In 2015, malaria alone was found to have resulted in about 438 000 under-five deaths globally with 85% of cases occurring in African children (WHO, 2015). Several intervention measures such as effective chemotherapy through the use of Arteminsinin-based Combination Therapy (ACTs), chemoprevention, use of insecticide treated nets, indoor residual spraying and rapid diagnosis adapted by the World Health Organization have seen a 48% reduction in mortality since 2000 (WHO, 2015). However the disease still poses enormous burden on mankind especially in sub-Saharan Africa where at least one in five childhood deaths can be attributed to malaria.

In Ghana, malaria has been reported as the major cause of poverty and low productivity, accounting for about 32.5 % of all OPD attendance. Children under five years account for 48.8% of all admissions in the major health facilities in the country (WHO, 2015). It is generally believed that malaria is the cause of the highest loss of number of days of healthy life in Ghana although reliable information on the impact of malaria on labour productivity and the economy is absent (Asenso-Okyere & Dzator, 1997).

Malaria is known to be caused by five species of plasmodium namely *P. falciparum, P. vivax, P. ovale, P. malarie* and *P. knowlesi*. These parasites are transmitted by the female anopheles mosquitoes, which act as vectors of the disease. *P. falciparum* is known to be the deadliest of the five species and it is responsible for about 91% all the mortality from malaria with the majority (86%) occurring in the African region (WHO, 2014).

Malaria can occur in less than two weeks in non-immune individuals after a mosquito bite and severe malaria may develop if not treated after 24hours of onset of symptoms (WHO, 2005). *P. falciparum* is the only species that is known to directly affect the central nervous system causing neurological deficits and cognitive sequelae, making cerebral malaria the deadliest form of severe malaria. Of the millions of children affected, an estimated 20 - 50% of the cases develop cerebral malaria and most deaths occur in the first 24 hours of hospitalization (English et al., 2004). Cerebral malaria collectively involves the clinical manifestations of *P. falciparum* malaria that induces changes in mental status and coma, a deep level of unconsciousness (Ozen et al., 2006). Cerebral malaria is therefore considered a medical emergency demanding urgent clinical assessment and treatment.

Between 5% and 30% of children who survive cerebral malaria have some neurological sequelae, which may take the form of cerebellar ataxia, hemiparesis, speech disorders, cortical blindness, behavioural disturbances, hypotonia or generalized spasticity. Epilepsy is a sequel that develops in up to 10% of children, usually not until several weeks or months after the initial illness (WHO, 2012).

Initially, cerebral malaria in children may present with fever (37.5–41°C), followed by poor oral intake of food and water. Vomiting and cough are also common. The history of symptoms preceding coma may be brief, usually 1 or 2 days. Other signs such as moderate hypotension

(systolic blood pressure 70–80mm Hg) has been observed in 10% of children with cerebral malaria (WHO, 2012). Even though rare, some studies have reported severe shock (systolic blood pressure < 50mm Hg), in about 2% of cases of severe malaria.

Low platelet count, referred to, as thrombocytopenia is the most common laboratory abnormality, followed by high bilirubin levels, hyperbilirubinemia, anaemia and elevated hepatic aminotransferase levels. The leukocyte count is usually normal or low, but neutrophilia with a marked increase in band forms (left shift) is present in the majority of cases. The erythrocyte sedimentation rate, C-reactive protein, and procalcitonin are almost invariably elevated. The severity of malaria corresponds to the degree of the laboratory abnormalities (Doherty et al., 1995).

It has been suggested that children with *Plasmodium falciparum* malaria are at risk of invasive bacterial infection (IBI). Blood stream infection, largely secondary to enteric Gram negative organisms (EGNOs), with a predominance of non-typhoidal salmonella (NTS) species, has been widely reported as a complication of severe malaria (Church & Maitland, 2014).

However, it remains uncertain whether malaria infection is a risk factor for invasive bacterial disease since the majority of children in malaria-endemic Africa are frequently infected by P. falciparum throughout childhood and only a minority will develop severe disease. A sub-analysis within a comprehensive systematic review of blood stream infections in Africa indicated that 6.5% of 11,814 malaria infections had concomitant bacteremia. Which children are at greatest risk of developing dual infection and whether this extends across the clinical spectrum (asymptomatic, mild and severe) remains unclear (Reddy et al., 2010)

One of the criteria that has been used for decades to describe severe malaria is hyper-parasitaemia (WHO, 2012). Several studies have seen as association between the parasite burden and common prognostic factors for severe malaria (Tangpukdee et al. 2012; McMaster et al. 2009; Ekvall 2003)

1.2 Problem statement

Severe anaemia, cerebral malaria, and metabolic acidosis are considered the three major clinical manifestations in severe childhood malaria. However, there is a remarkable shortage of clinical description of severe malaria in different endemic regions. The disease pattern and the relative contribution of individual symptoms to mortality differ with endemicity, geographic location, access to health services, and age, among other factors (Imbert et al., 1997).

Additionally, data on the prevalence of invasive bacterial infection in malaria among children is limited. Such information is important to health workers on how frequently antibacterial therapy may be indicated in children presenting with nonfocal febrile illness and malaria parasitaemia. However, there is growing body of evidence that bacterial bloodstream infections may account for hospital admissions with fever and a higher proportion of child deaths. Studies have shown that malaria deaths in children with bacteraemia are rapid (Berkley et al., 2005). Previous prospective studies have reported bacteremia due to a diversity of organisms in 5%–8% of African children with severe malaria (Enwere et al., 1998). These studies mainly included children with cerebral malaria (CM). A consistent association has been suggested between bacteremia due to non-typhoidal *Salmonellae* (NTS) and severe malarial anemia (SMA)(Brent et al., 2006) but no prospective study has reported the prevalence of bacteremia in children with cerebral malaria.

There is growing evidence that the parasite burden has an effect on the common prognostic factors of cerebral malaria (Tangpukdee et al., 2012). Different malaria parasites burden exhibited

important distinctive hematological parameters such as leukocyte and platelet counts (Kotepui et al. 2015).

1.3 Significance of the study

Cerebral malaria is a life-threatening disease that represents a global health problem particularly in tropical countries. The considerable morbidity and mortality in falciparum malaria is due to its protean manifestations, multi-organ involvement, delay in diagnosis and failure of administration of treatment promptly and adequately (Tamez et al., 2008). Given the necessary emphasis on the clinical assessment of children with severe malaria in resource-poor settings, such clinical indices might play a role in patient triage and may also reflect the underlying pathophysiology of the disease. Hence an evaluation of the demographic, clinical and laboratory profile is appropriate in this context. Also, a better understanding of the common causes of bacteraemia in children presenting with cerebral malaria could help guide antibiotic management in resource-poor regions, in which both infections are common, blood culture is usually unavailable, and antibiotic choice is limited (Reddy et al., 2010).

As it has been shown that clinical description of cerebral malaria differs in different endemic regions, knowledge on the prognostic factors of cerebral malaria patients in the Greater Accra region of Ghana will also help in a quicker identification, enhance treatment and improve clinical outcome.

1.4 Hypotheses

1. Study describes the demographic factors, clinical manifestations and laboratory findings in patients with cerebral malaria.
2. The study hypothesized that; parasitaemia significantly influences presenting clinical manifestations and laboratory findings in children with cerebral malaria.

1.5 Objectives

1.5.1 Main objective

To describe the clinical presentation of cerebral malaria and related complications among children in five health facilities in Accra, Ghana.

1.5.2 Specific Objectives

1. To describe the demographic characteristics of children presenting with cerebral malaria
2. To evaluate the clinical and lab presentation of children with cerebral malaria
3. To determine prevalence of bacterial co-infection in children with cerebral malaria
4. To assess the effect of parasite burden on clinical and laboratory findings

CHAPTER 2

LITERATURE REVIEW

2.1 Malaria Burden

The World Malaria Report of 2015 shows that approximately 3.2 billion people were at risk of malaria around the world and 214 million cases are estimated to have occurred. Africa alone accounts for 88% of malaria cases and 90% of malaria deaths (WHO, 2015). In 2014, malaria alone caused an estimated 306 000 under-five deaths globally and an estimated 292 000 African children died before their fifth birthday due to malaria in the same year (WHO, 2015). More than 90 countries are known to have ongoing malaria transmission and hence malaria is considered a public health problem (WHO, 2015). Despite the considerable public health efforts directed at treatment and control worldwide, malaria remains the most important parasitic disease globally with almost one-quarter of the world‘s population at risk (Hay et al., 2009).

In Ghana, there have been some giant strides after the implementation of the National Malaria Control Programme with a drop in malaria cases over the last 15 years. Whilst there was a drop in under-five case fatality from 2.8 per cent in 2011 to 0.6 per cent in 2012 and overall prevalence from 11.4 million in 2013 to 8.4 million in 2014 (GHS, 2016), there are still gaps in achieving the planned targets .

2.1.1 Socio-economic burden

Malaria situation remains high (between 25-65 death rate per 100,00 population) (Figure 2.1), with a prevalence of 67% of households reporting an episode of malaria every two weeks (Musah, 2013). It causes serious negative effects on health and lays an enormous economic and social burden on individuals, families, communities and societies in the poorest countries of the world

and has thus been labeled as a disease of poverty (Karunamoorthi, 2012). Studies have shown that repeated absenteeism at work places and farms in endemic regions is primarily caused by malaria and this results in short and long term losses in productivity as the main transmission periods coincide with the peak agricultural and harvesting seasons (Karunamoorthi & Bekele, 2009).

Jobs and school days are lost in regions where malaria thrives and productivity plummets and entire communities remain locked in an unbreakable cycle of disease and poverty (RBM, 2003). When it does not kill, the disease can lead to permanent neurological and cognitive damage in children, thus impeding education, reducing career opportunities and lowering productivity in adult age. The direct and indirect costs of malaria have been shown to be a major constraint to economic development with the direct costs being a combination of personal and public expenditures on both prevention and treatment of the disease.

At the micro level the personal expenditures include individual or family spending on insecticide-treated nets (ITNs), doctors' fees, anti-malarials, transport to health facilities and support for the patient and an accompanying family member during hospital stays (RBM, 2003). At the macro level the economic burden of malaria is estimated at an annual reduction in economic growth of 1.3% for those African countries with the highest burden (WHO, 2009). An estimated 12 billion USD loss to the African continent's Gross Domestic Product (GDP) annually as a result of malaria (RMB, 2013).

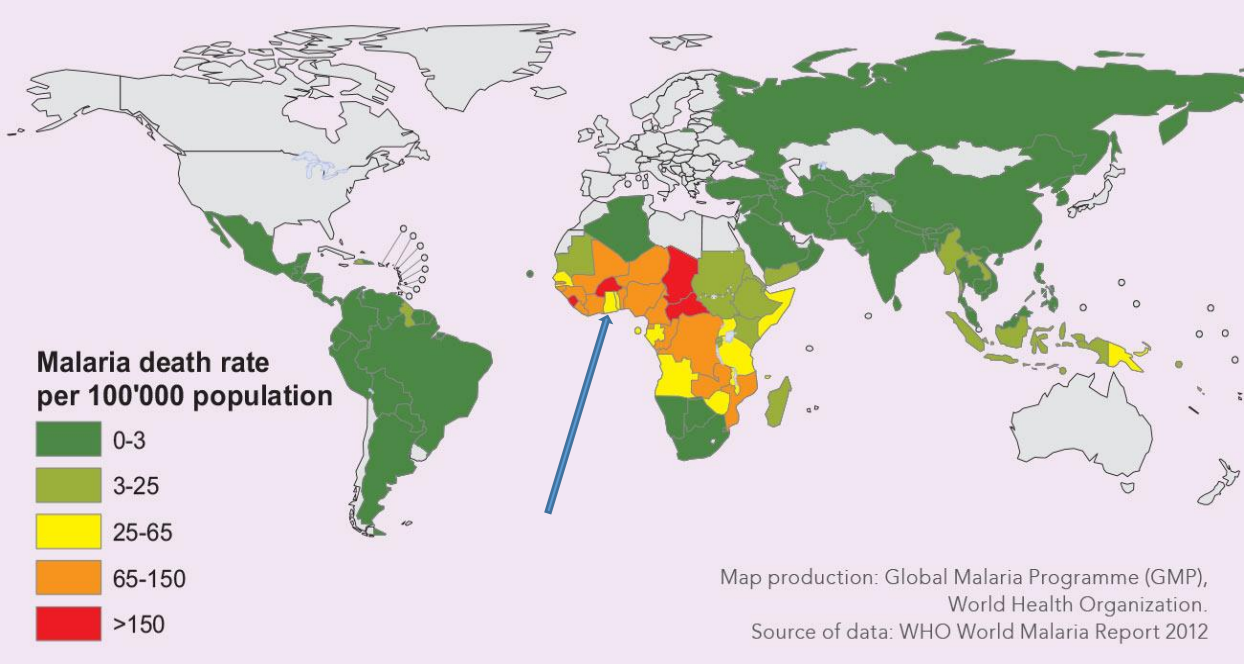


Figure 2.1 Malaria death rate per 100,000 population

2.2 Malaria parasite

Malaria is caused by infection of red blood cells by the protozoan parasite *Plasmodium.* There are five species of *Plasmodium* that infect humans (*falciparum*, *vivax*, *ovale*, *malariae* and *knowlesi*). These are transmitted by over 30 species of Anopheles mosquitoes. *P. falciparum* is known to be the most deadly among the five species and results in a number of different pathologies associated with specific organ systems. It is responsible for almost all the mortality from malaria and is the only species that appears to directly affect the central nervous system (CNS) causing neurologic deficits and cognitive sequelae. *Plasmodium falciparum* is also known to be the predominant species in sub-Saharan Africa where over 90% of all malaria deaths occur (WHO, 2012).

2.3 Malaria in children

Despite several breakthroughs in understanding the biology of the malaria parasite, including the sequencing of the *Plasmodium falciparum* genome (Gardner et al., 2002) and efforts to eradicate the mosquito vector through widespread insecticide campaigns, people (mostly children) are still dying from malaria. In the year 2000, malaria accounted for 18.0% of child deaths in sub-Saharan Africa, including populations not exposed to malaria (Rowe et al., 2006). The majority of malarial deaths occur in children under 5 years of age, as non-sterile immunity develops with increasing age and recurrent exposure to malaria (Marsh, 1992). However, it remains unclear why some children develop the severe manifestations of disease while others suffer only mild symptoms or remain asymptomatic (Rowe et al., 1995). Often, death may arise from acute episodes of severe falciparum malaria, severe anaemia resulting from repeated malaria infections and indirectly through increased susceptibility of child to other infections resulting from malaria infection (Nosten et al., 2004).

2.3.1 Severe malaria in children

When a child is inoculated with a plasmodium parasite, a number of clinical effects may arise. These may follow the sequence; infection, asymptomatic parasitaemia, uncomplicated illness, severe malaria and subsequently death. Many factors influence the disease manifestations and the likelihood of progression to the last two categories. These factors include the species of the infecting parasite, the levels of innate and acquired immunity of the host, and the timing and efficacy of treatment, if any.

A study by Idro et al., 2005, shows that in areas of intense malaria transmission, infection and clinical disease are rare in children up to age 6 months and in the few cases, symptoms are mild as a result of passive immunity from maternal antibodies. The study also showed that the main burden

of severe malaria is in infants in the first 2 years of their lives, and by age 4 years clinical disease is rare and typically mild whiles in areas with less intense transmission, the peak incidence of severe disease falls at a later age (Idro et al., 2005).

Almost all cases that progress to severe malaria are due to *P. falciparum*. In a patient with asexual *P. falciparum* parasitaemia and no other confirmed cause for their symptoms or signs, the presence of one or more of the clinical or laboratory features as stated below classifies that patient as suffering from severe malaria: Prostration/coma, respiratory distress, shock, jaundice, haemoglobinuria, severe anaemia (Hb <5g/dl). A child who requires parenteral treatment due to persistent vomiting may also be considered as having severe malaria (WHO, 2016) .

2.3.2 Severe malaria pathogenesis

Severe malaria develops due to the fact that parasites sequester themselves in various organs including heart, lung, brain, liver, kidney, subcutaneous tissues and placenta (Miller et al., 2002). It has been shown that one or more of these organs can be affected with different levels of severity and this can be classified as neurologic and renal dysfunction, haematologic, cardiovascular, and respiratory dysfunction, as well as hepatic and metabolic dysfunction depending on the organ affected (Mohapatra & Das, 2009).

These may be due to the fact that, during the malaria cycle, iRBCs circulating in the blood stream begin to lose their deformability (MacPherson et al., 1985) thus becoming potential targets for filtering and destruction by the spleen. To avoid this, the parasite expresses and exports adhesive proteins to the surface of the host iRBC, causing the cell to stick to microvascular endothelial cells in different organs, thereby preventing its clearance by the spleen. Available evidence suggests that organ dysfunction and severe pathology follow the accumulation of iRBCs at high density in particular organs (MacPherson et al., 1985).

2.3.3 Clinical manifestation of severe malaria

Severe malaria can mimic many other diseases that are also common in malaria-endemic countries. The most important of these are central nervous system infections, septicaemia, severe pneumonia and typhoid fever. The frequent presentations of severe *falciparum* malaria include cerebral malaria, metabolic malaria (hyperlactaemia, acidosis or respiratory distress) and severe anaemia (Miller et al., 2013). Most often than not, seizures, impaired consciousness, or metabolic acidosis presenting as respiratory distress or severe anaemia are usual manifestations of severe *falciparum* malaria in African children growing up in areas where malaria is endemic. African children rarely develop renal failure or pulmonary oedema (MacPherson et al., 1985).

2.3.4 Cerebral malaria

2.3.4.1 Cerebral malaria burden

Most malaria-related deaths are associated with cerebral malaria (CM) which is arguably one of the most common non-traumatic encephalopathies in the world and remains a major cause of morbidity (Mishra and Newton, 2009). Cerebral malaria is considered the most severe form of malaria and is caused by infection with *P. falciparum* parasites (Miller et al., 2002). *Plasmodium falciparum* parasite is responsible for almost all the neurological complications associated with malaria, although *P. vivax* causes seizures in children, and is also associated with coma in both children and adults (Mishra and Newton, 2009). It is also considered one of the most dangerous diseases due to the fact that up to 30% of patients who develop cerebral malaria can die (Adams et al., 2002). This severe form of malaria primarily affects young children who develop a potentially rapidly reversible encephalopathy. Peak incidence is recorded in preschool children where approximately 575000 children are affected with cerebral malaria annually (Breman, 2001). A study conducted by Gyan et al., 2009, showed that the mean age of cerebral malaria patients in Accra, was 5.2 years with young males being more affected (60%). In another study in the northern

part of Ghana, cerebral malaria was common in children 25–60 months old with approximately equal proportion of males and females (Oduro et al., 2007). An earlier study on brain swelling in Kenyan children with cerebral malaria also had more males affected (57%) (J C Newton et al., 1994).

2.3.4.2 Cerebral malaria pathogenesis

The clinical hallmark of cerebral malaria is coma and this collectively involves the clinical manifestations of *P. falciparum* malaria that induces changes in mental status (Idro et al., 2005). The commonly accepted clinical definition of CM is the neurological syndrome with patients in unarousable coma (Newton et al., 1990). The World Health Organization also defines cerebral malaria as a clinical syndrome characterized by coma at least 1 hour after termination of a seizure or correction of hypoglycemia, asexual forms of *Plasmodium falciparum* parasites on peripheral blood smears and no other cause to explain the coma (WHO, 2012) .

In African children, cerebral malaria can occur in less than two weeks after a mosquito bite and coma develops suddenly with seizure onset often after 1–3 days of fever (Rénia et al., 2012). A few children develop coma after progressive weakness and prostration (Idro et al., 2010).

Without treatment, cerebral malaria is invariably fatal. In children, parenteral anti-malarials (cinchonoids or artemisinin derivatives) are indicated, but even with this treatment, 15– 20% die (Idro et al., 2010). Although highly effective anti-malarial drugs are widely available, CM case fatality remains 15-20% globally. If a person is not treated, CM is fatal in 24 - 72 hours (Babikir, 2010)**.**

Earlier studies suggested that surviving patients fully recover (Muntendam et al., 1996) but over the past 20 years, it became clear that many children sustain significant brain injury; 11% are

discharged with gross neurological deficits and these may include weakness, spasticity, blindness, speech problems and epilepsy (Newton & Krishna, 1998). There is also evidence that suggests that some children who appear to have made a complete neurological recovery from cerebral malaria may develop significant cognitive problems (attention deficits, difficulty with planning and initiating tasks and language problems), which can adversely affect school performance and persist for years after the attack (Njuguna & Newton, 2004).

A key feature of the biology of *Plasmodium falciparum* is its ability to cause infected red blood cells (iRBCs) to adhere to the linings of small blood vessels. Such sequestered parasites cause considerable obstruction to tissue perfusion. In addition, in severe malaria there may be marked reductions in the deformability of uninfected RBCs (Dondorp et al., 2008).

2.3.4.3 Factors affecting prognosis of CM

Clinical presentations: Three consistent presenting complaints of cerebral malaria are fever, convulsion and loss of consciousness (Idro et al., 2010). The clinical picture of cerebral malaria is that of a diffuse encephalopathy with unarousable coma with focal signs relatively uncommon (Dondorp 2005). In young children, coma can develop rapidly, with a mean onset after only 2 days of fever, but sometimes just a few hours. The depth of coma is an important prognostic factor (Idro et al., 2005). Seizures manifesting as nystagmoid eye movements, irregular breathing, excessive salivation, and conjugate eye deviation have been reported in Kenyan children who were admitted with cerebral malaria (Crawley et al., 1996). Seizures have also been reported as repetitive and prolonged with episodes of status epilepticus and these have been associated with increased mortality (Dorovini-Zis et al., 2011). Studies have shown that, in more than 50% of pediatric cases, convulsions are mostly generalized tonic-clonic in nature but can also be Jacksonian type or focal (Islam & Rahman, 2015).

Laboratory findings: The severity of malaria has been shown to correspond to the degree of laboratory abnormalities (Trampuz et al., 2003). Laboratory findings of prognostic significance were hypoglycaemia, leukocytosis, hyperparasitaemia, elevated plasma concentrations of alanine and 5'–nucleo–tidase, and elevated plasma or cerebrospinal fluid lactate (Molyneux et al. 1989). World health organization classifies hypoglycaemia as a common and serious manifestation of cerebral malaria (WHO, 2016). Other studies have however reported hyperglycemia in cerebral malaria (Daas et al., 2010). Studies by Van Thien et al., 2001, have reported that cerebral malaria stimulates the production of glucose to a greater extent than other forms of malaria (van Thien et al., 2004). Hyperglycemia like in other critically ill patients could be a result of sepsis or a stress response to increased counter regulatory hormones.

Thrombocytopenia has been shown to be a key indicator of malaria in febrile patients (Erhart et al., 2004). Studies have reported between 56.7 - 60% of hospital admission due to cerebral malaria (Ladhani et al. 2002; D'Acremont et al. 2002; do Amaral et al. 2003).

Anaemia is considered a measure of the cumulative impact of malaria on an individual patient (Kotepui et al., 2015). Previous studies have shown anaemia in 86.7% (Amaral et al., 2003) and 30% (D'Acremont et al., 2002) in children presenting with cerebral malaria.

Factors associated with a fatal outcome in CM include deep breathing or acidosis (base excess below -8), hypotension (systolic blood pressure < 80mmHg), raised plasma creatinine (>80]tmolll), low oxygen saturation «90%), dehydration and hypoglycaemia 2.5 mmol/l) (Babikir, 2010).

Parasite burden: Hyper-parasitemia has been listed as one of the criterion of severe falciparum malaria for more than two decades (WHO, 2012). A correlation between parasite density and severity of malarial infections has been shown (Tangpukdee et al., 2012). Study by Kotepui et al.

2015, has shown that different malaria parasite burden exhibited important distinctive haematological parameters that is evident in changes in leukocyte count, platelet count and haemoglobin concentration during the infection. Similar studies also found a consistent positive relationship between leukocyte counts and parasite density in Plasmodium-infected patients (Mcmaster et al., 2005). Moreover, excessive haemolysis of parasitized RBCs in malaria infection may lead to anaemia (Ekvall, 2003).

2.4 Bacterial coinfection in children with cerebral malaria

Currently, malaria with all its life-threatening consequences is known to be co-endemic with many other diseases. In some cases, malaria may modify the process of another disease without being affected itself (Faure et al., 2015). Bacterial infection and malaria may overlap in terms of geographical distribution, age groups at greatest risk and seasonality of both diseases in the tropics (Takem et al. 2014). A positive correlation between invasive non-typhoidal disease and malaria found in a multicentre study in 13 sites (Park et al., 2016) exemplifies the need to consider other appropriate treatments in addition to malaria prevention and treatment. The prevalence of any bacteremia and of non-typhoidal *salmonellae* (NTS) was observe to be highest in children with severe malarial anaemia (11.7% and 7.6%) (Bronzan et al., 2007). There is enough epidemiological and preclinical evidence that supports the causal association between malaria and bacteraemia (Takem et al., 2014). Studies done by the Department of Pediatrics, University of Ghana Medical School, among newborns revealed neonatal bacteraemia of 22.2% with high fatality rate (Anyebuno & Newman, 1995).

Therefore, a better understanding of the common causes of bacteraemia in children presenting with severe malaria could help guide antibiotic management in resource-poor regions, in which both infections are common, blood culture is usually unavailable, and antibiotic choice is limited.

2.5 Seasonal variation of cerebral malaria

Seasonal variation is a cardinal feature of paediatric diseases in Africa. Malaria cases in general vary according to the rainfall patterns during the year. Studies have also found association between malaria transmission and rainfall patterns (Mabaso et al., 2007). A study in the northern part of Ghana showed that cerebral malaria incidence was seasonal with 71% occurring during the main raining season (Oduro et al., 2007). Other studies have shown that areas that have experienced decreased rainfall have recorded a decline in malaria transmission (Small et al., 2003). The intensity and pattern of transmission depends on the variations in altitude or rainfall, social and environmental factors and coverage of health services. In a 3-year prospective study of 9584 consecutive paediatric admissions to the Royal Victoria Hospital in Banjul, The Gambia, the impact of seasonal variation in childhood diseases was evaluated. It was found that falciparum malaria, pneumonia, gastro-enteritis and malnutrition all peaked in September to October following the rainy season. The mortality rate was also higher in the rainy season than in the dry season. Of the 1525 children with cerebral malaria, 83% were admitted during the extended rainy season from July to December (Brewster & Greenwood ,1993).

CHAPTER 3

METHODOLOGY

3.1 Study site

This descriptive study is based on secondary data collected in a longitudinal study into Severe Malaria conducted between 2012 and 2016 in five hospitals in Accra, Ghana. The hospitals are the La General, Ridge, Tema General, LEKMA (Ledzokuku Krowor Municipal Assembly) and the Princess Marie Louise hospitals. These hospitals are referral hospitals located within the Greater Accra region as seen in figure 3.1 below. They serve both poor and affluent communities around where they are located. They are all publicly-owned and under the Ghana Health Service.

Figure 3.1 Map of Greater Accra Region of Ghana showing study sites and residence of study participants

3.1.1 Princess Marie Louise (PML) Hospital

PML is Ghana's premier children's hospital located at the commercial centre of the capital city. It is dedicated to providing medical care, family planning and nutrition services. With a 74 bed capacity, PML is known to be the second largest paediatric facility in Accra (Tetteh et al., 2016). The facility provides both primary and secondary care for paediatric patients under the age of 18 years. Since its inception in 1926, it has served as a centre which caters for the needs of children suffering various effects of malnutrition and infectious diseases. In fact PML is the hospital where the terms 'kwashiorkor and marasmus' were first described by Cicely Williams in 1935 (Williams, 1935). The hospital receives Paediatric referrals from health centres, private clinics, government polyclinics and hospitals located in and around Accra including Korle Bu Teaching Hospital (largest tertiary referral unit in Ghana) with a small proportion patients being referred from outside Accra. Parents can bring their children to the hospital with or without a referral at any time (Tetteh et al., 2016). The facility also serves as a training centre for health personnel through contributions from research. Close to 200 children are treated at PML daily

3.1.2 Ledzokuku-Krowor Municipal Assembly (LEKMA) Hospital

LEKMA hospital is a government health facility established in 2010 to cater for the health needs of communities in the Ledzokuku-Krowor Municipal Assembly and beyond. The facility assists nurses-in-training and other health professional through internships and practical experience. The hospital has a 100-bed capacity that has all the units of a general hospital including special services, laboratory and radiological facilities. It also has a Malaria Research Centre and a Herbal Medicine Unit.

3.1.3 Tema General Hospital

Tema General Hospital was constructed in 1954 and is located in Tema, an industrial community that also houses the biggest harbour and the manufacturing companies in Ghana. The hospital runs 24 hour admission services with a 100 beds capacity and provides services such as specialist clinics including internal medicine, surgery, obstetrics and gynaecology, paediatrics, dental services, physiotherapy, laboratory X ray services as well as accident and medical emergencies. It serves different communities ranging from less privileged slums to the affluent communities including popular towns such as Ashaiman, Tema Newtown, Communities 1-22 and Michel Camp. The hospital is also a major referral point receiving cases from all over the coastal areas with over six hundred Out-Patient Department cases daily.

3.1.4 Ridge Hospital

Ridge Hospital is the Regional Hospital of Greater Accra Region located in the Ridge residential area of the Osu Klottey Sub-Metro. It was established in 1919 and was formerly referred to as 'European Hospital', built exclusively for the European colonial community during the Gold Coast era (Kiptoo, 2015). Until late 2016, the hospital was a 348 bed regional teaching and referral Centre in the Greater Accra Region providing general and specialized services for the population in the city and surrounding areas. The hospital acts as a regional referral point for other health facilities within the region and also serves communities including Nima, Mamobi and Accra Central which have the highest population density in the Greater Accra Region.

3.1.5 La General hospital

La General hospital was established in 1964 as a polyclinic to serve the people La, Osu and nearby communities along the coast. Like all other referral hospitals, La General also operates 24 hours

providing both accident and medical emergencies. It became a hospital in the 2004 with a bed capacity of 161. Out of the 200 OPD cases per day, 18.6% constitute malaria

3.2 Study type

This is a largely descriptive study with data from a severe malaria study in five referral Hospitals in Accra. Data from laboratory investigation were quantitatively analyzed whiles physical as well as demographic information were described to evaluate clinical presentations and complication of cerebral malaria. Demographic, physical and laboratory data on for children with confirmed cerebral malaria were available for this study.

3.3 Study population/Unit

The study was conducted in children between the ages of 1 and 12 years presenting with coma to the emergency unit of affiliated hospitals who were screened by paediatricians for cerebral malaria. Clinical data on each patient was recorded in both hospital folder and study chart/questionnaire (appendix 2). This was done between 2012 and 2016.

3.4 Sample size

Number of cases in the study was dependent on data available from a previous study carried out by the NMIMR in five hospitals in the greater Accra region. Eighty three (83) cerebral malaria cases were available for the study.

3.5 Sampling techniques

All children (1-12 years) who presented with coma to the emergency rooms of the study hospitals were screened by paediatricians using the study eligibility criteria (below). Informed consent was obtained from parents or guardians of children who qualified for the study. Recruitment of study participant was done between May, 2012 and August, 2016.

3.5.1 Specific Inclusion criteria

Children aged 1-12 years presenting with cerebral malaria i.e. unconscious with a score of ≤3 on the Blantyre Coma Scale (BCS) and having being in coma for at least 60 minutes were enrolled. Children were excluded if they had a record of recent severe head trauma or other causes of coma or neurological diseases including meningitis/encephalitis (as assessed by lumbar puncture).

Recovery from CM was defined as regaining of full consciousness (BCS of 5). The patient may or may not have neurologic sequelae, which was assessed by study physicians and any deficits were recorded and monitored for the duration of the study.

Enrolled children underwent thorough physical examination as part of routine emergency clinical care by the attending paediatricians. This included neurological assessment. A standard set of samples were taken for laboratory investigations. This included blood and cerebrospinal fluid. Along with information on the socio-demographic characteristics of enrolled children, the findings of clinical examination were recorded on specially-designed research case record forms.

3.6 Laboratory Investigations

Laboratory investigations on samples obtained were carried out in the hospitals' laboratories and in the laboratories at NMIMR. Investigations done at the Hospital laboratories were for routine and immediate care. This included blood smears for malaria parasite identification and estimation as well as complete blood counts. Results from the hospital laboratories were made available to the Clinicians and the study team. Analysis of samples for research purposes were performed at NMIMR and the procedures are described below.

3.6.1 Blood sample collection

Per routine clinical procedures, two millilitres (2ml) of venous blood was collected from each patient into EDTA tubes by trained phlebotomists for complete blood count (CBC), blood culture,

sickling test and blood smears for malaria parasite count. In addition 3ml of venous blood were collected into heparinized (1ml) and EDTA (1ml) tubes and blood culture bottles (1ml) for laboratory analysis and immunological assays. Cerebrospinal fluid (CSF) samples were obtained through lumbar puncture done by paediatricians.

3.6.2 Parasitological Evaluation

At the research laboratories at NMIMR, thick and thin blood smears were prepared from EDTA treated blood for confirmation of parasitaemia and determination of parasite densities. Blood smears were prepared according to WHO protocol. Both thick and thin films were prepared on the same slide as shown in Figure 3.2 below.

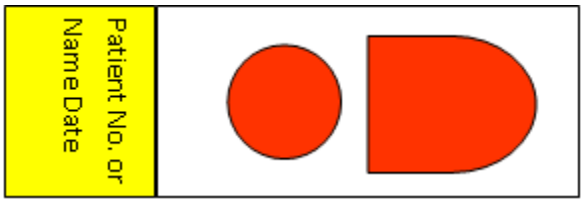


Figure 3.2 Thick and thin blood film

The thick smears; a drop of about 7µl of blood was placed at one end of a microscope glass slide and evenly spread in a circle to a diameter of about 1cm. For the thin blood film, about 4µl of blood was placed close to the middle of the microscope glass slide. A spreader slide was inclined at an angle of 45^{O} on the drop of blood. The blood was made to spread along the entire width of the spreader slide and pushed forward rapidly and smoothly. The prepared blood films were air-dried thoroughly. The thin blood film was fixed in absolute methanol for species identification. Both films were then stained with freshly prepared 10% Giemsa solution (in Phosphate buffer) and left to stain for 15 minutes, washed and examined under light microscope with immersion oil. Parasite densities were estimated using the WHO guidelines (WHO, 2010)

3.6.3 Haematological evaluation

For malaria patients recruited into the study, complete blood counts were done at the hospital laboratory on initial presentation and subsequent reviews. A haemotological analyzer was used to measure: haemoglobin levels, platelets counts, total white blood cell (WBC) counts, and total red blood cell (RBC) counts, mean corpuscular volume (MCV), haematocrit (HCT), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin content (MCHC).

3.6.4 Bacteraemia evaluation

Evaluation of bacteremia in patients was done according to (Cheesbrough, 1984). Briefly, blood sample (1ml) was obtained aseptically form each patient and added to 50ml broth medium. To reduce the risk of contamination, blood was collected from a peripheral vein by a qualified phlebotomist. Culture medium was kept in an incubator at 37^{o}C and observed daily for seven days for any signs of haemolysis, production of gas, coagulation of the broth and turbidity above the red cell layer. If there was a sign of bacterial growth, a subculture was done on solid media and examined after 24 hours for bacteria growth. However, if there was no sign of bacterial growth, the culture was examined daily for seven days.

Identification of bacteria growth was done by initially doing a Gram stain to differentiate Gram-positive from Gram-negative bacteria.

3.7 Data Management and Analysis

Data on case report forms are stored and locked at NMIMR. Each subject was given a coded non-identifying number. The coded clinical data and results of research study tests (demographic, clinical and laboratory) also captured using REDCap, web secure research data management center which was centrally-managed at Weill Cornell Medical Center (WCMC) in New York, USA. All

analyses were performed using only the coded data. The platform for data capture had in-built checks for consistency and completeness.

The data was exported to Microsoft Excel 2010 and STATA version 14 for data analysis. The analyses were largely descriptive.

3.8 Ethical considerations

Ethical approvals for data collection were obtained from the Institutional Review Board (IRB) of the Noguchi Memorial Institute for Medical Research (NMIMR IRB-CPN 030/08-09), the Ghana Health Service and Ghana Education Service (GHC-ERC 11/11/11) as well as Weill Cornell Medical College. Study participants were enrolled only after informed consent was obtained from their parents and guardians following their understanding of the objectives of the study (see appendix 1). Ethical approval for the work presented here was also obtained from the Institutional Review Board of the Ensign College of Public Health (see appendix 3).

3.9 Limitation

Since this was a retrospective study, data was limited to sample size of the original study.

CHAPTER 4

RESULTS

4.1 Background and demographics of study participants

4.1.1 Overall age and sex distribution

Eighty-three (83) children aged between 1 and 12 years who presented with cerebral malaria in the five hospitals from May 2012 to August 2016 were recruited into the study. Overall, the mean age was 5.5 (standard deviation-SD=2.8) years with 42.2% of patients aged from 5 to 8 years. There were twice as many males (65%) as females (35%) in the age groups as shown in Figure 4.1 below.

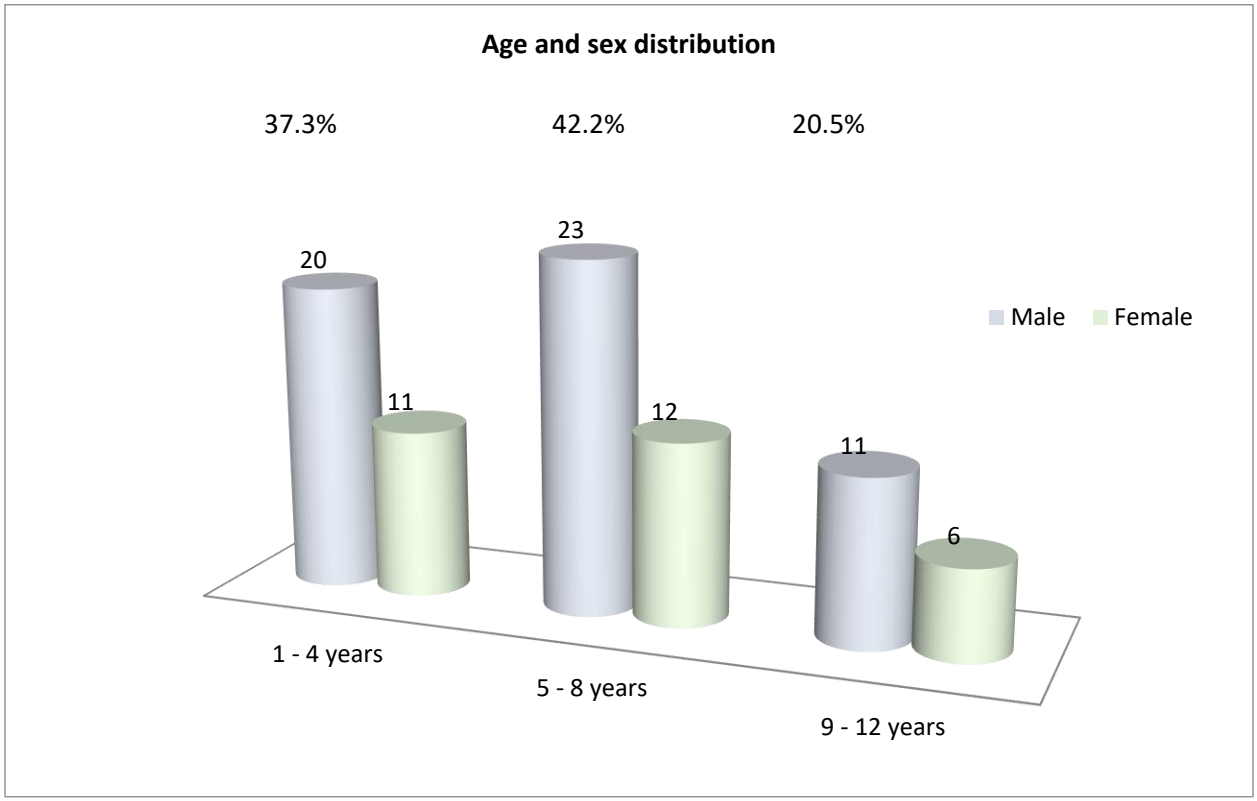


Figure 4.1 Age and sex distribution of study participants

4.1.2 Age and sex distribution per facility

Table 4.1 shows the age and the sex distribution of study participants recruitment in the different hospitals. Tema General Hospital recorded the highest number of males who had CM. The highest number of females who had CM were recorded in both PML and Tema general hospital as shown in table 4.1.

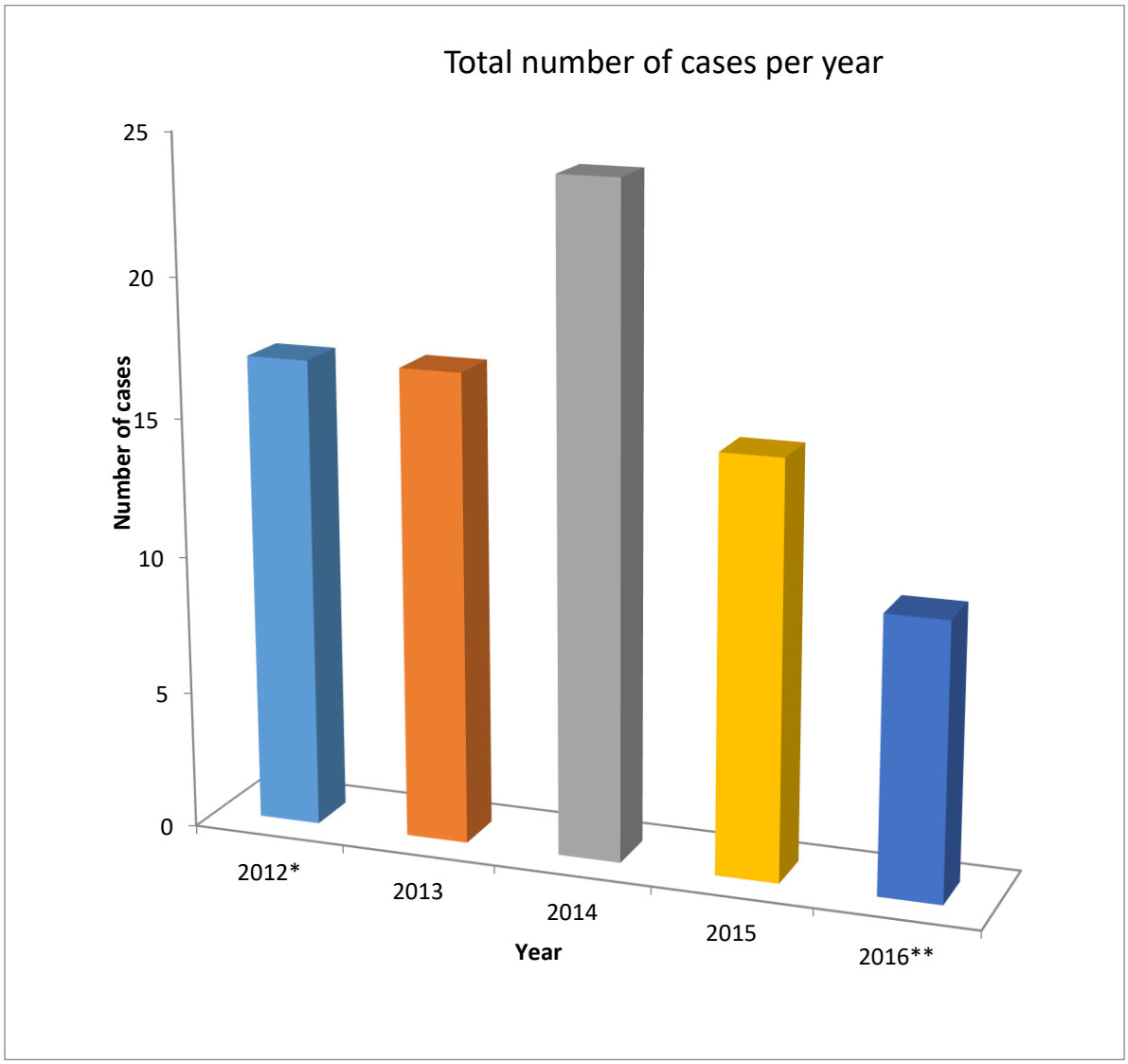
Table 4.1 Age and sex distribution of study participants per facility

		Age range (Years)			
Health Facility	**Sex**	1 - 4	5 - 8	9 - 12	**Total**
La General Hospital	Male	4	2	2	8
	Female	2	1	2	5
PML Hospital	Male	8	4	3	15
	Female	2	4	1	7
Ridge Hospital	Male	2	2	1	5
	Female	1	2	1	4
Tema General Hospital	Male	5	12	3	20
	Female	3	2	2	7
LEKMA Hospital	Male	1	4	2	7
	Female	3	2	0	5
Total		31	35	17	83

4.2 Cerebral Malaria cases

4.2.1 Cases per year

Figure 4.2 below shows total number of CM cases recorded per year over the study period. The number of CM cases peaked in 2014 and declined in 2015 and 2016.



*: recruitment between May and December **: Recruitment between January and August

Figure 4.2 Total number CM cases per year

4.2.2 Cases per facility

Figure 4.3 below shows the total number of cerebral malaria cases recorded in the five hospital between 2012 and 2016. Highest numbers of cases were recorded in 2012, 2013 and 2015 in LGH, PML and TGH respectively. No CM was recorded in LGH and Ridge hospital in 2015 and 2016

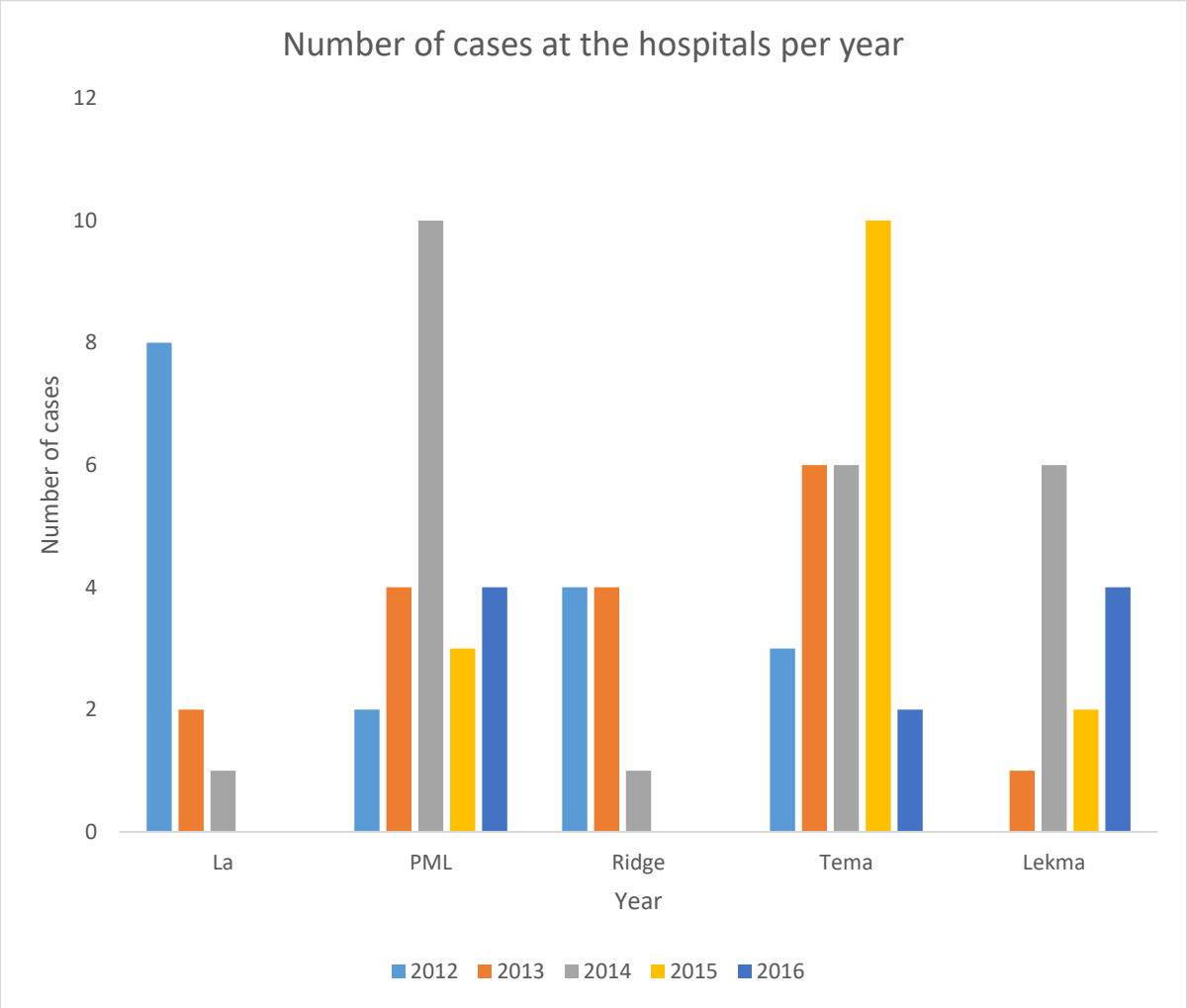


Figure 4.3 Yearly Cerebral Malaria cases per facility

4.3 Seasonal records of Cerebral Malaria

Figure 4.4 below shows the seasonal variations in the incidence of cerebral malaria between may, 2012 and August 2016. Most of the cases were recorded between May and October which coincides with the rainy season.

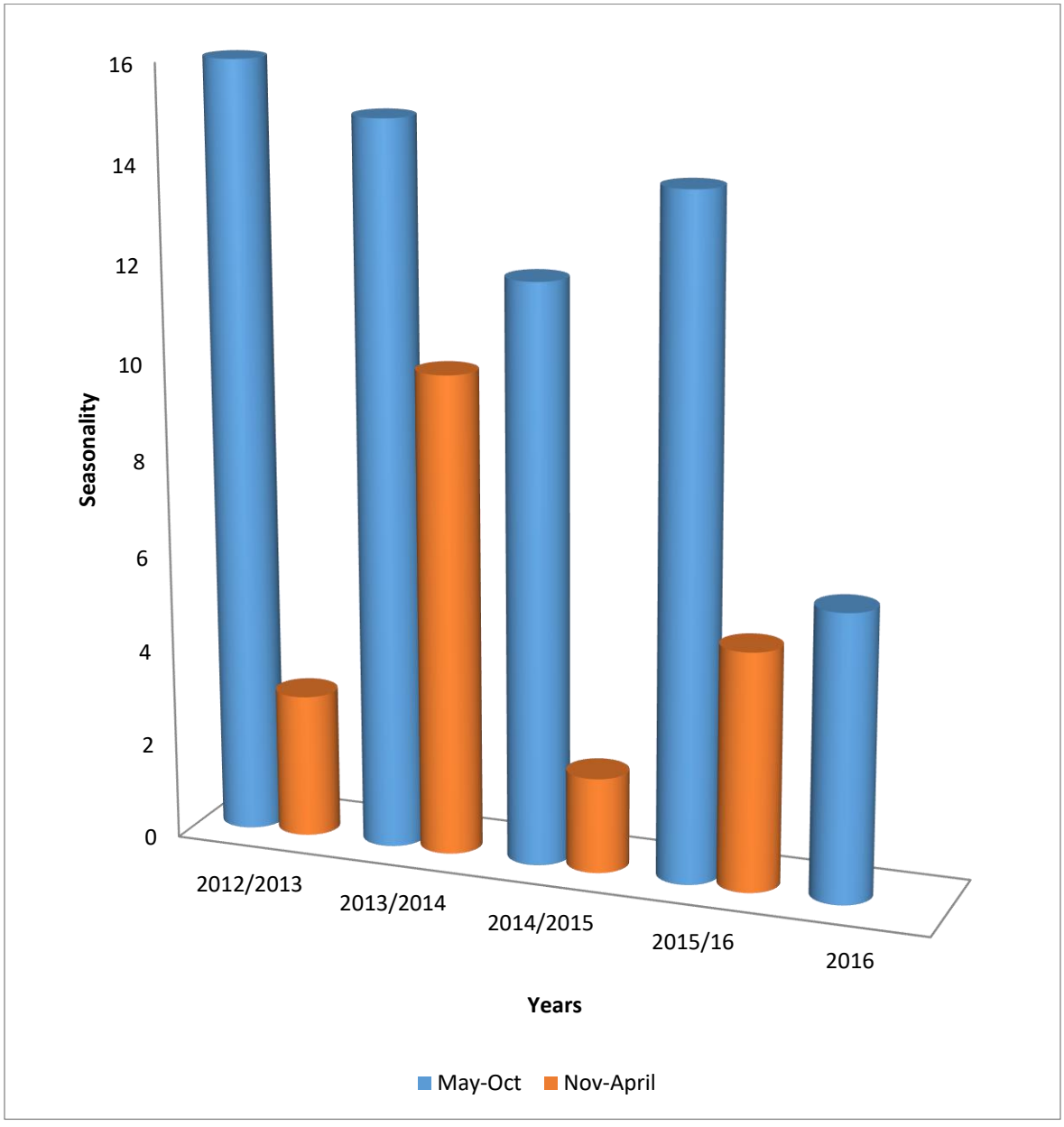


Figure 4.4 Yearly Cerebral malaria incidences in malaria and non-malaria seasons

4.4 Clinical outcome of cerebral malaria

Figure 4.5 below shows the clinical outcome of cerebral malaria cases between 2012 and 2016. Of the 83 patients in the study, majority recovered from the disease whiles 7% died.

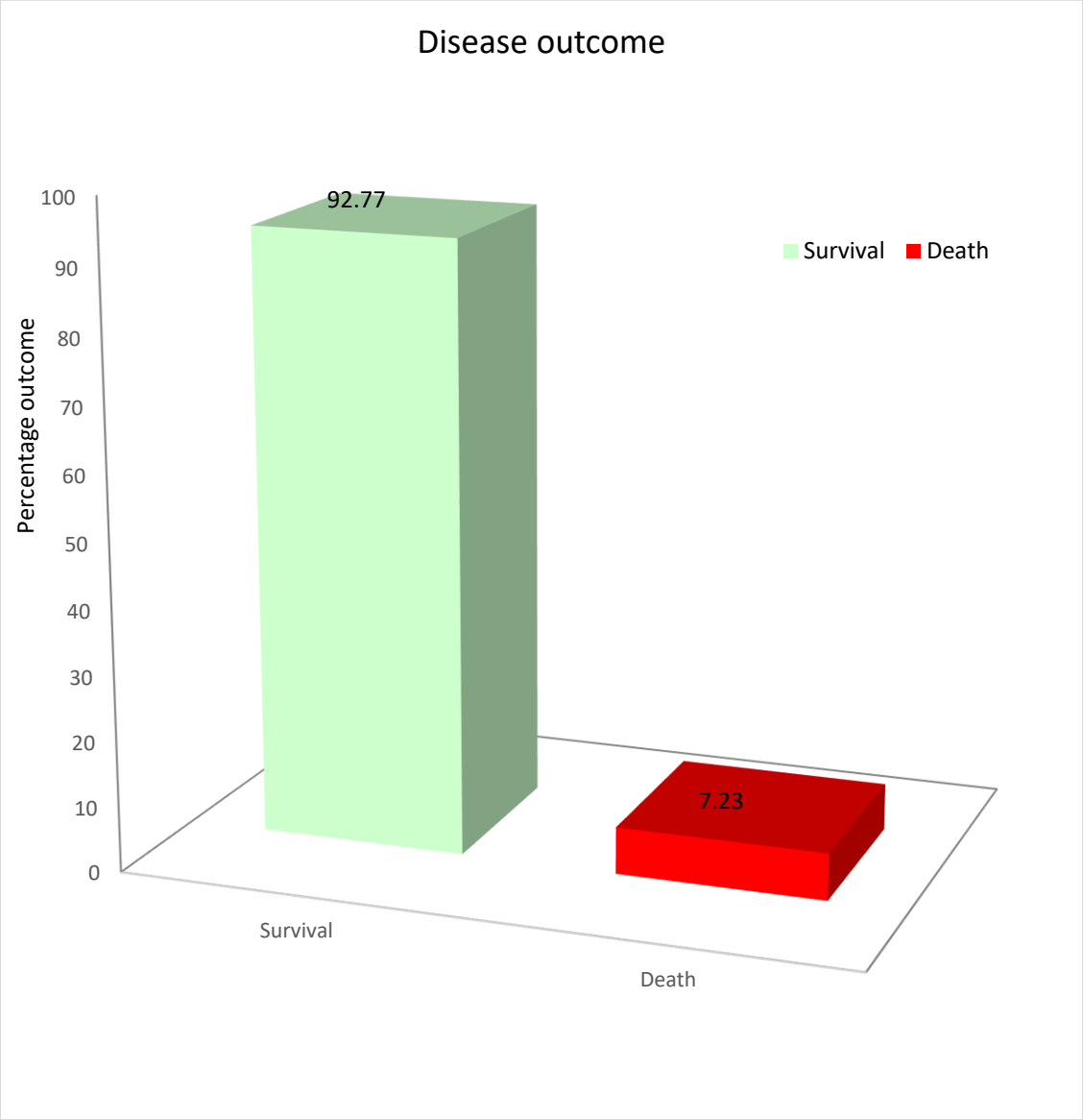


Figure 4.5 Clinical outcome of cerebral malaria

4.5 Signs and symptoms of Cerebral Malaria

4.5.1 Common presenting symptoms at initial presentation

Figure 4.6 below shows the percentage of study participant showing some common symptoms of cerebral malaria as reported by caregivers. All cerebral malaria cases had altered consciousness evidenced by Blantyre coma score of less than 3. More than half of the patients presenting with cerebral malaria also had fever.

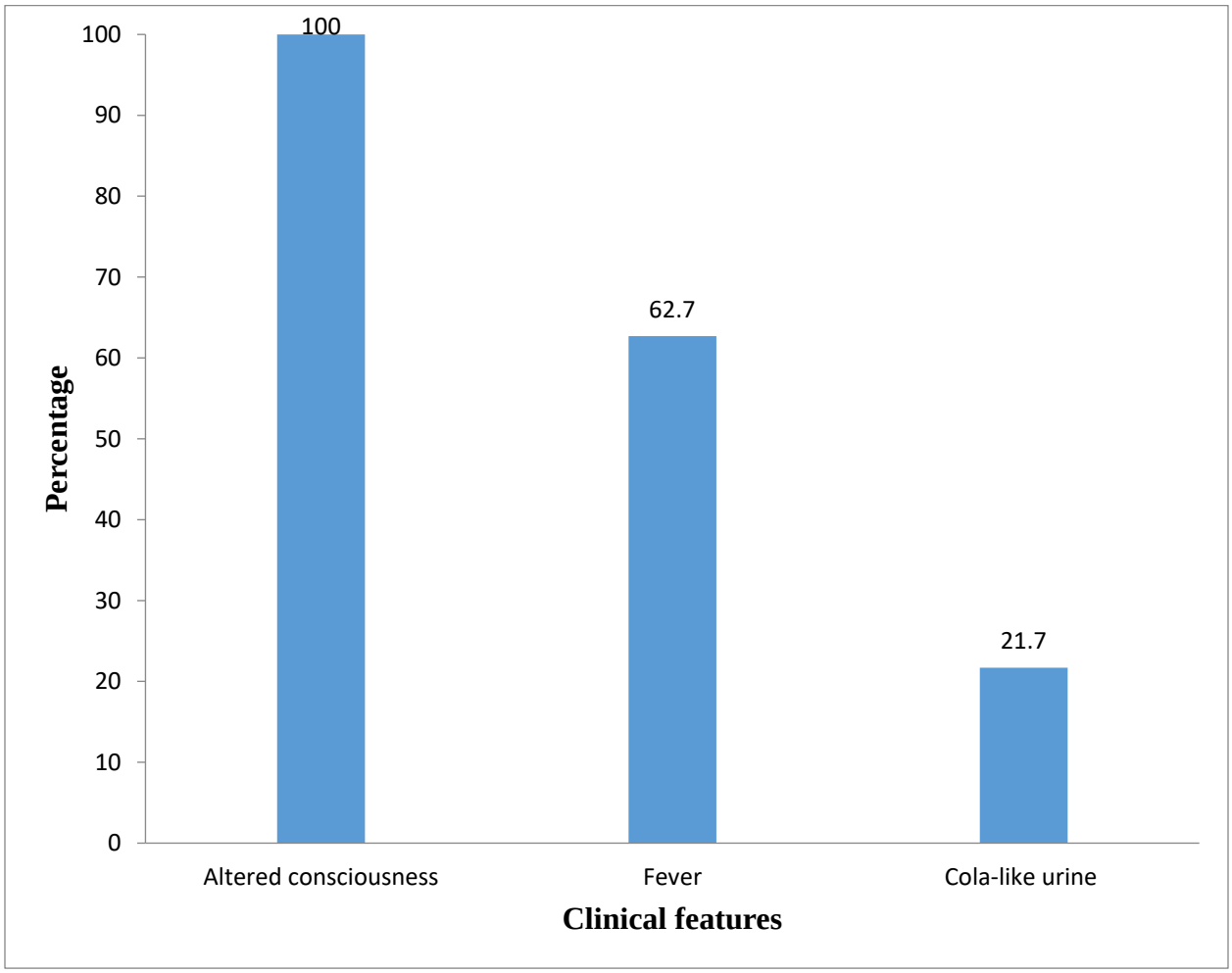


Figure 4.6 Percentage of children presenting with common symptoms of cerebral malaria

4.5.2 Common observed physical signs at initial presentation

Figure 4.7 below shows percentage of study participants showing common physical signs at initial presentation to the hospitals. Majority of the children studied had seizures.

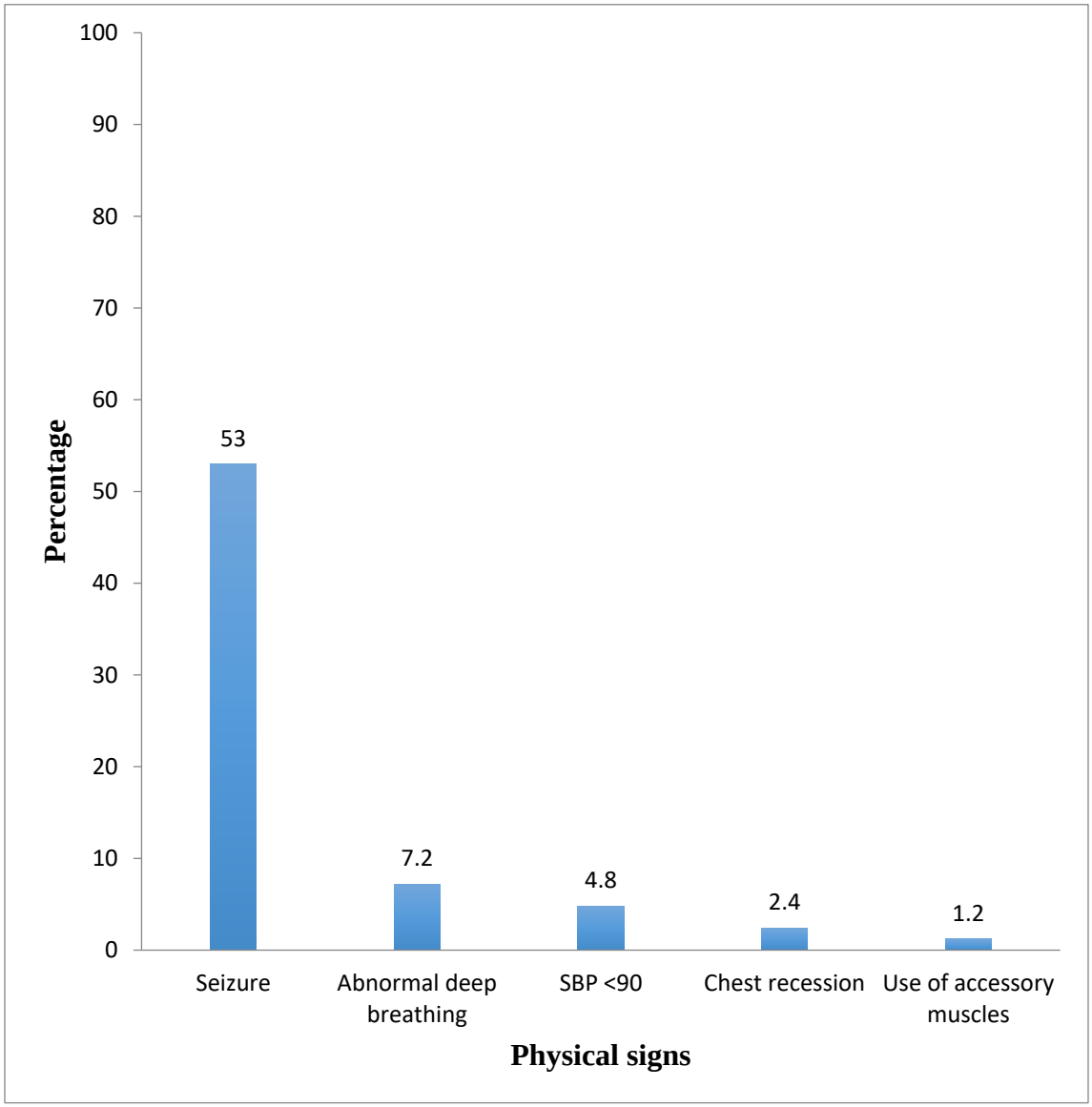


Figure 4.7 Percentage of children showing physical signs of Cerebral Malaria

4.6 Duration of coma in study participants

Figure 4.8 below shows the percentage of children and the duration of coma. Majority of the patients came recovered from coma between 24 and 48 hours.

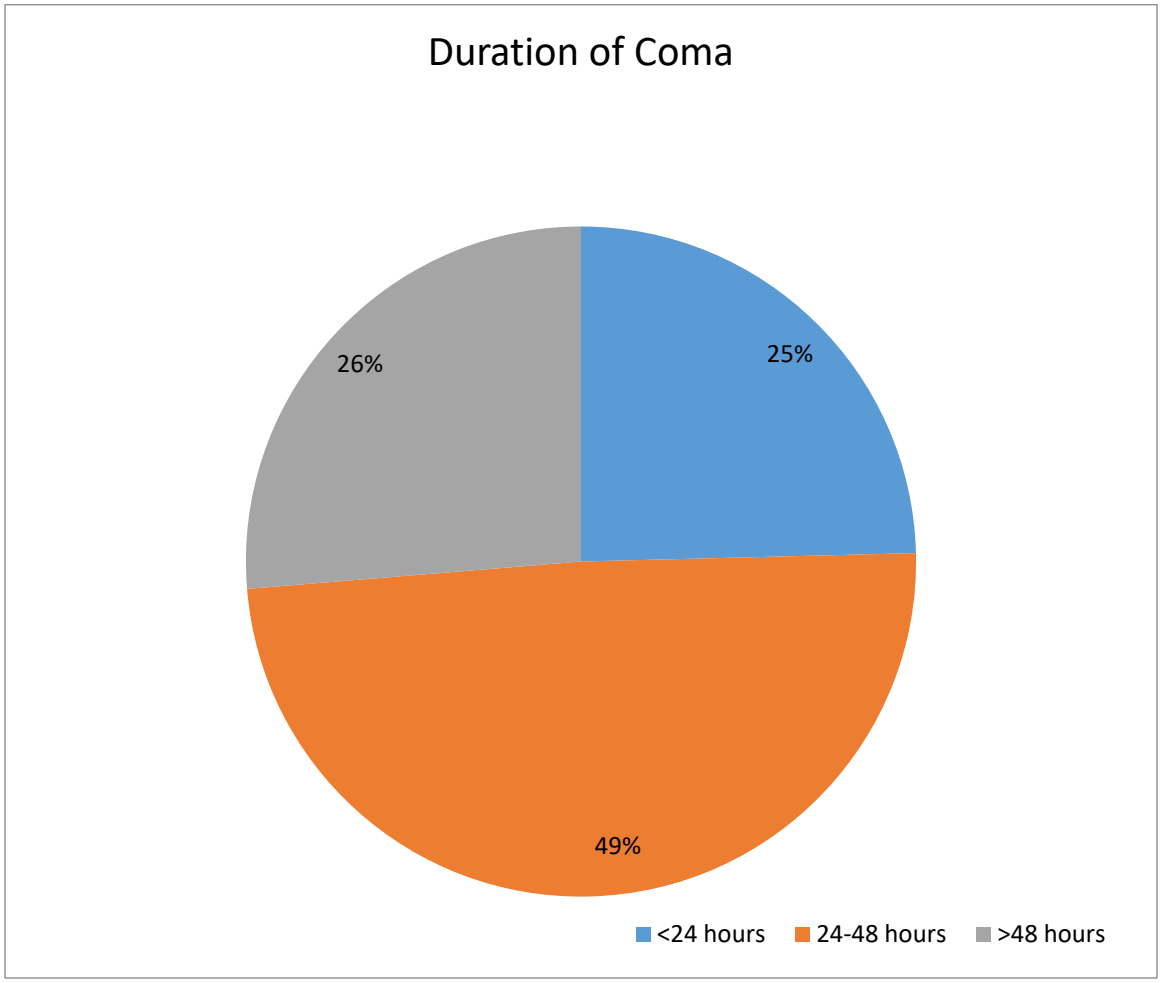


Figure 4.8 Duration of coma in study participants

4.7 Laboratory Characteristics

4.7.1 Baseline Laboratory characteristics

The below shows the baseline laboratory characteristics of patients at initial clinical presentations.

Table 4.2 Patient's baseline laboratory characteristics

Patient baseline laboratory characteristics		
	Mean ± SD	Range
N	83	
Random Blood Sugar	7.6 ± 2.7	1.6 - 13.4
Haemoglobin (g/dL)	8.9 ± 1.8	4.7 - 14.9
Platelet count ($x10^9$/L)	120.2 ± 161.2	10 - 930
WBC count ($x10^9$/L)	10.8 ± 4.8	2.8 - 33.0
Parasite density (/dL)	36113.3 ± 77747.4	16 - 312200

Data represents the mean and standard deviation as well as the range of the minimum and maximum values recorded in children presenting with cerebral malaria to five referral hospitals.

4.7.2 Clinical definitions of Laboratory findings

The following are the clinical definitions applied in the care of children in all five hospitals

Table 4.3 Clinical definitions

Haemoglobin level (Hb)	
Normal	>11g/dl
Mild anaemia	10 - 11g/dl
Moderate anaemia	7 – 9 g/dl
Severe anaemia	<7 g/dl
White Cell Count (WBC)	
Normal	4 – 10 (x 10^9 /L)
Leucopenia	< 4 x 10^9 /L
Leukocytosis	> 4 x 10^9 /L
Platelet count	
Normal	15- 400 (x 10^9 /L)
Thrombocytopenia	< 150 x 10^9 /L
Thrombocytosis	> 400 x 10^9 /L

4.7.2 Abnormal laboratory finding in study participants

Figure 4.9 below show the number of cerebral malaria cases with abnormal and normal laboratory findings at initial presentation to the hospitals. The commonest abnormal laboratory finding was thrombocytopenia (54.2%). Other abnormal findings that were observed in a significant number of cases include leukocytosis and moderate anaemia (32.5%).

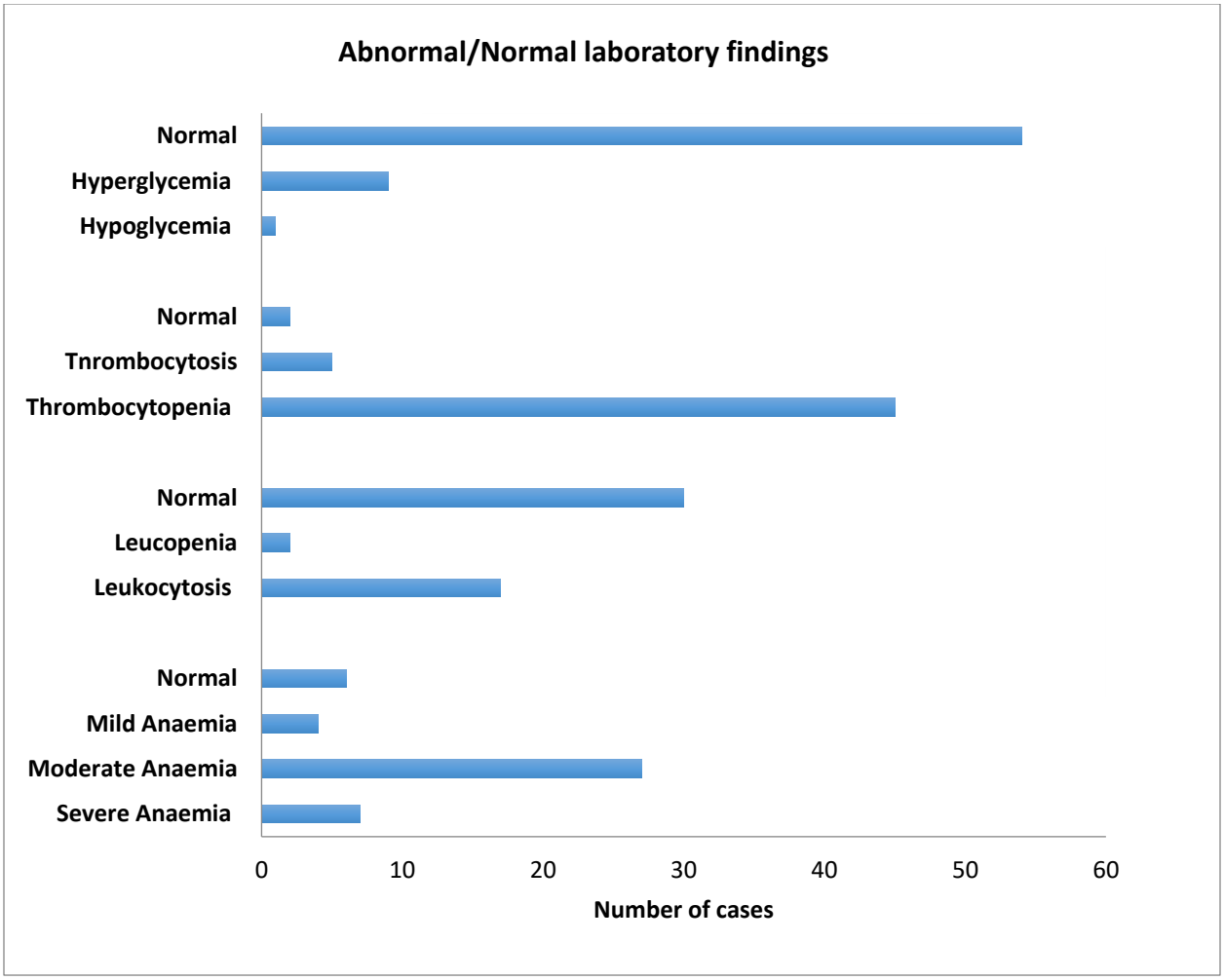


Figure 4.9 Cases showing abnormal/normal laboratory findings

4.8 Cerebral Malaria and bacterial co-infection

The majority (97.6%) of cases were single infections with malaria. Co-morbidity with bacterial infections was found in 2.4% of cases. (**Figure 4.10**)

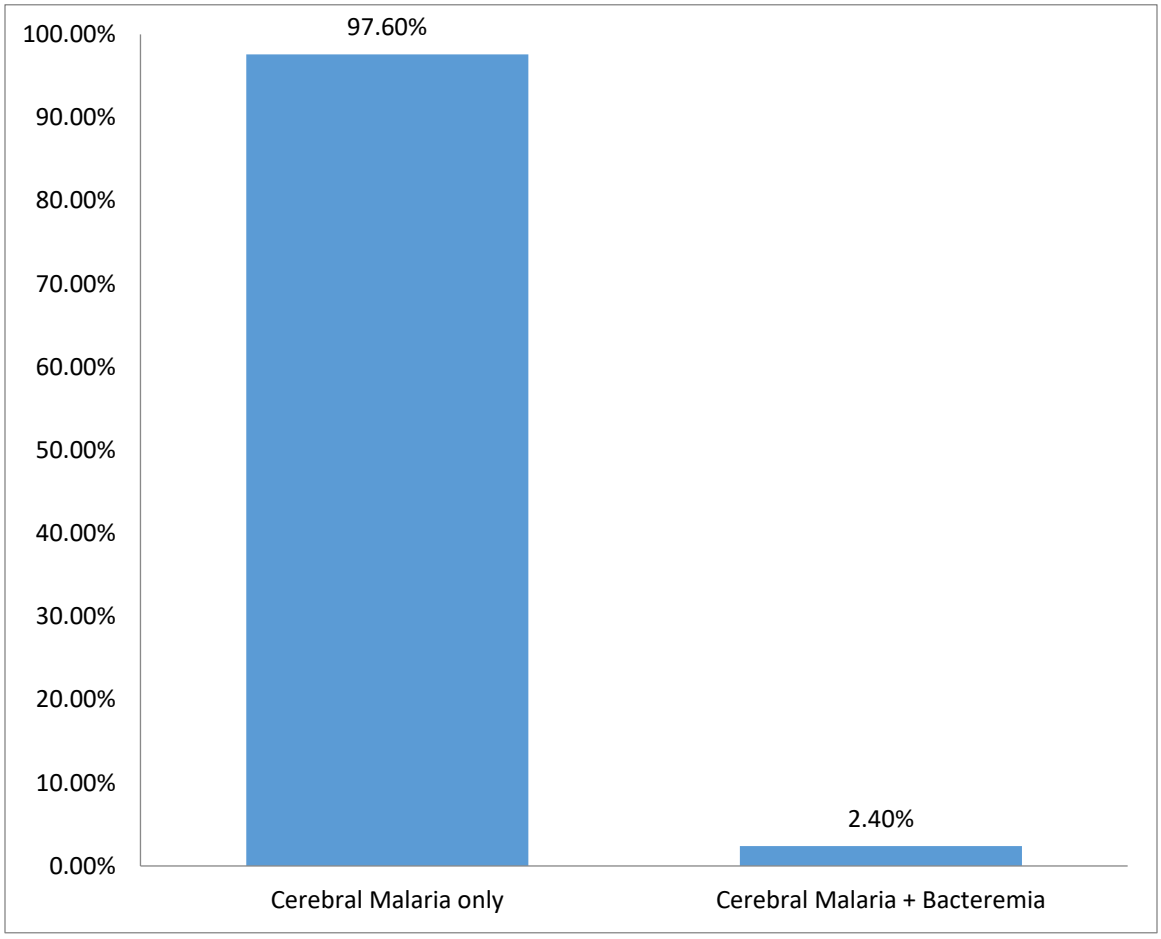


Figure 4.10 Percentage of cases of cases of cerebral malaria only and with bacteremia

4.9 Effect of parasite density on physical and laboratory findings

4.9.1. Parasite density and physical signs

The figure below shows the parasite densities recorded in some physical signs at initial evaluation of the patients recruited into the study. Parasite density in patients who stayed on coma for more than 48 hours were higher than those from 48 hours and below. Patients with fever had more parasites than those without fever.

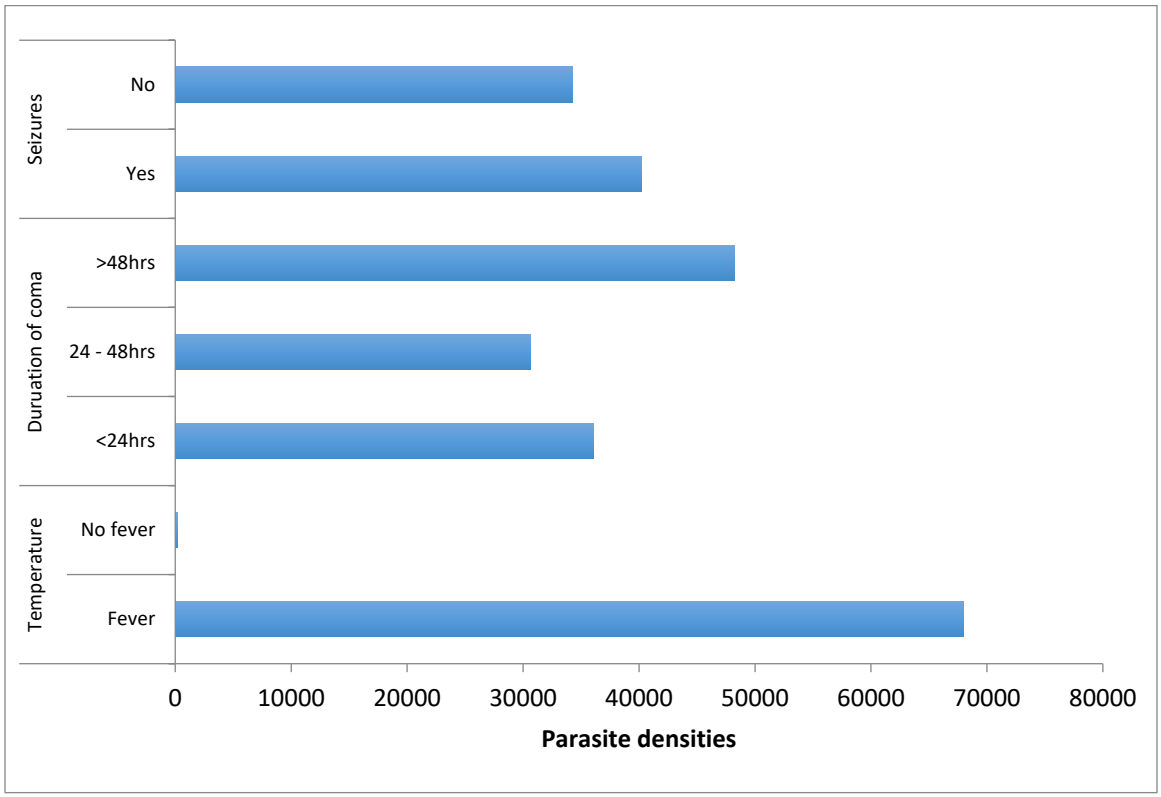


Figure 4.11 Physical signs and associated parasite densities

4.9.2 Parasite density and laboratory findings

Figure 4.12 below shows the parasite densities of patients with both abnormal and normal laboratory findings. Patients with low platelet count and severe to moderate anaemia recorded higher parasite densities.

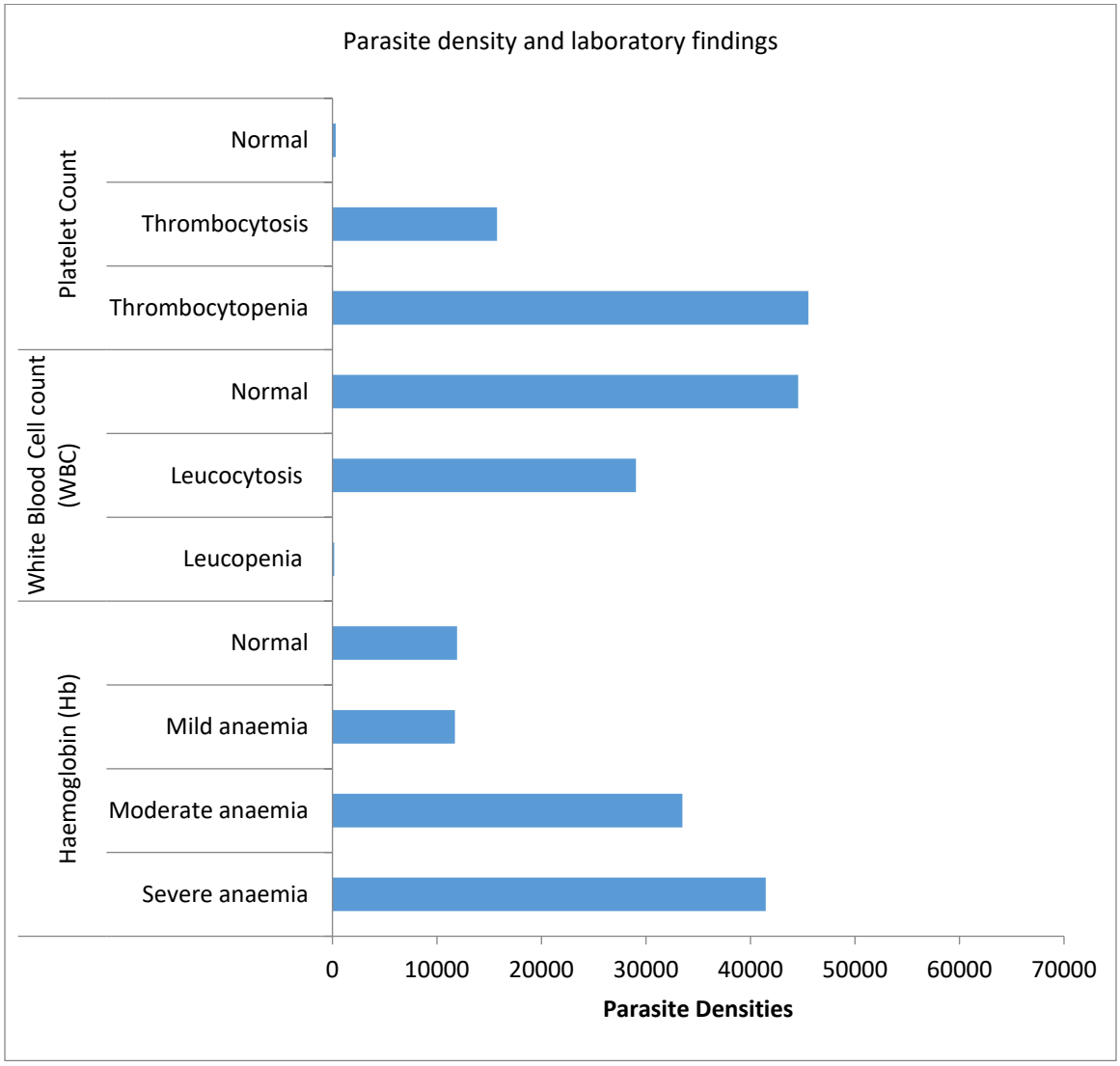


Figure 4.12 Laboratory findings and associated parasite densities

4.9.3 Parasite density per demographic characteristics and clinical presentation

The majority of demographic characteristic and parameters of clinical presentation explored did not demonstrate any effect by the extent of parasitaemia (P-value ≥ 0.05). The only exception was the duration of fever where the findings suggested that the greater the level of parasitaemia, the longer the duration (≥36hrs) of fever (P-value =0.02). (**Table 4.3**)

Table 4.3 Difference in mean levels of parasitemia by demographic characteristics and clinical presentations

Variable	Category	Frequency (%)	Mean parasitaemia	95% Conf. interval	P-value*
Sex	Male	48 (64.0)	39381.64	13090.8 - 65672.5	0.76
	Female	27 (36.0)	33129.00	7972.5 - 58285.5	
Age	< 5yrs	37 (50%)	41128.5	15173.6 - 67083.4	0.73
	≥5 yrs	37 (50%)	34429.7	5288.6 - 63570.7	
Reported Convulsion	Yes	88 (83.0%)	40202.93	18340.0 - 62066.9	0.78
	No	18 (17.0%)	34225.52	-7422.3 - 75873.32	
Liver	Palpable	34 (52.3%)	47860.71	2716.682 - 52788.61	0.34
	Not palpable	31 (47.7%)	27752.65	12721.43 - 83000	
Flaring of ala nasae	Yes	11 (15.1%)	62814.0	-12730 - 138358.4	0.26
	No	62 (84.9%)	32754.3	13815 - 51693.0	
Cola-like urine	Yes	10 (13.5%)	3302.2	-3892.4 -10496.9	0.16
	No	64 (86.5%)	42728.8	20960.5 – 64497.0	
Observed Convulsion	Yes	52 (70.3%)	35167.3	14858.0 – 559476.5	0.70
	No	21 (28.4%)	43599.6	-5032.2 – 92231.3	
Died	Yes	7 (9.6%)	37748.6	-19027.6 – 94524.7	0.98
	No	66 (90.4%)	37108.9	16695.5 – 57522.5	
Duration of fever	**< 36hrs**	**50 (52.1%)**	**11545**	**-3884.3 – 26974.3**	**0.02**
	≥36hrs	**46 (47.9%)**	**60598.9**	**17192.4**	
Peak Temp.	<39	68(55.7%)	32946.3	8350.6 – 57541.9	0.80
	≥39	54(44.3%)	38142.8	2109.3 – 74167.3	
Coma stay	<40hrs	60(52.6%)	42134.4	8950.6 – 75318.2	0.73
	≥40hrs	54(47.4%)	34251.7	2562.3 – 65941.0	
Number of convulsions	<3	88(83.0%)	41655.6	13584.6 – 69726.6	0.45
	≥3	18(17.0%)	18338.0	-9499.9 – 46275.8	

* Using two-tailed t-test for independent samples

CHAPTER 5

DISCUSSIONS

Malaria is curable when diagnosed and treated promptly with available antimalarial drugs. The disease may result in a variety of symptoms ranging from mild to complicated and even death if not treated promptly. Cerebral malaria, which is the severest form of malaria, involves the manifestation of Plasmodium falciparum infection that induces changes in the mental status and coma (Babikir, 2010). This study described the demographic profile and clinical presentations of children presenting with cerebral malaria in five referral facilities in the Greater Accra Region of Ghana. A total of 83 children between the ages of 1 and 12 years presenting with CM to these hospitals were recruited. This number of cases could be indicative of the incidence of CM within this region of Ghana (outside of Korle-Bu Teaching Hospital) during the study period as most cases of CM are referred to these five collaborating hospitals.

It is known that hospital admissions for malaria are concentrated in children under-5 years of age in all settings (Carneiro et al., 2010). Most of these hospital admissions result from malaria progressing from uncomplicated to severe malaria of which cerebral malaria forms the majority (Tetteh et al., 2016). In this current study, children from the age of 5 to 8 years and 1 to 5 years constituted 42.2% and 37.5% respectively of the total cases of cerebral malaria. About 20% of the children recruited were aged from 9 to 12 years. This study agrees with other findings that show that younger children are more vulnerable to cerebral malaria (Breman, 2001). A studies in the northern parts of Ghana also showed a significant decreasing trend towards increasing age in cerebral malaria cases (Oduro et al. 2007). It has also been reported that, in areas with less intense or seasonal transmission, cerebral malaria is frequent in slightly older children (Harrison & Matson 2003). This has implication for preventive measures that aim to address the worst effect of malaria. Studies have shown that precise age-specific pattern of disease depends upon intensity of

transmission in a given community (Nankabirwa et al., 2014). Greater Accra region is classified as a high malaria transmission population (WHO, 2016) and under conditions of intense transmission, younger children are more at risk from disease and death which usually results from the severe form such as cerebral malaria (Brooker et al., 2000).

Overall, 65% of the cases recruited in the study were males while 35% were females indicating a sex ratio of 1.7:1. Similar studies in Cameroun also reported almost twice as many males as females (sex ratio of 1.8: 1) (Monebenimp et al., 2010). In a study by (Gyan et al. 2009), more males (60%) than females (40%) were reported, which is consistent with this current study. However, similar studies in the Upper East region of Ghana reported 48.9% of cerebral malaria patients being males contrary to what was reported in this study and that of Gyan et al 2009 (Oduro et al. 2007). A 1:1 sex ratio has also been reported in similar studies in Nigeria (Oninia et al, 2015). Sex ratio in cerebral malaria could be dependent on demographic and socio economic factors in the communities where cerebral malaria cases are recorded. It has been suggested that females are more vulnerable to malaria as they participate more in household activities (Ayele et al. 2012). On the other hand, because they tend to be at home more often, malaria in females tend to be detected early and treated compared that in their male counterparts who are always out of home playing. The progression to severe malaria as seen in patients recruited for this study may therefore be quicker in males. This could explain why more males with cerebral malaria are recorded in deprived communities.

Even though several control measures are in place, Ghana still records greater than 1 case per 1000 population, thus being classified as a hyper-endemic malaria transmission zone (DHS ,2012). Several gains have been made with a decline in malaria incidence since 2007 (DHS, 2012). However such decline could not correspond with a decline in cerebral malaria cases as is observed

in this current study. Cerebral malaria incidence in the five major referral hospitals between during the study period did not show any significant reduction. A spike in cerebral malaria cases was observed in 2014.

Approximately 76% of cerebral malaria cases occurred between May and October during the study period. This coincides with the main rainy season in the southern part of Ghana. This is consistent with studies in the northern part of Ghana where cerebral malaria incidence was seasonal with 71% occurring during the main raining season there (June-October) (Oduro et al., 2007). Other studies have also found association between malaria transmission and rainfall patterns (Mabaso et al., 2007). Studies have shown that areas that have experienced decreased rainfall have seen a decline in malaria transmission (Small et al., 2003). However, as less attention is given to children most especially in the off-malaria season, spikes of severe malaria cases occur, as observed between November 2013 and April 2014 in this current study.

Cerebral malaria is invariably fatal if not treated promptly. Some studies have shown that the case fatality rate among children with cerebral malaria ranges from 15 – 20% even with treatment (Idro et al., 2010). Others have reported a case fatality rate of 8.9%. This current study report of a case fatality rate of 7.23%, which is lower than what was observed in other studies. In the study by Oduro et al, 2007, a case fatality of 29.8% was reported which is higher than what was reported in this study. Several factors may affect the case fatality rate in children with cerebral malaria and this may include early detection of clinical signs, accessibility to health facility as well as quality of care. The difference in the case fatalities reported in the two studies could be attributed to malaria intervention measures since 2007, easy accessibility and quality of care in Accra compared to the Upper East region of Ghana.

The clinical hallmark of cerebral malaria is coma, which is an alteration of the consciousness of the patient. All (100%) patients recruited into the study had altered consciousness with a Blantyre coma score below 4. Another common presenting sign observed in study participants was fever, which is defined by body temperature above 37.5ºC. Almost 63% of cases recorded in this study had fever upon initial clinical presentation. Other studies have reported other clinical presenting symptoms such as vomiting, diarrhoea and long duration of fasting (Monebenimp et al. 2010). Fasting can cause hypoglycemia and hence can deprive the child of energy which can lead to death (Monebenimp et al., 2010). Other common physical signs reported in this study included seizures (53%) and abnormal deep breathing (7.2%). Other studies have reported the presence of seizures in more than 50% of paediatric cases (Molyneux et al., 1989) which is consistent with findings in this current study.

The study showed that most (49%) of patients regained full consciousness between 24 to 48 hours. In a study by (Gordeax R et al., 1992), patients recovered between 24.1 and 43.1 hours after the correction of hypoglycemia. Survivors of cerebral malaria sustain brain injury and have long-term neurologic and cognitive deficits (Idro et al., 2006) and it has been shown that the longer the duration of coma the more severe or intense the neurological sequelae (Brewster et al., 1990). Therapies that would reduce the duration of coma will also reduce the severity of neurological sequelae after recovery from cerebral malaria.

This study reported a mean blood glucose level of 7.6mmol/L in the study participants. This is significantly higher ($p=0.001$) than the 6.2 mmol/L reported by Oduro et al, 2007 in a similar study in the Upper East region of Ghana. Children in Upper East often consume minimally processed whole grains compared to the milled and refined grains mostly consumed in southern Ghana. Milled and refined grains have a relatively higher glycemic index indicating a higher blood sugar

level. This could account for the differences in mean blood sugar levels observed in this current study and that done by Oduro et al, 2007.

Unlike other studies that have reported hypoglycemia as a common and serious manifestation of cerebral malaria (WHO, 2016), this current study showed more than 70% of cases having normal blood sugar levels and about 10% and 1% being hyperglycemic and hypoglycemic respectively. This is consistent with a study that reported 2 out of 162 cases of cerebral malaria with hyperglycemia (Dass et al., 2010). Some studies have reported association of hyperglycemia and severe malaria. For instance, Van Thien et al, 2001, reported that cerebral malaria stimulates glucose production to a greater extent than other forms of malaria. Hyperglycaemia in cerebral malaria, could also be a result of either associated sepsis or may be a stress response with increased counter regulatory hormones similar to those in the critically ill.

Thrombocytopenia has been identified as a key indicator of malaria in febrile patients (Erhart et al., 2004).This study recorded more than 50% of cases having thrombocytopenia. Other studies have also shown 56.7% of hospital admission due to cerebral malaria having thrombocytopenia (Ladhani et al., 2002) which is consistent with this study. A study by (D'Acremont et al., 2002) reported thrombocytopenia as the most common laboratory abnormality (60% of cases) in cerebral malaria. Another study on platelet in children admitted to hospitals showed that thrombocytopenia was common among children suffering from cerebral malaria (Amaral et al., 2003). About 6% showed thrombocytosis whereas less than 3% had normal platelet counts. This study has therefore confirmed the prominence of thrombocytopenia in cerebral malaria.

White blood cell (WBC) counts during malaria are generally characterized as being low to normal, a phenomenon that is widely thought to reflect localization of leukocytes away from the peripheral circulation. Data from CM patients showed 61% of cerebral malaria cases having normal WBC (4

to 10 x10^9L) with 35% having leukocytosis (WBC greater than 11 x 10^9 /L). Leukopenia was seen in 4% of cases. This is consistent with a study by (Khan & Malik, 1996) which showed sixty-eight patients having normal leukocyte count with 3 and 19 having leukopenia and leukocytosis respectively.

Anaemia is considered a measure of the cumulative impact of malaria on an individual patient (Kotepui et al., 2015). This study showed that most cerebral malaria cases had moderate anaemia (45%). Previous studies have shown anaemia in 86.7% (Amaral et al., 2003) and 30% (D'Acremont et al., 2002) in children presenting with cerebral malaria.

Studies have shown that in sub-Saharan Africa invasive bacterial infections are important concurrent infections in paediatric populations with severe malaria and other childhood illnesses (Berkley et al., 2005). In this study, 2.4% of cases had concurrent bacterial infection with cerebral malaria. In most cases, treatment is initiated at local health facilities or by guardians by administering antibiotics which could affect blood culture for concurrent bacteraemia determination in CM. Studies in Kenya and Malawi have shown 11.7% malaria co-infection with bacteraemia (Were et al., 2011). Other studies have suggested that there is ample evidence to suggest a causal association between malaria and NTS bacteraemia (Takem et al. 2014). A reduction in the burden of malaria is likely to simultaneously reduce the burden of NTS bacteraemia and should be a priority.

The World Health Organization (WHO) has listed hyper-parasitaemia as one of the criterion of severe falciparum malaria for more than two decades (WHO, 2016). Previous studies have shown that there is a correlation between parasite density and severity of malarial infections (Tangpukdee et al., 2012). Malaria patients with different malaria parasite burden exhibited important distinctive haematological parameters that are evident in changes in leukocyte count, platelet count and

haemoglobin concentration during the infection. These findings offer the opportunity to recognize and diagnose malaria and help support the treatment thereof, as well as relieve symptoms of cerebral malaria in endemic regions (Kotepui et al., 2015). In this current study, most of the clinical symptoms were not significantly affected by the parasite burden (see Table 4.3). Other factors including nitric oxide have been reported to have a significant effect on clinical signs and symptoms of CM (Clark et al., 1991). However, parasite densities in patients whose fever settled within 36 hours after initial clinical presentation were significantly lower than those whose fever lasted for more than 36 hours.

In the laboratory findings, higher parasite densities (>20000/uL) were observed in cerebral malaria patients which showed thrombocytopenia, leukocytosis, moderate and severe anaemia. In a study by (Kotepui et al., 2015), leukocyte counts, especially neutrophil granulocytes, were significantly higher in patients with high parasitaemia compared to those with low and moderate parasitaemia. Another study also found a consistent positive relationship between leukocyte counts and parasite density in Plasmodium-infected patients (McMaster et al. 2009; Kotepui et al. 2015).

In the same study by (Kotepui et al., 2015), a parallel trend in thrombocytopenia with parasitaemia where low platelet counts were associated with increased parasite density. Their study also suggested that persons with platelet counts < 150,000/L were 12−15 times more likely to have malaria than persons with platelet counts 150,000/L. Other studies have also shown that thrombocytopenia was strongly associated with percentage parasite (Ladhani et al., 2002). This association may be explained as resulting from sensitization induced by parasitized RBCs in platelets, with consequent increase in platelet sensitivity to adenosine diphosphate (ADP) and higher dense-granule secretion (Amireh et al., 2016). These alterations could promote platelet aggregation on the endothelium, such as found in cerebral malaria (Grau et al., 2003).

High parasitaemia due to *Plasmodium falciparum* infection takes a serious turn in anaemia (Bashawri et al., 2002). Moreover, excessive haemolysis of parasitized RBCs in malaria infection may lead to anaemia (Ekvall, 2003). In this current study, higher parasite densities were associated with moderate to severe anaemia. Other studies have also shown that at higher levels of parasitaemia and excessive haemolysis of parasitized RBCs may lead to anaemia (Kotepui et al., 2015). A previous study also showed that anaemia was observed in 72.4% of patients with a high parasite count, as well as an inverse relationship between parasite densities and haemoglobin levels (Ali et al., 2008) Other studies also showed that RBC counts and haemoglobin were significantly reduced in high parasitaemia patients, which is consistent with previous studies showing a significant increase in the prevalence of anaemia with increase in parasite density (Kitua et al., 1997)

Results from this study showed that most clinical features are independent of extent of level of parasitaemia. This suggests there are other mediators of the occurrence of cerebral malaria and not merely parasitaemia. Studies have shown that an array of pro- and anti-inflammatory cytokines, chemokines, growth factors, and effector molecules are released by the host in cerebral malaria (Perkins et al. 2011). Nitric oxide has long been known to be one of such mediators (Clark et al. 1992). Research is also on-going to identify immunological mediators of CM and the findings in this study confirm the need for such studies. Consideration of these mediators in future studies cannot be overemphasized.

Microscopy was the only method of detection for malaria parasites in this current study. A hallmark of the pathogenesis of cerebral malaria is the sequestration of infected RBCs in the brain vasculature. This means that the effect of malarial parasitaemia on haematological parameters may be underestimated. Moreover, studies have shown that high parasitaemia, even without

complications, can lead to high mortality, which can reach up to 50% in patients with parasitaemia greater than 10% in areas of low transmission (WHO, 2012).

Public health relevance of CM Study

Even though several efforts have been put in place to address the burden of malaria, not much has been achieved in reducing morbidity and mortality from cerebral malaria in children, especially in sub-Saharan Africa. The disease still progresses to the severe form which is associated with neurological sequelae including memory impairment, behavioural disturbances and other cognitive disorders. Early identification of the clinical and laboratory findings could help achieve the public health goal of secondary level of disease prevention which aims at reducing the impact of the severe form of malaria aforementioned. Hyperparasitaemia alone does not account for the clinical features of cerebral malaria as seen from this study. Studies into other factors which could influence the occurrence of these clinical features, especially immunological markers could be of immense importance to the extent that they could form the basis of improved therapeutic options to prevent the neurological complications.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The study has shown that cerebral malaria occurs more frequently in children below the age of 9 years and most common in young males than females. It has also shown that CM is seasonal and more frequent in the rainy season in the Greater Accra region of Ghana.

The study concludes that coma, fever and seizures are the commonest clinical presentations whiles thrombocytopenia and moderate anaemia are the commonest laboratory findings in children with cerebral malaria in the Greater Accra Region. Bacteremia in CM has also been shown to be less prevalent in cerebral malaria patients.

The study concludes that the common clinical presentations are not influenced by the parasite burden and that other mediators may play a major role in the clinical presentations observed in cerebral malaria cases. Low haemoglobin levels and low platelet counts are however associated with high parasite densities

6.2 Recommendations

The study recommends that:

1. Age-targeted strategies such as provision of bed nets and intermittent preventive treatment aimed at reducing the burden of malaria in children could be intensified as burden of cerebral malaria in the younger children may be underestimated.
2. Particular attention could be given to young males since it has been shown that CM is more prevalent in young males in the Greater Accra region of Ghana.
3. Preparedness towards the rainy season be intensified by:

a. Increasing human resource allocation to intensify public education on adopting more preventive measures.
b. Consideration of seasonal chemo prophylaxis by the Ministry of Health

4. Educating parents and guardians on early clinical signs such fever, altered consciousness (coma) and seizures, which have been shown to be common in cerebral malaria.
5. Medical officers intermittently checking on parasite burden on patients, as high parasitaemia has been associated with low platelet counts and anaemia which are considered prognostic to cerebral malaria.
6. Further studies into mediators that influence the clinical features of cerebral malaria is highly recommended.

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APPENDICES

Appendix 1: Consent for children with malaria

Title: Severe Malaria Study

Principal Investigator: Ben Gyan, PhD

Address: Department of Immunology, NMIMR, Box LG 581, Legon

Information: (To be read or translated to parents/guardians in their own mother tongue)

Dear Volunteer,

This consent form contains information about the research entitled *Circulating endothelial cells and the pathogenesis of malaria.* In order to be sure that you are informed about being in this research, we are asking you to read (or have read to you) this Consent Form. You will also be asked to sign it (or make your mark in front of a witness). We will give you a copy of this form. This consent form might contain some words that are unfamiliar to you. Please ask us to explain anything you may not understand.

Why this study is planned

Your child is being asked to participate in the above study in order to find out factors in the blood that may be of risk to severe malaria. Malaria is caused by a germ that is passed from one person to the other by the bite of a mosquito that carries the malaria germ. Malaria is a very serious health problem in Ghana, as it is in many African countries. We do not know why some children become severely ill from malaria or why some of those children die from malaria. To understand this problem we need to study children who come to the hospital with severe malaria and compare them to children who have less severe malaria, and to other children who are feeling well. The purpose of the study is to find out what factors they already have in their blood that may make them severely sick when they have malaria. If we can find the answer to this question, we hope to be able to suggest new ways of controlling such severe sicknesses in malaria.

General Information and your part in the study

For a child to qualify to be part of this study that child should be between the ages of 1 and 12 years. If your child/ward agrees to be in the study, we will collect venous blood sample for laboratory diagnosis and 2 ml (teaspoonful) for our research at the time of admission. We may contact you at home by phone, or in person to schedule another visit and to see if you still want to take part in the research. When this contact is made you will not be identified as being in this research.

Possible Benefits

There are no direct benefits to your child from this study. However, his/her participation may help us develop better malaria treatment. He/she will not be paid for participation in this study but you will be reimbursed for your time and travel during the follow up visits.

Possible Risks

The amount of blood collected is harmless, although there may be a slight pain and bruising at the bleeding site. All subjects will receive appropriate treatment as necessary. Sterile techniques and disposable, single-use equipment will be used at all times.

Withdrawal from study

We would like to stress that this study is strictly voluntary. Should the child decide not to participate; it will have no consequences for him/her. Should the volunteer, at any point during the study, decide that he/she do not wish to participate any further, you are free to terminate the participation, effective immediately. Any such decision will be respected without any further discussion. Your decision will not affect the health care you would normally receive.

Visits

If the child misses a scheduled visit, we may contact you at home by phone, or in person to schedule another visit and to see if you still want to take part in the research. When this contact is made you will not be identified as being in this research.

Confidentiality

All information gathered would be treated in strict confidentiality. We will protect information about your child taking part in this research to the best of our ability. The child will not be named in any reports. However, the staff of [list all groups that may access the research records] may sometimes look at his/her research records. If you have any questions, please feel free to ask the physician in charge. Someone from the IRB or Ethical Committee might want to ask you questions about being in the research, but you do not have to answer them. A court of law could order medical records shown to other people, but that is unlikely.

Contacts: If you ever have any questions about the research study or study-related problems, you may contact Prof. Ben Gyan of the Noguchi Memorial Institute for Medical Research (0244 726016) at any time. For questions about the ethical aspects of this study or your rights as a volunteer, you may contact Dr. Samuel Ayete-Nyampong, Chairman, Institutional Review Board, NMIMR, University of Ghana (0302 501178/9) or Chairman of the Ghana Health Service Ethical Committee (Tel. 0302 681109)

Your rights as a participant

This research has been reviewed and approved by the NMIMR IRB and Ghana Health Service Ethical Committee. An IRB or Ethical Committee is a committee that reviews research studies in order to help protect participants. If you have any questions about your rights as a research

participant you may contact [Dr. Samuel Ayete-Nyampong, Tel 0302-501-178/179 or Chairman of the Ghana Health Service Ethical Committee (Tel. 0302 681109)

VOLUNTEER AGREEMENT

The above document describing the benefits, risks and procedures for the research title *Circulating endothelial cells and the pathogenesis of malaria* has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree my child/ward to participate as a volunteer.

---------- ----------

Date Signature or Thumbprint of volunteer

If volunteer's Parent/Guardian cannot read the form themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer's Guardian/Parent has agreed to take part in the research.

---------- ----------

Date Signature or Thumbprint of witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

---------- ----------

Date Signature Person who obtained Consent

Appendix 2: Assessment forms/questionnaires patients

SEVERE MALARIA STUDY

CEREBRAL MALARIA COHORT

INITIAL ASSESSMENT/QUESTIONNAIRE

Initial Clinical Presentation

I.1 Study ID number .....|__|__|__|__|__|__|

I.2 Date of Admission ..... ___/___/___

1.2a Date of recruitment into study……..……__/___/___

I.3 Time of recruitment into study ..... ___:_(AM or PM)

I.4 Name……_____

1.5 Hospital name (circle) PML__Ridge__La General___ Tema General ___LEKMA

I.5a Hospital Folder #...…… |__|__|__|__|__|__|

General Exclusion Criteria

G.1 Pre-existing neurological disease (1=yes, 2 =no) .....|__|

G.2 Recent severe head trauma (within 3 months)(1 = yes 2 = no) .....|__|

G.3 Other cause of coma e.g. DM (1 = yes, 2 = no) .....|__|

G.4 Blantyre Coma Score>3 (1=Yes, 2=No) …… |__|

G.5 Blantyre Coma Score >3 **within 5 min of correction for hypoglycemia**(1=Yes,2=No)|__|

G.6 Duration of coma < 60 mins (1=Yes, 2=No) ……. |__|

G.7 Other febrile illness (1=Yes, 2=No) …… |__|

G.8 Recent severe bleeding i.e. within 3 months (1=yes 2=no) .....|__|

G.9 Other causes of anaemia including SCD and Sickle Cell trait (1= yes, 2=no) ..... |__|

G.10 Obvious clinical evidence of bacterial infection (1=yes, 2=no) .....|__|

G.11 Obvious clinical evidence of viral infection (1=yes, 2=no) .....|__|

G.12 History of Diabetes Mellitus (1=yes, 2=no) .....|__|

G.13 History of cardiovascular disease (1=yes, 2=no).....|__|

G.14 History of Hypertension (1=yes, 2=no) .....|__|

G.15 History of increased cholesterol (1=yes, 2=no)…… .....|__|

G.16 History of surgery within 1 month? (1=yes, 2=no).....|__|

G.17 History of bone fracture within 3 months (1=yes, 2=no).....|__|

G.18 History chronic viral infection (chronic weight loss, diarrhea) (1=yes, 2=no).....|__|

NOTE –exclude HIV patients

G.19 Major trauma (car accident, etc.) within 1 month (1=yes, 2=no) .....|__|

G.20 Transfusion within past 3 months (1=Yes, 2=No).....|__|

G.21 Severe pallor (1=yes, 2=no)…… |__|

G.22 *****CONSENT REQUIRED****** please indicate obtained, 1=Yes 2=No.....|__|

G.23 Ethnic origin.....|__|

(Akan=1, Ga-Adangme=2, Ewe=3, Hausa=4, Frafra=5, Dagomba=6

Non-Ghanaian=7, Other=8 Specify _____)

G.24 Area of residence/Direction to your house

_____

_____ ****Cell phone #_____

G.25 Sex (1=M, 2=F).....|__|

G.26 Age (Last half year passed).....|__|__|.|__|

G.27 Referral on the basis of a lab report positive for malaria parasites (1=Yes, 2=No)|__|

G.28 History of a febrile illness in the preceeding 2 weeks (1=Yes, 2= No) .....|__|

G.29 Duration of symptoms before presentation (Days same day = 1).....|__|

G.30 History of other antimalarial for this attack (1=yes, 2=no, 9=DK*) .....|__|

G.31 If yes specify: …….

G.32 Reported cola urine (1=Yes, 2=No 9=DK).....|__|

G.33 Observed cola urine (1=Yes, 2=No) .....|__|

G.34 Reported convulsions (1=Yes, 2=No 9=DK).....|__|

G.35 Already seen at this hospital for this attack? (1=Yes, 2=No) .....|__|

G.36 If yes initial antimalarial prescribed (**…….**)

Physical exam, vital signs and laboratory results

P.1 Best Motor Response (0-2).....|__|

P.2 Best Verbal Response (0-2).....|__|

P.3 Eye Movements (0-1) .....|__|

P.4 Total Coma Score (0-5) .....|__|

P.5 Duration of Coma (0=no coma, 1=0-60 mins, 2=60+ mins).....|__|

P.6. Observed Convulsions (1=Yes, 2=No).....|__|

P.7 Alar flare (1=Yes, 2=No).....|__|

P.8. Chest (subcostal, intercostal) Recession (1=Yes, 2=No) .....|__|

P.9 Abnormally deep breathing (1=Yes, 2=No).....|__|

P.10 Use of Accessory muscles (supraclavicular/suprasternal recessions) (1=Yes, 2=No)|__|

P.11 Fast breathing (1-4yr>40/min, >5yr>30/min) (1=yes, 2=no).....|__|

P.12 Respiratory Distress (1=Yes, 2=No)…… |__|

P.13 ∗Peripheral O_2 saturation (**for all patients with resp. distress**)..... |__|__|

P.14 Temperature.....|__|__|.|__|

P.15 Weight (in kgs) .....|__|__|.|__|

P.16 Height (in cms) ..... |__|__|__|

P.17 Blood Pressure (mmHg) .....|__|__|__|/|__|__|

P.18 Pulse..... |__|__|__|

P.19 State of hydration (1=normal, 2=impaired, i.e., ↓ skin turgor or dry mouth) .....|__|

P.20 Recruited into study (1=Yes, 2=No).....|__|

Samples (Please tick when taken):

S.1 ___ EDTApurple top (EPC sample) S.5 ___CXR (for respiratory distress)

S.2 ___ EDTA purple top (FBC-full blood count) S.6 ___CSF (for CM)

S.3 ____ Heparin tube(s)

S.4 ____ Blood Culture

S.5 _____PAX-gene tube (RNA)

Results initial studies at recruitment:

R.1aDate sample obtained (____/____/____)

R.1 RBS: Glucometer (mmol/L)..…… |__||__|.__|

R.2 Haemoglobin (Hb)..... |__|__|.|__|

R.3 WBC ($X10^9/\mu L$)…… |__|__|.|__|

RBC ____($X10^6/\mu L$) Hemoglobin (Hb)_____(g/dL) HCT____ (%) MCV_____(fL)

MCH_____(pg) MCHC____(g/dL) PLT_____ ($X10^3/\mu L$)

R.4 Blood film species (1=p.f.,2=p.m.,3=p.o., 4=p.v.,5=p.f.+p.m.,6=p.f.+p.o.).....|__|

R.5 Parasite density (per μL)_____

R.6 Relative abundance (negative smear, 1+, 2+, 3+, 4+)……|__|

R.7 Asexual stage, density per μl .....|__|__|__|__|__|__|__|

R.8 Sickling status (1=positive, 2=negative).....|__|

NOTE- If positive then exclude the from study

R.9 Blood culture results (1=positive, 2=negative .....|__|

R.10 If positive organism cultured_____

INPATIENT CM MONITORING CHART

See footnote(s)/codes end of form		**Day0** ***date_____**	**Day 1** ***date_____**	**Day 2** ***date_____**	**Day 3** ***date_____**
M.1 Asexual parasite count (per μL) AM/date (If initial smear negative)**Ψ**					
2 AM	M.27 Coma score†				
	M.28 Temp.^{o}C				
	M.29 BP				
	M.30 Pulse/Resp Rate	P___ /RR___	P___ /RR___	P___ /RR___	P___ /RR___
	M.31 Staff name				
6 AM	M.2 Coma score†				
	M.3 Temp. ^{o}C				
	M.4 BP				
	M.5 Pulse/Resp Rate	P___ /RR___	P___ /RR___	P___ /RR___	P___ /RR___
	M.6 Staff name				
10 AM	M.7 Coma score†				
	M.8 Temp. ^{o}C				
	M.9 BP				
	M.10 Pulse/Resp Rate	P___ /RR___	P___ /RR___	P___ /RR___	P___ /RR___
	M.11 Staff name				
2 PM	M.12 Coma score†				
	M.13 Temp.^{o}C				

	M.14 BP				
	M.15 Pulse/Resp Rate	P___ /RR___	P___ /RR___	P___ /RR___	P___ /RR___
	M.16 Staff name				
6 PM	M.17 Coma score†				
	M.18 Temp. ^{o}C				
	M.19 BP				
	M.20 Pulse/Resp Rate	P___ /RR___	P___ /RR___	P___ /RR___	P___ /RR___
	M.21 Staff name				
10 PM	M.22 Coma score†				
	M.23 Temp ^{o}C				
	M.24 BP				
	M.25 Pulse/Resp Rate	P___ /RR___	P___ /RR___	P___ /RR___	P___ /RR___
	M.26 Staff name				

HOSPITAL (INPATIENT) COURSE SUMMARY

CS.1a **Date of discharge*_____CS.1b MD/RN completing summary_____**

Clinical Course (Refer to page prior pages for clinical notes/information)

CS.1 Highest temperature first 24 hours..... |__|__|.|__|

CS.2 Time for fever to settle (hrs) |___|___|___|

(from admission till first time temp. falls to <37.5^{o}C for at least 48 hours)

CS.3 Time for coma score to reach 5 (hrs) |___|___|___|

CS.4 Antimalarial changed before discharge? (1=Yes, 2=No)……. .....|__|

CS.5 If yes; what was it changed to.....

CS.6 Day of change (Day 0 = Admission day).....|__|

CS.7 Any other drugs (1=Yes, 2=No) .....|__|

CS.8 If yes, specify _____

CS.9 Blood C/S (1=positive‡, 2=negative, 3=not investigated, 9=missing) .....|__|

CS.10 If positive organism cultured _____

CS.11 Antibiotics prescribed (1=yes, 2=no)…….....|__|

CS.12 Name of antibiotic given_____

CS.13 CSF C/S (1=positive‡, 2=negative, 3=not investigated, 9=missing) .....|__|

CS.14 If positive organism cultured=_____

CS.15 Antibiotics prescribed (1=yes, 2=no)……...|__|

CS.16 Antibiotic given 1=ceftriaxone 2=other, give name_____|__|

CS.17 HB ≤ 5 g/dL during admission (1=Yes, 2=No) .....|__|

CS.18 Blood transfusion (1=Yes, 2=No).....|__|

CS.19 If yes day of transfusion (day 0=day of admission).....|__|

CS.20 Coca cola urine (1=Yes, 2=No) .....|__|

CS.21 Respiratory distress (1=Yes, 2=No).....|__|

CS.22 Total number of convulsions.....|__|

CS.23 Duration of longest convulsion (minutes).....|__|

CS.24 Died (1=Yes, 2=No) .....|__|

CS.25 Interval between admission and death (hours) (10=NA)..... |__|__|__|

CS.26 Postmortem done (1=Yes, 2=No) (10=NA).....|__|

CS.27 Additional / Differential Diagnoses ....._____

Detailed information regarding address/contact_____

CS.28 Patient to continue in the study (1=Yes, 2=No)..…….|__|

CS.29 If excluded give reason _____

CS.29 Scheduled days/times for return:

CS.30 7 days post discharge=_____14 days post discharge=_____

CS.31 Name MD who discharge patient/completed form_____

Appendix 3: Institutional Review Board Approval

ENSIGN COLLEGE OF PUBLIC HEALTH - KPONG

OUR REF: ENSIGN/IRB/M2
YOUR REF:
Tel: +233 245762229
Email: irb@ensign.edu.gh
Website: www.ensign.edu.gh

P. O. Box AK 136
Akosombo
Ghana

21st November, 2016.

INSTITUTIONAL REVIEW BOARD SECRETARIAT

Patience Okantey
Ensign College of Public Health.

Dear Ms. Okantey

OUTCOME OF IRB REVIEW OF YOUR THESIS PROPOSAL

At a meeting of the INSTITUTIONAL REVIEW BOARD (IRB) of Ensign College of Public Health held on 16th and 17th November 2016, your proposal entitled "**Emerging trends of Severe Falciparum malaria and bacteremia in children in Accra over a 4 year period (2012-2016)** "was considered.

Your proposal has been approved for data collection in the following settings:

1. Obtain a secondary data with permission from Noguchi Memorial Institute for Medical Research.

We wish you all the best.

Sincerely,

Dr (Mrs) Acquaah-Arhin
(Chairperson)

Cc. Dean of Ensign College.
Cc: Ag. Academic Registrar, Ensign College.